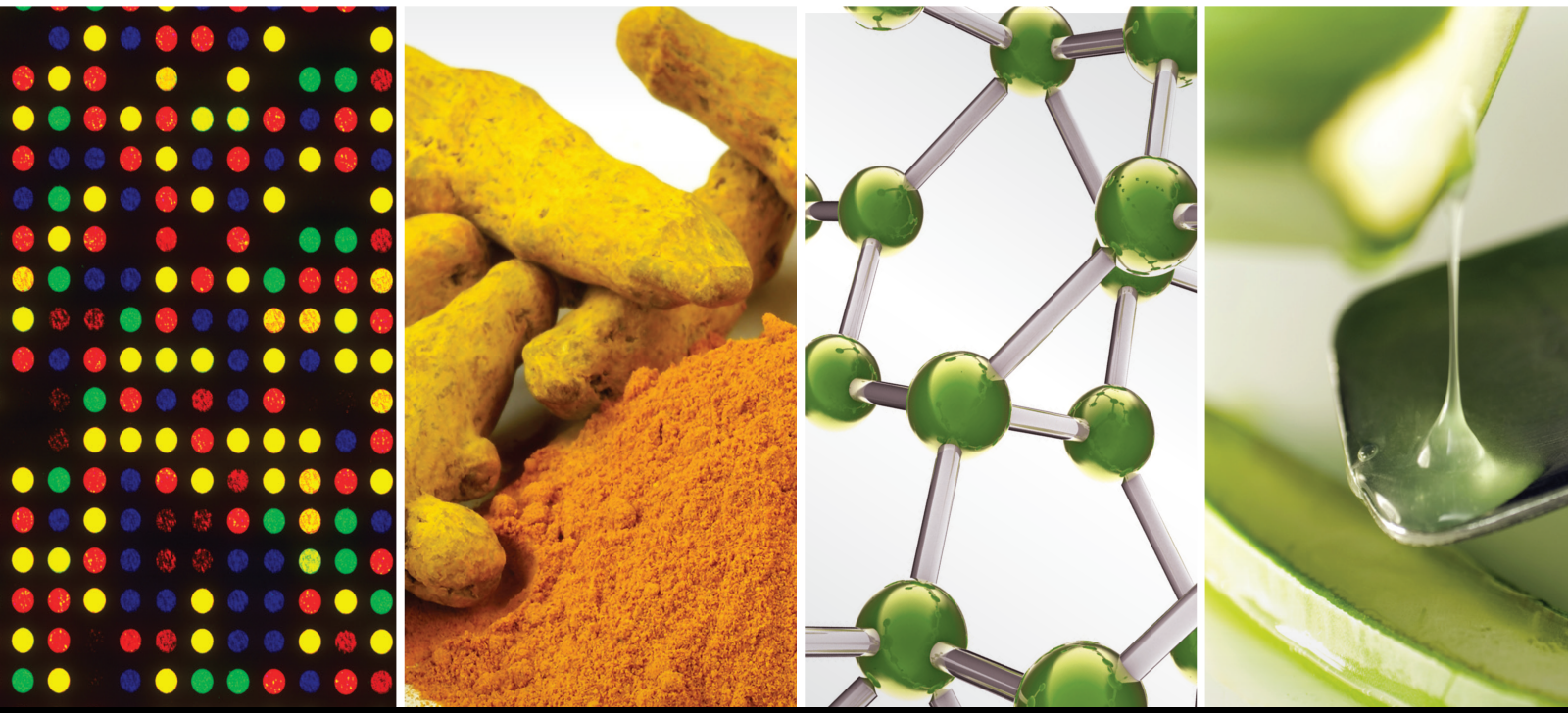
Evidence-Based Complementary and Alternative Medicine

Effects and Molecular Mechanisms of Non-Drug Therapy on Neural Plasticity and Repair after Stroke

Lead Guest Editor: Peng-Yue Zhang
Guest Editors: Yu-Long Bai, Yunping Deng, and Xiangjian Zhang



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Chief Editor

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Evidence-Based Complementary and Alternative Medicine

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[1] F. Gui, X. Yu, Y. Wu, C. Wu, and Y. Zhang, "Mechanism of LncHOTAIR Regulating Proliferation, Apoptosis, and Autophagy of Lymphoma Cells through hsa-miR-6511b-5p/ATG7 Axis," *Evidence-Based Complementary and Alternative Medicine*, vol. 2022, Article ID 2166605, 10 pages, 2022.

Hindawi
Evidence-Based Complementary and Alternative Medicine
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Retraction

Retracted: NEK2 Serves as a Novel Biomarker and Enhances the Tumorigenicity of Clear-CellRenal-Cell Carcinoma by Activating WNT/β-Catenin Pathway

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Hindawi
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https://doi.org/10.1155/2023/9829428

Retraction

Retracted: LncRNA-PAX8-AS1 Silencing Decreases Cell Viability, Enhances Apoptosis, and Suppresses Doxorubicin Resistance in Myeloid Leukemia via the miR-378g/ERBB2 Axis

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Hindawi
Evidence-Based Complementary and Alternative Medicine
Volume 2023, Article ID 9826952, 1 page
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Retraction

Retracted: Camrelizumab and Apatinib Combined with Radiotherapy Is Effective in Advanced Oligometastatic Non-Small-Cell Lung Cancer

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Hindawi
Evidence-Based Complementary and Alternative Medicine
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Retraction

Retracted: Effect of Percutaneous Nephrolithotomy Combined with Needle Nephrolithotomy on Renal Function and Complication Rate in Patients with Complex Renal Calculi

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Retraction

Retracted: Comparison of Iliac Bone Transplantation with Bone Transport in the Treatment of Femur Fracture and Bone Defect

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Hindawi
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Volume 2023, Article ID 9819238, 1 page
https://doi.org/10.1155/2023/9819238

Retraction

Retracted: miR-28-5p's Targeting of GAGE12I Inhibits Proliferation, Migration, and Invasion of Gastric Cancer in Vitro

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Volume 2023, Article ID 9819124, 1 page
https://doi.org/10.1155/2023/9819124

Retraction

Retracted: MiR-19b-3p Attenuates Chondrocytes Injury by Inhibiting MAPK/NF-Kb Axis via Targeting SOCS1

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Hindawi
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Volume 2023, Article ID 9813924, 1 page
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Retraction

Retracted: Carbetocin Controls Intraoperative Blood Loss and Thickness of Myometrium in Scar Uterus Cases

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Retraction

Retracted: Multivariate Analysis of Recurrence after Hysteroscopic Diagnosis and Treatment of Endometrial Polyps following IVF-ET Failure

Evidence-Based Complementary and Alternative Medicine

Received 20 June 2023; Accepted 20 June 2023; Published 21 June 2023

This article has been retracted by Hindawi following an investigation undertaken by the publisher [1]. This investigation has uncovered evidence of one or more of the following indicators of systematic manipulation of the publication process:

(1) Discrepancies in scope
(2) Discrepancies in the description of the research reported
(3) Discrepancies between the availability of data and the research described
(4) Inappropriate citations
(5) Incoherent, meaningless and/or irrelevant content included in the article
(6) Peer-review manipulation

The presence of these indicators undermines our confidence in the integrity of the article's content and we cannot, therefore, vouch for its reliability. Please note that this notice is intended solely to alert readers that the content of this article is unreliable. We have not investigated whether authors were aware of or involved in the systematic manipulation of the publication process.

In addition, our investigation has also shown that one or more of the following human-subject reporting requirements has not been met in this article: ethical approval by an Institutional Review Board (IRB) committee or equivalent, patient/participant consent to participate, and/or agreement to publish patient/participant details (where relevant).

Wiley and Hindawi regrets that the usual quality checks did not identify these issues before publication and have since put additional measures in place to safeguard research integrity.

We wish to credit our own Research Integrity and Research Publishing teams and anonymous and named external researchers and research integrity experts for contributing to this investigation.

The corresponding author, as the representative of all authors, has been given the opportunity to register their agreement or disagreement to this retraction. We have kept a record of any response received.

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[1] J. Xu, B. Liu, X. Feng, L. Shen, and Q. Qu, "Multivariate Analysis of Recurrence after Hysteroscopic Diagnosis and Treatment of Endometrial Polyps following IVF-ET Failure," *Evidence-Based Complementary and Alternative Medicine*, vol. 2022, Article ID 4140022, 8 pages, 2022.

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Retraction

Retracted: Paeoniflorin Protects H9c2 Cardiomyocytes against Hypoxia/Reoxygenation Induced Injury via Regulating the AMPK/Nrf2 Signaling Pathway

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References

[1] W. Yu, H. Sun, Y. Tan, and W. Zhang, "Paeoniflorin Protects H9c2 Cardiomyocytes against Hypoxia/Reoxygenation Induced Injury via Regulating the AMPK/Nrf2 Signaling Pathway," *Evidence-Based Complementary and Alternative Medicine*, vol. 2022, Article ID 7667770, 8 pages, 2022.

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Retraction

Retracted: Low-Dose Apatinib Improves the Prognosis of Patients with Recurrent High-Grade Gliomas

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[1] M. Zhang, L. Gao, X. Liu et al., "Low-Dose Apatinib Improves the Prognosis of Patients with Recurrent High-Grade Gliomas," *Evidence-Based Complementary and Alternative Medicine*, vol. 2022, Article ID 3181133, 6 pages, 2022.

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Retraction

Retracted: Elevated Plasma Interleukin-35 as a Prognostic Indicator in Localized Clear Cell Renal Cell Carcinoma

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[1] J. Zhang, X. Xu, Z. Chen, Z. Zhu, and J. Hou, "Elevated Plasma Interleukin-35 as a Prognostic Indicator in Localized Clear Cell Renal Cell Carcinoma," *Evidence-Based Complementary and Alternative Medicine*, vol. 2022, Article ID 6886590, 9 pages, 2022.

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Retraction

Retracted: Application of Meditation Relaxation Training and Rosenthal Effect in Patients with Adenoidectomy

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[1] W. Jun and Y. Tian, "Application of Meditation Relaxation Training and Rosenthal Effect in Patients with Adenoidectomy," *Evidence-Based Complementary and Alternative Medicine*, vol. 2022, Article ID 1420639, 5 pages, 2022.

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Review Article

Effect and Mechanism of Traditional Chinese Medicine Exercise Therapy on Stroke Recovery

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Stroke is currently the second largest contributor to disability-adjusted life years (DALYs) in developing countries, and it is the third largest contributor to DALYs in developed countries. It requires a large number of resources from the health care system every year, which places a great burden on society, families, and individuals. The treatment of traditional Chinese medicine exercise therapy (TCMET) during stroke recovery has become a hot topic of current research due to its few adverse events and high efficiency. This article sorts out the latest progress of TCMET on the recovery of stroke through the review method and explores its role and mechanism based on existing clinical and experimental studies. TCMET treatment of stroke recovery mainly includes Tai Chi, Baduanjin, Daoyin, Yi Jin Jing, five-fowl play, and six-character tips, which can effectively improve motor function, balance and coordination ability, cognitive dysfunction, nerve function, depression or emotional state, daily living ability, and so on after stroke. The mechanisms of stroke treated by TCMET are discussed, and deficiencies in the literature are discussed and analyzed. It is hoped that some guiding suggestions will be provided for future clinical treatment and experimental studies.

1. Introduction

Stroke disease is also known as stroke. It is a type of disease that is mainly manifested by sudden coma, unconsciousness, half-body failure, tongue tilting, and unfavorable speech [1]. It is equivalent to the cerebrovascular accident (CVA) in modern medicine. According to the pathological results, stroke is divided into ischemic stroke, cerebral hemorrhage, subarachnoid hemorrhage, and mixed stroke. According to the 2020 Chinese Stroke Association Guidelines for Clinical Management of Cerebrovascular Diseases, stroke is clinically divided into three distinct stages: the acute stage, the recovery period, and the sequelae period. The acute phase refers to the onset of illness within 1 month. The recovery period is 1 month to half a year after the onset of the disease, and half a year later is the sequelae period [2].

Stroke is currently the second largest contributor to disability-adjusted life years (DALYs) in developing countries, and it is the third largest contributor to DALYs in developed countries [3]. Globally, 132.1 million DALYs have been lost [4]. This requires significant resources from the healthcare system [5]. The incidence of stroke varies greatly according to the age structure of the population under study. With age, the incidence increases dramatically in both men and women. The risk of disease in children under 15 years of age is 1 in 100,000, while the risk of disease in people aged 85 years and older is 1 in 33. The incidence of stroke from the age of 55 to the age of 84 more than doubles every decade [6]. Stroke deaths in China in recent years have accounted for one-third of the total number of stroke deaths worldwide [7, 8]. There are certainly regional differences in the incidence of the disease, mostly in the northern and central regions. About 70% of stroke survivors have some degree of disability, including physical and mental disabilities, mobility restrictions, and participation restrictions [9]. This increases the burden of stroke-related disabilities in China and other countries around the world, putting enormous pressure on society, families, and individuals. With the advent of an aging population, these problems will become more pronounced in the future. How to solve the problem of

disability caused by stroke will become the direction of continuous exploration in this research field.

Traditional Chinese medicine exercise therapy (TCMET) is also known as Gong Fa. Under the guidance of holism in traditional Chinese medicine (TCM) and treatment based on syndrome differentiation, there are certain exercise principles and operating methods, and through the coordination and unification of exercise, breathing, consciousness, and self-tuina, that is, the combination of "body adjustment, heart adjustment, and pranayama," a kind of exercise therapy is used to prevent and treat diseases and strengthen the body. During the exercise, it is required to relax the body and mind, pay attention to softness and rigidity, and static braking. In the state of relaxation and naturalness, body movement is used to practice shape, and breathing exercise is used to practice qi, while mind guidance is used to restore the normal operation of qi and blood around the body, so that the body can recover. TCMET is an important part of TCM and belongs to a kind of aerobic exercise of active exercise, mainly including Tai Chi, Baduanjin, Daoyin, Yi Jin Jing, five-fowl play, six-character tips, Shaolin kung-fu, Relaxation exercise, and Standing pile exercise. For the study of stroke disease, the ancient Chinese physician Chao Yuanfang quoted many Daoyin methods in the "Health Care Formula Guide Method" in the "General Treatise on Causes and Manifestations of All Diseases" to treat stroke disease. Liu Wansu attaches great importance to the restoration of the motor function of stroke hemiplegia by TCM and early Daoyin tuina. TCMET is convenient, economical, and rarely causes adverse events; the safety, efficacy, and fall rates were also confirmed by literature studies [10–12]. Therefore, TCMET's treatment of stroke disease has attracted more and more attention.

2. Search Strategy and Literature Screening

2.1. Search Strategy. The literature was searched in PubMed, Web of Science, Embase, Cochrane library, China national knowledge infrastructure (CNKI), ChongqingVIP, and Wanfang database, from the establishment of the library to Aug 29, 2022, through the keywords "Traditional Chinese Medicine Exercise Therapy," "Tai chi," "Baduanjin," "Daoyin," "Yi Jin Jing," "five-fowl play," "six-character tips," "Shaolin kung-fu," "Health care exercises," "Relaxation exercises," "Standing pile exercises," and "Stroke" and adjusted according to different databases. The search strategy of the PubMed database is shown in Table 1.

2.2. Literature Screening. A total of 2571 articles were identified in the search, and 228 articles were deemed potentially relevant through the exclusion of the title and abstract. There were 71 articles through reading the full text to exclude repeated publications, research proposals, abstracts, case studies, and conference papers. The full text cannot be found and it is still in clinical trials. The literature clearly did not belong to the stroke recovery period. Among them, there are 24 articles on Tai Chi, 18 articles on Baduanjin, 9 articles on Daoyin, 5 articles on Yi Jin Jing, 2 articles on five-fowl play, 10 articles on six-character tips, and 3 articles on relaxation exercises (Figure 1).

3. Research Status

Judging from the published literature, the hot spots of research in the past decade have mainly focused on the treatment of the stroke recovery period with TCMET. By choosing this stage of treatment and the introduction of Western medical rehabilitation into China in the 1970s and 1980s and the gradual development of traditional Chinese medicine rehabilitation, the treatment of stroke has placed more and more emphasis on the grasp of the time window. Through clinical studies, the earlier the intervention in traditional Chinese and Western medicine rehabilitation, commonly known as early introduction, the more beneficial it is to the patient's later recovery. Multidisciplinary cross-fusion treatment has become a trend in stroke treatment. TCMET plays a role in the recovery period of stroke with its unique advantages. At present, more research studies are being conducted on Tai Chi, Baduanjin, Daoyin, Yi Jin Jing, five-fowl play, six-character tips, and Relaxation exercises. The summarized current status of TCMET in the recovery period of stroke is shown in Tables 2–7.

From Table 3, Tai Chi uses the simplified 24 forms issued by the General Administration of Sports of China in 2003 and improves on this basis, choosing a single type of exercise such as Tai Chi Yunshou or choosing the 8 postures that are most commonly used for stroke patients. The 8 postures are commencing, step back and whirl arms, knee hugging and twist step, wild horse mane, yunshou, hand waving pipa, picking the tail, ending posture, etc., which include moving forward, backward, left, and right from the fixed step. It also emphasizes the "waist as the axis" to drive the movement training of upper limbs, hips, knees, and ankles. During the practice, the waist and hips are required to be loose and heavy, with breathing exercises..

In the specific practice, attention is paid step by step to specific exercises, from easy to difficult, practiced in stages, and certain protective measures are taken. The intervention time was 20–60 minutes each time, 1-2 times a day and 2–5 times a week. Some literature studies used a combination of group exercises and individual exercises, and the total intervention time was half a month to 6 months. In terms of clinical efficacy, it mainly improved motor function, balance ability, cognitive impairment, neurological function, daily living ability, emotion regulation, cardiopulmonary function, safety, and fall prevention in stroke patients, and it was reported that with the extension of intervention time, the effect was more significant. From the perspective of literature quality, except for one piece of the literature that is unclear whether to use randomized control, the rest of the literature used randomized controlled trials, compared the baseline data of the literature, and recorded the shedding cases, so the results of the included literature studies were recommended.

From Table 4, Baduanjin refers to the "Fitness Qigong Baduanjin" standard issued by the General Administration of Sports of China in 2003 [83], and the Eighteen section brocade is a training method that combines Tai Chi, Baduanjin, and modern fitness exercises. The included literature mainly used Baduanjin or eighteen sections brocade

Table 1: Search strategy for the PubMed database.

	Search term
#1	"Taijiquan"[mesh]
#2	"Tai Chi" OR "Tai Ji" OR "Taiji" OR "Tai Chi Chuan"
#3	#1 OR #2
#4	"Baduanjin" (mesh)
#5	"Daoyin" (mesh)
#6	"Yi Jin Jing" (mesh)
#7	"Five-fowl play" (mesh)
#8	"six-character tips" OR "Liu Zi Jue qigong" (mesh)
#9	"Shaolin kung-fu" OR"Shaolin neigong" (mesh)
#10	"Health care exercises" (mesh)
#11	"Relaxation exercises" (mesh)
#12	"Standing pile exercises" (mesh)
#13	"Traditional Chinese medicine exercise therapy" (mesh)
#14	"Stroke" (mesh)
#15	#3 OR #4 OR #5 OR #6 OR #7 OR #8 OR #9 OR #10 OR #11 OR #12 OR #13
#16	#14 AND #15

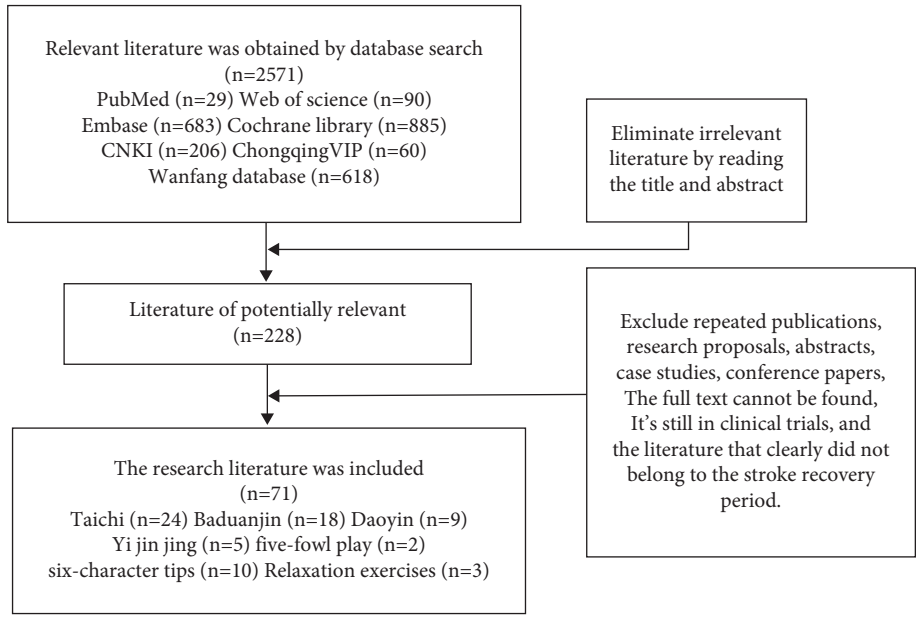

Figure 1: The flow chart of literature screening.

combined with other methods for the treatment of stroke, and the efficacy was significantly better than that of Baduanjin alone or eighteen section brocade. The improvement after stroke is mainly in motor function, balance function, cognitive function, depression after stroke, and daily living ability.

From the perspective of intervention time, compared with Tai Chi, the intervention time of Baduanjin is shorter, generally 20–30 minutes each time, generally once a day in the morning and evening, 40–50 minutes 3 times a week, and the intensity of exercise is in line with the aerobic activity intensity recommended by the American College of Sports Medicine and the American Heart Association for the health of the elderly in 2007 [84]. In terms of the evaluation of literature quality, the included literature all adopted the randomized control method, but there were certain confounding factors due to the combination of multiple methods. The insufficient sample size and a wide range of centers affected the results.

It can be seen from Table 5 that the choice of Daoyin is mainly caused by causes and manifestations of all diseases, which reflect the combination of breath, heart, and body adjustment. Sitting, standing, and horizontal training for different parts can be adopted. The curative effect of Daoyin combined with basic rehabilitation is better than simple basic rehabilitation. The curative effect of stroke is mainly on motor function, balance and coordination ability, daily living ability, and so on. The safety and fall prevention of stroke have not been found in the relevant literature. The total intervention time is relatively short, and the relationship between extended practice time and efficacy remains to be verified.

It is concluded from Table 6 that the six-character tips mainly adopt the fitness qigong six-character tips of the General

Table 2: General view of all therapeutic approaches.

Therapeutic approaches/sample number (n)	Specifications	Efficacy	Refs
Tai Chi ($n = 1716$)	Based on ancient Chinese philosophical ideas such as the theory of yin and yang and the principle of Tai Chi, it is a traditional boxing technique that integrates functions such as temperament, physical fitness, and technical confrontation	Treating the symptoms and signs during the recovery of stroke	[11, 13–35]
Baduanjin ($n = 1505$)	Baduanjin is a fitness method invented in ancient China. There are eight beautiful and smooth movements such as "Jin," which is a set of exercises for internal exercises of "Jing, Qi and Spirit" and external exercises for preventing and treating diseases, strengthening the body, and improving physical strength	Treating the symptoms and signs during the recovery of stroke	[36–53]
Daoyin ($n = 718$)	Daoyin is the earliest and most distinctive exercise method in many therapies of TCM, which is a way of organically combining breathing, body movement, idea guidance, and self-tuina, which plays a unique role in health care, disease treatment, and rehabilitation	Treating the symptoms and signs during the recovery of stroke	[54–62]
Six-character tips ($n = 828$)	six-character tips is a set of tuna health care methods handed down in ancient China, focusing on breathing and exhaling using "Xu, He, Hu, Xi, Chui, Xi," the specific pronunciation of the six characters, mouth training to affect the operation of different organ meridian qi and blood, supplemented by corresponding body movements and ideas, and then achieving the purpose of strengthening the body and maintaining health and rehabilitation	Treating the symptoms and signs during the recovery of stroke	[63–72]
Yi Jin Jing ($n = 601$)	Yi Jin Jing originated from the ancient Chinese health care guidance technique, through the use of subjective energy, a comprehensive exercise of self-body and mind, changing the bones and muscles, making them a strong set of traditional exercises. It uses a certain posture to induce breathing and gradually strengthens the function of the tendons and internal organs	Treating the symptoms and signs during the recovery of stroke	[73–77]
Five-fowl play ($n = 120$)	Five-fowl play is an ancient traditional exercise that imitates the movements of five animals, namely tigers, deer, bears, apes, and birds (cranes), and is a guiding method for health care and strengthening the body	Five-fowl play can significantly improve the motor function, balance function, and ability of daily living in patients with stroke hemiplegia	[78, 79]
Relaxation exercises ($n = 209$)	Relaxation exercises are a kind of meditation exercise, which organically combines the three practice methods of mind, breathing, and posture and adopts postures such as "standing, sitting, and lying down" to relax various parts of the body from the head to the toe, from the top to the bottom, to adjust the whole body to a comfortable, relaxed, and natural state	Treating the symptoms and signs during the recovery of stroke	[80–82]

Table 3: Summary of outcomes of Tai Chi interventions in stroke recovery.

Intervention methods	Course of disease	Number of cases	Randomized trial	Comparator	Intervention time	Positive findings
Chen styles of Tai Chi	0.25–12 months	40	Yes	Modern rehabilitation therapy	20–40 minutes/time, 5 times/week, 0.5 month	Chen styles of Tai Chi-style Tai Chi, fixed-foot stance Yunshou, modified Tai Chi, and Tai Chi. It can significantly improve patients' motor function, balance and coordination ability, cognitive function, nerve excitability, daily living ability, emotional regulation, and cardiopulmonary function and has a certain safety and prevention of falls. With the prolongation of intervention time, the effect is more obvious. The curative effect of Tai Chi combined with modern rehabilitation technology is better than that of modern rehabilitation technology alone, and the curative effect is improved with the prolongation of intervention time. Tai Chi has certain safety and prevents falls
Fixed-foot stance Yunshou	<6 months	60	Yes	Routine treatment and rehabilitation nursing	30 minutes/time, 5 times/week, 2 months	
Fixed-foot stance Yunshou combined with bobath handshake training	<6 months	59	Yes	Routine treatment and rehabilitation nursing	30 minutes/time, 5 times/week, 2 months	
Tai Chi Yunshou	<6 months	60	Yes	Routine treatment and rehabilitation nursing	30 minutes/time, 1 time/week, 5 days/week, 2 months	
Tai Chi Yunshou	>3 months	30	Yes	Basic therapy (conventional internal medicine and health education)	60 minutes/time, 1 time/day, 5 days/week, 3 months	
Modified Tai Chi	0.5–2 months	62	Yes	Stretching training	30 minutes/time, 1 time/day, 5 days/week, 1 month	
Modified Tai Chi	<6 months	73	Yes	Modern rehabilitation therapy	60 minutes/time, 1 time/day, 5 days/week, 1 month	
Modified Tai Chi	<6 months	22	Yes	Routine rehabilitation	60 minutes/time, 1 time/day, 5 days/week, 1 month	
Modified Tai Chi	<6 months	40	Yes	Routine rehabilitation	60 minutes/time,1 time/day,5 days/week,1 month	
Tai Chi Yunshou	>3 months	80	Yes	Routine rehabilitation	60 minutes/time, 1 time/day, 5 days/week, 3 months	
Tai Chi	≥3 months	17	Yes	Traditional rehabilitation	60 minutes/time, 1 time/day, 2 days/week, 6 months	
Tai Chi catwalk exercise combined with acupoint tuina	0.5–6 months	180	Yes	Traditional Chinese and western medicine in combination with comprehensive rehabilitation	30 minutes/time, 2 times/day, 6 months	
Taijiquan	>3 months	62	Yes	Modern comprehensive rehabilitation treatment	40 minutes/time, group training 3 times/week, individual training 2 times/week, 2 months	
Taijiquan	≥3 months	16	Yes	Traditional rehabilitation	60 minutes/time, group training 1 time/week, training in family 1 time/week, 6 months	
Taijiquan	>0.5 month	56	Yes	Routine rehabilitation	30 minutes/time, 1 time/day, 5 days/week, 1 month	
Tai Chi posture training	<6 months	110	Yes	Modern rehabilitation therapy	60 minutes/time, 1 time/day, 5 days/week, 1 month	
Tai Chi pile work	0.5–3 months	80	Yes	Physical therapy and homework therapy	20 minutes/time, 2 times/day, 3 months	
Modified 24 styles of Tai Chi	<6 months	60	Yes	Antidepressant drug treatment	60 minutes/time, 1 time/day, group training 1 day/week, 3 months	
Modified 24 styles of Tai Chi	<3 months	105	Not clear	Control or Baduanjin	40 minutes/time, group training 3 times/week, individual training 2 times/week, 2 months	
Body weight support (BWS)and Tai Chi	≥3 months	71	Yes	Conventional rehabilitation therapies	40 minutes/time, 3 times/week, 3 months, 20 minutes/BWS, 20 minutes/Taichi	
Community-basedYang-style Tai Chi	≥3 months	28	Yes	Usual care	60 minutes/time, 3 times/week,3 months, a 20 minutes warm-up period, 30 minutes of Tai Chi exercise, and a 10 minutes cool-down period	
Yang-style24-postureshort-form Tai Chi	≥3 months	145	Yes	SilverSneakers, usual care	60 minutes/time, 3 times/week, 3 months, a 10 minutes warm-up period, 40 minutes of Taichi exercise, and a 10 minutes cool-down period	
Tai Chi Yunshou exercise	≥3 months	244	Yes	Rehabilitation	60 minutes/time, 5 times/week, each session comprised 45 minutes of exercise plus a 15 minutes warm-up and cool down. 3 months	
Tai Chi	≥3 months	16	Yes	Traditional rehabilitation	6 months	

Note. The column of course of disease is based on month, and those less than 1 month are converted proportionally; in the column of intervention time, the time of each intervention is calculated as minutes, and the total time of intervention is calculated as months. Each exercise therapy status analysis in TCMET was expressed in such a discounted way in terms of the course of disease and intervention time.

Table 4: Summary of outcomes of Baduanjin interventions in stroke recovery.

Intervention methods	Course of disease	Number of cases	Randomized trial	Comparator	Intervention time	Positive findings
Baduanjin combined with Traditional Chinese medicine measures	<6 months	90	Yes	Traditional Chinese medicine measures	30 minutes/time, 1 time/day, 6 months	The combination of Baduanjin, eighteen section brocade, and other methods can effectively improve the overall clinical efficacy of stroke recovery patients; improve neurological function and limb function; reduce the degree of depression of patients; improve motor function, muscle strength, muscle tone, life independence ability, and balance ability; and improve overall cognitive function, cognitive dysfunction execution, attention, memory, and processing speed
Baduanjin combined with action observation therapy	<3 months	90	Yes	Routine treatment and nursing plan/ Baduanjin	20 minutes/time, 1 time/day, 6 days/ week, 1 month	
Baduanjin combined with balancing function training	<6 months	60	Yes	Balancing function training	20 minutes/time, 2 times/day, 1.5 months	
Baduanjin combined with balancing training	1–3 months	62	Yes	Balance training	20 minutes/time, 2 times/day, 5 days/ 6 days, 1.6 months	
Baduanjin combined with rehabilitation training	<6 months	224	Yes	Rehabilitation training	20 minutes/time, 2 times/day, 7 days/ week, 1.5 months	
Baduanjin exercise	≥2 months	48	Yes	Health education	40 minutes/time, 3 times/week, 6 months	
Baduanjin exercise	≥2 months	48	Yes	Health education	40 minutes/time, 3 times/week, 6 months	
Baduanjin	1–6 months	60	Yes	Control group	45 minutes/time, 3 times/week, a 5 minutes warm-up period, 5 minutes relaxation period two times, 1 month	
JieYu no. 1 recipe combined with Baduanjin	<3 months	80	Yes	Sertraline hydrochloride	20 minutes/time, 2 times/day, 5 days/ week, 1 month	
Eighteen-section brocade combined with routine care and rehabilitation	0.5–6 months	80	Yes	Routine care and rehabilitation	30 minutes/time, 2 time/day, a 5 minutes warm-up period, 2 months	
Eighteen-section brocade combined with rehabilitation	0.25–6.25 months	96	Yes	Rehabilitation	30 minutes/time, 2 time/day, 3 months	
Cluster needling technique at the head point and Baduanjin exercise	1–3 months	60	Yes	Cluster needling technique at the head points	20 minutes/time, 2 times/day, 5 days/ week, 1.5 months	
Five-element music therapy combined with Baduanjin	$6.35 \pm 1.69^*$ months	72	Yes	Baduanjin	30 minutes/time, 2 time/day, 1.3 months	
Acupuncture combined with eighteen-section brocade	<3 months	201	Yes	Routine therapy	30 minutes/time, 2 time/day, 1 months	
Zishen Yisui acupuncture based on the Baduanjin	3–12 months	80	Yes	Baduanjin exercise	40 minutes/time, 3 times/week, 2 months	
Baduanjin exercise	$6.58 \pm 2.14^*$ months	48	Yes	Health education sessions	40 minutes/day, 3 days/week, 6 months	
Baduanjin Qigong	>3 months	58	Yes	2 sessions of supervised conventional fitness training in the first week combined with home-based exercise practice	50 minutes/time, 3 times/week, a 10 minutes warm-up, 10 minutes cool down, 4 months	
Baduanjin exercise based on original medication and rehabilitation treatment	>3 months	48	Yes	Original medication and rehabilitation treatment	40 minutes/day, 3 days/week, 6 months	

*Note.**indicates that the course of the disease was found in baseline data and expressed as a mean, not clearly defined in the inclusion criteria.

Table 5: Summary of outcomes of Daoyin interventions in stroke recovery.

Intervention methods	Course of disease	Number of cases	Randomized trial	Comparator	Intervention time	Positive findings
Daoyin method of general treatise on causes and manifestations of all diseases	≤6 months	68	Yes	Routine rehabilitation	40 minutes/day, 5 days/week, 1.5 months	Daoyin can improve upper limb motor function, balance and coordination, daily living ability, and neurological function in stroke patients
Daoyin method of general treatise on causes and manifestations of all diseases	<6 months	60	Yes	Routine rehabilitation	30 minutes/time, 2 times/day, 5 days/week, 1.5 months	
Daoyin method of Chao Yuanfang	<12 months	130	Yes	Routine rehabilitation	5 days/week, 0.75 month	
Daoyin method of Chao Yuanfang	<6 months	40	Yes	Routine rehabilitation	20 minutes/time, 2 times/day, 6 days/week, 0.5 month	
Daoyin method of Chao Yuanfang combined with routine rehabilitation	<6 months	40	Yes	Routine rehabilitation	20 minutes/time, 2 times/day, 6 days/week, 0.5 month	
Daoyin method of Chao Yuanfang	<6 months	30	Yes	Routine rehabilitation	20 minutes/time, 2 times/day, 6 days/week, 0.5 month	
Daoyin therapy and routine rehabilitation training	1–6 months	60	Yes	Routine rehabilitation training	45–60 minutes/time, 1 time/day, 5 days/week, 1.5 months	
Daoyin therapy and routine rehabilitation training	0.5–2 months	200	Yes	Routine rehabilitation	45–60 minutes/time, 1 time/day, 1.5 months	
Upper limb guiding technique	$4.29 \pm 1.87^{*}$ months	90	Yes	Bobath therapy	30–45 minutes/time, 1.5 months	

*Note.** indicates the same comments as Table 4.

Table 6: Summary of outcomes of six-character tip interventions in stroke recovery.

Intervention methods	Course of disease	Number of cases	Randomized trial	Comparator	Intervention time	Positive findings
Six-character tips	>0.5 months	34	Yes	Routine treatment, nursing, and rehabilitation	18–24 minutes/time, 1 time/day, 7 days/week, 3 months	Six-character tips combined with routine rehabilitation, basic pronunciation training, and traditional breathing exercise can significantly improve pulmonary respiratory function, speech disorders, daily living ability, motor function, balance and coordination, cognitive function, nerve damage, depression, etc.
Six-character tips combined with conventional articulation training	0.5–6 months	157	Yes	Traditional breathing training and conventional articulation training	20 minutes/time, 1 time/day, 5 days/week, 0.5 month	
Six-character tips	<6 months	60	Yes	Routine care for depression after stroke	15–20 minutes/time, 2 times/day, 0.93 month	
Liu Zi Jue qigong with basic articulation training	0.5–6 months	60	Yes	Traditional breathing training + basic articulation training	20 minutes/time, 5 times/week, 0.75 month	
Six-character tips	≤3 months	84	Yes	Routine treatment and care	30 minutes/time, 1 time/day, 5 days/week, 3 months	
Six-character tips combined with the diaphragm muscle release technique	3–7 months	80	No	Routine rehabilitation such as inhalation training	30 minutes/time, 1 time/day, 5 days/week, 2 months	
Liu Zi Jue qigong combined with conventional rehabilitation training	0.5–6 months	160	Yes	Traditional core stability training combined with routine rehabilitation	15 minutes/time, 1 time/day, 5 days/week, 0.5 month	
Six-character tips combined with inspiratory muscle training	3.61 ± 2.35* months	75	Yes	Routine rehabilitation/routine inspiratory muscle training	30 minutes/time, 1 time/day, 5 days/week, 3 months	
Liu Zi Jue qigong combined with conventional breathing speech training	4–14 months	50	Yes	Conventional breathing speech training	20 minutes/time, 1 time/day, 5 days/week, 1 month	
Six-character formula respiratory gymnastics	1–12 months	68	Yes	Medical treatment, routine rehabilitation, and occupational therapy	30 minutes/time, 1 time/day, 5 days/week, 3 months	

Note. * indicates the same comments as Table 4.

Table 7: Summary of outcomes of Yi Jin Jing, five-fowl play, and relaxation exercise interventions in stroke recovery.

Intervention methods	Randomized trial	Comparator	Intervention time	Positive findings
Yi Jin Jing(Yi Jin Jing, modified Yi Jin Jing, Yi Jin Jing + Xingnao kaiqiao acupuncture, Yi Jin Jing + acupuncture)	Yes	Routine rehabilitation/group occupational therapy/basic treatment/ acupuncture	20–60 minutes/time, 1 time/day, 3–6 times/week, 0.7–3 months	Yi Jin Jing combined with acupuncture and routine rehabilitation can significantly improve motor function, daily living ability, cognitive function and emotional state of stroke patients
Five-fowl play	Yes	Routine rehabilitation	30–60 minutes/time, 5 times/ week, 1–5 months	It can significantly improve the motor function, daily living ability, and balance and coordination of patients with stroke hemiplegia
Relaxation exercises (relaxation exercises + rehabilitation and relaxation exercises + bobath therapy)	Yes	Routine rehabilitation/rehabilitation care and health education/bobath therapy + basic rehabilitation	20–30 minutes/time, 1 time/day, 1–2 months	Relaxation exercise combined with routine rehabilitation can improve motor function, neurological function impairment, depression and daily living ability of stroke patients with hemiplegia during recovery, and the effect is better than that of conventional rehabilitation alone

Administration of Sports of China [85], and some literature combines Daoyin on this basis [86], which can be exercised step by step in supine, sitting, and standing positions. The treatment of stroke is mainly to improve the patient's lung and respiratory function, speech disorders, and so on, and the efficacy of the combination of other methods is significantly better than that of other methods alone. One piece of literature on the source of the case involved a multicentre study [64], but there were limitations and the overall intervention time was short.

From Table 7, it can be concluded that Yi Jin Jing, five-fowl play, and Relaxation exercises are the three TCMETs with the least amount of literature research and the total intervention time is short, which mainly improves motor function and daily living ability of stroke patients. The efficacy of combination with other methods is better than that of other methods alone.

Subgroup analysis of each TCMET therapy is conducted in Tables 3–7. Different TCMETs have common effects on stroke recovery, which also have different characteristics. The summarized efficacy and frequency of TCMET on stroke recovery are shown in Figure 2.

It can be seen from Figure 2 that Taichi and Baduanjin have conducted extensive studies, and TCMET has improved motor function after stroke. Daoyin is mainly used to improve balance and coordination ability and daily living ability in addition to motor function. Besides, Baduanjin has conducted more studies on cognitive function, depression, and emotional state after stroke. Studies on six-character tips are mainly used in lung function and speech disorders. There is relatively little literature on Yi Jin Jing, five-fowl play, and relaxation exercises. Only Taichi and Baduanjin are involved in safety evaluation. However, only Taichi has a special literature study on fall prevention in TCMET.

From the above research and analysis of TCMET on the recovery period of stroke, its efficacy in combination with other therapies is worthy of recognition, which is a useful supplement of modern Western medicine rehabilitation in the recovery period of stroke and is worthy of promotion and application. Subsequent studies were provided in terms of the use, sample size, intervention time, and tendency of each therapy to facilitate function recovery during stroke recovery.

4. Mechanism Discussion

4.1. To Explore the Mechanism of TCMET from Brain Function Remodeling

4.1.1. From the Functional Structure of the Brain. From the cortex research, Chen Yiyun and Liu believe that stroke patients with hemiplegia actively and consciously practice such as Baduanjin can enhance the activity of the motor center of the human brain cortex and increase the excitability of nerve cells [87]. Liu and Li found that the cerebral cortex was in a special state during the Daoyin exercise, which may be one of the mechanisms of its influence on brain function. In addition, it was also found that skin temperature changes significantly in the process of Daoyin exercise, suggesting that Daoyin exercise can affect autonomic nervous function [88]. Zhu found that specific Daoyin techniques can activate specific areas of the cerebral cortex and promote plastic changes in the central nervous system, and it is believed that the active use of limbs can cause plasticity changes in the function of the brain's motor cortex [89]. From the study of brain gray matter, Ye is based on voxel morphological analysis, and by comparing MRI before and after treatment, it is believed that Baduanjin's effect on gray matter volume in patients with mild cognitive impairment (MCI) is significantly related to the changes in the gray matter structure in the right middle temporal gyrus, the right middle occipital lobe, and the right posterior cerebellar lobe [90]. Based on multimodal magnetic resonance imaging, Lu explored the effect of Tai Chi exercise on cognitive function and believed that Tai Chi tends to adjust the functional connectivity of the brain's default mode network and change the structure of white matter fibers [91]. In a study published in 2012 by Xue et al. in Professor Chen Lidian's team, participants were scanned by resting-state fMRI before and after the 12-week Tai Chi and Baduanjin practice interventions, and the memory function of the subjects was evaluated, aiming to explore whether they could improve memory function and regulate the resting function connection of the hippocampus. The results showed that both Tai Chi and Baduanjin could significantly enhance the memory function of the participants, and the strength of the functional connection between the bilateral hippocampus and the medial prefrontal cortex in the Tai Chi group increased significantly. This change in functional connectivity has a significant correlation with the improvement of memory function in participants, and the intrinsic effect mechanism of Tai Chi in improving memory function is partially illustrated at the level of the central nervous system [92].

4.1.2. Study the Effects of Brain Waves. Chen Chi's research on the effect of fitness qigong six-character tips on brain waves in patients with mild cognitive dysfunction, from the analysis of brain waves (EEG) before and after practice, shows that the approximate entropy is significantly reduced at the beginning of 1 month of practice, the EEG tends to be orderly, and the approximate entropy is still slightly reduced after 3 months of practice, indicating that insisting on practicing fitness qigong six-character tips can keep brain waves more orderly, manifested as memory loss and improvement, improved ability to respond to things, and a calm mood [93].

4.1.3. From Neuromodulation Studies. Zhao et al. found that the "Six-step" Daoyin exercises can stimulate the potential of the central nervous system, and the Daoyin therapy method can clearly improve the motor muscle signal intensity of the quadriceps, and under the guidance of this feedback technology, the patient's exercise program can be gradually reestablished. The study also used feedback techniques to show that Daoyin therapy can stimulate people's "thoughts"

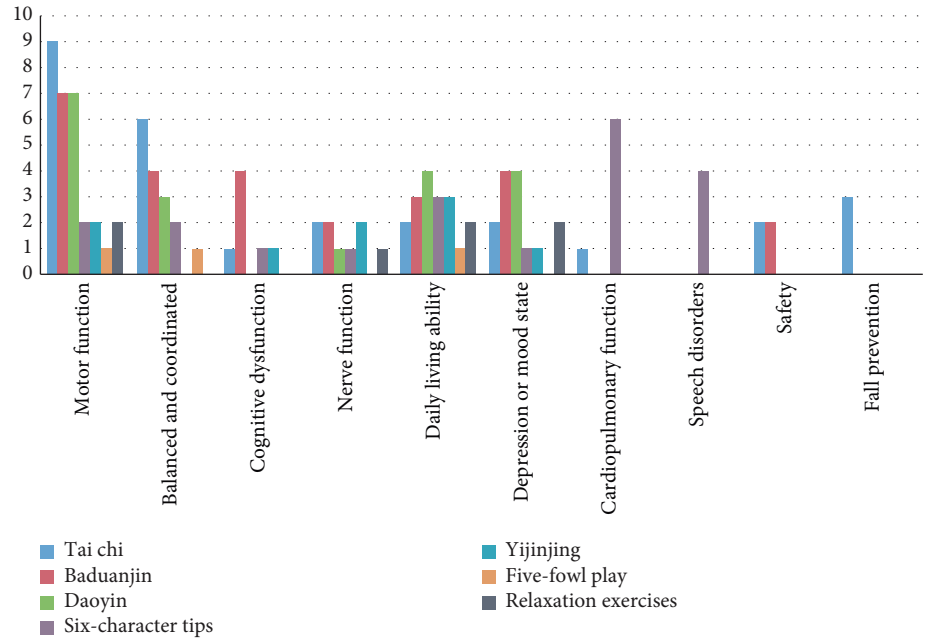

Figure 2: The therapeutic effect and the frequency figure of TCMET on recovery of stroke.

during the rehabilitation process, thereby promoting the rehabilitation of specified movements to a certain extent [94].

4.1.4. Blood Flow Studies from the Brain. Li et al. observed the circulatory state of brain blood circulation in patients after Daoyin therapy, and the cerebral blood flow chart of patients after Daoyin therapy showed that the blood inflow time was shortened and the tension of blood ducts decreased significantly, indicating that Daoyin therapy can improve the brain blood flow chart, improve blood duct elasticity, and increase cerebral blood volume, particularly vertebrobasilar volume [95].

4.1.5. Study on Changes in Cerebral Blood Oxygen. Qiang et al. measured the brain oxygen metabolism state of a senior Shaolin kung-fu exerciser during exercise by near-infrared spectrophotometry, and the amount of oxygenated hemoglobin was slightly reduced compared with that of the quiet sitting position during the Shaolin kung-fu exercise, and the total hemoglobin amount was almost unchanged. The ratio of oxygenated hemoglobin to oxyhemoglobin in the total hemoglobin volume during the Shaolin kung-fu exercise decreased slightly, and the amount of oxyhemoglobin increased slightly. In terms of oxygen saturation, compared with the quiet sitting position before the Shaolin kung-fu exercise, the Shaolin kung-fu exercise is only reduced by about 1-2%, which cannot be said to have changed significantly. In experiments with senior Shaolin kung-fu exercisers, despite the intensity of isotonic muscle contractions, the total amount of hemoglobin and oxygen saturation in the brain have been maintained within the physiological range. This suggests that the "Natural respiration" method practiced through long-term exercise can promote the very economical oxygen consumption of brain tissue, effectively inhibiting the increase in the amount of deoxyhemoglobin in the blood. Correct, moderate Shaolin kung-fu exercises do not affect the homeostasis of the human environment but can improve the oxygenation capacity of brain tissue [96].

4.2. To Explore the Mechanism of TCMET at the Molecular Level. Wang Qian et al. discussed the mechanism of eighteen-section brocade combined acupuncture treatment to improve exercise quality and motor function of patients from the levels of serum matrix metalloproteinases-9 (MMP-9), erythropoietin (EPO), and homocysteine (Hcy). In normal brain tissue, EPO is hardly expressed, but its content is significantly increased in stroke and can be used as a marker of brain damage [97]. HCY is a risk factor for ischemic stroke and atherosclerosis. The study found that MMP-9 is closely related to the structure of the blood vessel wall and the permeability of the blood-brain barrier, which can reflect the edema area and the degree of cerebral infarction in stroke patients. The serum MMP-9, EPO, and Hcy levels in the observation group were significantly lower than those in the control group, indicating that acupuncture combined with eighteen-section brocade could reduce brain injury by reducing serum MMP-9, EPO, and Hcy levels [49]. In addition, some researchers have significantly reduced the urine uric acid content before and after practicing standing pile exercises, which shows that the synthesis of nucleic acids is greater than the decomposition during the exercise, and then the synthesis of nuclear proteins can be introduced to be greater than the decomposition. Whether the resynthesis rate of nuclear protein is accelerated or the decomposition rate of nuclear protein is slowed down during practice, the

body will enter a state of energy storage. In turn, it will have a good impact on the function of various organs in the human body. It is believed that the clear-eyed, energetic self-proprioception that occurs after practicing is related to this good effect of influence [98].

4.3. To Explore the Mechanism of TCMET from the Changes in Lung Ventilation. Ding et al. explored the mechanism of six-character tips in the treatment of respiratory control ability in patients with motor speech disorder after stroke by comparing six-character tips with traditional breathing training. Its mechanism may be the same as the "six-character tips" which emphasizes the simultaneous training of breathing and pronunciation. Breathing pronunciation is gentle, slow, and uniform, in fact, a low-load, slow-rate breathing training. It is easier to activate the main respiratory muscle group composed of slow muscle fibers than simple respiratory muscle reinforcement training, and it is not easy to cause fatigue and does not cause excessive tension in the vocal cords [64]. In addition, the vocalization method of the "six-character tips" is conducive to the stabilization of subglottic pressure, providing sufficient airflow for vocal cord vibration, ensuring the duration and tone of laryngeal pronunciation, and gradually adjusting the patient's breathing mode to abdominal breathing during training, which helps to enhance the patient's respiratory function and provide continuous and stable respiratory airflow support for pronunciation [99]. Moreover, the "six-character tips" guided action can fully mobilize the respiratory muscles and increase the amplitude of abdominal movement before and after and the range of movement of the diaphragm up and down, which is conducive to increasing the volume of the chest cavity and lung capacity [99, 100]. Some studies have further shown that when training the "Xu" word in the six-character tips of breathing, the air tract and extrathoracic resistance of the body can be increased when exhaling, and the squeeze of the airway by the pressure in the chest can be reduced through this way. The collapse of the small airway and the premature closure of the bronchi are avoided, and the residual air volume in the lungs is reduced, so that the phenomenon of airflow obstruction is reduced, and the role of improving the lung function of the body can be achieved [72]. Some researchers have used Tai Chi to regulate post-stroke cardiopulmonary function by means that the heart rate variability (HRV) of long-term Tai Chi practitioners will be greatly improved. Secondly, the abdominal breathing of Qi Shen Dantian during the long-term practice of Tai Chi not only exercises the respiratory muscles, but also strengthens the depth of breathing, expands the lung capacity, improves the microcirculation system, improves the gas exchange capacity, and has a more obvious effect of increasing the velocity max [22].

In summary, the above studies explore the mechanism of TCMET in stroke treatment from the aspects of brain function remodeling, molecular mechanism, and lung ventilation change mechanism, and prove how TCMET plays a role in stroke treatment. However, the research on the mechanism of action is still relatively weak, and the research on the mechanism needs to be further strengthened if TCMET is to be promoted and applied during the recovery period of stroke.

5. Trend of Development

The characteristic of TCM is holism and treatment based on syndrome differentiation. TCMET such as Tai Chi, Baduanjin, Daoyin, Yi Jin Jing, six-character tips, Relaxation exercises, Shaolin kung-fu, and Standing pile exercises mentioned in this article reflect the advantages of TCM through body adjustment, heart adjustment, and pranayama, which can adjust the motor function, balance and coordination ability, and overall disease resistance and rehabilitation ability of stroke patients from multiple levels and angles, which is consistent with the overall rehabilitation and comprehensive rehabilitation goals of stroke rehabilitation and is worthy of further promotion and application. TCMET has achieved some success in the study of the stroke recovery period. However, there are still several issues that merit further in-depth study. For example, in the design of clinical trials, TCMET is difficult to blind, and most studies use evaluator blinding; the sample size of the trial is too small, and further large-sample studies are needed to further confirm the reliability of the data; in terms of inclusion standards, it is difficult for patients with poor motor function and balance function to participate in the trial. The measurement-efficiency relationship of intervention time needs further follow-up and study; there is no completely unified standard for TCMET intervention during stroke recovery. From the analysis of each subgroup in TCMET, most of them used TCMET combined with other drugs, Western medicine rehabilitation, and other methods, and there were many confounding factors and certain limitations. There is relatively insufficient research on safety evaluation and fall prevention; in terms of evaluation standards, most of them are scales, with certain subjectivity; the discussion of mechanism is relatively weak; and further research on the intrinsic characteristics and effect mechanisms of each TCMET, at the molecular level and even deeper levels, needs to be further developed.

In the future, TCMET's prevention and treatment of the stroke recovery period will need to overcome the shortcomings of existing research based on artificial intelligence and big data technology, combined with different TCMET characteristics and the development of different stages of multiexercise or partial stereotype integration of unified standards for TCMET to stroke recovery period research to provide evidence-based medical evidence, multilevel, large sample, multicenter research, and from stroke recovery period to subacute stage transition research, so that more stroke patients can receive timely and early intervention in TCMET treatment. Let this therapy become an effective method for the prevention and treatment of stroke diseases in countries around the world. The burden of stroke is shared by society, families, and individuals.

Data Availability

All data supporting the findings of this study are available within the article.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

Authors' Contributions

Cunxiao Guo and Xiantao Tai contributed to the conception and design of the review. Yuanwang Wang applied the search strategy. Cunxiao Guo, Shuang Wang, and Shouyao Zhang applied the selection criteria. Yuanwang Wang and Shouyao Zhang analyzed the data and interpreted data. Cunxiao Guo wrote this manuscript. Xiantao Tai and Cunxiao Guo edited this manuscript. Xiantao Tai is responsible for the overall project.

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Retraction

Retracted: Targeted Perioperative Nursing Combined with Propofol and Fentanyl for Gynecological Laparoscopic Surgery

Evidence-Based Complementary and Alternative Medicine

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This article has been retracted by Hindawi following an investigation undertaken by the publisher [1]. This investigation has uncovered evidence of one or more of the following indicators of systematic manipulation of the publication process:

(1) Discrepancies in scope
(2) Discrepancies in the description of the research reported
(3) Discrepancies between the availability of data and the research described
(4) Inappropriate citations
(5) Incoherent, meaningless and/or irrelevant content included in the article
(6) Peer-review manipulation

The presence of these indicators undermines our confidence in the integrity of the article's content and we cannot, therefore, vouch for its reliability. Please note that this notice is intended solely to alert readers that the content of this article is unreliable. We have not investigated whether authors were aware of or involved in the systematic manipulation of the publication process.

In addition, our investigation has also shown that one or more of the following human-subject reporting requirements has not been met in this article: ethical approval by an Institutional Review Board (IRB) committee or equivalent, patient/participant consent to participate, and/or agreement to publish patient/participant details (where relevant).

Wiley and Hindawi regrets that the usual quality checks did not identify these issues before publication and have since put additional measures in place to safeguard research integrity.

We wish to credit our own Research Integrity and Research Publishing teams and anonymous and named external researchers and research integrity experts for contributing to this investigation.

The corresponding author, as the representative of all authors, has been given the opportunity to register their agreement or disagreement to this retraction. We have kept a record of any response received.

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[1] X. Yun, S. Chen, and Q. Zheng, "Targeted Perioperative Nursing Combined with Propofol and Fentanyl for Gynecological Laparoscopic Surgery," *Evidence-Based Complementary and Alternative Medicine*, vol. 2022, Article ID 1257260, 5 pages, 2022.

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Research Article

Targeted Perioperative Nursing Combined with Propofol and Fentanyl for Gynecological Laparoscopic Surgery

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Objective. The aim of this study is to investigate the clinical effects of targeted perioperative nursing combined with propofol and fentanyl in gynecological laparoscopic surgery. *Methods*. Patients who were admitted to our hospital for gynecological laparoscopic surgeries from October 1, 2019 to November 30, 2021 were included in this retrospective study. Patients in group A received routine propofol and fentanyl. Patients in group B received targeted perioperative nursing on the basis of interventions in group A. The anesthetic effects, clinical indicators, mental health status, and adverse reactions were compared between the two groups. *Results*. A total of 84 qualified patients were retrieved. The total effective anesthesia rate, extubation time, operation time, consciousness recovery time, intraoperative blood loss, hospital stay, SAS score, SDS score, health status indicators, and adverse events in group B were all significantly better than those in group A ($P < 0.05$ for all comparisons). *Conclusion*. Combined intervention (propofol + fentanyl + targeted perioperative care) for gynecological laparoscopic surgery patients has a significant anesthesia effect, which can effectively improve the patient's clinical indicators and mental health status and can also reduce the occurrence of adverse events. It has good safety and can be widely used in clinical practice.

1. Introduction

With the continuous development of clinical minimally invasive and endoscopic techniques, laparoscopic surgery has been widely used in the gynecological field due to its advantages of fewer traumas, less pain, and faster recovery from surgery [1]. The advantages of propofol and fentanyl, such as the rapid onset of anesthesia and no accumulation of anesthetic effect, make them widely used in gynecological laparoscopic surgeries [2, 3]. The fast development of laparoscopic surgery also leads to higher requirements for nursing staff in clinical practice, so as to improve the quality of nursing services and promote a more harmonious relationship between doctors and patients, which is beneficial to postsurgical recovery to a certain extent [4, 5].

Anxiety and depression are commonly seen in postsurgical and cancer patients, especially in the elderly and females [6, 7]. Therefore, in this study, we retrieved patients who were admitted to our hospital and received gynecological laparoscopic surgeries and further analyzed the clinical effects and mental status after the combined postsurgical intervention (propofol + fentanyl + targeted perioperative care), aiming to provide a basis for clinical care plan in patients after gynecological laparoscopic surgeries.

2. Materials and Methods

Patients who received gynecological laparoscopic surgeries at our hospital from October 1, 2019 to November 30, 2021 were retrieved and divided into group A and group B. Patients in group A received routine surgical intervention, while patients in group B were given targeted perioperative care on the basis of intervention in group A. Inclusion criteria [8]: (1) all included patients met the corresponding criteria for gynecological laparoscopic surgery; (2) aged between 18 and 80 years old; (3) the clinical data of all included patients were complete. (4) Signed the informed consent form. Exclusion criteria [9]: (1) patients with severe

mental disorders or clouded consciousness; (2) patients with respiratory diseases; (3) patients with certain contraindications or allergic history to anesthetics. This study was approved by the Ethics Committee of our hospital

Anesthesia intervention: The patient was first given in of atropine 0.5 mg before surgery. Secondly, the clinical signs of the patient were monitored immediately after entering the operating room, and 0.04 mg/kg midazolam, 2 mg/kg propofol and, and 0.4 ug/kg fentanyl were used to induce anesthesia, and then tracheal intubation was performed to assist ventilation. Finally, anesthesia was maintained with 0.5 ug/kg/min fentanyl and 4 mg/kg/h propofol, which was terminated 30 min before the completion of surgery.

Patients in group A received routine surgical intervention, while patients in group B received targeted perioperative nursing on the basis of the intervention in group A [10]. The specific steps were: (1) Preoperative intervention: patients were prone to anxiety and depression and other adverse psychological emotions before surgery. Therefore, nursing staff should actively communicate with patients at this time and enhance their confidence in treatment by patiently informing patients of successful anesthesia cases. At the same time, nursing staff should also make sufficient preparations for surgery and prepare ECG monitors, ventilators, and all necessary surgical instruments before surgery. (2) Intervention during operation: after the patient enters the operating room, the nursing staff should provide psychological intervention with the patient in time to relieve their negative psychological emotions. A series of unexpected situations may occur during the operation, so the nursing staff should focus on monitoring the patient's physical indicators. At the same time, it is also necessary to timely solve the problems of aspiration and reflux that might occur during the operation. (3) Postoperative intervention: after the operation, the nursing staff should reassure the patient's psychological state, instruct the patients to remain in a supine position after returning to the ward, and pay close attention to their vital signs until they return to normal. At the same time, it is necessary to avoid slippage of the drainage tube and record the status, color, smell, and drainage volume of the drainage material in detail.

2.1. Evaluation of Anesthesia Effect. Significant effect: the patient's anesthesia induction state is stable, the depth of anesthesia maintenance is reasonable, and the state is stable during recovery; normal effect: the patient's anesthesia induction state is relatively stable, the depth of anesthesia maintenance is reasonable, and mild agitation occurs during recovery; terrible effect: the patient's anesthesia induction state unstable, unreasonable depth of anesthesia maintenance, severe agitation during recovery. Total effective effective anesthetic rate = (Significant + Normal)/total number of cases × 100% [11, 12].

2.2. Evaluation of Clinical Indicators. The extubation time, operation time, consciousness recovery time, intraoperative blood loss, hospitalization days, and adverse events of the two groups of patients were recorded and compared [13].

2.3. Assessment of Mental Health Status. The anxiety and depression status of the patients were assessed by the Self-rating Anxiety Scale (SAS) and Self-Rating Depression Scale (SDS) scores, respectively. A SAS score ≥50 indicated that the patient had anxiety, and a SDS score ≥53 points indicates that the patient had depression [14, 15].

2.4. Statistical Methods. Data were analyzed by SPSS21.0 (IBM, Armonk, USA). The enumeration data were represented by $n(\%)$ and analyzed by χ^2 test, and the measurement data were expressed by mean ± SD and analyzed by t-test, and the difference was determined as significant if a 2-sided $P < 0.05$.

3. Results

A total of 84 qualified patients were retrieved. The average age was (35.94 ± 5.56) years in group A ($n = 42$), and (36.14 ± 4.82) years in group B ($n = 42$). The clinical characteristics of the enrolled patients were detailed in Table 1, which showed no significant differences in age, weight, or primary disease composition between the two groups ($P > 0.05$).

3.1. Comparison of Anesthesia Effects. The total effective anesthetic rate of group B after this combined intervention was 88.12% (37/42), which was more significant than that of group A (73.81%, 31/42) ($P < 0.01$, Table 2).

3.2. Comparison of Clinical Indicators. There were significant differences in the extubation time (7.12 ± 2.32 min vs 5.32 ± 1.56 min), operation time (76.33 ± 11.39 min vs 100.76 ± 22.67 min), consciousness recovery time (10.32 ± 2.25 min vs 5.78 ± 1.64 min), intraoperative blood loss (98.53 ± 37.48 ml vs 115.51 ± 28.54 ml), hospital stay (7.45 ± 2.32 days vs 5.64 ± 1.64 days) between group A and group B ($P < 0.01$). Details are shown in Table 3.

3.3. Comparison of Mental Health Status. At admission, there were no significant differences in SAS score (70.38 ± 6.67 vs 71.21 ± 7.83) or SDS score (75.12 ± 7.56 vs 74.78 ± 8.34) between group A and group B. After intervention, there were significant differences in the SAS score (55.34 ± 3.45 vs 48.44 ± 3.12, $P < 0.05$) and SDS score (61.34 ± 5.41 vs 50.41 ± 3.26, $P < 0.01$) between group A and group B. See Table 4 for details.

3.4. Comparison of Adverse Events. There were no severe adverse symptoms in the two groups of patients after interventions, which indicated the safety of the intervention program. The total incidence of adverse events in group B

Table 1: Comparison of the clinical characteristics.

Group	Case	Age (years-old)	Weight (kg)	Primary disease (case)		
				Uterine fibroids	Ectopic pregnancy	Ovarian cyst
A group	42	35.94 ± 5.56	51.3 ± 1.85	8	13	21
B group	42	36.14 ± 4.82	50.4 ± 2.17	10	12	20
$\chi^2/t/u$		0.453	0.335		0.331	
P		0.521	0.572		0.632	

Table 2: Comparison of anesthesia effect [n (%)].

Group	Significant;	Normal	Terrible	Total effective rate
A group ($n = 42$)	18 (42.86)	13 (30.95)	11 (26.19)	31 (73.81)
B group ($n = 42$)	23 (54.76)	14 (33.33)	5 (11.91)	37 (88.12)
χ^2		—		6.985
P		—		<0.01

Table 3: Comparison of clinical indicators (days, mean ± SD).

Project	A group ($n = 42$)	B group ($n = 42$)	t	P
Duration of extubation (min)	7.12 ± 2.32	5.32 ± 1.56	10.764	<0.01
Operation time (min)	76.33 ± 11.39	100.76 ± 22.67	15.564	<0.01
Consciousness recovery time (min)	10.32 ± 2.25	5.78 ± 1.64	9.431	<0.01
Intraoperative blood loss (ml)	115.51 ± 28.54	98.53 ± 37.48	18.445	<0.01
Hospital days (d)	7.45 ± 2.32	5.64 ± 1.78	4.112	<0.01

Table 4: Comparison of mental health status of two groups of patients before and after intervention (mean ± SD).

Group	A group ($n = 42$)	B group ($n = 42$)	t	P
SAS score				
On admission	70.38 ± 6.67	71.21 ± 7.83	0.564	>0.05
After intervention	55.34 ± 3.45	48.44 ± 3.12	5.564	<0.01
SDS score				
On admission	75.12 ± 7.56	74.78 ± 8.34	0.575	>0.05
After intervention	61.34 ± 5.41	50.41 ± 3.26	7.563	<0.01

Table 5: Comparison of the occurrence of adverse events [n (%)].

Group	Shortness of breath	Nausea and dizziness	Mania	Total incidence
A group ($n = 42$)	3 (7.14)	3 (7.14)	6 (14.28)	12 (28.57)
B Group ($n = 42$)	1 (2.38)	1 (2.38)	2 (4.76)	6 (9.52)
χ^2		—		4.657
P		—		<0.01

was 9.52% (6/42), which was significantly lower than that of group A (28.57%, 12/42) ($P < 0.01$). See Table 5 for details.

4. Discussion

Laparoscopic surgery is a common minimally invasive surgery in clinical practice, and it has received widespread attention and recognition due to its small postoperative trauma, fewer complications, and faster recovery [16, 17]. Clinically, propofol and fentanyl are used to anesthetize patients with good effect. While propofol has a fast onset and strong controllability, and will not cause much impact on hemodynamics, fentanyl has a good analgesic effect [18, 19]. The application of targeted perioperative care in the perioperative period of surgical patients can improve the patient's compliance and complete the operation more smoothly [16, 20]. In this study, we found that the total effective anesthesia rate, extubation time, operation time, consciousness recovery time, intraoperative blood loss, hospital stay, SAS score, SDS score, health status indicators, and adverse events in group B were all significantly better than those in group A. This shows that propofol + fentanyl + targeted perioperative care is superior to routine surgical intervention.

Patients undergoing gynecological laparoscopic surgery usually have different degrees of negative psychological emotions, mainly because of the uncertainty of the implementation of the operation, which leads to a series of concerns and worries about the disease prognosis and recovery process. Patients are afraid of surgery, so effective

psychological intervention is of great significance to relieve the patient's negative emotions [21, 22]. In this study, there were no significant differences in SAS score or SDS score between the two groups at admission, but there were significant differences in SAS score or SDS score after intervention, which shows that the intervention program of group B can greatly improve the patient's mental health and speed up the recovery. The application of targeted perioperative care might be promising in more severe cases, such as brain injury, fulminant hepatitis, infection, and so on [23–30].

All in all, the combined intervention (propofol + fentanyl + targeted perioperative care) for gynecological laparoscopic surgery patients has a significant anesthetic effect, which can effectively improve the patient's clinical indicators and mental health status and reduce the occurrence of adverse reactions. It has good safety and can be widely used in clinical practice.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Disclosure

Xue-Yu Yun and Shu-Juan Chen are the co-first authors.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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Research Article

Influence of Information-Based Continuous Care on Disease Control and Treatment Compliance of Elderly Diabetic Patients

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Background. The incidence of diabetes is increasing year by year. For elderly diabetic patients, poor blood glucose control and worsening immune function greatly increase the risk of complications, which will seriously affect their quality of life. *Purpose*. This paper primarily clarifies the influence of information-based continuous care on disease control and treatment compliance of elderly diabetic patients. *Methods*. From December 2018 to December 2021, 106 elderly diabetic patients were selected, and their clinical data were retrospectively studied. Patients were grouped according to the type of care they received: an observation group (OG) comprising 56 cases receiving information-based continuous care and a control group (CG) including 50 cases treated with routine nursing. The two cohorts of patients were compared regarding disease control, treatment compliance, glucose and lipid metabolism (GLM), and self-management. *Results*. After analysis, it was found that the disease control and treatment compliance were statistically higher in OG compared with CG. OG also showed significantly reduced fasting blood glucose (FBG), 2-hour postprandial blood glucose (2hPG), total cholesterol (TC), and triglyceride (TG) after nursing that were all lower compared with CG. In terms of self-management, OG outperformed CG in diet, exercise, blood glucose monitoring, and adherence to medical regimens. *Conclusions*. Information-based continuous care has beneficial effects on disease control and treatment compliance of elderly diabetic patients and can help control blood sugar and optimize patients' self-management level, with high clinical promotion value.

1. Introduction

Diabetes mellitus (DM) is a chronic metabolic disease, with clinical experience indicating that approximately 95% of all diagnosed cases being type 2 diabetes mellitus (T2DM) and 5% being type 1 diabetes mellitus (T1DM) [1, 2]. Different DM types are generally considered to be related to insulin resistance due to the inability to produce insulin and improper use of glucose (T1DM) or the ability to produce hormones but inability to interact with its receptors (T2DM) [3]. Recent years have witnessed the gradual rise of the incidence of DM. However, the early onset of DM is insidious, and a cure is difficult to achieve after onset [4, 5]. It is shown that for elderly DM patients with vascular fragility, poor blood glucose (BG) control, and deteriorating immune function, there is a higher risk of infective complications, which seriously affects the quality of life of patients and their caregivers [6]. Choosing an appropriate care model is critical due to the great difficulty in curing the disease. This study mainly explores the influence of information-based continuous care on disease control and treatment compliance of elderly DM patients, aiming at optimizing the management mode of such patients, which is of great significance to improve patients' clinical symptoms and their self-management level.

Continuous nursing is a care method that aims to consolidate and maintain treatment effects and attempts to improve disease outcomes through long-term management of patients [7]. The gradual maturity and promotion of the Internet of Things technology makes continuous nursing based on information possible [8]. Under this mode, smart phones can be used as media to provide a series of automatic

functions for patients through software, and telephone consultants can also be utilized to provide effective continuity of care for elderly DM patients [9]. In the research of Xia [10], on the influence of information-based continuous nursing on colostomy patients, this nursing method shows obvious advantages in enhancing patients' self-efficacy and confidence, contributing to reduced complications and improved life quality. This report is enlightening. The routine nursing currently applied is not effective in controlling the disease in elderly DM patients, which is mainly because DM is difficult to cure and requires long-term blood glucose management, while long-termin-hospital routine nursing often causes a great economic burden to patients. Considering the above-mentioned conditions, this research is expected to provide a new reference for the management of elderly DM patients by comparing the differences in disease control and treatment compliance between information-based continuous care and routine care.

2. Data and Methods

2.1. General Information. This retrospective study selected 106 elderly DM patients presented between December 2018 and December 2021 to the Hunan Provincial People's Hospital. According to the type of care they received, they were assigned to two cohorts: an observation group (OG) comprising 56 cases receiving information-based continuous care and a control group (CG) including 50 cases treated with routine nursing.

The mean age and average disease course of OG were (69.45 ± 6.48) years and (7.28 ± 3.51) years, respectively, while those of CG were (68.76 ± 5.54) years and (7.08 ± 3.51) years, respectively. The two cohorts of patients were clinically comparable without statistical differences in general data ($P > 0.05$).

The Ethics Committee of Hunan Provincial People's Hospital approved this research without reservations, and all subjects provided informed consent.

2.2. Eligibility Criteria. Elderly patients who were in accordance with the diagnosis of DM and willing to receive follow-up and guidance, with normal communication and cognitive ability, and no mental illness or communication disorders were enrolled.

Those with severe heart, lung, kidney dysfunction, other serious chronic diseases, malignant tumors, or incomplete medical records, and those who refused to participate in this clinical trial were excluded.

2.3. Nursing Methods. CG was given routine nursing. Guidance on correct medication and dietary, as well as monitoring of patients' BG and timely management of related complications were given according to the patients' condition.

Information-based continuous care was used in OG: (1) a nursing plan was formulated according to each patient's specific condition. (2) DM health education manual and self-monitoring diary were distributed to each patient. (3) The Internet hospital medical service model was adopted. The Internet was used as an auxiliary tool of extended medical care to provide complete medical support for elderly DM patients in consultation, diagnosis and treatment, drug purchase, rehabilitation nursing, and health management. (4) An information platform was established, and new media such as QQ and WeChat were used to strengthen communication with patients and/or their relatives. (5) Home nursing intervention was also provided, mainly including psychological intervention (patiently listening to patients' demands and timely adjusting patients' state of mind), dietary guidance (guiding patients to calculate daily calories and determining appropriate recipes), and life and health care guidance (preventing colds and cleaning the vulva with warm water). (6) Patients were encouraged to do aerobic exercise (walking, jogging, swimming, Tai Chi, etc.) within 1 to 3 hours after meals at least once every two days. (7) Patients were instructed to correctly use the BG meter to self-monitor BG and to take antihypertensive and hypoglycemic drugs reasonably. The indications, contraindications, and possible adverse reactions of drugs were also introduced to patients. For those with poor compliance or memory, medication supervision was strengthened. (8) Importance was also attached to the follow-up. Home visits were performed at least once every two weeks during the first 3 months following discharge. During the fourth to sixth months, a telephone follow-up at least every half a month and a home visit every month were carried out so as to identify potential adverse factors in time.

2.4. Disease Control Efficacy Assessment. Marked improvement: it was defined as FBG <7.2 mmol/L, 2hPG <8.3 mmol/L, TC < 1.8 mmol/L, and TG < 5.3 mmol/L.

Improvement: it was defined as FBG <8.3 mmol/L, 2hPG <10.0 mmol/L, TC < 2.5 mmol/L, and TG < 6.5 mmol/L.

Ineffectiveness: if the patient's BG and glycation control indexes did not meet the above standards, it was classified as ineffectiveness.

2.5. Outcome Measures. Nursing efficacy. The assessment criteria for disease control are shown above. The total effective rate of disease control was the percentage of the total number of patients with significant improvement and improvement in the total number of cases.

Treatment compliance. Complete compliance: the patient takes the medicine on time and actively as instructed; compliance: the patient does not take the medicine on time but passively; noncompliance: coercive measures have to be taken to make the patient take medicine because the patient hides the drugs and refuses to take medicine.

Glucose and lipid metabolism (GLM). The information platform was utilized to supervise and guide patients to use BG meters to measure BG and to count the levels of FBG, 2hPG, TC, and TG.

Self-management. The self-management ability scale made by our hospital was used to evaluate the self-management ability of patients from four aspects: active

Table 1: General data of elderly diabetic patients (n (%), mean ± SEM).

Elements	Control group ($n = 50$)	Observation group ($n = 56$)	χ^2/t	P
Gender (male/female)	28/22	34/22	0.242	0.623
Age (years old)	68.76 ± 5.54	69.45 ± 6.48	0.586	0.559
Course of disease (years)	7.08 ± 3.51	7.28 ± 3.51	0.293	0.770
History of smoking (yes/no)	24/26	23/33	0.514	0.474
History of alcoholism (yes/no)	13/37	15/41	0.008	0.927
Exercise habit (yes/no)	10/40	13/43	0.161	0.689
Marital status (married/single)	34/16	39/17	0.033	0.855
Place of residence (urban/rural)	24/26	21/35	1.192	0.275

diet control, self-monitoring of BG, active exercise, and adherence to medical regimens.

2.6. Statistical Processing. This study used the SPSS21.0 software package for statistical analysis and GraphPad Prism 6 for visualization. The number of cases/percentage (n/%) and mean ± SEM were used to represent count data (e.g., sex and smoking history) and quantitative data (e.g., age and disease duration), respectively. As for the methods for comparisons, χ^2 test was used for count data, while the independent sample t-test and paired t-test were used to identify within-group and between-group differences of quantitative data, respectively, with $P < 0.05$ as the significance level for all tests.

3. Results

3.1. General Information of Elderly DM Patients. As shown in Table 1, the general data (sex, age, disease course, smoking/alcoholism history, exercise habits, marital status, place of residence, etc.) of the two groups were comparable, with no statistical significance ($P > 0.05$).

3.2. Disease Control of Elderly Diabetic Patients. As presented in Table 2, the nursing efficacy was evaluated as significant improvement in 27 cases, improvement in 24 cases, and ineffectiveness in 5 cases in OG, while in CG, the number of significant improvement, improvement, and ineffectiveness cases was 19, 20, and 13, respectively. OG had a statistically higher total effective rate of disease control than CG (91.07% vs. 78.00%, $P < 0.05$).

3.3. Treatment Compliance of Elderly Diabetic Patients. We evaluated patients' treatment compliance to analyze the influence of the two nursing methods on treatment compliance (Table 3); the data showed that the corresponding cases of complete compliance, partial compliance, and noncompliance in OG were 23, 30, and 3, respectively, while those in CG were 15, 25, and 10, respectively. The data revealed obviously higher treatment compliance in OG compared with CG (94.64% vs. 80.00%, $P < 0.05$).

3.4. GLM in Two Groups. We tested GLM indexes to compare and evaluate the impacts of two intervention models on GLM (Figure 1). Statistical significance was absent in GLM indexes between the groups prior to intervention ($P > 0.05$) while these indexes decreased to varying degrees after intervention, with significantly lower FBG, 2hPG, TC, and TG in OG versus CG ($P < 0.05$).

3.5. Self-Management Level of Elderly Diabetic Patients. We evaluated the self-management level of patients from the aspects of active diet control, active exercise, self-monitoring of BG, and adherence to prescribed medication (Table 4). It was found that the self-management level of OG was significantly better than that of CG in these four aspects ($P < 0.05$).

4. Discussion

Against the background of socioeconomic development and population aging trend, the incidence of DM in the elderly is increasing year by year [11]. It has been reported that elderly DM patients are more predisposed to osteoporosis and chronic cardiopulmonary diseases [12, 13]. According to statistics, the prevalence of osteoporosis in elderly T2DM patients is as high as 31.7%, which seriously hinders the normal life activities of such patients and greatly affects patients' as well as their families' quality of life [14]. Therefore, optimizing nursing methods is of great significance for improving the outcome and quality of life of elderly DM patients.

With the increase in the number of patients, the working pressure on nurses in hospital is also increasing [15]. For elderly DM patients with high risk of recurrence, it is necessary to maintain long-term continuous care after the initial stage of intensive treatment [16]. Among the several new approaches with favorable application that can provide continuity of care for patients, the information-based continuous care model provides patients with longer continuous care while actively engaging them, which may produce more positive outcomes for patients [17]. This study included 106 elderly DM patients as the research subjects and divided into an OG (information-based continuous care) and a CG (routine nursing care) according to the nursing models. In our research, markedly better disease control was observed in OG, indicating that information-based continuous care has a significant impact on the disease control of elderly DM patients. In the report on the application of information-based continuous nursing in patients with coronary heart disease, Zhou et al. [18] indicated that this nursing method can effectively reduce the risk of

Table 2: Disease control of elderly diabetic patients (n (%)).

Groups	n	Marked improvement	Improvement	Ineffectiveness	Total effective rate (%)
Control group	50	19 (38.00)	20 (40.00)	13 (26.00)	39 (78.00)
Observation group	56	27 (48.21)	24 (42.86)	5 (8.93)	51 (91.07)
χ^2 value	—	—	—	—	5.014
P value	—	—	—	—	0.025

Table 3: Treatment compliance of elderly diabetic patients (n (%)).

Groups	n	Complete compliance	Partial compliance	Non-compliance	Total compliance
Control group	50	15 (30.00)	25 (50.00)	10 (20.00)	40 (80.00)
Observation group	56	23 (41.07)	30 (53.57)	3 (5.36)	53 (94.64)
χ^2 value	—	—	—	—	5.264
P value	—	—	—	—	0.022

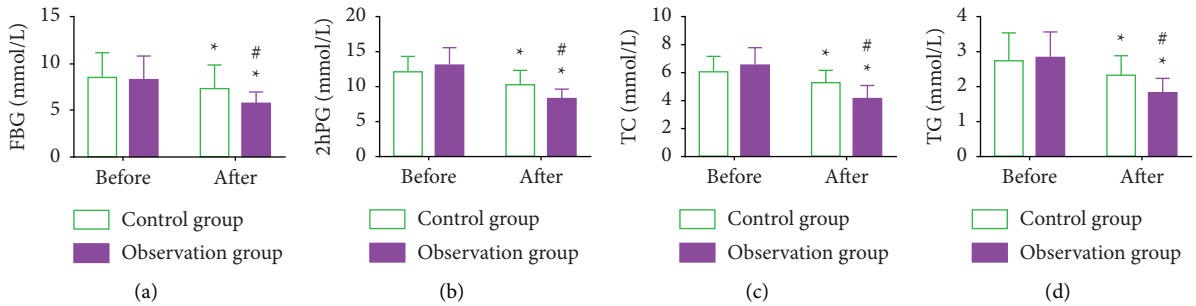

Figure 1: Glucolipid metabolism in two groups. (a) FBG of patients in both groups. (b) 2hPG of patients in both groups. (c) TC of patients in both groups. (d) TG of patients in both groups. Note: *means $P < 0.05$ compared with the level before treatment (intragroup); #means $P < 0.05$ between the observation group and the research group after treatment $P < 0.05$. FBG, fasting blood glucose; 2hPG, 2 hours postprandial blood glucose; TC, total cholesterol; TG, triglyceride.

Table 4: Self-management level of elderly diabetic patients (n (%)).

Groups	n	Active diet control	Active exercise	Self-monitoring of blood glucose	Adherence to prescribed medication
Control group	50	32 (64.00)	25 (50.00)	28 (56.00)	33 (66.00)
Observation group	56	50 (89.29)	52 (92.86)	56 (100.00)	48 (85.71)
χ^2 value	—	9.642	24.413	31.093	5.696
P value	—	0.002	<0.001	<0.001	0.017

disease under exposure to various risk factors and avoid disease recurrence and deterioration, which is consistent with the results of disease control in this study. Then, we detected GLM indicators in both groups of patients. The results determined statistically lower GLM indexes FBG, 2hPG, TC, and TG in OG, suggesting that it was more advantageous to adopt information-based continuous care in glucose and lipid control. FBG and 2hPG can directly reflect the BG level of patients, while TC and TG have been proved to be linked to the incidence of DM in previous studies, and the abnormal increase of the two is a risk factor for the onset of DM in elderly patients [19, 20]. The reduction of the above four indicators demonstrates that information-based continuous care exerts a positive impact on the disease control of elderly DM patients. In the nursing plan of the observation group, we guided patients to use the BG meter to detect the BG level through the information platform and supervised patients to take drugs reasonably, so the BG level of patients was well controlled. In addition, we also arranged aerobic exercise at least once every two days. In the report of Mehbodniya et al. [21], it was suggested that aerobic exercise could affect patients' lipid metabolism, which in turn effectively reduced patients' TC and TG levels, similar to our findings. Later, we evaluated patients' performance in terms of treatment compliance. After analysis, it was found that OG had markedly higher treatment compliance than CG (94.64% vs. 80.00%), which suggested that information-based continuous care is helpful to improve the treatment compliance of elderly DM patients. This may be due to the distribution of education manuals and self-test diaries to patients in the observation group to lay the foundation for patients' compliance with treatment; in

addition, medical staff maintain communication with patients and their families through the information platform and timely communicate with patients once they are found to have negative emotions, which all lead to the improvement of patients' treatment compliance. By evaluating the self-management level of the two groups of patients, we found that OG outperformed CG in the aspects of diet control, exercise, self-monitoring of BG, and adherence to prescribed medication, demonstrating that information-based continuous care can help optimize patients' self-management level. Mehbodniya et al. [22] pointed out in their study on information-based nursing for DM control and self-management that information-based medical management has a bright future in promoting self-health care and self-management.

The innovation of this research lies in the comprehensive analysis of the intervention effect of information-based continuous nursing from the aspects of disease control, treatment compliance, GLM, and self-management level of patients, which confirms its effectiveness in elderly DM patients and provides a new choice for nursing management of such patients. But there is still a room for improvement in this study. First of all, only 106 samples were included for analysis, so the number of subjects needs to be expanded to improve the accuracy of the conclusions. Second, this study is a single-center study, which is prone to information bias. Finally, due to the limitation of objective conditions, some corresponding indicators of elderly DM patients have not been studied. In the future, we will improve the research from the above perspectives.

Conclusively, information-based continuous care can better control the condition of elderly DM patients and effectively improve their treatment compliance and self-management level, which is conducive to disease management and is worth popularizing in clinics.

Data Availability

The labeled dataset used to support the findings of this study is available from the corresponding author upon request.

Conflicts of Interest

The authors declare no conflicts of interest.

Acknowledgments

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Retraction

Retracted: Paeoniflorin Protects H9c2 Cardiomyocytes against Hypoxia/Reoxygenation Induced Injury via Regulating the AMPK/Nrf2 Signaling Pathway

Evidence-Based Complementary and Alternative Medicine

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This article has been retracted by Hindawi following an investigation undertaken by the publisher [1]. This investigation has uncovered evidence of one or more of the following indicators of systematic manipulation of the publication process:

(1) Discrepancies in scope

(2) Discrepancies in the description of the research reported

(3) Discrepancies between the availability of data and the research described

(4) Inappropriate citations

(5) Incoherent, meaningless and/or irrelevant content included in the article

(6) Peer-review manipulation

The presence of these indicators undermines our confidence in the integrity of the article's content and we cannot, therefore, vouch for its reliability. Please note that this notice is intended solely to alert readers that the content of this article is unreliable. We have not investigated whether authors were aware of or involved in the systematic manipulation of the publication process.

Wiley and Hindawi regrets that the usual quality checks did not identify these issues before publication and have since put additional measures in place to safeguard research integrity.

We wish to credit our own Research Integrity and Research Publishing teams and anonymous and named external researchers and research integrity experts for contributing to this investigation.

The corresponding author, as the representative of all authors, has been given the opportunity to register their agreement or disagreement to this retraction. We have kept a record of any response received.

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[1] W. Yu, H. Sun, Y. Tan, and W. Zhang, "Paeoniflorin Protects H9c2 Cardiomyocytes against Hypoxia/Reoxygenation Induced Injury via Regulating the AMPK/Nrf2 Signaling Pathway," *Evidence-Based Complementary and Alternative Medicine*, vol. 2022, Article ID 7667770, 8 pages, 2022.

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Research Article

Paeoniflorin Protects H9c2 Cardiomyocytes against Hypoxia/Reoxygenation Induced Injury via Regulating the AMPK/Nrf2 Signaling Pathway

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Myocardial ischemia/reperfusion (MIR) injury contributes to the exacerbation of heart disease by causing cardiac arrhythmias, myocardial infarction, and even sudden death. Studies have found that paeoniflorin (PF) has a protective effect on coronary artery disease (CAD). However, the mechanism of PF in MIR has not been fully investigated. The purpose of this study was to investigate the functional role of PF in H9c2 cells subjected to hypoxia/reoxygenation (H/R). Here, PF treatment enhanced cell viability in H/R-stimulated H9c2 cells. In H9c2 cells, PF treatment reduced the formation of reactive oxygen species (ROS) induced by H/R. In H/R-stimulated H9c2 cells, PF also increased the activity of antioxidant enzymes such as superoxide dismutase and glutathione peroxidase. Furthermore, PF protected H9c2 cells against H/R-induced apoptosis, as demonstrated by increased Bcl-2 expression, decreased Bax expression, and decreased caspase-3 activity. Furthermore, PF increased the levels of p-AMPK and nuclear Nrf2 expression in response to H/R stimulation. AMPK inhibition, on the other hand, abolished the PF-mediated increase in Nrf2 signaling and the cardiac-protective effect in H9c2 cells exposed to H/R. These data suggest that PF protected H9c2 cells against H/R-induced oxidative stress and apoptosis through modulating the AMPK/Nrf2 signaling pathway. Our findings support the therapeutic potential of PF in myocardial I/R damage.

1. Introduction

Myocardial infarction is a common fatal and disabling disease [1]. Myocardial ischemia is caused by coronary artery obstruction or stenosis, resulting in insufficient myocardial blood supply, the imbalance between cardiac oxygen supply and oxygen demand, resulting in loss of myocardial cells and the formation of cardiac scar, ultimately leading to heart failure [2]. However, myocardial ischemia-reperfusion injury (MIR) often occurs after treatment of this disease, which leads to the death of a large number of myocardial cells and aggravation of myocardial injury [3]. At present, platelet regulation drugs, β–blockers, and calcium channel antagonists are used to treat this disease clinically [4–6]. Although modern medicine has made great progress in the treatment of myocardial ischemia, there are no effective drugs. Therefore, prevention and treatment of myocardial ischemia-reperfusion injury is an effective method to treat myocardial infarction. The pathophysiological mechanism of myocardial ischemia-reperfusion injury is complex. Studies have shown that myocardial ischemia can promote inflammation and oxidative stress translation and can lead to myocardial apoptosis through reperfusion, in which inflammatory factors can activate and chemotaxis leukocytes, which are also the products of activation of the leukocytes, which can aggravate myocardial injury [1, 7–9]. Superoxide dismutase (SOD) is essential to prevent oxidative stress, and it can effectively resist the damage of oxygen free radicals to the body through its antioxidant and antifree radical functions [10]. The surface level of malondialdehyde (MDA)

in the body can reflect the level of oxygen free radicals in the body, and then, reflect the damage degree of oxidative stress in the body [11]. Therefore, oxidative stress plays an important role in the occurrence and development of myocardial ischemia-reperfusion injury.

Paeoniflorin (PF) is a bioactive glycoside isolated from the root of Paeonia Alba. Paeoniflorin has been reported to have beneficial effects on the cardiovascular system (hypertension, atherosclerosis, and bleeding) and the nervous system (headaches, vertigo, dementia, and pain) [12]. Paeoniflorin by lowering lipid peroxidation products MDA and ROS generation level, reducing oxidative stress, and increase the glutathione (GSH) content, thus, reducing oxidative stress and inflammatory pathways, effectively avoiding the loss of neurons and microglia activation and cerebral white matter lesions, which caused by hypoxia-ischemia brain damage to play effective protection [12–14]. Previous studies showed that the mechanism of PF's ability to exert antioxidative stress injury may be related to improving the activity of SOD and other antioxidant enzymes in vivo, thus, alleviating the damage of oxygen free radicals to the body [15]. The effects of PF on I/R-mediated oxidative stress and apoptosis in cardiomyocytes, on the other hand, are unknown.

In this study, the hypoxia/reoxygenation injury model of myocardial cells was used to simulate the ischemic injury of ischemic heart disease in the process of hypoxia and the reperfusion injury in the process of reoxygenation after hypoxia. The levels of creatine kinase muscle/brain (CK-MB), lactate dehydrogenase (LDH), and MDA in cell culture medium at different time points during hypoxia/reoxygenation were measured to evaluate the degree of damage to myocardial cells, and then to evaluate the protective effect and mechanism of PF on hypoxia/reoxygenation injury of myocardial cells.

2. Materials and Methods

2.1. Cell Culture. The rat cardiomyocyte-derived H9c2 cell line was purchased from the cell bank of the Chinese Academy of Sciences. The H9c2 cells were gown in Dulbecco's modified Eagle's medium, added with 10% FBS at 37 in a 5% CO_2 atmosphere.

2.2. Establishment of Hypoxia/Reoxygenation (H/R) Model [16]. H9c2 cells were cultivated in a hypoxic environment with 1% O_2, 94% N_2, and 5% CO_2 in modular gas chambers for 24 h, and then, reoxygenated for 2 h at 37 C in a 21% O_2, 5% CO_2, and 74% N_2 incubator. Before H/R stimulation, cells were pretreated with or without PF for 2 h.

2.3. Cell Viability Assay. The 3-(4, 5-dimethylthiazol)-2, 5-diphenyltetrazolium bromide (MTT) assay kit was used to analysis cell viability. H9c2 cells were seeded in 96-well plates and grown for 24 h prior to H/R. H9c2 cells were cultured for 1 h with 10 ul MTT solution following various treatments. Then, the H9c2 cells were added with 200 ml of DMSO to dissolve the formazan crystals. A microplate reader (Varioskan Flash, Thermo, Finland) was measured to measure absorbance at 490 nm, and the absorbance values of control cells were adjusted to 100%.

2.4. Cell Cytotoxicity Assay. The culture supernatants were collected after 24 h of incubation with various concentrations of PF (0, 5, 10, 20, and 40 mM) to determine lactate dehydrogenase (LDH) leakage. The LDH detection kit was used to analyze the LDH content.

2.5. Measurement of Cellular ROS Production. Flow cytometry was used to examine the generation of intracellular ROS using dichlorofluorescin diacetate as the fluorescent probe. In brief, H9c2 cells were washed with PBS before being treated with 10 mM DCFH-DA at 37 for 30 min in the dark. The flow cytometer was then used to investigate H9c2 cells using a 488 nm excitation filter and a 525 nm emission filter.

2.6. Determination of SOD, MDA, and GSH. H9c2 whole cell lysates were collected according to the manufacturer's instructions using RIPA lysis buffer. The activities of SOD, MDA, and GSH were investigated using the corresponding kits. The MDA level was determined using the thiobarbituric acid method and MDA detection kit (A003-1-2; Jiancheng Bioengineering Institute). SOD and GSH activity were detected using the hydroxylamine method, and total SOD detection kit, and GSH detection kit (Jiancheng Bioengineering Institute).

2.7. Western Blot Analysis. Cells were washed with precooled PBS and added to the protein lysate. The supernatant was removed and obtained after centrifugation. The protein content was determined by the BCA method. Mix with protein loading buffer in proportion, heat at 100 to denaturate protein, and store at low temperature for later use. The proteins in the polyvinylidene fluoride (PVDF) gel were transferred to a polyvinylidene fluoride (PVDF) membrane by sodium dodecyl sulfate and polyacrylamide gel electrophoresis, and were sealed at 5% BSA at room temperature for 2 h. The B cell lymphoma-2 (Bcl-2), Bax, Caspase3, P-AMPK, AMPK, Nrf2, GAPDH, and lamin B2 primary antibody (Abcam) were added to a resistant shaker overnight. The PVDF membrane was washed by TBST for 3 times, 5 min each time, and placed into the rat secondary antibody (1 : 5000) for incubation for l ~ 2 h. The PVDF membrane was removed and washed by TBST for 3 times, 5 min each. The ECL kit was used for development, the chemiluminescence gel system analyzer was used for display, and photos were taken using VisionWorks 6.3.3. The gray values of protein bands were analyzed by image acquisition and analysis software with GAPDH as an internal reference. The experiment was repeated three times. The images were scanned for preservation and analyzed with ImageJ software, with the gray-scale value digitized on each special band.

2.8. Caspase-3 Activity. Caspase-3 activity of H9c2 cells was analyzed by using a Caspase-3 Colorimetric Assay Kit. In brief, H9c2 cell lysates were treated at 37 with caspase 3 substrates, Ac-DEVD-pNA and the released p-NA was quantified using a spectrophotometer at 405 nm.

2.9. Statistical Analysis. Graphpad software was used to analyze the results of three separate tests, which were reported as mean + SD. One-way ANOVA was used to assess group comparisons, followed by the least significant difference test. $P < 0.05$ was considered to be statistically significant. $^{*}P < 0.05$ denotes a significant change as compared to control H9c2 cells. $\#P < 0.05$ denotes a significant change as compared to H9c2 cells treated with H/R. denotes a significant difference when compared to the H/R + PF groups.

3. Results

3.1. PF Improves the Cell Viability and Injury in H/R Stimulated H9c2 Cells. To explore the effect of PF on H/R stimulated H9c2 cells, the cells were incubated with a series of concentration of PF (0, 50, 100, and 200 μM) for 24 h. The MTT assay demonstrated that H/R inhibited H9c2 cell viability. The different concentrations of PF (50, 100, and 200 μM) treatments markedly enhanced the cell viability in H/R induced H9c2 cells (Figure 1(a)). LDK leakage assay showed that H/R increased the LDK leakage and the different concentration of PF (50, 100, and 200 μM) treatments markedly decreased the LDK leakage in H/R induced H9c2 cells (Figure 1(b)). Besides, the H/R induced the production of CK-MB and the different concentration of PF (50, 100, and 200 μM) treatments reduced the CK-MB level in H/R induced H9c2 cells (Figure 1(c)). Therefore, PF effectively protected the cell viability and injured H/R stimulated H9c2 cells.

3.2. PF Represses Oxidative Stress in H9c2 Cells Exposed to H/R Treatment. As shown in Figure 2(a), the ROS level was higher in the H/R group than control, while PF markedly decreased the production of ROS in H/R stimulated H9c2 cells. Moreover, the activity of SOD and GSH were reduced in H/R group compared with control, PF markedly enhanced the SOD and GAH activities (Figures 2(b) and 2(c)); H/R-induced increase in the MDA activities, which was blocked by pretreatment with PF (Figure 2(d)). Thus, PF reduces oxidative stress in H9c2 cells exposed to H/R.

3.3. PF Inhibits Apoptosis in H9c2 Cells Exposed to H/R Treatment. Subsequently, cell apoptosis was assessed by detecting the expression levels of Bax and Bcl-2. As shown in Figures 3(a)–3(c), H/R treatment significantly increased the Bax protein expression and reduced the Bcl-2 protein expression in H9c2 cells, while PF prevented the change of Bax and Bcl-2 protein caused by H/R. In addition, the caspase-3 activity was significantly enhanced in H/R stimulated H9c2 cells; PF markedly inhibited the caspase-3 activity in H/R stimulated H9c2 cells (Figure 3(d)). Thus, PF reduced cell apoptosis in H9c2 cells exposed to H/R treatment.

3.4. PF Induced the Activation of AMPK/Nrf2 Signaling Pathway. The AMPK/Nrf2 signaling pathway has been discovered as a ROS-activated antioxidant signaling mechanism. We then looked at how PF affected AMPK/Nrf2 activation in H/R-exposed H9c2 cells. As shown in Figures 4(a)–4(c), the levels of p-AMPK and nuclear Nrf2 were inhibited in H/R-exposed H9c2 cells, PF increased the levels of p-AMPK, and nuclear Nrf2 in H/R-exposed H9c2 cells.

3.5. Treatment with Compound C Reserved the Effects of PF on Cell Viability, Oxidative Stress, and Apoptosis in H/R Stimulated H9c2 Cell. Compound C, an AMPK inhibitor, was employed to impede AMPK signaling in order to validate the involvement of AMPK/Nrf2. Compound C treatment resulted in the predicted reduction in nuclear Nrf2 expression in H9c2 cells (Figures 5(a)–5(c)). Furthermore, AMPK inhibition effectively reversed the regulatory effects of PF on cell survival (Figure 5(d)), ROS levels (Figure 5(e)), and caspase-3 activity (Figure 5(f)). These findings revealed that AMPK mediated the role of PF on Nrf2 signaling in H9c2 cells.

4. Discussion

At present, the basic treatment principle for ischemic heart disease is to restore reperfusion. The recovery of reperfusion not only improves the ischemic state, but also causes myocardial injury again–reperfusion injury. Myocardial cell hypoxia/reoxygenation model well simulated myocardial cell reperfusion injury [17]. It is generally believed that during hypoxia/reoxygenation, cardiomyocytes produce various oxygen free radicals, which react with the peroxidation of cell membrane and biological macromolecules and destroy the normal structure of the cell membrane [18]. Myocardial enzymes such as CK and LDH leak out of the cell with the destruction of the cell membrane, and the peroxide MDA of membrane lipid molecules is produced in large quantities, resulting in the lack of reoxygenation injury of cardiomyocytes [19]. Therefore, myocardial cell injury is the culprit of myocardial ischemia and ischemia-reperfusion injury, and the key to the treatment of such diseases is to combat myocardial cell injury. In this study, the H/R induced the production of CK-MB, and the different concentrations of PF (50, 100, AND 200 μM) treatments reduced the CK-MB level in H/R induced H9c2 cells. Therefore, PF effectively protected the cell viability and injured H/R stimulated H9c2 cells.

The caspase family is an important molecule that mediates cell apoptosis. Caspase-3 and Caspase-9 are involved in signal transduction of the death receptor apoptosis pathway and mitochondrial apoptosis pathway, respectively [20]. Finally, caspase-3 is activated and apoptosis is performed through cascade activation of multiple downstream caspase molecules [21, 22]. Paeoniflorin is the active

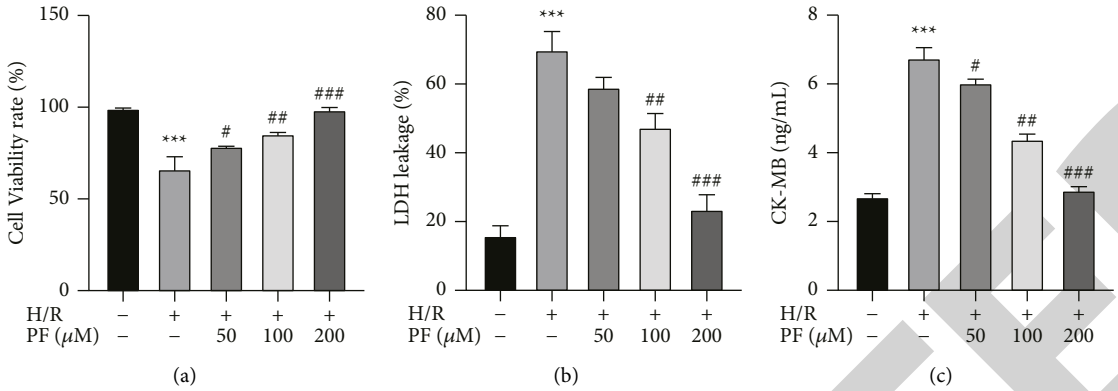

FIGURE 1: Effect of PF on cell viability, LDK and CK-MB level in H/R induced H9c2 cells. H9c2 cells were added with PF (50, 100, 200 μM) for 2 h and then subjected to H/R stimulation. (a) MTT assay was used to analyze the cell viability. (b) LDK leakage assay was used to assess the cytotoxic effect of PF in H9c2 cells. (c) ELISA was performed to assess the CK-MB levels in H9c2 cells. $N = 3$, $^{*}P < 0.05$ vs. control. $\#P < 0.05$ vs. H/R.

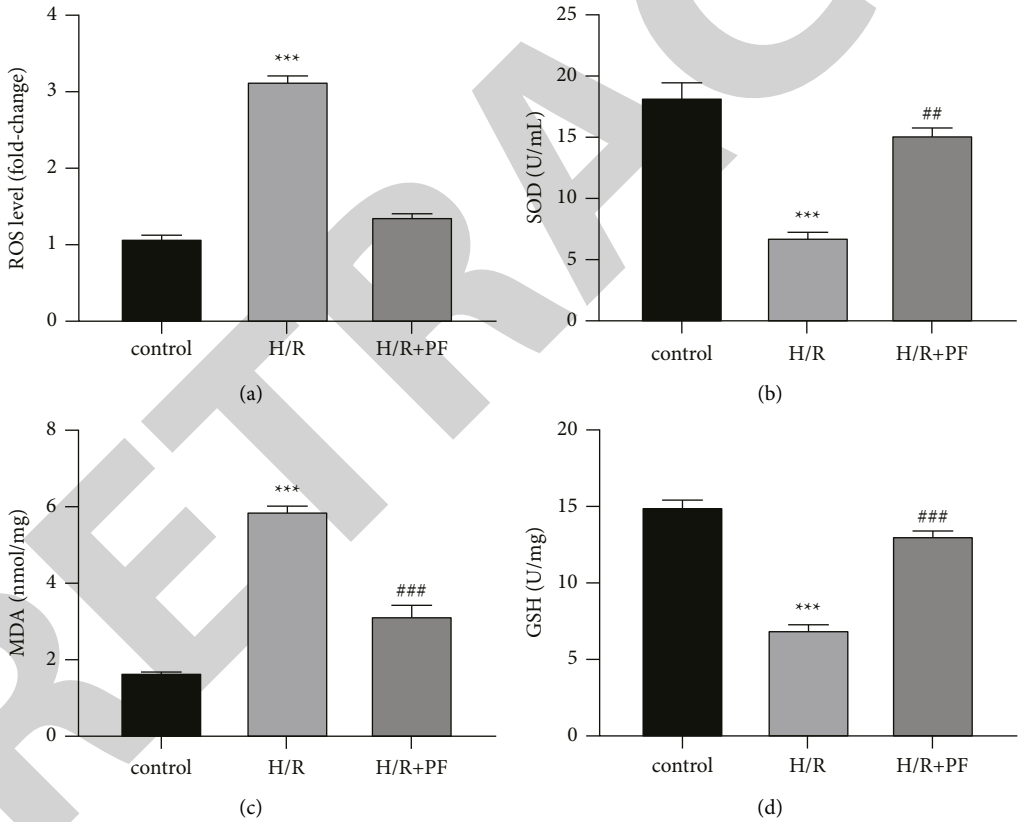

FIGURE 2: Effect of PF on oxidative stress in H9c2 cells. (a) Flow cytometry was used to assess ROS generation in H9c2 cells. (b–d) SOD, GSH, and MDA activities in H9c2 cells were measured by ELISA. $N = 3$, $^{*}P < 0.05$ vs. control. $\#P < 0.05$ vs. H/R.

ingredient of Paeonia lactiflora, which can protect cells from inflammation and oxidation [23]. In order to define the paeoniflorin effects on myocardial ischemia injury in the process of apoptosis, we measured caspase-3 activity and apoptosis gene expression quantity on the basis of the comparison. The results showed that paeoniflorin H9c2 cells in ischemia reperfusion, so paeoniflorin can inhibit myocardial ischemia injury in the process of cell apoptosis, and alleviate myocardial damage.

The nuclear factor E2-related factor 2 (Nrf2) is a key transcription factor widely existing in animals to defend against oxidative stress and can combine with antioxidant response elements (ARE) to activate downstream antioxidant genes, such as HO-1 and NQO1, so as to resist various

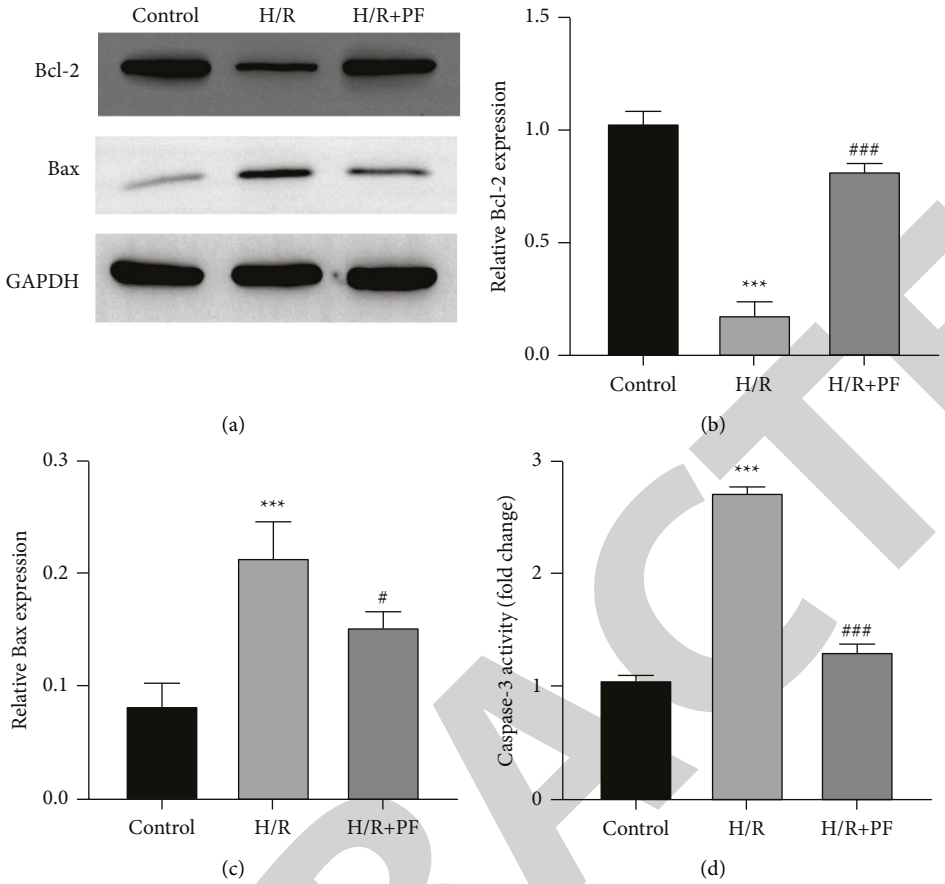

Figure 3: PF's effect on apoptosis in H9c2 cells. (a) Western blot analysis was used to determine the levels of expression of apoptosis-related proteins such as bax and bcl-2. Quantification of bax and bcl-2 (b and c). The caspase-3 activity with the substrate peptide Ac-DEVD-pNA was measured using a colorimetric technique (d).

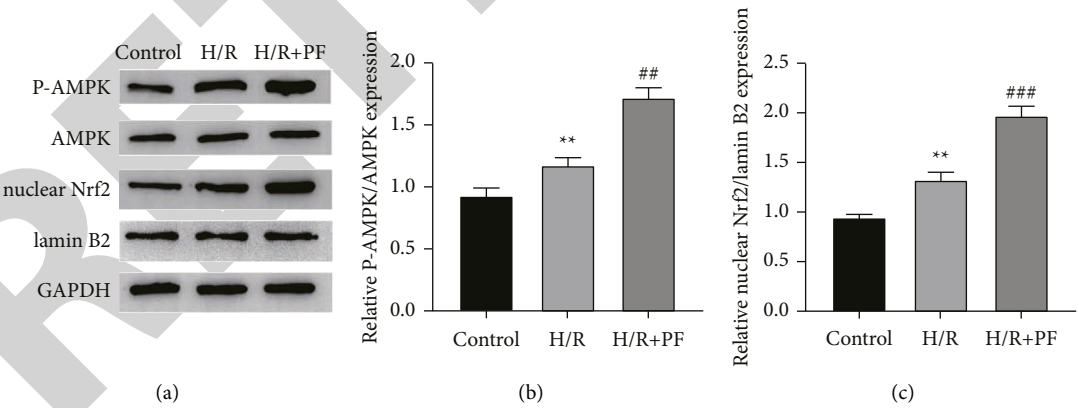

Figure 4: The effect of PF on the AMPK/Nrf2 signaling pathway in H9c2 cells activated with H/R. (a) Western blot was used to determine the levels of AMPK, p-AMPK, and nuclear Nrf2 expression. (b-c) AMPK, p-AMPK, and nuclear Nrf2 quantification analysis. $N = 3$, $^{*}P < 0.05$ vs. control. $\#P < 0.05$ vs. H/R.

protoplasts-induced intracellular oxygenation excitation states [24, 25]. Adenosine monophosphate-activated protein kinase (AMPK), a silk/threonate albuminase composed of three peptide chains, is an important regulator of human energy metabolism and is closely related to promoting catabolism, inhibiting anabolism, improving endothelial function, alleviating inflammatory response, and inhibiting oxygen-reduction reaction [26, 27]. It has been shown in previous studies that AMPK can activate Nrf2 through phosphorylation and generate downstream antioxidant genes such as HO-1 and NQO1 to play an antioxidative stress role [28]. Here, we found that PF induced the

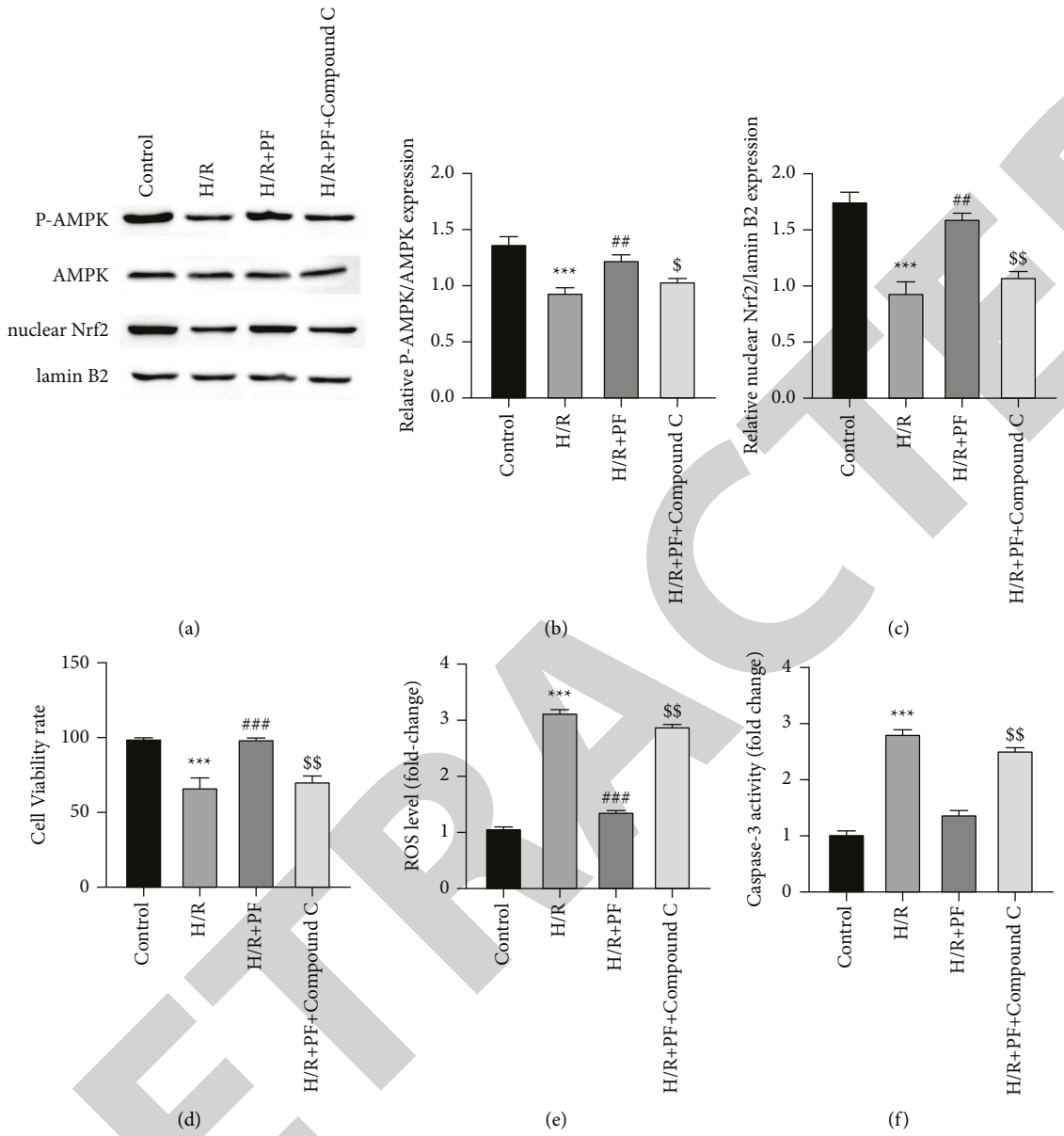

Figure 5: Effect of AMPK inhibition on PF-mediated Nrf2 signaling pathway activation in H/R induced H9c2 cells. H9c2 cells were exposed to H/R damage after being treated with PF in the presence of Compound C (10 M). (a) Western blot analysis of AMPK and Nrf2 nuclear protein expression. (b-c) P-AMPK and Nrf2 quantification analysis. (d) Cell viability in H9c2 cells. (e) ROS generation in H9c2 cells. (f) Caspase-3 activity in H9c2 cells, $^{*}P < 0.05$ vs. control. $\#P < 0.05$ vs. H/R, vs. H/R + PF group.

activation of the AMPK/Nrf2 signaling pathway in H/R stimulated H9c2 cells.

A previous study reported that Galanthamine improves myocardial I/R induced cardiac dysfunction by activating the AMPK/Nrf2 pathway in rats [29]. Galanthamine improves myocardial I/R-induced cardiac dysfunction by activating AMPK/Nrf2 pathway in rats [28]. Galanthamine improves myocardial I/R-induced cardiac dysfunction by activating the AMPK/Nrf2 pathway in rats [30]. Here, we found that PF induced the activation of the AMPK/Nrf2 signaling pathway in H/R stimulated H9c2 cells. Compound C, an AMPK inhibitor, was employed to impede AMPK signaling in order to validate the involvement of AMPK/Nrf2. AMPK inhibition dramatically reversed the regulatory effects of PF on cell survival, ROS levels, and caspase-3 activity. These findings revealed that AMPK mediated the control of PF on Nrf2 signaling in H9c2 cells.

5. Conclusions

In conclusion, our findings show that PF protects H/R stimulated H9c2 cells by inhibiting oxidative stress and apoptosis. The AMPK/Nef2 signaling pathway was activated to control the protective effects of PF. As a result, PF might be a potential therapeutic medication for the treatment of myocardial I/R damage.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Wen Yu designed the experiments. Huang Sun wrote the article. Yang Tan performed experiments. Wei Zhang analyzed this data. All the authors read and approved the final manuscript. The authors Wen Yu and Huang Sun contributed equally to this article.

Acknowledgments

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Research Article

Clinical Study on Blood Pressure Variability, Montreal Cognitive Assessment and Arteriosclerosis Index in Patients with Cerebral Small Vessel Disease Treated with Integrated Traditional Chinese and Western Medicine by Invigorating Kidney and Removing Blood Stasis

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Objective. To explore the clinical improvement in blood pressure variability, Montreal Cognitive Assessment, and angiosclerosis index in patients with cerebral small vessel disease treated with integrated traditional Chinese and Western medicine. *Methods*. A randomized controlled study of patients with cerebral small vessel disease who were treated in our hospital from November 1, 2018, to January 31, 2022. The enrolled patients were randomized into 2 groups according to the random numbers: an observation group treated with integrated traditional Chinese and Western medicine and a control group treated with Western medicine only. Blood pressure variability, Montreal Cognitive Assessment (MoCA), and angiosclerosis index were compared between the two groups. *Results*. There were 71 qualified cases in the observation group and 58 qualified cases in the control group. Before treatment, the indicators between the two groups were comparable ($P > 0.05$). After treatment, the mean values of systolic blood pressure (SBP) and diastolic blood pressure (DBP) were significantly decreased ($P < 0.05$); the decrease of 24hSBP-coefficient of variation (CV), daytime SBP (dSBP)-CV, 24hSBP-standard deviation (SD), and dSBP-SD in the observation group was significantly better than that in the control group; the MoCA scores of the observation group were significantly higher than those of the control group (($P < 0.05$); the ABI and PWV were significantly different between the two groups ($P < 0.05$); TC, TG, HDL-C, and LDL-C in observation group decreased after treatment, and HDL-C increased significantly ($P < 0.05$). *Conclusion*. Integrative traditional Chinese and Western medicine treatment can further reduce the blood pressure variability, especially systolic blood pressure; improve the MoCA score and cognitive function, increase the ankle-brachial index, reduce pulse wave velocity and the degree of arteriosclerosis; and improve lipid metabolism a comprehensive intervention role.

1. Introduction

With the aging of the population and changes in people's living habits, the prevalence of stroke in China is increasing year by year, which has become a major social problem. In the past, most of the understanding of cerebral apoplexy was based on two diseases that mainly damage the large blood vessels in the brain, but due to its extensive clinical use, it has been used in many cerebral small vessel diseases (CSVDs) [1–4]. Hypertension and aging are the most important independent risk factors for CSVD, and cognitive impairment and stroke are the final outcomes of the disease. At present, there is a lack of secondary prevention and treatment research on CSVD. Antihypertensive, antithrombotic, and statin lipid regulation are important measures for the

Table 1: General information of group 2.

Project	Observation group ($n = 71$)	Control group ($n = 5$)	t/χ^2	P
Male/Female (n)	39/32	31/27	3.053	0.985
Age (years)	63.24 ± 1.42	63.89 ± 1.68	1.176	0.241
Height (cm)	162.45 ± 11.27	163.83 ± 12.06	4.247	0.805
Weight (kg)	51.62 ± 4.33	52.60 ± 3.95	8.635	0.526
Smoking history	21 (29.58)	15 (25.86)	5.849	0.635
Alcoholism history	17 (23.94)	11 (18.97)	11.273	0.781
Course of disease (years)	3.79 ± 1.03	3.76 ± 1.15	7.492	0.893
Hypertension				
Grade I	25 (35.21)	20 (34.48)	16.845	0.847
Grade II	29 (40.85)	23 (39.66)		
Grade III	17 (23.94)	15 (25.86)		
Diabetes	24 (33.80)	19 (32.76)	9.724	0.846
Coronary heart disease	16 (22.54)	14 (24.14)	2.448	0.751
Hyperlipidemia	64 (90.14)	53 (91.38)	8.729	0.819

treatment of CSVD. Some recent studies have shown that, in addition to blood pressure amplitude, blood pressure variability (BPV) has a significant relationship with the development of CSVD [5]. As a quantitative index, BPV can independently predict changes in the heart, brain, and kidney. Target visceral lesions such as peripheral blood vessels and the risk of cardiovascular disease are the main clinical indicators of CSVD patients. Therefore, early intervention for BPV is very necessary, but there is still a lack of high-level clinical trials to prove it. In this randomized controlled trial (RCT), we analyzed the patients with cerebral small vessel disease treated in our hospital.

2. Materials and Methods

This is an RCT of patients with cerebral small vessel disease who were treated at our hospital from November 1, 2018, to January 31, 2022. The subjects were randomized into 2 groups according to the random numbers, one in the observation group (71 cases) and the other in the control group (58 cases); the observation group included 39 males and 32 females, with an average age of (63.24 ± 1.42) years; the control group included 31 males and 27 females, with an average age of (63.89 ± 1.68) years. This study was approved by the Medical Ethics Committee of our hospital (Approval no. 2018–084) and was enrolled in the China Clinical Trial Registry (Registration No. ChiCTR1800018873). The patients and their families were informed and agreed to the study and signed the consent form (Table 1).

2.1. Diagnosis, Inclusion, and Exclusion Criteria

(i) The diagnostic criteria of traditional Chinese medicine (TCM) syndrome types were as follows: (1) dizziness, sore waist, and weak knees; (2) dark purple tongue or ecchymosis; (3) tinnitus, forgetfulness

(ii) Diagnostic criteria were as follows: (1) Clinical manifestations: asymptomatic lacunar infarction, cerebral microbleeds, partial leukoaraiosis, various lacunar syndromes, and vascular cognitive dysfunction; and (2) imaging confirmed as lacunar infarction, perivascular gap enlargement, and white matter lesions

(iii) Inclusion criteria were as follows: (1) met the diagnostic criteria; (2) no macrovascular disease or intracranial tumor; (3) 40–80 years old; (4) MoCA score greater than 26 points; (5) signed informed consent

(iv) Exclusion criteria were as follows: (1) had other cerebral small vessel diseases in the past, such as amyloid angiopathy type, other hereditary disease-related small vessel disease, hypertensive emergency (SBP >180 mmHg, DBP > 110 mmHg), hypertensive crisis, and secondary obesity; (2) severe liver or kidney insufficiency; (3) allergic constitution or allergic to multiple drugs; (4) complicated with other serious diseases

Patients in both groups were treated for 12 weeks. Patients in the control group were given basic medical treatment such as antihypertensive, hypoglycemic, lipid-regulating, and antiplatelet aggregation. Patients in the observation group received the treatments as patients in the control group, combined with the Bushen Huayu Recipe, two doses a day taken in the morning and evening, 200 mL decoction each time. Recipe: 20 g of Cistanche deserticola, 12 g each of Pheretima aspergillum and Alpinia oxyphylla Miq, 10 g each of Salvia miltiorrhiza, and Radix Curcumae.

2.1.1. Observation Indicators Included

(i) Blood pressure and blood pressure variability including 24 h mean systolic blood pressure (24hSBP), 24 h mean diastolic blood pressure (24hDBP), daytime SBP (dSBP), daytime DBP (dDBP), nighttime SBP (nSBP), nighttime DBP (nDBP), 24 h systolic blood pressure variation (24hSBPv), and 24 h diastolic blood pressure variation (24hDBPv); standard deviation (SD) of 24hSBP (24hSBP-SD), 24hDBP-SD, dSBP-SD, dDBP-SD, nSBP-SD, and nDBP-SD; coefficient of variation (CV; CV = SD/mean) of 24hSBP (24hSBP-CV), 24hDBP-CV,

Table 2: Comparison of 24 h ambulatory blood pressure mean values before and after treatment in the two groups ($\overline{x} \pm s$, mmHg).

Group	Observation group ($n = 71$)		Control group ($n = 58$)		T	$P^{\#}$
	Before therapy	After treatment	Before therapy	After treatment		
24hSBP	146.73 ± 6.23	123.68 ± 3.48$^{\#}$	145.96 ± 6.34	130.52 ± 4.38$^{\#}$	7.482	0.035
24hDBP	81.56 ± 7.16	72.27 ± 6.33$^{\#}$	79.82 ± 8.36	71.36 ± 6.27$^{\#}$	4.394	0.873
dSBP	148.71 ± 8.43	123.62 ± 5.43$^{\#}$	147.82 ± 8.49	132.79 ± 6.11$^{\#}$	11.751	0.005
dDBP	81.57 ± 7.39	73.89 ± 6.28$^{\#}$	82.25 ± 7.42	72.43 ± 6.45$^{\#}$	8.463	0.497
nSBP	137.64 ± 17.58	122.89 ± 9.71$^{\#}$	138.39 ± 13.25	125.89 ± 9.06$^{\#}$	10.527	0.693
nDBP	77.34 ± 10.83	69.47 ± 7.56$^{\#}$	76.92 ± 9.68	67.89 ± 7.49$^{\#}$	15.263	0.749

Note. $^{\#}$Comparison between groups after treatment.

dSBP-CV, dDBP-CV, nSBP-CV, and nDBP-CV, which were detected by ambulatory blood pressure at 0 and 12 weeks of intervention. The respective change levels of subjects before and after intervention and between groups were compared.

(ii) Cognitive function: the Montreal Cognitive Assessment (MoCA Assessment) was assessed at 0 and 12 weeks of the intervention.

(iii) Degree of vascular arteriosclerosis: ankle-brachial index (ABI) and pulse wave velocity (PWV) were examined at 0 and 12 weeks of intervention.

(iv) Serum total cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) were detected at 0 and 12 weeks of intervention.

2.2. Statistical Methods. The data in this experiment were analyzed by SPSS21.0 (SPSS, Chicago, IL, USA), in which the χ^2(%) test was performed for the count data, and the t-test ($\overline{x} \pm s$) was performed for the measurement data. $P < 0.05$ (2-sided) can determine that this experiment has statistical significance.

3. Results

There were no significant differences in gender, age, or accompanying diseases between the two groups ($P > 0.05$, Table 1).

3.1. Comparison of the Mean Values of 24 h Ambulatory Blood Pressure. Before treatment, there were no significant differences in blood pressure parameters between the two groups ($P > 0.05$). The mean values of systolic blood pressure and diastolic blood pressure in the period after treatment were significantly decreased ($P < 0.05$). There were significantly lower 24hSBP (123.68 ± 3.48 mmHg vs. 130.52 ± 4.38 mmHg) and dSBP (123.62 ± 5.43 mmHg vs. 132.79 ± 6.11 mmHg) in the observation group ($P < 0.05$, Table 2).

3.2. Comparison of the Coefficient of Variation (CV) of Ambulatory Blood Pressure. Before treatment, there were no significant differences in the variation coefficients of ambulatory blood pressure between the two groups ($P > 0.05$). After treatment, the 24hSBP-CV, 24hDBP-CV, and dSBP-CV of the two groups decreased significantly ($P < 0.05$). There was a significant decrease in 24hSBP-CV (0.073 ± 0.018 mmHg vs. 0.091 ± 0.020 mmHg) and dSBP-CV (0.059 ± 0.021 mmHg vs. 0.084 ± 0.024 mmHg) in the observation group ($P < 0.05$, Table 3).

3.3. Comparison of MoCA Scores between the Two Groups before and after Treatment. There were no differences in MoCA scores between the two groups before treatment ($P > 0.05$). After treatment, the MoCA scores of the observation group were all significantly higher than those of the control group ($P < 0.05$, Table 4).

3.4. Comparison of ABI and PWV. Before treatment, there were no significant differences in ABI and PWV between the two groups ($P > 0.05$). After treatment, ABI (1.198 ± 0.081 vs. 1.136 ± 0.077) and PWV (1432.47 ± 191.62 vs. 1523.46 ± 196.73) in the observation group were significantly different than those in the control group ($P < 0.05$, Table 5).

3.5. Comparison of TC, TG, HDL-C, and LDL-C. Before treatment, there were no differences in TC, TG, HDL-C, and LDL-C between the two groups ($P > 0.05$). Compared with those before treatment, TC, TG, and LDL-C in the observation group decreased significantly after treatment, and HDL-C increased significantly ($P < 0.05$). Compared with the control group, the LDL-C in the observation group was significantly decreased ($P < 0.05$, Table 6).

4. Discussion

CSVD is a clinical cognitive disorder caused by various lesions in the small perforators, arterioles (40–200 um), capillaries, and venules in the brain, with the exception of individual monophyletic disorders. The radiological and pathological features are dispersed. CSVD mainly manifests as cerebral infarction, cerebral hemorrhage, cognitive, emotional, and overall dysfunction, and is mainly characterized by diseases such as lacunar infarction, lacunar infarction, lacunae, white matter, white matter, perivascular space enlargement, and intracerebral microbleeds [6–9]. The vascular lesions of CSVD are microaneurysms, microaneurysms, fatty hyaline degeneration, fibrinoid necrosis, microaneurysms, and hemorrhage of the deep cerebral cortex. The capillaries are tortuous and capillary distribution is significantly reduced.

Table 3: Comparison of the coefficient of variation of ambulatory blood pressure before and after treatment in the two groups ($\overline{x} \pm s$, mmHg).

Group	Observation group ($n = 71$)		Control group ($n = 58$)		T	$P^{\#}$
	Before therapy	After treatment	Before therapy	After treatment		
24hSBP-CV	0.117 ± 0.053	$0.073 \pm 0.018^{\#}$	0.115 ± 0.046	$0.091 \pm 0.020^{\#}$	8.347	0.035
24hDBP-CV	0.132 ± 0.195	$0.119 \pm 0.025^{\#}$	0.126 ± 0.103	$0.098 \pm 0.021^{\#}$	6.559	0.376
dSBP-CV	0.113 ± 0.035	$0.059 \pm 0.021^{\#}$	0.115 ± 0.041	$0.084 \pm 0.024^{\#}$	11.548	0.015
dDBP-CV	0.124 ± 0.043	0.105 ± 0.029	0.122 ± 0.038	0.113 ± 0.033	9.325	0.579
nSBP-CV	0.122 ± 0.194	0.085 ± 0.031	0.123 ± 0.096	0.087 ± 0.028	13.739	0.417
nDBP-CV	0.119 ± 0.042	0.115 ± 0.036	0.121 ± 0.043	0.117 ± 0.041	7.681	0.689

Note. $^{\#}$Comparison between groups.

Table 4: Comparison of MoCA scores between the two groups before and after treatment ($\overline{x} \pm s$, score).

Group	Observation group ($n = 71$)		Control group ($n = 58$)		t	$P^{\#}$
	Before therapy	After treatment	Before therapy	After treatment		
Total score	16.83 ± 4.07	$22.35 \pm 6.23^{\#}$	16.49 ± 4.13	18.15 ± 4.64	9.547	<0.001
Verbal fluency	0.95 ± 0.24	$5.69 \pm 1.34^{\#}$	0.89 ± 0.23	$3.12 \pm 0.87^{\#}$	11.619	0.005
Naming	1.21 ± 0.31	$5.26 \pm 1.31^{\#}$	1.23 ± 0.36	$3.39 \pm 0.92^{\#}$	8.539	0.003
Abstraction	0.83 ± 0.25	$1.96 \pm 0.42^{\#}$	0.82 ± 0.24	1.09 ± 0.38	6.247	0.031
Attention	2.97 ± 0.63	$5.89 \pm 1.27^{\#}$	3.04 ± 0.61	4.12 ± 1.03	16.394	0.015
Delayed recall	0.97 ± 0.33	$4.15 \pm 0.82^{\#}$	0.93 ± 0.31	1.25 ± 0.49	7.132	0.001
Orientation score	2.46 ± 0.42	$5.26 \pm 0.91^{\#}$	2.44 ± 0.39	3.51 ± 0.46	8.429	0.035
Visuoconstructional skills	1.77 ± 0.46	$5.38 \pm 1.29^{\#}$	1.81 ± 0.46	2.41 ± 0.59	13.762	<0.001

Note. $^{\#}$Comparison between groups.

Table 5: Comparison of ABI and PWV between the two groups before and after treatment ($\overline{x} \pm s$).

Group	Observation group ($n = 71$)		Control group ($n = 58$)		T	P
	Before therapy	After treatment	Before therapy	After treatment		
ABI	1.093 ± 0.074	$1.198 \pm 0.081^{\#}$	1.094 ± 0.071	$1.136 \pm 0.077^{\#}$	9.547	0.045
PWV	1661.53 ± 206.24	$1432.47 \pm 191.62^{\#}$	1660.47 ± 204.23	$1523.46 \pm 196.73^{\#}$	11.619	<0.001

Note. $^{\#}$Comparison between groups.

The epidemiology of CSVD in China is mostly based on cohort studies and community population surveys. Among patients with cerebral infarction in 4 major cities in China, lacunar strokes account for 42.3% of ischemic strokes [10], and 17.2% of the cases were caused by occlusion of small blood vessels [11, 12]. Our department innovatively proposed that the main pathogenesis of CSVD is kidney deficiency and blood stasis based on years of TCM experience and modern medical research. CSVD patients present a gradual decline of vitality after middle age, the disharmony of the five internal organs, the loss of kidney essence and qi, the daily drying up of body water, the emptiness of the sea of marrow, the dystrophy of cerebral collateral circulation, the stasis of brain vessels, the deterioration of visceral functions over time, and the disharmony of qi and blood. Endogenous phlegm and blood stasis intertwine, resulting in atherosclerosis of small cerebral blood vessels, and abnormal vasoconstriction regulation, thus increasing blood pressure and variability. Based on years of experience in the treatment of ischemic cerebrovascular diseases, our TCM recipe is based on kidney deficiency, blood stasis, and phlegm as the target, and we established a TCM recipe consisting of Rou Cong-Rong, leech, Dilong, salvia, turmeric, and Yizhiren, which is a kidney-tonifying and stasis-removing prescription. In the recipe, Rou Cong-Rong tonifies the kidney and strengthens yang, fills the essence, and nourishes the marrow, and the main ingredient is phenylethanoid glycosides, which have antiaging and neuroprotective effects, and improve cognitive function [13, 14]. The leech chases and breaks down bad blood, does not damage fresh blood, and does not damage qi when it enters the blood [15, 16]. Dilong promotes blood circulation and removes blood stasis, dispels wind and clears collaterals, nourishes internal organs, and externally unblocks meridians. The main thrombolytic components can improve microcirculation, which is anticoagulant without affecting hemostasis [17]. Danshen removes bad blood, clears blood vessels, activates blood and removes blood stasis, clears the heart, and removes vexation [18–20]. Curcuma purifies qi, activates blood and dissipates phlegm, nourishes the brain, nourishes the kidney, and tonifies kidney. The combination of various medicines has the effect of invigorating the kidney and

Table 6: Comparison of TC, TG, HDL-C, and LDL-C between the two groups before and after treatment ($\overline{x} \pm s$, mmol/L).

Group	Observation group ($n = 71$)		Control group ($n = 58$)		T	$P^{\#}$
	Before therapy	After treatment	Before therapy	After treatment		
TC	4.88 ± 1.18	$4.01 \pm 0.92^{\#}$	4.85 ± 1.14	$3.95 \pm 0.87^{\#}$	10.271	0.642
TG	1.64 ± 0.59	$1.08 \pm 0.41^{\#}$	1.62 ± 0.56	$1.12 \pm 0.38^{\#}$	8.446	0.703
HDL-C	1.18 ± 0.45	$1.43 \pm 0.67^{\#}$	1.19 ± 0.46	$1.28 \pm 0.52^{\#}$	6.382	0.492
LDL-C	2.91 ± 0.93	$1.91 \pm 0.36^{\#}$	2.93 ± 0.97	$2.25 \pm 0.74^{\#}$	9.734	0.035

Note. $^{\#}$Comparison between groups.

removing blood stasis, resolving phlegm, and dredging collaterals. A review of the results of this study showed that the mean value of ambulatory blood pressure and the coefficient of variation of ambulatory blood pressure at each time point in the observation group decreased significantly after treatment, and the MoCA score, ABI, and PWV detection were better than those of the control group. It will be interesting to test the efficacy of Bushen Huayu's integrated traditional Chinese and Western medicine treatment in other diseases affecting the cerebral vessels, such as fulminant hepatitis, hypoxia, and inflammation. [21–30].

In general, Bushen Huayu's integrated traditional Chinese and Western medicine treatment can further reduce the blood pressure variables of CSVD patients, improve the MoCA score and cognitive function, increase the ABI, reduce PWV, reduce the degree of arteriosclerosis, and improve lipid metabolism.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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Research Article

Early Identification of High-Risk Factors for Upper Gastrointestinal Bleeding

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Objective. To identify simple and accurate pre-endoscopy risk factors for early identification of high-risk upper gastrointestinal bleeding. *Methods*. Patients who were admitted to Suzhou Hospital of Integrated Traditional Chinese and Western Medicine from January 1, 2016, to December 31, 2019, due to upper gastrointestinal bleeding were retrieved, and the detailed clinical data of the above patients were collected. Patients with a definite diagnosis of bleeding from esophageal/and gastric varices were assigned to the high-risk group. Patients with bleeding not caused by varices were divided into a high-risk and a low-risk group according to the Forrest grading and scoring standard (high-risk group Forrest Ia-IIb, low-risk group Forrest IIc-III). Univariate analysis, *t*-test, chi-square test, binary logistic regression, ROC curve (Receiver-operating characteristic curve), etc. were employed for analysis in order to identify some simple and accurate risk factors for high-risk upper digestion tract bleeding before endoscopy. *Results*. A total of 916 patients were collected. Three risk factors among the screened risk factors (1) hemoglobin ≤ 85 g/L, (2) vomiting red blood, and (3) "red bloody stool" were analyzed by ROC curve analysis. The specificities of each factor were 78.4%, 94.5%, and 96.7%, respectively, and the sensitivities were 71.8%, 55.9%, and 23.1%, respectively. We also derived a risk prediction scoring system for the three factors that meet the high risk such as (1) hemoglobin ≤ 83 g/L, (2)vomiting red blood, and (3) "red bloody stool." The area under the ROC curve (AUROC), sensitivity, and specificity were 0.877, 0.904, and 0.746. *Conclusion*. Hemoglobin ≤ 85 g/L, vomiting red blood, and red bloody stool were included in a simple scoring standard for predicting high-risk UGIB patients before endoscopy. The new risk prediction scoring system requires only three indicators and has the advantages of high accuracy, short time-consuming, and easy application.

1. Introduction

Acute upper gastrointestinal bleeding (AUGIB) refers to the bleeding above the ligament of Trevor's, the main clinical manifestations are hematemesis, melena, etc. It is a common and potentially life-threatening emergency clinical disease. Its annual incidence rate is (100–180)/100,000 [1]. Among them, nonvariceal upper gastrointestinal bleeding accounts for the vast majority, about 80%–90%, and variceal bleeding accounts for about 10% [2]. Although variceal bleeding is a minority among all upper gastrointestinal bleeding (UGIBs), it has a high mortality rate of 30 percent in initial hospitalization, nearly 60 percent within 1 year [3], and rebleeding occurs in 70 percent of patients [4]. In patients with nonvariceal bleeding such as high-risk ulcer Forrest Ia, the rebleeding rate is as high as 90% [5]. The management and timing of endoscopy in patients with high-risk conditions in UGIB differs from other patients because it predicts higher rates of rebleeding and mortality [6]. Therefore, risk stratification is important for determining treatment indications and predicting clinical outcomes, and early identification of high-risk patients is essential for intensive treatment and early intervention. At present, many studies have shown that endoscopy is key to the diagnosis of upper gastrointestinal bleeding, and it is also the gold standard for identifying active bleeding. Drug combined with endoscopy is now the preferred treatment, no matter in variceal bleeding or nonvariceal bleeding [7, 8]. More studies have shown that advanced endoscopic therapy can significantly reduce the rebleeding rate and mortality of patients with

gastrointestinal bleeding, and improve the prognosis of patients [9]. Currently widely accepted pre-endoscopy scoring systems are Glasgow Blatchford Score (GBS), Rockall Score (RS), and AIMS65. Among them, GBS has a high predictive value in terms of intervention and mortality [10]. However, these criteria are complicated to calculate or need to be completed after endoscopy before further evaluation, making it difficult to apply them clinically. A study found that only about 50% of the surveyed 1,000 related doctors knew about these scoring systems, and 30% of them had used one of these scoring systems for patients with UGIB [11]. To date, there is no universally agreed scoring standard for predicting high-risk upper gastrointestinal bleeding populations, and the existing scoring systems have not been updated to adapt to changes in clinical characteristics. Our purpose is to screen out high-risk UGIB patients using a more accurate, simple, and convenient scoring standard, which reminds clinicians to take the next step of diagnosis and treatment as soon as possible.

2. Materials and Methods

Admitted patients were retrieved from the Gastroenterology Department of Suzhou Integrated Traditional Chinese and Western Medicine Hospital from September 1, 2016, to September 30, 2018. The included patients should have symptoms of active hematemesis or melena, with the gastroscopy-confirmed presence of explainable lesions leading to upper gastrointestinal bleeding. Endoscopy should be completed within 24–48 hours after admission. Exclude patients who were unwilling to take endoscopy or had malignant diseases, hematological disorders, mental illness, drug allergies, etc.

Detailed medical history, such as cardiovascular and cerebrovascular diseases, liver cirrhosis, hypertension, diabetes, recent drug (NSAIDs, hormone drugs) taking history, etc. were collected. Clinical manifestations included the color of hematemesis, the color of stool, cold sweat, palpitations, and syncope. Vital signs included systolic blood pressure, diastolic blood pressure, and heart rate.

The laboratory data of UGIB patients within 48 hours of hematemesis or melena were collected for analysis, including prothrombin time (PT), activated partial prothrombin time (APTT), hemoglobin (Hb), hematocrit (MCV), the lowest value of platelet count (PLT), the highest value of blood urea nitrogen (BUN), and serum creatinine (SCr).

Endoscopy results: gastroscopy should be performed within 24–48 hours after symptoms appear, and those with peripheral circulatory failure should be corrected first. Endoscopy includes evidence of esophageal and gastric variceal bleeding such as (1) active bleeding from varices; (2) "white papilla" overlying varices; and (3) variceal overlying blood clots or no other Varicose veins that are the underlying cause of bleeding. For nonvariceal bleeding, the Forrest grading scale was used. All endoscopic diagnostic criteria were jointly decided by three endoscopists. Patients were treated according to the consensus of the Asia-Pacific Working Group recommended by the international consensus [12]. The standard of care is as follows: start PPIs like pantoprazole, omeprazole, and esomeprazole for all patients with upper GI bleeding who are admitted to the hospital. Blood transfusion is required in the following situations: the patient presents with unstable hemodynamics, such as systolic blood pressure <90 mmHg, heart rate > 120 beats/min, and hemoglobin < 70 g/L. Principles of endoscopic treatment: the treatment of acute esophageal variceal bleeding (EVB) mainly includes conservative drug administration, endoscopic treatment, balloon tamponade, transjugular intrahepatic portal shunt (TIPS), and surgical treatment [13]. For nonvariceal upper gastrointestinal bleeding, submucosal injection of epinephrine, electrocoagulation, titanium clips, or a combination of these methods are used alone. Patients with esophageal and gastric varices are treated with endoscopic band ligation, tissue adhesive injection, combined treatment with multiple methods, and interventional surgery, which depends on the judgment of the endoscopic surgeon [14].

2.1. Statistical Methods. SPSS17.0 (Armonk, NY) was used for statistical analysis and processing of data. The description of measurement data was performed by mean + standard deviation ($X \pm \mathrm{SD}$), and the description of categorical variable data was performed by percentage. Univariate factor analysis was performed for measurement data using an independent samples t-test, and the chi-square test was used for categorical variable data. The factors with significant significance between the two groups of data were included in the binary logistic regression analysis, and the relevant high-risk factors were obtained. Then the obtained high-risk factors were combined to establish a regression equation model (inclusion level 0.05, exclusion level 0.10). The independent risk factors screened above were compared individually and also in combination. Statistically significant was set at a two-sided $P < 0.05$.

3. Results

3.1. Clinical Characteristics of 916 UGIB Patients. A total of 1079 UGIB patients were retrieved, of which 108 patients underwent endoscopic examination more than 48 hours beyond the initial bleeding, 53 patients had chronic anemia, and 2 patients had bleeding in other parts of the upper gastrointestinal tract (1 patient with biliary bleeding, 1 patient with pancreatic cancer involving duodenum hemorrhage). The remaining 916 patients with upper gastrointestinal bleeding were included in this study, and 372 patients were at high risk according to the Forrest classification, accounting for 40.61% of the study population. Among them, there were 17 Forrest Ia patients (4.57%); 56 Forrest Ib patients (15.05%), 205 Forrest IIa patients (55.11%), and 94 Forrest IIb patients (25.27%). There were 425 low-risk patients, accounting for 46.39% of the study population, including 74 Forrest IIc patients (8.08%) and 351 Forrest III patients (34.39%). There were 119 patients with EVB bleeding, accounting for 12.99% of the study population. Due to the high recurrence rate and mortality rate of

bleeding caused by EVB [15, 16], they were all included in the high-risk group. In summary, there were 491 patients in the high-risk group and 425 in the low-risk group. The average age of the patients was 57.13 ± 17.31 years old in the high-risk group, and 52.54 ± 18.19 years old in the low-risk group ($P < 0.05$). The ratio of males to females was 195 : 296 in the high-risk group, and 112 : 313 in the low-risk group ($P < 0.05$), as shown in Table 1. The etiology of UGIB in this study included peptic ulcer in 813 cases (88.76%), a gastrointestinal tumor in 33 cases (3.60%), dieulafoy ulcer in 14 cases (1.53%), acute erosive hemorrhagic gastritis in 11 cases (1.20%), and cardiac tear in 45 cases (4.91%).

Among the 916 patients, there were significant differences between the two groups in terms of previous underlying diseases, non-steroidal anti-inflammatory drugs use, hepatitis, liver cirrhosis, and hypertension ($P < 0.05$). Clinical manifestations include "vomiting red blood, red bloody stools, and shock," "diastolic blood pressure" in vital signs, and "prothrombin time, activated partial thromboplastin time, hemoglobin, hematocrit" in laboratory hematology indexes, platelet count, albumin," which had statistical differences between the two groups ($P < 0.05$). It is suggested that the above 16 indicators may be meaningful for predicting high-risk UGIB patients. The 16 factors were further analyzed below, and the specific data are shown in Table 1.

3.2. Logistic Analysis of Clinical and Laboratory Characteristics for High-Risk UGIB Patients. Further binary logistic regression analysis was performed on the 8 meaningful categorical variables of "gender, non-steroidal anti-inflammatory drugs use, hepatitis, liver cirrhosis, hypertension, vomiting red blood, solution of red bloody stool, and shock." The five indicators of "gender, liver cirrhosis, vomiting red blood, red bloody stool, and shock" ($P < 0.05$) were risk factors for high-risk UGIB patients, and their odds ratios were 0.582, −1.877, 3.058, 2.215, and 0.855. Logit = $-19.326 + 0.582 \times$ gender $- 1.877 \times$ cirrhosis $+ 3.058 \times$ vomiting red blood $+ 2.215 \times$ red bloody stool- $+ 0.855 \times$ shock. The Cox and Snell R^2 was 0.434, and the Nagelkerke R^2 was 0.580.

Further binary logistic analysis was performed on the 8 indicators of meaningful measurement variables of "age, diastolic blood pressure, prothrombin time, activated partial prothrombin time, hemoglobin, hematocrit, platelet count, albumin," and only hemoglobin, hematocrit, platelet, prothrombin time, and albumin were statistically significant ($P < 0.05$), which were regarded as risk factors for high-risk UGIB patients, with odds ratios of 0.891, 1.259, 0.995, 1.112, and 0.963. Logit = $4.501 - 0.115 \times$ hemoglobin $+ 0.230 \times$ hematocrit $- 0.005 \times$ platelet $+ 0.106 \times$ prothrombin time- $- 0.037 \times$ albumin. The Cox and Snell R^2 was 0.328, and the Nagelkerke R^2 was 0.438 (Table 2).

3.3. Logistic and ROC Analysis of Clinical and Laboratory Characteristics for High-Risk UGIB Patients. The 10 indicators of "gender, liver cirrhosis, vomiting of red blood, solution of red bloody stool, shock, hemoglobin, hematocrit, platelet, prothrombin time, and albumin" were combined and further analyzed, and the results showed that gender, shock, red blood cell pressure, the partial regression coefficients of blood clots, platelets, prothrombin time, and albumin were statistically significant ($P < 0.05$). Finally, three indicators of "liver cirrhosis, vomiting of red blood, solution of red bloody stool, and hemoglobin" were used as high-risk factors for predicting high-risk UGIB. The regression equation was: Logit = $2.136 + -0.018 \times$ hemoglobin $+ 2.812 \times$ vomiting red blood $+ 1.673 \times$ relieving red bloody stool, Cox & Snell R^2 was 0.405, Nagelkerke R^2 was 0.550, and the detailed data are shown in Table 3.

Table 1: Clinical characteristics of 916 UGIB patients.

Clinical factors	Low risk ($n = 425$)	High risk ($n = 491$)	P
Gender, male	112 (26.35%)	195 (39.71%)	< 0.001
Age, y	52.54 ± 18.19	57.13 ± 17.31	< 0.001
SBP (mmHg)	121.61 ± 17.37	119.32 ± 20.85	0.083
DBP (mmHg)	73.55 ± 11.32	69.00 ± 12.54	< 0.001
HR (beat/min)	85.12 ± 14.06	83.93 ± 16.92	0.077
Hb (g/L)	100.49 ± 21.88	73.63 ± 20.71	< 0.001
HCT (%)	30.09 ± 6.73	22.73 ± 6.12	< 0.001
PLT ($*$ 109)	198.26 ± 61.33	151.40 ± 81.45	< 0.001
PT (s)	13.62 ± 2.35	15.22 ± 2.94	< 0.001
APTT (s)	32.38 ± 9.21	34.69 ± 7.81	< 0.001
Albumin (g/L)	36.68 ± 5.34	31.06 ± 9.52	< 0.001
BUN (mmol/L)	10.29 ± 6.79	11.95 ± 3.14	0.115
Cr (umol/L)	73.23 ± 24.01	78.76 ± 75.04	0.146
NSAID	21.88%	15.89%	0.022
Corticosteroids	0.47%	1.63%	0.116
Liver cirrhosis	5.41%	18.33%	< 0.001
Hypertension	31.16%	41.88%	0.001
Hepatitis	2.11%	33.40%	< 0.001
Diabetes mellitus	13.65%	17.31%	0.144
Cardiovascular diseases	12.47%	10.79%	0.496
Weight loss	4.24%	2.65%	0.203
Red hematemesis	6.36%	64.77%	< 0.001
Red stool	3.76%	25.25%	< 0.001
Palpation	48.71%	50.92%	0.508
Cold sweat	42.12%	48.27%	0.063
Syncope	16.00%	16.29%	0.928
Shock	2.82%	12.83%	< 0.001

APTT = activated partial thromboplastin time; BUN = blood urea nitrogen; DBP = diastolic blood pressure; Hb = hemoglobin; HCT = hematocrit; HR = heart rate; NSAIDs = non-steroidal anti-inflammatory drugs; PLT = platelet cell; PT = prothrombin time; SBP = systolic blood pressure.

Table 2: Logistic analysis of clinical and laboratory characteristics for high-risk UGIB patients.

Characteristics	Equation	Cox and Snell R^2	Nagelkerke R^2	Exp (R^2)	P
Sex NSAID Corticosterioids Hepatitis Cirrhosis HBP Diabetes mellitus Cardiovascular disease Red hematemesis Red stool Weight loss Palpation Cold sweat Syncope Shock	$-19.326 + 0.582 \times \text{sex} - 1.877 \times \text{cirrhosis} + 3.058 \times \text{red hematemesis} \times + 2.215 \times \text{red stool} + 0.855 \times \text{shock}$	0.434	0.580	0.582 (sex) −1.877(cirrhosis) 3.058 (hematemesis) 2.215 (red stool) 0.855 (shock) −1.433 (constant)	< 0.001 (hematemesis) < 0.001 (red stool) 0.012 (shock) < 0.001 (constant)
Age SBP DBP HR Hb HCT PLT PT APTT Albumin BUN	$4.501 - 0.115 \times \text{Hb} + 0.230 \times \text{HCT} + 0.005 \times \text{PLT} + 0.106 \times \text{PT-0.0.037} \times \text{Albumin}$	0.328	0.438	0.891(Hb) 1.259 (HCT) 0.995 (PLT) 1.112 (PT) 0.963 (albumin) 3.055 (constant)	<0.001 (Hb) <0.001 (HCT) <0.001 (PLT) 0.048 (PT) 0.028 (albumin) < 0.001 (constant)

APTT = activated partial thromboplastin time; BUN = blood urea nitrogen; DBP = diastolic blood pressure; Hb = hemoglobin; HCT = hematocrit; HR = heart rate; NSAID = non-steroidal anti-inflammatory drug; PLT = platelet cell; PT = prothrombin time; SBP = systolic blood pressure.

Table 3: Logistic analysis of clinical and laboratory characteristics for high-risk UGIB patients.

Risk	ß	Exp (ß)	P	Cox & Snell R^2	Nagelkerke R^2
Red hematemesis	3.113	22.483	< 0.001		
Red stool	1.795	6.020	< 0.001	0.4498	0.665
Hb	−0.083	0.920	< 0.001		
Cirrhosis	1.449	4.259	0.002		

* Hb = hemoglobin.

The "hemoglobin" was used to make the ROC curve according to the risk level of VUGIB. The optimal critical value of hemoglobin was 85 g/L, and the specificity, sensitivity, positive likelihood ratio, negative likelihood ratio, Youden index, and AUROC were 0.772, 0.774, 3.395, 0.293, 0.546, 0.820 ($P < 0.05$) are shown in Figure 1 and Table 4.

The ROC curve evaluation of the four indicators of hemoglobin ≤ 85 g/L, vomiting of red blood, solution of red bloody stool, and history of liver cirrhosis showed that the sensitivity of the three were 0.772, 0.651, 0.255, and 0.329, and the specificity was 0.772, 0.651, 0.255, and 0.329.0.771, 0.936, 0.962, 0.979, AUROC were 0.772, 0.794, 0.608, 0.654.

The four high-risk factors screened above were further defined as "(1) hemoglobin ≤ 85 g/L, (2) red blood vomiting, (3) red bloody stool, and (4) history of liver cirrhosis", and the vomit color was "bright red, dark red or with "blood clot" is defined as 1 point, other is 0 point; stool color is "bright red, dark red or accompanied by blood clot" is defined as 1 point, other is defined as 0 points; hemoglobin ≤ 83 g/L is defined as 1 point scores, otherwise defined as 0 points; and history of liver cirrhosis defined as 1 point, otherwise defined as 0 points. Combinations of ①②, ①③, ①②③, and ①②③④ were constructed to form four risk prediction scoring systems, e.g., score1, score2, score3, and score 4, respectively. The Blatchford scoring system and the resulting new scoring systems were evaluated by the ROC curve. When the critical value was 0.5, the maximum ROC curve area was 0.877, the sensitivity was 0.904, the specificity was 0.746, and the Youden index was 0.650 ($P < 0.05$). The P values, sensitivity, specificity, and cutoff points of the two scoring systems and the BRS scoring system are shown in Figure 1(c)and Table 4.

A correlation analysis by Wuerth and Rockey [17] found that after 72 hours of successful endoscopic hemostasis, Forrest Ib had a very low rebleeding and rebleeding rate compared with Forrest Ia, Forrest IIa, and Forrest IIb patients. Therefore, we eliminated the 33 participants who were classified as Forrest Ib and passed them through univariate analysis (t-test, chi-square test), binary logistic regression, and ROC curve again, and still obtained "(1) vomiting red blood, (2) Red bloody stool, and (3) hemoglobin≤85 g/L" were three high-risk factors, and a new scoring system ①③ and ①②③ were constructed to form two risk prediction scoring systems of score3 and score4, respectively. The Blatchford scoring system and the resulting new scoring system were evaluated by the ROC curve. See Figure 1 and Table 3 for details.

4. Discussion

AUGIB refers to bleeding caused by diseases of the gastrointestinal tract above the ligament of Trevor, including pancreatic or bile duct bleeding and bleeding caused by diseases near the anastomotic stoma after gastrojejunostomy [15]. Due to advanced medical and endoscopic treatment, the hospitalization rate for upper gastrointestinal bleeding has decreased by 20% in the past decade, and the mortality rate has dropped from 4.5% to about 2.1%. Although the hospitalization rate and case fatality rate have decreased, the number of people is still larger for a larger population base. In fact, patients with variceal bleeding are considered a specific high-risk group [18]. Due to the high mortality rate and many complications of esophageal and gastric variceal bleeding, early identification of high-risk patients is extremely important, so it still has great research value.

In fact, patients with variceal bleeding are considered a specific high-risk group. A study by Cho SH [19] compared patients undergoing emergency endoscopy with regular gastroscopy, and there were significant differences in mortality, blood transfusion volume, and need for clinical intervention. Therefore, early identification of high-risk UGIB patients and early improvement of endoscopy can further improve the prognosis of patients. At present, a variety of scoring systems have been clinically used to assess the risk of upper gastrointestinal bleeding, each of which has different specificity, sensitivity, and predictive value for clinical observation indicators. The most commonly used risk assessment systems are the Forrest classification [20], BRS scoring standard, AIMS56 [21], and Rockall score, each of which has the characteristics of complex calculation and/or the need for endoscopy results, which makes the risk assessment system more complex for clinical application [20]. At present, researchers have proposed that accurate prediction of a series of risks in patients with UGIB is helpful for clinically selective management of these patients, and it is recommended that risk assessment should classify patients into high-risk and low-risk, because this may help clinicians make early decisions, such as the choice of endoscopy time, patient allocation, choice of level of care, and time to leave the hospital [22]. Early identification and evaluation of high-risk patients can improve the effectiveness of clinical treatment, shorten the time of treatment for patients, reduce the cost of treatment for patients, and improve clinical outcomes for patients.

Endoscopy is the key to diagnosing the etiology of upper gastrointestinal bleeding, and drug combined with endoscopy is now the preferred treatment [23]. Studies have shown that there is no solid basis for the benefit of earlier endoscopy. The Forrest classification has been used to identify high-risk endoscopic nonvariceal upper gastrointestinal bleeding. The Forrest grading is based on the results of endoscopy. Since the Forrest grading can better predict the risk of UGIB rebleeding, it can more intuitively

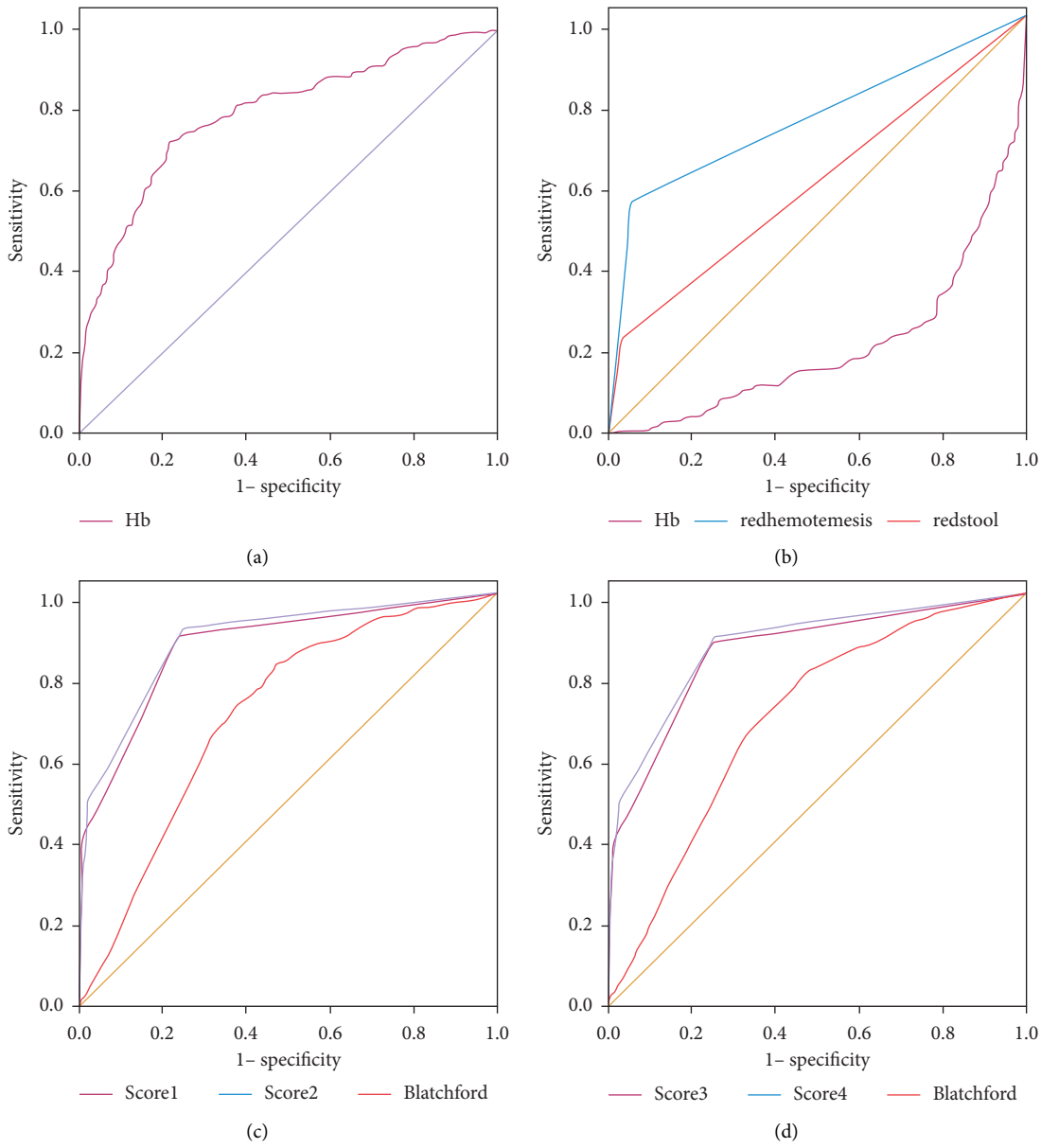

Figure 1: ROC curve of risk factors.

determine which UGIBs really need endoscopic intervention [24]. We took the Forrest classification as the gold standard for judging the severity of UGIB and divided UGIB patients into high-risk groups and low-risk groups according to the actual Forrest classification. The etiological analysis was consistent with the common etiology reported by the guideline [25]. We determined "hemoglobin" by ROC curve analysis, and the optimal critical value of hemoglobin was 83 g/L (see Table 4 and Figure 1(a) for details). Several clinical studies have suggested that hemoglobin between 80 and 85 g/L is a high-risk factor for acute upper gastrointestinal bleeding [5], and the conclusions drawn by our study were consistent with this. In the study of Forrest et al. [26], "vomiting bright red blood" was one of the high-risk indicators.

According to the correlation analysis by Zaragoza et al. [20], even after we excluded 33 participants who were classified as Forrest Ib, and through the aforementioned similar statistical methods, we still concluded that "(1) vomiting red blood, (2) red bloody stool, and (3) hemoglobin≤ 83 g/L" were three high-risk factors, and a new scoring system ①③ and ①②③ were constructed to form two risk prediction scoring systems of score3 and score4, respectively. It can be seen from Figure 1(d) that among the two newly constructed risk prediction scoring systems, the risk prediction score 3 has the largest AUROC area of 0.871 when the critical value is 0.5, and the sensitivity, specificity, and Youden index were 89.8%, 75.4%, and 75.4%, respectively. When the critical value of scoring system 4 was 0.5, the AUROC area was 0.885, and the sensitivity, specificity, and Youden index were 92.0%, 74.6%, and 0.665, respectively. The Blatchford scoring system and the new scoring system were used to evaluate the ROC curve, and the

Table 4: ROC analysis of clinic and laboratory for characteristics for high-risk UGIB patients.

Scoring system	Cutoff point	*P*	AC	Sensitivity	Specificity	PLR	NLR	Youden index
Hb (g/L)	85	< 0.001	0.820	0.744	0.772	3.395	0.293	0.546
Hb ≤ 85 (g/L)	0.5	< 0.001	0.772	0.772	0.771	3.371	0.296	0.543
Red hematemesis	0.5	< 0.001	0.794	0.651	0.936	10.172	0.373	0.587
Red stool	0.5	< 0.001	0.608	0.255	0.962	6.711	0.774	0.217
Liver cirrhosis	0.5	< 0.001	0.654	0.329	0.979	15.667	0.685	0.307
SCORE1	0.5	< 0.001	0.885	0.932	0.738	0.557	0.092	0.670
	1.5	< 0.001		0.491	0.969	15.839	0.525	0.460
SCORE2	0.5	< 0.001	0.830	0.817	0.757	3.362	0.242	0.574
	1.5	< 0.001		0.209	0.976	8.780	0.810	0.186
SCORE3	0.5	< 0.001	0.900	0.959	0.726	3,500	0.056	0.685
	1.5	< 0.001		0.589	0.948	11.327	0.433	0.537
	2.5	< 0.001		0.129	0.995	25.800	0,875	0.125
SCORE4	0.5	< 0.001	0.911	0.961	0.715	3.372	0.055	0.676
	1.5	< 0.001		0.659	0.941	11.169	0.362	0.600
	2.5	< 0.001		0.326	0.993	46.571	0.679	0.319
	3.5	< 0.001		0.060	1.000	—	0.940	0.060
Blatchford score		< 0.001						
		< 0.001						
		< 0.001						

Hb = hemoglobin; UGIB = upper gastrointestinal bleeding; PLR = positive likelihood ratio; ROC = receiver-operating characteristic.

results showed that the above two scoring systems were better than BRS, score1, and score2 in evaluating patients with the high-risk nonvariceal upper gastrointestinal bleeding system. The value of our scoring system should be evaluated in severe cases, such as fulminant hepatitis, inflammation, infections, hypoxia, or preterm birth [27–37].

In conclusion, this study showed that "hemoglobin ≤ 83 g/L, vomiting red blood, and red bloody stool" could be three independent high-risk factors in patients with UGIB, while the combined application of these three risk factors can be a good way to screen high-risk UGIB patients before endoscopy.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Disclosure

Xuesong Jin and Xiaohong Wang are the co-first authors.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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Retraction

Retracted: MiR-19b-3p Attenuates Chondrocytes Injury by Inhibiting MAPK/NF-Kb Axis via Targeting SOCS1

Evidence-Based Complementary and Alternative Medicine

Received 20 June 2023; Accepted 20 June 2023; Published 21 June 2023

This article has been retracted by Hindawi following an investigation undertaken by the publisher [1]. This investigation has uncovered evidence of one or more of the following indicators of systematic manipulation of the publication process:

(1) Discrepancies in scope

(2) Discrepancies in the description of the research reported

(3) Discrepancies between the availability of data and the research described

(4) Inappropriate citations

(5) Incoherent, meaningless and/or irrelevant content included in the article

(6) Peer-review manipulation

The presence of these indicators undermines our confidence in the integrity of the article's content and we cannot, therefore, vouch for its reliability. Please note that this notice is intended solely to alert readers that the content of this article is unreliable. We have not investigated whether authors were aware of or involved in the systematic manipulation of the publication process.

Wiley and Hindawi regrets that the usual quality checks did not identify these issues before publication and have since put additional measures in place to safeguard research integrity.

We wish to credit our own Research Integrity and Research Publishing teams and anonymous and named external researchers and research integrity experts for contributing to this investigation.

The corresponding author, as the representative of all authors, has been given the opportunity to register their agreement or disagreement to this retraction. We have kept a record of any response received.

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[1] L. Shi, L. Duan, D. Duan, Y. Fan, and H. Xu, "MiR-19b-3p Attenuates Chondrocytes Injury by Inhibiting MAPK/NF-Kb Axis via Targeting SOCS1," *Evidence-Based Complementary and Alternative Medicine*, vol. 2022, Article ID 5133754, 10 pages, 2022.

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Research Article

MiR-19b-3p Attenuates Chondrocytes Injury by Inhibiting MAPK/NF-Kb Axis via Targeting SOCS1

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In this study, miR-19b-3p was downregulated in osteoarthritic cartilage tissues and IL-1β-stimulated primary chondrocytes, and miR-19b-3p overexpression reversed the inhibitory effect of IL-1β on cell viability, the promotion effects of apoptosis, inflammatory factor secretion and extracellular matrix degradation, whereas the opposite effect was observed with miR-19b-3p inhibitor. Moreover, SOCS1 is a target gene of miR-19b-3p. Furthermore, SOCS1 overexpression enhanced cell injury compared with IL-1β alone treatment, whereas knockdown of SOCS1 restored cell damage caused by IL-1β. Further studies revealed that miR-19b-3p promoted chondrocyte injury repair by suppressing SOCS1 expression, and we found that was mediated by blocking the MAPK/NF-κB axis. Taken together, our findings may provide a new therapeutic strategy for osteoarthritis.

1. Introduction

Osteoarthritis is a progressive degenerative joint disease with a primary predilection for the elderly. The clinicopathological manifestations of osteoarticular cartilage degeneration include apoptosis of chondrocytes as well as disturbances in extracellular matrix (ECM) synthesis, catabolism and metabolism. The relevant epidemiological findings showed that symptomatic knee osteoarthritis was more common in middle-aged and elderly people, with an overall prevalence of 8.1%, and it was significantly higher in women than in men [1]. Concomitant with the accelerating ageing society, there is an increasing trend in the prevalence of osteoarthritis, with the incidence of knee osteoarthritis being as high as more than 50% in the population over the age of 65 years. At present, the main therapeutic measures against osteoarthritis are still to reduce pain and control symptoms, and there is no obvious means to reverse the progression of the disease, so research aimed at osteoarthritis prevention and treatment is becoming more urgent [2].

MicroRNAs (miRNAs), a kind of short chain ribonucleic acid without coding function, can exist in various living bodies. Mature miRNAs suppress the expression levels of corresponding proteins by interfering with the translation process of their target genes, thus achieving sink expression of target genes post transcriptionally [3, 4]. Numerous studies indicating that multiple chronic inflammatory diseases including osteoarthritis may be associated with dysregulation of miRNA expression. MiR-9 functions to regulate MMP-13, and overexpression of miR-9 effectively reduces MMP-13 expression and decreases the level of cartilage matrix degradation [5]. Similarly, knockdown of miR-98 or overexpression of miR-146 in isolated human chondrocytes reduced IL-1β-induced TNF-α production [6]. MiR-140-5p is an important small nucleic acid molecule involved in maintaining chondrocyte homeostasis, and studies have found that miR-140-5p expression decreases articular cartilage degeneration and aging, and miR-140-5p protect artilage may be achieved by downregulating the corresponding target gene expression, such as ADAMTS5, MMP-13, and IGFBP5 [7].

Recently, emerging evidence has suggested that miR-19b-3p may be involved in the progression of osteoarthritis. Li et al. showed that miR-19b-3p expression was upregulated in osteoarthritic cartilage tissue and IL-1β stimulated chondrocytes compared with the control group, and miR-

19b-3p mimic inhibited IL-1β induced chondrocyte apoptosis and ECM degradation [8]. In addition, a microarray analysis pointed to circulating miR-19b-3p as an independent factor for the risk of knee osteoarthritis, and the combined detection of the remaining miR-122-5p and miR-486-5p may serve as novel biomarkers for the diagnosis of osteoarthritis [9]. Independently, another study found that miR-19b-3p mimic significantly increased chondrocyte viability and inhibited apoptosis, it also increased the expression of type II collagen, aggrecan and glycosaminoglycan, and decreased the expression of MMP-1 and MMP-13 caused by IL-1β, suggesting that miR-19b-3p is able to attenuate osteoarthritis injury [10].

Therefore, we used miR-19b-3p as an entry point to deeply explore the potential mechanism of miR-19b-3p to inhibit chondrocyte matrix degradation and delay the progression of osteoarthritis, with a view to improving new references for osteoarthritis treatment.

2. Materials and Methods

2.1. Cell Culture. Articular cartilage tissue was collected and fully digested in DMEM with streptomycin and collagenase, and then the digested material was filtered to obtain the culture medium. Next, the medium was centrifuged (1000 r/min; 5 min) and the supernatant discarded, and DMEM (containing FBS, penicillin, and streptomycin) was added again. Cells were cultured in culture bottle (2×10^5) and rested in an incubator with 5% CO_2 at 37°C. The protocol of this study was approved by the ethics committee of Shaanxi Provincial People's Hospital, and written informed consent was obtained from each participant. The miR-19b-3p mimic, miR-19b-3p inhibitor and their negative control were performed from RiboBio Co., Ltd. The pcDNA-SOCS1 and vector were purchased from RiboBio Co., Ltd. The si-SOCS1 and scramble were purchased from Santa Cruz Biotechnology. SiRNAs targeting SOCS1 (sense 5′ UCG CCC UUA GCG UGA AGA U 3′; antisense 5′ AUC UUC ACG CUA AGG GCG A 3′), and scramble sequence siRNA (sense 5′ UCG AUU CUG CGA AGC CGA U 3′; antisense 5′ AUC GGC UUC GCA GAA UCG A 3′).

2.2. RT-qPCR. Cell Total RNA was isolated by the TRIzol. Single-stranded cDNA was synthesized with the PrimeScript Reagent Kit. Real-time qPCR was conducted by using a SYBR Premix Ex TaqTM Kit. Sangon Biotech designed and costained the primers in the study. MiR-19b-3p forward, 5′-CAC TGT TCT ATG GTT AG-3′, reverse, 5′-CAC TAC CAC AGT CAG TT -3′'; SOCS1 forward, 5′- CTT CCT CCT CTT CCT CCT C-3′, reverse, 5′- GCC ATC TTC ACG CTA AGG-3. The relative expression levels were normalized by using the $2^{-\Delta\Delta Ct}$ method.

2.3. Western Blotting. Cell protein were extracted by RIPA lysis buffer. Next, proteins were transferred to PVDF membranes and blocked for 2 h at room temperature and incubated at primary antibodies for 12 h. GAPDH (1 : 3000), SOCS1 (1 : 1000), cleaved caspase3 (1 : 500), MAPK (1 : 2000), MAPK (phospho Y322, 1 : 500), NF-κB (1 : 1500) and NF-κB (phospho S337, 1 : 1000). Then, the membranes were incubated with secondary antibody (1 : 2500, ab6721) for 1 h. Next, an exposure liquid is added and a visualized protein band is acquired in the instrument. The bands were visualized by using an ECL Plus Chemiluminescence Reagent Kit (Pierce, Rockford, IL, USA) and were photographed by a chemiluminescence imaging system. Image J software was used to quantify the band densities.

2.4. CCK-8 Assay. The cells were digested with 0.25% trypsin, and then the cell suspension (3×10^4/mL) was prepared again with DMEM, and then inoculated into 96-well plates (100 μL), and incubated for 4 h (37°C; 5% CO_2). The absorbance was measured by a microplate reader (450 nm).

2.5. ELISA. The secretion levels of IL-6, TNF-α, COX-2, MMP13, collagen II and ADAMTS5 were detected by ELISA kit (Solarbio, China).

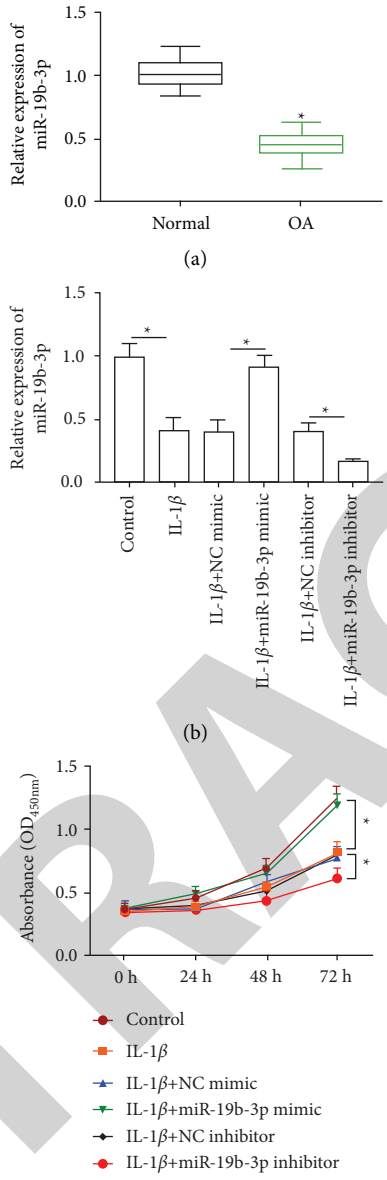
2.6. Flow Cytometry. The cell suspension (100 μL, 1×10^5 cells/mL) was prepared with PBS and transferred to the culture tube, and then cultured with annexin V-FITC and propidium iodide in a dark environment for 20 min. Finally, apoptotic cells were determined by flow cytometry.

2.7. Luciferase Reporter Gene Assay. First, SOCS1 wild- and mutant-type sequences were fused in a PGL3 luciferase reporter vector. Next, miR-19b-3p mimic and negative control were transfected into HEK293 cells together with luciferase reporter vectors, and after 48 h of culture, luciferase activity was measured by a microplate reader (MA, USA).

2.8. Statistical Analysis. The SPSS software was used to analysed all dates (ver. 21.0). The quantitative data derived from three independent experiments were expressed as mean ± SD. $P < 0.05$ means statistically significant.

3. Results

3.1. MiR-19 b-3p Alleviates the Damage of IL-1β-Treated Chondrocytes. The miR-19b-3p expression was down-regulated in osteoarthritic cartilage tissues (Figure 1(a)). Next, the transfection efficiency of miR-19b-3p is shown in Figure 1(b). Then, the cell viability was inhibited (Figure 1(c)) and apoptosis (Figure 1(d)), inflammatory factor secretion (Figure 1(e)), and extracellular matrix degradation was promoted after IL-1β treatment (Figure 1(f)), as indicated by increased secretion of MMP-13 and ADAMTS5 and decreased secretion of type 2 collagen, whereas overexpression of miR-19b-3p aggravated chondrocyte injury caused by IL-1β, and transfection of miR-19b-3p inhibitor had the opposite effect.



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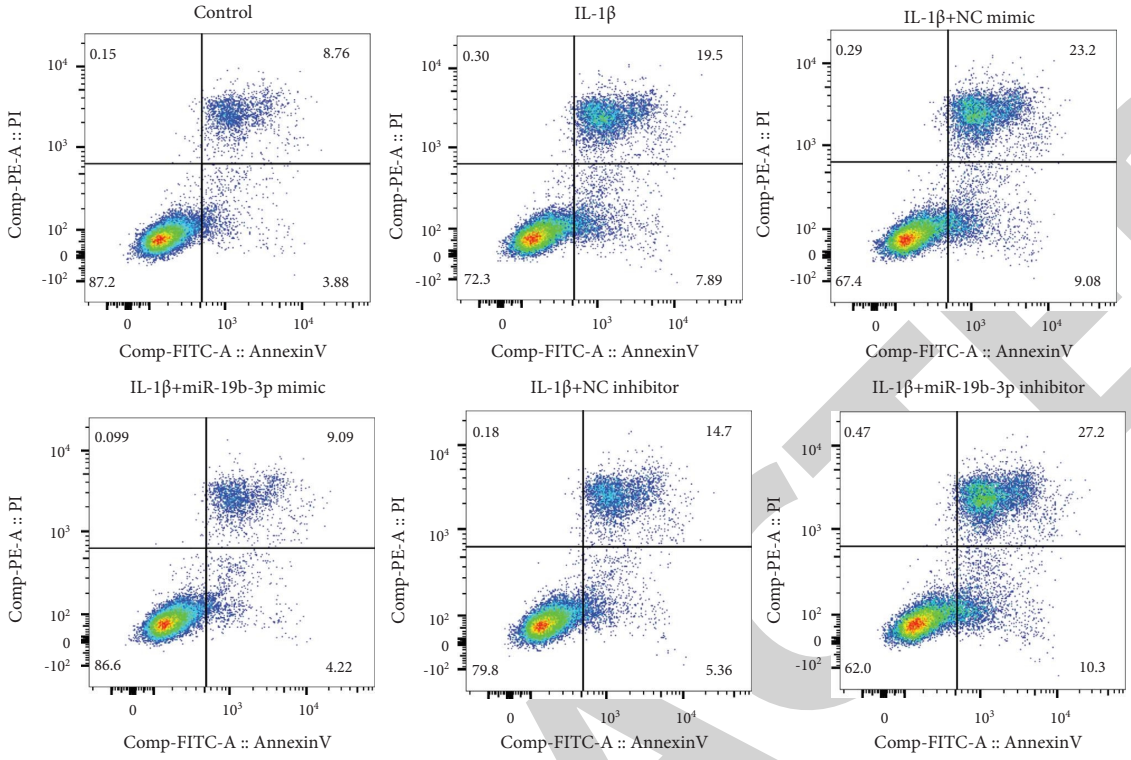

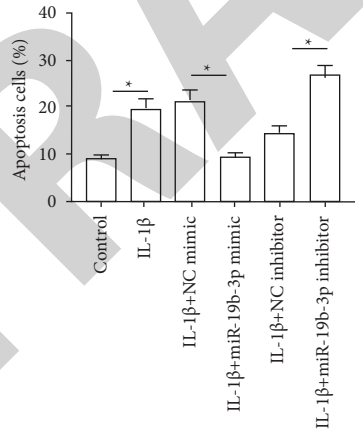

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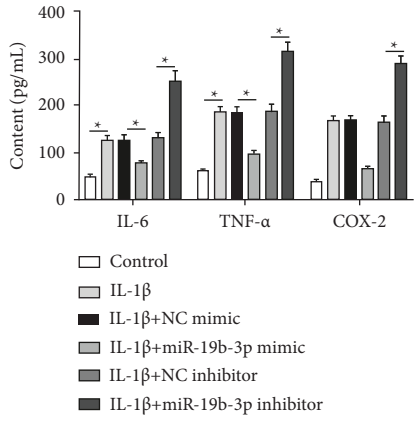

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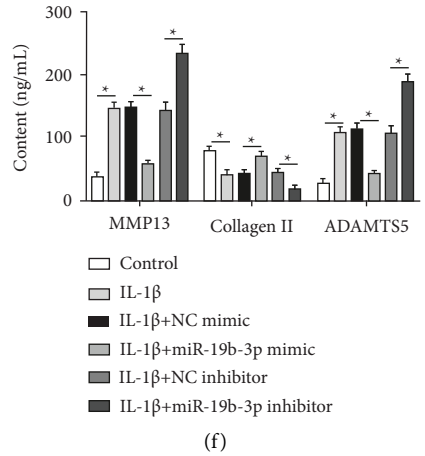


(f)

Figure 1: Effect of miR-19b-3p on chondrocytes (a) The miR-19b-3p expression in tissues (b) MiR-19 b-3p expression (c) Cell viability (d) Cell apoptosis (e) Levels of inflammatory factor secretion (f) The secretion levels of MMP-13, Collagen II and ADAMTS5. $^{*}P < 0.01$.

3.2. SOCS1 was a Direct Target of miR-19b-3p. Figure 2(a) showed the SOCS1 wild- and mutant-type sequences, respectively (https://starbase.sysu.edu.cn/). Next, miR-19b-3p mimic decreased the luciferase activity of SOCS1 wild-type reporter, instead of SOCS1 mutant reporter (Figure 2(b)). Moreover, miR-19b-3p mimic decreased SOCS1 mRNA and protein expression), and miR-19b-3p inhibitor increased SOCS1 mRNA (Figure 2(c)) and protein (Figure 2(d)) expression.

3.3. SOCS1 Aggravates the Damage of IL-1β-Treated Chondrocytes. The pcDNA-SOCS1 and si-SOCS1 were transfected into IL-1β-treated chondrocytes. IL-1β promoted SOCS1 protein expression, and SOCS1 overexpression enhanced the effect of IL-1β, whereas knockdown of SOCS1 expression had the opposite effect (Figure 3(a) and 3(b)). Moreover, compared with IL-1β treated, the pcDNA-SOCS1 inhibited cell viability (Figure 3(c)), promoted the expression of apoptosis related protein cleaved caspase3 (Figure 3(a) and 3(d)), inflammatory factors secretion (Figure 3(e)) and extracellular matrix degradation (Figure 3(f)), and si-SOCS1 alleviated cell damage caused by IL-1β.

3.4. MiR-19 b-3p Alleviates IL-1β-Induced Chondrocyte Injury by Inhibiting SOCS1. We observed that overexpression of miR-19b-3p inhibited SOCS1 expression compared with IL-1β treatment, whereas transfection of pcDNA-SOCS1 inhibited SOCS1 expression (Figure 4(a)). Furthermore, miR-19b-3p mimic enhanced cell viability (Figure 4(b)) and decreased cell injury (Figure 4(c), 4(d) and 4(e)) compared with IL-1β alone treatment, and pcDNA-SOCS1 reversed the protective effect.

3.5. MiR-19 b-3p Attenuates IL-1β-Induced Chondrocyte Injury via MAPK/NF-Kb Signaling Pathway. We found that IL-1β increased p38 MAPK phosphorylation levels, promoted NF-κB p65 protein expression, and miR-19b-3p overexpression reversed this effect, and C16-PAF (MAPK pathway) reversed the effect of miR-19b-3p (Figure 5(a) and 5(b)). Furthermore, miR-19b-3p mimic decreased apoptotic protein cleaved caspase3 expression (Figure 5(a) and 5(c)), inflammatory factor secretion (Figure 5(d)), and extracellular matrix degradation (Figure 5(e)), but this protective effect on chondrocytes was abolished by MAPK activator.

4. Discussion

Suppressors of cytokine signaling (SOCS) are a recently identified class of protein signaling molecules that function prominently in the immune system by inhibiting cytokine signaling through negative feedback loops. SOCS1 plays important roles in various physiopathological processes, such as cell differentiation, regulation of inflammation, participation in tumor immunity, and regulation of metabolism. He et al. showed that SOCS1 knockdown increased osteoarthritic chondrocytes viability [11]. In rheumatoid arthritis progression, silencing SOCS1 has been reported to promote cell proliferation and invasion, upregulate IL-1β and MMP expression, and activate the ERK pathway to alleviate synovial tissue damage [12].

Osteoarthritis is characterized by an imbalance in ECM synthesis and degradation, resulting in joint pain and progressive dysfunction. Numerous factors are involved in cartilage destruction in osteoarthritis, mainly cytokines or chemokines including TNF and IL, inflammatory mediators including PGE2 and NO, MMP-13 and components of degraded matrix including ADAMTS [13, 14]. The MMPs family consists of 20 different enzymes whose expression levels are significantly higher in osteoarthritis than in normal cartilage, and the expression of MMP-13 in normal cartilage is only one fourth of its expression in osteoarthritic chondrocytes. MMP-13, a member of the extracellular matrix degrading endopeptidase family, is a key MMP collagen hydrolase because it degrades collagen and a wide range of matrix molecules [15, 16].

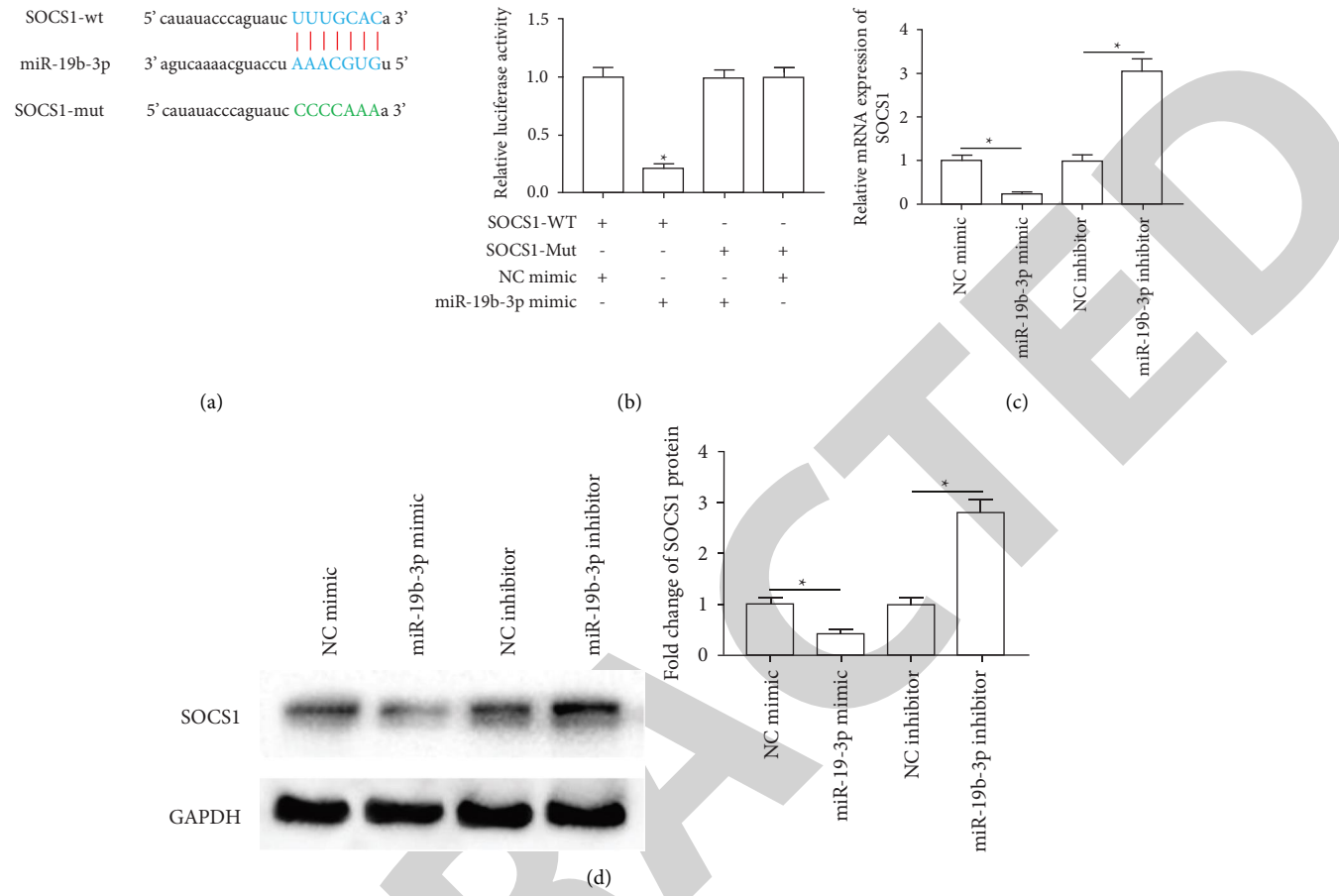
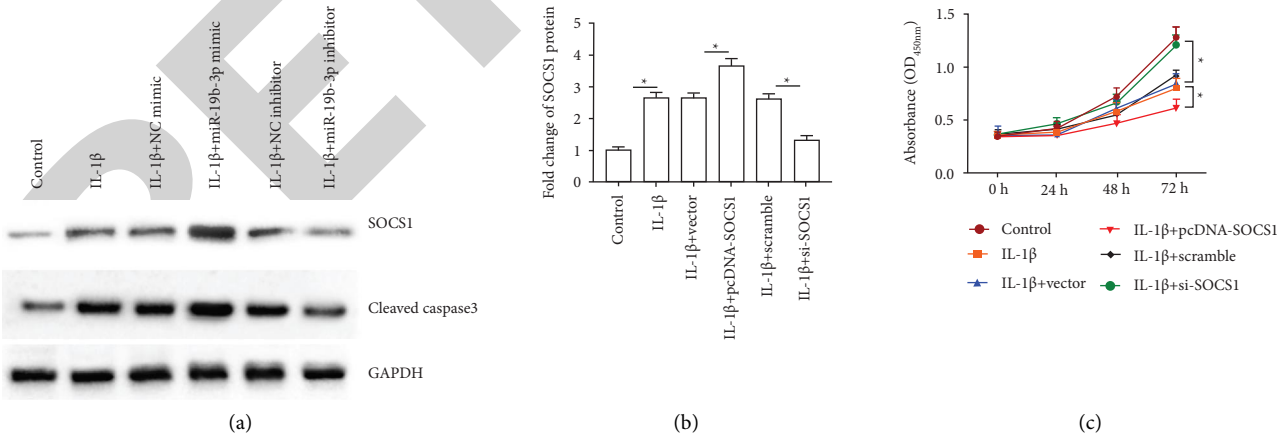


Figure 2: SOCS1 is a direct target of miR-19b-3p. (a). SOCS1 sequences (wild- and mutant-type). (b). Relative luciferase activity (wild- and mutant-type of SOCS1). C&D. SOCS1 mRNA and protein expression. $^*P < 0.01$.



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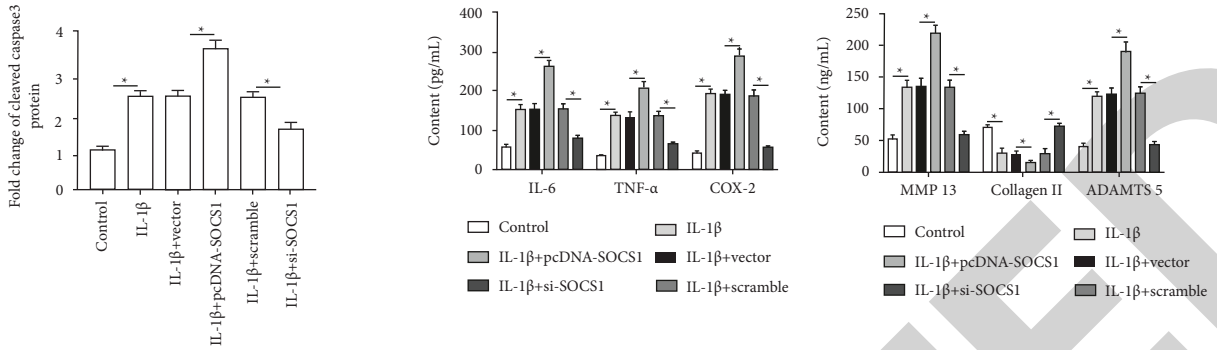
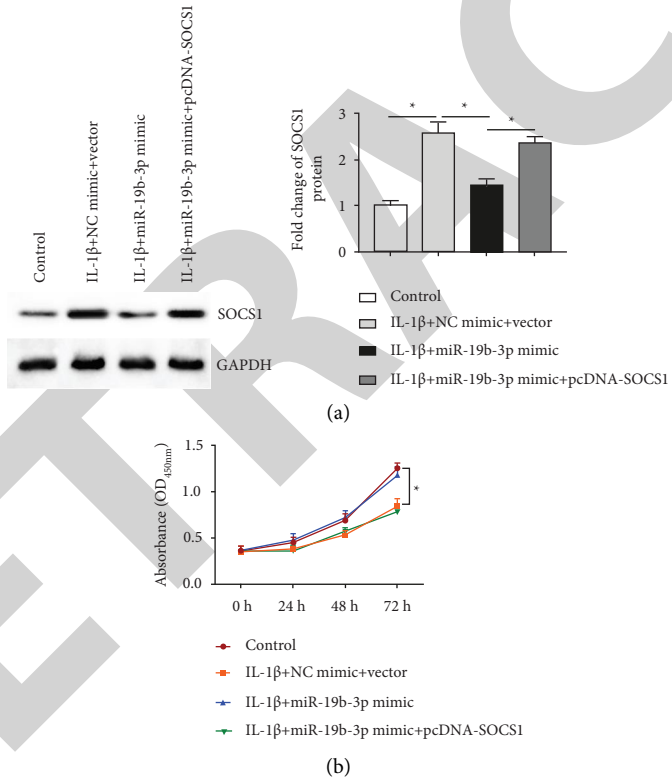


FIGURE 3: Effect of SOCS1 on chondrocytes. a&b. SOCS1 protein expression. (c) Cell viability. a&d. Cleaved caspase3 protein expression. (e) Levels of inflammatory factor secretion. (f) MMP-13, Collagen II and ADAMTS5 secretion levels. $^{*}P < 0.01$.



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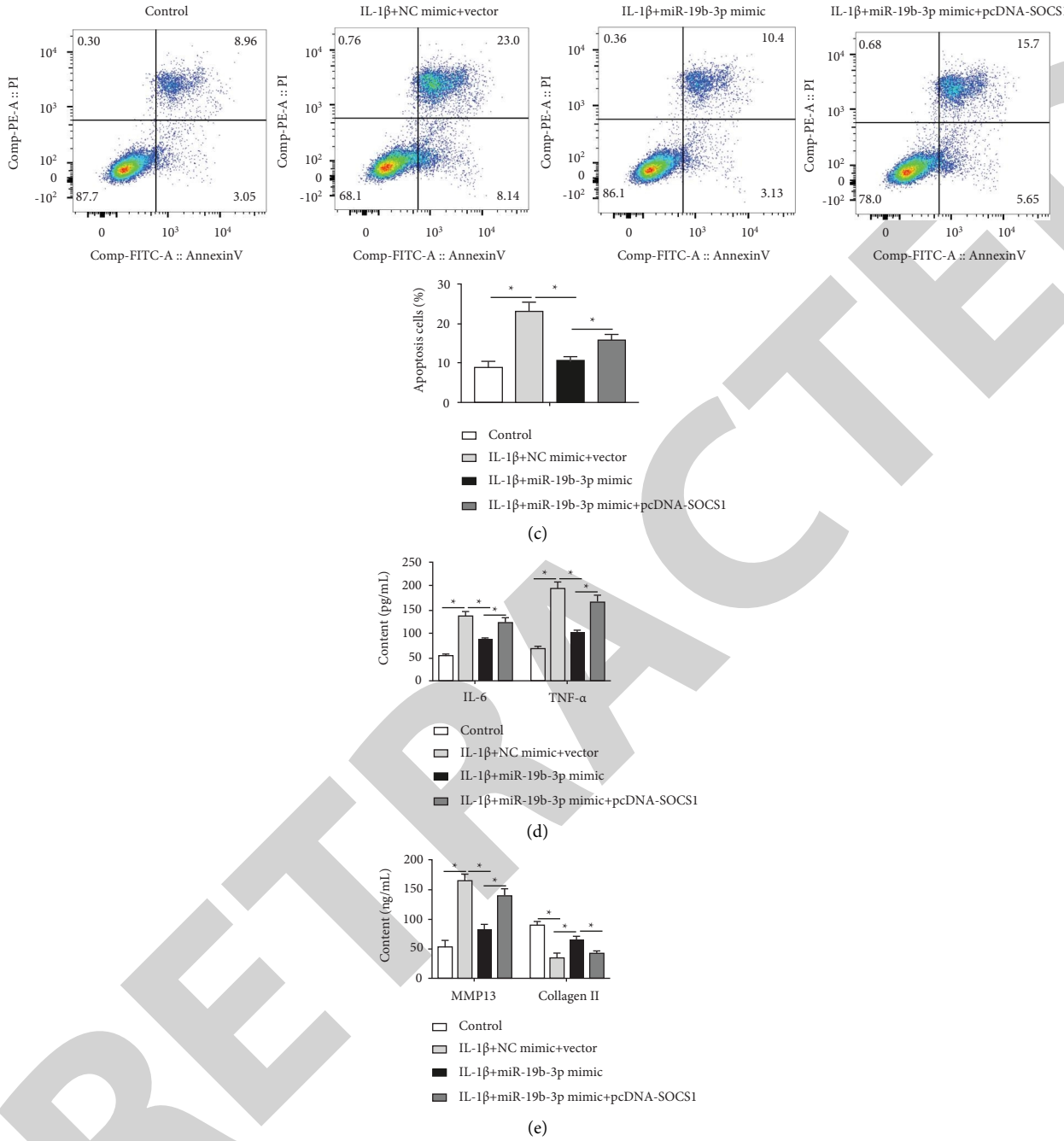

Figure 4: MiR-19 b-3p affects chondrocytes via SOCS1. (a) SOCS1 protein expression. (b) Cell viability. (c) Cell apoptosis. (d) Levels of inflammatory factor secretion. (e) MMP-13 and Collagen II secretion levels. $^{*}P < 0.01$.

As early as 1994, the p38 MAPK axis was found to be involved in the physiological response of yeast to stress stimuli in fungal studies, and it is also involved in the inflammatory response and stress stimuli in mammals. A variety of studies have now shown MAPKs signaling activation in osteoarthritic cartilage tissue, manifested by increased levels of p38 MAPK, ERK as well as JNK phosphorylation [17–20]. In addition, the NF-κB signaling pathway exists widely in various cells, mainly in the inactive p65 subunit form, and phosphorylation occurs when p65 is activated, whereas p65 transduces into the nucleus after dissociation from the phosphoproteasome, further activating the inflammatory response and secreting a series of proinflammatory cytokines [21]. In a word, miR-19b-3p promotes viability, inhibits apoptosis, and reduces inflammatory factor secretion and extracellular matrix degradation of injured chondrocytes by targeting and inhibiting SOCS1 expression, and this is mediated by blocking the MAPK/NF-κB axis.

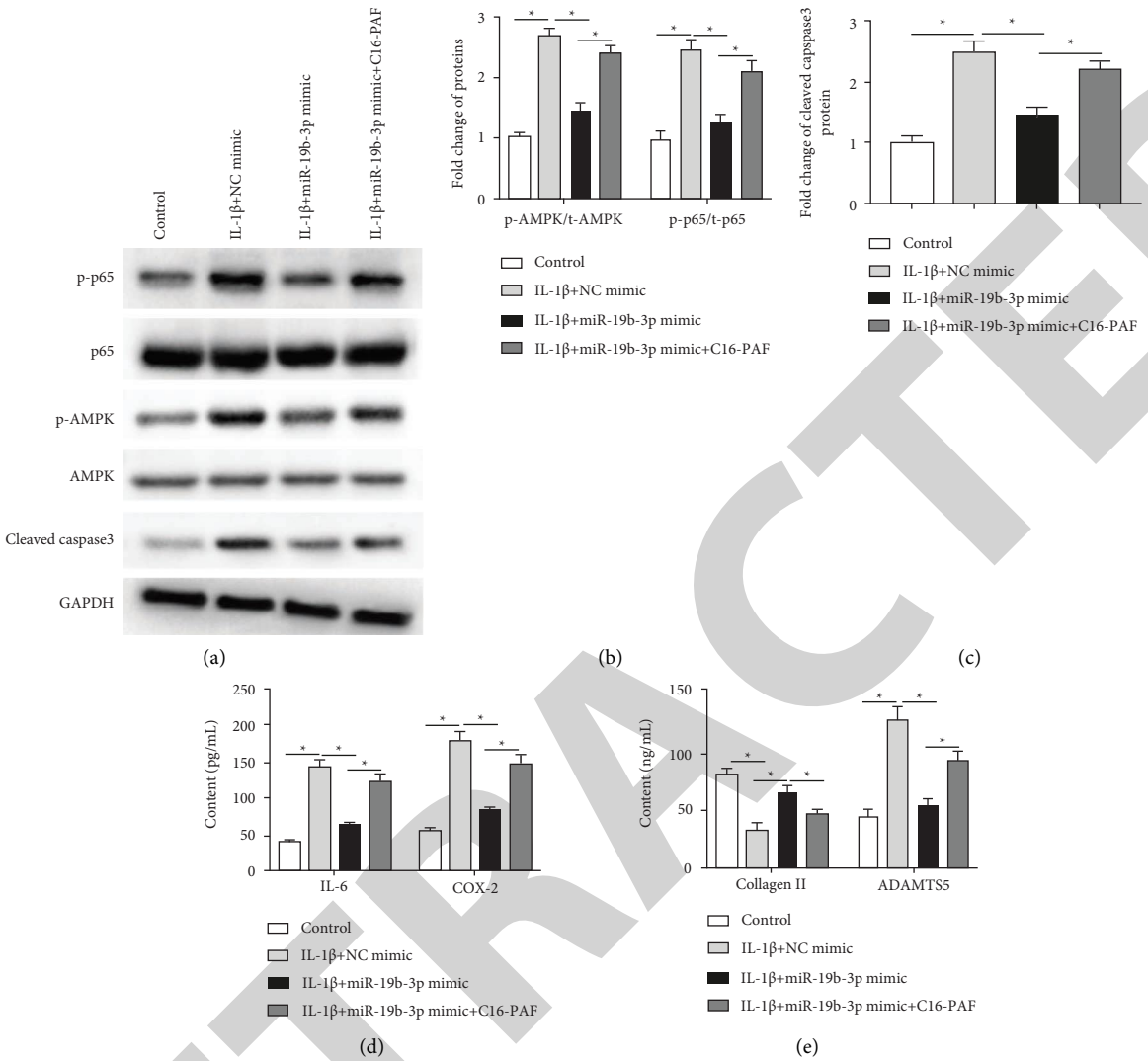

Figure 5: MiR-19 b-3p affects chondrocytes through the MAPK/NF-κB axis. a&b. The phosphorylation of p38 MAPK and NF-κB p65 protein. a&c. Cleaved caspase3 protein expression. (d) Inflammatory factor secretion levels. (e) The secretion levels of Collagen II and ADAMTS5. $^{*}P < 0.01$.

Data Availability

The labeled dataset used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

All authors declare that there are no conflicts of interest regarding this study.

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Retraction

Retracted: Identification of Prognostic LncRNAs Subtypes Predicts Prognosis and Immune Microenvironment for Glioma

Evidence-Based Complementary and Alternative Medicine

Received 20 June 2023; Accepted 20 June 2023; Published 21 June 2023

This article has been retracted by Hindawi following an investigation undertaken by the publisher [1]. This investigation has uncovered evidence of one or more of the following indicators of systematic manipulation of the publication process:

(1) Discrepancies in scope

(2) Discrepancies in the description of the research reported

(3) Discrepancies between the availability of data and the research described

(4) Inappropriate citations

(5) Incoherent, meaningless and/or irrelevant content included in the article

(6) Peer-review manipulation

The presence of these indicators undermines our confidence in the integrity of the article's content and we cannot, therefore, vouch for its reliability. Please note that this notice is intended solely to alert readers that the content of this article is unreliable. We have not investigated whether authors were aware of or involved in the systematic manipulation of the publication process.

Wiley and Hindawi regrets that the usual quality checks did not identify these issues before publication and have since put additional measures in place to safeguard research integrity.

We wish to credit our own Research Integrity and Research Publishing teams and anonymous and named external researchers and research integrity experts for contributing to this investigation.

The corresponding author, as the representative of all authors, has been given the opportunity to register their agreement or disagreement to this retraction. We have kept a record of any response received.

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[1] F. Chen, X. Peng, Z. Teng, H. Long, and H. Wu, "Identification of Prognostic LncRNAs Subtypes Predicts Prognosis and Immune Microenvironment for Glioma," *Evidence-Based Complementary and Alternative Medicine*, vol. 2022, Article ID 3709823, 13 pages, 2022.

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Research Article

Identification of Prognostic LncRNAs Subtypes Predicts Prognosis and Immune Microenvironment for Glioma

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Glioma is the most commonly occurring primary neuroepithelial neoplasm. Long noncoding RNAs (lncRNAs) are emerging as pivotal modulators of gene expression in the immune system and play critical roles in the growth, progression, and immune response of carcinomas. In this study, we performed univariate Cox regression analysis on survival data from TCGA and identified 20 prognostic lncRNAs. Moreover, we revealed that these prognosis-related lncRNAs (PRLnc) were dysregulated in glioma. Furthermore, we constructed a signature based on the expression levels of these prognosis-related lncRNAs based on 13 prognostic lncRNAs, including AGAP2-AS1, CYTOR, MIR155HG, LINC00634, HOTAIRM1, SNHG18, LINC01841, LINC01842, LINC01426, MIR9-3HG, TMEM220-AS1, LINC00641, LINC01270, and LINC01503. The Kaplan–Meier curves show that high-risk patients had a shorter survival time. Finally, the glioma samples were classified into 2 subgroups based on the median expression of prognosis-related lncRNAs in each sample. In summary, these findings suggest that PRLnc is associated with tumor-infiltrating immune cells in glioma and that subtype 2 patients may respond more positively to immunotherapy.

1. Introduction

Glioma is the most commonly occurring primary neuroepithelial neoplasm, mainly, occurring in the brain and arising in the glial tissue. The glioma family is consisting of ependymomas, astrocytomas (such as glioblastoma), oligodendrogliomas, mixed gliomas, and optic nerve, and brain stem gliomas, etc. [1]. The histology of these neoplasms varies greatly; from benign ependymal neoplasms to the most aggressive and fatal grade IV GBM [1, 2]. Although great advances have been made in the field of glioma therapies, comprising radiotherapy, chemotherapy, and targeted treatment, the treatment effect is still unsatisfactory, with a low success rate and 12–14 months survival period after treatment [3, 4]. Inaccurate disease progression prediction and unexpected treatment outcomes were probably due to complicated molecular mechanisms and inconsistent histopathological grading of glioma. At present, the standard treatment for patients with glioma is postoperative radiotherapy and adjuvant chemotherapy [5]. The need to uncover the molecules related to tumor development and to explore split-new methods for individualized treatment of patients with glioma is urgent [6].

Long noncoding RNAs (lncRNAs) exhibit important transcriptional activities, chromosome modification, and nuclear transport [7]. LncRNAs are widely distributed in various species and many kinds of human cells, with the features of stable structure, highly conservative, complex modulation, and tissue-specific expression [7]. There have been some studies showing that lncRNAs display as sponges to adsorb microRNAs (miRNAs) to modulate the expression of genes and exert an effect on regulating transcription and interfering with splicing mechanisms [8, 9]. More and more evidence showed that lncRNAs exhibited a pivotal role in tumor occurrence and metastasis [9, 10], including glioma. There were some studies demonstrating that lncRNAs played a role in glioma progression. For example, lncRNA HOXD-AS2 functioned as a promoter in glioma progression and was perhaps an outstanding target toward glioma's diagnosis and therapy [11]. LncRNA MT1JP mediated an inhibition of glioma cells growth, and metastasis via motivating the Akt signaling [12]. H19 imposed an effect on the

immune infiltration level, thereby affecting glioma patients' prognosis [7]. Wang et al. reported that lncRNA LINC00473 might be expressed as a competing endogenous RNA (ceRNA) to decrease the level of miR-195-5p, followed by raising the expression of YAP1 and TEAD1, which were the downstream targets of miR-195-5p [13]. This finding sheds light on the working mechanisms of LINC00473, causing the progression of glioma [13]. LncRNA LINC00174 induced glioma glycolysis via modulating miR-152-3p/SLC2A1 [14]. Zhang et al. discovered that lncRNAs worked as epigenetic mediator and predictor in the proliferation, migration, invasion, angiogenesis and metastasis in cell line/animal model of glioma [15]. In summary, the studies listed herein make a compelling case for further investigation of lncRNAs in glioma. More promising lncRNAs associated with glioma need to be unearthed.

Herein, we carried out integrated bioinformatics to systematically investigate the association between lncRNA expression levels and glioma patients' clinical characteristics and the prognostic value of lncRNAs. We identified prognostic lncRNAs in glioma utilizing two public databases, including the CGGA and TCGA datasets. Furthermore, based on the expression of these lncRNAs, a predictive signature and two molecular subtypes were developed and established. Additionally, the distribution of tumor-infiltrating immune cells in prognostic lncRNA-based glioma subtypes was estimated. We hoped that this study would aid in identifying patients who might benefit from immunotherapy and hence increase glioma patient survival.

2. Methods and Materials

2.1. Expression Profiles and Sample Information. The RNA-seq data, as well as clinical features of glioma patients, were downloaded and analyzed from TCGA data to discover changes in gene expression. Age, tumor status, surgery status, and grade were all documented along with the clinical data. No further ethics committee approval was required due to all data were acquired from TCGA.

2.2. Establishment of Risk Signature and Statistical Analysis. We identified lncRNAs with prognostic potential using a univariate Cox regression model. The candidate lncRNAs that were substantially connected to survival were then filtered out using the LASSO model [16], resulting in a risk signature. The risk score was computed based on the levels of 13 different lncRNAs. The "survival" R program was used to plot the receiver operating characteristic (ROC) curve, and the Area under Curve (AUC) value was calculated to measure the predictability of the results. SPSS 25.0 (IBM Corp., Armonk, N.Y., USA) and R software were used for all image creation and data analysis in this investigation.

2.3. Functional Enrichment Analysis. Functional enrichment analysis was performed to assess the potential roles of DEGs in different prognostic lncRNAs related subtypes with the DAVID system [17]. Significantly enriched function annotations were defined with a 2-sided P value of less than 0.01.

2.4. Identification of Molecular Subtypes of Glioma. To cluster prognosis-related lncRNAs, researchers utilized ConsensusClusterPlus [18] V1.48.0. Using the median values of prognosis linked lncRNAs expression; the Z-score was utilized to classify the TCGA dataset.

2.5. Estimation of Tumor-Infiltrating Immune Cells. We have used CIBERSORT algorithms [19] to quantify the immune infiltration differences between subtype 1 and subtype 2 groups to study the association between prognosis-related lncRNAs and tumor-infiltrating immune cells. The StromalScore, ImmuneScore, and microenvironment score were then calculated in the subtype 1 and subtype 2 groups to evaluate the tumor microenvironment difference between the 2 groups using CIBERSORT algorithms. The Spearman's correlation test was performed to assess the correlations between PRLnc score and tumor-infiltrating immune cells, which were further, explored using the CIBERSORT algorithm. Moreover, the expression patterns of immune checkpoint genes in the two groups were then compared using the "ggpubr" package of R software [20].

3. Results

3.1. Identification of Prognosis Related lncRNAs in Glioma. Here, we determine the prognostic value of lncRNAs in glioma. We identified 20 prognosis-related lncRNAs (PRLnc) using a univariate Cox regression model, which was present as a forest map (Figure 1(a)). As can be seen from the figure, AGAP2-AS1, CYTOR, MIR155HG, MIR4435-2HG, HOTAIRM1, SNHG18, LINC01841, LINC01842, LINC01426, TMEM220-AS1, LINC01270, and LINC01503, LINC01273 were high-risk lncRNAs, which were significantly upregulated in glioma and positively correlated to the progression of glioma (Figure 1(b)). Whereas LINC00634, SLC25A21-AS1, MIR9-3HG, and LINC00641 were low-risk lncRNAs, which were suppressed in glioma and negatively correlated to the progression of glioma (Figure 1(b)).

3.2. Confirmation of the Correlation between PRLnc Expression and Outcome in Glioma Using CGGA Database. To confirm the correlation between prognosis-related lncRNA expression and overall survival (OS) time in glioma, we analyzed the CGGA database. As present in Figure 2, we showed the higher levels of AGAP2-AS1, CYTOR, HOTAIRM1, MIR155HG, and SNHG18 were correlated to shorter OS in patients with glioma, indicating these lncRNAs may serve as tumor promoting genes (Figures 2(a)–2(e)). However, higher expression levels of LINC00641, LINC00634, and SLC25A21-AS1 were correlated to longer OS in patients with Glioma, indicating these lncRNAs may serve as tumor suppressing genes (Figures 2(f)–2(h)).

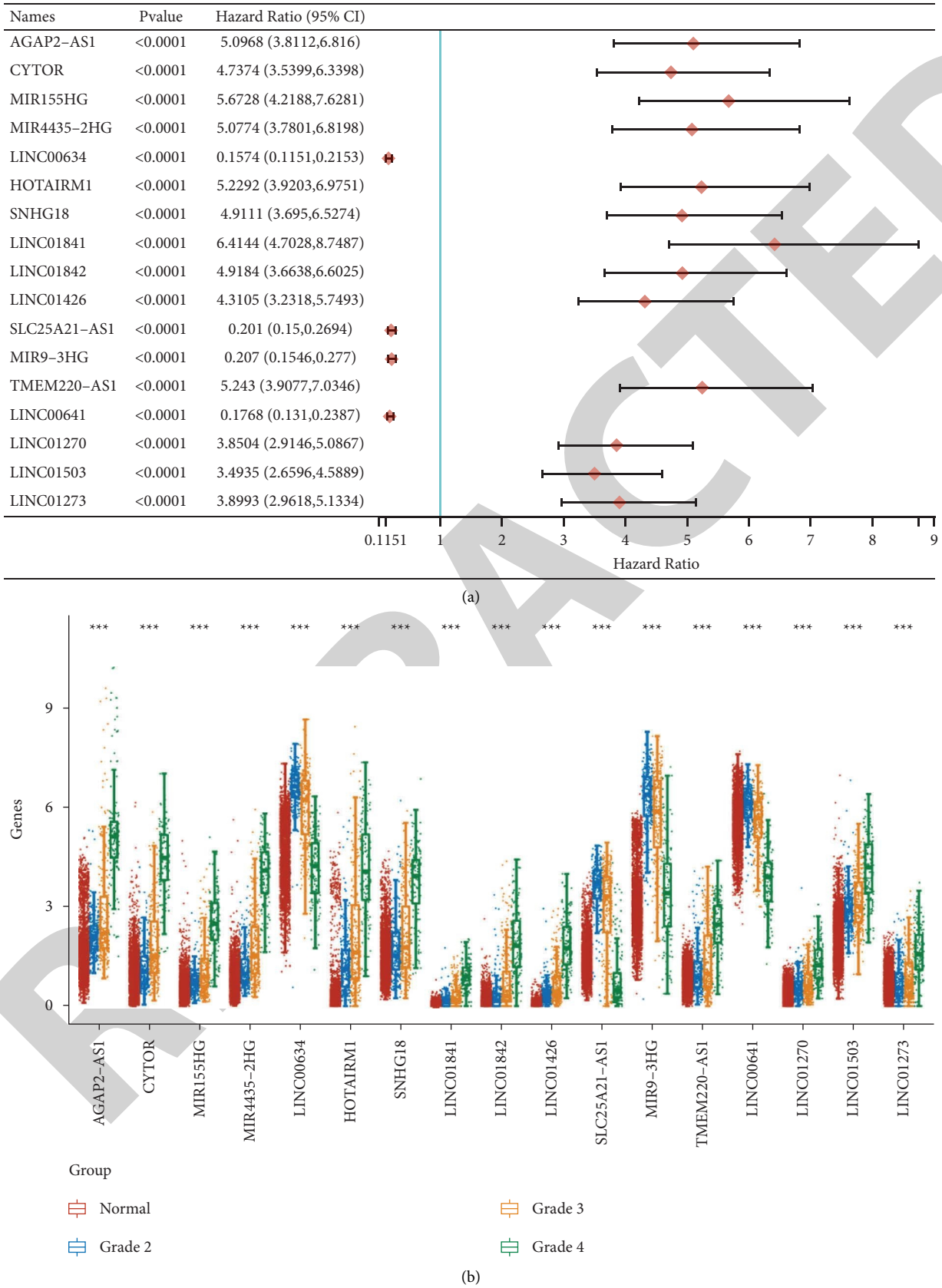


Figure 1: Identification of prognosis related lncRNAs in Glioma. (a) A forest map showed 20 prognosis related lncRNAs (PRLnc) in glioma using a univariate Cox regression model. (b) The expression levels of PRLnc in glioma were evaluated.

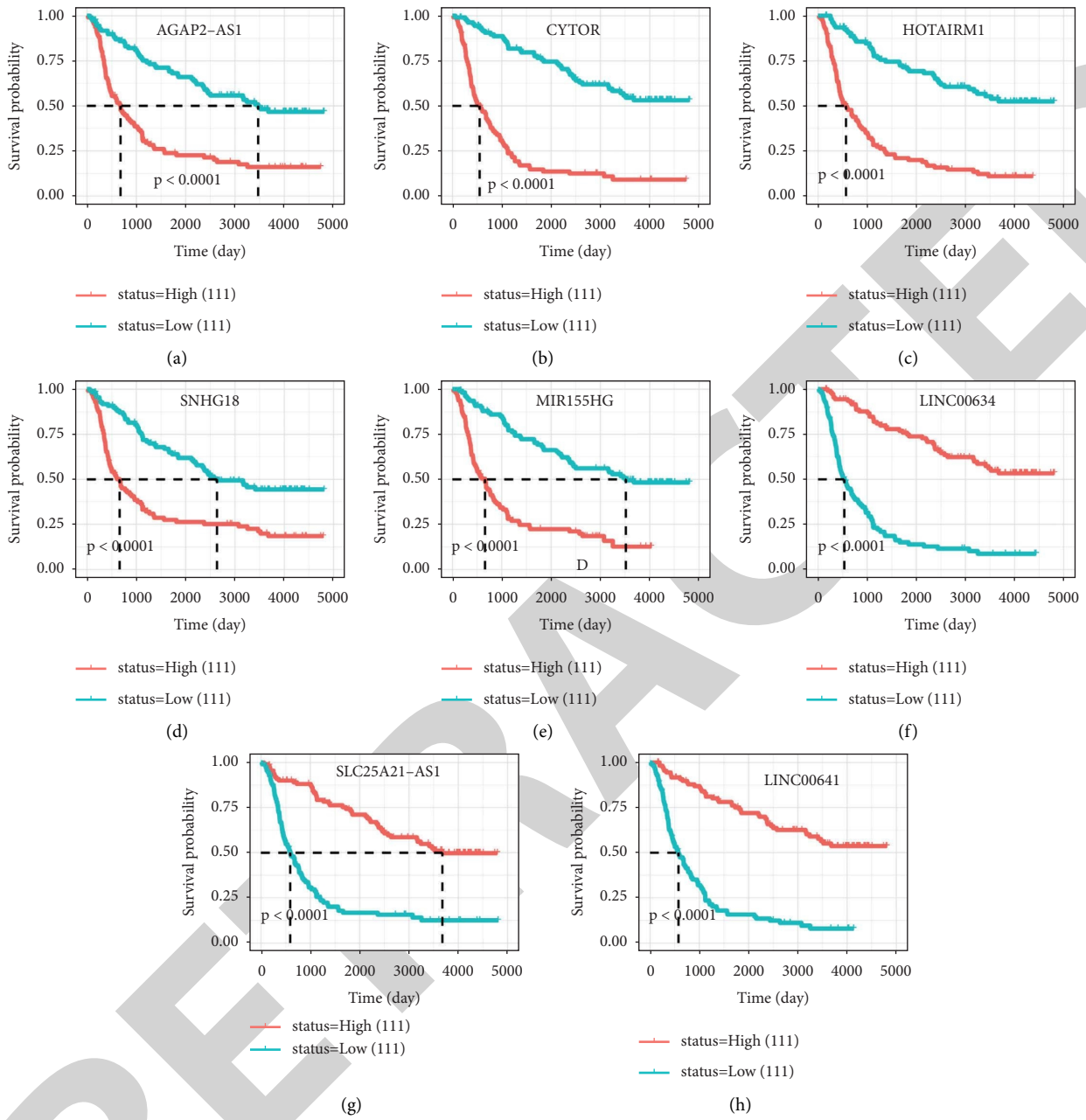


Figure 2: Confirmation of the correlation between PRLnc expression and survival time in glioma using CGGA database. (a–h) The higher levels of AGAP2-AS1, CYTOR, HOTAIRM1, SNHG18, and MIR155HG, were correlated to shorter OS, However, higher expression levels of LINC00634, SLC25A21-AS1, and LINC00641 were correlated to longer OS in patients with glioma.

3.3. Construction of an lncRNA Signature for Glioma. We next used LASSO regression analysis on these 20 lncRNAs to find the most promising candidates (Figure 3(a)). Finally, 13 lncRNAs were chosen as candidates for the lncRNA signature: AGAP2-AS1, CYTOR, MIR155HG, MIR4435-2HG, LINC00634, HOTAIRM1, SNHG18, LOC100506474, LINC01841, LINC01842, LINC01426, LINC01265, SLC25A21-AS1, LOC101928134, MIR9-3HG, TMEM220-AS1, LINC00641, LINC01270, LINC01503, and LINC01273 (Figure 3(a)).

The eight lncRNAs were combined to establish a risk score model for glioma patients, as follows: riskscore = (0.1434) ∗ AGAP2-AS1 + (0.1612) ∗ CYTOR + (4e − 04) ∗ MIR155HG + (−0.1641) ∗ LINC00634 + (0.0385) ∗ HOTAIRM1 + (0.0816) ∗ SNHG18 + (0.0141) ∗ LINC01841 + (0.0819) ∗ LINC01842 + (0.0016) ∗ LINC01426 + (−0.0282) ∗ MIR9-3HG + (0.0925) ∗ TMEM220-AS1 + (−0.1016) ∗ LINC00641 + (0.0768) ∗ LINC01270 + (0.0016) ∗ LINC01503 (Figure 3(a)). Individual risk scores were assigned to all patients based on the lncRNA signature. Figure 3(b) depicts the correlation between the lncRNA expression and the risk score. We found high-score glioma patients have a shorter OS than low-score glioma patients (Figure 3(c)). We then performed time-dependent ROC curve analysis to determine the

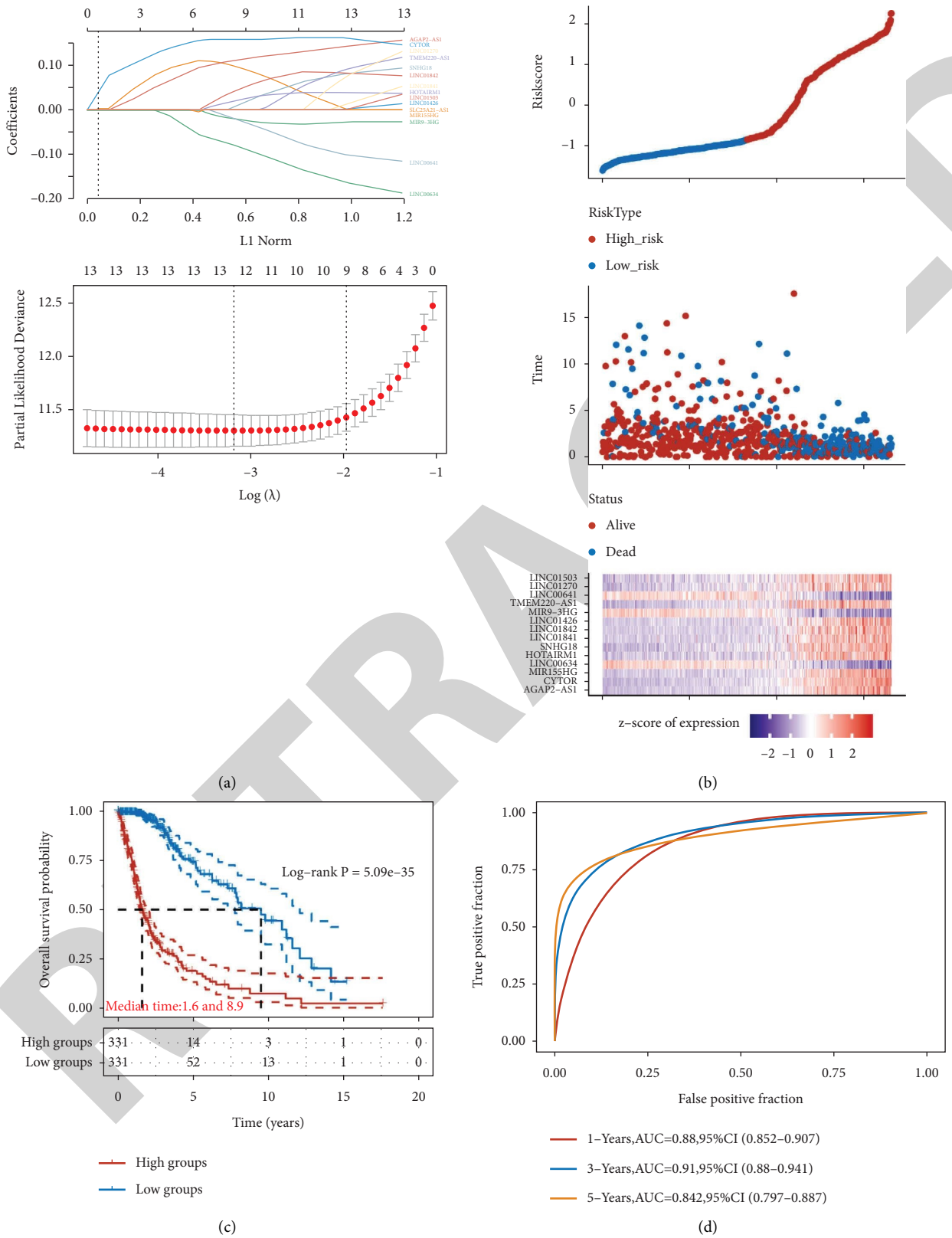


Figure 3: Construction of an lncRNA signature for glioma. (a) LASSO regression analysis on these 20 lncRNAs. (b) Figure 3(b) depicts the correlation between the lncRNAs expression and the risk score. (c) Kaplan–Meier analysis showed high-risk patients have a shorter OS than low-risk patients. (d) We then performed time-dependent ROC curve analysis to assess the ability of the PRLnc signature in predicting the OS of glioma patients.

PRLnc signature in predicting the OS of glioma patients. The AUC was 0.88, 0.91, and 0.842 for 1-, 3-, and 5-years, respectively (Figure 3(d)). Our findings imply that the lncRNA signature established in this study is an effective biomarker of prognosis in glioma.

3.4. Analysis of PRLnc Expression to Identify 2 Subtypes of Glioma. Then, glioma were classified as 2 subtypes based on the expression of PRLnc expression using consensus clustering when $K = 2$ (Figures 4(a) and 4(b)). The samples were classified into 2 subgroups based on prognosis-related lncRNAs in each glioma sample (Figures 4(c) and 4(d)). Figure 1 depicts PRLnc expression in the 2 subtypes of glioma (Figure 4(e)). Further examination of the correlations between the 2 subgroups and overall survival time revealed significant disparities in prognosis between the two subtypes. Those with subtype 1 glioma exhibited considerably shorter OS times than patients with subtype 2 glioma (Figure 4(f)).

3.5. Analysis of Differentially Expressed Genes between lncRNAs Related Subtypes in Glioma. We discovered DEGs between subtype 1 and subtype 2 glioma and created a volcano map to investigate the impact of prognosis-related lncRNA expression on malignancy. A total of 2398 DEGs were identified, with 1137 being induced and 1261 being suppressed (Figures 5(a) and 5(b)). The most significantly upregulated genes included CHI3L1, LTF, PDPN, MEOX2, TIMP1, IGFBP2, POSTN, EMP3, SAA1, METTL7B, RARRES2, FMOD, MOXD1, PLA2G2A, FABP5, NNMT, RBP1, and ANXA1. And the most significantly downregulated genes included SFRP2, SMOC1.

Figure 5 depicts the comparative values of the three datasets. The bioinformatics analysis showed upregulated DEGs were related to immune response, such as T cell activation, cellular response to IFN-γ, IFN-γ–mediated signaling pathway, neutrophil activation involved in immune response, neutrophil degranulation, and cell cycle, such as microtubule cytoskeleton organization, mitotic nuclear division, and mitotic sister chromatid segregation (Figure 5(c)). The bioinformatics analysis showed downregulated DEGs were related to axonogenesis, cognition, glutamate receptor signaling pathway, modulation of chemical synaptic transmission, neurotransmitter secretion, ion transmembrane transport, and membrane potential (Figure 5(d)).

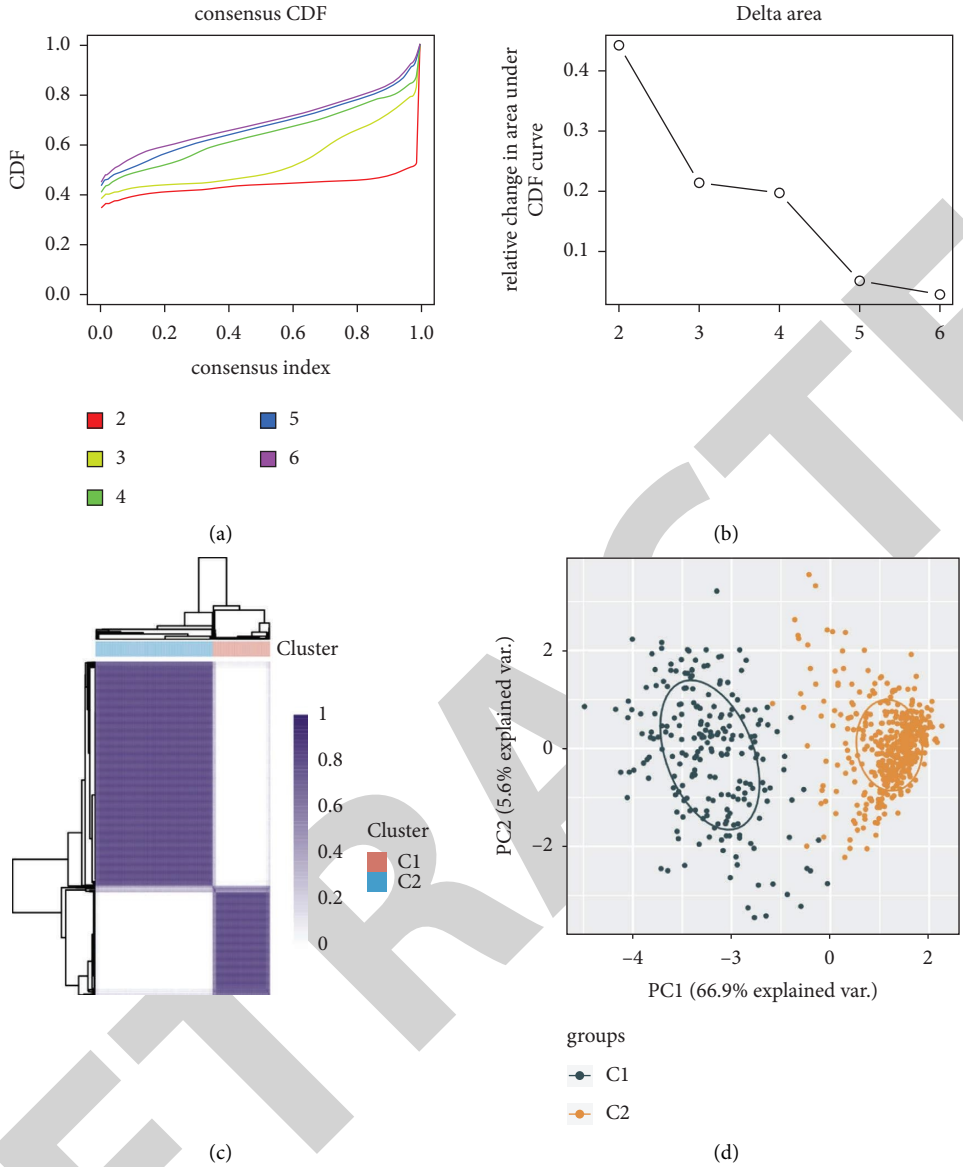
3.6. Analysis of Tumor-Infiltrating Immune Cells Distribution in PRLnc Subtypes. We have used CIBERSORT algorithms to quantify the immune infiltration differences between subtype 1 and subtype 2 groups to study the association between prognosis-related lncRNAs and tumor-infiltrating immune cells. Figure 6(a) depicts a heatmap of all significantly distinct immune responses. Comparative assessments of immune cell subpopulations indicated considerable disparities in immune cell infiltration levels between the subtype 1 and subtype 2 groups. We revealed the infiltrating levels of hematopoietic stem, endothelial cells, CD8+ naive T cells, common lymphoid progenitor, CD4+ Th2 T cell, macrophage, macrophage M1/2, monocyte, CD4+ memory T cells, CD4+ effector memory T cells, and CD8+ effector memory T cells were higher in subtype 1 than in subtype 2 groups. However, the infiltrating levels of NK cell, B cells plasma, CD4+ Th1T cells, myeloid dendritic cell activated, mast cell, T cells regulatory (Tregs), eosinophil, neutrophil, and T cell NK were higher in subtype 2 than in subtype 1 groups (Figure 6(a)).

StromalScore, ImmuneScore, and microenvironment score were then calculated in the subtype 1 and subtype 2 groups to evaluate the tumor microenvironment difference between 2 groups. The results showed that StromalScore, ImmuneScore, and microenvironment score were significantly higher in subtype 1 than in subtype 2 groups (Figures 6(b)–6(d)). Moreover, the correlations between PRLnc score and tumor-infiltrating immune cells were further explored, and the results showed that PRLnc score were significantly positively correlated to T cells CD8+ cells, neutrophil cells, macrophage cells, myeloid dendritic cells in glioma (Figure 6(e)).

The patterns of immune checkpoint genes in the two groups were then compared, and we observed several genes (CD274, CTLA4, HAVCR2, LAG3, PDCD1, PDCD1LG2, TIGIT, and SIGLEC15) were overexpressed in the subtype 1 group (Figure 6(f)). The TIDE algorithm was also utilized to determine whether PRlnc-related subtypes might predict immunotherapeutic benefit. Patients in the subtype 1 group showed considerably higher TIDE ratings than those in the subtype 2 group (Figure 6(g)), indicating that patients in the subtype 2 group might respond better to immunotherapy.

4. Discussion

Accompanied by the development of high throughput sequencing technology, studies towards the molecular basis of carcinoma have made progress, but the etiopathogenesis and biomarkers of glioma have not been completed [21, 22]. To ameliorate the curative effect and to clearly comprehend the pathogenesis of glioma, it is urgent to carry out more conclusive research to unearth the molecular biomarkers related to the initiation and progression of glioma and to identify potential therapeutic targets that are urgently needed. lncRNAs are a subset of RNA that is > 200 bps with limited or no protein coding ability. Increasing evidence shows that lncRNAs take part in glioma carcinogenesis, exhibiting as oncogenes or tumor suppressors. For instance, Ji et al. elaborated that lncRNA SChLAP1 stabilized ATN4 and stimulated NF-κB signaling to induce the development of GBM by forming a complex with HNRNPL [23]. Another study demonstrated that LINC01116 mediated the facilitation of proliferation and neutrophil recruitment via modulating IL-1β, furnishing a novel understanding of lncRNAs-mediated glioma progression [24]. Besides, emerging evidence revealed that lncRNAs were abnormally expressed and their dysregulation exerted an effect on the occurrence and development of glioma. For example, Gong and Huang clarified that downregulated lncRNA maternally expressed



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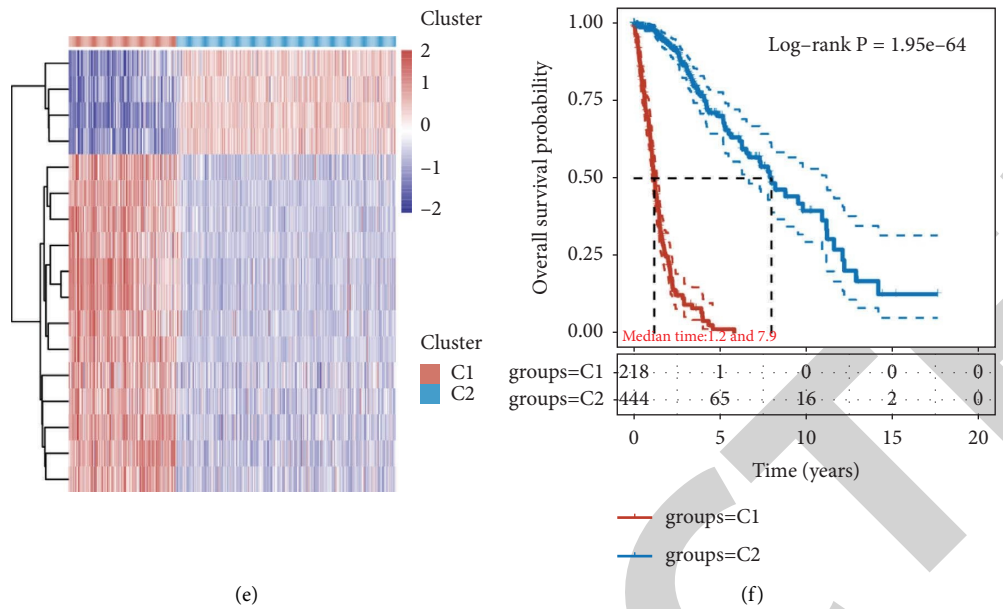
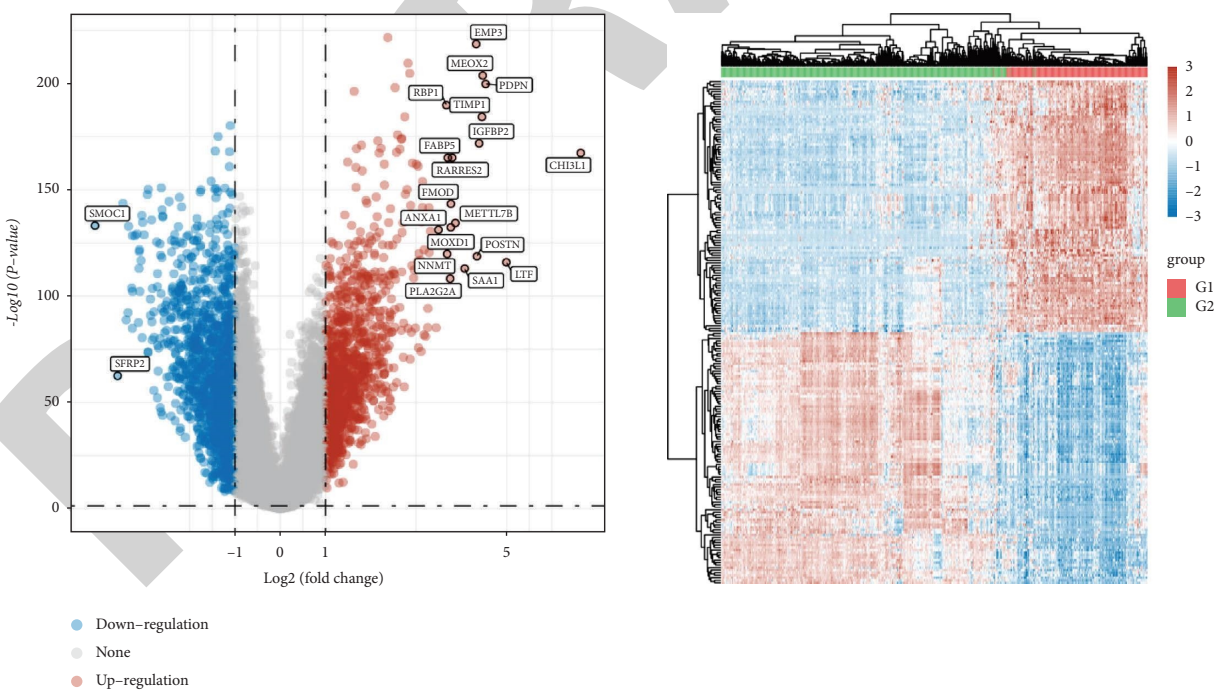


(e) (f)

Figure 4: Analysis of PRLnc expression to identify four subtypes of glioma. (a-b) Consensus clustering cumulative distribution function (CDF) and relative change in the area under the CDF curve (CDF Delta area) were analyzed. (c) The samples were classified into 2 subgroups based on prognosis-related lncRNAs in each glioma sample. (d) Principal component analysis (PCA) of 2 subgroups based on prognosis-related lncRNAs in each glioma sample. (e) The expression levels of PRLnc in 2 subgroups were presented. (f) Those with subtype 1 glioma exhibited considerably shorter OS times than patients with subtype 2 glioma.



(a) (b)

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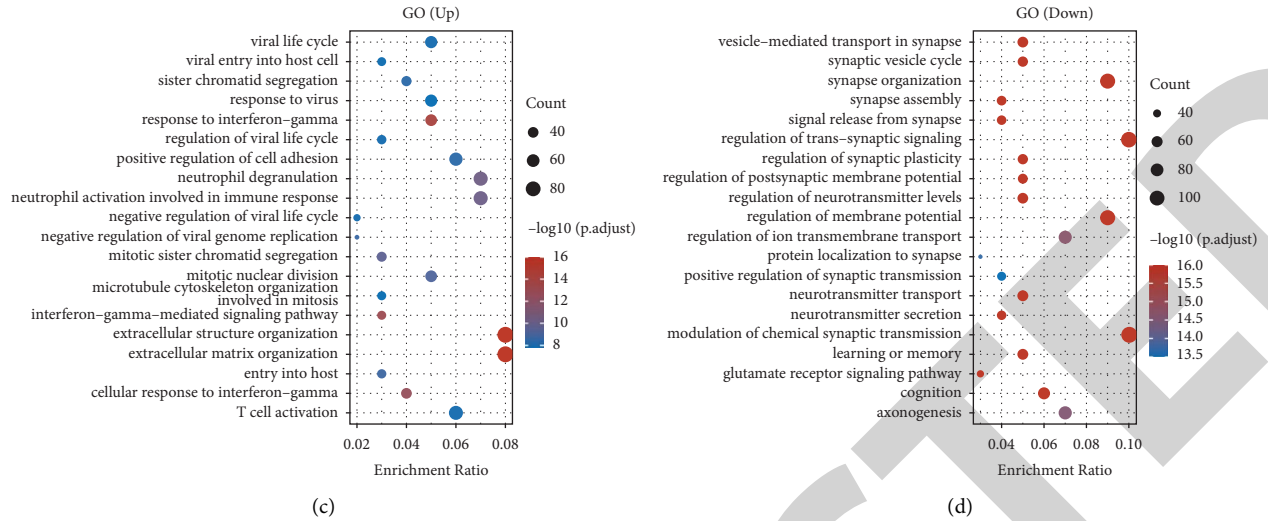
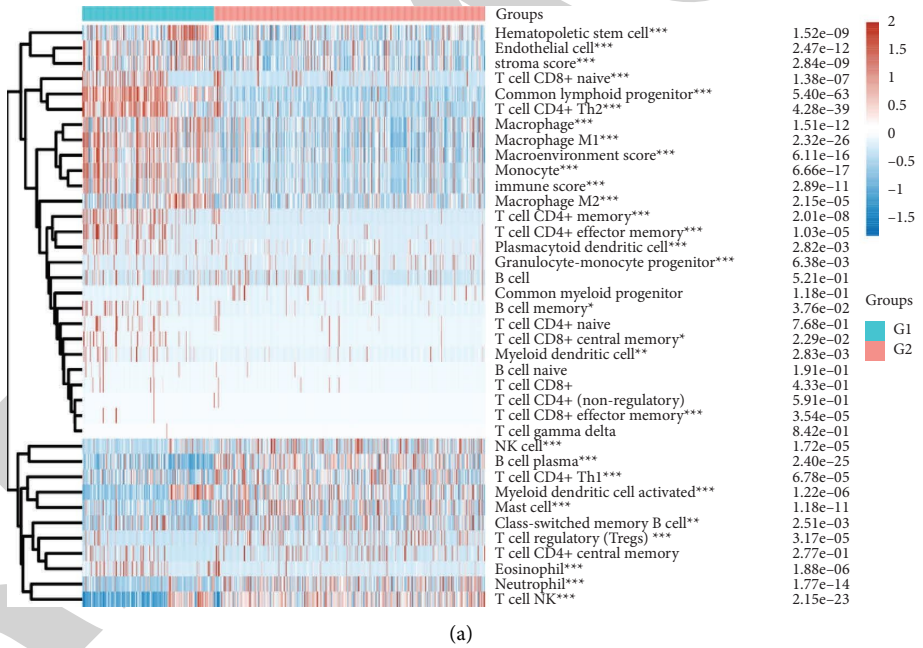


Figure 5: Analysis of differentially expressed genes between lncRNAs related subtypes in glioma. (a-b) volcano map and heatmap showed DEGs between subtype 1 and subtype 2 glioma samples. (c-d) GO analysis of upregulated and downregulated DEGs.



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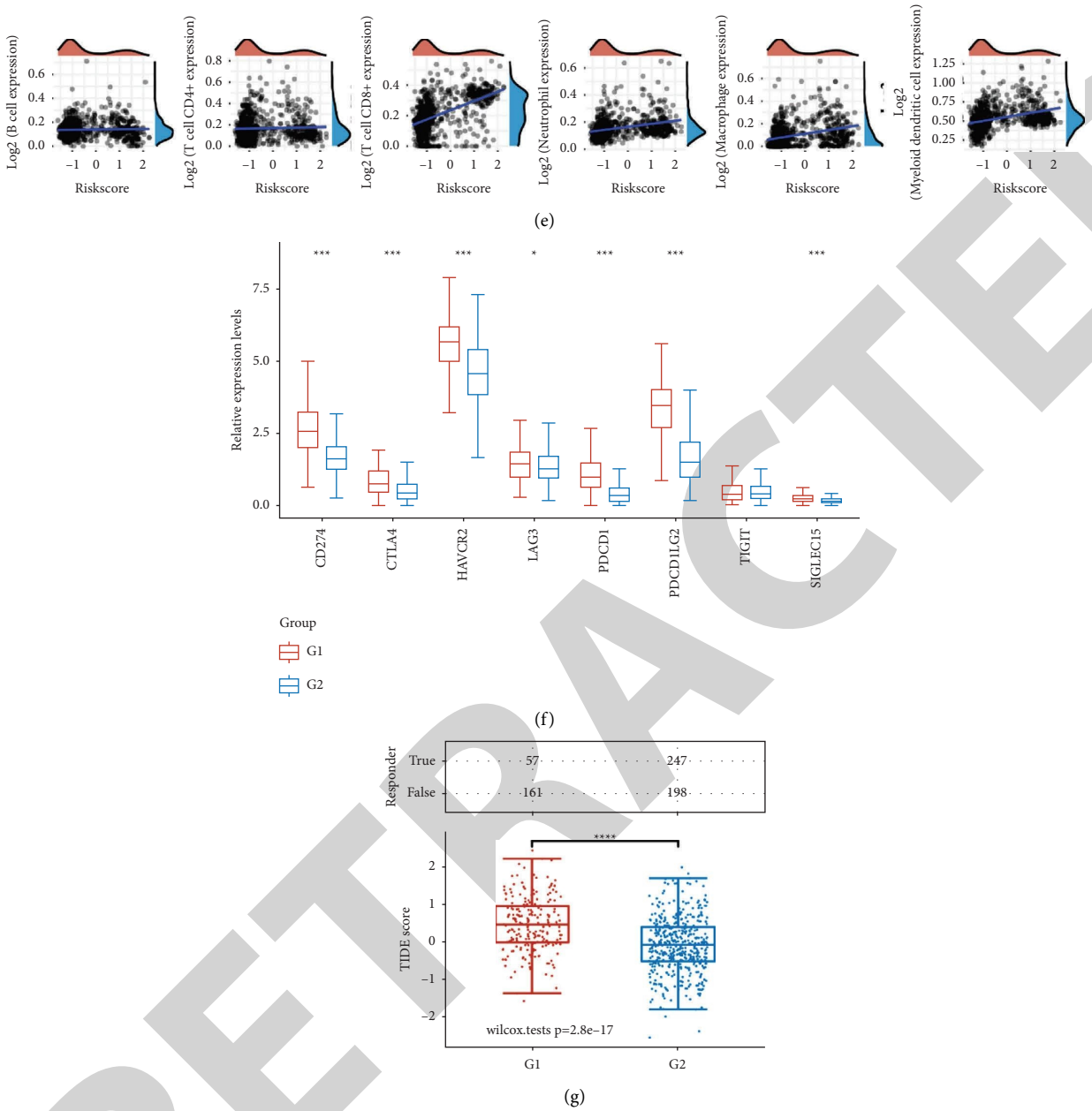

Figure 6: Comparison of PRLnc subtypes and existing immune molecular subtypes. (a) Heatmap was used to depicts of all significantly distinct immune infiltration levels by using CIBERSORT algorithms. (b–d) microenvironment score, StromalScore, and ImmuneScore, were then, examined in the two groups. (e) The correlations between PRLnc score and tumor-infiltrating immune cells were calculated. (f) The expression patterns of immune checkpoint genes in the two groups were then compared. (g) The TIDE algorithm was also utilized to determine whether PRlnc related subtypes might predict immunotherapeutic benefit.

gene 3 (MEG3) in glioma cells suppressed migration of glioma cells by modulating the miR-6088/SMARCB1 axis [25]. LncRNA LINC00909 was greatly raised in the tissues and cell lines of glioma, and it acted as a ceRNA to interact with miR-194 and thus up-regulate MUC1-C, further inducing cell proliferation and invasion of glioma [26]. Collectively, these findings demonstrate that lncRNAs play a crucial role in the occurrence and development of glioma and might act as a novel therapeutic target.

In our study, we identified 20 prognostic lncRNAs and revealed that these prognosis-related lncRNAs were dysregulated in glioma. It was suggested that AGAP2-AS1 overexpression was an unfavorable prognostic factor in many carcinomas, such as lung carcinoma and glioma [27–29]. In GBM, AGAP2-AS1 has been identified as an oncogenic gene that regulates GBM cell motility and invasion. Cytoskeleton regulator RNA (CYTOR), also known as LINC00152, is a new long intergenic noncoding RNA. As

previously described, it was suggested that CYTOR was increased in gastric carcinoma [30], renal cell carcinoma (RCC) and gallbladder carcinoma tissues. As compared to paired noncancerous tissues, high expression of CYTOR exhibited a positive association with poor overall survival [31]. CYTOR was up-regulated in the tissues of hepatocellular carcinoma (HCC), with circulating CYTOR was also highly expressed in plasma specimens of HCC patients, and could be considered as a potential biomarker for HHC's diagnosis [32]. Zhenget al. elaborated that CYTOR was upregulated in a variety of carcinoma types and is especially upregulated in GBM. The expression of SNHG18 exhibited a negative relation to the mutation of isocitrate dehydrogenase 1 (IDH1) [33]. Zheng et al. [33], revealed that SNHG18 promoted cell motility of glioma via disrupting nucleocytoplasmic transport of α-enolase. LINC01503 was greatly upregulated in the tissues and cells of glioma, and its overexpression exhibited a significant correlation with tumor size and WHO grade in patients with glioma. Mechanistic evaluation demonstrated that LINC01503 facilitated tumorigenesis and progression by activating Wnt/β-catenin signaling [34]. Recently, LINC01426 expression and function in human carcinomas have aroused great interest [35–37]. LINC01426 was upregulated in glioma, clear cell renal cell carcinoma, and lung adenocarcinoma, and the increased expression of LINC01426 was related to adverse clinicopathological characteristics. Functionally, LINC01426 played a pro-oncogenic role in these carcinomas, and it was reported to be implicated in regulating several neoplasms' biological phenotypes. Additionally, LINC01426 facilitated the progression of glioma by the PI3K/AKT signaling pathway and was an independent predictor of glioma patients' prognosis [37]. Little reports were shown towards these lncRNAs related to glioma, including LINC00634, LINC01842, LINC01265, LOC101928134, TMEM220-AS1, LINC00641, and LINC01270. However, there were other studies that revealed these lncRNAs displayed a relation to the process of other types of carcinomas. For instance, LINC01270 was shown to have a significant association with a worse outcome in breast cancer. This study further investigated LINC01270's functions and found that reduced LINC01270 dramatically inhibited cell viability, colony formation, and cell migration ability in triple-negative breast cancer (TNBC) cells. Li et al. suggested that inhibiting LINC01270 could give rise to the suppression of esophageal carcinoma (EC) progression via demethylation of GSTP1 [38]. Ablating LINC00634 resulted in a decrease in EC cell viability and an inducing in cell apoptosis levels [39]. In order to identify more accurate biomarkers for the prognosis of glioma, we aimed to construct a risk model based on the levels of these PRLnc. The 13 lncRNAs were combined to establish a molecular risk score model for patients with glioma. High-risk patients have a shorter life expectancy than their low-risk peers. We confirmed that the lncRNA signature established here is an effective predictor of overall survival in glioma patients. Based on AUC, the predictive performance of our risk score is better than in a previous study [40].

In 2016, the World Health Organization (WHO) incorporated molecular features into the classification of brain tumors for the first time to enable more accurate, "stratified" diagnoses, improve patient management, and more accurately estimate the likelihood of prognosis and treatment response. Based on their genetic profile, gliomas could be classified as IDH-mutant, 1p/19q-intact glioma. In this study, we identified molecular subtypes of glioma using prognosis-related lncRNAs expression in glioma samples. Finally, the glioma samples were classified into 2 subgroups based on the median expression of PRLncs in each sample. Those with subtype 1 glioma exhibited considerably shorter OS times than patients with subtype 2 glioma, indicating that the subtype 1 glioma patients may have a more aggressive cancer status.

Recently, research focusing on lncRNAs' function and acting mechanisms has been increasingly reported. LncRNAs play pivotal roles in the growth, progression, and immune system of carcinomas [41]. For instance, the lncRNA NeST was reported to have a relationship to T cell activation and was critical for the regulation of immune response [42]. The lncRNA NRON maintained T cells resting state via NFAT [43]. Carcinoma cell antigen presentation was downregulated by the oncogenic lncRNA LINK-A [44]. Li et al. found that the lncRNAs were related to immune cell infiltration and presented high tissue-specific expression [41]. Here, we identified several lncRNAs that are associated with immune pathways. We found that lncRNA-based subtype 1 and subtype 2 had distinct immune cell subpopulations. We found that the infiltrating levels of hematopoietic stem cells, endothelial cells, CD8+ naive T cells, common lymphoid progenitor, CD4+ Th2 T cells, macrophage, macrophage M1/2, monocyte, CD4+ memory T cells, CD4+ effector memory T cells, and CD8+ effector memory T cells were higher in the subtype 1 group than those in the subtype 2 groups. The TIDE algorithm analysis showed that patients in the subtype 1 group showed considerably higher TIDE ratings than those in the subtype 2 group, indicating that patients in the subtype 2 group might respond better to immunotherapy. Other noncoding RNAs might be valuable in the prediction of the prognosis of glioma [45–47].

In conclusion, we identified 20 PRLnc dysregulated in glioma. PRlnc is associated with tumor-infiltrating immune cells in glioma and that subtype 2 patients may respond more positively to immunotherapy. This study may help to identify glioma patients who will benefit from immunotherapy to improve their survival.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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Retraction

Retracted: LncRNA-PAX8-AS1 Silencing Decreases Cell Viability, Enhances Apoptosis, and Suppresses Doxorubicin Resistance in Myeloid Leukemia via the miR-378g/ERBB2 Axis

Evidence-Based Complementary and Alternative Medicine

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This article has been retracted by Hindawi following an investigation undertaken by the publisher [1]. This investigation has uncovered evidence of one or more of the following indicators of systematic manipulation of the publication process:

(1) Discrepancies in scope
(2) Discrepancies in the description of the research reported
(3) Discrepancies between the availability of data and the research described
(4) Inappropriate citations
(5) Incoherent, meaningless and/or irrelevant content included in the article
(6) Peer-review manipulation

The presence of these indicators undermines our confidence in the integrity of the article's content and we cannot, therefore, vouch for its reliability. Please note that this notice is intended solely to alert readers that the content of this article is unreliable. We have not investigated whether authors were aware of or involved in the systematic manipulation of the publication process.

In addition, our investigation has also shown that one or more of the following human-subject reporting requirements has not been met in this article: ethical approval by an Institutional Review Board (IRB) committee or equivalent, patient/participant consent to participate, and/or agreement to publish patient/participant details (where relevant).

Wiley and Hindawi regrets that the usual quality checks did not identify these issues before publication and have since put additional measures in place to safeguard research integrity.

We wish to credit our own Research Integrity and Research Publishing teams and anonymous and named external researchers and research integrity experts for contributing to this investigation.

The corresponding author, as the representative of all authors, has been given the opportunity to register their agreement or disagreement to this retraction. We have kept a record of any response received.

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[1] X. Song, Y. Chen, Y. Peng et al., "LncRNA-PAX8-AS1 Silencing Decreases Cell Viability, Enhances Apoptosis, and Suppresses Doxorubicin Resistance in Myeloid Leukemia via the miR-378g/ERBB2 Axis," *Evidence-Based Complementary and Alternative Medicine*, vol. 2022, Article ID 2295044, 19 pages, 2022.

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Research Article

LncRNA-PAX8-AS1 Silencing Decreases Cell Viability, Enhances Apoptosis, and Suppresses Doxorubicin Resistance in Myeloid Leukemia via the miR-378g/ERBB2 Axis

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Objective. Considering the role of lncRNAs reported as regulators in acute myeloid leukemia (AML) progression, the current research aims to investigate the role of PAX8-AS1 in chemo-resistant AML. *Methods*. Human AML cells HL60 and human doxorubicin (ADM)-resistant AML cells (HL60/ADM cells) were used to establish *in vitro* models of chemo-sensitive AML and refractory/recurrent AML, respectively. CCK-8 assay and flow cytometry were used to determine cell resistance to ADM, viability, and apoptosis. PAX8-AS1, miR-378g, and ERBB2 expressions in the models and/or AML patients were quantified via qRT-PCR or Western blot. The miRNA/mRNA axis targeted by PAX8-AS1 was analyzed using Starbase, TargetScan, or GEO and validated through a dual-luciferase reporter assay. The expressions of Bcl-2, Bax, and C Caspase-3 in cells were quantitated by Western blot. *Results*. The highly expressed PAX8-AS1 was observed in AML patients and HL60 cells, which was more evident in refractory/recurrent AML patients and HL60/ADM cells. Compared with that in ADM-treated parental HL60 cells, the viability of ADM-treated HL60/ADM cells remained strong. PAX8-AS1 overexpression increased viability and Bcl-2 expression, while diminishing apoptosis, Bax, and C Caspase-3 expressions in HL60 cells. However, the abovementioned aspects were oppositely impacted by PAX8-AS1 silencing in HL60/ADM cells. PAX8-AS1 directly targeted miR-378g, whose expression pattern is opposite to that of PAX8-AS1 in AML. MiR-378g upregulation abrogated the effects of PAX8-AS1 overexpression on HL60 cells. MiR-378g downregulation offset PAX8-AS1 silencing-induced effects on HL60/ADM cells. Moreover, ERBB2 was recognized as the target of miR-378g, with a higher expression in HL60/ADM cells than in HL60 cells. *Conclusion*. PAX8-AS1 silencing decreases cell viability, enhances apoptosis, and suppresses ADM resistance in AML via regulating the miR-378g/ERBB2 axis.

1. Introduction

Acute myeloid leukemia (AML) is characterized by clinical and biological heterogeneity and poor prognosis and is the most common subtype of acute leukemia in adults [1]. Uncontrolled proliferation and impaired differentiation of clonal mass of myeloid stem cells are considered to be highly related to the pathogenesis of AML [2] and can lead to a rapid onset of deadly infections, bleeding, or organ infiltration [3]. Currently, chemotherapy has emerged as the main therapeutic option for AML when compared to molecularly targeted drugs and allogeneic hematopoietic stem cell transplantation [4]. However, chemotherapy has a propensity to fail owing to the acquired resistance of leukemia cells to chemotherapeutic agents [4].

The development of multidrug resistance (MDR) involves multiple mechanisms [5], which are ATP-binding cassette (ABC) overexpression-induced drug efflux pumps that reduce intracellular drug concentrations [6], FLT3 mutation [7], DNA repair abnormalities [8], apoptosis tolerance [5], and bone marrow microenvironment changes [9]. Doxorubicin (ADM) is a first-line chemotherapeutic drug used in AML [10] and has been recorded to mediate caspase activation and apoptotic DNA fragmentation to induce death of AML cells [11]. Resistance to ADM involves upregulation of proteins from the ABC superfamily to cause efflux of the drug in AML cells [12], which remains a significant obstacle to the successful treatment of AML. Notably, an existing study has revealed that altered expressions of long noncoding RNAs (lncRNAs) are implicated in the ADM resistance of patients diagnosed with relapsed/refractory AML [13].

LncRNAs, a class of transcripts produced in mammals and other eukaryotes, are constituted by over 200 nucleotides without an open reading frame, possessing great functional diversity [14]. Considerable lncRNAs have been recognized to be biologically significant in many human diseases including malignant tumors [15, 16]. Aberrant expressions of lncRNAs can cause repercussions on cancer cell proliferation and apoptosis, thus altering drug resistance to eventually affect cancer progression [17, 18]. A report has shown that poor outcomes for AML patients are attributed to resistance to treatment [4]. Therefore, targeting lncRNAs with an intention to antagonize treatment resistance may be a promising approach to improve the result of AML patients. The lncRNA risk score system built for predicting survival of children with AML has uncovered that PAX8 antisense RNA 1 (PAX8-AS1) in combination with MYB-AS1 can serve as effective predictor of AML prognosis [19]. PAX8-AS1, an lncRNA located in the upstream region of the paired box 8 (PAX8) gene, modulates the expression of PAX8 gene [20], which is found to upregulate the Wilms' tumor gene 1 (WT1), an oncogene for AML [20, 21]. Previous data have also indicated that the polymorphisms of PAX8-AS1 are related to an increased risk of childhood AML [22]. These discoveries underlined that PAX8-AS1 may contribute to AML development and progression. In addition, since lncRNAs can modulate ADM resistance [13], which is critically related to the poor outcomes of AML patients [4], and PAX8-AS1 expression can reflect the poor prognosis of AML childhood, we hypothesized that PAX8-AS1 may be possibly implicated in ADM-resistant AML, thus impacting the prognosis of ADM-resistant patients. Accordingly, we investigated the specific molecules that might have an association with the poor prognosis of AML patients from the perspective of PAX8-AS1.

Furthermore, a well-known mechanism, through which lncRNAs can modulate cell biological behaviors, is the consequence of the process in which lncRNAs sponge microRNA (miRNA) to indirectly regulate gene expression [23]. Therein, miRNAs are those small noncoding RNAs, a great number of which are also found to be dysregulated along with their target genes in AML [24, 25]. Wang et al. have proposed that PAX8-AS1, whose overexpression leads to the development of gynecological cancers, may exert an oncogenic effect through constructing a PAX8-AS1-hsa-miR-4461-TNIK network in uterine corpus endometrial carcinoma (UCEC) [26]. Bioinformatic analyses conducted in the current study preliminarily predicted that miR-378g is a miRNA directly targeted by PAX8-AS1. A previous study has confirmed that miR-378g promotes the osteogenic differentiation of bone marrow mesenchymal stem cells after escaping from the inhibition caused by HOTAIR [27]. HOTAIR confers ADM resistance in AML [13]. Meanwhile, miR-378g has been also perceived as a suppressor in many types of cancers [28–30]. Taken together, we conjectured that miR-378g may participate in the ADM resistance of AML by inhibiting malignant progression.

This study seeks to propose a novel PAX8-AS1/miR-378g axis-induced lncRNA-miRNA-mRNA regulatory network and investigate the role of this network in the proliferation and apoptosis of ADM-resistant AML cells, so as to provide feasible therapeutic targets for refractory/recurrent AML.

2. Methods and Materials

2.1. Ethical Statement. The study has obtained ethic approval from the Ethics Committee of Zhejiang Provincial People's Hospital (approval number: 2021QT323). All the participants enrolled in our research agreed that their tissues would be used for clinical research, and signed the written informed consent.

2.2. Clinical Samples. Bone marrow samples were collected from chemo-sensitive AML patients ($n = 23$; male: 13, female: 10; 21~58 years old), refractory/recurrent AML patients ($n = 22$; male: 10, female: 12; 20~62 years old), and healthy volunteers ($n = 45$; male: 25, female: 20; 18~56 years old), all of whom were enrolled at Zhejiang Provincial People's Hospital in 2020. Inclusion criteria: the patients with refractory/recurrent AML were insensitive to chemotherapy and were pathologically confirmed according to the published criteria [31]. Exclusion criteria: patients with myelodysplastic syndrome, previously known as malignancy; and patients with hepatic and renal insufficiency. After aspiration, the bone marrow samples were instantly preserved at −80°C.

2.3. Cell Culture and Treatment. Human AML cell line HL60 and human doxorubicin (ADM)-resistant AML cell line HL60/ADM were obtained as gifts from the Institute of Hematology Affiliated with Chinese Academy of Medical Sciences (Tianjin, China). All the cells were cultured in Dulbecco's modified Eagle's medium (DMEM, A4192101, ThermoFisher, Waltham, Massachusetts, USA) blended with 10% bovine calf serum (BCS, F8687, Sigma–Aldrich, St. Louis, Missouri, USA) at 37°C with 5% CO_2.

ADM (D1515) was procured from Sigma–Aldrich (USA). HL60 or HL60/ADM cells were treated with DMEM containing ADM with gradually increasing concentrations

(0, 0.01, 0.03, 0.07, 0.15, 0.3, 0.6, 1.2 and 2.4 μg/mL) at 37°C with 5% CO_2 for 24 hours (h) before cell viability determination.

2.4. Cell Transfection. PAX8-AS1 overexpression plasmid was structured using pcDNA3.1 vector (V79520, ThermoFisher, USA). Small interfering RNA targeting PAX8-AS1 (si-PAX8-AS1, sense: 5′-AGTTAAACAAGTTCTTTTCGG-3′, antisense: 5′-GAAAAGAACTTGTTTAACTAA-3′) was synthesized by RIBOBIO (Guangzhou, China). MiR-378g mimic/inhibitor (miR10018937-1-5/miR20018937-1-5) and mimic/inhibitor control (miR1N0000001-1-5/miR2N0000001-1-5) were also purchased from RIBOBIO (China).

With the help of Lipofectamine 3000 transfection reagent (L3000015, ThermoFisher, USA), parental HL60 cells were transfected with PAX8-AS1 overexpression plasmid or miR-378g mimic alone or in combination, while HL60/ADM cells were transfected with si-PAX8-AS1 or miR-378g inhibitor alone or in combination. Specifically, HL60 or HL60/ADM cells (3×10^4) were seeded to achieve 90% confluence. Opti-MEM (31985062, ThermoFisher, USA) was used to dilute Lipofectamine 3000 transfection reagent, PAX8-AS1 overexpression plasmid, si-PAX8-AS1, and miR-378g mimic/inhibitor. P3000 reagent was added into gene solutions except for the diluted si-PAX8-AS1. Subsequently, the gene solutions were mixed with the diluted lipofectamine 3000 transfection reagent and incubated at room temperature for 10 minutes (min). Later, the incubated solution, which appeared as the gene-lipid complex, was incubated with the cells at 37°C for 48 h.

2.5. Cell Counting Kit-8 (CCK-8) Assay. CCK-8 reagent (20140419, Beyotime, Beijing, China) was employed to assess the sensitivity of transfected HL60 or HL60/ADM cells to ADM. HL60 and HL60/ADM cells, which were either transfected with or without PAX8-AS1 overexpression plasmid or si-PAX8-AS1 were diluted to 1×10^4 cells/mL and inoculated in 96-well plates (265300, ThermoFisher, USA). The cell solution was sequentially incubated at 37°C overnight to adhere to the wall and treated with ADM at the indicated concentrations for 24 h. CCK-8 reagent was diluted by DMEM at the ratio of 10 : 1. The HL60 or HL60/ADM cells in each well were added with 100 μL of the diluted CCK-8 reagent and incubated at 37°C for 2 h. A spectrophotometer (GENESYS 140/150, ThermoFisher, USA) was used to read the absorbance at 450 nm.

2.6. Annexin V-FITC and Propidium Iodide (PI) Staining. The apoptosis of HL60 or HL60/ADM cells was evaluated via Annexin V-FITC apoptosis detection kit (C1062S, Beyotime, China). Following the transfection as described above or the treatment with ADM at indicated concentration for 24 h, HL60 or HL60/ADM cells (5×10^5) were rinsed with phosphate buffer saline (PBS, P5493, Sigma–Aldrich, USA), detached using trypsin (T1426, Sigma–Aldrich, USA) and centrifuged at 1,000 × g for 5 min. Again, the HL60 or HL60/ADM cells were resuspended in PBS and centrifuged at 1,000 × g for 5 min. Subsequently, the HL60 or HL60/ADM cells were resuspended in 195 μL Annexin V-FITC solution, mixed with 5 μL Annexin V-FITC solution, and stained with 10 μL of PI, followed by incubation at 20°C for 20 min without light. CytoFLEX flow cytometer and CytExpert software (ver. 2.2.0.97), both of which were available from Beckman Coulter (Brea, CA, USA) were used to analyze and quantify cell apoptosis.

2.7. Bioinformatics Analyses. The Venn diagrams were adopted to screen out the targets of miR-378g from a range of mRNAs, which include AML-associated differentially expressed mRNAs obtained through the analysis of GPL19956 from GSE142700 in the GEO database and include potential miR-378g-targeted mRNAs predicted through Starbase and TargetScan. Then, Starbase (https://www.lncrnablog.com/tag/starbase-v2-0/) and TargetScan (https://www.targetscan.org/mamm_31/) were applied to perform the sequence alignment between PAX8-AS1 and miR-378g and between miR-378g and erb-b2 receptor tyrosine kinase 2 (ERBB2), respectively.

2.8. Dual-Luciferase Reporter Assay. The sequences of wild-type PAX8-AS1 (5′-CACGGGCCCAGCATCCGAGA-3′)/mutant PAX8-AS1 (5′-CACGGGACCAGCAGCCCAGA-3′) and sequences of wild-type ERBB2 (5′-CCTCCTCCTGCCTTCAGCCCAGC-3′)/mutant ERBB2 (5′-CCTCCTCCTGCCTTCATACCCGC-3′) were separately cloned onto pmirGLO vectors (pmirGLO, E1330, Promega, Madison, Wisconsin, USA) to construct the corresponding reporter plasmids. HL60 cells and HL60/ADM cells (3×10^4 cells/well in 96-well plates) were seeded to achieve 70% confluence and were cotransfected with the reporter plasmids and miR-378g mimic/inhibitor or mimic control/inhibitor control using Lipofectamine 3000 transfection reagent for 6 h.

48 hours after the cotransfection, the change of the luciferase activity of the HL60 cells was measured using the dual-luciferase reporter assay system (E1910, Promega, USA). Briefly, following the lysis by Lysis Buffer (16189, ThermoFisher, USA), HL60 cells were added with Luciferase Assay Reagent II and Stop & GLo Reagent to determine the reaction intensities of firefly luciferase and Renilla luciferase in the dark. The ratio of the two reaction intensities was calculated to indicate the expressions of the target genes.

2.9. Quantitative Reverse–Transcription Polymerase Chain Reaction (qRT-PCR). Bone marrow samples were homogenized by a homogenizer (TissueLyser-96, Thunder Sci, Shanghai, China). The total RNA and total miRNA from HL60 cells, HL60/ADM cells, and homogenate of bone marrow samples were extracted using TRIzol lysis buffer (15596018, ThermoFisher, USA) and RNAiso for Small RNA kits (9753Q, TaKaRa, Liaoning, China), respectively. The lysates of RNA or miRNA were added with 200 μL chloroform (48520-U, Sigma–Aldrich, USA) and centrifuged at 12,000 × g for 15 min at 4°C. 500 μL isopropanol (W292907,

Sigma–Aldrich, USA) was added into the upper water phase, followed by centrifugation at 12,000 × g for 10 min at 4°C. Then, the precipitate of RNA or miRNA was obtained and washed with 1 mL 75% ethanol (32205, Sigma–Aldrich, USA). After being centrifuged (10,000 × g) at 4°C for 5 min, the precipitate was dissolved in 50 μL nonRNase water (10977023, Sigma–Aldrich, USA). The purified RNA or miRNA was reversely transcribed into cDNA using RevertAid First Strand cDNA Synthesis Kits (K1621, ThermoFisher, USA). PCR was conducted on a real-time PCR machine (Applied Biosystems, Foster City, CA, USA) and TB Green Premix Ex Taq II (Tli RNaseH Plus) (RR820Q, TAKARA, China), with the indicated conditions as follows: activation (95°C for 10 min) and 40 cycles of denaturation (95°C for 15 seconds (s)), annealing (60°C for 30 s), and extension (60°C for 1 min). The sequences of primers used were listed in Table 1. The relative gene expressions were determined by the $2^{-\Delta\Delta CT}$ method. The experiment was carried out in triplicate.

2.10. Western Blot. The total proteins from HL60 and HL60/ADM cells with or without transfection were extracted using RIPA Buffer (89900, ThermoFisher, USA), following which the concentration of protein sample was quantitated by a BCA kit (A53227, ThermoFisher, USA). 30 μg protein and 4 μL marker (PR1910, Solarbio, Beijing, China) were separately loaded, electrophoresed using 12% SDS-PAGE gel (P0053A, Beyotime, China) for 1 h, and then transferred onto polyvinylidene difluoride (PVDF) membranes (P2438, Sigma–Aldrich, USA). The membranes were blocked by 5% skimmed milk in Tris-buffered saline with 1% Tween 20 (TBST, TA-125-TT, ThermoFisher, USA) at room temperature for 1 h, and incubated with the following primary antibodies (Abcam, Cambridge, MA, USA) at 4°C overnight, including those against Bcl-2 (ab59348, 26 kDa, 1 : 1000), Bax (ab32503, 21 kDa, 1 : 1000), C Caspase-3 (ab2302, 17 kDa, 1 : 500), ERBB2 (ab237715, 180 kDa, 1 : 1000) and housekeeping control GAPDH (ab8245, 36 kDa, 1 : 10000). Next, the membranes were washed with TBST and cultured with the secondary antibody goat antimouse IgG (A32723, 1 : 1000, ThermorFisher, USA) or goat antirabbit IgG (A32731, 1 : 1000, ThermorFisher, USA) at room temperature for 2 h. The proteins were detected with the help of an enhanced chemiluminescence reagent (WP20005, ThermoFisher, USA) on an imaging System (iBright CL1500A, ThermoFisher, USA). The grey values of the protein bands were quantified using ImageJ (v. 1.52s, National Institutes of Health, Bethesda, MA, USA).

2.11. Statistical Analysis. All the results were analyzed using SPSS (v. 12.0, SPSS, Chicago, Illinois, USA), and data from three independent trials were presented as mean ± standard deviation. Comparison of gene expression changes among healthy volunteers, refractory/recurrent patients, and chemo-sensitive patients was performed by independent t-test. Differences between two groups were evaluated by Student's t-test, and those among multiple groups were analyzed using One-Way ANOVA followed by Turkey's posthoc test. All the experiments were repeated in independent triplicate. Statistical significance was defined as two-sided $P < 0.05$.

3. Results

3.1. PAX8-AS1 Was Highly Expressed in Refractory/Recurrent AML Patients and ADM-Resistant AML Cells. Based on the analyses via qRT-PCR, it was evident that PAX8-AS1 expression level was pronouncedly upregulated in AML patients, in comparison with that in the healthy volunteers ($P < 0.001$, Figure 1(a)), and such upregulation was more notably in refractory/recurrent AML patients than in chemo-sensitive AML patients ($P < 0.001$, Figure 1(b)). Next, the CCK-8 assay was conducted to determine the sensitivity of AML cells to ADM. As depicted in Figure 1(b), the viability of ADM-resistant HL60 (HL60/ADM) cells was stronger than that of HL60 cells after the treatment of ADM at concentrations of 0.3, 0.6, 1.2, and 2.4 μg/mL ($P < 0.05$). Then, PAX8-AS1 expression level in HL60/ADM cells and HL60 cells was quantified via qRT-PCR. The relevant results suggested that PAX8-AS1 expression was upregulated in HL60/ADM cells compared to that in parental HL60 cells ($P < 0.001$, Figure 1(c)). These discoveries collectively demonstrated that the resistance to ADM is associated with a high expression level of PAX8-AS1.

3.2. PAX8-AS1 Regulated the Viability and Apoptosis of Parental AML Cells and ADM-Resistant AML Cells. PAX8-AS1 was overexpressed or silenced in HL60/ADM cells and HL60 cells via transfection. After being transfected with PAX8-AS1 overexpression plasmid, HL60 cells exhibited an increased expression level of PAX8-AS1 in comparison with NC-transfected HL60 cells ($P < 0.001$, Figure 2(a)), and si-PAX8-AS1 transfection induced a decreased expression level of PAX8-AS1 in HL60/ADM cells, relative to siNC transfection ($P < 0.001$, Figure 2(b)). Then, the CCK-8 assay indicated that PAX8-AS1 upregulation increased the viability of HL60 cells treated with ADM (0.6, 1.2, and 2.4 μg/mL) ($P < 0.001$, Figure 2(c)), while PAX8-AS1 downregulation decreased the viability of HL60/ADM cells treated with ADM (0.3, 0.6, 1.2 and 2.4 μg/mL) ($P < 0.01$. Figure 2(d)). Subsequently, the flow cytometry analysis proved that the apoptosis of HL60 cells was inhibited after the overexpression of PAX8-AS1, compared to that of NC-transfected cells ($P < 0.001$, Figures 2(e) and 2(f)), but the apoptosis of HL60/ADM cells was promoted by PAX8-AS1 silencing, compared to that of siNC-transfected cells ($P < 0.001$, Figures 2(g) and 2(h)). To further verify the relation between PAX8-AS1 expression and apoptosis, the expressions of apoptosis-related factors were analyzed by Western blot, the result of which revealed that PAX8-AS1 overexpression raised Bcl-2 protein expression while lowering the protein expressions of Bax and C Caspase-3 in HL60 cells ($P < 0.01$, Figures 3(a) and 3(b)). PAX8-AS1 silencing, however, dwindled Bcl-2 protein expression, but elevated protein expressions of Bax and C Caspase-3 in HL60/ADM cells ($P < 0.001$, Figures 3(c) and 3(d)). These discoveries reflected that the resistance of AML cells to ADM was attributed to increased viability and inhibited apoptosis caused by PAX8-AS1 overexpression.

Table 1: Primers used in a quantitative reverse transcription-polymerase chain reaction for related genes.

Gene	Species	Forward	Reverse
PAX8-AS1	Human	5′-GCTGCAGAGTCTTTGTTGGC-3′	5′-GGTTGGTGTCCGTGAAAAGC-3′
miR-378g	Human	5′-ACACTCCAGCTGGGGAAGACTGAGGTTC-3′	5′-CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGAGCCCAGT-3′
EPOR	Human	5′-TGGAGGACTTGGTGTGTTTCT-3′	5′-GCAACTCTAGGGGCACGAA-3′
CDC25B	Human	5′-ACGCACCTATCCCTGTCTC-3′	5′-CTGGAAGCGTCTGATGGCAA-3′
GNG12	Human	5′-AGCAAGCACCAACAATATAGCC-3′	5′-AGTAGGACATGAGGTCCGCT-3′
NOTCH2	Human	5′-CCTTCCACTGTGAGTGTCTGA-3′	5′-AGGTAGCATCATTCTGGCAGG-3′
ERBB2	Human	5′-TGCAGGGAAACCTGGAACTC-3′	5′-ACAGGGGTGGTATTGTTCAGC-3′
U6	Human	5′-CCTGCGCAAGGATGAC-3′	5′-GTGCAGGGTCCGAGGT-3′
GAPDH	Human	5′-GAAGGTCGGAGTCAACGGAT-3′	5′-CCTGGAAGATGGTGATGGG-3′

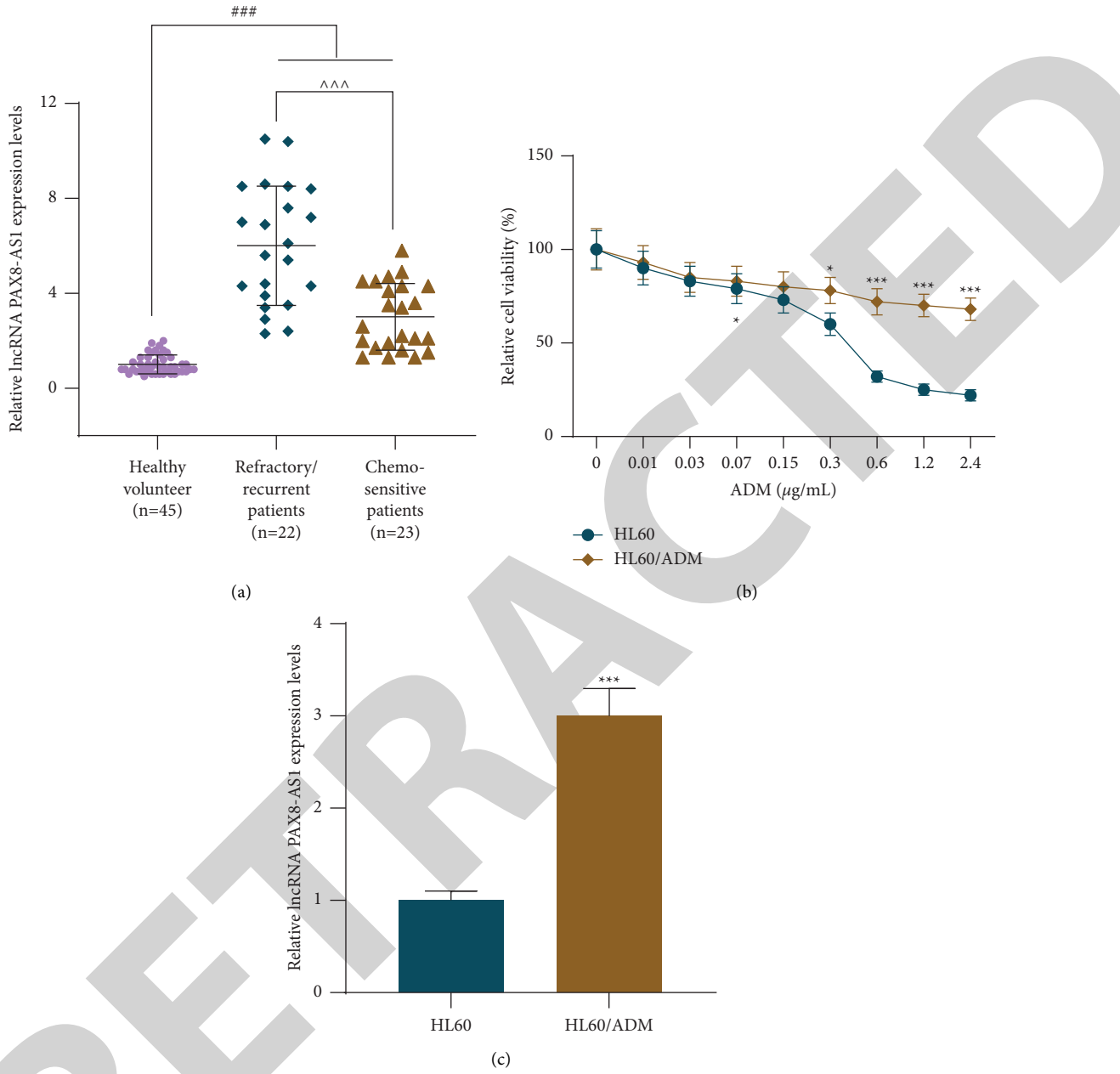


Figure 1: PAX8-AS1 expression was upregulated in refractory/recurrent AML patients and ADM-resistant AML cells. (a) PAX8-AS1 expression in bone marrow samples from refractory/recurrent AML patients, chemo-sensitive AML patients, and healthy volunteers analyzed by qRT-PCR. (b) The sensitivity of HL60 and HL60/ADM cells to ADM was assessed by CCK-8 assay. (c) PAX8-AS1 expression in HL60 and HL60/ADM cells was quantified by qRT-PCR. $^{*}P < 0.05$; $^{\#\#\#}P$ or $^{\wedge\wedge\wedge}P$, or $^{***}P < 0.001$; $^{\#}$vs. healthy volunteer; $^{\wedge}$ vs. refractory/recurrent patients; $*$ vs. HL60 (AML: acute myeloid leukemia; ADM: doxorubicin; qRT-PCR: quantitative reverse transcription-polymerase chain reaction; CCK-8: Cell counting kit-8; PAX8-AS1: PAX8 antisense RNA 1).

3.3. MiR-378g Was Directly Targeted by PAX8-AS1, and Lowly Expressed in Refractory/Recurrent AML Patients and ADM-Resistant AML Cells. Starbase-based analyses identified that PAX8-AS1 had binding sites complementary to miR-378g, which was additionally confirmed by dual-luciferase reporter assay (Figure 4(a)). It was shown that the transfection of miR-378g mimics suppressed the luciferase activity of HL60 cells containing wild-type PAX8-AS1, compared to transfection of mimic control ($P < 0.001$, Figure 4(b)), whereas the luciferase activity of wild-type PAX8-AS1 was enhanced in HL60/ADM cells after the transfection of miR-378g inhibitor ($P < 0.001$, Figure 4(c)). Next, miR-378g expression was analyzed in healthy volunteers, chemo-sensitive AML patients, and refractory/recurrent AML patients as well as in HL60 cells and HL60/ADM cells. The relevant analyses via qRT-PCR revealed that miR-378g expression was lower in AML patients than in healthy volunteers ($P < 0.001$, Figure 4(d)), and miR-378g

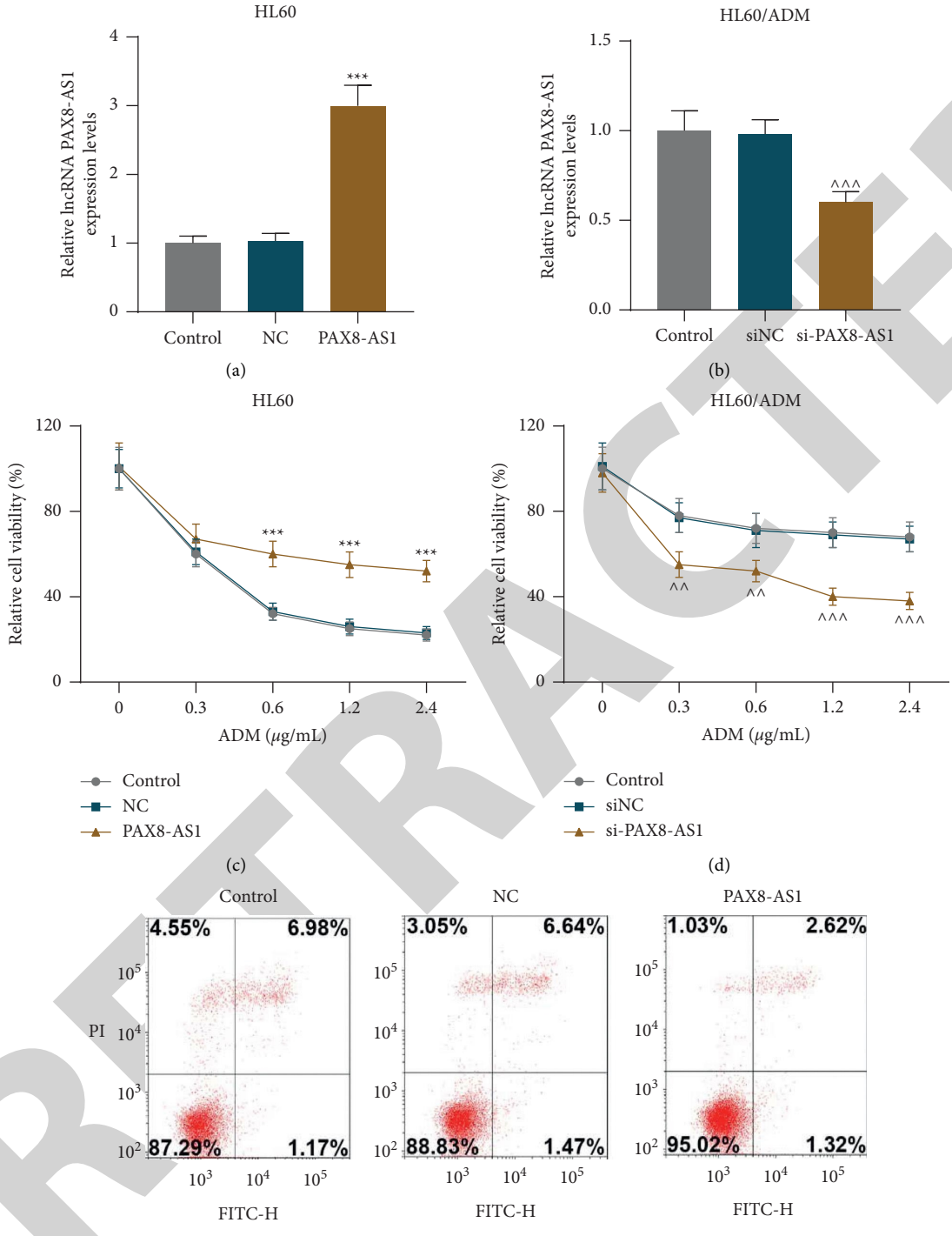


Figure 2: Continued.

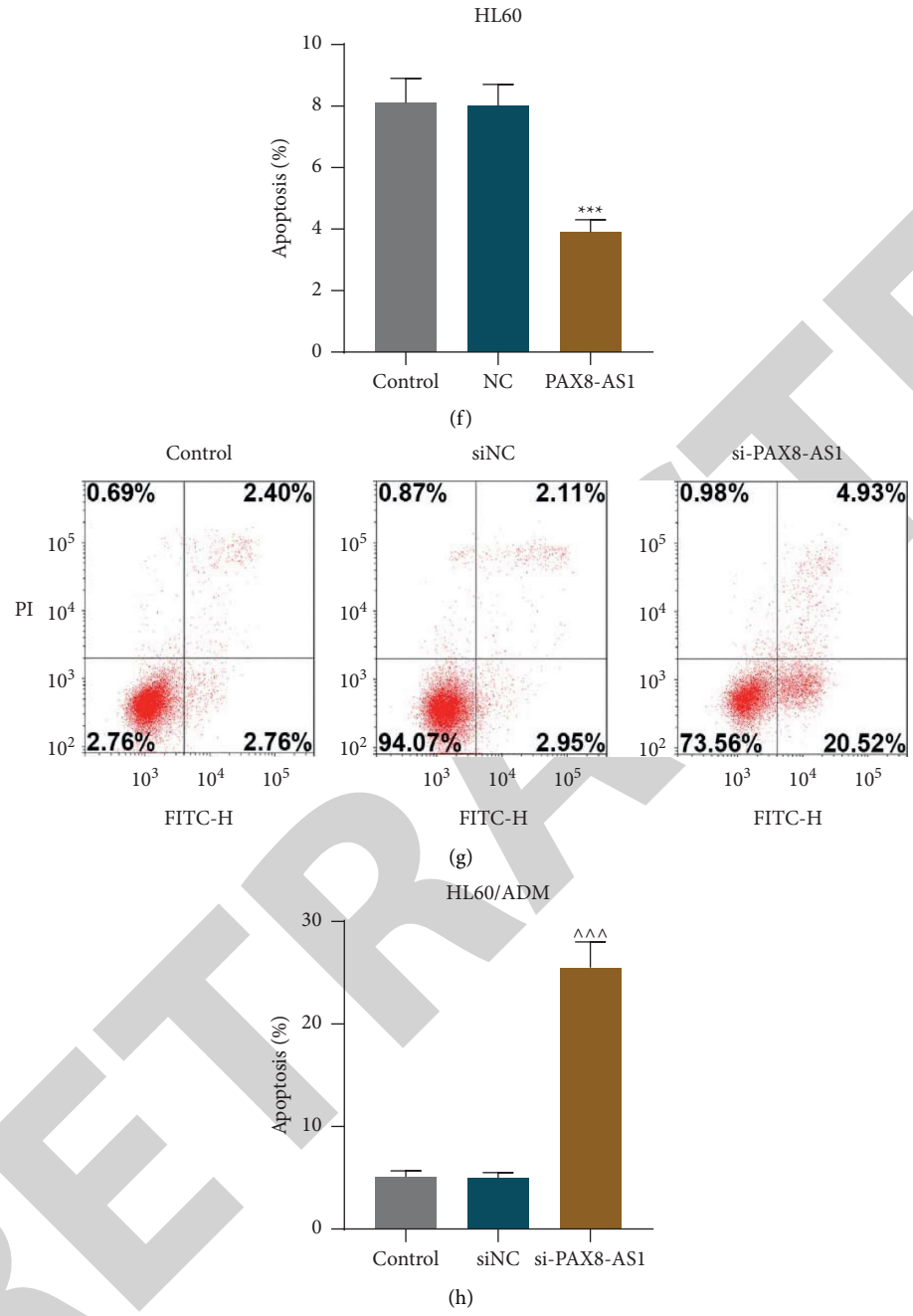


Figure 2: PAX8-AS1 regulated the viability and apoptosis of AML cells and ADM-resistant AML cells. (a). PAX8-AS1 expression in HL60 cells transfected with PAX8-AS1 overexpression plasmid was analyzed by qRT-PCR. (b). PAX8-AS1 expression in HL60/ADM cells transfected with si-PAX8-AS1 was evaluated by qRT-PCR. (c). The viability of HL60 cells transfected with PAX8-AS1 overexpression plasmid was measured by CCK-8 assay. (d). The viability of HL60/ADM cells transfected with si-PAX8-AS1 was detected by a CCK-8 assay. ((e) and (f)) The apoptosis of HL60 cells transfected with PAX8-AS1 overexpression plasmid was tested by flow cytometry. ((g) and (h)) The apoptosis of HL60/ADM cells transfected with si-PAX8-AS1 was measured by flow cytometry. $^{\wedge\wedge}P$ or $^{**}P < 0.01$; $^{\wedge\wedge\wedge}P$ or $^{***}P < 0.001$; $*$ vs. NC; $\wedge$ vs. siNC (AML: acute myeloid leukemia; ADM: doxorubicin; qRT-PCR: quantitative reverse transcription-polymerase chain reaction; NC: negative control; siNC: siRNA-negative control; si-PAX8-AS1: siRNA-PAX8-AS1; CCK-8: Cell counting kit-8; PAX8-AS1: PAX8 antisense RNA 1).

expression was much lower in refractory/recurrent AML patients and HL60/ADM cells than in chemo-sensitive AML patients and HL60 cells, respectively ($P < 0.001$, Figures 4(d) and 4(e)). These findings manifested that the resistance of AML patients and AML cells to ADM involves miR-378g downregulation caused by PAX8-AS1 upregulation.

3.4. PAX8-AS1 Regulated the Apoptosis of AML Cells and ADM-Resistant AML Cells through Interacting with miR-378g. As the abovementioned results confirmed a high PAX8-AS1 expression-related ADM resistance and demonstrated the direct interaction between PAX8-AS1 and miR-378g in AML. Subsequently, miR-378g mimic and

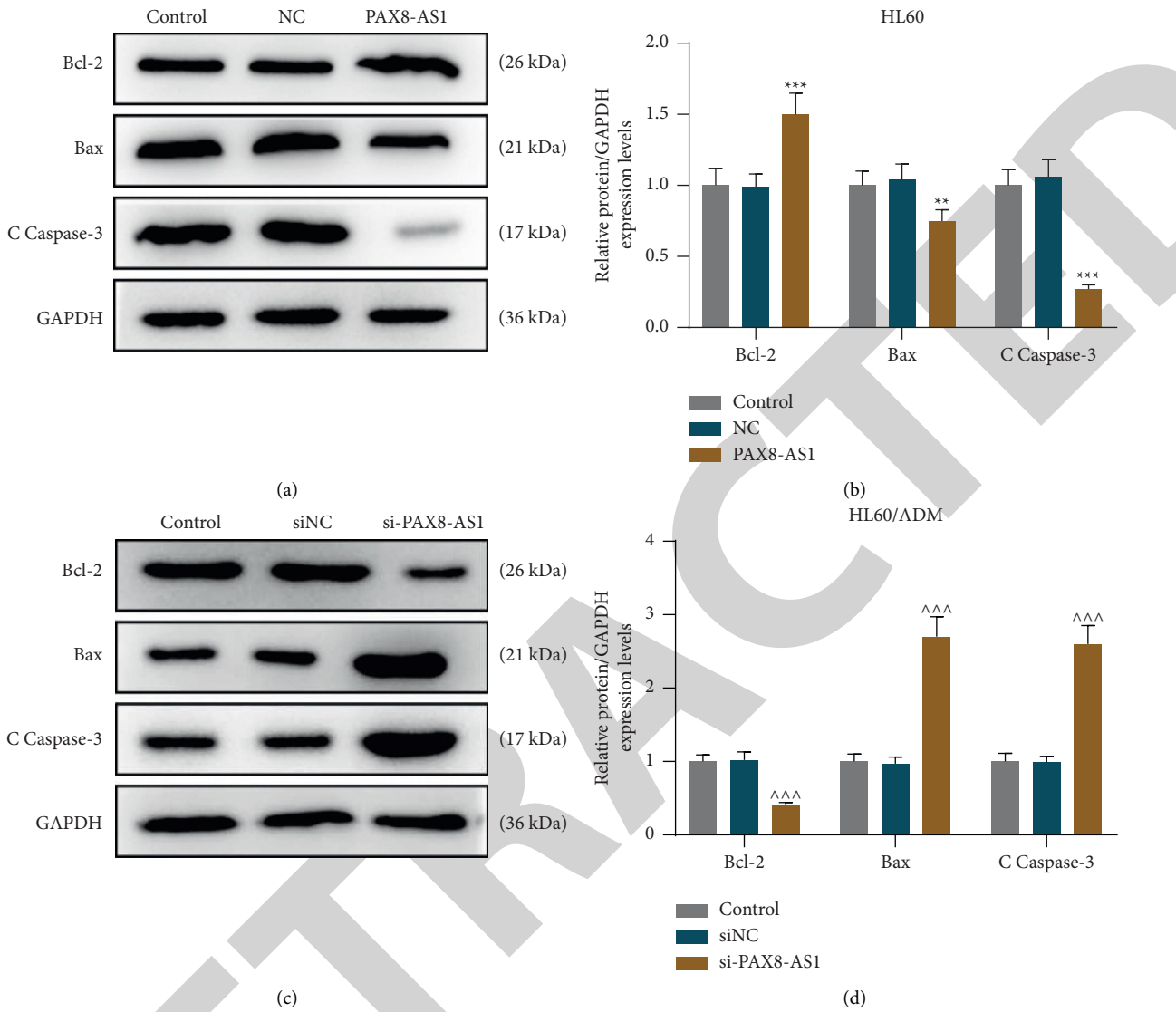
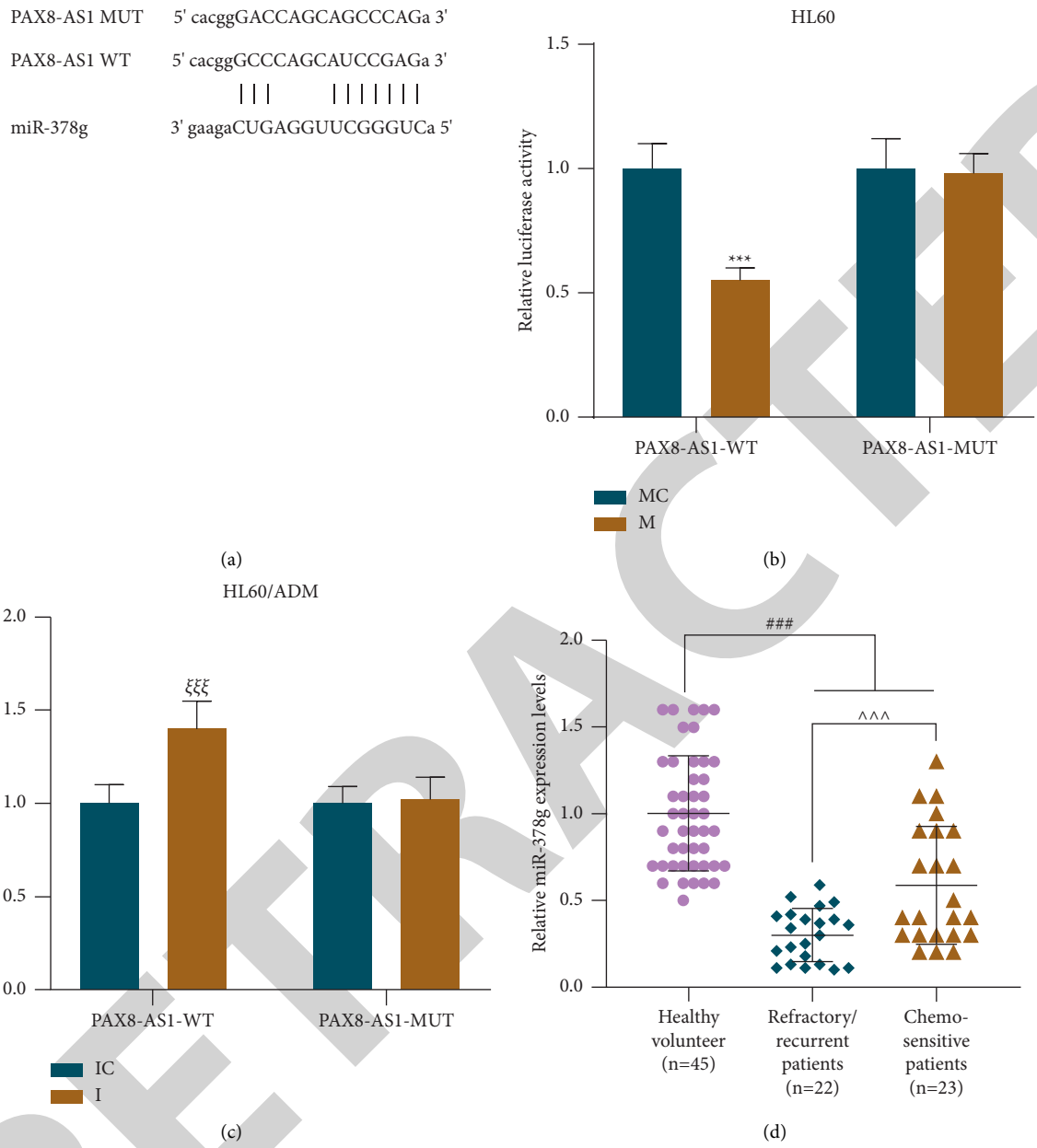


Figure 3: PAX8-AS1 regulated apoptosis-related marker expressions in AML cells and ADM-resistant AML cells. (a) and (b) The protein expressions of Bcl-2, Bax, and C Caspase-3 in HL60 cells transfected with PAX8-AS1 overexpression plasmid were analyzed by Western blot, with GAPDH serving as a reference gene. (c) and (D) The protein expressions of Bcl-2, Bax, and C Caspase-3 in HL60/ADM cells transfected with si-PAX8-AS1 were quantitated by Western blot, with GAPDH serving as a reference gene. $^{**}P < 0.01$; $^{\wedge\wedge\wedge}P$ or $^{***}P < 0.001$; $*$ vs. NC; $^{\wedge}$ vs. siNC (AML: acute myeloid leukemia; ADM: doxorubicin; NC: negative control; PAX8-AS1: PAX8 antisense RNA 1; siNC: siRNA-negative control; si-PAX8-AS1: siRNA-PAX8-AS1).

inhibitor were used to investigate the role of miR-378g in PAX8-AS1-mediated antiADM-resistant activity in AML cells. Through qRT-PCR analysis, we found that when compared to the NC + MC groups, the expression of miR-378g was decreased in PAX8-AS1 + MC group, while it was increased in the NC + M group ($P < 0.001$, Figure 5(a)). As expected, miR-378g expression was higher in PAX8-AS1 + M group than that in PAX8-AS1 + MC group, but it was lower than that in NC + M group ($P < 0.001$, Figure 5(a)). Besides, in HL60/ADM cells, siNC + I group displayed downregulation of miR-378g level but si-PAX8-AS1 + IC group displayed upregulation of miR-378g expression when compared to the siNC + IC groups ($P < 0.001$, Figure 5(b)), while miR-378g expression was lower in si-PAX8-AS1 + I group than that in si-PAX8-AS1+IC group, but it was higher than that in siNC + I group ($P < 0.001$, Figure 5(b)).

Moreover, the analysis of flow cytometry revealed that PAX8-AS1 overexpression significantly inhibited apoptosis of HL60 cells, but upregulation of miR-378g promoted the apoptosis and partially reversed the suppressive effect of PAX8-AS1 overexpression on the apoptosis of HL60 cells ($P < 0.001$, Figures 5(c) and 5(e)). Meanwhile, in HL60/ADM cells, silencing of PAX8-AS1 promoted the apoptosis of HL60/ADM cells, but downregulation of miR-378g inhibited the apoptosis and partially reversed the promotive effect of PAX8-AS1 silencing on the apoptosis of HL60/ADM cells ($P < 0.001$, Figures 5(d) and 5(f)).



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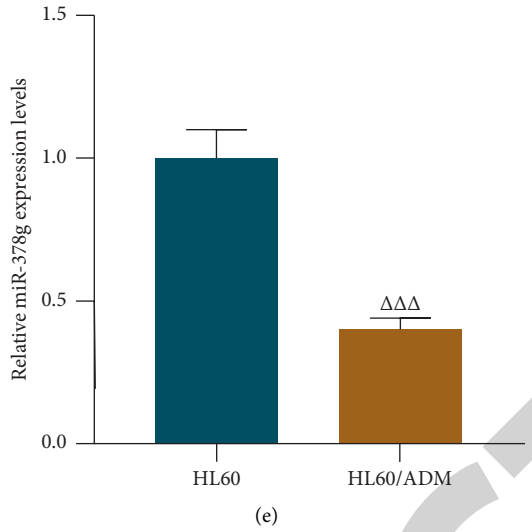
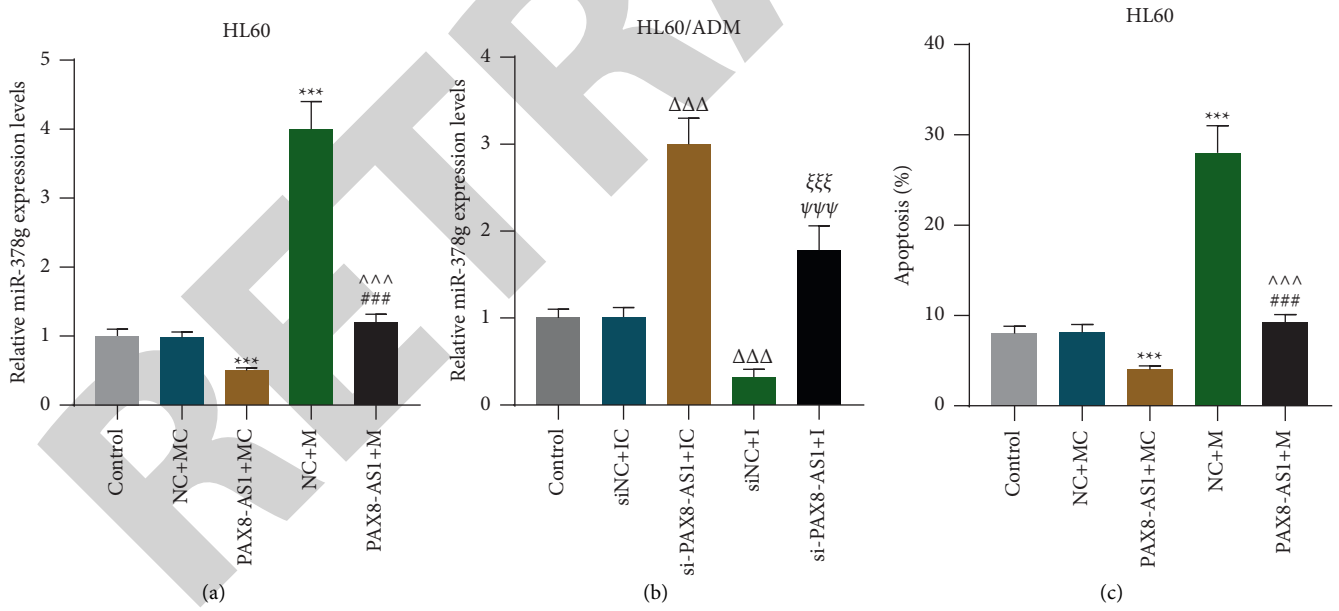


Figure 4: MiR-378g was directly targeted by PAX8-AS1, and lowly expressed in refractory/recurrent AML patients and ADM-resistant AML cells. (a) The sequence alignment between miR-378g and PAX8-AS1 was conducted using Starbase. (b) and (c) The interaction between miR-378g and PAX8-AS1 was validated by a dual-luciferase reporter assay. (d) MiR-378g expression in bone marrow samples from refractory/recurrent AML patients, chemo-sensitive AML patients, and healthy volunteers was analyzed by qRT-PCR. (e) MiR-378g expression in HL60 and HL60/ADM cells was analyzed by qRT-PCR. $^{***}P$ or $^{\xi\xi\xi}P$ or $^{\#\#\#}P$ or $^{\wedge\wedge\wedge}P < 0.001$; * vs. MC; $^{\xi}$ vs. IC; $^{\Delta}$ vs. HL60; $^{\#}$ vs. healthy volunteer; $^{\wedge}$ vs. refractory/recurrent patients (AML: acute myeloid leukemia; ADM: doxorubicin; WT: wild-type; MUT: mutant type; qRT-PCR: quantitative reverse transcription-polymerase chain reaction; MC: miR-378g mimic; M: mimic control; IC: miR-378g inhibitor; I: inhibitor control; PAX8-AS1: PAX8 antisense RNA 1).



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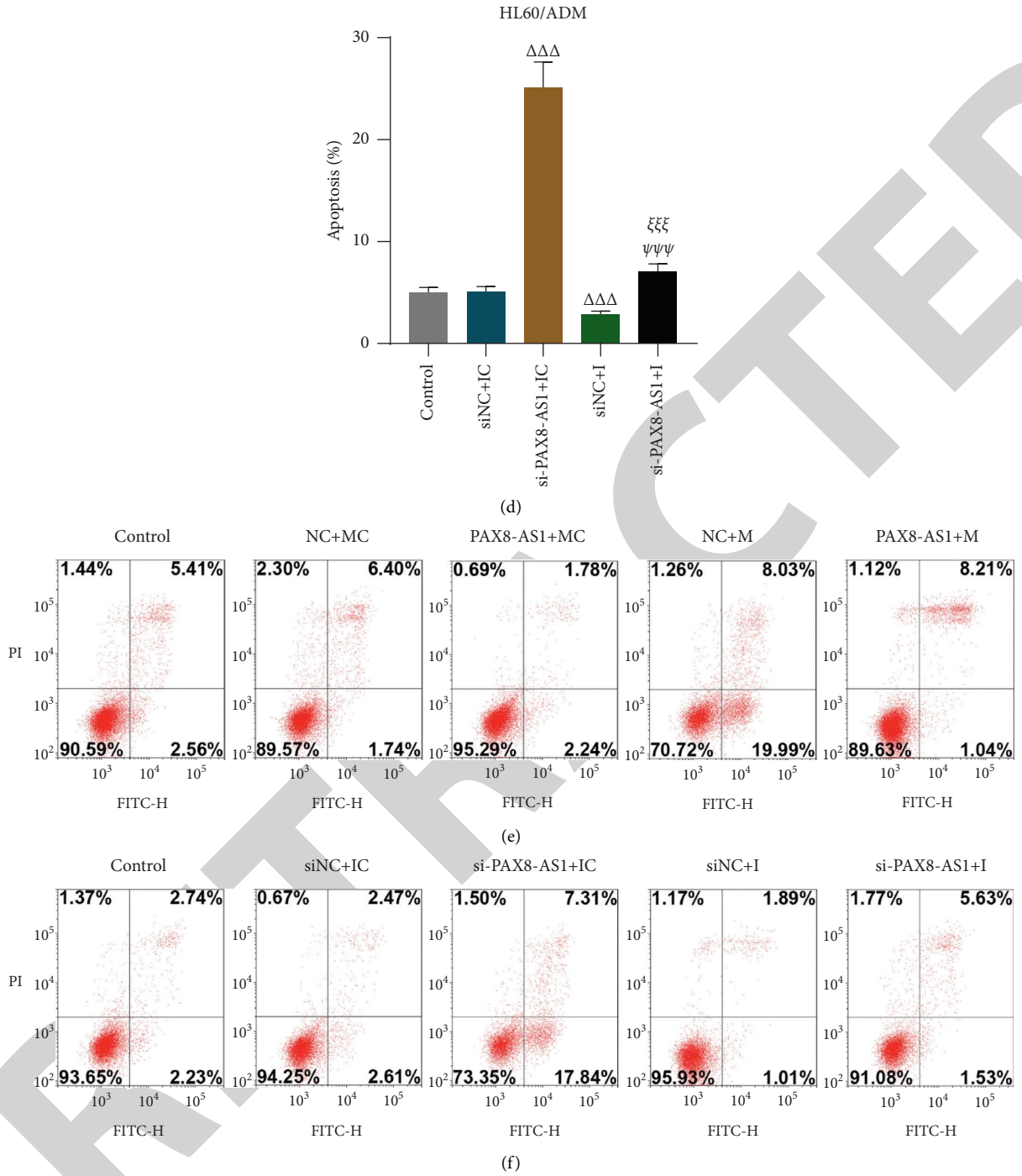


Figure 5: PAX8-AS1 modulated the apoptosis of AML cells and ADM-resistant AML cells by interacting with miR-378g. (a). MiR-378g expression in HL60 cells transfected with PAX8-AS1 overexpression plasmid or miR-378g mimic alone or in combination was analyzed by qRT-PCR. (b). MiR-378g expression in HL60/ADM cells transfected with si-PAX8-AS1 or miR-378g inhibitor alone or in combination was quantified by qRT-PCR. (c) and (e) The apoptosis of HL60 cells transfected with PAX8-AS1 overexpression plasmid or miR-378g mimic alone or in combination was evaluated by flow cytometry. (d) and (f) The apoptosis of HL60/ADM cells transfected with si-PAX8-AS1 or miR-378g inhibitor alone or in combination was measured by flow cytometry. $^{***}P$ or $^{\wedge\wedge\wedge}P$ or $^{\#\#\#}P$ or $^{\Delta\Delta\Delta}P$ or $^{\xi\xi\xi}P$ or $^{\Psi\Psi\Psi}P < 0.001$; $*$ vs. NC + MC; $\wedge$ vs. NC + M; $^{\#}$ vs. PAX8-AS1+MC; $^{\Delta}$ vs. siNC + IC; $^{\xi}$ vs. siNC + I; $^{\Psi}$ vs. si-PAX8-AS1+IC (AML: acute myeloid leukemia; ADM: doxorubicin; qRT-PCR: quantitative reverse transcription-polymerase chain reaction; NC: negative control; siNC: siRNA-negative control; PAX8-AS1: PAX8 antisense RNA 1; si-PAX8-AS1: siRNA-PAX8-AS1; M: miR-378g mimic; MC: mimic control; I: miR-378g inhibitor; IC: inhibitor control).

Furthermore, the expression changes of apoptosis-related factors were analyzed in response to miR-378g mimic/inhibitor. Western blot analyses demonstrated that in contrast to NC + MC group, NC + M group exhibited downregulation of Bcl-2 protein expression, and upregulation of Bax and C Caspase-3 protein expressions, PAX8-AS1+MC group exhibited the upregulated Bcl-2 protein expression yet the downregulated Bax and C Caspase-3 protein expressions ($P < 0.001$, Figure 6(a)). However, Bcl-2 protein expression was elevated yet Bax and C Caspase-3 protein expressions dwindled in PAX8-AS1 + M group, as compared to those in the NC + M group ($P < 0.001$, Figure 6(a)). In addition, in HL60/ADM cells, the protein expression level of Bcl-2 was augmented and those of Bax and C Caspase-3 were lessened in siNC + I groups, relative to those in the siNC + IC group ($P < 0.001$, Figure 6(b)). The protein expression level of Bcl-2 was decreased and those of Bax and C Caspase-3 were increased in si-PAX8-AS1 + IC groups, relative to those in the siNC + IC group ($P < 0.001$, Figure 6(b)). Bcl-2 protein expression was lowered but Bax and C Caspase-3 expressions were augmented in si-PAX8-AS1 + I group, when compared to those in the siNC + I group ($P < 0.001$, Figure 6(b)). These discoveries mirrored that the resistance of AML cells to ADM might be attributed to the inhibited apoptosis caused by PAX8-AS1 overexpression-induced miR-378g downregulation.

3.5. ERBB2 Was Directly Targeted by miR-378g and Highly Expressed in ADM-Resistant AML Cells. AML-associated differentially expressed mRNAs were obtained based on the analysis of the GPL19956 dataset from the GSE142700 database in the GEO, which was followed by the selection of mRNAs up-regulated with a fold change absolute value greater than 1. These selected mRNAs are defined as set 1 (Figure 7(a)). Meanwhile, Starbase and TargetScan were used to predict potential miR-378g-targeted mRNAs, which are defined as set 2 and 3. The intersection of the three sets presented fourteen overlapping mRNAs, among which the mRNAs with the five highest scores in TargetScan were selected as subjects (EPOR, CDC25B, GNG12, NOTCH2 and ERBB2) for qRT-PCR analysis (Figure 7(a)). The results of qRT-PCR unveiled that ERBB2 expression was downregulated (or upregulated) the most among the above-mentioned five mRNAs after HL60 cells (or HL60/ADM cells) were transfected with miR-378g mimic (or inhibitor) ($P < 0.001$, Figures 7(b) and 7(c)). Later, the binding sites complementary to ERBB2 on miR-378g were shown through TargetScan-based analysis (Figure 7(d)). Furthermore, validation via dual-luciferase reporter assay displayed that the luciferase activity of HL60 cells containing wild-type ERBB2 was suppressed by transfection with miR-378g mimic ($P < 0.001$, Figure 7(e)), and the luciferase activity of HL60/AMD cells containing wild-type ERBB2 was promoted by the transfection of miR-378g inhibitor ($P < 0.001$, Figure 7(f)). Next, ERBB2 expression was analyzed in HL60 cells and HL60/ADM cells. QRT-PCR and Western bot analyses both revealed that HL60/ADM cells exhibited a higher ERBB2 level than HL60 cells ($P < 0.001$, Figures 7(g) and 7(h)). These data corroborated that the resistance of AML cells to ADM involves activation of the PAX8-AS1-miR-378g-ERBB2 regulatory network.

4. Discussion

Poor response to intensive chemotherapy renders AML patients with a particularly gloomy outlook [32]. This poor response is strongly developed mainly due to ABC-induced drug efflux [2] in secondary AML which harbors characteristics such as upregulation of antiapoptotic proteins and MDR proteins [33–36]. Resistance to ADM, which arises from upregulation of the ABC superfamily proteins, results in less accumulation of ADM in AML cells [12], thereby impeding anticancer activities in AML. Therefore, the identification of novel therapeutic targets for ADM resistance is of great importance to decrease chemoresistance and recurrence rate. Accumulating pieces of evidence have documented that lncRNAs are key players in cellular processes including apoptosis through interacting with miRNA/mRNA axis in AML [37, 38]. A prior study has revealed that these lncRNA-mediated regulatory networks are correlated with the development of drug resistance in AML [39]. Our study discovered that lncRNA-PAX8-AS1 participated in the ADM resistance of AML cells via the miR-378g/ERBB2 axis by regulating cell viability and apoptosis.

The expression profile screening and bioinformatics analysis have identified dysregulated lncRNAs, some of which can be used to predict clinical outcomes, exist in ADM-resistant cells [40], and strengthen the relation between lncRNAs and ADM resistance in AML. ADM resistance indicates a poor prognosis of refractory/recurrent AML patients [10, 41], and can be aggravated by AML-associated oncogenic genes, such as HOTAIR [13, 42]. PAX8-AS1 regulates PAX8 [20], which is a physiological regulator that causes upregulation of certain oncogenic genes in AML [43]. PAX8-AS1, which has been previously found to be highly expressed in UCEC, plays an oncogenic role in gynecological cancers [26]. Similar to the expression of PAX8-AS1 in UCEC, PAX8-AS1 was discovered in this study to be higher expressed in AML patients compared to that in healthy volunteers, signifying that PAX8-AS1 may function as an oncogene in AML. Meanwhile, PAX8-AS1 overexpression is related to the poor recurrence-free survival (RFS) in UCEC [26] and thyroid cancer [44], suggesting that PAX8-AS1 contributes to disease recurrence in these cancers. Similarly, PAX8-AS1 is an lncRNA whose polymorphisms are risk factors for childhood AML [22]. Based on the findings above, we surmised that PAX8-AS1 exerts an oncogenic effect and confers ADM resistance in AML.

In our study, PAX8-AS1 expression was detected to be higher in AML patients. Based on further comparison, we found that the upregulation level of PAX8-AS1 was more pronounced in refractory/recurrent AML patients than in chemo-sensitive AML patients. Following that, the *in vitro* experiment was conducted with HL60/ADM cells. We found that HL60/ADM cells displayed stronger viability after ADM treatment, which was in accord with the differences between the performances of ADM-resistant THP-1(THP-1/ADM)

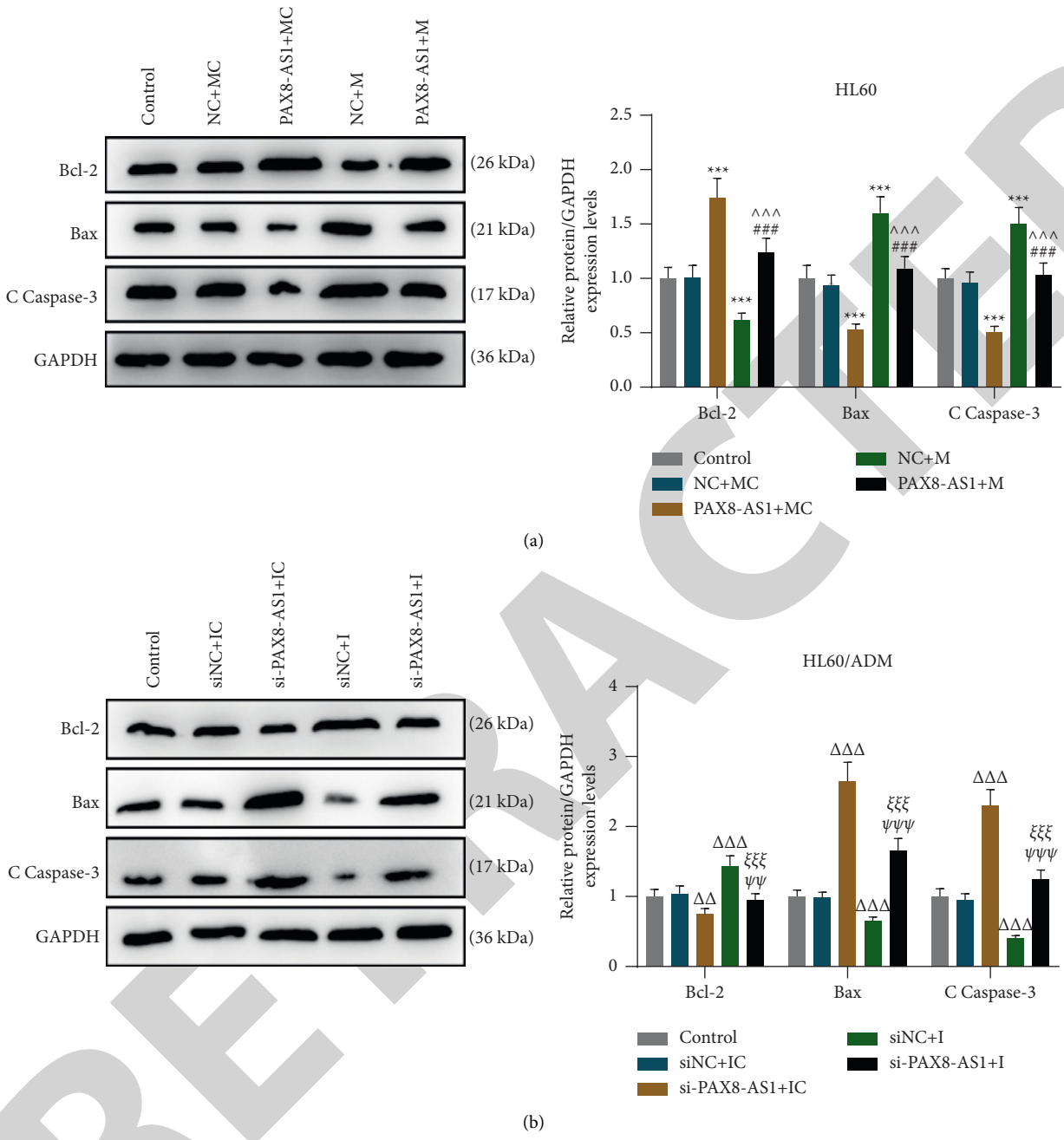
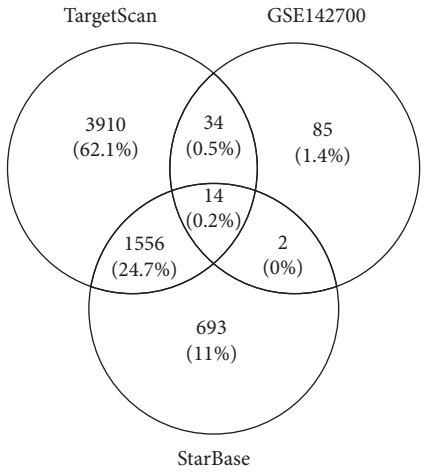


Figure 6: PAX8-AS1 regulated apoptosis-related marker expressions in AML cells and ADM-resistant AML cells through interacting with miR-378g. (a) The protein expressions of Bcl-2, Bax, and C Caspase-3 in HL60 cells transfected with PAX8-AS1 overexpression plasmid or miR-378g mimic alone or in combination were analyzed by Western blot, with GAPDH serving as a reference gene. (b) The protein expressions of Bcl-2, Bax, and C Caspase-3 in HL60/ADM cells transfected with si-PAX8-AS1 or miR-378g inhibitor alone or in combination were quantitated by Western blot, with GAPDH serving as a reference gene. $^{***}P$ or $^{\wedge\wedge\wedge}P$ or $^{\#\#\#}P$ or $^{\Delta\Delta\Delta}P$ $^{\xi\xi\xi}P$ or $^{\Psi\Psi\Psi}P < 0.001$; * vs. NC + MC; $^{\wedge}$ vs. NC + M; $^{\#}$ vs. PAX8-AS1+MC; $^{\Delta}$ vs. siNC + IC; $^{\xi}$ vs. siNC + I; $^{\Psi}$ vs. si-PAX8-AS1+IC (AML: acute myeloid leukemia; ADM: doxorubicin; NC: negative control; siNC: siRNA-negative control; PAX8-AS1: PAX8 antisense RNA 1; si-PAX8-AS1: siRNA-PAX8-AS1; M: miR-378g mimic; MC: mimic control; I: miR-378g inhibitor; and IC: inhibitor control).

cells and THP-1 cells subsequent to the ADM treatment [45]. This discovery indicates that HL60/ADM cells are qualified to establish an *in vitro* drug-resistant AML model. Moreover, as compared to HL60 cells, HL60/ADM cells exhibited highly expressed PAX8-AS1, suggesting a positive relationship between high PAX8-AS1 expression and ADM resistance to AML cells. Yu's study has disclosed that PAX8-AS1 activation reduces cell viability in breast cancer [46]. Contrary to the result caused by PAX8-AS1 activation in Yu's study, our study showed that overexpressed PAX8-AS1 boosted the viability of ADM-treated HL60 cells. More importantly, we detected that PAX8-AS1 silencing led to



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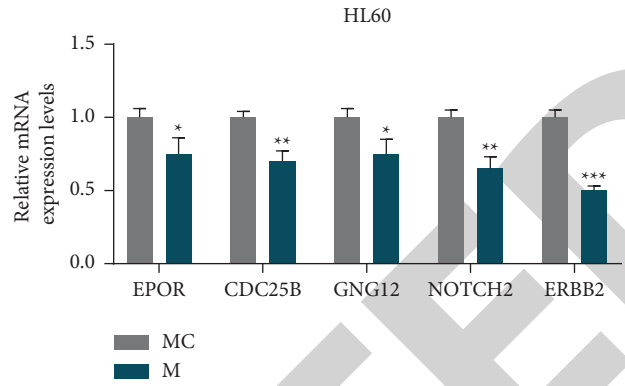

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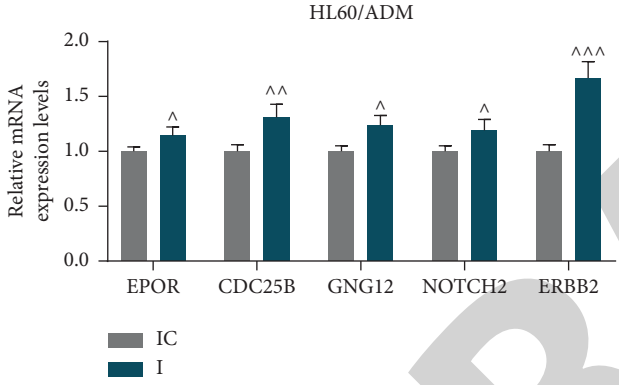

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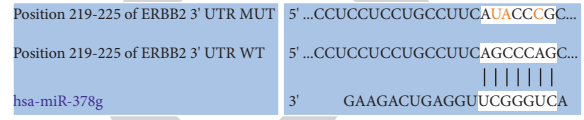

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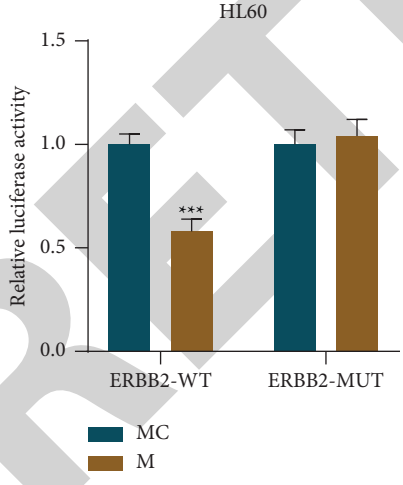
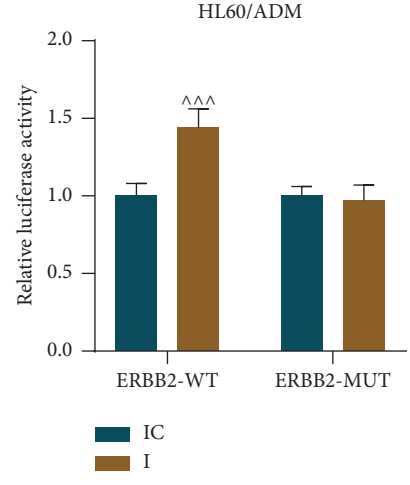


(e)



(f)

Figure 7: Continued.

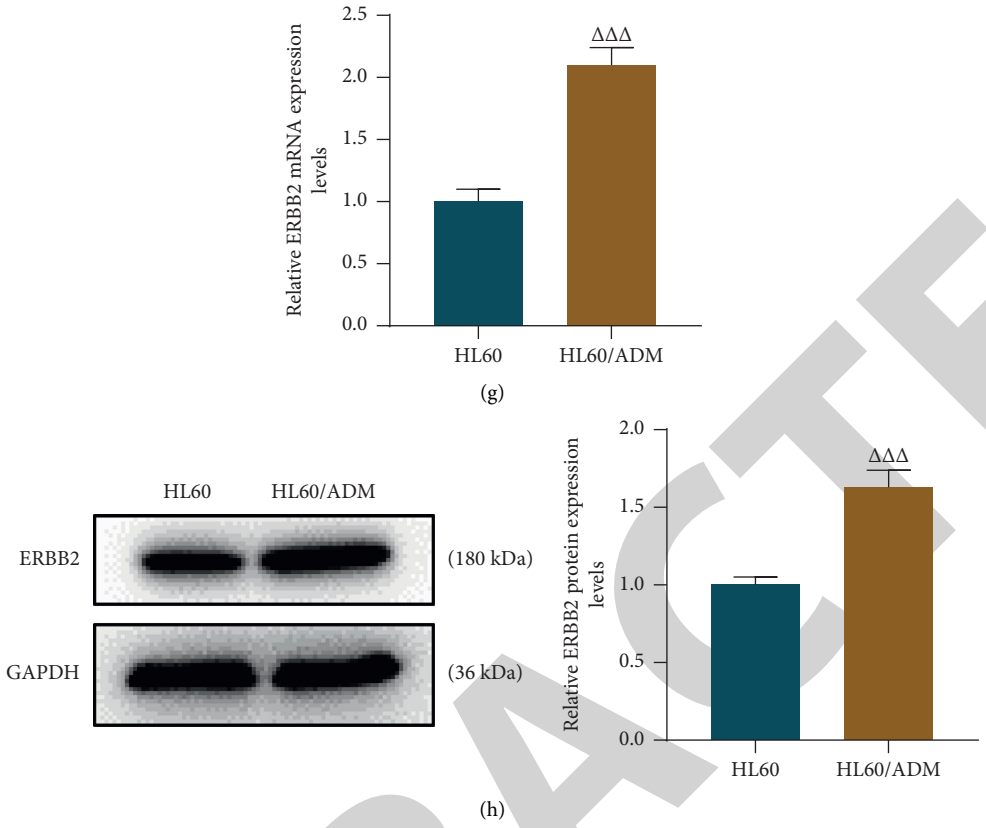


Figure 7: ERBB2 was directly targeted by miR-378g and highly expressed in ADM-resistant AML cells. (a). Venn diagram-based analyses of miR-378g-targeted mRNAs which were predicted through GPL19956 from GSE142700 in GEO predicted from Starbase, and predicted from TargetScan (b) and (c) The expressions of potential miR-378g-targeted mRNAs (EPOR, CDC25B, GNG12, NOTCH2, and ERBB2) in HL60 and HL60/ADM cells were analyzed by qRT-PCR. (d) The sequence alignment between miR-378g and ERBB2 was conducted using TargetScan. (e) and (f) The interaction between miR-378g and ERBB2 was validated by a dual-luciferase reporter assay. (g) and (h). ERBB2 expression in HL60 and HL60/ADM cells was analyzed by qRT-PCR (g), and by Western blot (h), with GAPDH serving as a reference gene. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P$ or $^{\wedge\wedge\wedge}P$; or $^{\Delta\Delta\Delta}P < 0.001$; * vs. MC; $^{\wedge}$ vs. IC; $^{\Delta}$ vs. HL60 (AML: acute myeloid leukemia; ADM: doxorubicin; WT: wild type; MUT: mutant type; qRT-PCR: quantitative reverse transcription-polymerase chain reaction; M: miR-378g mimic; MC: mimic control; I: miR-378g inhibitor; IC: inhibitor control).

decreased viability of HL60/ADM cells. Our findings demonstrated that PAX8-AS1 positively regulates AML cell viability to promote ADM resistance in AML.

ADM resistance mainly causes apoptosis failure in cytostatic treatment of haemoblastosis, leading to chemoresistance in AML [47]. Zhou's study recorded that PAX8-AS1 positively correlates with the apoptosis of papillary thyroid carcinoma cells [48]. In some way, our results contradict Zhou's finding by demonstrating that PAX8-AS1 overexpression inhibited apoptosis of HL60 cells. Also, we discovered that PAX8-AS1 silencing enhanced apoptosis of HL60/ADM cells. Collectively, our results indicated that PAX8-AS1 expression negatively regulated apoptosis to induce ADM resistance in AML. Meanwhile, chemoresistance-associated apoptosis inhibition is driven by upregulated level of antiapoptotic protein, Bcl-2 [49, 50]. Bcl-2 upregulation impedes the eradication of AML cells during ADM treatment [51]. Besides, Bax activation is also an initial step in apoptosis induction [52], and is found to be released to trigger apoptosis induced by the synergy of ADM plus panobinostat in acute leukemia cells [53]. Upregulation of C Caspase-3, which is essential to initiate and execute the apoptotic process [54], is detected to attenuate ADM resistance in HL60 cells [55]. In our study, the inhibited apoptosis caused by PAX8-AS1 overexpression in HL60 cells was accompanied by the higher expression of Bcl-2 and the lower expressions of Bax and C Caspase-3, and the promoted apoptosis resulted from PAX8-AS1 silencing in HL60/ADM cells was concurrent with the downregulation of Bcl-2 levels and upregulation of Bax and C Caspase-3, which indicated that PAX8-AS1 could affect the result of AML patients through apoptotic mechanism-mediated drug resistance.

LncRNAs can impact the drug resistance-related biological processes of AML cells through modulating miRNAs [39]. In our study, we predicted that miR-378g could be a target of PAX8-AS1, which was later validated via dual-luciferase reporter assay. Existing research has revealed that miR-378g is lowly expressed in various kinds of cancers, such as ovarian cancer [30], oral squamous cell carcinoma [29], colon cancer [56], and nasopharyngeal carcinoma (NPC) [57]. Furthermore, miR-378g is discovered to be related to the activation of apoptosis-related signaling

pathways in stage II colon cancer [56], and enhanced radiosensitivity of NPC cells [57]. However, the impact of miR-378g on AML remained unknown. Our current study unraveled that miR-378g expression was downregulated in AML patients, and this downregulation was more evident in refractory/recurrent AML patients and HL/60/ADM cells than in chemo-sensitive AML patients and parental HL/60 cells, respectively. Besides, consistent with the proapoptotic role of miR-378g revealed in a previous cancer study [57], our study found that miR-378g was positively related to apoptosis as well as apoptosis-related protein expression changes in both parental HL/60 cells and HL/60/ADM cells, and counteracted the inhibiting effect of PAX8-AS1 on apoptosis of these cells.

Furthermore, to figure out the PAX8-AS1-induced lncRNA-miRNA-mRNA regulatory network in drug-resistant AML, three databases including GEO, Starbase, and TargetScan were utilized to predict the target(s) of miR-378g. After validation via dual-luciferase reporter assay, our study proved that ERBB2 was directly targeted by miR-378g. Mutations of ERBB2 as an oncogene are an event with a high incidence rate in numerous tumor types such as the bladder (9.4%), small bowel (7.1%), ampullar (6.5%), and skin nonmelanoma (6.1%) [58]. During chemotherapy against AML, mubritinib, an ERBB2 inhibitor, fulfills a potent antileukemic effect [59]. Our study uncovered a significantly higher ERBB2 expression level in HL/60/ADM cells than in parental HL/60 cells, implicating that inhibiting ERBB2 can be a valid approach to antagonize ADM resistance in AML. However, resistance to mubritinib is still developed in those AML patients bearing highly expressed homeodomain-containing transcription factor HOXA9 and other HOX-network genes [59]. Therefore, our study suggested that ERBB2 inhibition resulting from the binding of ERBB2 to miR-378g may alleviate refractory/recurrent AML in patients without highly expressed HOX-network genes. Furthermore, the study should put more effort to define the effective range within which ERBB2 inhibition can generate an antileukemic effect and find solutions to drug resistance caused by aberrant HOX-network gene expressions in AML.

In conclusion, this study discovers the upregulated PAX8-AS1 and the downregulated miR-378g in both *in vivo* and *in vitro* samples of ADM-resistant AML when compared to those in chemo-sensitive AML. Besides, this study also demonstrates that PAX8-AS1 expression is negatively associated with cell apoptosis but positively associated with viability in ADM-resistant AML cells via targeting the miR-378g/ERBB2 axis. Collectively, our current study provides a potential regulatory network-based target for antagonizing chemoresistance in AML.

Data Availability

The analyzed data sets generated during the study are available from the corresponding author on reasonable request.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

Authors' Contributions

Xiaolu Song and Yirui Chen contributed equally to this work.

Acknowledgments

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Review Article

A Systematic Review and Meta-Analysis of the Effectiveness of High-Intensity Interval Training in People with Cardiovascular Disease at Improving Depression and Anxiety

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Background. To assess the effects of high-intensity interval training (HIIT) on depression and anxiety symptom in people with cardiovascular diseases (CVDs) compared with usual care (UC) and traditional aerobic continuous training (CT). *Methods*. Randomized controlled trials (RCTs) that investigated the effectiveness of HIIT on depression and/or anxiety outcomes before and after treatment in people with CVDs were included. A systematic search of database containing PubMed, Web of Science, Cochrane Central Register of Controlled Trials (CENTRAL), EMBASE, SPORTSDiscus, and CINAHL (EBSCOhost) was performed up to December 2021. The analyses of study characteristics, heterogeneity, and forest plot in analyses analogous were conducted via the pooled standardized mean difference (SMD) in random- or fixed-effect models as the measure of effectiveness. *Results*. Twelve independent studies (515 participants) were included. One study was rated as low quality, and four studies were evaluated as high quality. The other studies were rated as moderate quality. Visual interpretation of funnel plots and Egger test indicated no evidence of publication bias. There was a statistically significant reduction in the severity of depression (12 studies, SMD = −0.42 [Random], 95% CI, −0.69 to −0.16, $p = 0.002$, $I^2 = 52\%$) rather than that of anxiety symptoms (8 studies, SMD = −0.14 [Fixed], 95% CI, −0.35 to 0.06, $p = 0.18$, $I^2 = 0\%$) following HIIT compared with UC and CT control groups. Subgroup analysis revealed that high-intensity treadmill training significantly improved ($p = 0.01$) the depression symptom instead of training with a cycle ergometer ($p = 0.07$) and strength training ($p = 0.40$). *Conclusions*. High-intensity interval treadmill training can significantly improve symptoms of depression rather than anxiety in cardiovascular patients compared to usual care and conventional aerobic continuous training.

1. Introduction

Patients with cardiovascular diseases (CVDs) are at high risk of mental disorders such as depression and anxiety. Depression is a condition of general emotional dejection and withdrawal that can affect a person's thoughts, behavior, feelings, and sense of well-being. Anxiety often cooccurs with depression in children and adolescents [1]. Depression and anxiety are the two most common mental disorders, often referred internalizing disorders, which seriously contribute to the global health burden [2]. 31–45% of patients with CVDs suffer from clinically significant symptoms of depression, including those with stable coronary artery disease (CAD), chronic heart failure (HF), unstable angina, or myocardial infarction (MI) [3]. A large number of epidemiological studies have confirmed that depression and anxiety significantly affect the course of disease, clinical manifestations, and recurrence of vascular events in patients with CVDs [4, 5].

Antidepressants such as selective serotonin reuptake inhibitors (SSRIs) remain the most common treatment choice and are considered the first-line option. Unfortunately, there are one-third patients with depression remaining drug-resistant and less than one-third achieving remission after initial treatment with antidepressant [6]. Moreover, patients taking antidepressants often suffer from many side effects, including cardiovascular events, gastrointestinal disorders, falls, epilepsy, and increased risk of all-

cause mortality [7, 8]. Accumulating evidence demonstrates that aerobic exercise contributes to establishing recovery and preventing relapse of depression symptoms [9]. However, people with depression engage in low levels of physical activity and high levels of sedentary behavior [10]. To date, contribution of exercise and behavioral therapy gained notable attention in preventing and treating mental illnesses. The components of exercise protocol include frequency, intensity, time(or duration), and type (or modality), known as FITT principle [11]. Compared with the study using a single index of exercise intensity alone, the study using exercise frequency and intensity index showed a stronger correlation with depressive symptoms [12]. High-intensity interval training (HIIT) is a new strategy that maximizes exercise intensity through short bursts of concentrated effort alternated with low activity or rest. HIIT has been applied in healthy adults [13] and patients with CVDs, including CAD [14], heart transplantation [15], and HF [16]. HIIT is proved to be superior compared to conventional moderate-intensity continuous training (MICT) for improving cardiopulmonary function [17] and walking ability [18], which have important implications for the improvement of mental health, well-being, and quality of life.

Clinically, HIIT has been found to improve depression and anxiety in a variety of chronic diseases. Wu et al. [19] reported that 8-week HIIT significantly improved the depression and anxiety symptoms in 24 patients with chronic schizophrenia. However, Choi et al. [20] showed that HIIT improved depression in 44 patients with myocardial infarction whereas MICT did not, and HIIT was more effective in improving depression rather than anxiety. Given the existence of these results, evidence about the efficacy of different modes of exercise (HIIT versus MICT) in prevention and treatment of depression or anxiety in patients with CVDs is needed. The present review aimed to summarize and critically assess the impact of HIIT on depression and anxiety compared with usual care (UC) or continuous training (CT). A secondary aim was to review whether the observed changes in mental health were mediated by exercise patters (i.e., cycle ergometer, treadmill, and strength training).

2. Methods

2.1. Study Selection Procedure. Electronic database search of MEDLINE via PubMed (1966 to December, 2021), Web of Science (1900 to December, 2021), EMBASE (1988 to December, 2021), Cochrane Central Register of Controlled Trials (CENTRAL; December, 2021), CINAHL (EBSCOhost; 1982 to December, 2021), and SPORTSDiscus (EBSCOhost; 1949 to December, 2021) were performed. "High-intensity interval training" is not a MeSH term; therefore, the full electronic search strategy for a detailed description of HIIT was conducted using the following terms in titles and abstracts: HIIT, HIIE, "high intensity interval," "high-intensity interval," "sprint interval," "aerobic interval," "high intensity intermittent," "high-intensity intermittent," and depress*, anxiety, anxious, anxiousness, dysthymia, dysthymic, mood, stress, panic, emotions, phobic, despair, phobia, nervousness, obsession, apprehension, fear, schizo*, posttraumatic, mental health, mental disorders, obsessive compulsive disorder, obsessive-compulsive disorder, posttraumatic stress disorder, and PTSD. We further expanded our search strategy in reference lists of retrieved articles to assess additional eligible publications.

2.2. Inclusion and Exclusion Criteria. Titles and abstracts of returned articles were preliminarily read and the full text was further thoroughly assessed based upon the following inclusion or exclusion criteria: (1) study design: all randomized controlled trials (RCT) and clinical controlled trials; (2) patients: CVDs are a series of diseases involving the circulatory system, including CAD, angina, arrhythmias, atherosclerosis, cardiomyopathy, HF, hypertension, stroke, and MI; (3) intervention: exercise intensity refers to how hard the body works while engaging in physical activity. There are several ways to monitor exercise intensity, such as HR, rate of oxygen consumption (VO_2), rating of perceived exertion (RPE), watts, and walking speed/incline [21]. High intensity is often identified as 60–84% HRR/VO_2peak, 70–89% HRmax, or 14–16 Borg RPE (6–20 scale) [22]. HIIT is always performed on devices (e.g., cycle ergometer, treadmill, and strength training) or through other activities such as swimming or walking. If HIIT was used in combination with other interventions such as psychotherapy and physical factor therapy, the study was excluded. Studies using antidepressants during treatment were also excluded; (4) control: continuous training, namely low to moderate intensity continuous training, is defined as less than 60% HRR/VO_2 peak or 70% HRmax, or 14 RPE. Usual care, includes usual physical activities such as balance training or not include any exercise intervention; (5) outcomes: studies must have included at least one or more well-being measures of depression or anxiety before and after the exercise intervention using validated scales, including, but not limited to the 2-and 9-item, Patient Health Questionnaires (PHQ-2 and PHQ-9), the Beck Depression Inventory (BDI), Hospital Anxiety and Depression Scale (HADS), the Hare-Davis Cardiac Depression Scale (HDCDS), the Hamilton Rating Scale for Depression (HRSD), or Profile of Mood States (POMS). When provided, adjusted effect sizes (e.g., 95% CI and/or p values) were included. Reviews, expert opinions, abstracts, case reports, and studies without available data were excluded.

2.3. Risk of Bias. Risk of bias was evaluated according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) recommendations by two authors (TG & PH) using the Cochrane risk of bias assessment tool. The risk of bias in each subcategory was classified as high, low, or unclear according to the Cochrane Handbook definition. Decisions were compared and discussed to achieve consensus.

2.4. Data Extraction. A data extraction database was developed by the primary reviewer (TG) and checked by the secondary reviewer (PH) in Microsoft Excel based on the

Cochrane Handbook for Systematic Reviews before commencement of the review and included the following:

(1) Study characteristics: author, year and type of publication, and country.

(2) Participant: the number of participants, mean age, and type of disease.

(3) Interventions: doses of exercise, including frequency, intensity, duration, and mode according to the American College of Sports Medicine (ACSM) [23].

(4) Control group: low- to moderate-intensity continuous training, usual care, no any exercise, or other control.

(5) Outcomes: pre- and posttreatment depression or anxiety scores in PHQ, BDI, HADS, HDCDS, or POMS.

If the article only provides preintervention and postintervention data, estimate the change data according to the guidelines in the Cochrane Handbook for Systematic Reviews of Intervention. Moreover, if the study reported results at multiple time points, we chose the final follow-up data according to the following reasons. Firstly, the previous study suggested that it may need more time and duration to elicit psychological benefits for behavioral change. Secondly, there was no obvious comparable time point across studies due to heterogeneity. If more than one well-being measures were available, then the most common scale was chosen.

2.5. Statistical Analysis. The software Review Manager (RevMan) Version 5.4 was used for the meta-analyses. Stata V.14.0 was used to conduct the meta-analyses. Standardized mean difference (SMD) with 95% CI were analyzed as summary statistics due to the fact that anxiety and depression were continuous variables measured by similar but not identical instruments across studies. Both the fixed effects model and the random effects model were considered in the analysis depending on the I-squared. If the I-squared was more than 50%, a random effects model was used to calculate the parameters. Otherwise, a fixed effects model was applied. Heterogeneity was assessed with the I-squared statistics for each analysis, which was classified as low, moderate, or high according to an I^2 values of 25%, 50%, and 75%, respectively.

Two analyses with subgroups were planned: control group (HIIT versus usual care and HIIT versus continuous training), mode (i.e, cycle ergometer, treadmill, and strength training) of intervention in the experimental group. To assess the robustness of our results, sensitivity analysis was subsequently performed: (1) computing the effects using fixed effects model or random effects model; (2) exploring the source of heterogeneity using trim-and-fill computation.

Publication bias was assessed by a visual inspection of funnel plots using the Begg and Egger tests, and $p < 0.1$ was defined as significant publication bias. A probability value of $p < 0.05$ was considered statistically significant.

3. Results

3.1. Studies Retrieved. A PRISMA flow chart detailing the study selection process is presented in Figure 1. The initial search returned a total of 3064 articles, of which 1864 were original studies. After records screened by titles and abstracts, 1624 were discarded. The full text of a total of 240 citations was examined. Among these, 228 did not fulfil the inclusion criteria, and 12 studies were finally included in quantitative synthesis. Full details of characteristics of the 12 studies were summarized in Table 1.

3.2. Characteristics of Included Studies. There were 12 RCTs involving 515 participants included in the review. The age range of participants was 50–70 years, and the average age of most participants was 60 years. The type of diseases in the studies included chronic heart failure ($n = 4$), heart transplantation ($n = 4$), myocardial infarction ($n = 1$), coronary artery disease ($n = 1$), Parkinson's disease ($n = 1$), and stroke ($n = 1$). Among these studies, 12 reported the change of depression symptom and 8 reported the change of an anxiety symptom. The scales used to assess depression were varied as follows: HADS ($n = 9$), ZDRS ($n = 1$), HDCDS ($n = 1$), and GDS ($n = 1$). Anxiety was assessed using the HADS ($n = 8$). Among the 12 controlled trials, 6 (44%) had a UC group, and 8 (56%) had a CT group. Most studies ($n = 10$) adopted HIIT, 2 other studies adopted HIIT but did not report interval intensity and time. The exercise modes of HIIT and CT varied greatly. Most of HIIT intervention included cycle ergometer ($n = 5$), treadmill ($n = 5$), and strength training ($n = 2$). Intensity of exercise was defined by HRR, HRmax, HRpeak, VO_2 peak, and work rate (WR). The intensity in the experimental group ranged from 70% to 100% HRmax/VO_2 peak. In most studies ($n = 9$), the intensity of high-intensity training exceeds 80% HRmax/VO_2 peak. The interval time (30 s–3 min), total time (18–60 min), weekly frequency (2–5 times per week), and duration (8–16 weeks) varied widely among the studies. CT intervention ranged from 28 to 45 min per session at intensities between 60% and 70% VO_2 peak/HRmax. Antidepressants and antianxiety medication usage was not reported in any study. There were statistically significant differences in the baseline of depression symptom between the HIIT and control group in three studies [15, 27, 30, 33]. We used the mean change between pre- and postintervention and standard deviation (SD), rather than final values according to the guidelines from the Cochrane Handbook for Systematic Reviews of Interventions [34] in four studies.

3.3. Publication Bias and Risk of Bias. We assessed the risk of bias for all included studies (Figure 2). Only two studies were at high risk of bias in random sequence generation because of allocating participants according to the time of hospitalization and condition of patients. Reports on allocation concealment, participant blinding, and assessor blinding were mostly unclear, and only 4 of the 12 studies have been fully described that. The rest of the literature had an unknown risk of bias because of unclear information

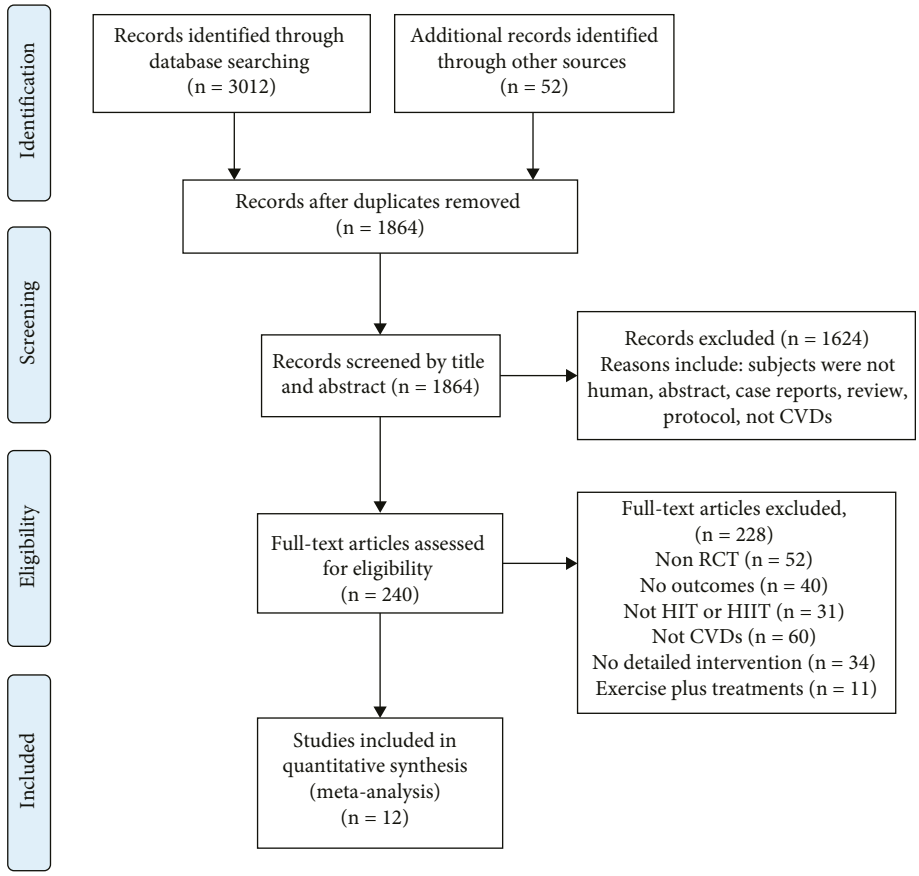

Figure 1: A PRISMA flow chart detailing the study selection process.

concerning blinding of assignments and assessor. Risk of bias due to complete outcome data and nonselective reporting was low overall ($n = 12$). Four studies that were rated as low risk and met at least six of the seven criteria were ultimately considered high quality. And one study which met less than three of the criteria was rated as low quality. The remaining studies that met three to five criteria were rated as moderate quality.

Visual interpretation of funnel plots for depression suggested no obvious evidence of asymmetry (Figure 3(a)). In this analysis there was no publication bias on the Egger test ($p = 0.745$), indicating no evidence of publication bias. Visual interpretation of funnel plots for anxiety suggested no obvious evidence of asymmetry (Figure 3(b)). In this analysis there was no publication bias on the Egger test ($p = 0.535$), indicating no evidence of publication bias.

3.4. Effects of HIIT on Depression Compared with Other Treatments

3.4.1. Main Analysis. The pooled analysis of 12 studies (with a total of 515 participants) showed that there was a significant effect size between the HIIT group and control group, and the heterogeneity of depression scores between studies was not statistically significant (SMD = −0.42 [Random], 95% CI, −0.69 to −0.16, $p = 0.002$). The heterogeneity between the trials was moderately indicated by the I-squared test (I-squared = 52%, Tau-squared = 0.11, $p = 0.02$). The heterogeneity decreases to 0% after one study (Chrysohoou et al. [28]) was excluded, and the results still showed a significant effect size ($p = 0.004$, SMD = −0.20 [Fixed], 95% CI, −0.47 to −0.09). Results were robust when random or fixed effects model was applied in the analysis (Figure 4).

3.4.2. Subgroup Analysis. We preformed two subgroup analysis in the control group (HIIT versus usual care and HIIT versus continuous training) and mode (i.e., cycle ergometer, treadmill, and strength training) of intervention in the experimental group.

Compared to the UC group, HIIT intervention showed statistically significant difference (SMD = −0.51 [Random], 95% CI, −0.95 to −0.06, $p = 0.02$) in the analysis of six studies (with a total of 277 participants). The heterogeneity between the trials was substantial indicated by the I-squared test (I-squared = 68%, Tau-squared = 0.21, $p = 0.008$). Results were robust when the analyses were performed using the random or fixed effects model. The heterogeneity decreases to 0% after one study (Chrysohoou et al. [28]) was excluded, while the results showed no significant effect size ($p = 0.06$, SMD = −0.27 [Fixed], 95% CI, −0.55 to 0.01); compared to the CT group, HIIT intervention elicited statistically significant difference (SMD = −0.31 [Random], 95% CI, −0.59 to −0.02, $p = 0.03$) in the analysis of six studies (with a total of 238 participants). The heterogeneity between

Table 1: Studies included in the analysis.

Study	Participants			HIIT protocol						Control group	Outcomes
	Country	Disease	Age (years)	Number	High intensity	Recovery	Time	Duration	Mode		
Chrysohoou et al. [24]	Greece	CHF	60 years	nHIIT = 33 nUC = 39	30 s at 100% max workload	30 s rest	45 min	3 days a week, 12 weeks	Cycle ergometer	Usual care	ZDRS
Yardley et al. [25]	Norway	HTx	50 years	nHIIT = 21 nUC = 20	4 × 4 min at 85–95% HRmax	3 min at 6–20 RPE	38 min	3 days a week, 8 weeks	Treadmill	Usual care	HADS
Dall et al. [15]	Denmark	HTx	52 years	nHIIT = 16 nCT = 16	1,2,4 min at >80% VO_2 peak	2 min at 60% VO_2 peak	30 min	3 days a week, 12 weeks	Cycle ergometer	CT 45 minutes at 60–70% VO_2 peak	HADS
Choi et al. [20]	Korea	MI	53 years	nHIIT = 23 nCT = 21	4 × 4 min at 85–100% HRmax	3 min at 50–60% HRmax	38 min	1–2 days a week, 9–10 weeks	Strength training	CT 28 min at 60–70% HRmax	HADS
Freyssin et al. [26]	France	CHF	54 years	nHIIT = 12 nCT = 14	12 × 30 s at 60–80% the maximal power	1 min rest	18 min	5 days a week, 8 weeks	Cycle ergometer	CT 45 minutes at the heart rate in VT1	HADS
Smart et al. [27]	Australia	CHF	60 years	nHIIT = 10 nCT = 13	60 s at 70% VO_2 peak	60 s rest	60 min	3 days a week, 16 weeks	Cycle ergometer	CT 30 minutes at 60–70% VO_2 peak	HDCDS
Christensen et al. [28]	Denmark	HTx	53 years	nHIIT = 14 nUC = 13	>80% VO_2 peak	-	60 min	3 days a week, 8 weeks	Treadmill	Usual care	HADS
Isaksen et al. [29]	Norway	CHF	66 years	nHIIT = 19 nUC = 11	4 × 4 min at 85% HRmax	3 min active recovery	60 min	3 days a week, 12 weeks	Treadmill	Usual care	HADS
Pedersen et al. [30]	Denmark	CAD	62 years	nHIIT = 26 nUC = 29	90% VO_2 peak	-	-	3 days a week, 12 weeks	Cycle ergometer	Usual care	HADS
Uc et al. [31]	America	PD	65 years	nHIIT = 18 nCT = 17	3 min at 80–90% of HRmax	3 min at 60–70% HRmax	45 min	3 days a week, 6 weeks	Treadmill	CT 45 minutes at 70–80% HRmax	GDS
Gjellesvik et al. [32]	Norway	Stroke	58 years	nHIIT = 36 nUC = 34	4 × 4 min at 85%–95% HRpeak	3 min at 50%–70% HRpeak	38 min	3 days a week, 8 weeks	Treadmill	Usual care	HADS
Nytrøen et al. [33]	Norway	HTx	50 years	nHIIT = 37 nUC = 41	4 × 4 min at 85%–95% HRpeak	3 min at 50%–70% HRpeak	43 min	3 days a week, 8–12 weeks	Strength training	CT 40 minutes at 60–80% HRpeak	HADS

CHF, chronic heart failure; HTx, heart transplantation; MI, myocardial infarction; CAD, coronary artery disease; PD, Parkinson's disease; HIIT, high-intensity interval training; UC, usual care; CT, continuous training group; HRmax, maximum heart rate; VO_2peak, peak oxygen consumption; RPE, rated perceived exertion; ZDRS, zung depression rating scale; HADS, hospital anxiety and depression scale; HDCDS, the Hare–Davis cardiac depression scale; GDS, geriatric depression scale.

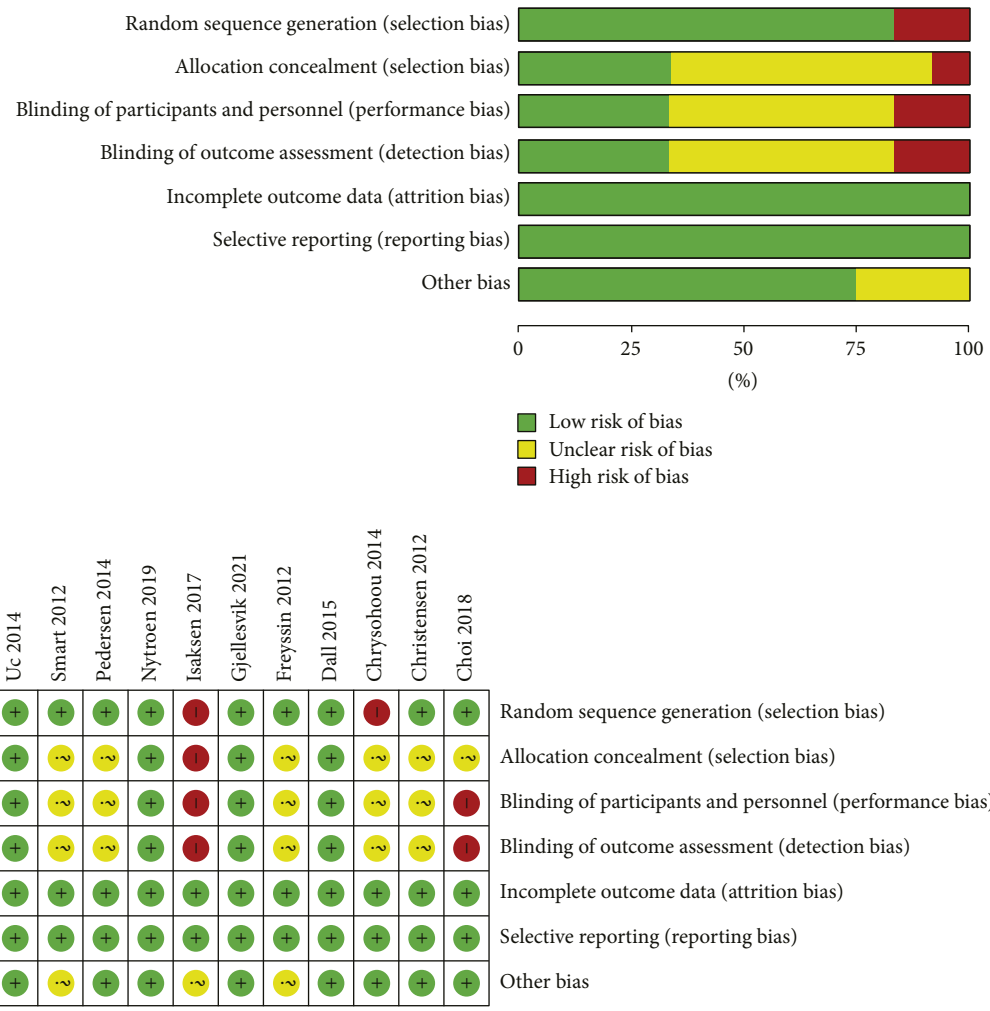

Figure 2: The risk of bias in individual studies.

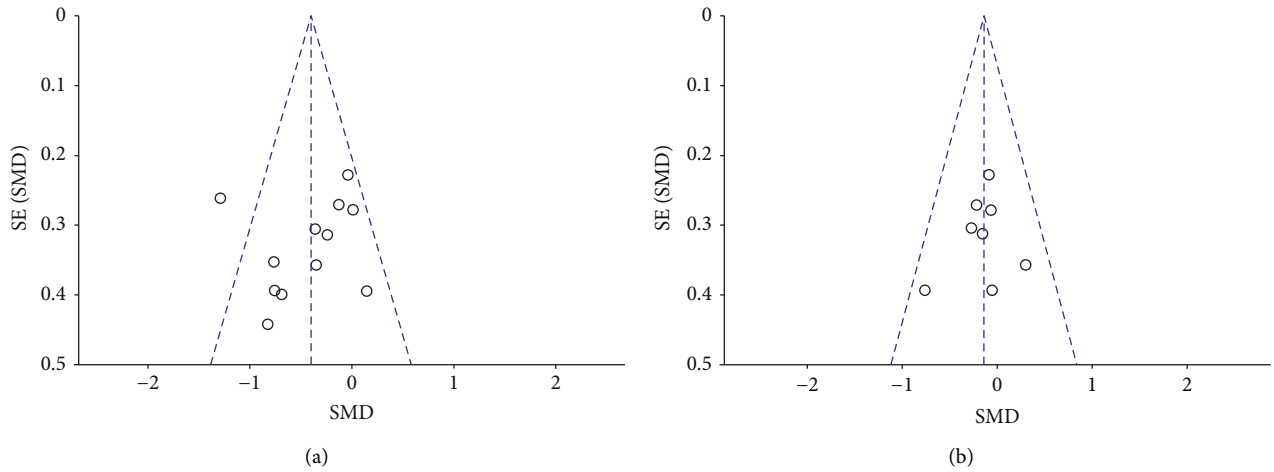

Figure 3: (a) Visual interpretation of funnel plots in depression; (b) visual interpretation of funnel plots in anxiety.

the trials was low indicated by the I-squared test (I-squared = 15%, Tau-squared = 0.02, $p = 0.32$). Results were robust when the analyses were performed the using random or fixed effects model (Figure 5).

Subgroup analysis revealed that high-intensity treadmill training significantly improved the depression symptom (SMD = −0.42 [Random], 95% CI, −0.75 to −0.09, $p = 0.01p = 0.01$, $I^2 = 17\%$) in the analysis of five studies

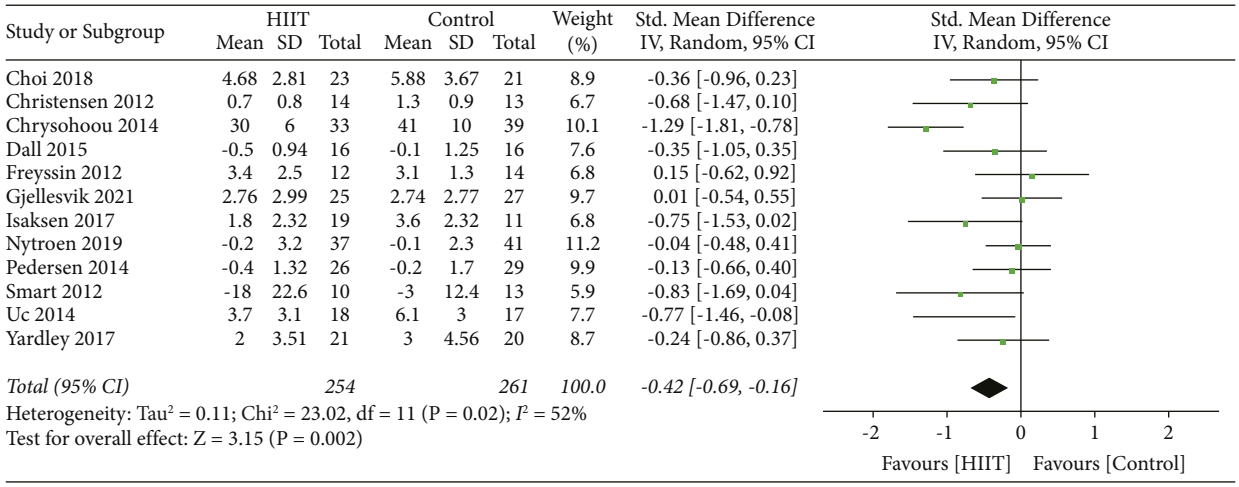

Figure 4: Summary effect sizes between the HIIT and control group for depression.

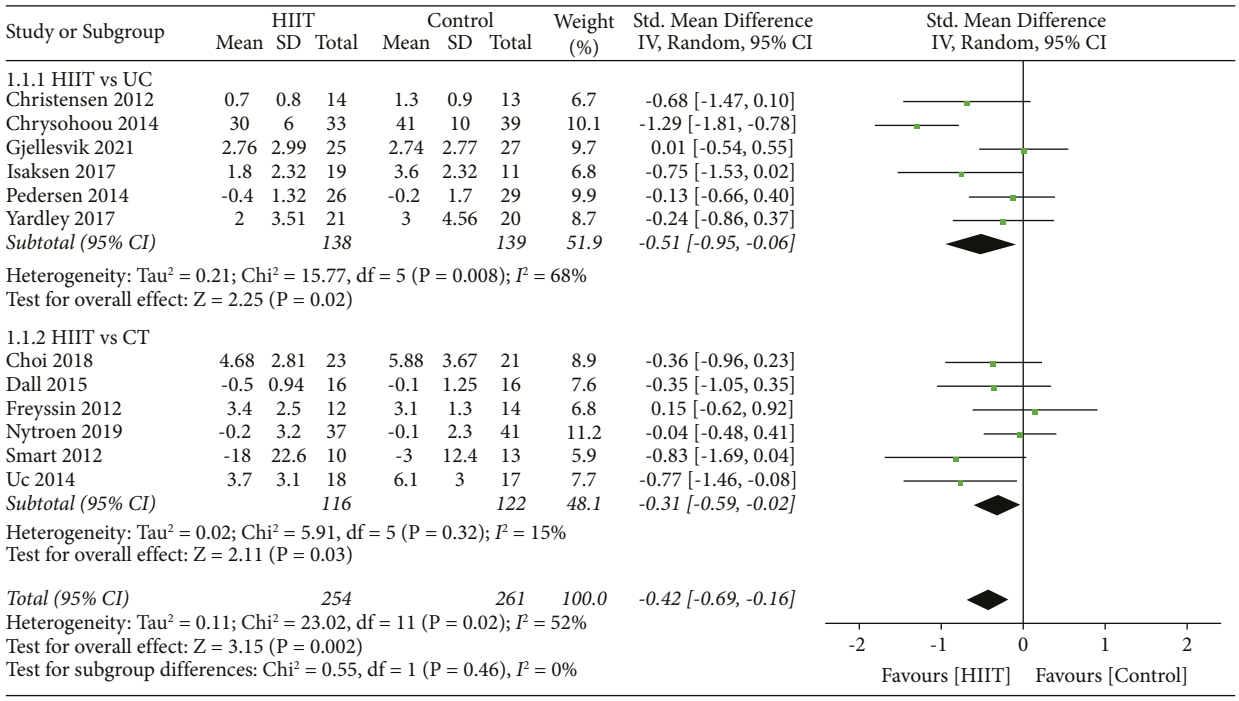

Figure 5: Subgroup analysis for the effects on depression after HIIT compared to UC and CT.

(with a total of 185 participants) rather than high-intensity training with a cycle ergometer (SMD = −0.50 [Random], 95% CI, −1.06 to 0.05, $p = 0.07$, $I^2 = 72\%$) in the analysis of five studies (with a total of 208 participants) and strength training (SMD = −0.15 [Random], 95% CI, −0.51 to 0.20, $p = 0.40$, $I^2 = 0\%$) in the analysis of two studies (with a total of 122 participants) (Figure 6).

3.5. Effects of HIIT on Anxiety Compared with Other Treatments

3.5.1. Main Analysis. The pooled analysis of 8 studies (with a total of 358 participants) revealed that there was no significant effect size between the HIIT group and control group, and the heterogeneity of anxiety scores between the studies was not statistically significant (SMD = −0.14 [Fixed], 95% CI, −0.35 to 0.06, $p = 0.18$). The heterogeneity between the trials was low indicated by the I-squared test (I-squared = 0%, $p = 0.73$) (Figure 7).

3.5.2. Subgroup Analysis. We preformed two subgroup analysis in the control group (HIIT versus usual care and HIIT versus continuous training) and mode (i.e., cycle ergometer, treadmill, and strength training) of intervention in the experimental group.

Compared to the UC group, HIIT intervention showed no significant difference (SMD = −0.24 [Fixed], 95% CI, −0.53 to 0.06, $p = 0.12$) in the analysis of four studies (with

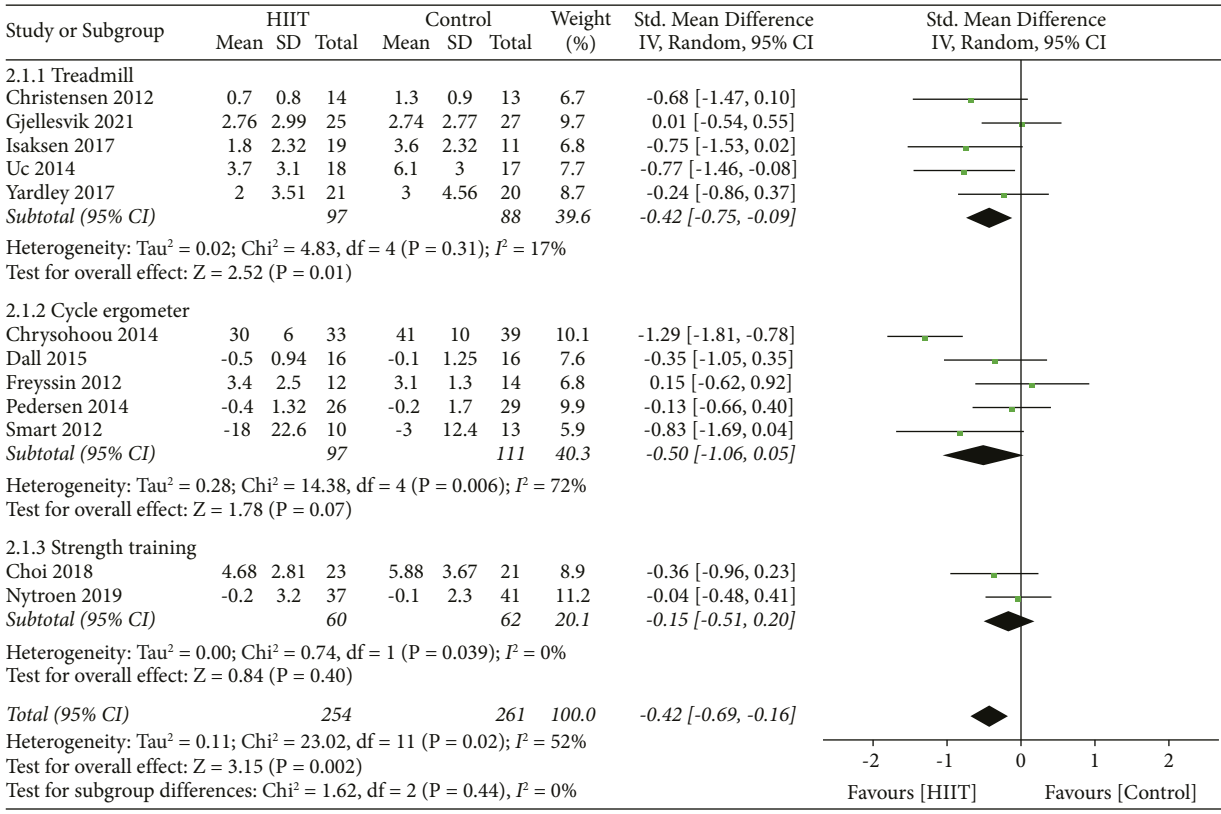

Figure 6: Subgroup analysis for the effects on depression after HIIT using treadmill, cycle ergometer, or strength training.

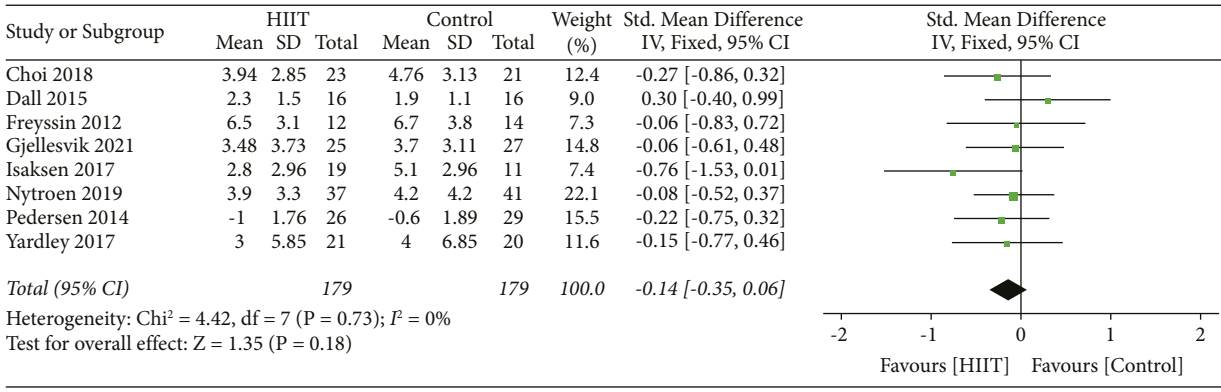

Figure 7: Summary effect sizes between the HIIT and control group for anxiety.

a total of 178 participants). The heterogeneity between the trials was low indicated by the I-squared test (I-squared = 0%, $p = 0.53$). Results were robust when the analyses were performed using random or fixed effects model; compared to the CT group, HIIT intervention showed no statistically significant difference (SMD = −0.06 [Fixed], 95% CI, −0.35 to 0.24, $p = 0.71$) in the analysis of four studies (with a total of 180 participants). The heterogeneity between the trials was low indicated by the I-squared test (I-squared = 0%, $p = 0.69$). Results were robust when the analyses were performed using the random or fixed effects model (Figure 8).

Subgroup analysis revealed there was no significant difference in anxiety symptom whether high-intensity training was performed by cycle ergometer (SMD = −0.03 [Fixed], 95% CI, −0.40 to 0.34, $p = 0.86$, $I^2 = 0\%$) in the analysis of three studies (with a total of 113 participants) or treadmill (SMD = −0.25 [Fixed], 95% CI, −0.61 to 0.11, $p = 0.18$, $I^2 = 9\%$) in the analysis of three studies (with a total of 123 participants) or strength training (SMD = −0.15 [Fixed], 95% CI, −0.50 to 0.21, $p = 0.42$, $I^2 = 0\%$) in the analysis of two studies (with a total of 122 participants) (Figure 9).

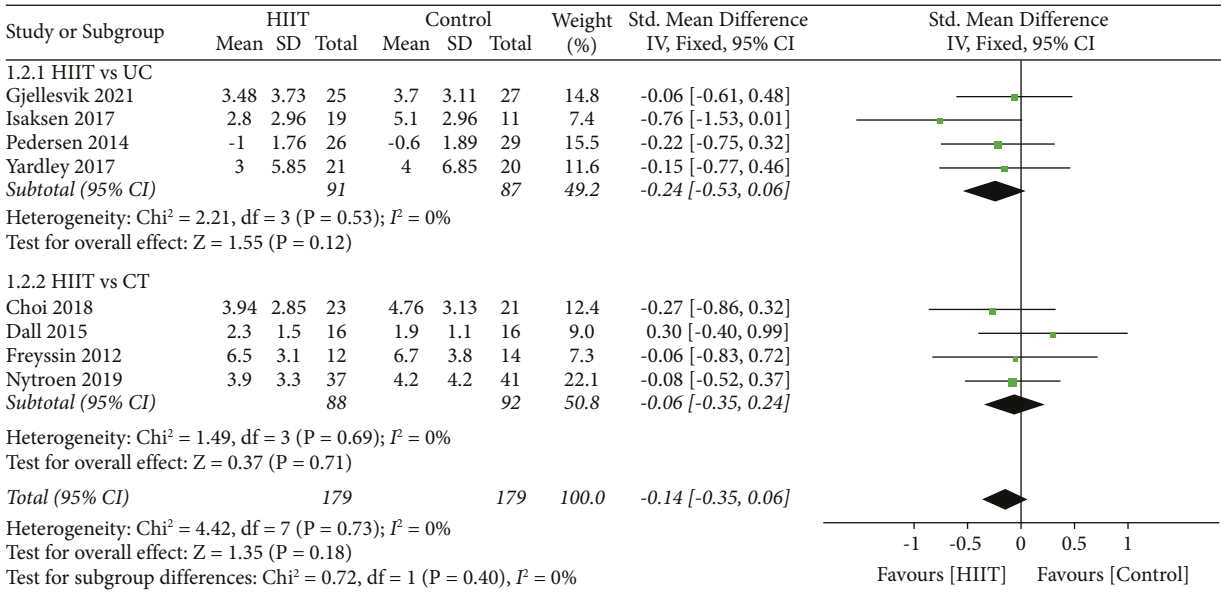

Figure 8: Subgroup analysis for the effects on anxiety after HIIT compared to UC and CT.

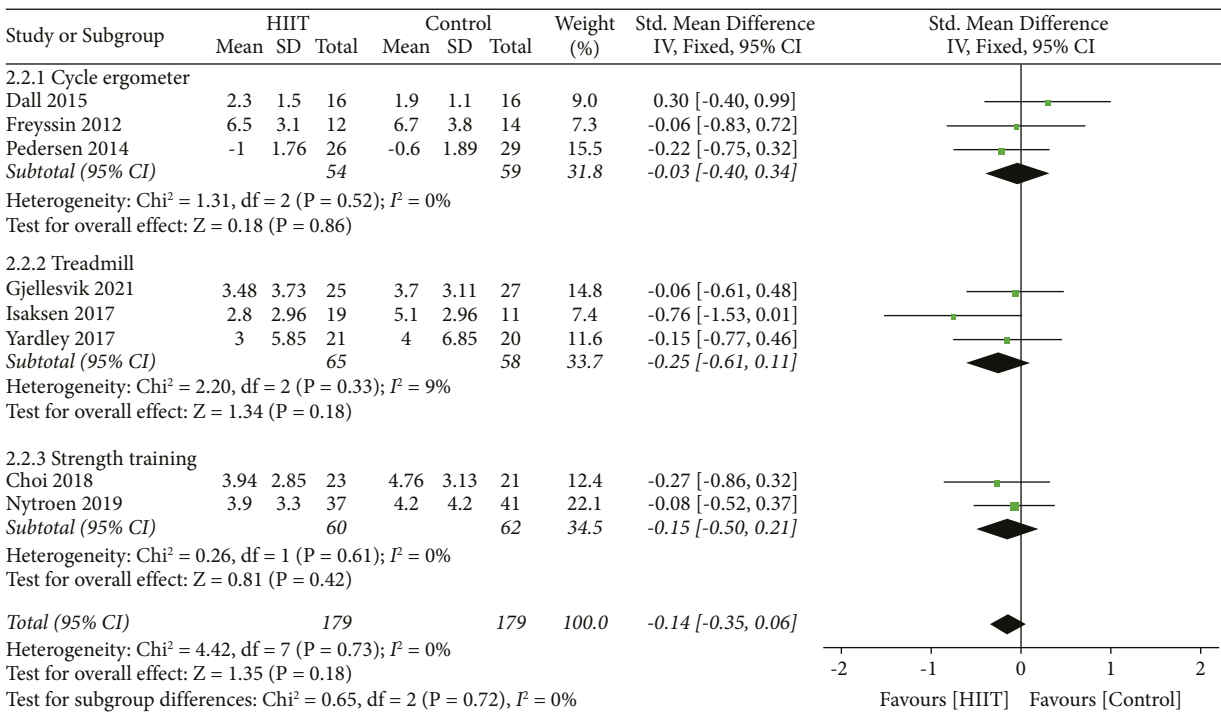

Figure 9: Subgroup analysis for the effects on anxiety after HIIT using treadmill, cycle ergometer, or strength training.

4. Discussion

To the best of the authors' knowledge, this is the first systematic review and meta-analysis that has specifically focused on examining the efficiency of HIIT for improving depression and anxiety in people with CVDs, compared with conventional low- to moderate-intensity continuous training or usual care. Our study found that HIIT can significantly improve symptoms of depression rather than anxiety symptoms in cardiovascular patients compared to usual care and conventional aerobic continuous training.

Physical inactivity, or lack of regular exercise, can increase risk of morbidity and mortality in patients with CVDs [35–37]. A recent meta-analysis found that sedentary behaviors increased the risk of depression by 25% in the general population [38]. A cross-sectional study found a negative correlation where increased depressive symptoms were associated with significantly decreased step counts [39].

It is estimated that higher physical capacity would reduce the global CVD-related burden by 6% [40]. There is a stronger correlation between increased physical activity and reduced rates of depression and anxiety [41, 42]. Our study showed that the HIIT intervention can be considered an effective nonpharmacological treatment for depression. Similar to other literature [19, 27], HIIT can significantly improve depressive symptom compared to usual care or non-exercising. It is noteworthy that the efficiency of HIIT in improving depression and anxiety in chronic diseases (such as heart disease and CAD) might be smaller than that in populations only with anxiety or mood disorders. Depression or anxiety may be caused by poor physical conditions or potential side-effects of medical treatments in these chronic disease patients, and the symptoms of depression and anxiety are hard to be relieved before the primary affections get treated. In addition, patients with chronic pain or other diseases have lower levels of anxiety or depression before treatment, and the improvement degree of after treatment may be relatively less than those with high levels of anxiety or depression before treatment.

Compared to conventional low- or moderate-intensity continuous training, however, the HIIT intervention failed to make a significant difference in the improvement of anxiety symptoms. The research results of HIIT on improving anxiety caused by other diseases are also different. There are significant differences in fibromyalgia [43] and Parkinson's disease [31], and no significant differences in chronic obstructive pulmonary disease [44] and cancer [45]. We think there may be two reasons for our result. One is that the number of studies is insufficient, resulting in insufficient evidence for the analysis. The second is the degree of anxiety in each study are different. But it does not mean that we cannot consider replacing CT with HIIT. For people with coronary artery disease [46, 47], myocardial infarction [48], and heart failure [49, 50], HIIT has shown significant improvements in aerobic capacity and endothelial function [51], left ventricular (LV) [52] and overall myocardial function [53], and specific blood pressure (BP) dynamics [53], compared with CT. The majority of participants in the studies included have a mean age of 50 years, suggesting the evidence base for young people is scarce. In spite of this, the HIIT intervention can be considered an effective non-pharmacological treatment for depression in older adults due to the fact that late-life depression is becoming a major social burden with increased healthcare costs and risk of suicide and morbidity.

Several theories have been proposed as the mechanism by which exercise may lead to improved mood, including the following: (1) anti-inflammatory effects: inflammatory cytokines (e.g., C-reactive protein (CRP) and interleukin-6 (IL-6)) can predict cardiovascular mortality and disease progression in healthy people [54] and patients with CAD [55] and HF [56]. In patients with or without history of heart disease, depression is also associated with elevated cytokine levels (especially CRP, IL-1, and IL-6) [57]. Physical activity (PA) could decrease the levels of proinflammatory markers and promote the secretion of anti-inflammatory factors, such as interleukin-1 beta increased in hippocampal volume and serum with symptom improvement [58]; (2) effects on neurogenesis: autonomic nervous system dysfunction plays an important role in the connection between depression and outcomes in HF [59]. PA increases the brain-derived neurotrophic factor (BDNF) levels in hippocampus leading to neural plasticity [60, 61]; (3) hormonal changes: PA could increase the levels of some monoamine neurotransmitters (e.g., dopamine, noradrenaline, and beta-endorphins) and elicit neuroendocrine effects on the hypothalamic-pituitaryadrenal axis and insulin sensitivity [62, 63]; (4) oxidative stress: PA can make an increment in the levels of antioxidant markers and a reduction in the levels of prooxidative markers [64], as well as differences in cortical activity and structure [65].

5. Limitations

Although there was only one study rated as low quality, majority of studies were rated as moderate quality due to the unclear risk with insufficient information about allocation concealment, blinding of participants and outcome assessment, which limited the reliability of results. Begg's Test and Egger test indicated no evidence of publication bias in the pooled analysis. But we need to be cautious about it due to the small amount of included studies and high heterogeneity between different control groups. There were differences in participants, scales of assessment, types of interventions across studies, and the heterogeneity indicated significant in some comparisons. Therefore, it is questionable whether it is appropriate to use a combined study in this meta-analysis. Sensitivity analyses could also be performed in the limited comparison (type of disease and exercise mode), which showed that the high heterogeneity could be caused by few studies in some comparison. The sensitivity analysis showed that there was no statistically significant difference between the random effects model and fixed effects model. Although we have tried to minimize the effects of heterogeneity, results in this review should still be treated with caution.

6. Clinical Application

At present, the symptoms of depression and anxiety associated with chronic cardiovascular disease are mainly treated with antidepressants. However, antidepressants have many side effects. Studies have shown that the long-term use of antidepressants has certain links with the development of depressive symptoms [66]. HIIT is proven to be safe, effective, and time-saving in the rehabilitation of cardiovascular disease. Therefore, the combination of HIIT and antidepressants can be used clinically to cure symptoms of depression and anxiety; or the antidepressants can be applied in the early stages, and then gradually be replaced with HIIT.

7. Suggestions for Future Research

Our study found that HIIT can significantly improve the symptoms of depression rather than anxiety in cardiovascular patients compared to usual care and conventional

aerobic continuous training. Therefore, more research is needed to explore the effects of two types of exercise on the comorbidity of depression, anxiety, and CVDs. Second, there is a lack of research exploring the effect of HIIT on depression and anxiety versus antidepressants. The study failed to predict which component of the HIIT affected the efficacy of antidepressants. Thirdly, long-term effects of HIIT on depression in people with CVD needed to be explored in the future.

8. Conclusions

Despite the limitations, we have demonstrated that HIIT is beneficial for mental health. In the case of improving depression symptom rather than anxiety symptom after CVDs, HIIT may be superior to the effect of usual care and continuous training, especially for the elderly. More studies are needed to explore the long-term effects of HIIT on depression and anxiety symptom compared to the conventional low- to moderate-intensity continuous training and to understand the mechanism in the future.

Data Availability

All data analyzed and presented in this study are available from the corresponding author on reasonable request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Tingting Gu and Pengli Hao have contributed equally to this study.

Acknowledgments

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Retraction

Retracted: Mechanism of LncHOTAIR Regulating Proliferation, Apoptosis, and Autophagy of Lymphoma Cells through hsa-miR-6511b-5p/ATG7 Axis

Evidence-Based Complementary and Alternative Medicine

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This article has been retracted by Hindawi following an investigation undertaken by the publisher [1]. This investigation has uncovered evidence of one or more of the following indicators of systematic manipulation of the publication process:

(1) Discrepancies in scope

(2) Discrepancies in the description of the research reported

(3) Discrepancies between the availability of data and the research described

(4) Inappropriate citations

(5) Incoherent, meaningless and/or irrelevant content included in the article

(6) Peer-review manipulation

The presence of these indicators undermines our confidence in the integrity of the article's content and we cannot, therefore, vouch for its reliability. Please note that this notice is intended solely to alert readers that the content of this article is unreliable. We have not investigated whether authors were aware of or involved in the systematic manipulation of the publication process.

Wiley and Hindawi regrets that the usual quality checks did not identify these issues before publication and have since put additional measures in place to safeguard research integrity.

We wish to credit our own Research Integrity and Research Publishing teams and anonymous and named external researchers and research integrity experts for contributing to this investigation.

The corresponding author, as the representative of all authors, has been given the opportunity to register their agreement or disagreement to this retraction. We have kept a record of any response received.

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[1] F. Gui, X. Yu, Y. Wu, C. Wu, and Y. Zhang, "Mechanism of LncHOTAIR Regulating Proliferation, Apoptosis, and Autophagy of Lymphoma Cells through hsa-miR-6511b-5p/ATG7 Axis," *Evidence-Based Complementary and Alternative Medicine*, vol. 2022, Article ID 2166605, 10 pages, 2022.

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Research Article

Mechanism of LncHOTAIR Regulating Proliferation, Apoptosis, and Autophagy of Lymphoma Cells through hsa-miR-6511b-5p/ATG7 Axis

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Objective. To explore the role of LncHOTAIR in apoptosis and autophagy in lymphoma. *Methods*. The interaction between LncHOTAIR and miR-6511b-5p, as well as between miR-6511b-5p and ATG7, was verified by a dual luciferase assay. LncHOTAIR overexpression lentivirus was transducted and siATG7s were transfected into Raji and BJAB lymphoma cells, and the efficiency was verified by qPCR. Lymphocyte proliferation was detected by the cell counting kit-8 (CCK8) test, and autophagy was detected by transmission electron microscopy. The protein expressions of ULK1, Beclin1, ATG7, LC3, Bax, cleaved-caspase 3, and Bcl-2 were detected using Western blots. *Results*. There was a targeting relationship between LncHOTAIR and miR-6511b-5p and between miR-6511b-5p and ATG7. LncHOTAIR overexpression promoted the proliferation and autophagy of Raji and BJAB cells, significantly upregulated ATG7, Beclin1, ULK1, Bcl-2, and LC3-II/LC3-I levels, and downregulated Bax and cleaved-caspase3 levels. siATG7 significantly inhibited the proliferation and autophagy of Raji and BJAB cells and promoted their apoptosis. *Conclusion*. LncHOTAIR/hsa-miR-6511b-5p/ATG7 could regulate the proliferation, apoptosis, and autophagy of Raji and BJAB lymphoma cells.

1. Introduction

Lymphoma is a systemic malignant tumor that originates from the lymphohematopoietic system, accounting for 3%–4% of all malignant tumors. Most lymphomas originate from B cells, whereas other lymphomas are derived from T cells or natural killer (NK) cells [1]. Burkitt lymphoma (BL) belongs to B-cell lymphoma, which leads to poor prognosis due to unclear early symptoms and a lack of effective treatment [2, 3]. Therefore, it is key to explore and identify new biomarkers for early detection and effective treatment.

Long noncoding RNA (e.g., LncRNA) is defined as longer than 200 nucleotides, and many lncRNAs are abnormally expressed in different diseases, especially cancer. The imbalance of lncRNA mainly promotes cancer progression by promoting the malignant biological behavior of tumor cells (such as proliferation, invasion, or metastasis). LncRNA can be used as tumor markers to provide a basis for early diagnosis of cancer because some lncRNA have abnormal expression in various types of human tumors.

LncHOTAIR is abnormally expressed in solid tumors, acute leukemia, and lymphoma, and is closely related to tumor proliferation, apoptosis, and migration [4, 5]. Autophagy plays a role in "tumor inhibition" in the occurrence, development, and malignant evolution of various cancers, including gastric cancer, glioma, and pancreatic cancer [6]. ATG7 is an E1-likeubiquitin-activating enzyme, and it is needed during autophagy as well as cytoplasmic-to-vacuolar transportation. However, no study has confirmed the regulatory relationship between LncHOTAIR and autophagy.

Therefore, to explore the regulatory mechanism between LncHOTAIR and autophagy in BL cells, we predicted that hsa-miR-6511b-5p may be involved in the regulation between LncHOTAIR and ATG7 using bioinformatics. We explored the molecular mechanisms of LncHOTAIR and

ATG7 regulating proliferation, apoptosis, and autophagy in Raji and BJAB cells, which provided a theoretical basis for understanding how autophagy is involved in the pathogenesis of BL lymphoma.

2. Materials and Methods

2.1. Cell Culture. The Raji and BJAB cells (cultured in RPMI1640 + 10% FBS) and 293T cells (cultured in DMEM + 10% FBS) were obtained from ATCC and cultured at 37°C with 5% CO_2.

2.2. Dual Luciferase Reporter Gene Assay. miR-6511b-5p′s interaction with LncHOTAIR and ATG7 were tested using pRL-SV40 containing LncHOTAIR and ATG7 3′-UTR luciferase reporter gene plasmids as a previously publication [7]. Sea kidney luciferase was used for standardization. miR-6511b-5P mimic sequences CUGCAGGCAGAAGUGGGG CUGACA,UGUCAGCCCCACUUCUGCCUGCAG and NC sequences UCACAACCUCCUAGAAAGAGU AGA,UCUACUCUUUCUAGGAGGUUGUGA.

2.3. Quantitative Rt-PCR. Total RNA was extracted from cells using the RNeasy kit (Qiagen) and reverse transcribed into cDNA according to the kit's instructions. The rt-PCR was performed on an ABI StepOne Plus system using SYBR Green qPCR Master Mix (Thermo Fisher Scientific, USA). The reaction was set at 95°C for 10 min for activation, then 95°C for 5 s, and 60°C for 40 s, with 40 cycles. β-Actin or U6 were employed as internal controls, and the amount of mRNA was calculated by the $2^{-\Delta\Delta CT}$ method. The primer sequences were summarized in Table 1.

2.4. Cell Proliferation. The logarithmic growth phase cells were harvested from plates by trypsin digestion, added into plates (1×10^4 cells per well of 96-well) for overnight incubation, then 10 μl of cell counting kit-8 (CCK8) solution, which was purchased from Beyotime (Shanghai, China), was added, followed by a 4 h incubation [8]. The OD_{450} was measured with a SmartReader 96 Microplate Absorbance Reader (Benchmark Scientific, USA; $n = 6$ per group).

2.5. Apoptosis Determination. The cells were collected and washed with ice-coldphosphate-buffered saline (PBS), followed by apoptosis detection by the AnnexinV-FITC Analysis Kit on a NovoCyte™ flow cytometry according to the instructions.

2.6. Cell Cycle Analysis. The cells were collected, washed with PBS, fixed with 70% ethanol at 4°C, followed by ribonuclease treatment, and 200 μl of 50 μg/ml of propidium iodide (PI) were added. After 30 minutes of incubation in the dark, samples were loaded for flow cytometry analysis [9].

2.7. Transmission Electron Microscope (TEM). The samples were fixed and dehydrated, embedded with acetone embedding solution, and solidified in an oven. The blocks were cut into 70 nm slices with an ultramicrotome. The slices were observed after 7.3% uranium acetate-lead citrate double staining under JEOL JEM-1230 (80 KV) TEM.

Table 1: Primers in this study.

Gene	Sequences (5′-3′)
LncHOTAIR *F*	GGTAGAAAAAGCAACCACGAAGC
LncHOTAIR *R*	ACATAAACCTCTGTCTGTGAGTGCC
ATG7 *F*	AGCAGCTCATCGAAAGCCAT
ATG7 *R*	AGTGCAGGGTCCGAGGTATT
β-actin*F*	TGGCACCCAGCACAATGAA
β-actin*R*	CTAAGTCATAGTCCGCCTAGAAGCA
U6 F	CTCGCTTCGGCAGCACA
U6 R	AACGCTTCACGAATTTGCGT
miR-6511b-5P *F*	CTGCAGGCAGAAGTGGGG
miR-6511b-5P *R*	AGTGCAGGGTCCGAGGTATT
miR-6511b-5P	GTCGTATCCAGTGCAGGGTCCGAGG
RT	TATTCGCACTGGATACGACTGTCAG

Table 2: siRNA sequences.

siRNA	Sequences
siATG7-1	CCAACACACUCGAGUCUUUTT AAAGACUCGAGUGUGUUGGTT
siATG7-2	GCUCUUCCUUACUUCUUAATT UUAAGAAGUAAGGAAGAGCTT
siATG7-3	GCGUGAGACACAUCACAUUTT AAUGUGAUGUGUCUCACGCTT
siATG7 NC	UUCUCCGAACGUGUCACGUTT ACGUGACACGUUCGGAGAATT

2.8. Western Blot. RIPA buffer was used for cell lysis. Lysates were centrifuged at 4°C (10000 rpm) for 10 min, and a BCA Protein Assay Kit (ThermoFisher, USA) was used to measure concentrations of the supernatant. Protein lysates were mixed with loading buffer, denatured at 95°C for 10 minutes, and separated (50 μg/lane) by electrophoresis in SDS-PAGE. Then, proteins were transferred to a PVDF membrane, which was washed and blocked using blocking buffer, followed by primary antibody incubation at 4°C overnight, rinsed, and the secondary antibody incubation for 1 h at room temperature, then immunoblotted by an enhanced chemiluminescence kit (Perkin-Elmer Inc.), and finally the Quantity One software was used for quantification.

2.9. Transduction and Transfection. The LncHOTAIR overexpression and control lentivirus were prepared by [7] and were transduced as previously described [10]. The transfection was conducted with the Lipofectamine 3000 kit (Thermo Fisher Scientific) in a control group, an siATG7 NC group, and an siATG7 group. The siRNA sequences are shown in Table 2.

2.10. Statistical Analyses. The data were analyzed by SPSS 20.0 (IBM, Armonk, NY, USA). One-way ANOVA was used to calculate the differences among groups. A 2-sided$P < 0.05$ was used to determine statistical significance.

3. Results

3.1. Targeting Relationship of LncHOTAIR/miR-6511b-5p/ATG7. LncHOTAIR WT bound with miR-6511b-5p mimic and significantly reduced luciferase activity (Figure 1(a)), ATG7 WT bound with miR-6511b-5p mimic and also significantly reduced luciferase activity (Figure 1(b)). These showed that LncHOTAIR and miR-6511b-5p, and miR-6511b-5p and ATG7 had a targeting relationship, respectively.

3.2. Verification of LncHOTAIR Lentivirus Overexpression. In Figure 2, in Raji and BAJB cells, the level of LncHOTAIR increased significantly after transduction with the LncHOTAIR overexpression lentivirus vector compared with those in the control group and empty NC group ($P < 0.05$), which suggested that LncHOTAIR overexpression lentivirus transduction was successful.

3.3. Effect of LncHOTAIR Overexpression on the Viability of Lymphoma Cells. As shown in Figure 3, LncHOTAIR overexpression significantly promoted the proliferation of Raji and BJAB cells compared to that of the control group and NC group ($P < 0.05$).

3.4. Effect of LncHOTAIR Overexpression on the Autophagy of Lymphoma Cells. The autophagy of Raji and BAJB cells was observed by TEM (Figure 4). Compared with the control group and NC group, the autophagy bodies significantly increased in the LncHOTAIR overexpression group. These results suggested that LncHOTAIR overexpression promoted autophagy in Raji and BAJB cells.

3.5. Effects of LncHOTAIR Overexpression on Autophagy and Apoptosis Related Proteins. The LncHOTAIR overexpression significantly increased the levels of autophagy related proteins ATG7, Beclin1, LC3-II/LC3-I, ULK1 and the antiapoptotic protein Bcl-2 in Raji and BAJB cells, and the levels of proapoptotic proteins were significantly decreased, such as Bax and cleaved-caspase3 (Figure 5).

3.6. ATG7 Interference Verification. The expression level of ATG7 in the siATG7-1, siATG7-2 and siATG7-3 groups decreased significantly in Raji and BAJB cells compared with that of the control group and NC group ($P < 0.05$), especially in the siATG7-1 group (Figure 6). Therefore, siATG7-1 was selected as the interference vector of ATG7 in subsequent experiments.

3.7. Effect of siATG7 on the Viability of Lymphoma Cells. ATG7 significantly decreased the viability of Raji and BAJB cells (Figure 7, $P < 0.05$).

3.8. Effect of siATG7 on Apoptosis and Cell Cycle. In Raji and BAJB cells, siATG7 significantly promoted cell apoptosis. It blocked BJAB and Raji cells in $G0/G1$ phase, which indicates that siATG7 could affect $G0/G1$ phase and then affect apoptosis (Figure 8).

3.9. Effect of siATG7 on the Autophagy of Lymphoma Cells. The autophagy of Raji and BAJB cells was observed by TEM after siATG7 treatment (Figure 9). The autophagy bodies significantly decreased in the siATG7 group. These results suggested that siATG7 inhibited the autophagy of Raji and BAJB cells.

3.10. Effects of siATG7 on Autophagy and Apoptosis Related Proteins. The siATG7 significantly inhibited autophagy-related proteins and antiapoptotic proteins in Raji and BAJB cells, and significantly increased proapoptotic proteins (Figure 10).

4. Discussion

Lymphoma is a malignant tumor that is primarily located in lymph nodes or extranodal lymphoid tissue. Its incidence rate is increasing and seriously threatens human health. In B-cell lymphoma, the content of BIC RNA/miR-155 increased significantly, and it was different among patients, suggesting that miR-155 may be transcribed and regulated by BIC RNA [11]. LncHOTAIR was upregulated in diffuse large B-cell lymphoma (DLBCL). Its expression level was significantly correlated with tumor characteristics. Knockdown of LncHOTAIR in vitro may lead to tumor cell growth inhibition, cell cycle arrest, and apoptosis through the PI3K/AKT/NF-κB pathway [12]. LncHOTAIR expression in DLBCL may induce H3K27me3 through EZH2-related PRC2 activation [13]. In this study, LncHOTAIR overexpression could significantly promote the proliferation of Raji and BAJB cells, which is consistent with Yan et al.'s results [12].

Bax promotes apoptosis, whereas Bcl-2 inhibits apoptosis [14]. When Bax expression increases, a large number of Bax homodimers are formed, which induces the release of Cyt-C into the cytoplasm and activates caspase-9. Then, caspase-9 digests caspase-3 zymogen and activates caspase-3, promotes the cleavage of caspase-3, starts a caspase cascade reaction, and induces apoptosis [15]. In colorectal cancer (CRC), siLncHOTAIR could significantly bring down Bcl-2 and bring up Bax and cleaved-caspase 3 protein expression [7], which was confirmed in our study in Raji and BAJB lymphoma cells.

Autophagy, also known as a special type of programmed cell death, participates in many pathological processes as well as biological growth and development. LncHOTAIR and ATG7 were upregulated and autophagy was significantly increased during liver injury. However, after LncHOTAIR expression was knocked down, autophagy induced by hydrogen peroxide in isolated hepatocytes was attenuated, and LncHOTAIR regulated autophagy in liver injury [16]. This study predicted that hsa-miR-6511b-5p may be involved in the

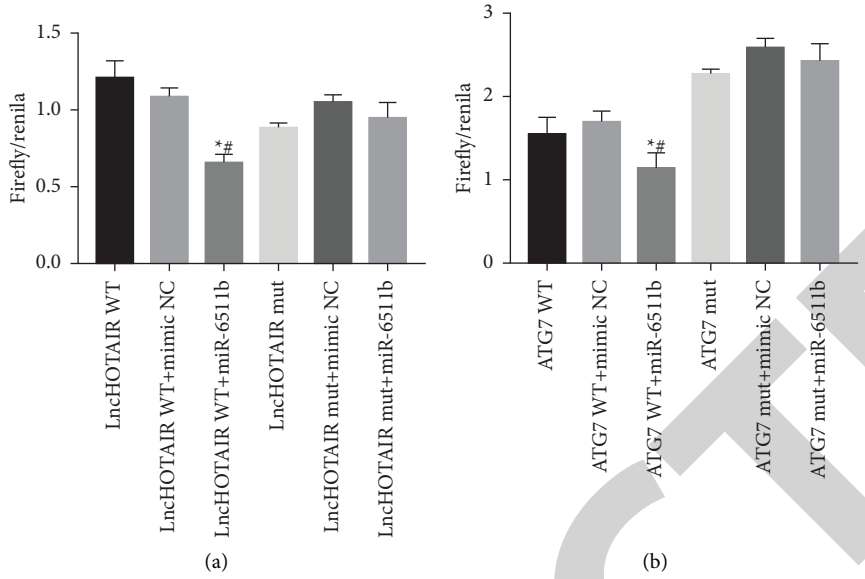

Figure 1: The targeting relationship between LncHOTAIR and miR-6511b-5p, miR-6511b-5p, and ATG7 was verified by dual luciferase assay. (a) Verification of LncHOTAIR and miR-6511b-5p targeting relationship; (b) Verification of miR-6511b-5p and ATG7 targeting relationship. $^{*}P < 0.05$ vs. LncHOTAIR WT or ATG 7; $^{\#}P < 0.05$ vs. LncHOTAIR WT + mimic NC or ATG7 WT + mimic NC.

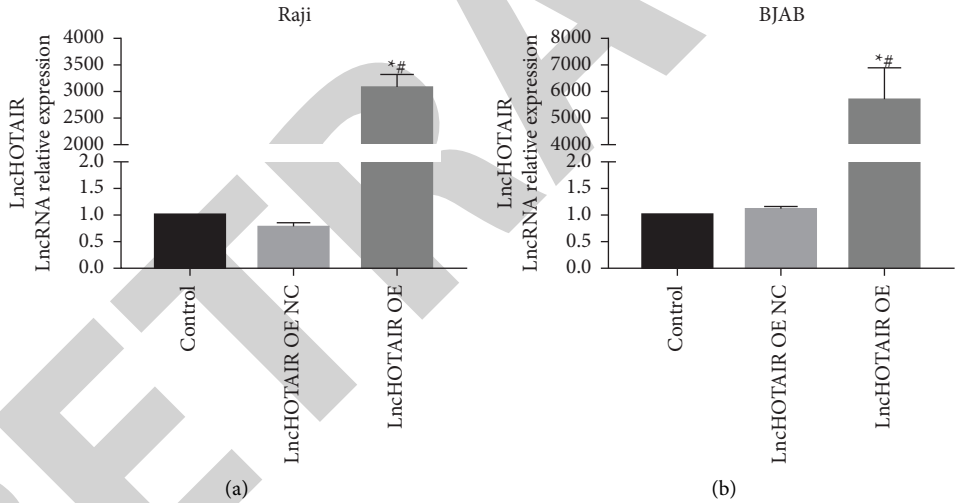

Figure 2: The expression level of LncHOTAIR in Raji and BAJB cells was detected by RT-qPCR. (a) Raji cell; (b) BAJB cell. $^{*}P < 0.05$ LncHOTAIR overexpression (OE) vs. control; $^{\#}P < 0.05$ LncHOTAIR OE vs. LncHOTAIR OE negative control (NC).

regulation of LncHOTAIR and ATG7 using the bioinformatic databases of TargetScan and Starbase, and proved that hsa-miR-6511b-5p had target sites for LncHOTAIR and ATG7 by dual luciferase reporter gene assay. Overexpression of LncHOTAIR could significantly upregulate ATG7 and increase the number of autophagy bodies in Raji and BAJB cells.

Autophagy is related to nutritional status, energy status, oxidative stress, ischemia, and hypoxia. It is regulated by multiple mechanisms, such as the ULK1 pathway, Beclin1 pathway, p53 pathway, AMP-activated protein kinase AMPK pathway, and so on. The Beclin1 pathway is the downstream regulatory signal of ULK1. ULK1 complex can phosphorylate Beclin1-Ser14 and Vps34Ser249, promote Beclin1-Vps34 complex formation, and thus promote the occurrence of autophagy [17]. LC3 is the earliest marker for autophagy, whose precursor excises the carboxyl terminal to generate LC3I, followed by covalent binding with phospholipids on the autophagy body membrane to form LC3II [18]. In this study, LncHOTAIR overexpression significantly promoted the expression levels of proteins related to autophagy, such as LC3-II/LC3-I, Beclin-1, and ULK1, which was consistent with the previous results [12, 16].

ATG7 is closely related to abnormal proliferation and drug resistance of a variety of tumors [19–24]. ATG7 deficiency completely inhibited the occurrence and development of mouse intestinal epithelial cell tumors and promoted the body's antitumor immune response [25]. In the mouse model, knockout of the ATG7 gene significantly

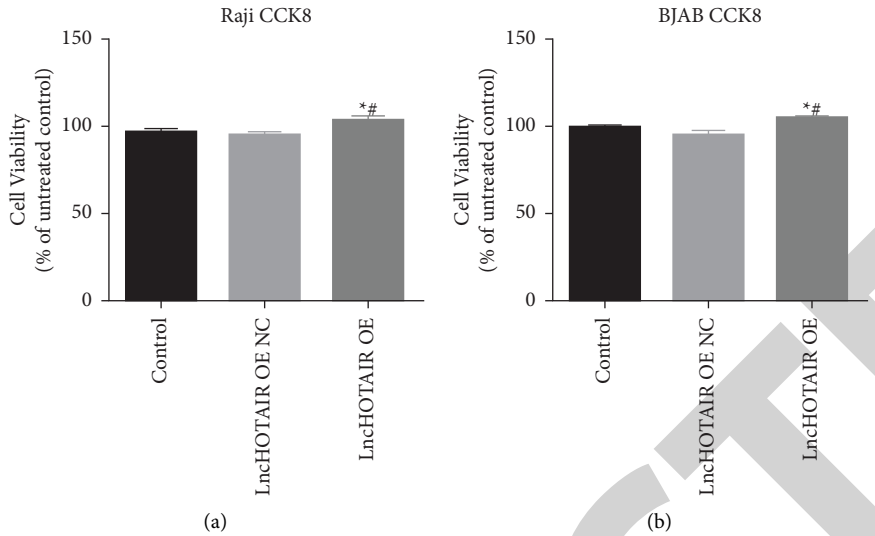

Figure 3: The proliferation of Raji and BAJB cells was detected by CCK8. (a) Raji cells; (b) BAJB cells. $^{*}P < 0.05$ vs. control; $^{\#}P < 0.05$ vs. LncHOTAIR OE NC.

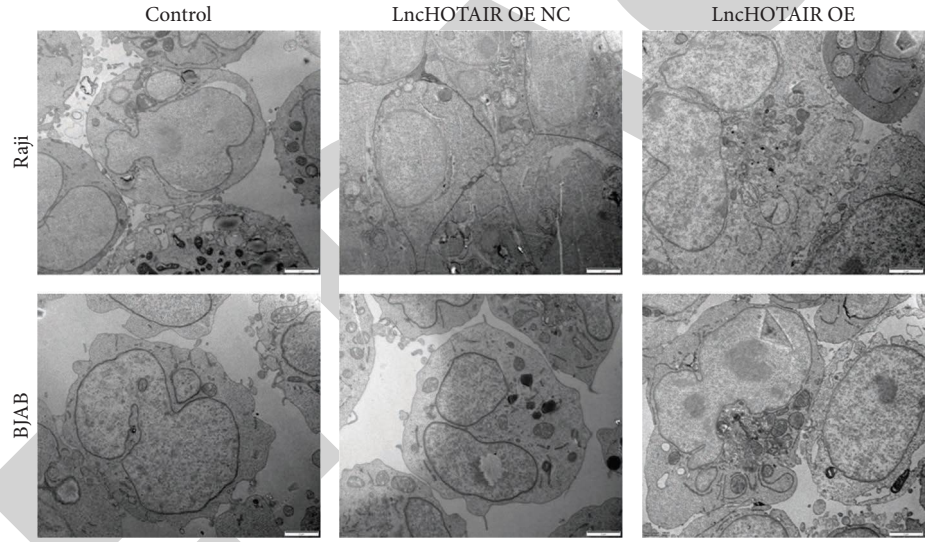

Figure 4: Effect of LncHOTAIR overexpression on the autophagy of lymphoma cells.

reduced the tumorigenicity of tumor cells and promoted the transformation of lung cancer cells into benign tumors [26]. In this study, siATG7 significantly inhibited Raji and BAJB lymphoma cell proliferation. In bladder cancer, ATG7 played a role in cell invasion, and ATG7-specific therapy had certain development potential [27]. ATG7 can also promote angiogenesis in the brain [28], regulate the activity of caspase-9, and regulate the process of apoptosis [29]. In SH-SY5Y cells, knockdown of ATG7 significantly decreased Bcl-2 while increasing Bax and Caspase-3, which proved that ATG7-mediated autophagy could not only promote cell proliferation but also inhibit cell apoptosis [30]. In this study, siATG7 significantly downregulated Bcl-2 and upregulated Bax and caspase3. The knockdown of the ATG7 gene could inhibit the conversion from LC3-I to LC3-II [30], which was consistent with the results of this study.

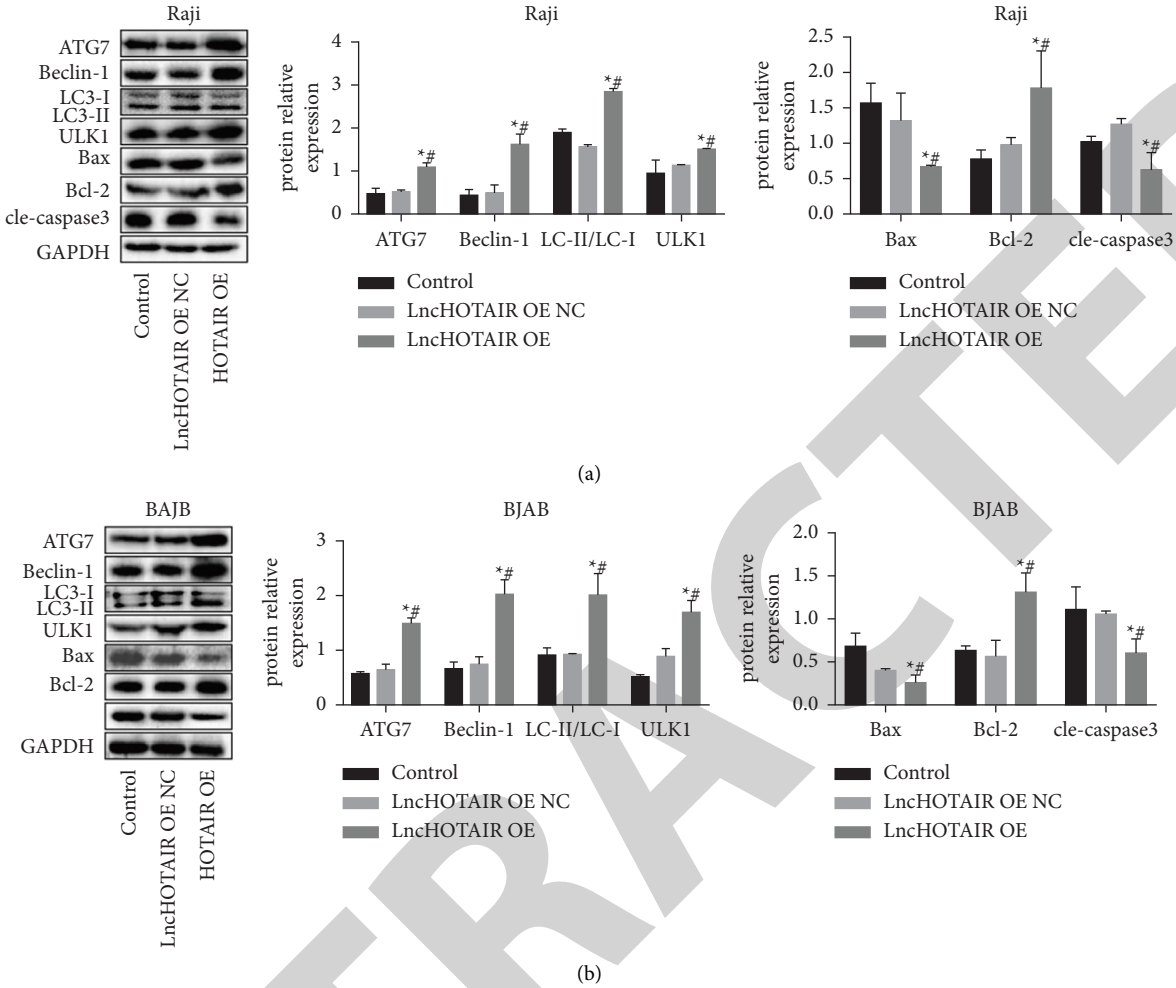

Figure 5: Western blotting results of autophagy and apoptosis related proteins in Raji and BAJBcells. (a) Raji cells; (b) BAJB cells. $^{*}P < 0.05$ vs. control; $^{\#}P < 0.05$ vs. LncHOTAIR OE NC.

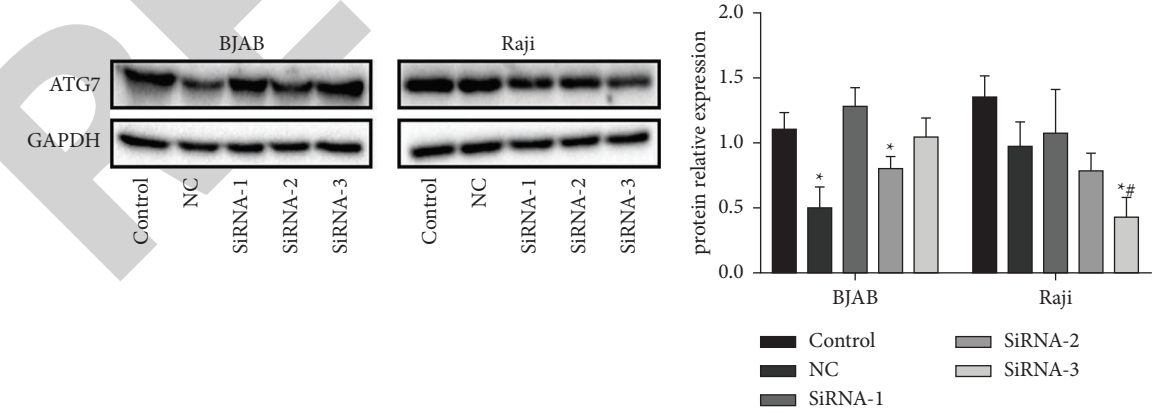

Figure 6: ATG7 expression in Raji and BAJB cells after siATG7 treatment. (a) Raji cells; (b) BAJB cells. $^{*}P < 0.05$ vs. control; $^{\#}P < 0.05$ vs. siATG7 NC.

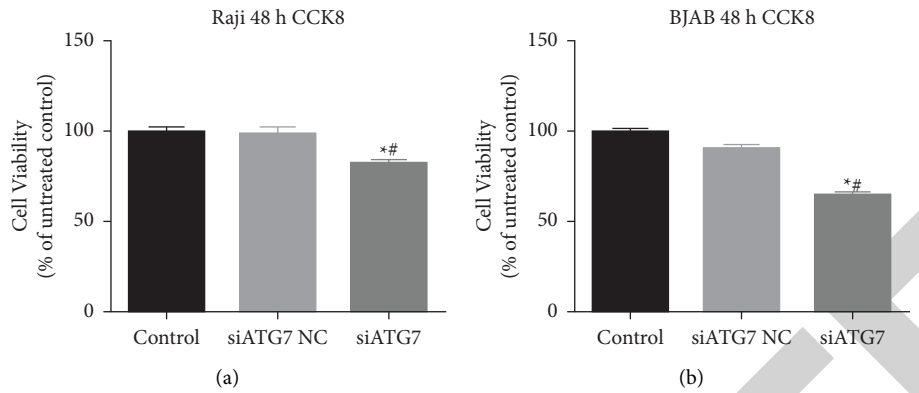

FIGURE 7: The viability of Raji and BAJB cells was detected by CCK8. (a) Raji cells; (b) BAJB cells. $^{*}P < 0.05$ vs. control; $^{\#}P < 0.05$ vs. siATG7 NC.

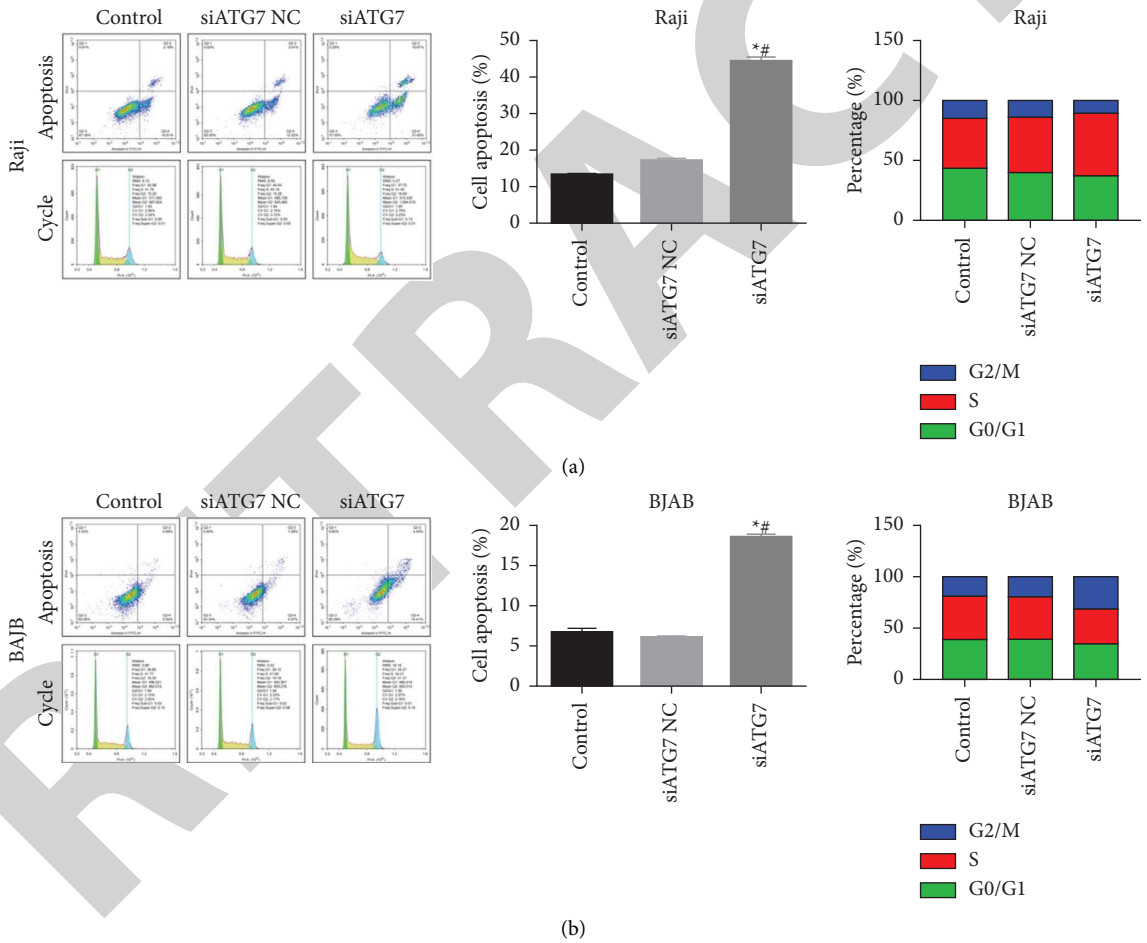

FIGURE 8: The apoptosis and cell cycle detected by flow cytometry. (a) Raji cells; (b) BAJB cells. $^{*}P < 0.05$ vs. control; $^{\#}P < 0.05$ vs. siATG7 NC.

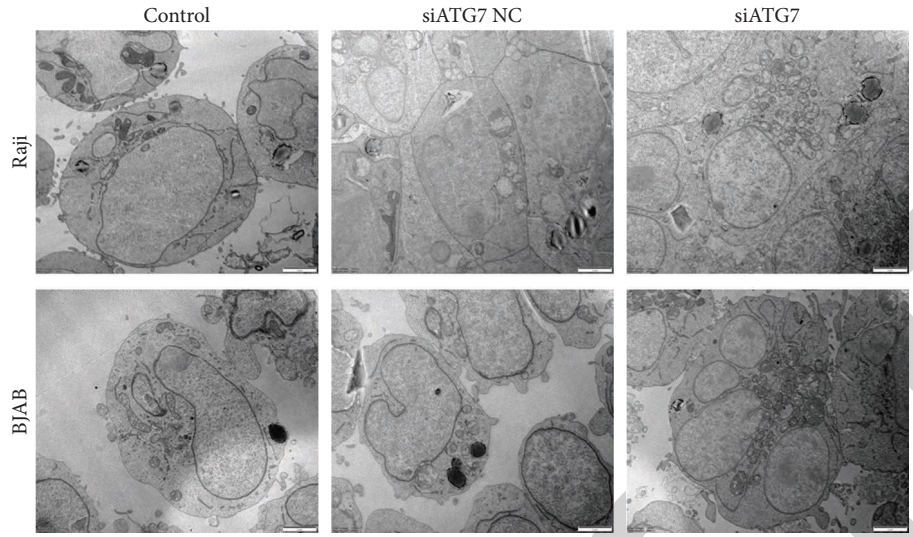

Figure 9: Effect of siATG7 on the autophagy of lymphoma cells.

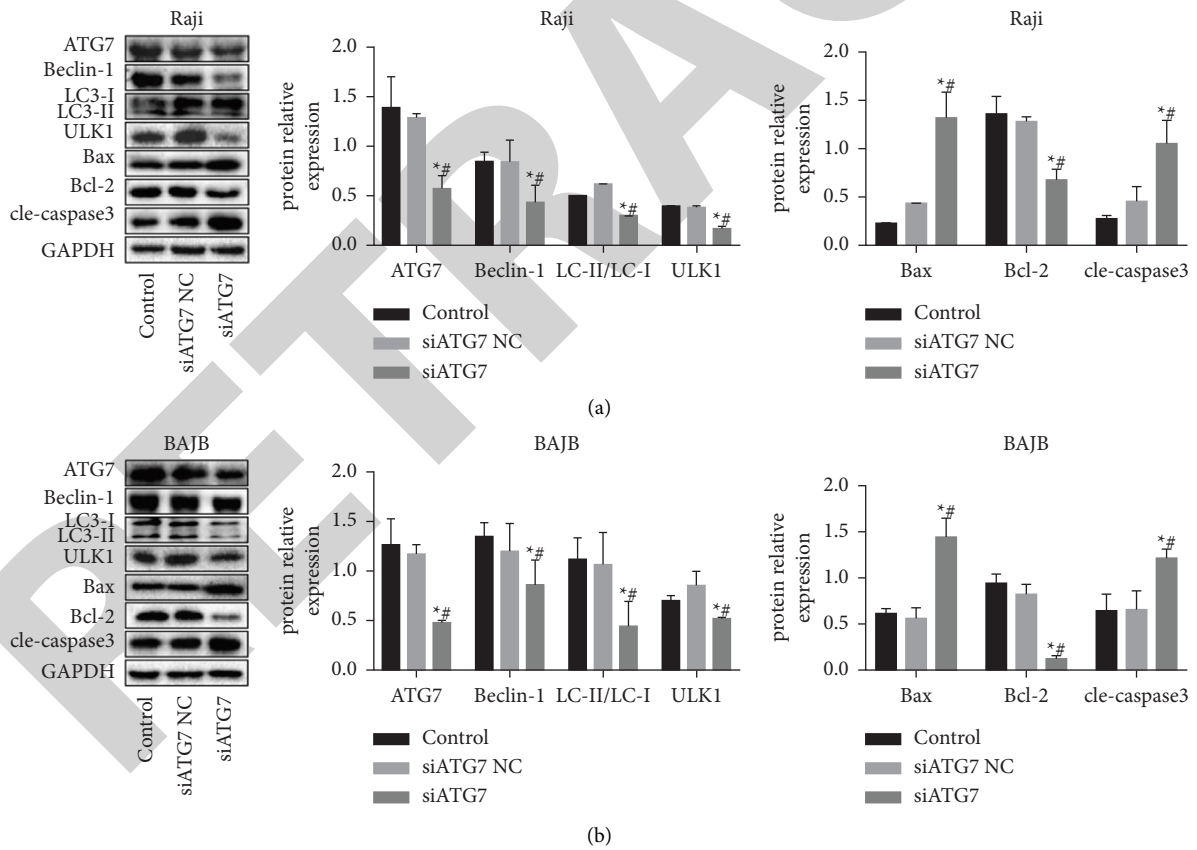

Figure 10: Western blotting results of autophagy and apoptosis related proteins in Raji and BAJBcells. (a) Raji cells; (b) BAJB cells. $^{*}P < 0.05$ vs. control; $^{\#}P < 0.05$ vs. siATG7 NC.

5. Conclusions

In conclusion, this study found that the proliferation, apoptosis, and autophagy of Raji and BJAB lymphoma cells induced by the LncHOTAIR overexpression may be realized by autophagy-related protein ATG7. In BL lymphoma cells, ATG7 may promote abnormal cell proliferation and inhibit apoptosis by mediating autophagy.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Fu Gui and Xinyi Yu are equal contributors.

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Retraction

Retracted: Carbetocin Controls Intraoperative Blood Loss and Thickness of Myometrium in Scar Uterus Cases

Evidence-Based Complementary and Alternative Medicine

Received 20 June 2023; Accepted 20 June 2023; Published 21 June 2023

This article has been retracted by Hindawi following an investigation undertaken by the publisher [1]. This investigation has uncovered evidence of one or more of the following indicators of systematic manipulation of the publication process:

(1) Discrepancies in scope
(2) Discrepancies in the description of the research reported
(3) Discrepancies between the availability of data and the research described
(4) Inappropriate citations
(5) Incoherent, meaningless and/or irrelevant content included in the article
(6) Peer-review manipulation

The presence of these indicators undermines our confidence in the integrity of the article's content and we cannot, therefore, vouch for its reliability. Please note that this notice is intended solely to alert readers that the content of this article is unreliable. We have not investigated whether authors were aware of or involved in the systematic manipulation of the publication process.

In addition, our investigation has also shown that one or more of the following human-subject reporting requirements has not been met in this article: ethical approval by an Institutional Review Board (IRB) committee or equivalent, patient/participant consent to participate, and/or agreement to publish patient/participant details (where relevant).

Wiley and Hindawi regrets that the usual quality checks did not identify these issues before publication and have since put additional measures in place to safeguard research integrity.

We wish to credit our own Research Integrity and Research Publishing teams and anonymous and named external researchers and research integrity experts for contributing to this investigation.

The corresponding author, as the representative of all authors, has been given the opportunity to register their agreement or disagreement to this retraction. We have kept a record of any response received.

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[1] D. Sun, "Carbetocin Controls Intraoperative Blood Loss and Thickness of Myometrium in Scar Uterus Cases," *Evidence-Based Complementary and Alternative Medicine*, vol. 2022, Article ID 5477432, 5 pages, 2022.

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Research Article

Carbetocin Controls Intraoperative Blood Loss and Thickness of Myometrium in Scar Uterus Cases

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Objective. To study the effect of carbetocin on intraoperative blood loss and thickness of myometrium during cesarean section with the scarred uterus at term pregnancy. *Methods*. Pregnant women with full-term gestational scar uterus who underwent cesarean section from March 1, 2021, to April 30, 2022, were retrospectively collected and divided into a reference group (using oxytocin) or a study group (using carbetocin). The clinical data of the two groups were retrospectively analyzed, and the operation time, intraoperative blood loss, hospital stay, uterine contraction effect, changes in the myometrium, and complications were compared between the two groups. *Results*. A total of 103 pregnant women were retrieved. There were 44 cases in the reference group and 59 cases in the study group. There were significant differences in operation time, intraoperative bleeding, hospital stay, postoperative adverse events, uterine fundus wall thickness, anterior wall thickness, posterior wall thickness, and uterine contraction effect between the two groups (p = 0.0001, 0.005, 0.006, 0.001, 0.0004, 0.003, 0.001, and 0.005, respectively). There were no significant differences in estradiol (E2), luteinizing hormone (LH), or follicle-stimulating hormone (FSH) between the two groups before the surgery (p = 0.596, 0.840, and 0.940, respectively), but there were significant differences after the surgery (p = 0.011, 0.001, and 0.005, respectively). *Conclusion*. The use of carbetocin in the cesarean section of a full-term scar uterus is significantly effective in shortening the operation time, reducing the amount of intraoperative blood loss, and promoting the recovery of the uterus.

1. Introduction

The scar in the uterine wall area after cesarean section or myomectomy is defined as the uterine scar. A second cesarean section is generally performed after the scarred uterus is pregnant, because the elasticity of the muscle fibers in the scar area located in the lower uterus will be significantly reduced, which will lead to weak uterine contractions, resulting in postpartum hemorrhage. At the same time, the scar will also cause certain negative effects on the recovery of the uterus. This leads to endometrial and pelvic infections, which aggravate postpartum morbidity and pain in mothers. Therefore, drugs that can increase uterine contractions should be used to reduce the risk of postpartum hemorrhage [1–3]. Therefore, we showed in this study the effect of carbetocin on intraoperative blood loss and thickness of myometrium during a cesarean section of full-term pregnancy scar uterus.

2. Materials and Methods

Pregnant women with full-term gestational scar uterus who underwent cesarean section from March 1, 2021, to April 30, 2022, were retrospectively collected and divided into a reference group (using oxytocin) or a study group (using carbetocin). Inclusion criteria were as follows: full-term pregnancy; scarred uterus; cesarean section required; and no related treatment was performed prior to inclusion in the study. Exclusion criteria were as follows: coagulation dysfunction; malignant tumors; chronic diseases such as diabetes, pregnancy-induced hypertension, etc.; mental illness; or allergic to drugs.

The clinical data of the two groups were retrospectively collected, and the operation time, intraoperative blood loss, hospital stay, uterine contraction effect, changes in the myometrium, and complications were compared between

the two groups. All patients in this study gave informed consent. This study was approved by the institutional ethical committee of our hospital.

All cases underwent cesarean section of the lower uterine segment after spinal anesthesia. The control group was given 100 micrograms of oxytocin, and the study group was given 100 micrograms of carbetocin during c-section.

2.1. Statistical Methods. Data were analyzed by SPSS21.0 ((SPSS, Chicago, IL, USA), in which the ^{2} (%) tests were performed for the count data, and the t-test ($x \pm s$) tests were performed for the measurement data. A two-sided $p < 0.05$ was determined as the threshold of statistical significance.

3. Results

3.1. General Information of the Two Groups of Patients. There was no significant difference in clinical characteristics between the two groups (Table 1).

3.2. Comparison of Operative Time, Intraoperative Bleeding, and Hospitalization Time. There were significant differences in operation time (82.18 ± 10.01 vs 104.28 ± 10.33), intraoperative bleeding (210.36 ± 25.89 vs 316.27 ± 20.57), hospital stay (5.86 ± 2.51 vs 7.86 ± 2.57) between the study group and the reference group ($p = 0.0001$, 0.005, and 0.006, respectively). See Table 2 for details.

3.3. Comparison of Adverse Events. There was a significant difference in the incidence of postoperative adverse events between the study group and the control group ($p = 0.001$). See Table 3 for details.

3.4. Comparison of Uterine Thickness. There were significant differences in uterine fundus wall thickness (9.18 ± 1.01 vs 7.28 ± 1.33), anterior wall thickness (8.36 ± 1.89 vs 6.27 ± 1.57), and posterior wall thickness (8.86 ± 1.51 vs 6.86 ± 1.57) between the study group and the control group ($p = 0.0004$, 0.003, and 0.001, respectively). See Table 4 for details.

3.5. Comparison of Uterine Contraction Effect. There was a significant difference in obvious uterine contraction effect between the study group (53/59) and the reference group (36/44, $p = 0.005$, Table 5).

3.6. Comparison of Sex Hormone Levels. Before c-section, there were no significant differences in estradiol (E2, (75.03 ± 9.15 vs 74.13 ± 9.07), luteinizing hormone (LH, 3.65 ± 4.51 vs 13.52 ± 4.42), or follicle-stimulating hormone (FSH, 5.52 ± 1.51vs 5.54 ± 1.35) between the study group and the reference group ($p = 0.596$, 0.840, and 0.940, respectively). However, after c-section, there were significant differences in E2 (60.96 ± 12.35 vs 66.37 ± 10.07), LH (7.65 ± 2.21 vs 9.25 ± 3.35), FSH (2.08 ± 0.79 vs 3.59 ± 0.51) between the study group and the control group ($p = 0.011$, 0.001, and 0.005, respectively, Table 6).

4. Discussion

In recent years, due to the continuous development of anesthesia and cesarean section technology, the incidence of cesarean section in China has been as high as 80% in some places. With the implementation of two- and three-child policies, the cicatricial uterus is more common in obstetrics [4]. Evidence shows that when the uterus contracts during the delivery process, the pressure in the uterine cavity will increase, which may cause the rupture of the uterine scar. Furthermore, the elastic plasticity and contractility of the local muscles will be reduced in the scarred uterus, resulting in postpartum hemorrhage. By 6 weeks after a natural delivery, the uterine lining has completely healed, and the whole uterus will return to normal [5–7]. However, this procedure is significantly slowed down in the scarred uterus, and the lochia is difficult to be excreted completely, which affects the contractility of the uterus, prolongs the recovery time of the uterus, and causes poor uterine involution.

Because uterine atony is an important factor leading to massive postpartum hemorrhage, oxytocin-family drugs are used in the routine cesarean section to decrease the risk of postpartum hemorrhage. Oxytocin works quickly, but its half-life is generally 3–4 minutes, requiring multiple or continuous applications. When the dose exceeds 60 U, there will be obvious receptor saturation, which significantly weakens the effect on the lower uterine muscle group. In addition, taking large doses of oxytocin can also cause side effects such as hypotension and arrhythmia. Our study showed that carbetocin has a good hemostatic effect, and the effect is rapid and long-lasting [8–10]. This method has been well applied in the cesarean section of pregnancy with a uterine scar.

Because fibrous scars significantly reduce the contraction and elongation of the uterus, the scarred uterus is likely to have uterine rupture during pregnancy, vaginal delivery is not recommended. Cesarean section is a common gynecological operation, and its surgical methods and procedures have become mature [11]. But postpartum hemorrhage remains a serious surgical complication that can cause maternal death. In a scarred uterus, the contractile function of the uterine muscle fibers is disrupted, thereby increasing the tension on the surrounding muscle fibers. Therefore, postpartum hemorrhage is common. Improving uterine contractile function is an important measure to prevent postpartum hemorrhage [12, 13]. Oxytocin, misoprostol, ergometrine, etc. are all drugs that can promote uterine contractions. Oxytocin is currently the most often used drug in Obstetrics. It binds to the receptors in the upper uterine segment, and after the receptor site is saturated, it promotes the contraction of the upper uterine segment, but a further increase in the dosage is ineffective anymore. Misoprostol, a derivative of prostaglandins, has a strong effect on smooth muscle contraction, but can also cause side effects such as fever, nausea, and vomiting. Carbetocin is a synthetic, long-acting oxytocin analog with stimulant effects. It can also

Table 1: General information of patients.

Generally	Study group	Reference group	t/χ^2	p
n	59	44		
Age	28.43 ± 4.39	27.51 ± 4.92	1.812	0.074
Parity			10.853	0.791
1	33	25		
>1	26	19		
Nationality			4.927	0.858
Han	56	42		
Other	3	2		
Education level			15.864	0.749
Primary school	11	5		
Junior high school	13	9		
High school	17	11		
University and above	18	19		

Table 2: Comparison of bleeding between two groups of patients ($\overline{x} \pm s$).

Group	Operation time (min)	Intraoperative blood loss (ml)	Hospital stay (d)
Reference group ($n = 44$)	104.28 ± 10.33	316.27 ± 20.57	7.86 ± 2.57
Study group ($n = 59$)	82.18 ± 10.01	210.36 ± 25.89	5.86 ± 2.51
T	7.943	11.274	9.538
p	0.001	0.005	0.006

Table 3: Comparison of adverse reactions between the two groups of patients ($\overline{x} \pm s$).

Group	Headache	Dizziness	Nausea	Vomit	Total incidence
Reference group ($n = 44$)	3	4	3	1	11 (25.00)
Study group ($n = 59$)	2	1	2	0	5 (8.47)
t					11.139
p					0.001

Table 4: Comparison of uterine thickness between two groups of patients ($\overline{x} \pm s$).

Group	Bottom wall (mm)	Front wall (mm)	Back wall (mm)
Reference group ($n = 44$)	7.28 ± 1.33	6.27 ± 1.57	6.86 ± 1.57
Study group ($n = 59$)	9.18 ± 1.01	8.36 ± 1.89	8.86 ± 1.51
T	10.153	9.365	11.427
P	0.004	0.003	0.001

Table 5: Comparison of uterine contraction effect between two groups of patients (n, %).

Group	Obvious contractions	Subtle contractions
Study group ($n = 59$)	55 (93.22)	4 (6.78)
Reference group ($n = 44$)	35 (79.55)	9 (20.45)
chi-square test		4.274
p		0.039

Table 6: Changes in T, LH, and FSH levels before and after birth ($\overline{x} \pm s$).

Group	n	E2 (ng/dl)		LH (mIU/ml)		FSH (mIU/ml)	
		Prenatal	Postpartum	Prenatal	Postpartum	Prenatal	Postpartum
Study group	59	75.03 ± 9.15	60.96 ± 12.35a	13.65 ± 4.51	7.65 ± 2.21a	5.52 ± 1.51	2.08 ± 0.79
Reference group	44	74.13 ± 9.07	66.37 ± 10.07a	13.52 ± 4.42	9.25 ± 3.35a	5.54 ± 1.35	3.59 ± 0.51
t	—	0.532	12.583	0.202	8.934	0.075	10.033
p	—	0.596	0.011	0.840	0.001	0.940	0.005

combine with oxytocin receptors to cause uterine smooth muscle contraction, and hemostasis can be achieved by squeezing blood vessels in the muscle layer [14, 15]. In this study, the operation time, intraoperative bleeding, and hospital stay of the study group were all less than those of the control group. The incidence of postoperative adverse events in the study group was also lower than that in the control group. The uterus in the study group was thicker than that in the control group, and the uterine contraction effect was also more obvious than that in the control group, which further showed the superiority of carbetocin. However, the application of carbetocin in other severe conditions [16–23], as well as its efficacy compared with other choices [24] still needs to be validated.

To sum up, the use of carbetocin in the cesarean section of the full-term scarred uterus has a significant effect, which can effectively shorten the operation time, reduce the amount of intraoperative blood loss, and promote the recovery of the thickness of the anterior and posterior uterine walls and the myometrium, with fewer adverse events.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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Retraction

Retracted: Comparison of Iliac Bone Transplantation with Bone Transport in the Treatment of Femur Fracture and Bone Defect

Evidence-Based Complementary and Alternative Medicine

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This article has been retracted by Hindawi following an investigation undertaken by the publisher [1]. This investigation has uncovered evidence of one or more of the following indicators of systematic manipulation of the publication process:

(1) Discrepancies in scope
(2) Discrepancies in the description of the research reported
(3) Discrepancies between the availability of data and the research described
(4) Inappropriate citations
(5) Incoherent, meaningless and/or irrelevant content included in the article
(6) Peer-review manipulation

The presence of these indicators undermines our confidence in the integrity of the article's content and we cannot, therefore, vouch for its reliability. Please note that this notice is intended solely to alert readers that the content of this article is unreliable. We have not investigated whether authors were aware of or involved in the systematic manipulation of the publication process.

In addition, our investigation has also shown that one or more of the following human-subject reporting requirements has not been met in this article: ethical approval by an Institutional Review Board (IRB) committee or equivalent, patient/participant consent to participate, and/or agreement to publish patient/participant details (where relevant).

Wiley and Hindawi regrets that the usual quality checks did not identify these issues before publication and have since put additional measures in place to safeguard research integrity.

We wish to credit our own Research Integrity and Research Publishing teams and anonymous and named external researchers and research integrity experts for contributing to this investigation.

The corresponding author, as the representative of all authors, has been given the opportunity to register their agreement or disagreement to this retraction. We have kept a record of any response received.

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[1] W. Huang, W. Zhu, and W. Lu, "Comparison of Iliac Bone Transplantation with Bone Transport in the Treatment of Femur Fracture and Bone Defect," *Evidence-Based Complementary and Alternative Medicine*, vol. 2022, Article ID 5358923, 6 pages, 2022.

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Research Article

Comparison of Iliac Bone Transplantation with Bone Transport in the Treatment of Femur Fracture and Bone Defect

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Objective. To compare the curative effect of iliac bone transplantation with the bone transport in the treatment of femur fracture complicated with a bone defect. *Methods*. Patients with femur fractures and defects who were admitted to our hospital from January 1, 2020, to January 31, 2022, and met the inclusion criteria were retrospectively selected and allocated into an iliac bone transplantation group or a bone transport group. The treatment effect and quality of life of the two groups were compared. *Results*. A total of 98 patients who met the inclusion standards were enrolled, including 50 cases in the iliac bone transplantation group and 48 cases in the bone transport group. There were no significant differences in IL-6, IL-8, TNF-α, visual analog scale (VAS) score, or Japanese Orthopedic Association (JOA) score between the two groups on postsurgical day 1 ($p = 0.051$, 0.150, 0.102, 0.564, and 0.826 respectively), but there were significant differences in the above index on postsurgical day 7 (all $p < 0.01$). There were no significant differences in social function, physical function, role function, and cognitive function between the two groups one week after the operation ($p = 0.245$, 0.051, 0.102, and 0.067, respectively), but there were significant differences in the above parameters at one month after operation ($p = 0.001$, 0.005, 0.005, and 0.001, respectively). The total effective rate of the bone transplantation group was significantly better than that of the iliac bone transplantation group ($p = 0.026$). The number of postoperative complications in the bone removal group was significantly fewer than that of the iliac bone graft group ($p = 0.001$). *Conclusion*. Bone transport is effective in treating femur fractures complicated with bone defects, with fewer postoperative complications.

1. Introduction

A femur fracture is a relatively common disease, mainly caused by traffic accidents and falls from height, often accompanied by bone defects. The treatment methods for such patients are complicated; the results are not ideal; and the incidence of disability is high. During surgery, large pieces of sequestrum need to be removed, and local bone defects will also occur during the removal, which has a certain impact on the postoperative recovery of patients [1]. At present, for such patients, autologous iliac bone transplantation is mostly used. Although this method has a certain osteogenic effect, the operation is complicated and the complication rate is high. Bone transplantation is based on the principle of "tension-stress law." Extracorporeal puncture technology has been used to fix steel nails on the bone at the fracture site and gradually elongate the bone through the force of osteotomy. Both the extension and the compression zone allow callus formation until the bone defect heals [2, 3]. Here, we showed the efficacy of autologous iliac bone transplantation vs. bone transport in the treatment of femur fractures and bone defects.

2. Materials and Methods

2.1. Methods. Patients with femur fractures and defects who were admitted to our hospital from January 1, 2020, to January 31, 2022, who met the inclusion criteria were

retrospectively selected. All patients in this study gave informed consent, and the patients themselves or their representatives signed the relevant consent forms. The inclusion criteria were as follows: femur fracture with the bone defect; surgical treatment required; and no relevant treatment was performed prior to inclusion in the study. The exclusion criteria were as follows: accompanied by malignant diseases, severe infection, organ failure, malignancy, mental illness, or drug allergy. The study protocol was approved by the institutional ethical committee of our hospital.

Patients in the iliac bone transplantation group received autologous iliac bone transplantation, whereas patients in the bone transport group received surgical bone transport [4]. Patients in both groups were treated with broad-spectrum antibiotics within 2 days of surgery to prevent swelling of the limbs. One week after the operation, the observation group continued to stretch for 5 days on the 8th day, and X-ray examinations were taken to observe the changes in osteotomy distraction. After 5 days of traction, the traction was carried out at a speed of 0.75 mm/day starting from 4 days. An X-ray examination was performed 4 weeks after the operation in the treatment group.

2.2. Evaluation of Efficacy. Visual analog scale (VAS) pain scale [5]: 0 is the best, no pain; 1–3 points or less mild pain, tolerable; 4–6 points or the pain is more severe, slightly affecting sleep; and 7–10 points or unbearable severe pain, inability to sleep seriously affects the normal life. The Japanese Orthopedic Association (JOA) score was used to evaluate the treatment efficacy, including subjective symptoms, clinical signs, and daily activities limitation. The total score is 0 to 29 points, and the higher the score, the better the function. The quality of life score (out of 100) was used, including social function, role function, cognitive function, and physical function [6]. The higher the score, the better the quality of life.

2.3. Statistical Methods. The data in this experiment need to be verified by SPSS21.0 software (SPSS, Chicago, IL, USA), in which the count data (n, %) were analyzed by χ^2 test, and the continuous data (mean ± SD) were analyzed by t-test. $p < 0.05$ (2-tailed) was set as the threshold of statistical significance.

3. Results

A total of 98 patients who met the inclusion standards were enrolled. All patients in this study gave informed consent, and the patients themselves or their representatives signed the relevant consent forms. The baseline data of the included subjects are detailed below (Table 1).

3.1. Comparison of Inflammatory Factors. On the 1st day after surgery, there was no significant difference in IL-6, IL-8, and TNF-α between the study group ((45.85 ± 3.64) (74.41 ± 5.37) and (67.23 ± 6.21)) and the control group ((46.63 ± 2.82), (75.45 ± 5.18) and (67.52 ± 6.31)) ($t = 2.315$, 2.175, 2.452, $p = 0.051$, 0.150, 0.102). Seven days after surgery, there were significant differences in IL-6, IL-8, and TNF-α between the study group ((22.83 ± 1.35), IL-8 (45.53 ± 3.82) and (42.37 ± 5.36)) and the control group ((28.58 ± 3.26), IL-8 (54.32 ± 4.27) and (55.85 ± 6.24)) ($t = 11.001$, 11.014, 10.310, $p = 0.001$, 0.001, 0.000). See Table 2 for details.

3.2. Comparison of VAS Score and JOA Score between the Two Groups before and after Surgery. There was no significant difference in the VAS score (7.58 ± 2.13) and JOA score (12.57 ± 0.38) of the study group compared with the control group (7.47 ± 2.25) (12.26 ± 0.50), before surgery ($t = 1.673$, 1.538, $p = 0.564$, 0.826). After surgery, the VAS score (4.41 ± 0.56) and JOA score (19.50 ± 0.51) of the study group were significantly different from those of the control group (4.36 ± 0.89) (19.41 ± 0.69) ($t = 12.274$, 8.379, $p = 0.005$, 0.000). See Table 3 for details.

3.3. Comparison of Quality of Life. One week after operation, there was no significant difference in social function, physical function, role function, and cognitive function between the study group ((63.80 ± 3.62), (64.23 ± 5.51), (67.00 ± 6.02), and (62.97 ± 4.28)) and the control group ((65.61 ± 2.80), (64.35 ± 5.08), (66.53 ± 6.25), and (64.33 ± 3.14)) ($t = 2.019$, 1.631, 1.461, 2.130, $p = 0.245$, 0.051, 0.102, and 0.067, respectively). One month after operation, there were significant differences in social function, physical function, role function, and cognitive function between the study group ((82.84 ± 1.15), (84.50 ± 3.80), (83.30 ± 5.38), and (80.61 ± 4.85)) and the control group ((79.13 ± 3.20), (75.44 ± 4.26), (76.82 ± 6.34), and (74.35 ± 3.52)) ($t = 15.943$, 12.005, 13.325, 10.142, $p = 0.001$, 0.005, 0.005, and 0.001, respectively). See Table 4 for details.

3.4. Comparison of Treatment Effects. The total effective rate of the bone transplantation group was 93.75% (45/48), which was significantly better than that of the iliac bone transplantation group 78.00% (39/50, $\chi^2 = 4.961$, $p = 0.026$, Table 5).

3.5. Comparison of Postoperative Complications. Postoperative complications occurred in patients in the bone removal group: delayed fracture union (3 cases), osteoarthritis (6 cases), infection (3 cases), pulmonary embolism (1 case), and the total incidence of complications was 27.08%. Compared with the iliac bone graft group: delayed fracture union (5 cases), osteoarthritis (7 cases), infection (6 cases), pulmonary embolism (3 cases), and the total incidence of complications was 42.00%, there was a significant difference between the two groups ($\chi^2 = 2.405$, $p = 0.121$). See Table 6.

4. Discussion

Evidence published over the past few decades has led to some consensus on the surgical management of femur fractures. However, in daily clinical practice, the exact choice of the implant is still unclear to the individual surgeon,

Table 1: General information of patients.

Characteristics	Iliac bone graft group	Bone transport group	t/χ^2 value	p value
Number of cases	50	48		
Age	36.43 ± 5.43	35.28 ± 6.81	1.812	0.074
Gender			0.397	0.529
Male	26	28		
Female	24	20		
Nationality			2.124	0.000
Han nationality	48	43		
Other	2	5		
Whether they have other bone diseases in the past			0.201	0.654
Yes	0	0		
No	45	45		
Bone defect site			0.544	1.421
Femur condyle	31	27		
Mid femur	19	21		
Smoking			0.521	0.000
Never	34	33		
Have	16	15		
Alcohol drinking			0.142	0.003
Never	21	23		
Have	29	25		
Hypertension/diabetes/cerebrovascular disease			1.244	0.012
None	20	26		
Have	30	22		

Table 2: Comparison of inflammatory factors ($\overline{x} \pm s$).

Group	Case	IL-6 (μg/L)		IL-8 (pg/L)		TNF-α (ng/L)	
		Day 1 after surgery	Day 7 after surgery	Day 1 after surgery	Day 7 after surgery	Day 1 after surgery	Day 7 after surgery
Iliac bone graft group	50	46.63 ± 2.82	28.58 ± 3.26	75.45 ± 5.18	54.32 ± 4.27	67.52 ± 6.31	55.85 ± 6.24
Bone transport group	48	45.85 ± 3.64	22.83 ± 1.35	74.41 ± 5.37	45.53 ± 3.82	67.23 ± 6.21	42.37 ± 5.36
T		2.315	11.001	2.175	11.014	2.452	10.310
p		0.051	0.001	0.150	0.001	0.102	0.000

Table 3: Comparison of VAS scores and JOA scores between the two groups ($\overline{x} \pm s$).

Group	VAS score		JOA score	
	Before surgery	After surgery	Before surgery	After surgery
Bone transport group ($n = 48$)	7.58 ± 2.13	3.10 ± 0.18	12.57 ± 0.38	23.42 ± 0.36
Iliac bone graft group ($n = 50$)	7.47 ± 2.25	4.41 ± 0.56	12.26 ± 0.50	19.50 ± 0.51
T	1.673	12.274	1.538	8.379
p	0.564	0.005	0.826	0.000

necessitating the use of an easy-to-use, evidence-based surgical approach that covers all types of femur fractures. Many articles recommend treatment of some aspect of surgery, but only a few authors have published a more or less explicit decision tree algorithm for surgical management of proximal femur fractures [5, 7, 8]. In some Western European countries, national guidelines for many aspects of hip fracture management have evolved over the past decade, including recommendations for surgical options for implants.

In car accidents, femur fracture combined with the bone defect is most common, and in accidents such as falls from height and heavy object crushing, due to the high risk of femur fracture combined with bone defect, the treatment is complicated, the prognosis is poor, and the disability rate is high. Bone displacement is based on bone extension, and 1 mm of bone movement is evidenced every day so that the soft tissue is in a state of tension. Vascular endothelial cells will gradually move to polymorphic mesenchymal cells and to some extent evolve into osteoblasts [9–12].

Table 4: Comparison of quality of life between the two groups of patients ($\overline{x} \pm s$).

Group	Social function		Physical function		Role function		Cognitive function	
	1 week after surgery	1 month after surgery	1 week after surgery	1 month after surgery	1 week after surgery	1 month after surgery	1 week after surgery	1 month after surgery
Iliac bone graft group	65.61 ± 2.80	79.13 ± 3.20	64.35 ± 5.08	75.44 ± 4.26	66.53 ± 6.25	76.82 ± 6.34	64.33 ± 3.14	74.35 ± 3.52
Bone transport group	63.80 ± 3.62	82.84 ± 1.15	64.23 ± 5.51	84.50 ± 3.80	67.00 ± 6.02	83.30 ± 5.38	62.97 ± 4.28	80.61 ± 4.85
T	2.019	15.943	1.631	12.055	1.461	13.325	2.130	10.142
p	0.245	0.001	0.051	0.005	0.102	0.005	0.067	0.001

Table 5: Comparison of clinical efficacy between the two groups of patients (cases (%)).

Group	Effective rate	Ineffective rate
Iliac bone graft group ($n = 50$)	39 (78.00)	11 (22.00)
Bone transport group ($n = 48$)	45 (93.75)	3 (6.25)
χ^2	4.961	
p	0.026	

Table 6: Comparison of postoperative complications between the two groups (cases, %).

Group	Delayed fracture union	Osteoarthritis	Infection	Pulmonary embolism	The total incidence of complications
Iliac bone graft group ($n = 50$)	5 (10.00)	7 (14.00)	6 (12.00)	3 (6.00)	21 (42.00)
Bone transport group ($n = 48$)	3 (6.25)	6 (12.50)	3 (6.25)	1 (2.08)	13 (27.08)
χ^2			—		2.626
p			—		0.105

With the elongation of bone, the callus site changes to a certain extent, and the central fibroblasts gradually differentiate into a parallel structure similar to the bone. Femur fractures and bone defects can be effectively treated by shaping the trabecular plate [13]. Bone grafting can completely eliminate sequestrum and has a traction effect on osteogenesis without artificial bone grafting. Autologous iliac bone transplantation technology builds a three-dimensional structure in the human body through bone tissue, induces bone conduction through osteogenesis, and realizes the fusion of bone junctions. Because the iliac bone contains a large amount of cancellous bone and medullary cavity and because the site where it is located is easy to cut, a good bone connection is formed after transplantation [14, 15]. However, different from the traditional artificial pelvis transplantation, after the autologous iliac bone flap transplantation, the soft tissues such as skin flaps and tendons in the donor and recipient areas must be removed, leading to many incisions, complicated procedures, and long operation times. The amount of bleeding during surgery increases, the incidence of postoperative trauma increases, and the probability of postoperative complications also increase. In the surgical treatment of femur fracture and bone defect, if the wound is large and there are multiple wounds, the blood flow at the fracture will be aggravated, and the surrounding soft tissue will be damaged, easily leading to postoperative infection, and the surrounding tissue will be necrotic or fractured. Healing in this situation is slow or complete recovery is not possible [16, 17]. Because of its simple operation, small trauma, and faster bone repair, bone transplant surgery is used in clinical practice. In this study, the surgical effect of the patients in the bone transport group was better than that in the iliac bone transplantation group, and the recovery indices were also faster than those in the iliac bone transplantation group. The applications of bone transport in other conditions are infections, wound healing problems, or neurological disorders [18–23].

In conclusion, bone transport is effective in the treatment of femur fracture and bone defects, and the incidence of postoperative complications is low.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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Review Article

Tongue Acupuncture for the Treatment of Poststroke Aphasia: A Systematic Review and Meta-Analysis

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Objective. This review evaluated the efficacy of tongue acupuncture for the clinical treatment of poststroke aphasia. *Methods*. PubMed, Medline, Cochrane, Embase, CNKI, VIP, and Wanfang databases were searched from their inception to 1st June 2022. The dataset included randomized controlled trials (RCTs) with tongue acupuncture for the treatment of poststroke aphasia. Data aggregation and risk of bias evaluation were conducted on Review Manager Version 5.4.1 and Stata16.0. The main outcome measures included the Aphasia Battery of Chinese (ABC), the Chinese Functional Communication Profile (CFCP), the Boston Diagnostic Aphasia Examination (BDAE), and clinical efficiency. Then, comparing the effectiveness of tongue acupuncture, tongue acupuncture combined with conventional therapies, conventional therapies with head acupuncture, language training, body acupuncture, and Jie Yu Dan. *Results*. A total of 20 studies with 1355 patients were included. Meta-analysis showed that compared with conventional treatments, tongue acupuncture has a significant improvement in clinical efficacy score (MD = 1.25, 95% CI (1.09, 1.43), $P = 0.001$) and CFCP of poststroke aphasia (MD = 39.78, 95% CI (26.59, 52.97), $P < 0.00001$), but was not significant in improving ABC (MD = 5.95, 95% CI (2.85, 9.04), $P = 0.06$). Compared to the conventional treatments, tongue acupuncture combined with conventional therapies promoted the ABC (MD = 11.48, 95% CI (2.20, 20.75), $P < 0.00001$), clinical efficacy score (MD = 1.22, 95% CI (1.14, 1.30), $P < 0.00001$), and CFCP score (MD = 29.80, 95% CI (19.08, 40.52), $P < 0.00001$) of poststroke aphasia. *Conclusion*. This systematic review indicated that tongue acupuncture or tongue acupuncture combined with conventional treatments was an effective therapy for treating poststroke aphasia. However, stricter evaluation standards and rigorously designed RCTs are needed.

1. Background

Stroke is one of the most common neurovascular diseases, with a global prevalence of 101.5 million (2021, latest statistics on heart disease and stroke), among which ischemic stroke accounts for 76 percent (77.2 million) [1]. Ischemic stroke refers to ischemic necrosis or softening of localized cerebral tissue caused by cerebral blood circulation disorder, ischemia, hypoxia, and corresponding symptoms of neurological dysfunction [2]. Ischemic stroke is one of the serious diseases with the highest morbidity and mortality in the world. Stroke is the second leading cause of death among the top ten causes of death in the world, released by the World Health Organization [3]. It is also one of the main causes of severe disability and cognitive impairment worldwide [4].

However, the only effective treatment in the acute phase of ischemic stroke is the application of thrombolytic drugs. Although the emerging endovascular thrombectomy has extended the time window to 24 hours [5], most survived patients still have severe neurological symptoms. Poststroke aphasia (PSA) is one of the most devastating symptoms among the various functional disabilities caused by stroke [6]. More than one-third of stroke survivors suffer from aphasia, in which 30–43% remain chronically affected [7].

The main treatment measures recommended in the "Guidelines for the Diagnosis and Treatment of Cerebral

Infarction in China (2017)" include syndrome differentiation and treatment combined with acupuncture and moxibustion [8]; in these guidelines, acupuncture therapy was recommended as a clinical physical therapy method with the exact curative effect on central nervous system diseases [9]. In recent years, acupuncture has made significant progress in the treatment of PSA. There are hypoglossal nerve, glossopharyngeal nerve, trigeminal nerve, and facial nerve distributed in the tongue, as well as rich peripheral nerve; the stimulating tongue can enhance the excitability of the central nervous system, promote nerve reflex, regulate the cortex-thalamus-cortex, balance the specific conduction and nonspecific conduction of the body, rebuild the neural circuit of language activity, and promote the recovery of language [10]. Many studies have shown that when the tongue is stimulated, the quiescent state of the language function of the cerebral cortex can be activated; then, the local stimulation acts as a communication circuit, which forms conditioned reflexes and improves language ability.

In the theory of traditional Chinese medicine, stroke damages the brain and spinal cord in the blood vessels, and its occurrence and recovery involve the dynamic pathological changes in multiple links of "blood-vessel-heart-spirit." The heart opens at the tongue, and the heart is the official of the monarch. The heart area of the tongue is mainly used to treat diseases related to the heart, blood vessels, nerves, and the mental system, such as the Juquan acupoint is in the spleen and the stomach area of the tongue. The spleen manages the transportation and transformation of blood and qi. Therefore, stimulating specific acupoints of the tongue may have positive curative effects through the dynamic pathological changes of "blood-pulse-heart-spirit."

Tongue acupuncture and its combination therapies (head acupuncture, language training, body acupuncture, and Jie Yu Dan) are gaining more and more attention from researchers, and some clinical studies have also described the clinical application of tongue acupuncture in the treatment of poststroke aphasia, but their quality has not been systematically evaluated. In addition, there was no meta-analysis for tongue acupuncture of poststroke aphasia in the past 10 years.

This study aims to critically evaluate the efficacy and safety of tongue acupuncture in the treatment of poststroke aphasia to provide an evidence base for the clinical practice of acupuncture.

2. Methods

2.1. Data Aggregation Method. We aggregated all the data about tongue acupuncture treatment after stroke aphasia RCTs from Chinese databases Chinese journal full-text database (CNKI), China biomedical literature database (CBM), VIP database (VIP), Wanfang data, and English databases PubMed, Cochrane, and Embase. The data aggregation time is from the establishment of the database to the present. Search terms include "stroke," "cerebrovascular accident," "cerebrovascular apoplexy," "brain vascular accident," "cerebrovascular stroke," "cerebral stroke," "acute stroke," "acute cerebrovascular accident," "CVA (cerebravascular accident)," "aphasia," "post-stroke aphasia," "randomized controlled trial," "random allocation," "randomization," and "RCT." The subject heading was combined with a free word search. The database retrieval strategy on CNKI are as follows: (1) Stroke AND acupuncture AND aphasia; (2) Stroke AND needle AND aphasia; (3) Cerebral hemorrhage AND needle AND aphasia; (4) Stroke AND acupuncture AND aphasia (in Chinese).

2.2. Inclusion Criteria. The inclusion criteria were as follows:

(1) Randomized controlled trials (RCTs) of tongue acupuncture in the treatment of poststroke aphasia published in China and abroad

(2) Patients who meet the diagnosis of poststroke aphasia, with comparable baselines. The treatment group used tongue acupuncture or combined tongue acupuncture based on the intervention measures in the control group.

(3) The main efficiency measurements are the ABC score and clinical efficacy; the secondary efficiency measurement is the CFCP score.

(4) The clinical efficacy observations and the experimental research on patients with tongue acupuncture as the main treatment

(5) Reasonable control group, control group intervention measures (body acupuncture, head acupuncture, language training, and Jie Yu Dan)

(6) The acupuncture points and methods are clearly exposed in the text

2.3. Exclusion Criteria. The exclusion criteria were as follows:

(1) Studies not in line with the diagnosis of motor aphasia after stroke

(2) The clinical treatment method is not mainly based on tongue acupuncture

(3) Animal experiments, literature review, and meta-analysis literature

(4) For repeated publications or repeated selections, keep one of them

2.4. Data Extraction. We imported the obtained literature data into Note Express software and used its automatic review function combined with manual review to remove duplicate literature. We reviewed all abstracts and full texts to make the first selection and carried out data extraction on the selected papers (basic research information, research methods, intervention measures, treatments, performance indicators, and literature quality evaluation).

2.5. Evaluation of the Quality of Research Methodology. We used Cochrane Handbook 5.4.1 "Risk of Bias Assessment Tool" to evaluate the selected studies, including random assignment, hidden grouping, blinding, incomplete data, selective reporting, and other sources of bias.

2.6. Statistical Methods. Statistical analysis was performed by using RevMan5.4.1 software provided by the Cochrane Collaboration. Publication bias analysis and sensitivity analysis were performed by using Stata16.0. The heterogeneity among the studies was tested through I^2 and P tests. If $P > 0.1$ and $I^2 < 50\%$, a fixed effect model was used; otherwise, a random effects model was used.

For dichotomous variables, the relative risk (RR) and 95% confidence interval (CI) were used to represent the efficacy analysis statistics; for continuous data, weighted mean difference (MD) and 95% confidence interval (CI) were used to express efficacy analysis statistics. Potential publication bias was analyzed by using an "inverted funnel" diagram, and bias in included trials was discussed. Funnel plot and Egger's test were used to analyze the publication bias of the primary outcome indicators.

3. Results

3.1. Search Results. A total of 267 papers were found, among which 165 independent studies were deduplicated with the Note Express software. By browsing the abstract and reading the full text, they were screened according to the inclusion and exclusion criteria, and finally, 20 RCTs were included [11–30], with a total of 1355 patients, all studies having a comparable baseline. The literature screening process is shown in Figure 1. The basic characteristics of the included studies are listed in Table 1.

3.2. Quality of the Included Literature. Cochrane risk of bias assessment was performed on the included literature, as shown in Figures 2 and 3. Three studies [13, 17, 21] used the random number table method, and the remaining studies [11, 12, 14–16, 18–20, 22–30] did not describe the specific randomization method. Four studies [19, 23, 29, 30] had no follow-up visits and dropouts. None of the studies have published reports, so it is impossible to judge whether the outcome of the choice to report exists. It is not clear whether other biases exist.

3.3. Meta-Analysis Results

3.3.1. ABC Score. Nine studies [13–15, 18, 19, 22, 27, 28, 30] reported ABC scores with large heterogeneity between studies ($P < 0.00001$, $I^2 = 96\%$). Subgroup analysis based on the intervention methods is shown in Figure 4.

The results of subgroup analysis showed that in the 3 studies comparing tongue acupuncture with conventional treatments [15, 27, 28] (MD = 5.95, 95% CI (2.85, 9.04), $P = 0.06$), the difference between the two groups was not significant. Six studies comparing tongue acupuncture combined with conventional therapies and conventional therapies [13, 14, 18–20, 22] combined effect results (MD = 11.48, 95% CI (2.20, 20.75), $P < 0.00001$), indicating tongue acupuncture combined with conventional therapies increased the ABC score more compared with tongue acupuncture. Sensitivity analysis found no significant reversal of meta-analysis results, indicating that the results were robust. See Figure 5 for details.

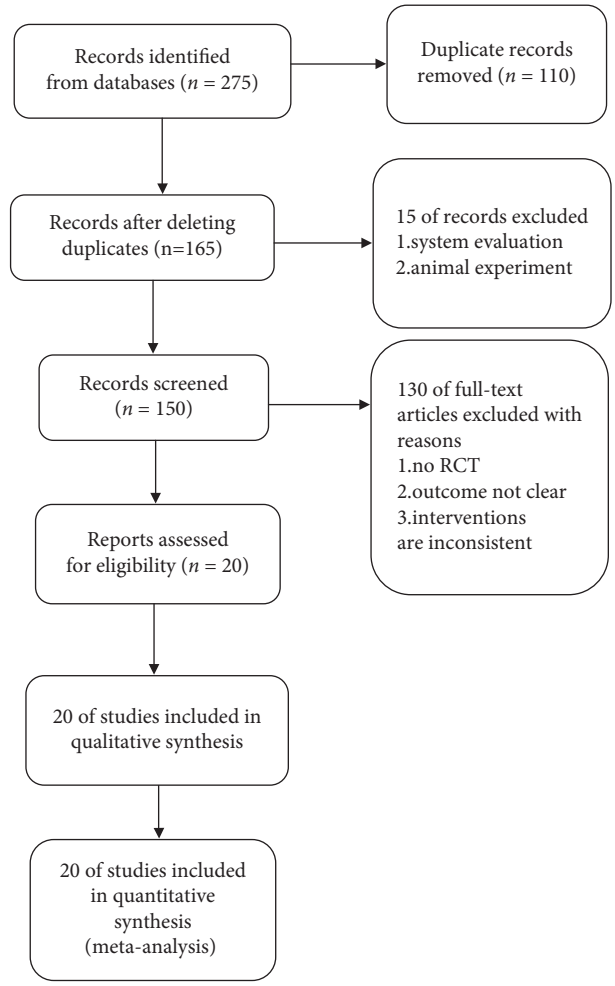

Figure 1: The flowchart of the screening process.

3.3.2. Clinical Efficacy. Fifteen studies [11–14, 16, 19–21, 23–27, 29, 30] reported clinical efficacy, with subgroup analyses by intervention methods. The results of subgroup analysis showed that the difference between tongue acupuncture and conventional treatments [12, 27, 29] was statistically significant ($P = 0.001$).

The 12 studies that compared tongue acupuncture combined with conventional therapies to conventional therapies [11, 13, 14, 16, 19–21, 23–26, 30] showed good homogeneity ($P = 0.96$, $I^2 = 0\%$), the difference was statistically significant (MD = 1.23, 95% CI (1.14,1.30), $P < 0.00001$), and the comparison of the two groups shows that tongue acupuncture combined with conventional therapies improved the clinical efficacy is clear. Details are shown in Figure 6. The results of sensitivity analysis showed that no studies had significantly reversed the results of meta-analysis, indicating that the above results were reliable. See Figure 7 for details.

3.3.3. CFCP Score. Three studies [13, 15, 23] reported CFCP scores, and there was heterogeneity between studies ($P = 0.29$, $I^2 = 20\%$), and subgroup analyses were performed according to different interventions (Figure 8). The results of

Table 1: Basic characteristics of the included literature.

Study	Patients number		Interventions		Treatment (days)	Results criteria
	Therapy group	Control group	Therapy group	Control group		
Xu et al. [11]	40	40	Tongue acupuncture + head acupuncture	Head acupuncture	60	⑤
Li et al. [12]	30	30	Jinjin, Yuye	Body acupuncture	20	⑥
Wang et al. [13]	35	35	Tongue acupuncture + head acupuncture + language training	Head acupuncture + language training	24	①②⑥
Xu et al. [14]	25	25	Tongue acupuncture + language training	Language training	40	②⑥
Li et al. [15]	30	30	Tongue acupuncture	Body acupuncture	20	①②③
Xu et al. [16]	25	25	Tongue acupuncture + language training	Language training	20	⑦
Zhong et al. [17]	60	60	Tongue acupuncture + head acupuncture + language training	Language training	30	②⑤
Wang er al. [18]	35	32	Tongue acupuncture + Schuell	Schuell stimulation	18	③
Wu et al. [19]	21	22	Tongue acupuncture + head acupuncture + language training	Language training	15	③④⑥
Gao et al. [20]	40	40	Tongue acupuncture + body acupuncture	Body acupuncture	15	④⑥
Pan et al. [21]	32	32	Tongue acupuncture + Jie Yu Dan	Jie Yu Dan	10	⑤
He et al. [22]	28	28	Tongue acupuncture + head acupuncture + Jie Yu Dan	Head acupuncture + Jie Yu Dan	24	②
Song et al. [23]	31	30	Tongue acupuncture + Schuell	Schuell stimulation	15	①③⑥
Li et al. [24]	28	28	Tongue acupuncture + language training	Language training	10	②⑥
Wang et al. [25]	30	30	Tongue acupuncture + language training	Language training	20	⑥
He et al. [26]	30	30	Tongue acupuncture + Schuell	Schuell stimulation	20	⑦
Li et al. [27]	30	30	Tongue acupuncture	Body acupuncture	10	③⑥
Jiang et al. [28]	40	36	Tongue acupuncture	Body acupuncture	20	②
Liao et al. [29]	52	48	Jinjin, Yuye	Body acupuncture	20	⑦
Mi et al. [30]	46	38	Tongue acupuncture + body acupuncture	Body acupuncture	30	②⑥

①, CFCP; ②, ABC; ③, BDAE; ④, NIHSS; ⑤, CADL; ⑥, clinical efficacy.

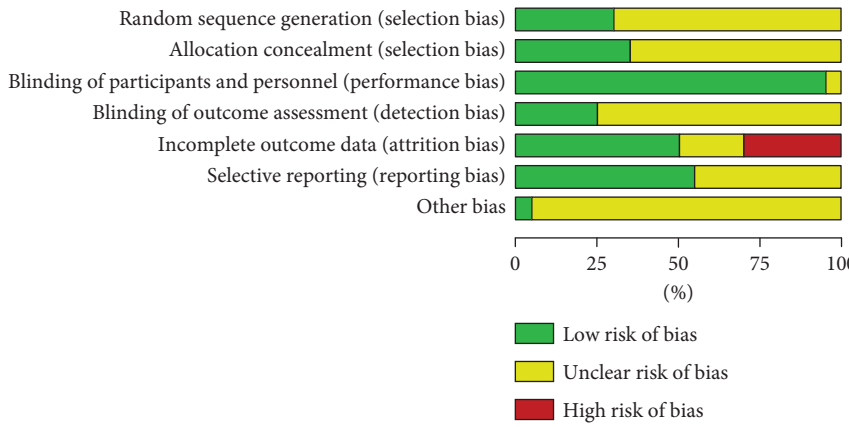

Figure 2: Risk of bias percentile bar graph.

subgroup analysis showed that the difference between tongue acupuncture and conventional treatments [15] was statistically significant (MD = 39.78, 95% CI (26.59, 52.97), $P < 0.00001$), indicating that tongue acupuncture can improve the CFCP score more compared with the conventional treatments.

There were two studies comparing tongue acupuncture combined with conventional therapies to conventional therapies [13, 23]. The difference between the studies was statistically significant (MD = 29.80, 95% CI (19.08, 40.52), $P < 0.00001$), indicating that tongue acupuncture combined with conventional therapies is more effective than conventional treatments in increasing the CFCP score.

3.4. Publication Bias Assessment. In this study, the funnel plot method was used to evaluate the publication bias of the primary outcome indicators, ABC score, and clinical efficacy.

3.4.1. ABC Score Publication Bias. Publication bias was evaluated for the 9 included studies [13–15, 18, 19, 22, 27, 28, 30]. The funnel plots show that two references comparing tongue acupuncture combined with conventional treatments to conventional treatments may present some publication bias (Figure 9. Egger's test was used for quantitative analysis; In Egger's test, $P = 0.135 > 0.05$, indicating no publication bias.

3.4.2. Clinical Efficacy Publication Bias. The 15 included studies [11–14, 16, 19–21, 23–27, 29, 30] were evaluated for publication bias, and the funnel plot was roughly symmetrical, indicating that there may be no publication bias, as shown in Figure 10. Egger's test was used for quantitative analysis; In Egger's test, $P = 0.201 > 0.05$, indicating no publication bias.

4. Discussion

This systematic review confirmed the effectiveness of tongue acupuncture in the treatment of poststroke aphasia and indicated that tongue acupuncture combined with other therapies is effective in improving ABC scores, CFCP scores, and clinical efficacy.

Tongue acupuncture has a strong acupuncture sensation, and most of the clinical acupoints are mainly tongue acupoints, emphasizing the effect of near treatment. The tongue, as one of the main sound organs, can dredge the meridians and regulate qi and blood by stimulating the meridians and acupoints related to the tongue and sound. Tongue acupuncture is a microneedle therapy based on the theory of acupuncture and moxibustion in traditional Chinese medicine and modern bioholographic theory. It is a special acupuncture method created by Zhengzhou Guan, a famous acupuncture expert, based on the theory of the relationship between tongue and viscera meridians in Huangdi Neijing and his decades of clinical experience [31]. The 24 tongue acupoints relate to certain zang-fu organs. The distribution of tongue acupoints involves the relationship of mutual generation and constraining of five elements. It has become the basic acupoint of tongue acupuncture therapy. The commonly used tongue acupuncture includes the following steps: gargle with 3% hydrogen peroxide solution before acupuncture and ask the patient to extend the tongue naturally. By using disposable sterile acupuncture needles, twist the thumb evenly back and forth 10 times and insert the needles about 1–3 mm. For acupoints on the base of the tongue, the surgeon uses the left hand to fix the anterior 1/3 of the tongue with sterile gauze, so that the tongue is rolled up to expose the acupoints, and routine disinfection is performed. Then, use a long needle to puncture Jinjin and Yuye and choose 2 acupoints each time with bleeding.

In clinical application, tongue acupuncture can also be used in conjunction with other treatments, such as head acupuncture. Head acupuncture is a therapy to acupuncture the corresponding head area to treat diseases, which is based on the theory of zang-fu organs and meridians, combined with the functional localization principle of the cerebral cortex. The acupoints are generally emphasized less but more precisely. Head acupuncture can regulate the mind and restore consciousness to speed up the repair of the language function area of the cerebral cortex. Body acupuncture emphasizes holistic treatment and has the characteristics of combining syndrome differentiation with meridian differentiation and regulating the mind and qi. Puncture bloodletting therapy has the characteristics of the

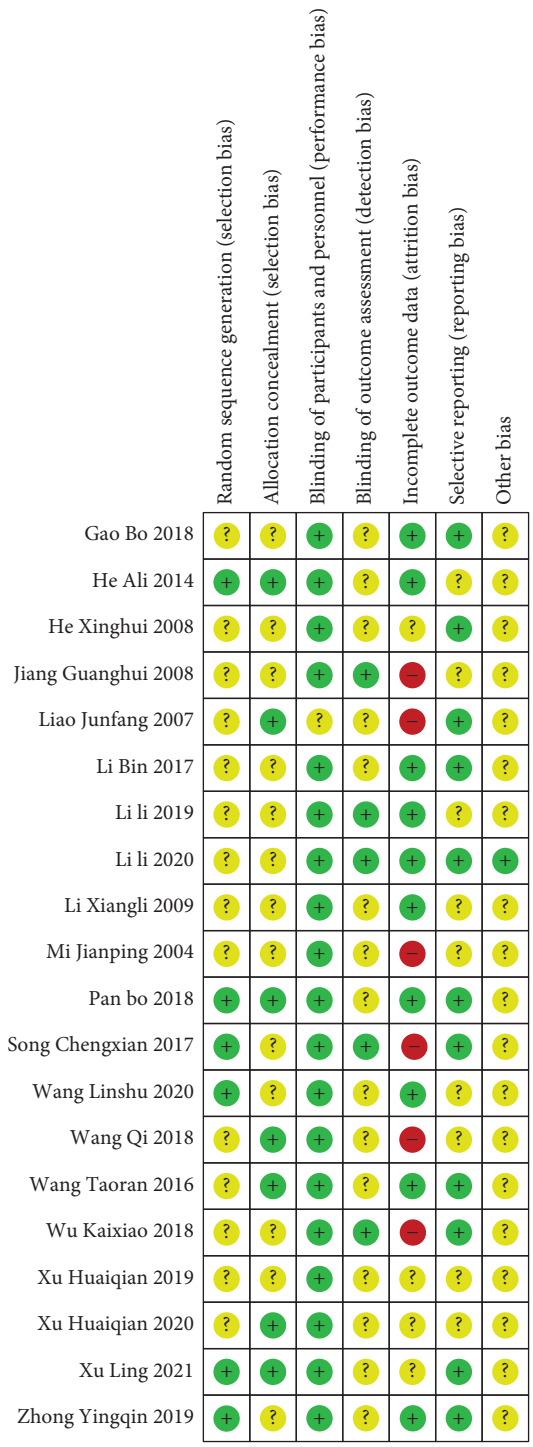

Figure 3: Risk of bias summary graph.

quick effect, remarkable curative effect, low cost, and few adverse reactions, but single bloodletting therapy is difficult to achieve long-term stimulation of the lingual nerve. Language training can re-establish the patient's language function through repeated stimulation from the aspects of oral movements, induced pronunciation, listening comprehension, and word writing. However, a single language training has a very long recovery period and needs to cooperate with clinical interventions to promote the recovery of patients, such as tongue acupuncture. Jie Yu Dan includes Qiang Huo and Quan Xie, which can relieve wind and dredge collaterals [21] and help the monarch medicine *Gastrodia elata* to dispel wind and phlegm; Quan Xie can also play an antispasmodic effect, which has a better prognosis for patients with cerebral infarction. Tongue acupuncture combined with Jie Yu Dan, the synergistic effect of the two, can enhance the efficacy of the drug to help patients improve speech function. Our results of meta-analysis confirmed that tongue acupuncture combined with the above therapies could make up for the limitations of each method alone, thus improving the clinical efficacy of aphasia patients.

The results of this meta-analysis showed that tongue acupuncture is beneficial to PSA. However, the underlying mechanism of tongue acupuncture action against PSA remains unclear. In recent years, many clinical studies and animal experiments have shown that acupuncture significantly improved clinical symptoms and the quality of life of patients by reducing the size of cerebral infarction, improving cerebral blood circulation, inhibiting apoptosis, and promoting cell proliferation and differentiation. Acupuncture therapy also has high safety with few adverse reactions and contraindications [32]. Some studies have suggested [33] that tongue acupuncture can reduce neuronal decay in the hippocampal CA1 region, rebuild cerebral nerve function, and play a positive role in restoring patients' language function. Tongue acupuncture can rapidly establish cerebrovascular collateral circulation [34], increase blood flow, improve cerebral circulation, and rebuild cerebral nerve activity after stroke by regulating the central nervous system. Studies have shown that [35] tongue acupuncture can significantly improve the blood perfusion of brain tissue at the lesion site more than body acupuncture and shrink the lesion site to varying degrees, thereby improving brain function. Tongue acupuncture can also reduce blood viscosity, improve microcirculation, prevent thrombosis, and enhance brain metabolism and blood supply to promote the repair of damaged brain tissue [19]. However, there is still a lack of research on the related signal pathways of tongue acupuncture in the treatment of poststroke aphasia, which should be the goal of future research.

A total of 20 studies were included in this study, and subgroup analysis was performed on CFCP scores [13, 15, 23] and ABC scores [13–15, 18, 19, 22, 27, 28, 30] according to intervention measures. The results showed that the difference between the tongue acupuncture and the conventional therapy group was statistically significant, but the heterogeneity was high, which may be related to the different acupoint selection and acupuncture intensity of the two groups, and less literature was included; the stability of the results is poor. In the ABC score publication bias, the funnel plots show that two references comparing tongue acupuncture combined with conventional treatments may present some publication bias, which may be due to the small number of cases included in the clinical control and cause shedding. More studies are needed to further analyze and verify the results. The analysis of tongue acupuncture combined with conventional therapy indicated that tongue

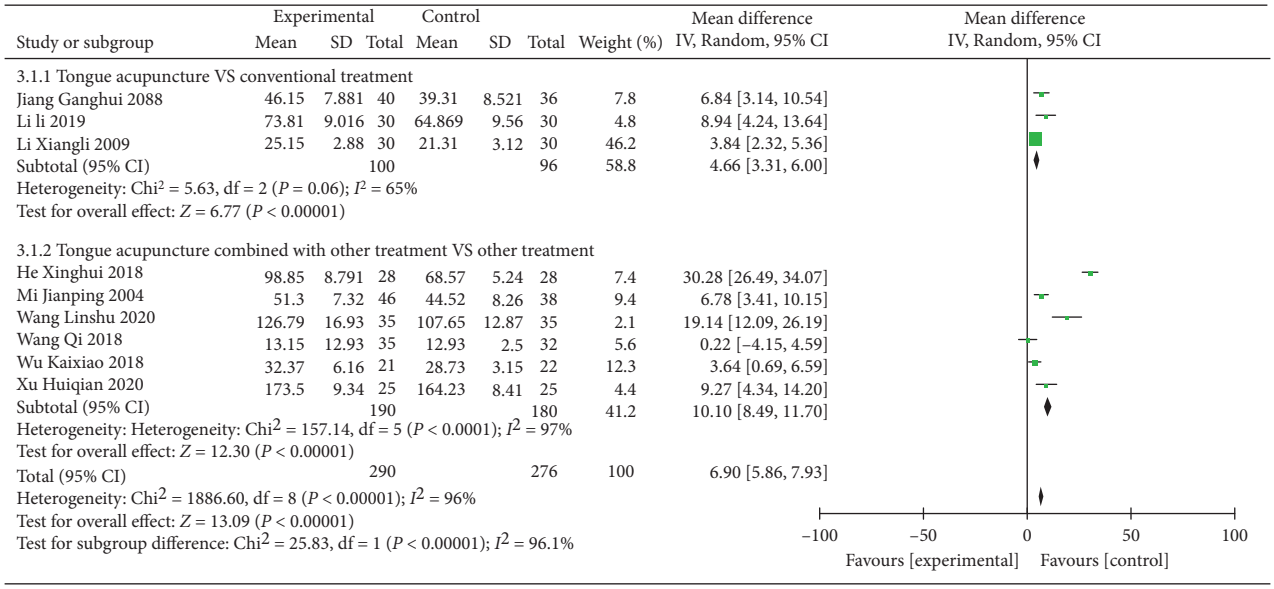

Figure 4: ABC score subgroup analysis.

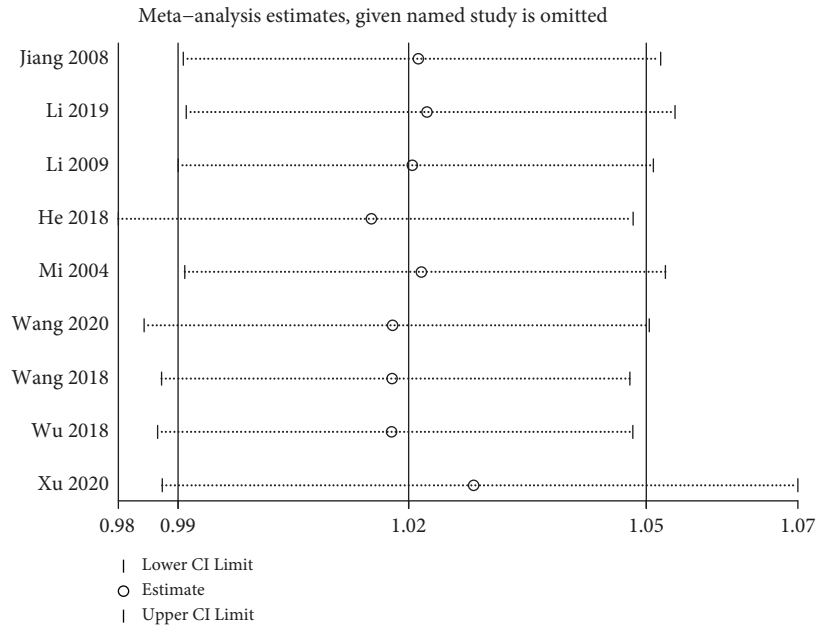

Figure 5: Sensitivity analysis ABC score.

acupuncture combined with conventional therapy for poststroke aphasia was superior to conventional therapy in improving CFCP score, ABC score, and clinical efficacy.

This review had certain limitations. The first limitation is the scarcity of studies, and the methodologically low to moderate quality of the primary data precludes us from drawing confirmative conclusions. Most of the included studies had an unclear risk of bias for blinding, random sequence generation, and allocation concealment; therefore, a preponderance of positive results was observed. The second limitation is that the number of studies is unevenly distributed in the different types of acupuncture, leading to a limited sample size for the CFCP score study. Meanwhile, the included studies also had limitations. The first limitation is that although mentioned randomization in all the 20 included studies, only 4 studies [12, 15, 16, 21] described specific randomization methods, and most of the included studies had unclear bias risks in blinding, random sequence generation, and allocation hiding. Although it is difficult for blind therapists who perform acupuncture, attempts should be made to blind patients, other care providers, and outcome evaluators to minimize trial outcome and evaluation bias. The second limitation is that the sample size included in the study was small, with the largest 120 cases [17] and the smallest 43 cases [19], and no sample size estimation was performed. A third limitation is that we did not perform

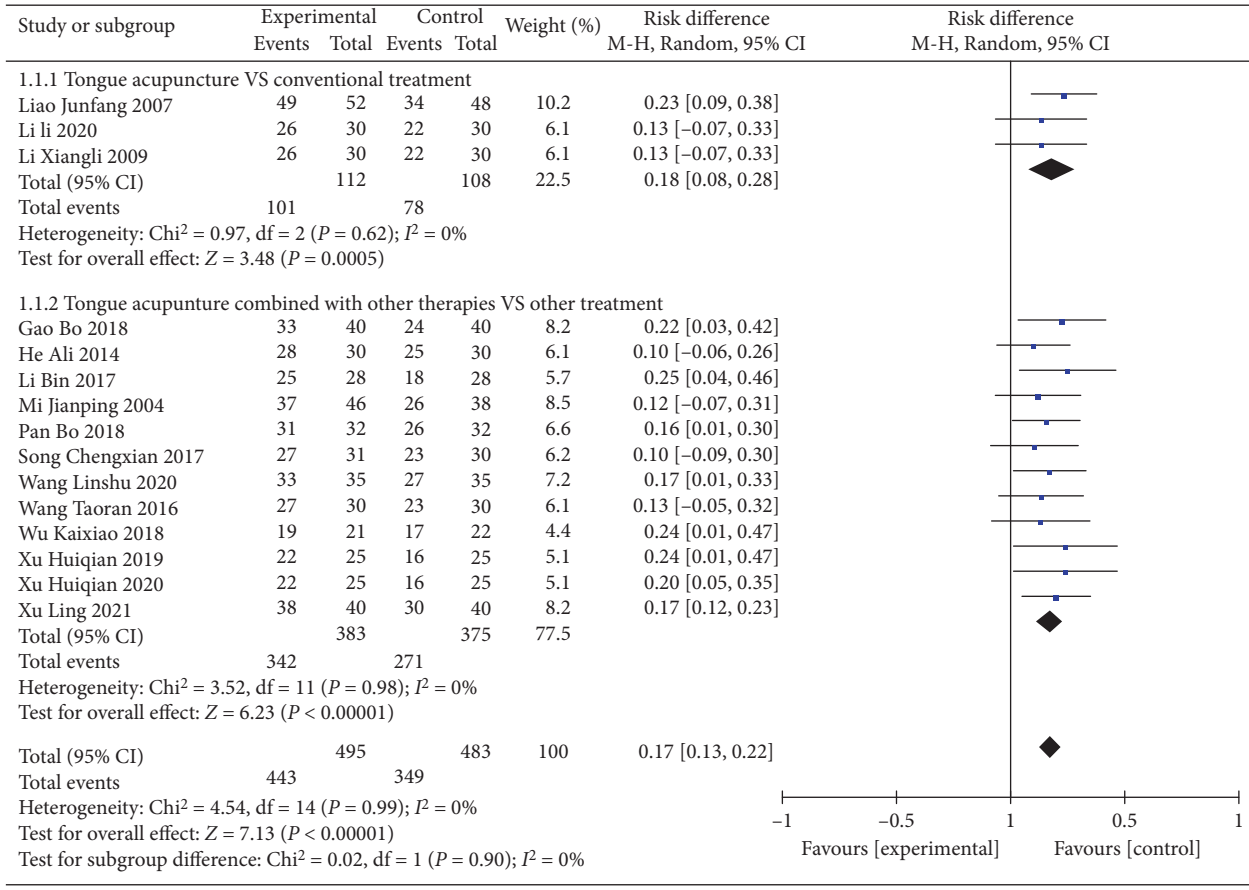

Figure 6: Clinical efficacy score of subgroup analysis.

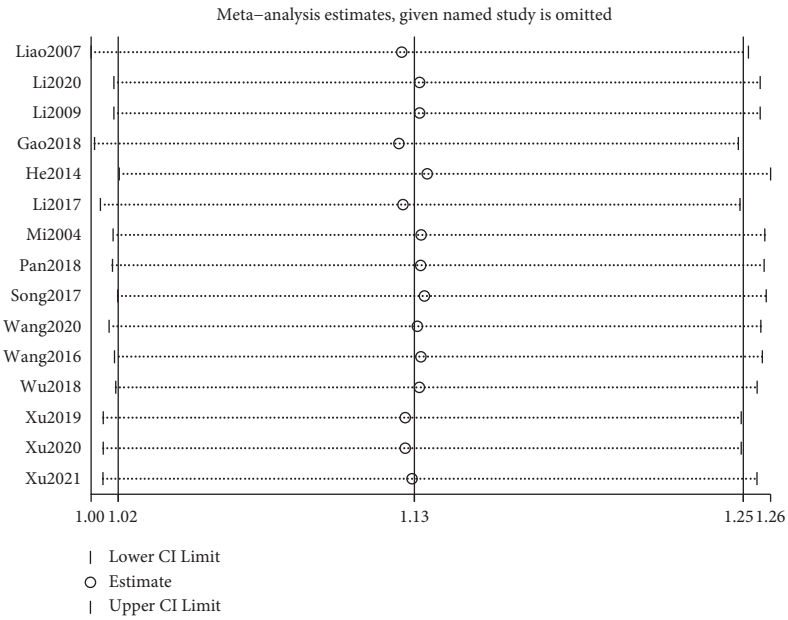

Figure 7: Sensitivity analysis of clinical efficacy.

a subgroup analysis of stroke duration, aphasia type, and treatment course, which may be a potential source of bias. These potential sources of clinical heterogeneity should be considered in future studies. This review also has some limitations. The first limitation is the scarcity of studies, and the methodologically low to moderate quality of the primary data precludes us from drawing confirmative conclusions. Most of the included studies had an unclear risk of bias for

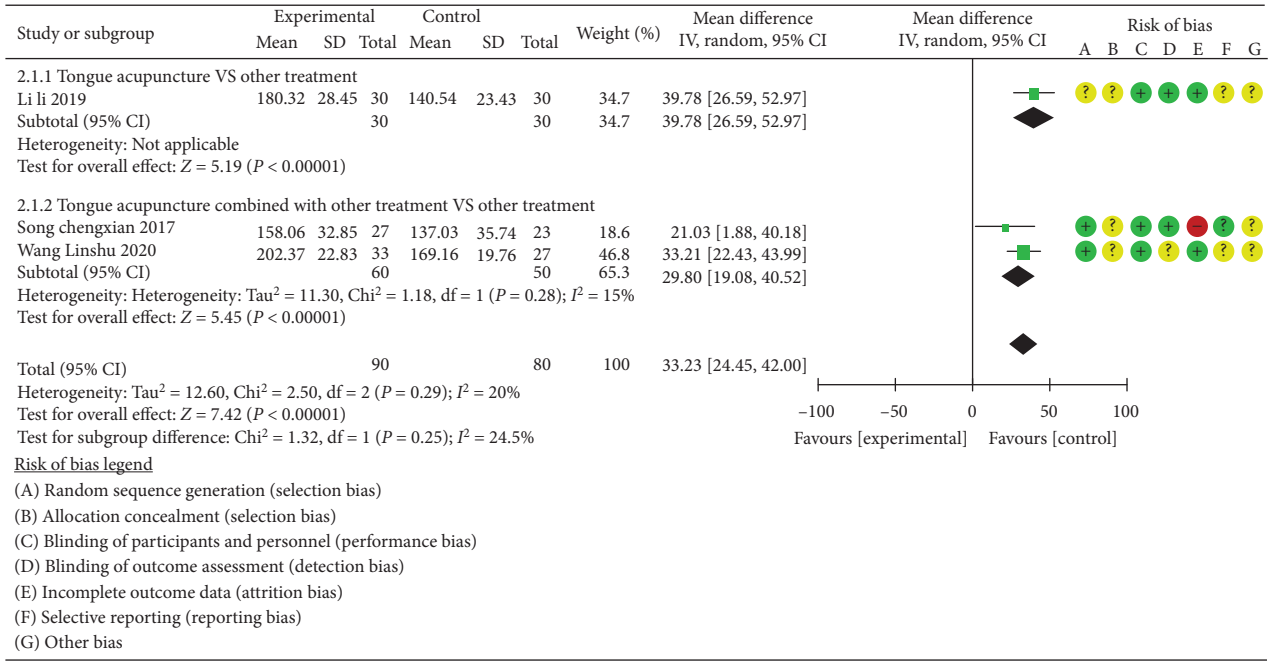

Figure 8: CFCP score of subgroup analysis.

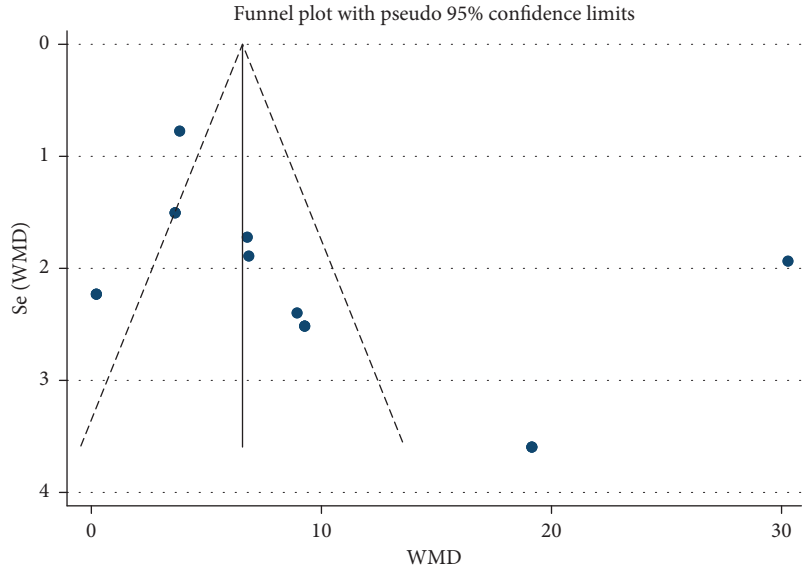

Figure 9: ABC score funnel chart.

blinding, random sequence generation, and allocation concealment; therefore, a preponderance of positive results was observed. The second limitation is that the number of studies is unevenly distributed in the different types of acupuncture, leading to a limited sample size for the CFCP score study.

In the future, researchers should pay attention to the implementation of randomization, describe the

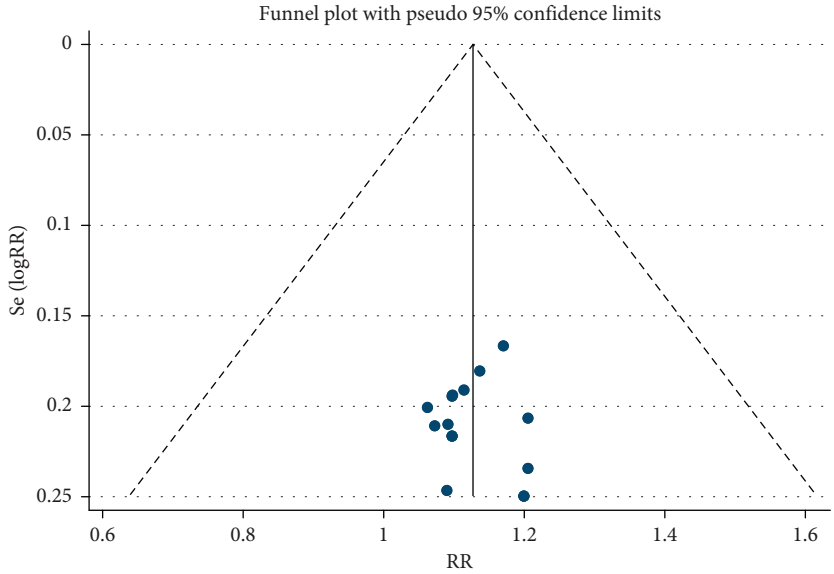

Figure 10: Clinical efficacy funnel chart.

randomization method in detail, improve the blind method and allocation concealment, standardize acupuncture therapy, focus on the records of adverse reactions and follow-up, try to select internationally recognized indicators, and improve the repeatability, quality, and reliability of research.

5. Conclusion

In conclusion, tongue acupuncture is effective and safe in the treatment of poststroke aphasia. Further exploration for tongue acupuncture of poststroke aphasia requires stricter evaluation criteria and high-quality RCT design.

Data Availability

The datasets analyzed during the current study are available from the corresponding author upon request.

Disclosure

Shengping Yang and Li Li are the co-first authors.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

YSP and LL participated in the research design. LL screened data extraction. YSP conducted literature search, analyzed the data, performed statistical analysis, and wrote the manuscript. YSP, JR, DHY, XF, KP, and GLL participated in the correction of the manuscript. All authors reviewed, read, and approved the final version of the manuscript.

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Retraction

Retracted: miR-28-5p's Targeting of GAGE12I Inhibits Proliferation, Migration, and Invasion of Gastric Cancer in Vitro

Evidence-Based Complementary and Alternative Medicine

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This article has been retracted by Hindawi following an investigation undertaken by the publisher [1]. This investigation has uncovered evidence of one or more of the following indicators of systematic manipulation of the publication process:

(1) Discrepancies in scope
(2) Discrepancies in the description of the research reported
(3) Discrepancies between the availability of data and the research described
(4) Inappropriate citations
(5) Incoherent, meaningless and/or irrelevant content included in the article
(6) Peer-review manipulation

The presence of these indicators undermines our confidence in the integrity of the article's content and we cannot, therefore, vouch for its reliability. Please note that this notice is intended solely to alert readers that the content of this article is unreliable. We have not investigated whether authors were aware of or involved in the systematic manipulation of the publication process.

Wiley and Hindawi regrets that the usual quality checks did not identify these issues before publication and have since put additional measures in place to safeguard research integrity.

We wish to credit our own Research Integrity and Research Publishing teams and anonymous and named external researchers and research integrity experts for contributing to this investigation.

The corresponding author, as the representative of all authors, has been given the opportunity to register their agreement or disagreement to this retraction. We have kept a record of any response received.

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[1] R. Xu, Q. Guo, P. Zhao, H. Lu, and B. Zhang, "miR-28-5p's Targeting of GAGE12I Inhibits Proliferation, Migration, and Invasion of Gastric Cancer in Vitro," *Evidence-Based Complementary and Alternative Medicine*, vol. 2022, Article ID 6946051, 6 pages, 2022.

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Research Article

miR-28-5p's Targeting of GAGE12I Inhibits Proliferation, Migration, and Invasion of Gastric Cancer in Vitro

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GAGE12I is a tumor metastasis-promoting factor, which can induce gastric cancer cells to invade and migrate. We investigated the effect of miR-28-5p targeting GAGE12I on proliferation, invasion, and migration of human gastric cancer cell lines SGC-7901, AGS, and MGC-803. The expression levels of miR-28-5p and GAGE12I were detected by real-time PCR and western blot, respectively. Cell proliferation, migration, and invasion were measured by MTT and Transwell chamber. The interaction between miR-28-5p and GAGE12I was investigated by bioinformatics analysis and luciferase assay. Results showed that the expression of miR-28-5p in human gastric cancer cell lines was lower than that in normal gastric epithelial cells ($P < 0.05$). Overexpression of miR-28-5p suppressed cell proliferation, invasion, and migration ($P < 0.05$). GAGE12I was confirmed as a target of miR-28-5p. Cell proliferation, invasion, and migration were decreased in cells transfected with shGAGE12I compared with those of the scrambled group ($P < 0.05$). Collectively, miR-28-5p negatively regulated GAGE12I and reduced the proliferation, invasion, and migration of gastric cancer cells.

1. Introduction

Gastric cancer is one of the most common malignant tumors, with very complicated pathogenesis. Metastasis of gastric cancer is an important cause of death. The invasion and migration of gastric cancer cells are regulated by a variety of genes. It is important to study the molecular pathogenesis of gastric cancer cells for the treatment of cancer [1]. MicroRNAs (miRNAs) are a class of highly conserved single-stranded RNA, which can induce or inhibit the translation of mRNA by binding to the target gene's mRNA molecules [2]. miR-28-5p is one of the miRNAs existing in human tissues, which is involved in the occurrence of tumors. It has been found that the expression of this miRNA in renal and colon cancer is downregulated, suggesting that miR-28-5p may play an inhibitory role in tumors [3, 4]. However, the role and mechanism of miR-28-5p in gastric cancer remain largely unknown. GAGE12I is a tumor metastasis-promoting factor, which can induce gastric cancer cells to invade and migrate [5]. In this study, we investigated the expression level of miR-28-5p in gastric cancer cells, and studied the effect and mechanism of miR-28-5p on proliferation, invasion, and migration of gastric cancer cells, in order to provide a reference for clarifying the mechanism of gastric cancer progression.

2. Materials and Methods

2.1. Material. Human gastric cancer cell lines SGC-7901, AGS, and MGC-803 [6] were purchased from Shanghai Cell Bank of the Chinese Academy of Sciences; normal gastric epithelial cells GES-1 was purchased from ATCC of USA; a reverse transcription kit was purchased from Thermo Fisher of USA; GAGE12I antibody was purchased from Abcam of USA; SYBR Green real-time PCR kit was purchased from Shanghai Saobao Biotechnology; miR-28-5p mimics and mimics control was purchased from Balmico Biotechnology; and GAGE12I shRNA and shRNA control from Beijing Ao Rui Dongyuan Biotechnology. All experiments were done in triplicates.

2.2. Real-Time PCR Assay. Trizol reagent was added to human gastric cancer cell lines SGC-7901, AGS, and MGC-803 and normal gastric mucosal epithelial cells GES-1 for RNA extraction. RNA was stored in a refrigerator at −80°C. The reverse transcription kit was used to get cDNA. The primers for RT-PCR reaction were: miR-28-5p forward primer: 5′-GCG CAT TGC ACT TGT CTC G-3′, reverse primer: 5′-AGT GCA GGG TCC GAG GTA TT-3′. The U6 was used as control (forward primer: 5′-CTC GCT TCG GCA GCA CATA-3′, reverse primer: 5′-CGA ATT TGC GTG TCA TCCT-3′). The PCR reaction consisted of a 2 × SYBR Green mix of 10 μL, RT product of 2 μL, forward primer and reverse primer of 0.8 μL each, and ddH_2O of 6.4 μL. The real-time quantitative PCR was performed with the CFX96 real-time PCR instrument (Bio-Rad, USA). The PCR program was: 95°C 20 s, 95°C 10 s, 60°C 20 s, and 72°C 10 s, with a total circulation of 40 times. The quantification was done using the comparative Ct ($2^{-\Delta\Delta Ct}$) method.

2.3. Cell Transfection. SGC-7901 cells were seeded into 6 well plates, each containing 5×10^5 cells, and incubated at 37°C under 5% CO_2 overnight. When cell density was 50%, cell transfection was performed. miR-28-5p mimics, mimics control, GAGE12I shRNA, or shRNA control were added to Opti-MEM containing 245 μL and incubated at room temperature for 20 minutes. Opti-MEM was incubated at 37°C for 4 hours. Opti-MEM was removed and cells were washed twice with PBS. RPMI1640 medium containing 10% FBS was added and incubated at 37°C for 48 hours. The SGC-7901 cells transfected with miR-28-5p mimics, mimics control, GAGE12I shRNA, or shRNA control were recorded as miR-28-5p, miR-NC, shGAGE12I, or scrambled group, respectively.

2.4. MTT Assay. miR-28-5p and miR-NC groups cells were inoculated into a 96-well plate at a concentration of 6×10^4/mL in 100 μL cell culture medium per well and incubated for 24 h, 48 h, or 72 h. Methylthiazolyl diphenyl-tetrazolium bromide (MTT) solution of 10 μL was added into each hole and cells were incubated at 37°C for an extra 4 h. The supernatant solution in the well was absorbed, and 150 μL dimethyl sulfoxide (DMSO) solution was added. The supernatant was shaken in a low-speed shaker for 10 min. OD value at 570 nm was measured for enzyme-linked immunosorbent assay (ELISA). Cell viability was calculated according to the manufacturer's manual [7].

2.5. Transwell Chamber Assay. Invasion experiment: Matrigel was dissolved at 4°C, and the RPMI1640 medium was preheated at 4°C and diluted with Matrigel. Then the residual liquid was collected out of the well. miR-28-5p and miR-NC groups cells were inoculated into the well compartment and 5×10^4 cells were added to each well. After incubation for 24 hours, the cells that had not penetrated the membrane were wiped off and the number of invasive cells was counted under a 200-fold microscope.

2.6. Prediction and Identification of Target Genes. According to the bioinformatics analysis by TargetScan and Pitar software, the target gene of miR-28-5p may be GAGE12I. Wild-type (WT) and mutant-type (MUT) luciferase reporter vectors were constructed according to GAGE12I 3-UTR binding sites and were cotransfected into SGC-7901 cells with miR-28-5p mimics, or mimics control, respectively, then detected by measurement of luciferase activity.

2.7. Western Blot. miR-28-5p and miR-NC groups cells were collected with the addition of RIPA buffer (Thermo Fisher, USA). The cells were lysed on ice for 1 h and centrifuged at 4°C for 15 minutes. The concentration of the supernatant of cell lysate was measured using the BCA kit (Thermo Fisher, USA). Each lysate sample containing 30 μg protein was mixed with SDS loading buffer and boiled for 10 minutes, followed by loading onto a 5% concentrating +12% separating gel. The initial voltage was 80 V at the concentrating gel and then adjusted to 120 V when the samples entered the separating gel. The electrophoresis was continued for about 1 h, and the proteins were transferred onto a PVDF at 100 V voltage for 1 h. The PVDF membrane was incubated in 5% bovine serum albumin for 1 h, then washed with TBST three times, added with GAGE12I antibody (1 : 100), or GAPDH antibody (1 : 100) as a loading control, left at 4°C overnight, washed with TBST three times, and then incubated with the secondary antibody for 1 h. After being washed with TBST, the ECL chemiluminescent substrate reagent kit (Thermo Fisher, USA) was used for luminescence and the image was taken by gel imager. The gray value of each band was analyzed, and the protein expression level was semi-quantitatively analyzed using ImageJ.

2.8. Statistical Analysis. All the experimental data were analyzed by the SPSS21.0 software (IBM Corp., Armonk, N.Y., USA). The measurement data were expressed by (means ± SD). The statistical significance of the difference between the two groups was analyzed by the *t*-test. A 2-sided$P < 0.05$ was considered significantly different.

3. Results

3.1. Low Expression of miR-28-5p in Gastric Cancer Cells. The expression of miR-28-5p in human gastric cancer cell lines SGC-7901, AGS, and MGC-803 were significantly lower than that in normal gastric epithelial cells GES-1 (Figure 1). SGC-7901 cells with the lowest level of miR-28-5p were used for the following analyses in vitro.

3.2. Overexpression of miR-28-5p Inhibits Proliferation, Migration, and Invasion of Gastric Cancer Cells. SGC-7901 cells were transfected with miR-28-5p mimics or mimics control, named as miR-28-5p or miR-NC group, respectively. The results of the real-time PCR assay showed that the expression of miR-28-5p was significantly increased in a miR-28-5p group compared with that in a miR-NC group (Figure 2(a)).

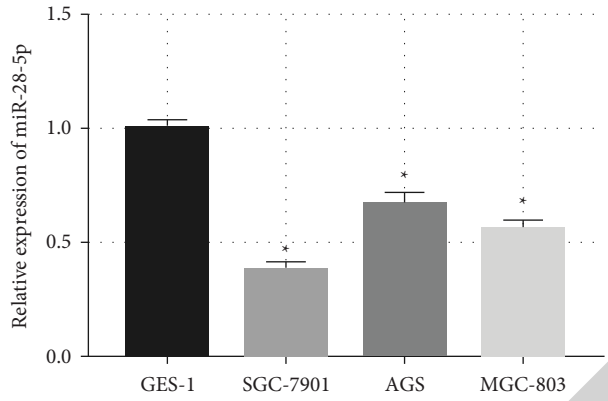

Figure 1: Low expression of miR-28-5p in gastric cancer cells. Note: compared with GES-1, $^*P < 0.05$.

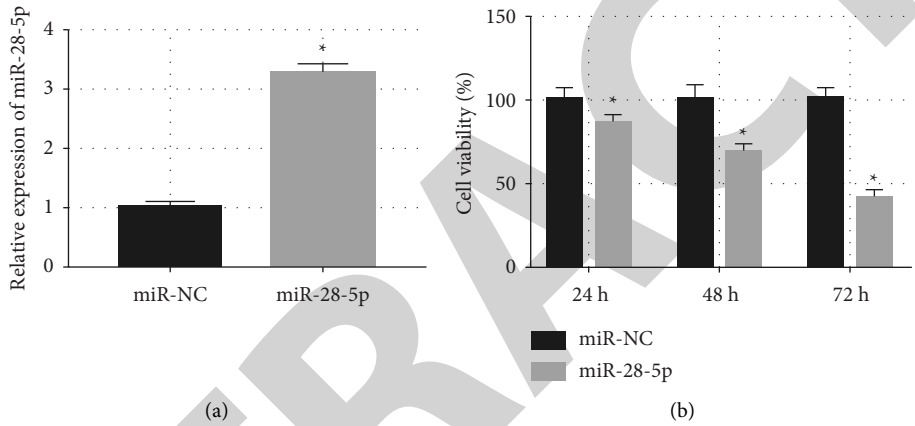

Figure 2: Overexpression of miR-28-5p inhibits proliferation of gastric cancer cells. (a) Overexpression of miR-28-5p in SGC-7901 cells; compared with miR-NC, $^*P < 0.05$. (b) Overexpression of miR-28-5p inhibited the proliferation of SGC-7901 cells. Compared with miR-NC, $^*P < 0.05$.

Moreover, the MTT assay revealed that the survival rate of SGC-7901 cells overexpressing miR-28-5p at 24 h, 48 h, and 72 h was significantly lower than that of the cells in the miR-NC group (Figure 2(b)). In addition, the migration and invasion were significantly lower in gastric cancer cells overexpressing miR-28-5p than those in cells transfected with mimics control (Figures 3(a) and 3(b)).

3.3. GAGE12I is a Target of miR-28-5p. Bioinformatics analysis using TargetScan and Pitar software provided the binding sites of miR-28-5p and GAGE12I, indicating that GAGE12I might be a target of miR-28-5p (Figure 4(a)). Luciferase assay was performed to validate this prediction, showing that the activity of luciferase decreased after cotransfection of miR-28-5p mimics and GAGE12I-WT (Figure 4(b)). The expression level of GAGE12I protein was significantly decreased in SGC-7901 cells after overexpression of miR-28-5p (Figures 4(c) and 4(d)).

3.4. Effect of GAGE12I Knockdown on Proliferation, Migration, and Invasion of Gastric Cancer Cells. After transfection of GAGE12I shRNA or shRNA control into SGC-7901cells, the expression of GAGE12I protein was effectively decreased in the shGAGE12I group compared with that in the scrambled group (Figures 5(a) and 5(b)). Furthermore, downregulation of GAGE12I significantly decreased cell proliferation (Figure 5(c)). Besides, after GAGE12I knockdown, the number of invasion and migration of gastric cancer cells were notably decreased (Figures 6(a) and 6(b)).

4. Discussion

MiRNAs regulate a variety of life activities in organisms and are important for cell differentiation, metabolism, and proliferation. At present, it has been found that miRNAs play regulatory roles in tumorigenesis. These miRNAs play inhibitory or promotive roles in tumor growth and metastasis [8–10]. MiRNAs do not have the function to encode proteins, but can affect life activities by regulating the expression and transcription of target gene mRNA [11]. In this study, we found that miR-28-5p can bind to GAGE12I-3′-UTR, and negatively regulate the expression of GAGE12I, indicating that GAGE12I is the target gene of miR-28-5p.

miR-28-5p is currently found to be closely related to tumor progression and is downregulated in liver cancer and

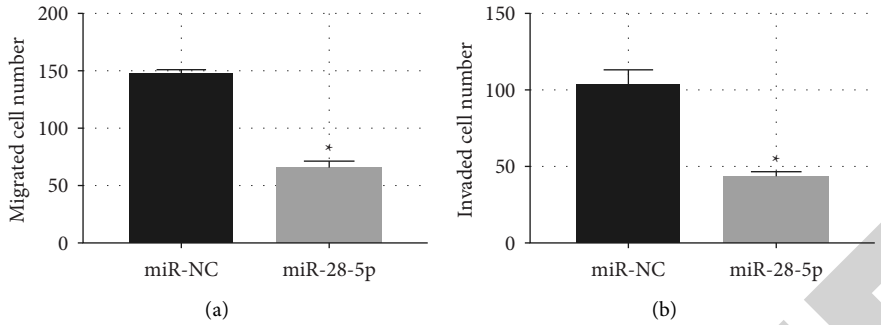

Figure 3: Overexpression of miR-28-5p inhibits invasion and migration of gastric cancer cells. (a) Cell migration test, $^{*}P < 0.05$ compared with miR-NC. (b) Cell invasion test; compared with miR-NC, $^{*}P < 0.05$.

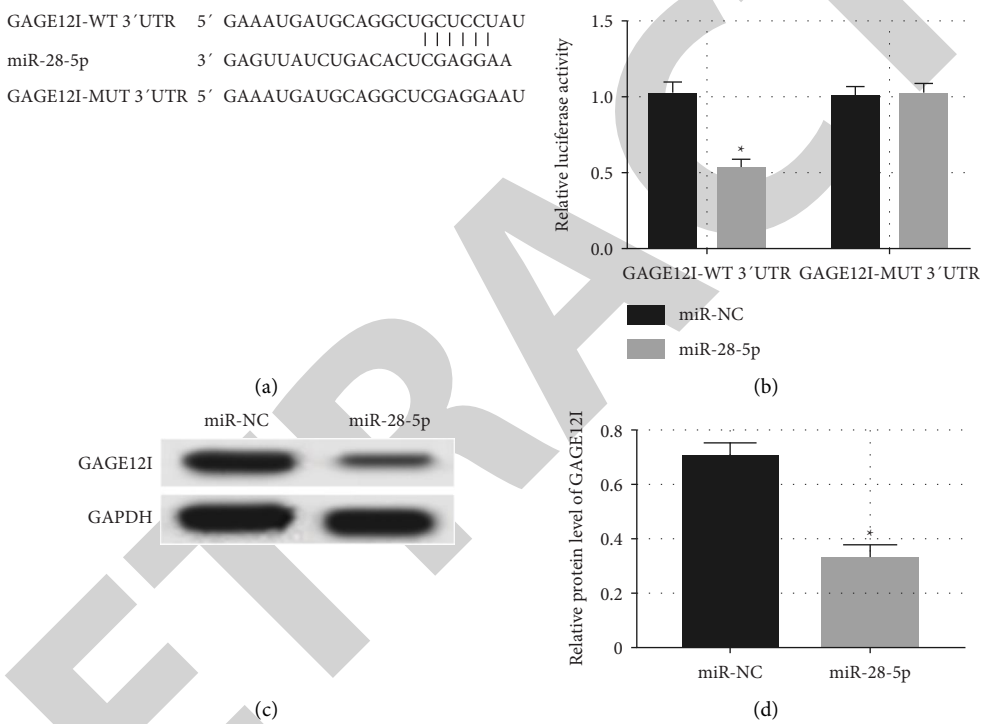

Figure 4: miR-28-5p targets GAGE12I. (a) The 3′UTR of GAGE12I contains a nucleotide sequence complementary to miR-28-5p. (b) Luciferase reporter gene test; compared with miR-NC, $^{*}P < 0.05$. (c) Western blot was used to determine the expression of GAGE12I protein in gastric cancer cells after overexpression of miR-28-5p. (d) GAGE12I protein expression. Compared with miR-NC, $^{*}P < 0.05$.

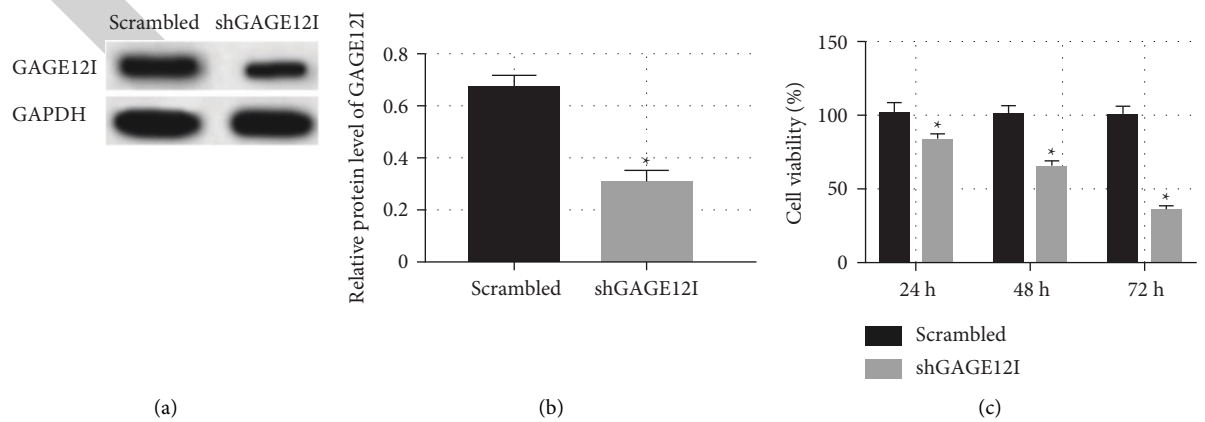

Figure 5: Knockdown GAGE12I inhibits the proliferation of gastric cancer cells. (a) Western blot was used to determine the knockdown effect. (b) GAGE12I protein expression; compared with scrambled, $^{*}P < 0.05$. (c) Knocking down GAGE12I on the survival rate of gastric cancer cells. Compared with scrambled, $^{*}P < 0.05$.

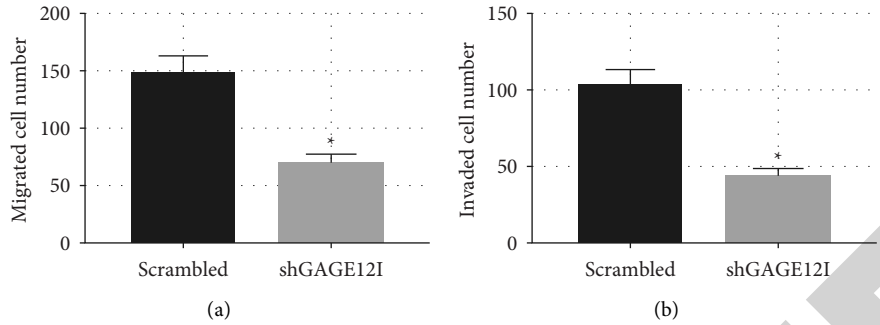

Figure 6: Knockdown GAGE12I inhibits invasion and migration of gastric cancer cells. (a) Knocking down GAGE12I on gastric cancer cell migration; compared with scrambled, $^*P < 0.05$. (b) Knockdown of GAGE12I on gastric cancer cell invasion. Compared with scrambled, $^*P < 0.05$.

B-cell lymphoma studies, which have shown that miR-28-5p may be a tumor suppressor [12, 13]. Studies in gastric cancer suggested that the expression of miR-28-5p is decreased in gastric cancer tissues, and the expression level of miR-28-5p is closely related to tumor metastasis and invasion [14]. The results of this study showed that the expression of miR-28-5p in gastric cancer cells was significantly lower than that in normal gastric epithelial cells, indicating that the expression of miR-28-5p in gastric cancer was downregulated, which was consistent with the above results [14]. This study also confirmed that overexpression of miR-28-5p could inhibit the proliferation, migration, and invasion of gastric cancer cells, indicating the potential therapeutic value of miR-28-5p in cancers. We hypothesized that might be associated with the cell cycle process, apoptosis, and epithelial-mesenchymal transition, which should be explored in the future. The function of miRNA is achieved by regulating its target expression. This study first suggested GAGE12I as a target of miR-28-5p in gastric cancer cells, which was confirmed by luciferase activity assay.

GAGE12I is a member of the GAGE family. With the increase of the metastatic potential of gastric cancer, the expression level of GAGE12I is gradually elevated [5, 15–17]. At present, there are few studies on GAGE12I. GAGE family genes are related to the prognosis of ovarian cancer and are also involved in the chemotherapy tolerance of ovarian cancer. GAGE7 plays an antiapoptosis role in cervical cancer. GAGE12B can promote the metastasis and growth of gastric cancer cells [18–22]. We found that the downregulation of GAGE12I could inhibit the invasion and migration of gastric cancer cells and decrease the proliferation of gastric cancer cells, indicating that GAGE12I is a promoter of gastric cancer progression. We also demonstrated that miR-28-5p regulated gastric cancer progression by targeting GAGE12I. To better understand, the role and mechanism of miR-28-5p in gastric cancer, in vivo experiments in more complicated conditions should be performed in the future [23–27].

In summary, the expression of miR-28-5p is downregulated in gastric cancer cells. Overexpression of miR-28-5p can suppress the proliferation, invasion, and migration of gastric cancer cells. The regulation of gastric cancer cell progression by miR-28-5p is related to the targeting of the GAGE12I gene, which is important for the study of the mechanism of gastric cancer genesis and metastasis.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

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Retraction

Retracted: NEK2 Serves as a Novel Biomarker and Enhances the Tumorigenicity of Clear-CellRenal-Cell Carcinoma by Activating WNT/β-Catenin Pathway

Evidence-Based Complementary and Alternative Medicine

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(3) Discrepancies between the availability of data and the research described
(4) Inappropriate citations
(5) Incoherent, meaningless and/or irrelevant content included in the article
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The presence of these indicators undermines our confidence in the integrity of the article's content and we cannot, therefore, vouch for its reliability. Please note that this notice is intended solely to alert readers that the content of this article is unreliable. We have not investigated whether authors were aware of or involved in the systematic manipulation of the publication process.

In addition, our investigation has also shown that one or more of the following human-subject reporting requirements has not been met in this article: ethical approval by an Institutional Review Board (IRB) committee or equivalent, patient/participant consent to participate, and/or agreement to publish patient/participant details (where relevant).

Wiley and Hindawi regrets that the usual quality checks did not identify these issues before publication and have since put additional measures in place to safeguard research integrity.

We wish to credit our own Research Integrity and Research Publishing teams and anonymous and named external researchers and research integrity experts for contributing to this investigation.

The corresponding author, as the representative of all authors, has been given the opportunity to register their agreement or disagreement to this retraction. We have kept a record of any response received.

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[1] J. Zhou, J. Lai, Y. Cheng, and W. Qu, "NEK2 Serves as a Novel Biomarker and Enhances the Tumorigenicity of Clear-Cell-Renal-Cell Carcinoma by Activating WNT/β-Catenin Pathway," *Evidence-Based Complementary and Alternative Medicine*, vol. 2022, Article ID 1890823, 9 pages, 2022.

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Research Article

NEK2 Serves as a Novel Biomarker and Enhances the Tumorigenicity of Clear-CellRenal-Cell Carcinoma by Activating WNT/β-Catenin Pathway

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Objective. Currently, cumulative evidence has shown that loss of NEK2 function suppresses tumor growth. However, complete studies on the regulatory role of NEK2 in clear-cellrenal-cell carcinoma (ccRCC) are rarely reported. *Methods*. The GEPIA database was used for information mining to analyze the gene expression differences between ccRCC tumor and normal tissues. At the same time, we analyzed the protein expression of NEK2 in clinical ccRCC samples and ccRCC cell lines. We detected the effect of NEK2 on the biological behavior of ccRCC at the cell level and further verified the biological effect of NEK2 on ccRCC cells in vivo by nude mouse tumorigenesis experiment. The expression of WNT/β-cateninpathway-related proteins and downstream proteins related to cell function were detected by Western blotting. *Results*. Using the GEPIA database, we observed that NEK2 expression level in ccRCC tissues was significantly higher than that in normal kidney tissues and was also related to tumor grade. The survival time of patients with ccRCC with high NEK2 expression was shorter than that of patients with low NEK2 expression. Compared with adjacent carcinoma and normal renal tubular epithelial cells, NEK2 levels were highly expressed in ccRCC tissues and ccRCC cell lines. NEK2 interference restrained ccRCC cell growth, migration, and invasion. NEK2 regulated the malignant behavior of ccRCC cells through the WNT/β-catenin pathway. Nude mouse tumorigenesis assay results showed that the transplanted tumors from NEK2 silenced mice grew more slowly and were smaller in size than those from control mice. *Conclusions*. NEK2 elevation may be associated with poor prognosis in ccRCC, and NEK2 enhances ccRCC cell proliferation, migration, and invasion ability by activating the WNT/β-catenin signaling pathway.

1. Introduction

Renal-cell carcinoma (RCC) is one of the ten most common malignant tumors in the world, and the incidence of RCC is second only to bladder cancer [1]. Clear-cellrenal-cell carcinoma (ccRCC) is the most common histological type of RCC, accounting for 75% of all renal cancers and the leading pathological type of death in renal cancer patients [2]. Therefore, it is of great significance to study the occurrence and development of ccRCC. Because clear-cellrenal-cell carcinoma is insensitive to both chemotherapy and radiotherapy, surgery is the preferred treatment for early renal cancer, and targeted therapy is the main treatment for advanced renal cancer [3]. Recently, with the development of targeted therapeutics, more and more targeted agents have been used in the clinical treatment of advanced or metastatic ccRCC. The results of clinical experiments showed that the targeted drugs significantly inhibited renal cancer cell growth and contributed to the improved prognosis of ccRCC patients [4]. But about 20% of patients treated with targeted drugs in the clinic will develop early resistance, resulting in poor treatment outcomes and poor patient outcomes [5–7]. There are studies statistically applying VEGF targeted agents to metastatic ccRCC with a median survival time of only

18.8 months [8, 9]. Therefore, it is important to elucidate the underlying molecular mechanisms of ccRCC progression and discover new highly effective and sensitive prognostic markers for the clinical diagnosis of ccRCC as well as develop new targeted therapeutics.

Never in mitosis gene A related kinase (NIMA) is a family of serine/threonine kinases representing NEK1-NEK11, located in the cytoplasm and mitochondria of ciliated centrosomes [10]. Accumulated evidence reveals that the NIMA-related kinase family (NEKs) is involved in various cellular functions and is associated with target pathways relevant to cancer development [11]. Thus, the members of NEKs have become drug targets of great interest. NEK2, a centrosomal serine/threonine kinase encoded by the NEK2 gene, is an essential enzyme in cell cycle progression, especially in mitotic regulation through the phosphorylation of different substrates [12]. NEK2 plays an important role in centrosome separation, microtubule organization, chromatin condensation, and the spindle assembly checkpoint. Upregulation of NEK2 leads to centrosome abnormalities and unipolar spindle formation and promotes aneuploidy by disrupting the control of mitotic checkpoints, leading to cell cycle disorders. [13, 14]. An increasing number of studies show that NEK2 expression is increased in tumor tissues, and NEK2 upregulation is closely associated with multiple types of tumor progression, drug resistance, and poor prognosis [15–17]. Meanwhile, a previous study demonstrated that deregulation of NEK2 protein is associated with poor prognosis in human ccRCC [18], but to date, there have been no reports on the regulatory mechanism of NEK2 protein on ccRCC.

In this study, we intend to detect the expression difference of NEK2 in ccRCC tissues and ccRCC cell lines, analyze the impact of NEK2 expression difference on the clinical prognosis of NEK2 patients, and finally explore the impact of NEK2 on the biological function of NEK2 in vivo and in vitro.

2. Materials and Methods

2.1. Patients and Tissue Specimens. In this study, 30 cases of clear-cellrenal-cell carcinoma (ccRCC) and adjacent noncancerous tissue specimens (normal tissues with the distance of 3 cm from the renal cancer tissues) removed by radical nephrectomy in Shaanxi Provincial People's Hospital from June 2016 and May 2020 were collected. All patients did not receive any radiotherapy, chemotherapy, or targeted therapy before the operation. Fresh ccRCC and adjacent noncancerous tissue specimens were collected during the operation, which was washed with PBS and immediately frozen in liquid nitrogen. Fresh ccRCC and adjacent noncancerous tissue specimens were collected intraoperatively and immediately frozen in liquid nitrogen after washing with PBS for Western blot analysis.

2.2. Cell Culture and Transfection. A498, Caki-1, and 786-O, as the most commonly used ccRCC cell lines and human proximal tubule epithelial cells (HK-2), were purchased from the cell resource center of the Shanghai Institutes of Biological Sciences, Chinese Academy of Sciences. 10% FBS was mixed with RPMI 1640 and then added into all cell plates and maintained in a 5% CO_2 incubator at 37°C with 2–3 changes of medium per week. Subsequently, 786-O and Caki-1 cells in the logarithmic growth phase were harvested, digested and resuspended, and seeded in 6-well plates at 5×10^5 cells/well and cultured overnight for cell transfection when the cell confluence reached approximately 80%. According to the Lipofectamine 2000 reagent instructions, shRNA targeting NEK2 (sh-NEK2: 5′-CCTGTATTGAGT GAGCTGAA-3′) and their negative control (sh-NC: 5′-TTC TCCGAACGTGTCACGT-3′) were transfected into the two cell lines, which were cultured for 48 h.

2.3. Western Blot Assay. ccRCC tissues and cells were collected and quickly loaded into 1.5 mL EP tubes with the addition of a well-configured RIPA lysis solution. The supernatant obtained by centrifugation after lysis was carefully aspirated, and the protein content was tested by the BAC method. Protein samples (20 μg) were applied to 10% SDS-PAGE gels to separate and transferred to PVDF membranes by a wet rotation. The PVDF membranes were immersed in 5% nonfat milk powder sealant and sealed for 2 h. Reference to the recommended ratio in the instructions, 5% nonfat milk was used to dilute primary antibodies, including NEK2, E-cadherin, GAPDH, c-Myc, MMP-9, GSK3β, and β-catenin (1 : 1000) for overnight incubation at 4°C. After washing the membrane three times with TBST, the PVDF membranes were immersed in secondary antibody diluent and incubated for 1 h at room temperature on a shaker. Finally, the luminescent solution was prepared, and the protein bands were soaked slightly in the luminescent solution, put into the luminescent machine, and then, luminescence development was performed and pictures were retained by Image J software.

2.4. CCK-8 Assay. The cells were suspended in complete medium and the concentration of cell suspension was adjusted to 2×10^4/mL. A uniform concentration of cell suspension (100 μL/well) was seeded in 96-well plates. After seeding, the 96-well plates were incubated in a 5% CO_2 incubator at a constant temperature for the corresponding planned time (24, 48, 72, and 96 h). At the planned assay time point, the 96-well plate was removed, 10 L of CCK-8 solution was added to the well of the cell to be tested, and continue to put the 96-well plate into the cell culture incubator for 1 h of incubation. Finally, the 96-well plate was removed, placed in the detection rack of the microplate reader, and the absorbance value (OD) of each well was detected at 450 nm.

2.5. 5-Ethynyl-2′-Deoxyuridine (EdU) Immunofluorescence Staining. EdU Kit (Ribobio) was utilized to assess cell proliferation. The 786-O and Caki-1 cells were suspended in complete medium and the concentration of cell suspension was adjusted to 1.5×10^5/mL. A uniform concentration of

cell suspension (200 μL/well) was seeded into 48-well plates, and then 50 μM of EdU marketing solution was added to each well to incubate with cells for 8 h. Subsequently, the cells were fixed with 70% ethanol, infiltrated with Triton X-100 and stained with Apollo reaction solution. After washing the cells three times with PBS, DAPI was used for nuclear staining. The images were captured under a fluorescence microscope (Olympus).

2.6. Cell Cycle Assay. Cell Cycle Staining Kit (MultiSciences Biotech Ltd., China) was utilized to cell cycle. 786-O and Caki-1 cells were suspended in complete medium and the concentration of cell suspension was adjusted to 5×10^5 cells/mL. Ethanol at a concentration of 70% was added to the cultured cells for overnight fixation, subsequently, PBS was added to wash, and PI was added to avoid light staining for 30 min. Finally, the percentage of the cells in different phases was measured with flow cytometry.

2.7. Transwell Assays. After the serum-free medium and Matrigel were diluted at a ratio of 7 : 1 according to the instructions, they were evenly spread over the transwell upper chambers for invasion assays. For the migrated assay, the transwell upper chambers did not contain Matrigel. 786-O and Caki-1 cells (5×10^5 cells/mL) were added to the upper chamber along with serum-free medium to 200 μL. The lower chamber was then filled with 600 μL of complete medium. After 24 h of incubation at 37°C in 5% CO_2, the chambers were removed, and the cells on the Matrigel were gently wiped off using a cotton swab, and the PBS working solution was used to wash chambers repeatedly three times. The chambers were then fixed in 4% paraformaldehyde for 20 min, and washed repeatedly three times with PBS working solution. Next, after staining with crystal violet dye solution for 20 min, the chambers were washed repeatedly thrice with PBS and left to air dry at room temperature. Eventually, the number of invaded or migrated cells within five random fields was counted under an IX51 microscope.

2.8. Immunohistochemistry. Tissue samples of 5 μm-thick paraffin sections were conventionally dewaxed, dehydrated by gradient ethanol, repaired with EDTA (pH = 9.0) for 3 min, sealed with 3% methanol hydrogen peroxide, and added with Ki67 primary antibody (1 : 200) at 4°C overnight. Next, slides were incubated with HRP-secondary antibodies, visualized with DAB, counterstained with hematoxylin, routinely dehydrated, and transparent. Images were captured by a light microscope.

2.9. Animal Experiments. Twenty BALB/c nude mice (5-week-old, 15–22 g) were purchased from the experimental Animal Center of Xi'an Jiaotong University, which were randomly divided into sh-NEK2 and sh-NC groups. 786-O cells transfected with sh-NEK2 or sh-NC were resuspended with PBS, and the cell concentration was adjusted to 3×10^7 cells/mL. 100 L of the above cell suspension was respectively pipetted and subcutaneously injected into the left axilla of mice. Nude mice status and subcutaneous tumorigenesis were observed weekly after inoculation. Six weeks after tumor formation, the nude mice were sacrificed to exfoliate the tumor, and the tumor was tested and weighed. Tumor volumes were calculated according to the formula: $[\text{length} \times \text{width}^2]/2$. Part of the tumor was stored in formalin for immunohistochemical experiments.

2.10. Statistical Analysis. The results of the measurement data were expressed as mean ± standard deviation (SD) from three independent experiments. The Shapiro-Wilk test was utilized to verify that the measurement data were normally distributed. The *t*-test was applied to compare the measurement data in accordance with normal distribution. SPSS 20.0 was used for data analysis. $P < 0.05$ considered that the difference was statistically significant.

3. Results

3.1. The Expression of NEK2 Was Overexpressed in ccRCC and Correlated with Clinical Parameters. NEK2 has been reported to play an oncogene role in other tumors. Thus, we want to further study its role in ccRCC progression. Using the GEPIA database, we found that NEK2 expression was upregulated in kidney clear renal cell carcinoma (KIRC) tumor tissues (Figure 1(a)). Surprisingly, compared with stages III and IV, the expression of NEK2 was remarkably differentially expressed in TNM stages I and II (Figure 1(b)). The results of the GEPIA database also revealed that elevated NEK2 was negatively correlated with the shortening of overall survival and disease-free survival (Figures 1(c) and 1(d)). To study whether the levels of NEK2 in ccRCC patients were consistent with the results of bioinformatics analysis, we collected 30 pairs of ccRCC tissues and adjacent noncancerous tissues to estimate NEK2 expression by Western blotting. The results revealed that a high level of NEK2 was found in ccRCC tissues (Figure 1(e)). Subsequently, we also demonstrated that NEK2 was elevated in ccRCC cell lines (A498, Caki-1, and 786-O) in comparison with the human proximal tubule epithelial cell line HK-2 (Figure 1(f)).

3.2. Interference of NEK2 Reduced ccRCC Cell Growth and Induced Cell Cycle Arrest. To investigate whether NEK2 regulated the malignant biological behavior of ccRCC cells, we constructed sh-NEK2 or sh-NC and transfected them into 786-O and Caki-1 cells and then used them for subsequent experiments. Western blotting confirmed that NEK2 protein expression was effectively decreased in sh-NEK2 transfected cells (Figure 2(a)). CCK-8 assays indicated that the inhibition of NEK2 by sh-NEK2 prominently impaired 786-O and Caki-1 cell proliferation (Figure 2(b)). Similarly, the sh-NEK2 group had fewer cell proliferation than the sh-NC group, as demonstrated by the EDU assay (Figure 2(c)). Through flow cytometry, we demonstrated that NEK2 silencing resulted in prominently increased cell cycle arrest at the G0/G1 stage and prominently decreased cell cycle arrest at the S phase (Figure 2(d)).

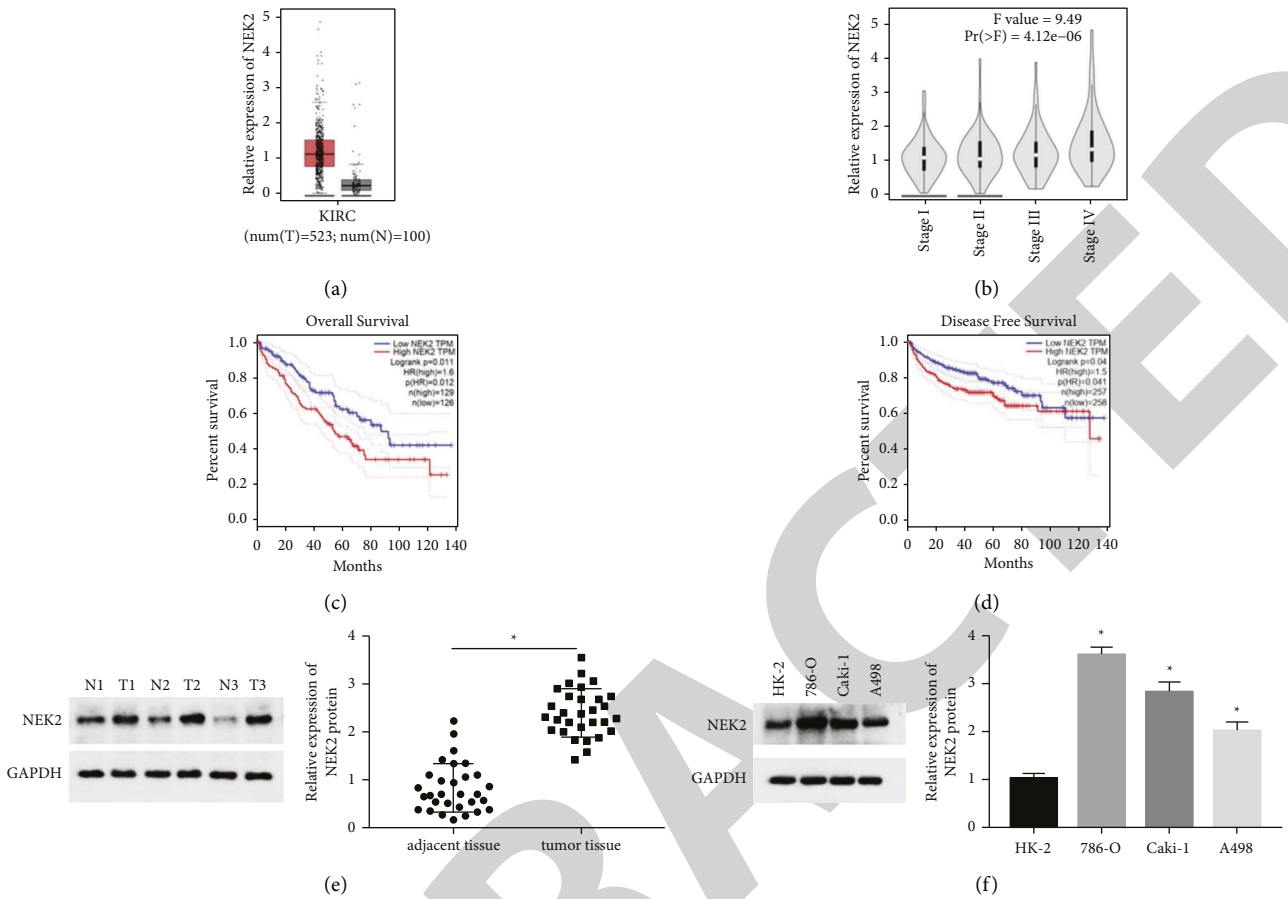
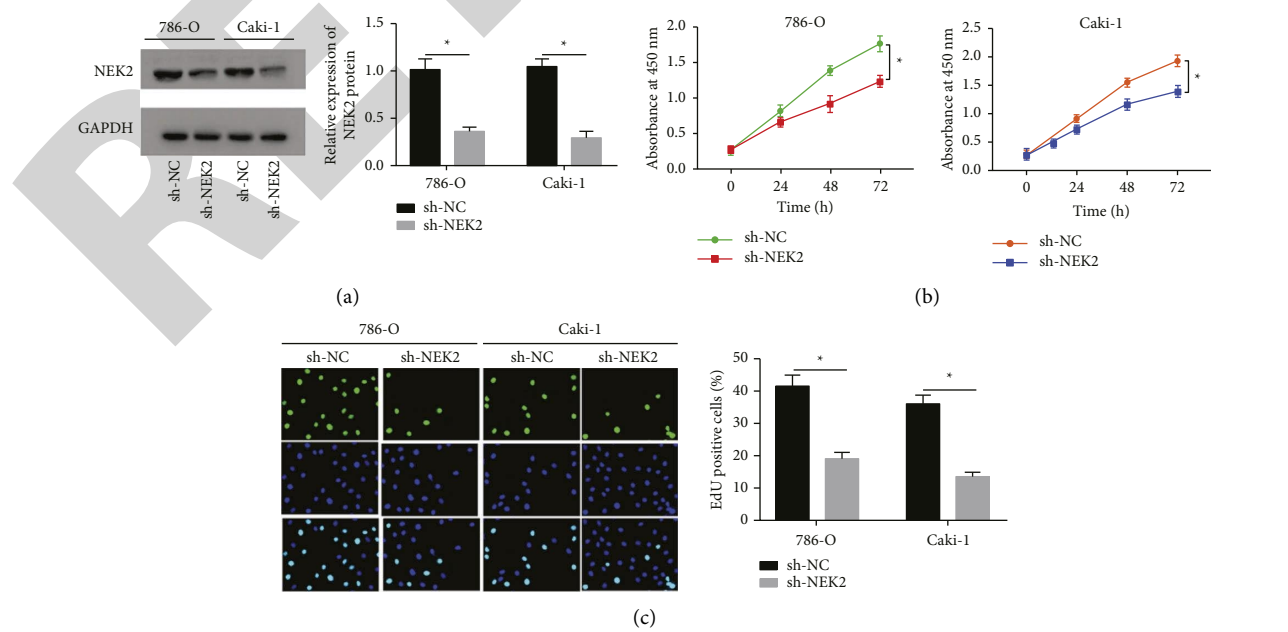


Figure 1: Elevated NEK2 was found in ccRCC tissues and cell lines. (a): NEK2 expression was predicted in kidney clear renal cell carcinoma (KIRC) samples and normal sample by the GEPIA database. (b): NEK2 expressions at TNM stages I, II, III, and IV were predicted by the GEPIA database. (c, d) Overall survival and progression-free survival of ccRCC patients with high or low expression of NEK2 was predicted by the GEPIA database. (e) NEK2 expression in tumor and adjacent issues collected from ccRCC patients ($n = 30$). (f): NEK2 expression in 786-O, Caki-1 and A498, and HK-2 cells. $^*P < 0.05$.



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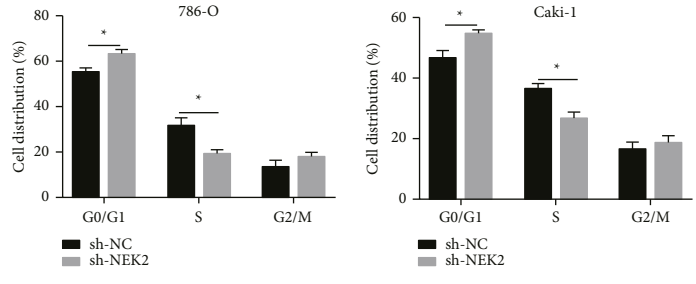


(d)

Figure 2: NEK2 silencing restrained cell growth. (a) Western blotting detection of NEK2 level in ccRCC cells after transfection. (b, c) CCK-8 and EDU assays detection of ccRCC cell proliferation after transfection. (d) Flow cytometry detection of the ccRCC cell cycle after transfection. $^{*}P < 0.05$.

3.3. Knockdown of NEK2 Reduced Migration and Invasion of ccRCC Cells. Through the analysis of the GEPIA database, we found that the elevation of NEK2 was prominently correlated with the prognosis and TNM stage of ccRCC. Therefore, we speculated that NEK2 may have a hand in the invasion and metastasis of ccRCC. We first examined the effect of NEK2 on the migration of ccRCC cells in vitro. In 786-O and Caki-1 cells, the migration ability of cells was significantly decreased, and NEK2 silencing triggered a decline in the number of cells passing through the chamber (Figure 3(a)). Transwell assays further demonstrated that NEK2 interference effectively suppressed the invasion ability of cells and caused a marked decrease in the number of invaded cells (Figure 3(b)). Of note, MMP-9 and E-cadherin are key factors in tumor invasion and metastasis. To investigate whether MMP-9 and E-cadherin participate in the invasion and migration of ccRCC promoted by NEK2, we detected MMP-9 and E-cadherin levels in NEK2 silenced cells and negative control cells. As predicted, the results of Western blotting indicated that NEK2 silencing prominently augmented the E-cadherin protein level and remarkably reduced the MMP-9 protein level (Figures 3(c) and 3(d)).

3.4. NEK2 Was Involved in the Malignant Behavior of ccRCC Cells via Wnt/β-Catenin Pathway. After confirming that NEK2 can affect the biological behavior of ccRCC, we further investigated the signaling pathway in which NEK2 may be involved. The Wnt/β-catenin signaling pathway has been reported to regulate ccRCC progression [19–21]. To determine whether NEK2 modulated the Wnt/β-catenin pathway to enhance the malignant behavior of clear-cellrenal-cell carcinoma, WNT pathway-related protein levels, including GSK3β, β-catenin, and c-Myc, in NEK2-depleted 786-O and Caki-1 cells and negative control cells were tested by Western blotting. The results demonstrated that the expression of phosphorylated GSK3β, β-catenin, and c-Myc was decreased (Figures 4(a)–4(c)).

3.5. Knockdown of NEK2 Restrained Tumor Growth In Vivo. To further validate the effect of NEK2 on ccRCC, we performed subcutaneous tumorigenesis in nude mice by applying the constructed 786-O cells with stable interference of NEK2 and the negative control cells to observe the tumor cell growth in vivo. The results uncovered that NEK2 interference resulted in inhibition of tumor growth, as demonstrated by a significant reduction in tumor size and weight in mice (Figures 5(a)–5(c)). Western blotting indicated that the NEK2 level in tumor tissues was abated in the sh-NEK2 group (Figure 5(d)). Of note, immunohistochemical analysis of Ki67 levels in tissues from tumors displayed that the sh-NEK2 group exhibited an effectively reduced Ki67 expression intensity in comparison with the sh-NC group (Figure 5(e)).

4. Discussion

In the current work, using bioinformatics analysis, we found that NEK2 expression was higher in ccRCC tissues than in adjacent noncancerous tissues. Furthermore, the results of survival analysis showed that patients with ccRCC in the NEK2 high-expression group had a significantly shorter survival time than patients with ccRCC in the NEK2 low-expression group. At the same time, the level of NEK2 protein expression in ccRCC tissues using Western blot analysis suggested that NEK2 protein expression was elevated in ccRCC tissues, and these results were consistent with the previous information analysis. We simultaneously examined NEK2 protein levels in ccRCC cell lines, namely, 786-O, Caki-1, A-498, and HK-2 cells, and the results suggested that NEK2 was more highly expressed in ccRCC cell lines than in HK-2 cells and that NEK2 expression was effectively increased in ccRCC cell lines, which also further corroborated previous examinations at the cellular and tissue levels.

Previous studies have identified abnormal levels of NEK2 proteins in a series of tumors, and NEK2 is involved in cell growth and apoptosis, enhances the ability of tumor invasion, and reduces the sensitivity of tumor cells to chemotherapeutic agents, thereby contributing to the tumorigenic capacity of malignant tumors [22, 23]. Through the inactivation of the AKT pathway, NEK2 silencing hindered gastric cancer cell growth by inducing autophagic cell death and suppressing aerobic glycolysis [24]. In the study of NSCLC, NEK2 not only mediated tumor angiogenesis and M2 polarization of macrophages but also contributes to tumor cell proliferation and invasion, thus promoting the

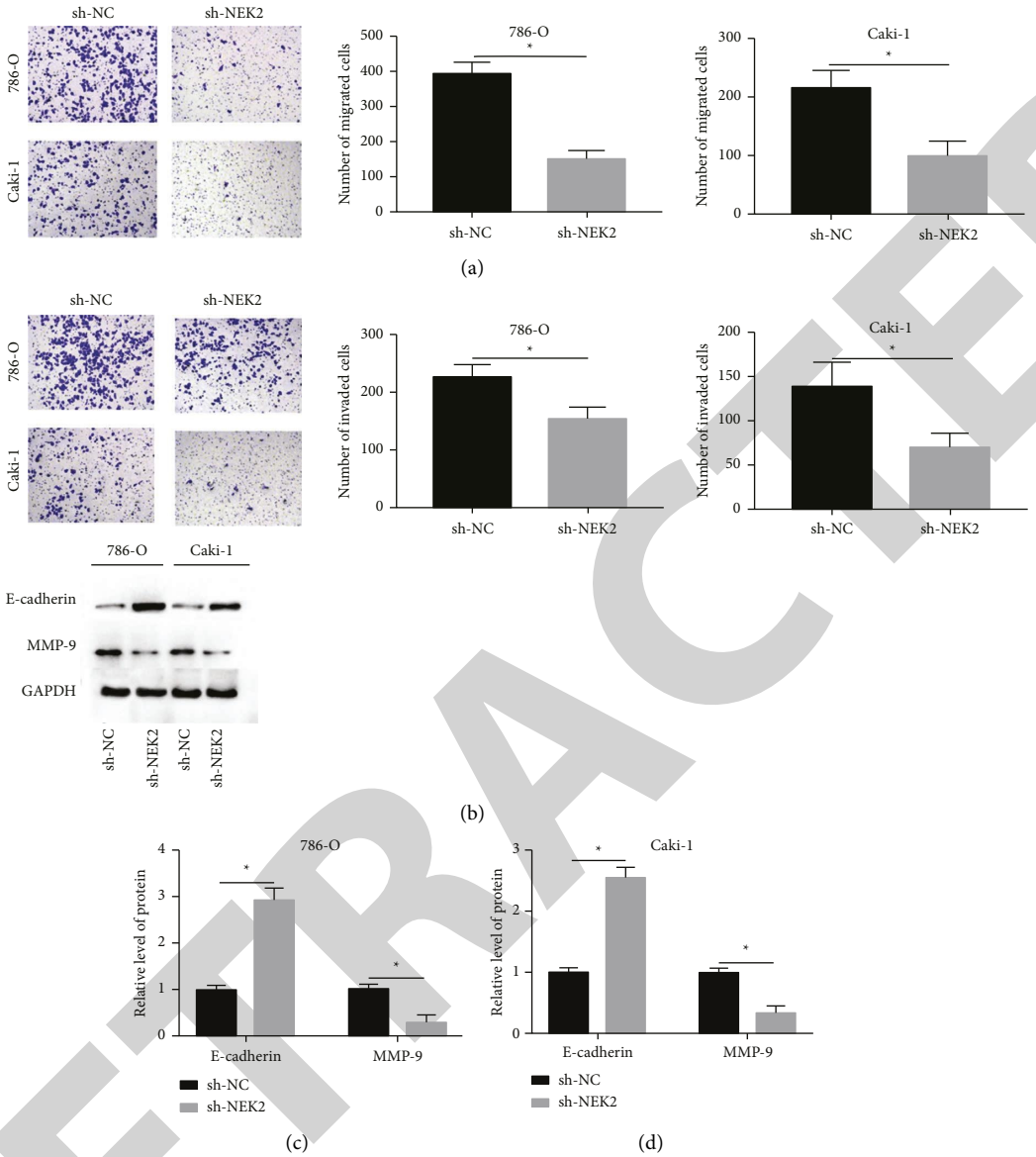


Figure 3: NEK2 silencing restrained ccRCC cell migration and invasion. sh-NEK2 or sh-NC was transfected into 786-O and Caki-1 cells, respectively. (a) Transwell migration assay detection of ccRCC cell migration. Scale bar: 100 μm. (b) Transwell invasion assay detection of ccRCC cell invasion. Scale bar: 100 μm. (c) Western blotting detection of E-cadherin in ccRCC cells. (d) Western blotting detection of E-cadherin and MMP-9 level in ccRCC cells. $^*P < 0.05$.

occurrence of nonsmall-cell lung cancer [25]. TP53 deletion elevated NEK2 expression by inducing NEK2 amplification, thereby inhibiting the ability of proliferation, drug resistance development, and tumorigenesis of multiple myeloma cells [26]. Given the above, NEK2 may serve as a molecular target in ccRCC research. In this study, we constructed NEK2 knockdown cells and xenotransplantation tumor models and conducted cell function and animal experiments after confirming their stable expression. Knockdown of NEK2 resulted in retarded ccRCC cell growth and significantly decreased cell migration and invasion abilities. Besides, NEK2 knockdown clearly inhibited tumor cell growth in vivo with reduced tumorigenic capacity.

WNT/β-catenin is a class of signaling pathways that have been highly conserved across species evolution and that play an important role in embryogenesis, organ formation, and regulation of homeostasis [27, 28]. Much tumorigenesis and progression are associated with mutations in key proteins in this signaling pathway, leading to aberrant activation of signaling. WNT proteins, through their interaction with cell surface specific receptors, cause the accumulation of β-catenin proteins in the cytoplasm, ultimately leading to the proliferation and metastasis of cancer cells [29]. β-Catenin protein has various cellular functions, where it can interact with E-cadherin at cell junctions, thereby playing an adhesive role [30]. Additionally, MMP-9 is one of the Wnt pathway target genes, which can be transcriptionally

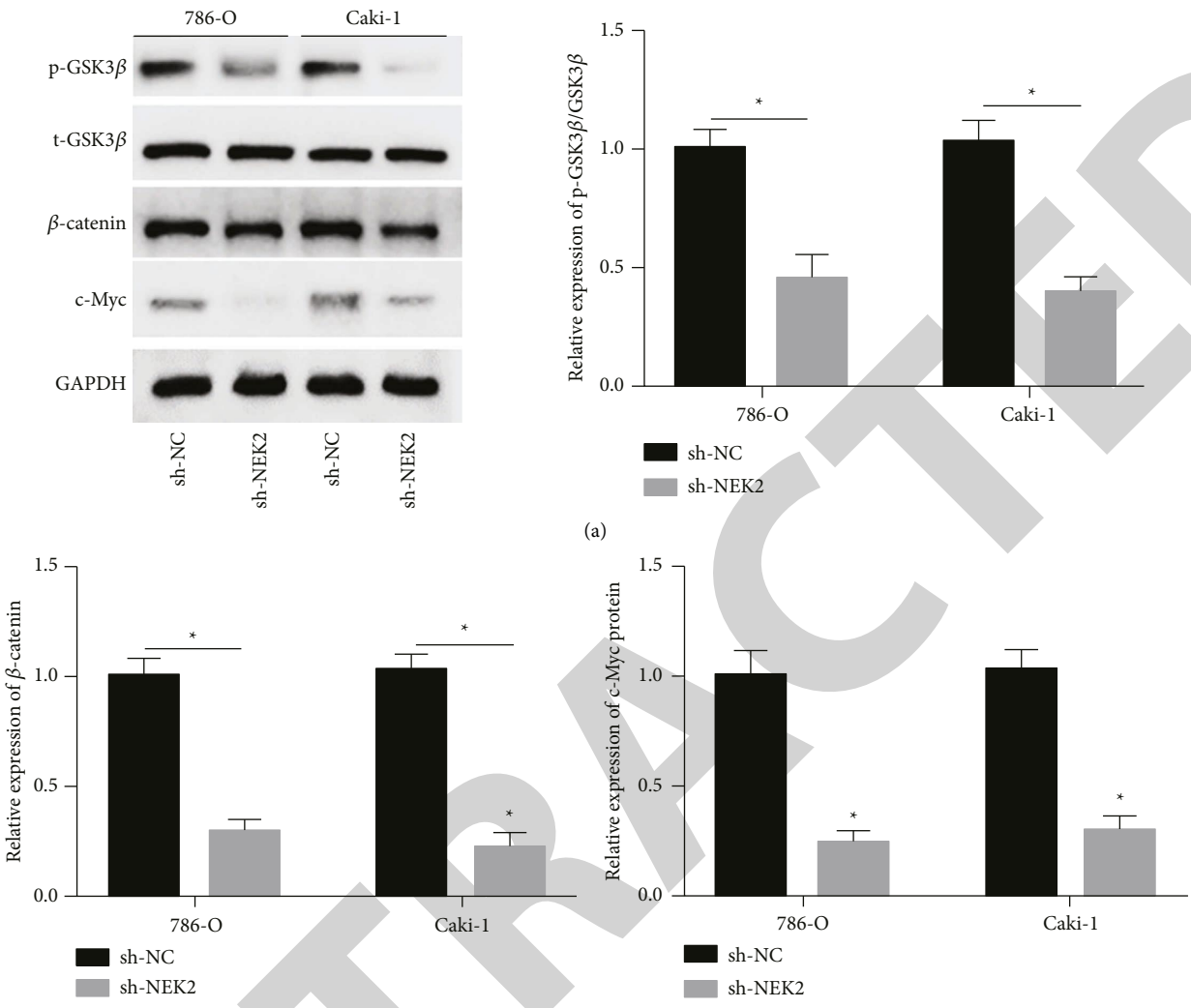
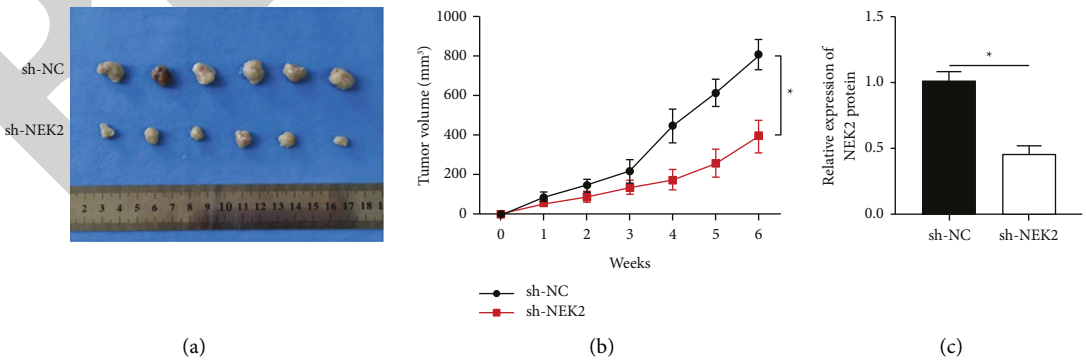


Figure 4: NEK2 silencing blocked Wnt/β-catenin pathway activation. sh-NEK2 or sh-NC was transfected into 786-O and Caki-1 cells, respectively. (a–c) Western blotting detection of WNT pathway-related protein (GSK3β, β-catenin, and c-Myc) level in ccRCC cells after transfection. $^{*}P < 0.05$.



(a) (b) (c)

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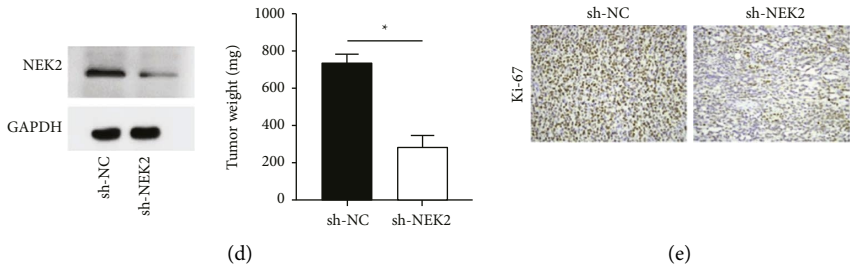

Figure 5: NEK2 silencing restrained cell growth in ccRCC in vivo. NEK2 stable knockdown (sh-NEK2) and negative control (sh-NC) 786-O cells were utilized to construct a xenograft model in mice. (a) Observation of tumor growth following NEK2 interference. (b) Detection of mouse tumor volume following NEK2 interference. (c) Detection of mouse tumor weight following NEK2 interference. (d, e) Western blotting detection of NEK2 level following NEK2 interference. (e) Immunohistochemical detection of Ki67 level following NEK2 interference. $^*P < 0.05$.

regulated [31]. According to some substantial studies, the WNT/β-catenin pathway is hyperactivated in ccRCC, and targeted inhibition of the WNT/β-catenin pathway may become a feasible therapeutic strategy for tumors [32–34]. Here, our results showed that NEK2 silencing could obviously downregulate the protein expression levels of key proteins, such as p-GSK-3β, β-catenin, and the downstream regulator c-Myc, proving that NEK2 silencing could inhibit the WNT/β-catenin signaling pathway.

In conclusion, NEK2 was upregulated in ccRCC tumors, and the degree of expression is closely related to tumor prognosis, which may provide a basis for early diagnosis and prognosis. NEK2 interference obviously repressed ccRCC cell growth and concurrently restrained invasion and migration. On a mechanistic level, our results suggest that NEK2 may act by repressing Wnt/β-catenin pathway to suppress ccRCC growth. The result of this experiment will provide insight into the molecular mechanism of NEK2 in ccRCC and lay a foundation for further exploration of it as a candidate for the therapy of ccRCC.

Data Availability

The labeled dataset used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that they have no conflicts interests.

Acknowledgments

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Research Article

Effect of Vacuum Sealing Drainage on Soft Tissue Injury of Traumatic Fracture and Its Effect on Wound Recovery

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Purpose. The current work is mainly to explore the effect of vacuum sealing drainage (VSD) on soft tissue injury (STI) caused by traumatic fractures (TFs) and its effect on wound recovery. *Methods*. We first selected 90 patients with TF STI from May 2019 to May 2021, of which 40 patients (control group) received routine treatment, and the other 50 patients (observation group) were treated with VSD. The curative effect, rehabilitation (changing dressing frequency, healing time, and hospitalization time), pain severity, patient comfort, and complications were evaluated and compared. *Results*. The observation group exhibited a higher total effective rate, lower dressing change frequency, complication rate, and shorter healing time and hospital stay than the control group, which are statistically significant. Statistically milder pain sensation and better patient comfort were also determined in the observation group. *Conclusions*. VSD is effective and safe in the treatment of TF-induced sexually transmitted infections, which can effectively accelerate wound recovery while reducing pain sensation and improving patient comfort, with clinical promotion value.

1. Introduction

All infections and injuries of soft tissues are no small challenge for patients and medical staff. Soft-tissue disorders are essentially a manifestation of systemic health that requires proper diagnosis, resuscitation, management, and support [1]. Traumatic fractures (TFs), often accompanied by open injuries, are prone to occur in all kinds of soft tissues in various sports [2–4]. These soft tissue injuries (STIs) can easily lead to the reduction of soft tissue coverage and the destruction of local blood supply that adversely affect fracture healing, resulting in some patients experiencing wound infection and necrosis due to wound contamination [5]. From an ergonomic point of view, the mechanical behavior of the whole tissue will change dramatically if the soft tissue is damaged, which in turn suggests that biomechanical changes caused by STIs can be used to detect the severity of the injury [6, 7]. In any event, all kinds of STIs must be considered when detecting and treating TFs [8]. Hence, research on the treatment of STIs and wound recovery has important practical value for improving patient outcomes and avoiding wound infection and necrosis.

Vacuum sealing drainage (VSD) has a wide range of applications in wound care as well as clinical treatment at present [9]. In the management of various diseases, including open abdominal wounds, burns, pressure sores, sternal wounds, and obese patients after bariatric surgery, VSD is often used [10, 11]. This technique plays an important role in preventing wound infections of closed incisions as well as surgical site complications [12]. The treatment begins with the construction of a closed, sealing system that applies negative pressure (suction) to the wound surface. The wound is then covered or wrapped with an open-cell foam or gauze dressing and sealed with a sealing cover. And subsequently, intermittent or continuous suction is maintained by connecting a suction pipe from the wound

dressing to a vacuum pump and a waste liquid collector [13]. In this paper, we will explore the curative effect of VSD in treating STIs resulted from TFs and its impact on wound recovery.

2. Methods

2.1. General Information. We selected 90 patients with STIs attributed to TFs admitted from May 2019 to May 2021, 40 of whom received routine treatment and were included in the control group, and the other 50 cases were additionally treated with VSD and used as the observation group. The control and observation groups were not statistically different in general data ($P > 0.05$).

Inclusion criteria were as follows: (1) diagnosis of TFs by the presence of clinical presentations, medical history, computerized tomography (CT) or ultrasound examination, and surgical examination [14]; (2) Gustilo classification type II or III [15]; (3) mentally normal with no history of mental illness; (4) ability to clearly express the discomfort, without communication barrier. Exclusion criteria were as follows: (1) serious primary diseases of heart, liver, kidney, and other VITAL organs; (2) serious diseases of the blood system; (3) history of anti-infection, immunosuppressants, hormones, and other drugs within one month before treatment.

Informed consent was provided by patients or their families, and the study was conducted after obtaining approval from the Hospital Ethics Committee.

2.2. Therapies. The control group received routine treatment. In the emergency operating room, the wound was precleaned with clean water and the necrotic tissue was removed, followed by disinfection with iodophor and hydrogen peroxide. In the case of wound degloving, a sharp knife was used to puncture the mesh for cleaning and disinfection; any foreign bodies in the wound were removed before wound cleaning and disinfection. After debridement, one-stage fracture repair was performed, and an appropriate amount of wound secretions were collected for bacterial culture. Drug sensitivity tests were performed on infected patients, and anti-infection dressings were selected based on the results of bacteria and drug sensitivity culture. Then, appropriate saline and antibiotics mixed with wet gauze were cut, gently applied to the wound surface, and wrapped up with cotton bandages. Dressing change was conducted 1 day later. Thereafter, the dressing was changed every 1–3 days according to the healing condition of the wound until the wound could be closed by second-stage surgery (suturing or skin grafting, skin flap repair, etc.). On this basis, the observation group was treated with VSD. The preoperative treatment, operation, bacterial culture, and drug sensitivity test of patients with primary fracture repair were the same as those of the control group, followed by 75% ethanol disinfection. The VSD dressing was cut according to the size and shape of the wound, and the translucent membrane was pasted to seal the wound (3 cm longer than the VSD dressing). Then the two drainage tubes were connected, one of which maintained VSD negative pressure at 26.6–59.8 kPa, and the other was continuously irrigated with anti-infective drugs +500 mL normal saline according to the results of bacteria and drug sensitivity culture. The dressing was changed once every 5–7 days in patients with turbid drainage fluid and once every 7–10 days in those with clear drainage fluid. The duration of VSD was determined according to the patient's wound size and the infection situation until the second-stage operation can be performed to close the wound (suture or skin grafting, skin flap repair, etc.)

2.3. Measurement Indicators

2.3.1. Rehabilitation. The rehabilitation of the two cohorts of patients, evaluated from dressing change frequency, healing time, and length of hospital stay (LOS), was compared.

2.3.2. Pain Severity and Comfort Status. The pain severity and patent comfort, assessed by the Visual Analog Scale (VAS) and Kolcaba's General Comfort Questionnaire (GCQ) [16, 17], respectively, were counted in both cohorts.

2.3.3. Total Efficiency. The total effective rate was also statistically compared. Evaluation criteria [4]were as follows: cured: the wound healed and the epidermis survived well without the need for dressing changes; effective: obviously reduced wound area, with partially survived skin flap, reduced secretions, but the need for dressing changes; ineffective: little relief of symptoms and signs before and after treatment, with no obvious change in wound surface and secretions. The total effective rate = (cure + effective) cases/the total number of cases in this group × 100%.

2.3.4. Complication Rate. The incidence of complications (infection, skin necrosis, amyotrophy, and osteomyelitis) was statistically compared.

2.4. Statistics and Methods. SPSS22.0 (Asia Analytics Formerly SPSS China) was employed for synthetic data analysis. Categorical variables ($n(\%)$) were tested using the χ^2 test, and continuous variables ($X \pm S$) were compared by the t-test before and after surgery within the group. $P < 0.05$ was the significance threshold.

3. Results

3.1. General Data. As shown in Table 1, the two cohorts were comparable in gender, age, body mass index (BMI), and other general data ($P > 0.05$).

3.2. Rehabilitation. Comparing patients' postoperative rehabilitation (Figure 1), we can see that the dressing change frequency, healing time, and LOS were significantly less in the observation group than in the control group ($P < 0.05$).

Table 1: General data.

Classification	Observation group ($n = 50$)	Control group ($n = 40$)	t/χ^2	P
Sex			0.02	0.887
Male	27 (54.00)	21 (52.50)		
Female	23 (46.00)	19 (47.50)		
Age (years old)	40.12 ± 5.75	39.08 ± 6.85	0.78	0.436
BMI (kg/m^2)	20.62 ± 3.26	20.42 ± 4.19	0.25	0.800
Gustilo classification			0.24	0.627
II	30 (60.00)	26 (65.00)		
III	20 (40.00)	14 (35.00)		
Site of injury			0.03	0.985
Upper limbs	12 (24.00)	9 (22.50)		
Thigh	28 (56.00)	23 (57.50)		
Lower leg	10 (20.00)	8 (20.00)		
Smoking			0.38	0.538
Yes	40 (80.00)	34 (85.00)		
No	10 (20.00)	6 (15.00)		
Drinking			0.15	0.701
Yes	37 (74.00)	31 (77.50)		
No	13 (26.00)	9 (22.50)		

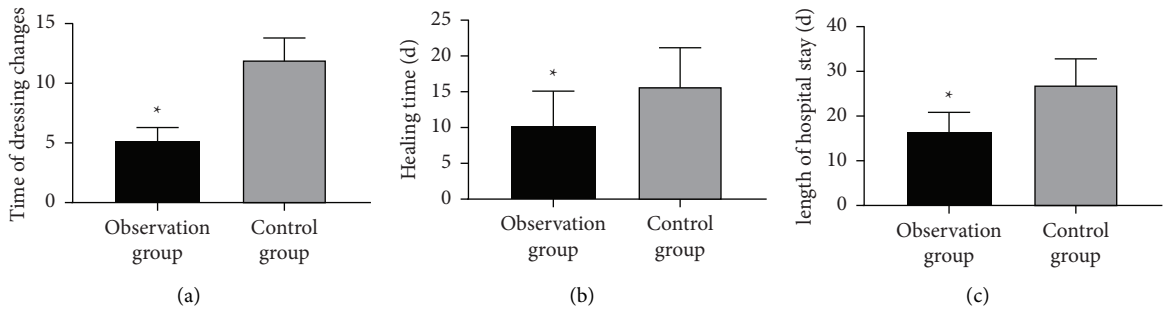

Figure 1: Comparison of rehabilitation: (a) times of dressing changes: the observation group had fewer times of dressing changes than the control group ($P < 0.05$); (b) healing time: the observation group had a shorter healing time than the control group ($P < 0.05$); (c) length of hospital stay: the observation group had less length of hospital stay than the control group ($P < 0.05$). Note * means that compared with the control group, $P < 0.05$.

3.3. Pain Severity and Comfort Status. The intergroup comparison (Figure 2) of postoperative pain severity and comfort status revealed obviously alleviated pain and improved patient comfort in both cohorts. And in comparison with controls, the observation group had a lower VAS score and a higher GCQ score ($P < 0.05$).

3.4. Total Effective Rate. Statistics on overall treatment efficacy (Table 2) revealed an evidently higher total effective rate in the observation group ($P < 0.001$).

3.5. Complication Rate. The statistics on postoperative complications (Table 3) identified a higher complication rate in the control group as compared to the observation group ($P < 0.05$).

4. Discussion and Conclusion

Fractures that occur after high-energy trauma are usually accompanied by various intra-articular lesions such as ligamentum teres injury, loose body, cartilage injury, and trauma of soft tissue like the lip, which can cause ischemic necrosis, post-traumatic osteoarthritis, and even long-term disability in severe cases [18–20]. Therefore, for STIs of TFs, it is necessary to use the correct treatment. In this section, we will study the effectiveness of VSD on TF-induced STIs through various indicators.

This study found significant changes in the postoperative dressing change frequency, healing time, and LOS in both cohorts, with fewer times of dressing changes, and shorter healing time and LOS in the observation group using VSD. VSD has been proven to be effective in treating chronic and complex wounds, with the wound dehiscence rate approximately halved with this technology [21]. VSD renders benefits to various types of surgeries. In breast surgery with easy recurrence and multiple complications, for example, the use of VSD can reduce seroma and its sequelae [22, 23]. As an auxiliary treatment method for open wounds, VSD will apply controllable negative pressure to the wounds through various equipment and professional dressings, and transfer wound liquid to appropriate containers, which can create a wound environment conducive to healing by removing infected materials and exudates, reducing edema, and

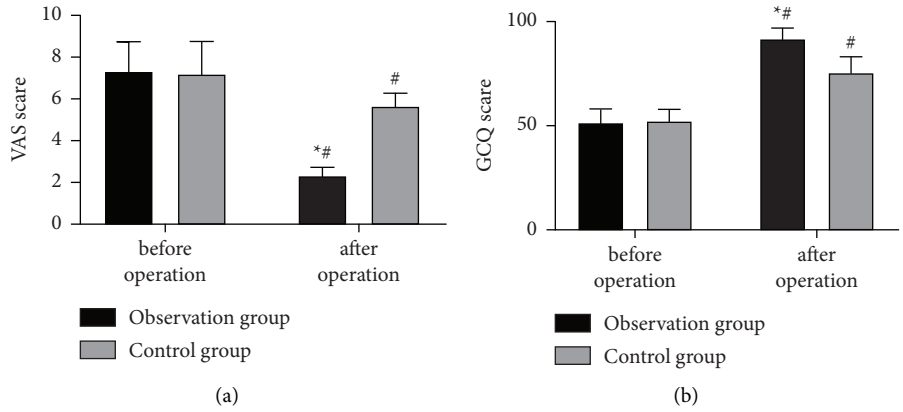

Figure 2: Comparison of rehabilitation: (a) VAS score: reduced postoperative VAS scores were observed in both cohorts, with a lower score in the observation group versus the control group ($P < 0.05$); (b) GCQ score: elevated postoperative GCQ scores were determined in both cohorts, with a more significant increase in the observation group compared with the control group ($P < 0.05$). Note * means $P < 0.05$ compared with the control group; # means $P < 0.05$ compared with the postoperative score within the group.

Table 2: Total effective rate of two groups of patients.

Classification	Observation group ($n = 50$)	Control group ($n = 40$)	χ^2	P
Cured	25 (50.00)	12 (30.00)	—	—
Effective	23 (46.00)	16 (40.00)	—	—
Ineffective	2 (4.00)	12 (30.00)	—	—
Total effective rate	48 (96.00)	28 (70.00)	11.44	<0.001

Table 3: Complications in two groups.

Classification	Observation group ($n = 50$)	Control group ($n = 40$)	χ^2	P
Infection	2 (4.00)	4 (10.00)	—	—
Skin necrosis	1 (2.00)	4 (10.00)	—	—
Amyotrophy	0 (0.00)	2 (5.00)	—	—
Osteomyelitis	0 (0.00)	4 (10.00)	—	—
Incidence of complications (%)	3 (6.00)	14 (35.00)	12.20	<0.001

promoting perfusion and granulation [24, 25]. Therefore, compared with the control group which only used conventional treatment, the wound healing of the observation group was far better with a higher total effective rate because of the use of VSD. Similarly, the complication rate in the observation group was not as high as that in the control group due to the effect of VSD in reducing inflammation and infection. From the above, it is clear that VSD, as an auxiliary treatment method, can greatly improve the wound healing effect and reduce the occurrence of various complications in patients. This has been confirmed in other researchers' analyses. For example, in the study of Cai et al. [26], VSD validly promoted postoperative wound healing in patients with closed calcaneal fractures, shortened the wound healing time, and lowered the incidence of wound complications, which is consistent with our findings. And as reported by Zhang et al. [27], compared with conventional dressing change intervention, orthopedic trauma patients treated with VSD had a higher total effective rate, lower dressing change frequency, and shorter wound healing time with certain security, which contributed to effectively reduced risk of adverse events such as postoperative infections and lower extremity deep vein thrombosis, similar to the results of our study.

This study also found evidently changed VAS and GCQ scores in both cohorts, with a lower VAS score and a higher GCQ score in the observation group. Consistently, Jiao et al. [28] reported significantly reduced pain severity during dressing change by VSD technique in patients with sural neurocutaneous flap transplantation in the foot and ankle. This technique has also been applied to examine patients with nonspecific hip, knee, and low back pain. Studies have found that associated soft tissues are affected following similar fractures and a range of similar orthopedic diseases [29]. This type of soft tissue defect of the skin in various parts of the body caused by external trauma is very common in clinical treatment [30]. Since some parts of the body (forearm, hand, knee joint, skin, and soft tissue in front of tibia and foot) have thinner skin texture, less subcutaneous tissue, and are located in the exposed parts of the limbs, ineffective repair of any defect will predispose patients to long-lasting pain and abnormal discomfort, which will exert a great impact on patient's mobility and life quality [31–33]. Combined with the results of this study, we can draw

a conclusion that, for patients in the observation group who used VSD, there were faster recoveries and better curative effects, contributing to less pain and discomfort compared with the control group.

This study is unique in that it comprehensively analyzes the clinical efficacy and safety of the VSD technique from multiple aspects such as efficacy, rehabilitation, pain degree, patient comfort, and complications, and confirms its effectiveness in the treatment of patients with TFs and STIs, which provides a new choice for the management of such patients. But it still has room for improvement. This time, we failed to explore patients' postoperative life quality, nor have we investigated their degree of cooperation during the operation and the improvement of psychological state. These survey indicators will be taken into account in future studies to continuously improve the clinical treatment plan.

Taken together, VSD is effective in treating TF-induced STIs, contributing to effectively accelerated wound recovery while ensuring patient safety, which deserves clinical popularization.

Data Availability

The labeled dataset used to support the findings of this study is available from the corresponding author upon request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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Research Article

Clinical Application of Multi-Index Combined Risk Assessment in Early Pregnancy for Screening of Preeclampsia

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Objective. To explore the predictive value of single-index screening or multi-index combined screening for preeclampsia. *Methods*. From January 1, 2019, to December 31, 2021, pregnant women with a singleton pregnancy who had been regularly checked in each center since the first trimester (between 11 and 14 weeks of gestation) were retrieved from multiple participating centers. The risk calculation software LifeCycle 7.0 was used to calculate the risk values before 32 weeks, 34 weeks, and 37 weeks of gestation, and through a receiver operating characteristic (ROC) curve analysis, the predictive values of pregnancy-associated protein A (PAPP-A), the placental growth factor (PLGF), the mean arterial pressure (MAP), the uterine artery pulsatility index (UTPI), or a combined multi-index were calculated for preeclampsia. *Results*. Finally, 22 pregnant women developed preeclampsia, and the area under the ROC curve of the PAPP-A + PLGF + MAP + UTPI combined screening program was greater than that of other screening programs before 37 weeks of gestation (AUC = 0.975, 0.946, or 0.840 for <32 weeks, <34 weeks, or <37 weeks, respectively). At 32 weeks, the Youden index was at its maximum. *Conclusion*. PAPP-A + PLGF + MAP + UTPI combined screening is the optimal screening mode for preeclampsia screening before 37 weeks of gestation, and the combined prediction using multiple indicators in early pregnancy is more suitable for predicting the risk of early-onset preeclampsia.

1. Introduction

Preeclampsia refers to elevated blood pressure and proteinuria after 20 weeks of gestation, which may be accompanied by symptoms such as damage to multiple organs or systems of the whole body. It is an important cause of maternal death, fetal growth restriction, and premature fetal birth. Studies have shown that taking aspirin before 16 weeks of gestation in high-risk women can significantly reduce the risk of preeclampsia [1]. Therefore, monitoring the occurrence of preeclampsia in the first trimester has significant social significance for ensuring maternal and fetal safety and reducing the incidence of birth defects. There are many screening programs for preeclampsia, and traditional screening is based on maternal factors with low reliability. In order to evaluate the reliability of preeclampsia screening before 37 weeks of gestation, this study combined pregnancy-associated protein A (PAPP-A), placental growth factor (PLGF), mean arterial pressure (MAP), and uterine artery pulsatility index (UTPI) in the first and second

trimesters of pregnancy. Multiple index screening methods are used to predict preeclampsia, and each screening index alone or the combined indexes were evaluated for preeclampsia screening. This study aims to provide a basis for the establishment of screening programs for preeclampsia in early pregnancy.

2. Materials and Methods

2.1. General Information. This study was conducted by Anhui Maternal and Child Health Hospital in collaboration with Lu'an Jin'an District Maternal and Child Health Hospital, Fuyang Women and Children's Hospital, Lu'an Maternal and Child Health Hospital, Bozhou Maternal and Child Health Hospital, and Chuzhou Maternal and Child Health and Family Planning Service Center. The study protocol was approved by the ethical committee of all participating hospitals. In this collaborative multicenter study on serological screening for preeclampsia, pregnant women with singleton pregnancies who started regular obstetric examinations at various hospitals from January 1, 2019, to December 31, 2021, since the first trimester (11–14 weeks of gestation) were retrieved. Inclusion criteria were: pregnant women with a singleton pregnancy who received early-trimester serological screening for Down syndrome at various hospitals at 11–14 weeks of gestation. Exclusion criteria were: (1) multiple pregnancies; (2) maternal complications that may affect the occurrence of gestational hypertension symptoms and comorbidities; (3) fetal malformation and chromosomal disease; and (4) miscarriage, fetal death, or termination of pregnancy before 28 weeks of gestation. A total of 9000 pregnant women were enrolled, of which 2862 were excluded for the following reasons: hypertension not related to preeclampsia ($n = 747$), immune diseases ($n = 459$), gestational diabetes mellitus ($n = 253$), miscarriage, termination of pregnancy ($n = 615$), and lost to follow-up ($n = 788$). Finally, 6138 pregnant women were included in the study, followed up until 37 weeks of gestation, and all pregnant women signed the informed consent form after consultation.

2.2. Observation Indicators and Evaluation Criteria. Fasting venous blood samples (2-3 ml) of the pregnant women who participated were collected. The serological indexes PAPP-A and PLGF were detected using the Auto DELFIA 1235 automatic time-resolved fluorescence immunoassay analyzer (PerkinElmer, USA). The placental growth factor determination kit and pregnancy-related protein A detection kit were used according to the manufacturer's manual [2].

Auxiliary diagnostic test indicators included: (1) the MAP was measured by an electronic sphygmomanometer, and the systolic and diastolic blood pressures were recorded (the difference between the diastolic blood pressures on both sides was less than 6 mm·Hg, and the difference in systolic blood pressure was less than 10 mm·Hg), and the average was taken. MAP = (systolic blood pressure + diastolic blood pressure × 2)/3; and (2) UTPI was checked by Doppler ultrasonography. The blood flow index of bilateral uterine arteries was detected at the point where the branch of the internal iliac artery crosses the uterine artery above the external iliac blood vessel, and each pregnant woman's bilateral uterine artery blood flow index was measured. The uterine artery Doppler blood flow spectrum was performed three times, and five complete, clear, consistent, and stable blood flow spectra were obtained each time. Finally, the average value of bilateral uterine artery blood flow, UTPI, was used for risk calculation.

Preeclampsia diagnostic criteria: systolic blood pressure ≥140 mm·Hg and/or diastolic blood pressure ≥90 mm·Hg after 20 weeks of gestation, accompanied by any of the following: urine protein quantitative >0.3 g/24 h, or random urine protein (+); no proteinuria but with any of the following organ or system involvement: eg., thrombocytopenia, hepatic or renal impairment, pulmonary edema, new-onset central nervous system abnormalities, or visual disturbances; preeclampsia with severe manifestations. In this study, those who met the above criteria were regarded as the preeclampsia group, and those who did not meet the above criteria were regarded as the control group.

2.3. Statistical Methods. SPSS 25.0 (SPSS, Chicago, IL, USA) was used for data processing, and the measurement data conforming to the normal distribution were expressed as the mean ± standard deviation ($\bar{x} \pm s$); otherwise, the nonparametric test was used, and the median and upper and lower quartiles were expressed. The values of PAPP-A, PLGF, MAP, and UTPI were analyzed using the LifeCycle7.0 risk assessment software developed by PerkinElmer (USA) to assess the risk of preeclampsia [3]. The effectiveness of each screening program in predicting preeclampsia was analyzed by the area under the curve (AUC) in the ROC curve, sensitivity, specificity, and the Youden index. The application value of the combined prediction scheme of PAPP-A, PLGF, MAP, and UTPI in predicting preeclampsia was analyzed, and the statistical difference was based on a 2-sided$P < 0.05$.

3. Results

3.1. Comparison of Basic Information of Two Groups of Pregnant Women. Among the 6138 pregnant women included in this study, there were 22 pregnant women in the preeclampsia group and 6116 pregnant women in the control group. The average expected delivery age of pregnant women was (27.85 ± 3.72) years old in the preeclampsia group and (28.10 ± 3.54) years old in the control group. The average weight of pregnant women was (60.27 ± 9.18) kg in the preeclampsia group and (56.93 ± 9.21) kg in the control group. The average height of pregnant women was (160.18 ± 4.85) cm in the preeclampsia group and (160.69 ± 4.96) cm in the control group. There was no statistical difference in prenatal age, weight, or height between the preeclampsia group and the control group ($P > 0.05$), as shown in Table 1.

Table 1: Basic information of pregnant women participating in preeclampsia screening.

	Control group ($n = 6116$)	Preeclampsia group ($n = 22$)	F/Z	P
Expected age (years)[#]	28.10 ± 3.54	27.85 ± 3.72	0.11	0.74
weight (kg)[#]	56.93 ± 9.21	60.27 ± 9.18	2.88	0.90
height (cm)[#]	160.69 ± 4.96	160.18 ± 4.85	0.23	0.63
MAP[#]	1.01 ± 0.11	1.14 ± 0.12	32.69*	<0.01
PAPP-A[#]	1.13 ± 0.69	0.68 ± 0.36	9.34*	<0.01
PLGF[$]	0.77 (0.59~1.02)	0.38 (0.30~0.55)	−5.01*	<0.01
UTPI[$]	0.80 (0.67~0.96)	0.92 (0.73~1.10)	−2.37*	0.02

[#]: Indicates that the normal distribution is obtained by the KS normality test, and the F test is used; [$]: indicates that the nonnormal distribution is obtained by the KS normality test, and the Mann–Whitney test is used; *: indicates that the result was significantly different.

The mean values of the multiple of the median (MoM) of each index of pregnant women in the control group and the preeclampsia group were compared. The Kolmogorov-Smirnov test (KS) normality test showed that the MoM values of MAP and PAPP-A were normally distributed. The mean value of PAPP-A MoM value (1.14 ± 0.12) in the preeclampsia group was higher than that of the control group (1.01 ± 0.11, $P < 0.01$), and the mean value of PAPP-A MoM value of pregnant women in the preeclampsia group (0.68 ± 3.60) was lower than that of the control group (1.13 ± 0.69, $P < 0.01$). Significant differences. The MoM values of PLGF and UTPI showed a nonnormal distribution. A nonparametric test was used. The average value of PLGF MoM in the preeclampsia group (0.38) was lower than that in the control group (0.77, $P < 0.011$), and the UTPI MoM value of the preeclampsia group (0.92) was higher than that of the control group (0.80, $P = 0.018$).

3.2. Analysis of the Predictive Value of Different Screening Programs for Preeclampsia. The predictive values for the risk of preeclampsia using PAPP-A, PLGF, MAP, and UTPI programs (PAPP-A + PLGF + MAP + UTPI, PAPP-A + PLGF + MAP, PAPP-A + PLGF, PLGF + MAP, PAPP-A) before 32 weeks, 34 weeks, or 37 weeks of gestation were analyzed by the ROC curve.

When the PAPP-A + PLGF + MAP + UTPI quadruple screening program was used and the overall false positive rate was 15%, the screening positive rate before 32 weeks of gestation, 34 weeks of gestation, and before 37 weeks of gestation were 1.16%, 3.31%, and 15.08%, respectively, and the detection rates could reach 90%, 80%, and 63.64%, respectively, see Figure 1(d). The AUC of the PAPP-A + MAP + PLGF + UTPI scheme was 0.975, which was the largest among all schemes. The AUC of the UTPI scheme is 0.758, which is the smallest among all schemes.

3.3. Multi-Index Combined Risk Assessment Is More Suitable for Predicting Early-Onset Preeclampsia. The ROC curve was used to analyze the sensitivity and specificity of each screening program in predicting the occurrence of preeclampsia before 32 weeks, 34 weeks, and 37 weeks of gestation, and the Youden index (sensitivity + specificity -1) of each coordinate point was calculated. The Youden index of each screening program for predicting preeclampsia showed a downward trend after 32 weeks of gestation, indicating that all screening programs had the greatest sensitivity and specificity for predicting early-onset preeclampsia, as shown in Figure 2.

4. Discussion

The incidence of preeclampsia is as high as 2% to 8% [4], and it seriously affects the safety of mothers and babies. More and more experts are aware of the importance of screening for preeclampsia [4–7]. The traditional screening program for preeclampsia is mainly based on maternal factors for risk assessment, including a previous history of gestational hypertension, chronic hypertension, chronic kidney disease, diabetes, or autoimmune diseases. However, in practice, most patients with preeclampsia do not present with the above factors. More and more evidence shows that the serological indicators PAPP-A and PLGF in the first trimester are related to the risk of preeclampsia, but the predictive value of a single indicator is not ideal [8–12]. Therefore, combinations of multiple indicators were used in this study to predict the risk of preeclampsia.

We found that before 37 weeks of gestation, the most effective screening regimen for preeclampsia was the PAPP-A + PLGF + MAP + UTPI quadruple regimen, which was similar to the previous study [13]. In addition, the UTPI prediction scheme alone was the worst among the screening schemes and was significantly different from other screening schemes. This may be related to the technical requirements of UTPI, which not all clinicians have mastered.

PAPP-A is a macromolecular glycoprotein secreted by placental trophoblast and decidual cells. PAPP-A levels decrease in pregnant women with preeclampsia due to placental dysfunction. PLGF promotes the maturation of the placental vasculature and is mainly expressed locally in the placenta. The level of PLGF affects placental vascular endothelial cells and trophoblast cells, and its decreased level induces preeclampsia. We found that after 32 weeks of gestation, with the increase of gestational weeks, the Youden index of the ROC curve of each screening program for preeclampsia showed a downward trend, which is similar to the conclusion of previous studies [14–16] that PAPP-A and PLGF are more closely related to the onset of early-onset preeclampsia.

Studies have shown [17] that the level of cell-free fetal RNA (cffRNA) in maternal peripheral blood is abnormal in the peripheral blood of pregnant women with preeclampsia.

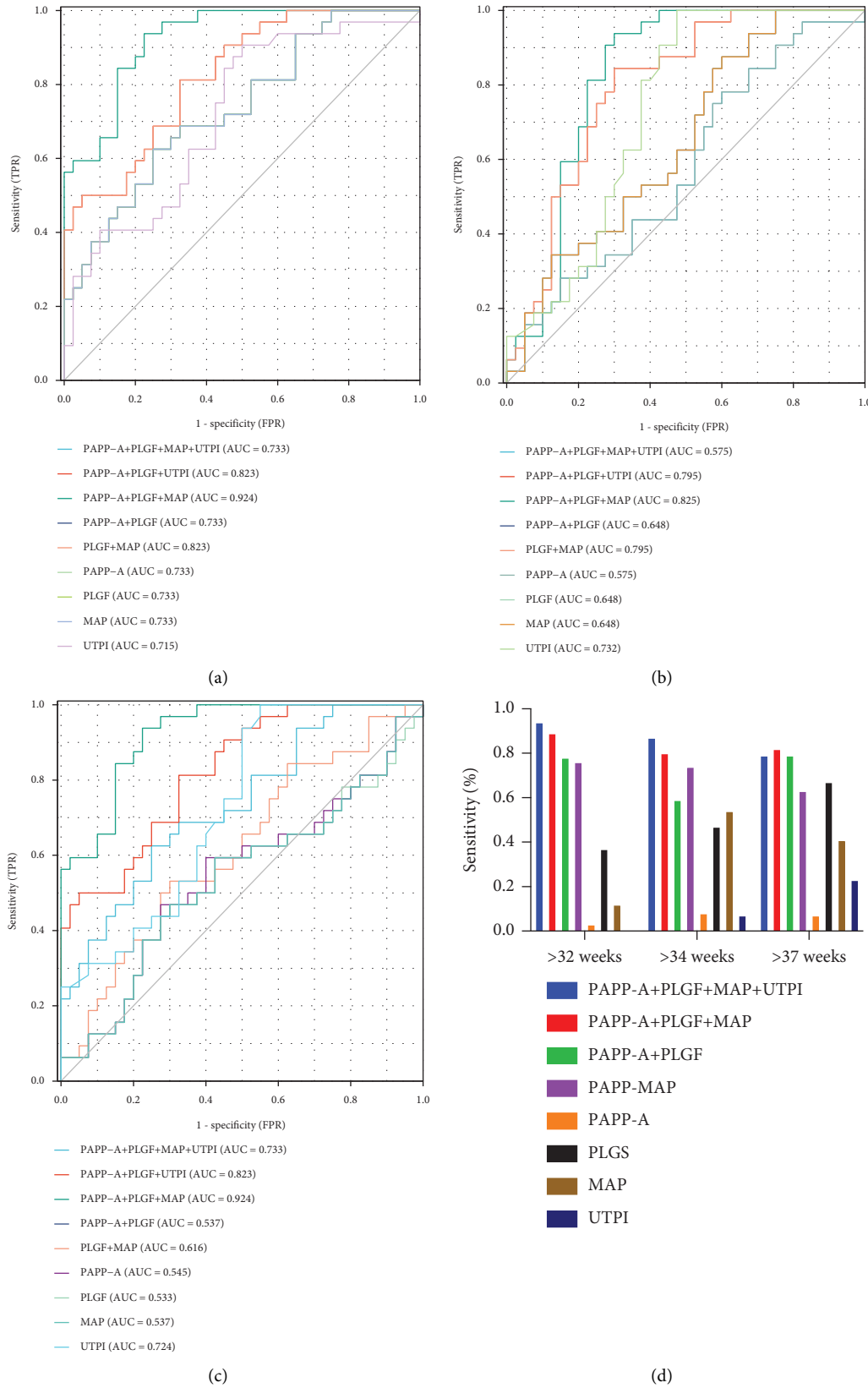

Figure 1: The ROC curves of different screening programs for predicting the risk of preeclampsia before 37 weeks of gestation. (a), the ROC curve of different screening programs for predicting the risk of preeclampsia before 32 weeks of gestation; (b), the ROC curve of different screening programs for predicting the risk of preeclampsia before 34 weeks of gestation; (c), the ROC curve of different screening programs for pregnant women for predicting the risk of developing preeclampsia before 37 weeks; (d), a summary of the sensitivity of different screenings of preeclampsia (<32 weeks, <34 weeks, and <37 weeks sum of the false positive rates).

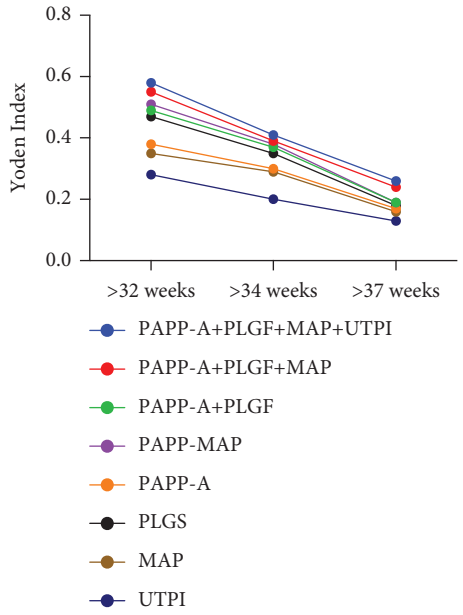

Figure 2: The changing trend of the Youden index of each screening program at different gestational weeks.

The placenta is the main source of cffRNA in maternal peripheral blood, and cffRNA is dependent on fetal sex. Given that cffRNA level directly reflects fetal gene expression pattern and can be noninvasively detected, cffRNA is considered as a potential biomarker for preeclampsia screening, but the time window of cffRNA detection is uncertain. For the reliability of preeclampsia screening, distinguishing gestational hypertension from preeclampsia, especially early-onset preeclampsia, is still needed. Other factors should also be considered when predicting preeclampsia [18–24].

In conclusion, we found that the optimal program for preeclampsia screening in pregnant women before 37 weeks of gestation is PAPP-A + PLGF + MAP + UTPI, which is extremely valuable in the prediction of early-onset preeclampsia.

Data Availability

Data will be made available upon request to the corresponding author.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

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Retraction

Retracted: Application of Meditation Relaxation Training and Rosenthal Effect in Patients with Adenoidectomy

Evidence-Based Complementary and Alternative Medicine

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This article has been retracted by Hindawi following an investigation undertaken by the publisher [1]. This investigation has uncovered evidence of one or more of the following indicators of systematic manipulation of the publication process:

(1) Discrepancies in scope
(2) Discrepancies in the description of the research reported
(3) Discrepancies between the availability of data and the research described
(4) Inappropriate citations
(5) Incoherent, meaningless and/or irrelevant content included in the article
(6) Peer-review manipulation

The presence of these indicators undermines our confidence in the integrity of the article's content and we cannot, therefore, vouch for its reliability. Please note that this notice is intended solely to alert readers that the content of this article is unreliable. We have not investigated whether authors were aware of or involved in the systematic manipulation of the publication process.

In addition, our investigation has also shown that one or more of the following human-subject reporting requirements has not been met in this article: ethical approval by an Institutional Review Board (IRB) committee or equivalent, patient/participant consent to participate, and/or agreement to publish patient/participant details (where relevant).

Wiley and Hindawi regrets that the usual quality checks did not identify these issues before publication and have since put additional measures in place to safeguard research integrity.

We wish to credit our own Research Integrity and Research Publishing teams and anonymous and named external researchers and research integrity experts for contributing to this investigation.

The corresponding author, as the representative of all authors, has been given the opportunity to register their agreement or disagreement to this retraction. We have kept a record of any response received.

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[1] W. Jun and Y. Tian, "Application of Meditation Relaxation Training and Rosenthal Effect in Patients with Adenoidectomy," *Evidence-Based Complementary and Alternative Medicine*, vol. 2022, Article ID 1420639, 5 pages, 2022.

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Research Article

Application of Meditation Relaxation Training and Rosenthal Effect in Patients with Adenoidectomy

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Objective. This study aims to explore the application effect of meditation relaxation training and the Rosenthal effect in patients with adenoidectomy. *Methods*. This study included 94 children who underwent adenoidectomy in our hospital from April 2020 to May 2022 and were divided into a study group and a control group. The control group was given routine care, and the study group was given meditation relaxation training and the Rosenthal effect on the basis of the control group. The negative emotions, treatment compliance, complication rates, and nursing satisfaction of children's family members before and after the intervention were compared between the two groups. *Results*. The results of this study showed that after the intervention, the CDI and SCARED scores of the children in the study group were significantly lower than those in the control group. The treatment compliance in the study group was significantly higher than that in the control group, and the incidence of complications was significantly lower than that in the control group. *Conclusion*. The intervention of meditation relaxation training and the Rosenthal effect on children with adenoidectomy can relieve their negative emotions, improve treatment compliance, reduce the incidence of complications, and the children's family members are more satisfied.

1. Introduction

Adenoid hypertrophy is a common disease in children, mainly, inflammation of the nasopharynx and adjacent parts, as well as pathological hyperplasia caused by repeated stimulation of adenoids by adenoid autoinflammation, including pharyngeal discomfort, nasal obstruction, snoring, mouth breathing, nasal congestion, and other symptoms [1, 2]. Conventional conservative treatment of adenoid hypertrophy can alleviate the disease, but it is difficult to completely eradicate the lesions, which may easily lead to repeated attacks. Adenoidectomy is an important minimally invasive treatment for this disease, with the advantages of fewer traumas, less blood loss, and faster postoperative recovery. However, children with adenoid hypertrophy are younger and prone to nervousness and fear during treatment, resulting in poor treatment compliance and unfavorable surgical treatment [3, 4]. Therefore, it is highly important to provide effective nursing intervention during adenoidectomy treatment [5].

Routine care only focuses on disease treatment and lacks sufficient attention to the patient's psychological state, resulting in poor results [6]. Meditation relaxation training is an open self-conscious attention process, which is an important clinical psychological intervention. It is simple to operate, easy to master, and is of great significance for regulating physical and mental states [7]. The Rosenthal effect is also an important clinical intervention model, mainly through positive psychological cues such as praise, trust, and expectation to improve individual self-esteem and self-confidence, change their behavior habits, and encourage them to face treatment positively [8].

This study aimed to select 94 children with adenoidectomy in our hospital to explore the combined intervention value of meditation relaxation training and

Rosenthal effect in groups, in order to provide new ideas for the clinical intervention of the disease.

2. Materials and Methods

2.1. General Information. A total of 94 children with adenoidectomy in our hospital from April 2020 to May 2022 were enrolled in this study. The inclusion criteria for this study were the following. (1) All patients underwent adenoidectomy under general anesthesia in our hospital. (2) The child's family members have good communication skills. The exclusion criteria for this study were (1) those with organic lesions of the kidney, liver, heart, and other organs. (2) Those with language communication impairment and cognitive dysfunction. (3) Those with congenital deformities. (4) Patients who underwent oral and laryngeal surgery 6 months before enrollment. According to the simple random number table method, patients were divided into the study and the control groups, with 47 cases in each. There were 26 males and 21 females in the study group; age 5–10 years, mean (6.54 ± 1.19) years; disease duration 0.5–4.5 years, mean (2.51 ± 1.37) years; body mass index 18.3–24.5 kg/m^2, average (21.39 ± 3.02) kg/m^2. In the control group, there were 29 males and 18 females; age 5–10 years, mean (6.29 ± 1.24) years; disease duration 0.5–5 years, mean (2.63 ± 1.51) years; and body mass index 18.1–24.8 kg/m^2, average (21.56 ± 2.96) kg/m^2. The clinical data of the two groups were balanced and comparable ($P > 0.05$). The study protocol was approved by the ethics committee of the second affiliated hospital of Harbin Medical University. All family members of patients signed the informed consent forms.

2.2. Treatment. The patients in the control group were given daily nursing care and assisted in the preoperative examination. One day before the operation, the operation process, anesthesia method, postoperative discomfort symptoms, and corresponding treatment methods were introduced. Within 2 hours after the operation, local cold compresses or ice cubes containing sodium chloride were used for analgesia, and atomization treatment was carried out in strict accordance with the doctor's instructions to relieve postoperative pain. Instruct patients to eat cold liquid food 4–6 hours after surgery, and encourage patients to exercise independently with the assistance of medical staff or family members.

On the basis of the control group, the patients in the study group used meditation relaxation training and the Rosenthal effect. Soundproof doors and windows and carpets were placed in the ward to reduce noise, and health posters related to sample excision were posted prominently in the ward. Actively communicate with patients and their families. Through the psychological intervention of nurses, we guide patients to accept their own disease and recovery process and help patients establish healthy and positive recovery behaviors through self-motivation and positive psychological suggestions. Reasonably match negative emotions and better emotional states, subtly changed the physical and mental states of patients with poor emotional states and reduced their fears and other psychology. Timely assess the patient's condition and physical and mental state during the intervention period, discuss the corresponding treatment measures immediately after discovering the problem and optimize the nursing intervention plan. Before relaxation, meditation relaxation training first guides the patient to choose the meditation object. In the first stage, the body is relaxed for 5 minutes to ensure the quietness of the ward, assist the patient to lie supine, and effectively relax the muscles. In the second stage, adjust the breathing for 5 minutes, guide the patient to self-regulate breathing according to the instructions, breathe slowly, and feel the feeling of the gas passing through the nasal cavity and oral cavity. At this stage, you need to pay attention to the patient's breathing frequency. After adjusting the breathing several times, guide the patient to adjust the breathing rate. Focus on what happened before the meditation. The patient is then instructed to close his eyes slightly and visualize himself in the picture, while guiding the patient's fantasy to extend the picture.

2.3. Observation Indicators. Negative emotions before and after the interventions in the two groups were counted. According to the evaluation of the Children's Self-rating Depression Inventory (CDI) and the Screening Scale for Children's Anxiety and Mood Disorders (SCARED), the total score of the CDI was 54 points, and the total score of SCARED was 82 points. High depression and anxiety are more intense. The treatment compliance of the two groups was counted, and the self-made treatment compliance scale (after the pretest, the internal consistency reliability of this scale was 0.93, and the validity coefficient was 0.91) to evaluate, with a total of 10 points. 9–10 points for complete compliance, 7-8 points for partial compliance, less than 7 points for noncompliance, treatment compliance = (complete compliance + partial compliance)/total number of cases × 100%. The incidence of complications in the two groups was calculated. The nursing satisfaction of the families of the children in the two groups was calculated, and the Newcastle Nursing Satisfaction Scale (NSNS) was used to evaluate the scores, which were divided into very satisfied, satisfied, generally satisfied, dissatisfied, and very dissatisfied. Total satisfaction = (very satisfied + satisfied)/total number of cases × 100%.

2.4. Statistical Analysis. The data were analyzed by SPSS 22.0 (IBM SPSS Statistics, USA). The measurement data were presented as mean ± standard deviation (SD). The differences were determined by the two-sided unpaired Student's *t*-test. $P < 0.05$ was considered statistically significant.

3. Results

3.1. Negative Emotions. As shown in Table 1, before the intervention, there were no significant differences in the CDI (42.89 ± 5.07 vs. 41.56 ± 6.11) and SCARED (69.54 ± 8.16 vs. 67.24 ± 9.05) points between the study and control groups ($P > 0.05$). After the intervention, the scores of CDI

Table 1: Comparison of negative emotion scores between the two groups (min).

Time	Group	Number of cases	CDI	SCARED
Before intervention	Study	47	42.89 ± 5.07	69.54 ± 8.16
	Control	47	41.56 ± 6.11	67.24 ± 9.05
	t value		1.148	1.294
	P value		0.254	0.199
After intervention	Study	47	21.65 ± 3.77	33.08 ± 3.38
	Control	47	27.11 ± 4.54	38.34 ± 4.62
	t value		6.343	6.299
	P value		<0.001	<0.001

(21.65 ± 3.77) and SCARED (33.08 ± 3.38) in the study group were significantly lower than those in the control group (CDI: 27.11 ± 4.54 and SCARED: 38.34 ± 4.62) ($P < 0.05$).

3.2. Treatment Compliance. As shown in Table 2, the treatment compliance in the study group (93.62%) was significantly higher than that in the control group (78.72%) ($P < 0.05$).

3.3. Complication Rate. As shown in Table 3, the incidence of complications in the study group (2.13%) was significantly lower than that in the control group (17.02%) ($P < 0.05$).

3.4. Nursing Satisfaction of Children's Family Members. The nursing satisfaction of the families of children in the study group (95.74%) was significantly higher than that in the control group (82.98%) ($P < 0.05$, Table 4).

4. Discussion

Adenoid hypertrophy can lead to insufficient lung expansion and poor breathing in children. In severe cases, it can cause thoracic deformity and abnormal growth hormone secretion, which will affect growth and development to varying degrees [9]. Adenoidectomy is an important measure in the clinical treatment of adenoid hypertrophy. With the aid of endoscopy, lesions can be effectively removed and clinical symptoms can be improved. However, due to the patient's young age, fear of surgery, and poor compliance with treatment, it will affect the smooth progress of surgery and increase the risk of complications [10, 11]. Therefore, it is of great significance to take effective nursing measures to intervene in pediatric adenoidectomy.

Routine nursing measures are mainly based on disease observation, surgical cooperation, and explanation of relevant precautions, which are difficult to meet the comprehensive needs of patients, and the overall effect, is difficult to achieve an ideal state [12]. Meditation and relaxation training is an important clinical intervention. It is mainly a self-regulation method in which individuals consciously maintain their attention on the current external/internal experience without any evaluation. It can effectively downregulate the key hormone cortisol content in stress response. Relief from a negative emotional state can also improve the individual's ability to regulate emotions to a certain extent. In addition, the individual is in a state of physical and mental relaxation during the intervention period, which is conducive to helping the individual effectively cope with and deal with related negative emotions [13–15]. The Rosenthal effect is also a commonly used clinical psychological adjustment measure. It is also known as the "expectation effect". It mainly improves individual behavior through motivation, praise, trust, and expectation. It is a new nursing model. Expectation-action-acceptance in the process of internalization, positive psychological hints are given by affirmation and encouragement, to achieve the purpose of adjusting the physical and mental state [16].

There are no clinical reports on the specific application value of meditation relaxation training and the Rosenthal effect in pediatric adenoidectomy. The results of this study showed that after the intervention, the CDI and SCARED scores of the children in the study group were significantly lower than those in the control group. The treatment compliance in the study group was significantly higher than that in the control group, and the incidence of complications was significantly lower than that in the control group. It is feasible and effective for children with adenoidectomy to be combined with the aerial effect to help relieve their negative emotional state and encourage them to accept adenoidectomy with a positive attitude so as to ensure the effectiveness and safety of surgical treatment and reduce the incidence of complications. The main reasons are as follows; the meditation relaxation training can increase the activity of the left prefrontal brain area and strengthen positive emotions. At the same time, the amygdala is the initiation area of negative emotions, and the medial prefrontal lobe layer is driven by the amygdala. The bridge of bad emotions, through meditation and relaxation training, one can affect the corresponding areas of the dorsal medial/lateral prefrontal cortex to play a role in stress response inhibition and cultivate their inner attention to the current, not subjectively judge emotional changes and perceptions, and truthfully accept the current reality [17–20]. The Rosenthal effect attaches great importance to children's psychological feelings, focusing on relieving children's negative emotional state and reducing psychological stress through anticipation, trust, praise, and encouragement, which is conducive to children's active venting of negative emotions and reducing physical and mental burden, and the dried cranberry exercises taken during the intervention period can adjust the individual's cognition of their own diseases and treatment through the power of mindfulness, avoid falling into blind psychological troubles, and prevent excessive psychological reactions.

In addition, from the results of this study, it can also be known that the nursing satisfaction of the children in the study group (95.74%) was higher than that in the control group (82.98%) after the intervention ($P < 0.05$), indicating that the meditation relaxation training combined with the Rosenthal effect intervention mode is also effective. To a certain extent, it can deepen the recognition of the clinical work by the families of children with adenoidectomy, which is of great significance to reducing disputes between nurses and patients and establishing the image of high-quality

Table 2: Comparison of treatment compliance between the two groups (%).

Group	Number of cases	Full compliance	Partial compliance	Non-compliance	Treatment compliance
Study	47	29 (61.70)	15 (31.91)	3 (6.38)	44 (93.62)
Control	47	21 (44.68)	16 (34.04)	10 (21.28)	37 (78.72)
χ^2 value					4.374
P value					0.036

Table 3: Comparison of the incidence of complications between the two groups (%).

Group	Number of cases	Nasal bleeding	Adenoid residual hyperplasia	Infection	Total incidence
Study	47	1 (2.13)	0 (0.00)	0 (0.00)	1 (2.13)
Control	47	3 (6.38)	2 (4.26)	1 (2.13)	8 (17.02)
χ^2 value					4.424
P value					0.035

Table 4: Comparison of nursing satisfaction of family members of the two groups of children (%).

Group	Number of cases	Very satisfied	Satisfied	Generally satisfied	Dissatisfied	Very dissatisfied	Total satisfaction
Study	47	33 (70.21)	12 (25.53)	1 (2.13)	1 (2.13)	0 (0.00)	45 (95.74)
Control	47	26 (55.32)	13 (27.66)	4 (8.51)	3 (6.38)	1 (2.13)	39 (82.98)
χ^2 value							4.029
P value							0.045

nursing services in the hospital. The physical and mental state of the patient can encourage him to actively cooperate with the treatment, thereby reducing the risk of complications, improving the effectiveness and safety of the treatment, relieving his clinical symptoms, and shortening the recovery process of the child, so the family members of the child are more satisfied.

To sum up, the intervention of meditation relaxation training and the Rosenthal effect on children with adenoidectomy can relieve their negative emotions, improve treatment compliance, reduce the incidence of complications, and improve the children's family satisfaction.

Data Availability

All data are included within the article.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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Retraction

Retracted: Camrelizumab and Apatinib Combined with Radiotherapy Is Effective in Advanced Oligometastatic Non-Small-Cell Lung Cancer

Evidence-Based Complementary and Alternative Medicine

Received 20 June 2023; Accepted 20 June 2023; Published 21 June 2023

This article has been retracted by Hindawi following an investigation undertaken by the publisher [1]. This investigation has uncovered evidence of one or more of the following indicators of systematic manipulation of the publication process:

(1) Discrepancies in scope
(2) Discrepancies in the description of the research reported
(3) Discrepancies between the availability of data and the research described
(4) Inappropriate citations
(5) Incoherent, meaningless and/or irrelevant content included in the article
(6) Peer-review manipulation

The presence of these indicators undermines our confidence in the integrity of the article's content and we cannot, therefore, vouch for its reliability. Please note that this notice is intended solely to alert readers that the content of this article is unreliable. We have not investigated whether authors were aware of or involved in the systematic manipulation of the publication process.

In addition, our investigation has also shown that one or more of the following human-subject reporting requirements has not been met in this article: ethical approval by an Institutional Review Board (IRB) committee or equivalent, patient/participant consent to participate, and/or agreement to publish patient/participant details (where relevant).

Wiley and Hindawi regrets that the usual quality checks did not identify these issues before publication and have since put additional measures in place to safeguard research integrity.

We wish to credit our own Research Integrity and Research Publishing teams and anonymous and named external researchers and research integrity experts for contributing to this investigation.

The corresponding author, as the representative of all authors, has been given the opportunity to register their agreement or disagreement to this retraction. We have kept a record of any response received.

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[1] W. Ye, Z. Song, and Z. Lin, "Camrelizumab and Apatinib Combined with Radiotherapy Is Effective in Advanced Oligometastatic Non-Small-Cell Lung Cancer," *Evidence-Based Complementary and Alternative Medicine*, vol. 2022, Article ID 5067402, 5 pages, 2022.

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Research Article

Camrelizumab and Apatinib Combined with Radiotherapy Is Effective in Advanced Oligometastatic Non-Small-Cell Lung Cancer

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Objective. To investigate the effect of camrelizumab + apatinib combined with radiotherapy on the expression of TRIM27, SCC-Ag, and CYFRA21-1 in advanced oligometastatic non-small-cell lung cancer (NSCLC). *Methods*. A retrospective analysis of patients with oligometastatic NSCLC who were treated at our hospital from January 1, 2021, to March 31, 2022. Patients who met the inclusion criteria were summarized into an observation group (camrelizumab on the basis of the control group), or a control group (radiotherapy combined with oral apatinib). The disease control rate, immune function, changes in the levels of TRIM27, SCC-Ag, CYFRA21-1, and the occurrence of adverse effects were compared between the two groups. *Result*. There were 86 patients who met the inclusion criteria, with 53 cases in the observation group and 33 cases in the control group. There were significant differences in complete remission (CR, 25/53 vs. 10/33), partial remission (PR, 17/53 vs. 12/33), disease control (DC, 7/53 vs. 4/33), disease progression (DP, 4/53 vs. 7/33), and disease control rate (49/53 vs. 26/33) between the observation group and the control group. There was no significant difference in immune function between the two groups before treatment ($p > 0.05$). After treatment, the levels of $CD3^+$, $CD4^+$, $CD4^+/CD8^+t$ cells, and NK cells in the observation group were higher ($p = 0.015$, 0.035, 0.003, 0.001, respectively), while the level of $CD8^+t$ cells was lower ($p < 0.001$). There were no significant differences in TRIM27, SCC-Ag, or CYFRA21-1 between the two groups before treatment ($p > 0.05$). After treatment, the observation group had lower levels of TRIM27 ($p = 0.035$), SCC-Ag ($p = 0.045$), and CYFRA21-1 ($p = 0.003$). There was no significant difference in the occurrence of adverse events between the two groups ($p < 0.05$). *Conclusion*. Treatment of camrelizumab + apatinib combined with radiotherapy is effective for advanced oligometastatic NSCLC, with mild adverse effects.

1. Introduction

Non-small-cell lung cancer (NSCLC) is a malignant tumor with high mortality, accounting for about 85% of all lung cancer patients in the United States and Europe. Most patients have distant metastases at the time of diagnosis, thus losing the window of complete cure [1–4]. The idea of oligometastases was first proposed in 1995 [5], which believed that cancer in this stage was only limited to one or a few targeted metastases. At present, there is no standard for the definition of oligometastases, but the European Society of Oncology and some recent studies believe that as long as the location of the metastases and the number of metastatic tissues (< 5), oligometastases can be confirmed by a complete radiological examination [6, 7]. Oligometastasis is a transitional period in which the local primary tumor spreads to a larger area, and it has the potential to be cured. Squamous cell carcinoma antigen (SCC-Ag) is a tumor-related antibody produced when tumor cells are abnormally differentiated, and it can accelerate the proliferation of their DNA. Cytokeratin 19 fragment antigen 21-1 (CYFRA21-1) is a major cytoskeletal protein, which can accelerate the

adhesion of cancer cells by inhibiting apoptosis and increasing its content and pass [8]. This study retrospectively analyzed the oligometastatic NSCLC patients treated at our hospital.

2. Materials and Methods

2.1. General Information. A retrospective analysis of patients with oligometastatic NSCLC who were treated in our hospital from January 1, 2021, to March 31, 2022. This study was approved by the Institutional Ethical Committee of Shandong Provincial Third Hospital. All patients in this study signed the informed consent forms. The inclusion criteria were as follows: (1) pathologically confirmed stage VI NSCLC; (2) signed informed consents; and (3) with complete data. The exclusion criteria were as follows: (1) patients with mental disorders; or (2) severe organ failure; or (3) lost to follow-up.

Patients in the control group were given radiotherapy combined with oral apatinib (Jiangsu Hengrui Medicine Co., Ltd., approved by H20140103), with an initial dose of 250 mg/d. If no adverse effects, it will be increased to 500 mg/d, 28 days as a cycle, and the treatment will be continued for 4 cycles. Patients in the observation group were treated with camrelizumab (Suzhou Shengdia Biopharmaceutical Co., Ltd., S20190027) on the basis of the observation group, given by iv., 200 mg each time, once every 3 weeks, for continuous treatment of 4 cycles.

(1) After 4 cycles of treatment, the curative effect of patients was evaluated according to the World Health Organization tumor curative effect evaluation standard [9]. Complete remission (CR): complete disappearance of all target lesions in the patient. Partial remission (PR): the longest diameter of the target lesion was reduced by 20% to 30% compared with the time of admission. Disease progression (DP): the longest diameter of the target lesion increased by 20% to 30% compared with admission. Disease Control (DC): between CR and DP. Disease control rate is the sum of CR rate, PR rate, and DC rate;

(2) Before treatment and after 4 cycles of treatment, an American FACS-type flow cytometer was used to detect immune function indicators, including $CD3^+$, $CD4^+$, $CD8^+$, $CD4^+/CD8^+$, and natural killer (NK) cell levels;

(3) Enzyme-linked immunosorbent assay (ELISA) was used to detect serum SCC-Ag and CYFRA21-1 levels; TRIM27 was detected by the RT-PCR method [10].

(4) Record the occurrence of adverse effects during the treatment, including gastrointestinal discomfort, abnormal liver and kidney function, leukopenia, and thrombocytopenia.

2.2. Statistical Methods. The data in this experiment need to be verified by SPSS21.0 (SPSS, Chicago, IL, USA) software. The data by count were tested by the χ^2 test, and the data by measurement were tested by t-test. $p < 0.05$ (2-tailed) was used as the threshold for statistical significance.

3. Results

3.1. General Information. In total, 489 patients with stage IV NSCLC were treated in our hospital from January 1, 2021, to March 31, 2022, including 86 patients with oligometastatic NSCLC who met the inclusion criteria and were summarized into 2 groups according to the treatment method. The observation group (53 cases, including 29 males and 24 females, aged 43.05 ± 1.39 years) and the control group (33 cases, including 18 males and 15 females, aged 42.87 ± 1.92 years). There were no significant differences in gender, age, and BMI between the two groups ($p > 0.05$, Table 1).

3.2. Comparison of Disease Control Rates between the Two Groups. There were significant differences in CR (25/53 vs. 10/33), PR (17/53 vs. 12/33), DC (7/53 vs. 4/33), DP (4/53 vs. 7/33), and disease control rate (49/53 vs. 26/33) between the observation group and the control group ($p < 0.001$). See Table 2 for details.

3.3. Comparison of Immune Function between the Two Groups. There was no significant difference in immune function between the two groups before treatment ($p > 0.05$). After treatment, there were significantly higher $CD3^+t$ cells (59.29 ± 3.31 vs. 51.89 ± 2.41), $CD4^+t$ cells (32.79 ± 2.81 vs. 25.23 ± 2.66), $CD4^+/CD8^+t$ cell (1.41 ± 0.59 vs. 1.14 ± 0.52), and NK cells (18.26 ± 3.51 vs. 14.26 ± 3.14) and lower $CD8^+t$ cell (23.63 ± 1.31 vs. 23.97 ± 1.36) in the observation group. See Table 3 for details.

3.4. Comparison of the Levels of TRIM27, SCC-Ag, and CYFRA21-1 between the Two Groups. There was no significant difference in the index levels between the two groups before treatment ($p > 0.05$). After treatment, there were lower TRIM27 (0.35 ± 0.03 vs. 0.51 ± 0.04), SCC-Ag (1.29 ± 0.34 vs. 1.51 ± 0.36), CYFRA21-1 (1.93 ± 0.31 vs. 2.39 ± 0.47) ($p = 0.035$, 0.045, and 0.003, respectively). See Table 4.

3.5. Comparison of Adverse Effects between the Two Groups. There was no significant difference in the occurrence of adverse effects between the two groups ($p < 0.05$). See Table 5.

4. Discussion

In the early stage of NSCLC, there may be no obvious clinical signs for early diagnosis. With the latent development of the disease, most patients will have abnormalities in CT or chest X-ray examinations. However, they are in the late stage of cancer, missing the opportunity for treatment and radiation therapy, and the 5-year survival rate is only 20% [11–14]. The occurrence of NSCLC is the main cause of local pain, compression symptoms, and mass effect, which increases the

Table 1: General information of the two groups.

Group	Male/Female (example)	Average age (years)	BMI (kg/m^2)
Observation group ($n = 53$)	29/24	43.05 ± 1.39	24.73 ± 3.44
Control group ($n = 33$)	18/15	42.87 ± 1.92	23.96 ± 3.32
t/χ^2	8.257	10.371	4.374
p	0.391	0.853	0.389

Table 2: Comparison of disease control rates between the two groups (cases, %).

Group	CR	PR	DC	DP	Disease control rate
Observation group ($n = 53$)	25 (47.17)	17 (32.08)	7 (13.21)	4 (7.55)	92.45
Control group ($n = 33$)	10 (30.30)	12 (36.36)	4 (12.12)	7 (21.21)	78.79
χ^2	—	18.736			
p	—	<0.001			

Table 3: Comparison of immune function between the two groups ($\overline{x} \pm s$).

Group	Observation group ($n = 53$)		Control group ($n = 33$)		t	p
	Before therapy	After treatment	Before therapy	After treatment		
$CD3^+$ (%)	56.36 ± 2.43	59.29 ± 3.31	56.39 ± 2.46	51.89 ± 2.41	10.649	0.015
$CD4^+$ (%)	28.06 ± 2.75	32.79 ± 2.81	28.13 ± 2.72	25.23 ± 2.66	7.287	0.035
$CD8^+$ (%)	26.83 ± 1.62	23.63 ± 1.31	26.78 ± 1.66	23.97 ± 1.36	9.468	0.003
$CD4^+/CD8^+$	1.23 ± 0.32	1.41 ± 0.59	1.25 ± 0.34	1.14 ± 0.52	9.521	0.001
NK cells (%)	16.47 ± 3.32	18.26 ± 3.51	16.39 ± 3.32	14.26 ± 3.14	13.739	<0.001

Table 4: Comparison of TRIM27, SCC-Ag, and CYFRA21-1 levels between two groups ($\overline{x} \pm s$).

Group	Observation group ($n = 53$)		Control group ($n = 33$)		T	p
	Before therapy	After treatment	Before therapy	After treatment		
TRIM27	0.83 ± 0.07	0.35 ± 0.03	0.82 ± 0.06	0.51 ± 0.04	16.798	0.035
SCC-Ag (μg/L)	1.76 ± 0.46	1.29 ± 0.34	1.75 ± 0.42	1.51 ± 0.36	11.619	0.045
CYFRA21-1 (μg/L)	3.95 ± 0.83	1.93 ± 0.31	3.97 ± 0.86	2.39 ± 0.47	8.549	0.003

Table 5: Comparison of adverse effects between the two groups (cases, %).

Group	Gastrointestinal discomfort	Abnormal liver and kidney function	Leukopenia	Thrombocytopenia
Observation group ($n = 53$)	10 (18.87)	9 (16.98)	7 (13.21)	11 (20.75)
Control group ($n = 33$)	6 (18.18)	5 (15.15)	5 (15.15)	7 (21.21)
χ^2	11.781	16.392	9.284	14.163
p	0.824	0.856	0.733	0.945

tumor burden and greatly reduces the quality of life. However, in patients with oligometastatic NSCLC, due to the transition period from primary metastasis to large-scale metastasis, the metastatic site and metastatic tissue structure are relatively simple, and the tumor burden is relatively light during this period. In combined therapy, there is good tolerance and a good prognosis.

The pathogenesis of NSCLC is still unclear, but the abnormal expression of tumor proteins related to NSCLC may cause abnormal proliferation of epithelial cells, thereby causing changes in their transcription. SCC-Ag is a glycoprotein secreted in dividing tumors, and its expression may be increased during rapid proliferation or marked differentiation. CYFRA21-1 is an active substance with enhanced tumor cell morphology, and its role is to enhance tumor cell infiltration into the basement membrane and epithelial-mesenchymal transition [15]. TRIM27 can activate the NF-κB signaling pathway and activate the transcription of the downstream target genes DKK1 and c-Myc of the Wnt/β-catenin pathway, thereby promoting their proliferation and activation. TRIM27 can also promote the proliferation of NSCLC, thereby activating the expression of ERK and JNK signal transduction pathways, thereby enhancing the infiltration of NSCLC [16–18].

The apatinib used in this study, as a new drug with its own patent, was first approved as third-line chemotherapy in November 2014. Clinical trials have shown that it has a significant efficiency. In addition, apatinib is an oral

tyrosine kinase inhibitor (TKI), which has the advantages of simple use, small side effects, safety, and reliability. Due to more and more relevant clinical trials, apatinib is gradually being applied to the clinical treatment of advanced NSCLC. Camrelizumab is a new type of NSCLC drug newly developed in China. It can be combined with PD-1 to inhibit its interaction with apoptosis receptors and improve the body's immunity [19, 20]. In patients with NSCLC, the long-term use of drugs and the weakening of the immune system will cause the decline of the immune system, thereby aggravating the general condition of NSCLC [21]. Therefore, it is very necessary to strengthen immunotherapy for non-small-cell lung cancer. Retrospective analysis of the combined treatment methods adopted by the observation group in this study achieved excellent clinical effects, effectively reduced the levels of TRIM27, SCC-Ag, and CYFRA21-1, and improved the immune function of the subjects. At the same time, there was no significant difference in the statistics of adverse effects between the two groups. It might need further investigation for those therapies in complicated cases [22–27].

In general, camrelizumab + apatinib combined with radiotherapy for advanced oligometastatic non-small-cell lung cancer has good efficacy, mild adverse effects, high safety, and can be widely used.

Data Availability

The data can be obtained by direct contact with the corresponding author.

Disclosure

Wei Ye and Zhonghua Song are the co-first authors.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Wei Ye and Zhonghua Song contributed equally to this article.

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Retraction

Retracted: Effects of Circ_0109046 Regulating Mir-338-3p on the Malignant Behavior of A2780 Cells

Evidence-Based Complementary and Alternative Medicine

Received 20 June 2023; Accepted 20 June 2023; Published 21 June 2023

This article has been retracted by Hindawi following an investigation undertaken by the publisher [1]. This investigation has uncovered evidence of one or more of the following indicators of systematic manipulation of the publication process:

(1) Discrepancies in scope
(2) Discrepancies in the description of the research reported
(3) Discrepancies between the availability of data and the research described
(4) Inappropriate citations
(5) Incoherent, meaningless and/or irrelevant content included in the article
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The presence of these indicators undermines our confidence in the integrity of the article's content and we cannot, therefore, vouch for its reliability. Please note that this notice is intended solely to alert readers that the content of this article is unreliable. We have not investigated whether authors were aware of or involved in the systematic manipulation of the publication process.

In addition, our investigation has also shown that one or more of the following human-subject reporting requirements has not been met in this article: ethical approval by an Institutional Review Board (IRB) committee or equivalent, patient/participant consent to participate, and/or agreement to publish patient/participant details (where relevant).

Wiley and Hindawi regrets that the usual quality checks did not identify these issues before publication and have since put additional measures in place to safeguard research integrity.

We wish to credit our own Research Integrity and Research Publishing teams and anonymous and named external researchers and research integrity experts for contributing to this investigation.

The corresponding author, as the representative of all authors, has been given the opportunity to register their agreement or disagreement to this retraction. We have kept a record of any response received.

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[1] Y. Zhao, H. Diao, J. Li et al., "Effects of Circ_0109046 Regulating Mir-338-3p on the Malignant Behavior of A2780 Cells," *Evidence-Based Complementary and Alternative Medicine*, vol. 2022, Article ID 4655939, 6 pages, 2022.

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Research Article

Effects of Circ_0109046 Regulating Mir-338-3p on the Malignant Behavior of A2780 Cells

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Objective. The objective is to explore the action and mechanism of circ_0109046 on the malignant phenotypes of ovarian cancer cells. *Methods*. Circ_0109046 and miR-338-3p expression were detected by quantitative real-time polymerase chain reaction (qRT-PCR). In vitro assays were conducted to investigate the action of circ_0109046 and miR-338-3p on ovarian cancer cell growth and metastasis. Western blotting was utilized to investigate the contents of apoptosis-related markers. The binding between circ_0109046 and miR-338-3p was validated using dual-luciferase reporter assay. *Results*. Circ_0109046 was increased, while miR-338-3p content was decreased in ovarian cancer tissues. Deficiency of circ_0109046 or the upregulation of miR-338-3p was observed to weaken cell proliferative, migratory, and invasive abilities and elevated cell apoptosis rate in ovarian cancer. Circ_0109046 targetedly suppressed miR-338-3p. Down-regulation of miR-338-3p was able to reverse the repressing impacts of circ_0109046 silencing on ovarian cancer growth and mobility. *Conclusion*. Circ_0109046 silencing impaired the proliferation, migration, and invasion of ovarian cancer cells through negatively regulating miR-338-3p in vitro, indicating the potential implication of circ_0109046 in ovarian cancer progression.

1. Introduction

Ovarian cancer is a common malignant tumor affecting women's life, health, and safety. Its pathogenesis is still unclear, and there is a lack of effective treatment [1]. Therefore, it is of great significance to find effective therapeutic targets to prevent ovarian cancer.

Circular RNA (circRNA) is a kind of noncoding RNA, which is highly stable due to its closed structure. A vast array of abnormally expressed circRNAs, such as circ_0005276 [2], circ_0004390 [3], and circPIP5K1A [4], have been discovered in ovarian cancer, which are closely related to the clinicopathological features and prognosis of ovarian cancer patients; moreover, functional experiments further confirmed their involvement in the tumorigenesis of ovarian cancer, implying that circRNAs may be promising candidates for the development of ovarian cancer therapeutic method. Recently, research showed that circ_0109046 was highly expressed in endometrial cancer and predicted poor prognosis; functionally, circ_0109046 lack inhibited the proliferation and invasiveness of endometrial cancer cells by miR-105 [5]. However, the action and mechanism of circ_0109046 on the ovarian cancer cell malignant phenotypes remain vague. MicroRNA (miRNA) is also a kind of noncoding RNA, and circRNA can act as a sponge for miRNA to affect the malignant progression of cancer cells [6]. In our study, preliminary online prediction revealed that circ_0109046 has complementary sequences of miR-338-3p. A previous study showed the decreased miR-338-3p in ovarian cancer, which was related to the bad survival rate [7]. However, the effect of miR-338-3p and the relationship between circ_0109046 and miR-338-3p are also unknown yet.

This study used the A2780 cells to explore the functions of circ_0109046 and miR-338-3p on ovarian cancer cell malignant phenotypes and further investigated whether circ_0109046 could exert its effects by miR-338-3p in ovarian cancer.

2. Materials and Methods

2.1. Patient Samples. A total of 57 patients (45.26 ± 6.59 years) with ovarian cancer who were hospitalized in our hospital from May 2017 to May 2020 were selected as the research objects. During the operation, the cancer tissues and adjacent normal tissues of the patients were collected and then stored in liquid nitrogen. Inclusion criteria are confirmed by pathological diagnosis for the first time. Exclusion criteria are preoperative radiotherapy, chemotherapy, and other treatments, combined with other malignant tumors. The study was approved by the ethics committee of our hospital in accordance with the Declaration of Helsinki, and all patients provided written consent forms.

2.2. Cell Culture. A2780 cells were purchased from Shanghai Cell Bank, Chinese Academy of Sciences (Shanghai, China), and then grew in RPMI-1640 medium (Solarbio, Beijing, China) supplemented with 10% fetal bovine serum (FBS), 100 U/mL of penicillin, and 100 μg/mL of streptomycin (Solarbio) with 5% CO_2 at 37°C.

2.3. Cell Transfection. The short hairpin RNA (shRNA) against circ_0109046 (sh-circ_0109046), miR-338-3p mimic (miR-338-3p), or inhibitor (anti-miR-338-3p) and negative control (sh-NC, miR-NC, or anti-NC) were obtained from Sangon Biotech (Shanghai, China). Cell transfection was carried out with reference to the Lipofectamine 2000 kit (Invitrogen, Camarillo, CA, USA) with 2.5 mL A2780 cells (5.0 × 104/mL). 24 h later, the expression of circ_0109046 or miR-338-3p was detected in the A2780 cells to verify the transfection efficiency.

2.4. Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR). Total RNA was prepared using the RNeasy Mini Kit as per the protocol (Qiagen, Crawley, UK). Thereafter, the obtained RNAs were subjected to reverse-transcription to synthesize cDNA using the PrimeScript RT polymerase (Takara, Otsu, Japan), and then qRT-PCR was performed by SYBR real-time PCR mixture (Takara). The conditions were programmed as follows: 42°C 5 min, 95°C 10 s, followed by 40 cycles at 95°C for 5 s, and 60°C 30 s. The gene expression was processed using the $2^{-\Delta\Delta Ct}$ method, and U6 or glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as the control. The primers for qRT-PCR are listed in Table 1.

2.5. Cell Counting Kit-8 (CCK-8) Assay. Transfected A2780 cells (5.0×10^4 cells/mL) were seeded into a 96-well plate and cultured for 24 h, followed by reacting with CCK-8 solution (10 μL, Beyotime, Shanghai, China) for another 2 h. Then, the optical density values at 450 nm were measured.

2.6. Flow Cytometry. After transfection, 2.5 mL A2780 cells of each group (5.0×104 cells/mL) were collected and resuspended in 500 μL binding buffer (1×). Then, cell apoptosis was analyzed by flow cytometry after staining orderly with 10 μL Annexin V-fluorescein isothiocyanate (FITC) and 5 μL propidium iodide (PI) (Life Technologies, Scotland, UK).

2.7. Transwell Assay. After transfection, 100 μL A2780 cells (5.0×10^4/mL) in each group suspended in serum-free medium were added to the top chambers of 24-well Transwell chambers (Corning, Cambridge, MA, USA) that were precoated without (for migration) or with Matrigel (Solarbio) (for invasion), and 500 μL of serum-containing medium was added to the lower chamber for 24 h culture. Finally, migrated and invaded cells were observed and counted by a microscope (Bio-Rad, Hercules, CA, USA) after crystal violet staining for 30 min.

2.8. Western Blotting. A2780 cells in each group were split by RIPA reagent (Yeasen, Shanghai, China) for 30 min on ice. Equal amounts of samples (20 μg) were separated by 10% SDS-PAGE and then electrophoretically shifted onto PVDF membranes, followed by blocking with 5% skim milk powder for 2 h. The membranes were interacted with primary antibodies against B-cell lymphoma-2 (Bcl-2) (1 : 2000, ab182858, Abcam, Cambridge, MA, USA) and BCL2-associated X (Bax) (1 : 1000, ab32503, Abcam) and GAPDH (1 : 5000 m ab8245, Abcam) all night at 4°C. Following secondary incubation at 37°C for 1 h, protein signals were quantified by an ECL reagent (Beyotime).

2.9. Dual-Luciferase Reporter Assay. The fragments of circ_0109046 carrying the wild-type (WT) binding site of miR-338-3p or mutated (MUT) sequences were cloned into pmirGLO vectors (Solarbio) to establish luciferase reporter vectors (WT/MUT-circ_0109046), which were then transfected into A2780 cells together with miR-NC or miR-338-3p mimics, and the luciferase activity in each group was tested 48 h later by dual-luciferase reporter kit (Solarbio).

2.10. Statistical Analysis. The experimental results were expressed as mean ± standard deviation (SD). SPSS 22.0 software was used for statistical analysis. The *t*-test was used for group comparison. $P < 0.05$ indicated statistically significant.

3. Results

3.1. The Expression of Circ_0109046 and MiR-338-3p in Ovarian Cancer. As shown in Figure 1(a), circ_0109046 expression was higher in ovarian cancer tissues (II) than those in adjacent normal tissues (I). However, miR-338-3p expression was opposite that was lowly expressed in ovarian cancer tissues (II) (Figure 1(b)).

3.2. Effects of Circ_0109046 on Ovarian Cancer Cell Malignant Phenotypes. As shown in Table 2, deficiency of circ_0109046 suppressed A2780 cell proliferation, migration, and invasion compared with the sh-NC group. Besides, circ_0109046 silencing induced apoptosis in A2780 cells relative to sh-NC

Table 1: The primers for qRT-PCR.

Name	Forward	Reverse
circ_0109046	5′-TCTTCCAGACACGATTCCGC-3′	5′-AGGGGAGGGATAGCACACAT-3′
GAPDH	5′-GTCTCCTCTGACTTCAACAGCG-3′	5′-ACCACCCTGTTGCTGTAGCCAA-3′
miR-338-3p	5′-GCCGAGTCCAGCATCAGTGAT-3′	5′-CAGTGCGTGTCGTGGAGT-3′
U6	5′-CTCGCTTCGGCAGCACATATA-3′	5′-AACGCTTCACGAATTTGCGT-3′

transfection, evidenced by increased apoptosis rate and Bcl-2 expression, as well as decreased Bax expression (Table 2 and Figures 2(a) and 2(b)).

3.3. Circ_0109046 Acted as a Sponge for MiR-338-3p. According to the prediction of CircInteractome, circ_0109046 possesses the binding site of miR-338-3p (Figure 3(a)). Furthermore, miR-338-3p mimic was found to reduce the luciferase activity of WT-circ_0109046 group in A2780 cells, but not the MUT-circ_0109046 group compared with the control group (Figure 3(b)). Besides, sh-circ_0109046 transfection in A2780 cells led to an increase of miR-338-3p expression level (Figure 3(c)).

3.4. Effects of MiR-338-3p on Ovarian Cancer Cell Malignant Phenotypes. As shown in Table 3 and Figures 4(a) and 4(b), miR-338-3p elevation impaired A2780 cell proliferation, migration, and invasion but evoked cell apoptosis compared with the miR-NC groups; besides, miR-338-3p overexpression decreased Bcl-2 expression as well as increased Bax expression in A2780 cells.

3.5. MiR-338-3p Inhibition Abolished the Effects of Circ_0109046 Knockdown on Ovarian Cancer Cell Malignant Phenotypes. As exhibited in Table 4 and Figures 5(a) and 5(b), down-regulation of miR-338-3p expression could attenuate circ_0109046 knockdown-mediated inhibition of cell proliferative, migratory, and invasive abilities, as well as the promotion of cell apoptotic rate in A2780 cells.

4. Discussion

CircRNAs exist widely in eukaryotes, and the circRNA/miRNA axis has been shown to be implicated in regulating the malignant behaviors of tumor cells that has an important impact on the genesis and progression of malignancies [8]. Studies have shown a variety of circRNAs in modulating ovarian cancer cell malignant behaviors. For instance, circ_0026123 silencing could inhibit the proliferative and migratory capacities of ovarian cancer cells by miR-124-3p/Enhancer of zeste homolog 2 (EZH2) axis, thereby inhibiting tumor malignant process [9]. An up-regulated circ_0007841 was discovered in ovarian cancer, and circ_0007841 enhanced cancer cell metastasis and growth *in vitro* and in nude mice by up-regulating Mex-3 RNA Binding Family Member C expression through competitively adsorbing miR-151-3p, indicating an oncogenic role of circ_0007841 in ovarian cancer [10]. Besides, upregulation of circ_0007874 inhibited the migration and proliferation of ovarian cancer cells by competitively adsorbing miR-760 and up-regulating

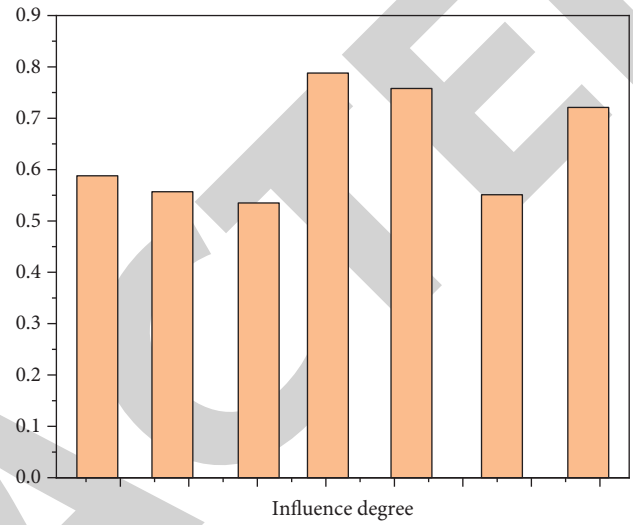

Figure 1: The expression of circ_0109046 and miR-338-3p in ovarian cancer. (a-b) The expression of circ_0109046 and miR-338-3p in ovarian cancer tissues and adjacent normal tissues detected by qRT-PCR. Note. (I) adjacent normal tissues; (II) ovarian cancer tissue. $^{*}P < 0.05$.

suppressor of cytokine signaling 3 (SOCS3) expression [11]. Circ_0109046 is a stable circRNA, while the effects and mechanism of circ_0109046 on ovarian cancer have not been elucidated. In this study, circ_0109046 was showed to be increased in ovarian cancer, suggesting the potential promoting action of it in the development of ovarian cancer. Functionally, reduction of circ_0109046 reduced the proliferative, migratory, and invasive abilities of A2780 cells and at the same time promoted apoptosis by enhancing the content of pro-apoptotic Bax protein and declining the level of anti-apoptotic Bcl-2 protein, indicating that circ_0109046 lack inhibited the malignant progression of ovarian cancer cells *in vitro*. Subsequently, we further elucidated by which knockdown of circ_0109046 hindered the malignant biological behaviors of ovarian cancer cells, and we identified the circ_0109046/miR-338-3p axis in cancer cells.

MiR-338-3p has been found to be abnormally expressed in various cancers and involved in cancer development. For example, down-regulation of miR-338-3p expression expedited the proliferation of lung cancer cells *in vitro* and *in vivo* [12]. MiR-338-3p upregulation could hinder hepatocellular carcinoma cell metastasis by targeting Zinc Finger E-Box Binding Homeobox 2 (ZEB2) [13]. A decreased miR-338-3p was found in clear cell renal cell carcinoma, which resulted in the promotion of cancer cell proliferation, migration, and invasiveness through targetedly inhibiting ETS Proto-Oncogene 1, Transcription Factor (ETS1) expression [14]. In

Table 2: Effects of circ_0109046 knockdown on A2780 cell proliferation, apoptosis, migration, and invasion ($\overline{x} \pm s$, $n = 9$).

Groups	Circ_0109046	% Cells				Bax	Bcl-2
		Proliferation	Apoptosis	Migration	Invasion		
Sh-NC	1.00 ± 0.00	0.00 ± 0.00	7.61 ± 0.48	161.56 ± 9.83	218.11 ± 11.62	0.20 ± 0.06	0.76 ± 0.06
Sh-circ_0109046	0.41 ± 0.05*	49.01 ± 3.32*	21.60 ± 1.20*	82.00 ± 4.81*	114.11 ± 6.35*	0.64 ± 0.05*	0.32 ± 0.04*
t	35.400	44.286	32.473	21.810	23.562	16.901	18.305
P	0.000	0.000	0.000	0.000	0.000	0.000	0.000

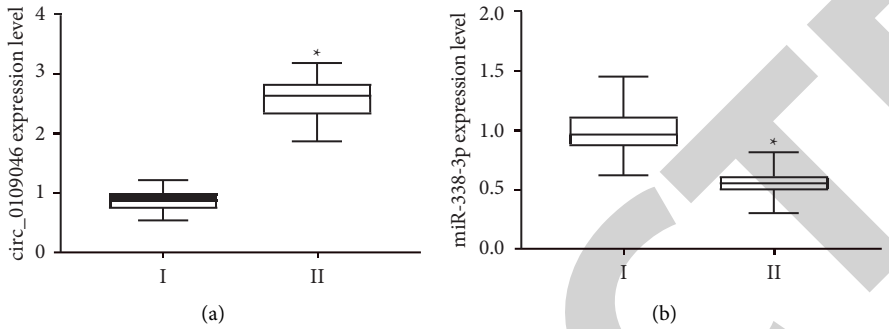

Figure 2: Effects of circ_0109046 on ovarian cancer cell apoptosis. (a) Flow cytometry for A2780 cell apoptosis after circ_0109046 knockdown. (b) Western blotting for the levels of Bax and Bcl-2 in A2780 cells after circ_0109046 knockdown. $^*P < 0.05$.

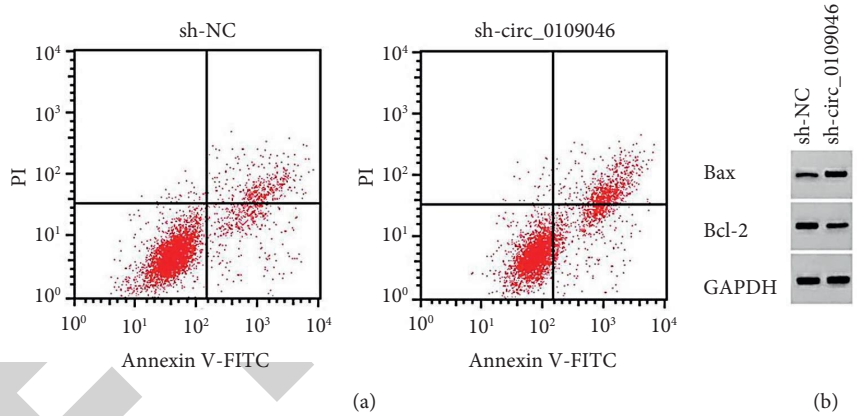

Figure 3: Circ_0109046 acted as a sponge for miR-338-3p. (a) The potential binding sites between circ_0109046 and miR-338-3p. (b) The interaction analysis by dual-luciferase reporter assay. (c) Increased miR-338-3p level in A2780 cells after circ_0109046 knockdown. $^*P < 0.05$ compared with miR-NC group; $^{\#}P < 0.05$ compared with sh-NC group.

Table 3: Effects of miR-338-3p overexpression on A2780 cell proliferation, apoptosis, migration, and invasion ($\overline{x} \pm s$, $n = 9$).

Groups	miR-338-3p	% Cells				Bax	Bcl-2
		Proliferation	Apoptosis	Migration	Invasion		
miR-NC	1.00 ± 0.00	0.00 ± 0.00	7.67 ± 0.72	162.89 ± 12.47	212.56 ± 14.70	0.21 ± 0.02	0.74 ± 0.08
miR-338-3p	3.20 ± 0.09*	54.77 ± 3.75*	23.00 ± 1.54*	65.78 ± 4.31*	92.11 ± 4.15*	0.72 ± 0.06*	0.25 ± 0.02*
t	73.333	43.816	27.053	22.081	23.657	24.191	17.826
P	0.000	0.000	0.000	0.000	0.000	0.000	0.000

addition, miR-338-3p was also declined in glioblastoma [15] and pancreatic cancer [16] and performed anticancer action to affect the development of these tumors. Importantly, miR-338-3p also had anti-growth and anti-metastasis effects on ovarian cancer cells [17]. Consistent with previous findings, this study also showed a lowly expressed miR-338-3p in ovarian cancer. Functionally, upregulation of miR-338-3p was found to reduce the mobility and growth in A2780 cells. At the same time, we also found that miR-338-3p deficiency reduced the suppressive effects of circ_0109046 lack on ovarian cancer cells, further suggesting that circ_0109046 affected ovarian cancer tumorigenesis by miR-338-3p.

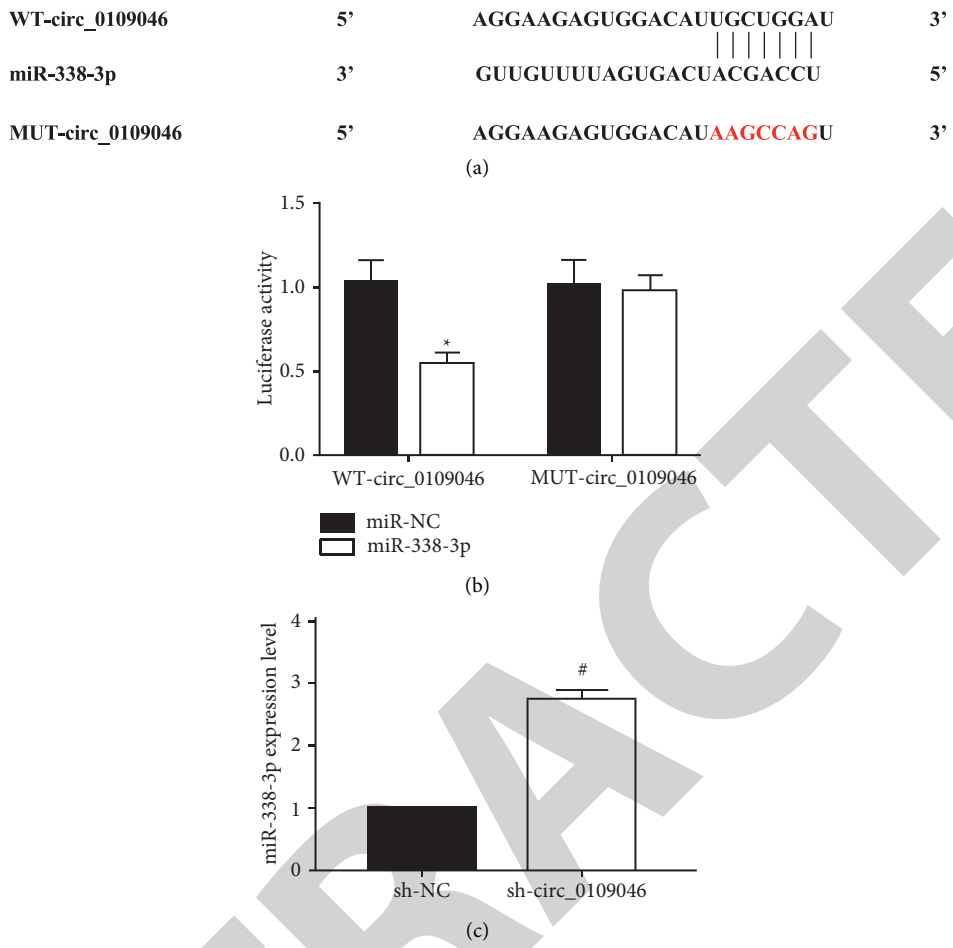

Figure 4: Effects of miR-338-3p on ovarian cancer cell apoptosis. (a) Flow cytometry for A2780 cell apoptosis after miR-338-3p overexpression. (b) Levels of Bax and Bcl-2 in A2780 cells after miR-338-3p overexpression by western blotting. $^{*}P < 0.05$.

Table 4: Effects of circ_0109046/miR-338-3p on A2780 cell proliferation, apoptosis, migration, and invasion ($\overline{x} \pm s$, $n = 9$).

Groups	miR-338-3p	%		Bax	Bcl-2	Cells	
		Proliferation	Apoptosis			Migration	Invasion
Sh-circ_0109046 + anti-miR-NC	1.00 ± 0.00	49.24 ± 3.31	21.38 ± 1.17	0.63 ± 0.06	0.30 ± 0.04	78.89 ± 5.38	114.67 ± 5.87
Sh-circ_0109046 + anti-miR-338-3p	0.39 ± 0.05*	22.34 ± 0.81*	12.32 ± 0.65*	0.35 ± 0.03*	0.59 ± 0.05*	143.67 ± 9.51*	183.00 ± 10.54*
t	36.600	23.682	20.307	12.522	13.587	17.786	16.991
P	0.000	0.000	0.000	0.000	0.000	0.000	0.000

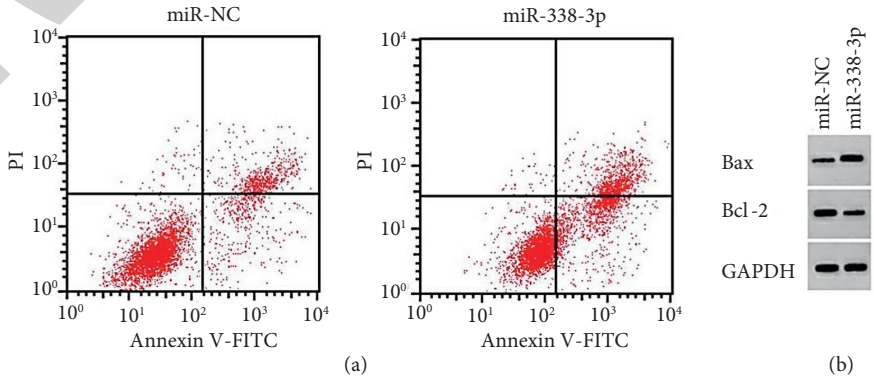

Figure 5: Effects of circ_0109046/miR-338-3p on ovarian cancer cell apoptosis. After co-transfection of sh-circ_0109046 and anti-miR-338-3p, (a) flow cytometry for A2780 cell apoptosis; (b) levels of apoptosis-related markers in A2780 cells by western blotting. $^{*}P < 0.05$.

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Retraction

Retracted: Effect of Percutaneous Nephrolithotomy Combined with Needle Nephrolithotomy on Renal Function and Complication Rate in Patients with Complex Renal Calculi

Evidence-Based Complementary and Alternative Medicine

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Research Article

Effect of Percutaneous Nephrolithotomy Combined with Needle Nephrolithotomy on Renal Function and Complication Rate in Patients with Complex Renal Calculi

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Objective. To investigate the effect of percutaneous nephrolithotomy combined with needle nephrolithotomy on renal function and incidence of complications in patients with complex renal calculi. *Methods*. From March 2020 to March 2022, 88 patients with complex renal calculi were enrolled and divided into two groups. Percutaneous nephrolithotomy (PCNL) was performed in the control group, and percutaneous nephrolithotomy combined with needle nephrolithotomy was performed in the study group. Perioperative conditions, renal function parameters such as blood urea nitrogen (BUN), serum creatinine (Scr), and cystatin C (CysC) levels, inflammatory factors such as C-reactive protein (CRP) and procalcitonin (PCT) levels before surgery and 1 day after surgery were determined. The incidence of complications was statistically analyzed between two groups. *Results*. There was no significant difference in stone clearance rate between the two groups. The operation time and hospital stay in the study group were shorter than those in the control group. The intraoperative blood loss was less than that in the control group. The VAS score was lower than that in the control group. BUN, Scr, and CysC in the study group were not significantly different from those in the control group. CRP and PCT in the two groups at 1 d after surgery were higher than those before surgery, but CRP and PCT in the study group were lower than those in the control group. The incidence of complications in the study group was lower than that in the control group. *Conclusion*. Percutaneous nephrolithotomy combined with needle nephrolithotomy is effective and safe in the treatment of complex renal calculi.

1. Introduction

The incidence of kidney stones is high. Complex kidney stones are mainly staghorn-shaped or multiple and large stones with a diameter of ≥2.5 cm. In general, stones are numerous, widely distributed, and difficult to remove, posing a great threat to the quality of life and physical and mental health of patients [1, 2]. Therefore, timely, safe, and effective treatment for patients with complex kidney stones is of great significance. Surgery is an important treatment for complex kidney stones. Traditional open surgery is more traumatic and has a higher incidence of postoperative complications, which gradually makes it difficult to meet the actual clinical needs [3–5]. Percutaneous nephrolithotomy (PCNL) is also an important treatment for complex kidney stones. Compared with laparotomy, PCNL has less trauma and faster recovery of physical function after surgery, but there may still be related complications such as organ damage, infection, and bleeding after surgery [6–8]. Needle nephroscope is a new type of PCNL device, which mainly includes a needle handle and a puncture outer sheath. The tail of the needle handle is a three-way device, which can be connected to a titanium laser fiber (200 μm), a video introduction fiber, and a liquid perfusion device, which is of great significance for improving the efficacy and safety of the treatment [9]. Based on this, this study planned to select 88 patients with complex renal calculi in our hospital, and grouped to explore the intervention value of percutaneous nephrolithotomy combined with needle nephrolithotomy.

2. Materials and Methods

2.1. General Information. Eighty-eight patients with complex renal calculi in our hospital from March 2020 to March 2022 were enrolled. The inclusion criteria were as follows: (1) confirmed by CT, urography, and transabdominal ultrasonography; (2) multiple or calculi with long diameter ≥2.5 cm and surface area >500 mm^2; (3) aware of this study and signed the consent form; and (4) single renal calculi. The exclusion criteria of this study were as follows: (1) Patients with coagulation disorders; (2) Patients with diabetes, hypertension, and chronic nephritis; (3) Patients with immune system diseases; (4) Patients with malignant tumors; (5) Patients with a history of surgical treatment of urinary calculi; (6) Lactating and pregnant women. They were divided into the study group and the control group according to simple random number table, 44 cases for each group. The study group consisted of 26 males and 18 females, aged 34~69 years, with mean one of (51.38 ± 12.71) years; affected side: left kidney in 27 cases, right kidney in 17 cases; stone type: multiple in 22 cases, cast in 16 cases, staghorn in 6 cases. The control group consisted of 28 males and 16 females, aged 32~69 years, with mean one of (50.81 ± 14.04) years; affected side: 25 cases of left kidney and 19 cases of right kidney; stone types: multiple in 18 cases, cast in 18 cases, and staghorn in 8 cases. There were no significant differences in clinical data between two groups ($P > 0.05$). The protocol of this study was approved by the Ethics Committee of Zhenjiang Hospital of Chinese Traditional and Western Medicine Urology Surgery.

2.2. Treatment. For the patients in the control group, PCNL was adopted, general anesthesia was given, lithotomy position was taken, the ureterorenoscope (F9.8) was inserted into the F5 ureteral catheter through the bladder, it was inserted into the renal pelvis, the ureteral catheter was properly fixed, an appropriate amount of normal saline was injected for those without hydronephrosis, abdominal pad height, the renal condition was identified, the puncture point was selected, puncture treatment through the puncture needle (18 G) of the target calyx was performed, incise the skin at the puncture site, the fascial dilator was placed, it was dilated to 16 F, F 18 soft sheath was set, the channel was dilated one by one through the metal coaxial dilator to F 21, F 24 nephroscope short sheath was placed, the dilator was removed, the percutaneous nephroscope channel was constructed, the corresponding film sheath was placed, the nephroscope and lithotripsy device was placed, the renal calculi through a clear imaging system was identified, the hydraulic perfusion pump flow was adjusted to 300~400 L/min, the upper limit of pressure is 200~300 mmHg, pneumatic ballistic channel was implemented; the larger broken calculi was removed through the ureteral forceps, the small calculi was washed through the perfusion pump and retrograde catheter, lithotripsy was completed, the normal saline was inserted, the lithotripsy was removed, the lithotripsy sheath was inserted again, and the lithotripsy sheath was removed, remove the lithotripsy sheath, remove the lithotripsy, remove the lithotripsy sheath, remove the lithotripsy sheath, remove the lithotripsy, remove the lithotripsy Routine hold of double-J tube.

For the patients in the study group, ercutaneous nephrolithotomy combined with needle nephrolithotomy was performed. First, the standard channel was constructed. The specific measures were the same as the control group. After the completion, lithotripsy was performed by ultrasonic lithotripter. The lithotripsy was crushed into small pieces. The large stones were removed by lavage fluid and stone extraction forceps. With the assistance of B ultrasound, needle nephrolithotomy was performed and inserted into the calyces that were difficult to be touched by standard channel PCNL. The stones were broken by a titanium laser fiber (200 um) and washed out by water. No double-J tube was required to be shelved.

2.3. Outcome Measures. (1) The perioperative conditions of the two groups were statistically analyzed, including the length of operation, intraoperative blood loss, stone clearance rate, length of hospital stay, and degree of pain, and the degree of pain was assessed according to the VAS scale, with a total of 10 points, and the lower the score, the better. (2) The levels of renal function (blood urea nitrogen (BUN), serum creatinine (Scr), and cystatin (C) were measured before and 1 d after operation in both groups. (3) The levels of inflammatory factors (C-reactive protein (CRP), procalcitonin (PCT)) before surgery and 1 d after surgery were counted in both the groups. (4) The incidence of complications in the two groups was counted.

2.4. Statistical Methods. The data were analyzed by SPSS 22.0 (IBM SPSS Statistics, USA), the data were expressed as mean ± standard deviation (SD). The differences were determined by *t*-test. The two-sided$P < 0.05$ indicated statistically significant differences.

3. Results

3.1. Perioperative Conditions. There was no significant difference in stone clearance rate between the study group (95.45%) and the control group (90.91%) ($P > 0.05$). The study group was shorter in operation time (42.26 ± 7.03) min, hospital stay (6.09 ± 1.28) d, intraoperative blood loss (12.35 ± 2.43) ml than the control group, and VAS score (2.92 ± 0.96) points than the control group ($P < 0.05$, Table 1).

3.2. Renal Function Indicators. There was no significant difference in BUN (6.06 ± 1.45) mmol/L, Scr (75.54 ± 8.48) μmol/L, and CysC (490.11 ± 20.37) μg/L between the study group and the control group before operation ($P > 0.05$). The serum levels of Scr, BUN, and CysC in the two groups at 1 d after operation were higher than those before operation ($P < 0.05$), but BUN (7.51 ± 1.14) mmol/L, Scr (84.68 ± 10.68) μmol/L, and CysC (585.16 ± 32.19) μg/L in

Table 1: Comparison of perioperative conditions between the two groups.

Group	Number of cases	Procedure duration (min)	Intraoperative blood loss (ml)	Stone clearance (n (%))	Length of stay (d)	VAS (points)
Study group	44	42.26 ± 7.03	12.35 ± 2.43	42 (95.45)	6.09 ± 1.28	2.92 ± 0.96
Control group	44	61.38 ± 10.35	39.97 ± 8.91	40 (90.91)	9.71 ± 3.44	3.87 ± 1.05
T/χ^2 value		10.137	19.838	0.179	6.542	4.429
P value		<0.001	<0.001	0.672	<0.001	<0.001

the study group were lower than those in the control group ($P < 0.05$), see Table 2.

3.3. Inflammatory Factors. There was no significant difference in CRP (17.65 ± 9.24) mg/L and PCT (0.43 ± 0.15) ng/L between the study group and the control group before operation ($P > 0.05$). One day after operation, CRP and PCT in the two groups were higher than those before operation ($P < 0.05$), but CRP (26.69 ± 10.04) mg/L and PCT (0.66 ± 0.17) ng/L in the study group were lower than those in the control group ($P < 0.05$), see Table 3.

3.4. Complications. The incidence of complications in the study group (4.55%) was lower than that in control group (18.18%) ($P < 0.05$), see Table 4.

4. Discussion

Because complex renal calculi have complex distribution, irregular shape, and large stone diameter, they are easy to cause urinary tract obstruction, infection, and other related complications, causing renal failure, kidney injury, etc., which are a great threat to the life and health of patients [10, 11]. Traditional open surgery requires incision of the renal parenchyma, which is easy to cause serious trauma, has a high incidence of postoperative complications, and is easy to residual stones, so the overall effect is difficult to achieve clinical expectations [12, 13]. Therefore, how to safely and effectively treat complex renal calculi is still a research hotspot.

With the improvement of medical technology, PCNL has gradually become the main treatment for renal calculi, with less trauma and higher safety. However, staged and multichannel surgery is usually required for complex renal calculi to achieve ideal results, but it will increase the medical costs and surgical risks to varying degrees [14–16]. The study pointed out that with the continuous improvement of endoscopic technology, the concept of needle-like nephroscope was developed in clinical practice combined with early ultrasound-assisted puncture needle puncture of parallel calyceal stones and pushing them to the renal pelvis to perform nephroscopic lithotripsy, so as to prevent redilatation and reduce the number of percutaneous renal channels. It can perform direct powdered lithotripsy through laser after successful puncture of the target calyceal calyx without reconstructing the skin renal channel, which can improve the success rate of stone lithotripsy and has a higher safety [17, 18]. At the same time, the treatment procedure was assisted by needle nephroscope, and the stones could be rapidly discharged by high-pressure lavage during the implementation of superior lithotripsy, without the influence of ureteral factors, and the stone clearance effect was satisfactory [19].

The results of this study showed that there was no significant difference in stone clearance rate between the study group and the control group, but other perioperative-related indicators and renal function-related indicators were superior to the control group, and the incidence rate of complications (4.55%) was lower than the control group (18.18%) ($P < 0.05$), indicating that percutaneous nephrolithotomy combined with needle nephrolithotomy has high application value in complex renal calculi, can reduce surgical trauma, help ensure renal function, and the inflammatory response caused by surgical invasive treatment is mild, the incidence rate of postoperative complications is low, and the safety is guaranteed. The main reasons for this analysis are as follows: in needle nephrolithotomy, the diameter of visual fiber is only 0.7 mm, which can transmit high-definition images in real time and clearly, assist physicians to implement relevant treatment operations, try to avoid blood vessels and prevent damage, and break stones through titanium laser during lithotripsy, which can improve the accuracy of surgical treatment and reduce body damage. At the same time, titanium laser is a solid pulse laser, which can produce photothermal effect during application and trigger the thermochemical reaction of stones, thus breaking and clearing stones, and the penetration depth of titanium laser tissue is small, usually no more than 0.5 mm, without obvious side damage to the tissue, ensuring the safety. In addition, not indwelling a double-J tube after surgery can also prevent infection, bleeding, and other related complications caused by the second extubation, in order to reduce the degree of pain and shorten the time of postoperative physical function rehabilitation.

PCT and CRP are commonly used indicators for clinical evaluation of inflammatory response, and their serum content is low under normal physiological conditions, but if the body is injured, it will be abnormally increased, so they can be used to assess the degree of surgical trauma. The results of this study showed that the serum PCT and CRP levels in the study group were lower than those in the control group at 1 d after operation ($P < 0.05$), and it was further confirmed from a microscopic point of view that the combination of percutaneous nephrolithotomy and needle nephrolithotomy for complex renal calculi was feasible and effective, with less trauma, which could reduce the degree of inflammatory response caused by surgical invasive treatment procedures. The main reasons for this analysis are as follows: accurate localization of stone location during

Table 2: Comparison of renal function indicators between the two groups.

Time	Group	Number of cases	BUN (mmol/L)	Scr (μmol/L)	CysC (μg/L)
Before surgery	Study group	44	6.06 ± 1.45	75.54 ± 8.48	490.11 ± 20.37
	Control group	44	6.42 ± 1.71	72.77 ± 9.09	485.88 ± 22.65
	T value		1.065	1.478	0.921
	P value		0.290	0.143	0.360
1 d after surgery	Study group	44	7.51 ± 1.14	84.68 ± 10.68	585.16 ± 32.19
	Control group	44	8.78 ± 1.65	93.15 ± 11.79	659.13 ± 38.38
	T value		4.201	3.532	9.795
	P value		<0.001	0.001	<0.001

Table 3: Comparison of inflammatory factors between the two groups.

Time	Group	Number of cases	CRP (mg/L)	PCT (ng/L)
Before surgery	Study group	44	17.65 ± 9.24	0.43 ± 0.15
	Control group	44	19.04 ± 8.78	0.41 ± 0.16
	T value		0.723	0.605
	P value		0.471	0.547
1 d after surgery	Study group	44	26.69 ± 10.04	0.66 ± 0.17
	Control group	44	35.77 ± 11.38	0.87 ± 0.20
	T value		3.969	5.307
	P value		<0.001	<0.001

Table 4: Comparison of complications between the two groups [n (%)].

Group	Number of cases	Fever	Urine leakage	Hemorrhage	Organ damage	Total occurrence
Study group	44	1 (2.27)	1 (2.27)	0 (0.00)	0 (0.00)	2 (4.55)
Control group	44	3 (6.82)	2 (4.55)	2 (4.55)	1 (2.27)	8 (18.18)
X^2 value						4.062
P value						0.044

surgery is an important prerequisite to ensure the pertinence and effectiveness of treatment. Conventional PCNL requires substantial swing to find stones, which easily produces different degrees of mechanical damage to peripheral organs and blood vessels. Needle nephroscope is small in size, light in weight, and can locate the stone location without substantial swing, so as to effectively reduce the damage to the patient's kidney and reduce the degree of stress response.

In summary, percutaneous nephrolithotomy combined with needle nephrolithotomy is effective in the treatment of complex renal calculi, which can reduce the damage to the body and renal function, with less inflammatory reactions caused by surgery, and a lower incidence of complications, with safety.

Data Availability

Data will be available from the corresponding author under reasonable requests.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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Retraction

Retracted: Multivariate Analysis of Recurrence after Hysteroscopic Diagnosis and Treatment of Endometrial Polyps following IVF-ET Failure

Evidence-Based Complementary and Alternative Medicine

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This article has been retracted by Hindawi following an investigation undertaken by the publisher [1]. This investigation has uncovered evidence of one or more of the following indicators of systematic manipulation of the publication process:

(1) Discrepancies in scope
(2) Discrepancies in the description of the research reported
(3) Discrepancies between the availability of data and the research described
(4) Inappropriate citations
(5) Incoherent, meaningless and/or irrelevant content included in the article
(6) Peer-review manipulation

The presence of these indicators undermines our confidence in the integrity of the article's content and we cannot, therefore, vouch for its reliability. Please note that this notice is intended solely to alert readers that the content of this article is unreliable. We have not investigated whether authors were aware of or involved in the systematic manipulation of the publication process.

In addition, our investigation has also shown that one or more of the following human-subject reporting requirements has not been met in this article: ethical approval by an Institutional Review Board (IRB) committee or equivalent, patient/participant consent to participate, and/or agreement to publish patient/participant details (where relevant).

Wiley and Hindawi regrets that the usual quality checks did not identify these issues before publication and have since put additional measures in place to safeguard research integrity.

We wish to credit our own Research Integrity and Research Publishing teams and anonymous and named external researchers and research integrity experts for contributing to this investigation.

The corresponding author, as the representative of all authors, has been given the opportunity to register their agreement or disagreement to this retraction. We have kept a record of any response received.

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[1] J. Xu, B. Liu, X. Feng, L. Shen, and Q. Qu, "Multivariate Analysis of Recurrence after Hysteroscopic Diagnosis and Treatment of Endometrial Polyps following IVF-ET Failure," *Evidence-Based Complementary and Alternative Medicine*, vol. 2022, Article ID 4140022, 8 pages, 2022.

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Research Article

Multivariate Analysis of Recurrence after Hysteroscopic Diagnosis and Treatment of Endometrial Polyps following IVF-ET Failure

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Objective. To explore the risk factors affecting the recurrence of endometrial polyps (EPs) after hysteroscopic diagnosis and treatment of EPs following in vitro fertilization-embryo transfer (IVF-ET) failure by multivariate analysis. *Methods*. The clinical data of 369 patients with EPs hysteroscopically treated in our department due to IVF-ET failure from January 2017 to January 2020 were retrospectively analyzed, including the number and size of polyps, postoperative treatment, endometriosis (EM), hydrosalpinx (HSP), and polycystic ovarian syndrome (PCOS), and the effects of these factors on EP recurrence were observed. *Results*. Of the patients enrolled, 184 cases (49.9%) were treated by curettage, and 185 cases (50.1%) by electrotomy. A total of 72 cases (19.5%) of postoperative recurrence were determined, including 34 cases (9.2%) without postoperative medication, 31 cases (8.4%) with one month of postoperative Didroxyprogesterone (DG) administration, and 7 cases (1.9%) with three months of postoperative DG administration. Surgical methods, 3 months of postoperative medication, PCOS, and polyp number and size significantly influence the recurrence of EPs, which were all the influencing factors of polyp recurrence. After controlling for other factors, the risk of EP recurrence after electrotomy was found to be lower than that after curettage, with an odds ratio (OR) (95% confidence interval (CI)) of 0.354 (0.163–0.767); the risk of EP recurrence after 3 months of postoperative medication was lower than that without postoperative medication, with an OR (95% CI) of 0.024 (0.005–0.104); the risk of EP recurrence in patients with PCOS was higher than that without PCOS, and the OR (95% CI) was 2.505 (1.113–5.639); patients with multiple polyps (≥2) were at an increased risk of recurrence than those with a single polyp, with an OR (95% CI) of 66.552 (14.711–301.084); patients with polyp diameter ≥ 2 cm had a higher risk of recurrence than those with polyp diameter < 2 cm, and the OR (95% CI) was 1084.76 (148.743–7910.999). *Conclusions*. PCOS patients are at an elevated risk of EP recurrence than non-PCOS patients. In patients with multiple polyps, those with a diameter ≥ 2 cm have an increased risk of polyp recurrence compared with those with polyp diameter < 2 cm; electrotomy is associated with a lower recurrence risk of EPs than curettage. The risk of EP recurrence in patients treated with postoperative progesterone for 3 months is lower than that of patients without postoperative medication.

1. Introduction

Endometrial polyps (EPs) are benign pathological entities induced by localized overgrowth of basal endometrium [1]. As one of the most common gynecological diseases in women of childbearing age, it is also an important reason for the failure of assisted reproduction [2]. Currently, the pathogenic mechanism of EPs is not yet completely clear, but it is shown to be associated with excessive estrogen, hypertension, and obesity [3]. EPs are prevalent with an incidence of about 7.8%–34.9% and the clinical presentations of abnormalities, uterine bleeding, menorrhagia, infertility, etc. [4, 5]. Routine hysteroscopy of infertile women abroad revealed an incidence of EPs as high as 17.6% [6]. And the incidence of EPs in primary infertility is 3.8%–38.5%, higher than that in secondary infertility (1.8%–17%) [7]. In addition, EPs have also been found to be a risk factor for pregnancy loss, with a prevalence rate of 15–50% among patients with recurrent abortion [8]. Big data studies at home and abroad have also shown that EPs are the most common endometrial lesions in

patients with infertility or even repeated implantation failure [9, 10]. Therefore, it is of great significance to optimize treatment options for EPs to ameliorate repeated implantation failure.

With the development of minimally invasive techniques, transcervical resection of polyp (TCRP) has become the preferred treatment for EPs, with many advantages such as high accuracy, low blood loss, and postoperative recurrence rate [11]. Among them, hysteroscopic curettage as a traditional surgical method can temporarily control polyp bleeding, but it may cause abnormal uterine bleeding because polyps are easy to remain in the uterine cavity [12]. In addition, this surgical approach results in an increased risk of postoperative recurrence due to the curettage of EPs from the pedicle [13]. Hysteroscopic electrotomy, on the other hand, is a surgical method for the treatment of EPs through the electroresection of the pedicled and basal parts [14]. This procedure has been widely applied in clinical gynecological diseases such as perimenopausal abnormal uterine bleeding, intrauterine fibroids, and benign uterine lesions and plays a positive role in improving clinical symptoms, sex hormone levels, and reducing complications [15–17]. However, this treatment also has a certain risk of recurrence, with a postoperative recurrence rate of 13.3% [18], greatly compromising the therapeutic effect. Therefore, it is the current research focus of gynecologists to explore the causes of postoperative recurrence in EPs patients and reduce the recurrence rate. In this study, the high-risk factors of postoperative EP recurrence in patients undergoing hysteroscopic TCRP after in vitro fertilization-embryo transfer (IVF-ET) failure were retrospectively analyzed to provide evidence for clinical diagnosis and treatment.

2. Materials and Methods

2.1. Data Source. A total of 369 patients with hysteroscopically diagnosed EPs who underwent hysteroscopic TCRP due to IVF-ET failure in the Department of Gynecological Endocrinology of the Chongqing Health Center for Women and Children were enrolled. Inclusion criteria were (1) age 20–40 years; (2) EPs were suggested by transvaginal ultrasound and confirmed by postoperative pathology; (3) complete case data [19]; (4) no use of hormone drugs within the last month; (5) no history of hypertension, cardio-cerebrovascular diseases, high-risk factors of thrombosis, migraine, liver and kidney dysfunction, gallbladder diseases, submucosal uterine fibroids, nor malignant tumors. Exclusion criteria were (1) acute reproductive system infection; (2) severe organ dysfunction; (3) uterine diseases such as hysteromyoma, adenomyosis, endometrial tumor, and uterine malformation; (4) history of treatment with sex hormone drugs in recent 3 months; (5) cervical hardness and inability to fully dilate due to cervical scar; (6) coagulation dysfunction and tuberculosis; (7) malignant tumors of the reproductive system; and (8) recent history of uterine surgery. The specific diagnostic criteria for the identification of EPs by vaginal B-ultrasound were endometrial thickening, inhomogeneity, intrauterine space-occupying lesions or strong echogenic masses, and the presence of dotted blood vessels. This study was approved by the Ethics Committee of our hospital, and the study subjects were informed and provided informed consent.

2.2. Methods

2.2.1. Surgical Methods. Preoperative examinations were rounded out 3–7 days after menstruation to eliminate the contraindications. Hysteroscopic TCRP was performed under total intravenous anesthesia, with anesthesia induced by 2.5 mg/kg propofol injection (0.5 mg/kg/h) followed by continuous infusion of propofol injection for maintenance of anesthesia. (1) All patients completed various preoperative examinations and were explained about the operation-related matters. Usually, the operation time was scheduled within 3–7 days after menstruation, with 8–10 h of preoperative fasting. (2) Among the enrolled patients, 185 patients were treated with hysteroscopic electrotomy, with the methods described as follows: Patients received general intravenous anesthesia in the lithotomy position, and 0.9% normal saline was used as the uterine distention solution administered at a pressure of 100 mmHg. The cervix was explored, and a resectoscope was placed after the cervix was dilated to determine the position, number, size, and pedicle site of the polyps. Then the root of polyps was completely removed through the ring electrode to reach the basal layer. Thereafter, a curette was inserted into the uterine cavity, and reasonable scratching and curettage were carried out for residual polyp tissue to ensure its complete removal. Hysteroscopy was used again to check for residual polyps. The rest 184 underwent hysteroscopic curettage, and the methods were as follows: EPs were hysteroscopically observed. Then, a curettage spoon was used to scratch and scrape intrauterine polyps, especially those parts that could not be found or were not easily removed by the ring electrode, and careful examination and treatment were given. The curette was then inserted into the uterine cavity, and reasonable scratching and scraping were carried out for residual polyp tissue to ensure its complete removal. The presence of any residual micropolyp was observed by the hysteroscope again, and a small scissor was used to cut off if any. (3) All tissues removed during the operation were sent to the Department of Pathology for examination. (4) Postoperative treatment: no medication or medication (Didroxyprogesterone (DG), SFDA Approval No. H20090470), per os, 20 mg/d, for 1 month or 3 months). The experimental flowchart is shown in Figure 1.

2.3. Outcome Measures. The postoperative EP recurrence rate of patients with two different surgical modalities and between patients with and without medication (1 or 3 months of medication) were compared. Factors influencing EP recurrence were determined using the multivariate analysis.

2.4. Follow-up. We followed up with the subjects by means of telephone visits, visits, reexaminations, and inquiring

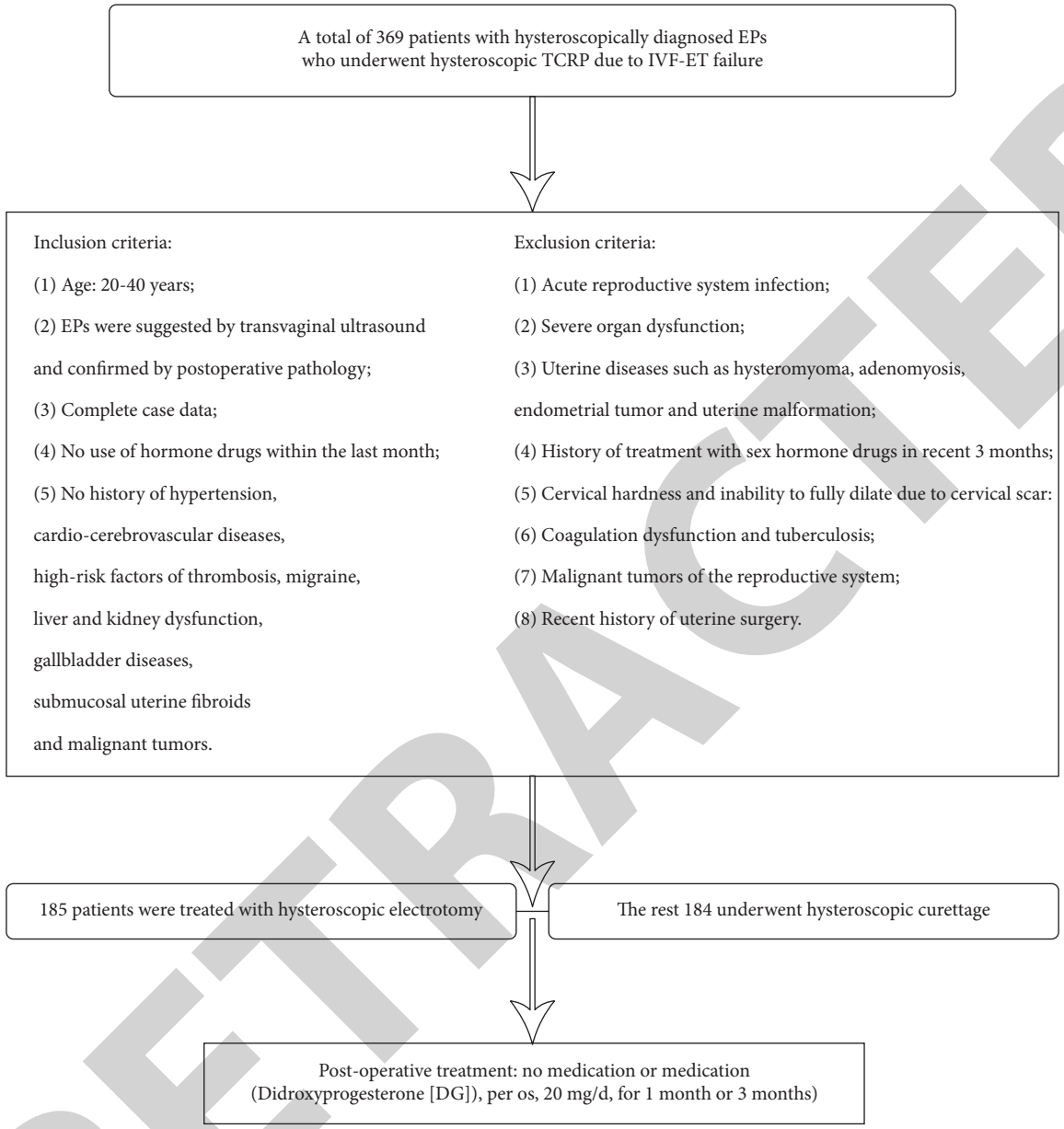

Figure 1: Experimental flowchart. Note. EPs, Endometrial polyps; TCRP, transcervical resection of the polyp; IVF-ET, in vitro fertilization-embryo transfer; DG, Didroxyprogesterone.

about the case system to record patients' recurrence. The enrolled patients underwent B-ultrasound every 1 and 3 months after surgery. Excluding 4 (1.07%) losses to follow-ups, 369 cases were eventually included. During the follow-up, recurrence was diagnosed if the B-ultrasound suggested EPs.

2.5. Statistical Analysis. SPSS 23.0 statistical software (IBM Corp., Armonk, NY, USA) was used for analysis. Quantitative data following normal distribution were represented by $(\overline{x} \pm s)$, and those of non-normal distribution were recorded as median (M) (interquartile range (IQR)). In this paper, binary Logistic regression (LR) was used to evaluate the influence of surgical methods, polyp number, polyp size, postoperative treatment, and complications on polyp recurrence. Those with statistical significance were further analyzed by binary LR to calculate the odds ratio (OR) value. P values < 0.05 were considered statistically significant.

3. Results

3.1. Basic Information. The median age of 369 patients with EPs was 31 years old. Among them, most cases (316 (85.6%)) had a body mass index (BMI) between 18.5 and 24.9 kg/m^2. There were 193 cases (52.3%) with endometriosis (EM), 121 cases (32.8%) with hydrosalpinx (HSP) and 118 cases (32%) with the polycystic ovarian syndrome (PCOS). Single polyp was found in 192 cases (52%) and multiple polyps in 177 cases (48%). The polyp diameter was less than 2 cm in 337

Table 1: Basic data of EPs patients (M (IQR), n (%)).

Parameters	No. of cases
Age	31
BMI (kg/m^2)	
< 18	18 (4.9)
18.5–24.9	316 (85.6)
25–29.9	34 (9.2)
> 30	1 (0.3)
Complicated with endometriosis	193 (52.3)
Complicated with hydrosalpinx	121 (32.8)
Complicated with PCOS	118 (32.0)
Single polyp	192 (52.0)
Multiple polyps	177 (48.0)
Polyp diameter < 2 cm	337 (91.3)
Polyp diameter ≥ 2 cm	32 (8.7)
Curettage	184 (49.9)
Electrotomy	185 (50.1)
Postoperative recurrence	72 (19.5)
Recurrence without postoperative medication	34 (9.2)
Postoperative recurrence after one month of DG administration	31 (8.4)
Postoperative recurrence after three months of DG administration	7 (1.9)

Note. BMI, body mass index; PCOS, polycystic ovarian syndrome; DG, Didroxyprogesterone; EPs, Endometrial polyps; *M*, median; IQR, interquartile range.

Table 2: Comparison of data between recurrent and nonrecurrent patients after surgery for EPs (n (%), mean ± SD).

Factors	n	Recurrence group ($n = 72$)	Nonrecurrence group ($n = 297$)	χ^2/t	P
Age (years old)	369	30.94 ± 4.44	30.52 ± 4.73	0.684	0.495
BMI (kg/m^2)				4.290	0.232
< 18	18	4 (5.56)	14 (4.71)		
18.5–24.9	316	60 (83.33)	256 (86.20)		
25–29.9	34	7 (9.72)	27 (9.09)		
> 30	1	1 (1.39)	0 (0.00)		
Complicated with endometriosis	193	47 (65.28)	146 (49.16)	6.036	0.014
Combined with hydrosalpinx	121	35 (48.61)	86 (28.96)	10.158	0.001
Complicated with PCOS	118	31 (43.06)	87 (29.29)	5.046	0.025
Number of polyps				6.192	0.013
Single polyp	192	28 (38.89)	164 (55.22)		
Multiple polyps	177	44 (61.11)	133 (44.78)		
Polyp size				7.219	0.007
< 2 cm in diameter	337	60 (83.33)	277 (93.27)		
≥ 2 cm in diameter	32	12 (16.67)	20 (6.73)		
Surgical methods				6.768	0.009
Curettage	184	26 (36.11)	158 (53.20)		
Electrotomy	185	46 (63.89)	139 (46.80)		
Postoperative treatment				12.710	0.002
No postoperative medication	62	34 (47.22)	28 (9.43)		
1 month of DG administration	200	31 (43.06)	169 (56.90)		
3 months of DG administration	107	7 (9.72)	100 (33.67)		

Note. BMI, body mass index; PCOS, polycystic ovarian syndrome; DG, didroxyprogesterone; EPs, endometrial polyps.

cases (91.3%); 184 cases (49.9%) were treated by curettage and 185 cases (50.1%) were treated by electrotomy; 72 cases (19.5%) of postoperative recurrence were identified, including 34 cases without postoperative medication (9.2%), 31 cases with one month of postoperative DG administration (8.4%), and 7 cases with three months of postoperative DG administration (1.9%), Table 1.

3.2. Comparison of Data between Recurrent and Nonrecurrent Patients after Surgery for EPs. Through analysis, we found no significant difference in age and BMI between patients with and without recurrence of EPs ($P > 0.05$), but the presence of statistical differences in EM, HSP, PCOS, polyp number, polyp size, surgical methods, and postoperative treatment ($P < 0.05$), Table 2.

3.3. Univariate and Multivariate Logistic Regression Analysis. We assigned the variables with significant differences between recurrent and nonrecurrent patients after surgery for EPs as follows (Table 3).

Table 3: Variable assignments of univariate and multivariate Logistic regression analysis of influencing factors of polyp recurrence.

Variables	Assignments
Surgical methods	Curettage = 0, electrotomy = 1
Number of polyps	Single = 0, ≥2 = 1
Size of polyps	<2 cm in diameter = 0, and ≥2 cm in diameter = 1
Postoperative treatment	No medication = 0, medication for 1 month = 1, medication for 3 months = 2
Complicated with endometriosis	Without = 0, with = 1
Complicated with hydrosalpinx	Without = 0, with = 1
Complicated with PCOS	Without = 0, with = 1

Note. PCOS, polycystic ovarian syndrome.

Table 4: Univariate analysis of endometrial polyp recurrence.

Related factors	β	S.E.	OR (95% CI)	Wald value	P value
Surgical methods (with curettage as reference)					
Electrotomy	−0.076	0.263	0.927 (0.552–1.552)	0.083	0.773
Number of polyps (with single lesion as reference)					
Multiple	1.445	0.297	4.244 (2.373–7.589)	23.757	<0.001
Polyp size (with < 2 cm as reference)					
≥2 cm	3.556	0.513	35.040 (12.832–95.681)	23.757	<0.001
Postoperative treatment (with nonmedication as reference)					
1 month after medication	−1.48	0.289	0.863 (0.489–1.521)	0.260	0.610
3 months after medication	−1.857	0.438	0.156 (0.066–0.369)	17.931	<0.001
Complicated with endometriosis	0.459	0.268	1.582 (0.935–2.676)	2.926	0.087
Complicated with hydrosalpinx	1.131	0.270	3.099 (1.826–5.258)	17.572	<0.001
Complicated with PCOS	0.602	0.270	1.825 (1.075–3.098)	4.965	0.026

Note. PCOS, polycystic ovarian syndrome; OR, odds ratio; CI, confidence interval.

Table 5: Multivariate analysis of endometrial polyp recurrence.

Related factors	β	S.E.	OR (95% CI)	Wald value	P value
Surgical methods	−1.038	0.394	0.354 (0.163–0.767)	6.929	0.008
Postoperative treatment					
1 month after medication	−0.82	0.395	0.922 (0.425–1.998)	0.043	0.836
3 months after medication	−3.744	0.758	0.024 (0.005–0.104)	24.414	<0.001
Complicated with PCOS	0.918	0.414	2.505 (1.113–5.639)	4.917	0.027
Number of polyps	4.198	0.770	66.552 (14.711–301.084)	29.714	<0.001
Size of polyps	6.989	1.014	1084.76 (148.743–7910.999)	47.532	<0.001

Note. PCOS, polycystic ovarian syndrome; OR, odds ratio; CI, confidence interval.

3.4. Univariate Analysis of EP Recurrence. The univariate analysis results of EP recurrence are shown in Table 4. With $\alpha = 0.05$ as the test level, the univariate analysis indicated that the p values of the number of polyps, polyp size, postoperative treatment, EM, HSP, and PCOS were all under 0.05, indicating statistical significance. Patients with multiple polyps were more likely to experience postoperative recurrence than those with single polyp; and compared with patients with polyp diameter < 2 cm, those with polyp diameter ≥ 2 cm were at an increased risk of postoperative EP recurrence. Besides, complications such as EM, PCOS, and HSP increased the risk of postoperative relapse. Patients were less likely to suffer from relapse after 3 months of medication than those without medication. Although no statistical significance was observed in EP recurrence between the postoperative medication (with vs. without) and between surgical methods (curettage vs. electrotomy), the two may be the influencing factors of postoperative polyp reexamination in a professional sense, so they were still included in the multivariate analysis model as classified variables.

3.5. Multivariate Analysis of EP Recurrence. Multifactor analysis variables were screened by the forward LR method. Although the univariate analysis of surgical methods and postoperative treatment methods were suggested $P > 0.05$, they were still included in the multivariate analysis from professional considerations. The final model results are shown in Table 5. The stepwise regression results indicated significance in surgical methods, postoperative medication for 3 months, PCOS, polyp number, and polyp size, suggesting their roles as influencing factors of polyp recurrence. While postoperative medication for 1 month, HSP and EM were excluded. After controlling for other factors, the risk of polyp recurrence after electrotomy was found to be lower than that after curettage, with an OR (95% confidence interval [CI]) of 0.354 (0.163–0.767); The risk of polyp

recurrence after 3 months of postoperative medication was lower than that without postoperative medication, with an OR (95% CI) of 0.024 (0.005–0.104); The risk of polyp recurrence in patients with PCOS was higher than that of non-PCOS patients, and the OR (95% CI) was 2.505 (1.113–5.639); The polyp recurrence risk in patients with multiple polyps (number ≥ 2) was higher than that of patients with a single polyp, and the OR (95% CI) was 66.552 (14.711–301.084); patients with polyp diameter ≥ 2 cm had a higher risk of recurrence than those with polyp diameter < 2 cm, and the OR (95% CI) value was 1084.76 (148.743–7910.999).

4. Discussion

Hysteroscopic TCRP is an accurate, safe, and effective procedure operated under direct vision, which is therefore the preferred treatment for EPs [20]. The results of this study showed that the postoperative recurrence rate was 19.5%, which was similar to previous literature [21]. At present, the causes of recurrent EPs in women of childbearing age are still elusive, but it is considered to be related to age, obesity, PCOS, estrogen stimulation, inflammation, etc [22]. In this research, 193 cases (52.3%) of EM, 121 cases (32.8%) of HSP, and 118 cases (32%) of PCOS were identified; There were 192 cases (52%) of a single polyp and 177 cases (48%) of multiple polyps; polyps smaller than 2 cm in diameter were determined in 337 cases (91.3%) and those larger than 2 cm in diameter were identified in 32 cases (8.7%); 184 cases (49.9%) were treated by curettage, and 185 cases (50.1%) underwent electrotomy; 72 cases (19.5%) had a postoperative relapse, among which 34 cases (9.2%) recurred without medication, 31 cases (8.4%) recurred after one month of DG administration, and 7 cases (1.4%) relapsed after three months of DG administration. Univariate and multivariate analysis showed that surgical methods, 3 months of postoperative medication, PCOS, and polyp number and size were predictive factors for the recurrence of EPs.

EM was found in 193 cases (52.3%) in this study. Lin et al. [23] reported that the incidence of EPs in stages 1–4 EM was 42.44%, 40.69%, 55.89%, and 51.52%, respectively, which were similar to our cohort data. As both EM and EPs are estrogenic diseases, the correlation between their occurrence has always been a hotspot in clinical research. It has been reported that most infertile patients with EM are accompanied by EPs, suggesting that EM is related to the occurrence of EPs [24]. Evidence has shown that EM syndrome in infertile patients is positively correlated with the occurrence of EPs [25], indicating that there may be mutual promotion between the pathological changes of EM and EPs in such patients.

Hysteroscopic TCRP is recommended as the most ideal treatment for EPs, as well as the gold standard for surgical diagnosis and treatment of polyps, which can not only significantly reduce bleeding in patients but also eliminate malignant lesions through polyp biopsy. The methods of hysteroscopic TCRP include unipolar electrotomy, bipolar electrocoagulation system, endoscopic scissors or graspers, and uterine curettage devices. Hysteroscopic treatment of multiple EPs mainly includes hysteroscopic electrotomy and hysteroscopy positioning curettage of polyps. Compared with curettage, hysteroscopic electrotomy can effectively remove polyps in the base and surrounding tissues. In addition, the recurrence rate of EPs by curettage is high, mainly because simple curettage cannot remove the base of polyps [19]. In this study, the recurrence of EPs in patients undergoing hysteroscopic electrotomy was lower than that of patients with curettage, which is consistent with the literature [26]. However, the thermal damage of electrotomy cannot be ignored as electrosurgical resection of polyps may lead to endometrial damage and intrauterine adhesion, and there is currently no definite evidence for its applicability to patients with fertility requirements.

Obesity and PCOS are high-risk factors for recurrence [27]. Since EP is a hormone-dependent disease, its recurrence may be related to high estrogen levels in patients. The conversion of peripheral adipose to estrogen increases in obese patients, and adipose tissue can increase estrogen storage. Serhat et al. showed that obesity, an independent risk factor for EPs, was not associated with diabetes and hypertension [28]. Hyperandrogenism is present in PCOS patients. Androgens can be converted to estrogen through the peripheral adipose tissue, which can increase estrogen storage and lead to long-term high estrogen effect in the endometrium, resulting in the imbalance of estrogen receptor (ER) and progesterone receptor (PR) in the endometrium [29]. Due to the decrease of PR expression in EPs, the endometrium shows a reduced sensitivity to progesterone response and even no response to progesterone, which leads to excessive hyperplasia of local endometrium tissues. Under the continuous stimulation of estrogen, the local endometrium of PCOS patients experienced excessive hyperplasia due to long-term anovulation and lack of progesterone antagonism, resulting in the formation of EPs. In addition, there are not only ER and PR in the endometrium but also insulin receptors. The combination of insulin and receptors can promote endometrial hyperplasia, so the increase in insulin level plays an important part in endometrial dysplasia [30]. Obese and PCOS patients often have insulin resistance and chronic inflammation, thus increasing the risk of EP recurrence. The results of this study confirm once again that PCOS is a high-risk factor for EPs. On the other hand, Gu et al. [21] also pointed out in their study that the risk of EP recurrence in patients with multiple EPs was about three times higher than that in patients with a single EP, suggesting that the number of EPs was an independent risk factor for EP recurrence, which was consistent with our research results. The novelty of this study and the contribution to the subject area is that by analyzing the clinical data of the included EPs patients who failed IVF-ET treatment, it was confirmed that PCOS, multiple polyps, polyp diameter ≥ 2 cm, curettage, and no postoperative medication were all risk factors for the recurrence of EPs, providing new references and predictive factors for the postoperative management and recurrence prevention of EP patients with IVF-ET failure. This study still needs to be improved on the following aspects. First, since this is a single-center study, there may be inevitable information

bias. Second, the number and size of polyps and other factors were not further subdivided to analyze the influence of these factors on postoperative recurrence of EPs after IVF treatment failure. Last, potential risk factors such as estrogen stimulation and inflammation were not included to verify their role in the postoperative recurrence of EPs after failed IVF treatment. In the future, this research will be gradually improved from the above perspectives.

5. Conclusion

EPs are a kind of most common endometrial lesions in infertile women. At present, most studies suggest that EPs have negative impacts on fertility through mechanical interference and inflammatory stress. For infertile women, hysteroscopic polypectomy can improve the pregnancy rate of both natural pregnancy and assisted reproduction. However, due to the lack of research in this area and low data quality, it is still controversial whether polypectomy should be regarded as a standard treatment before assisted reproduction. Postoperative recurrence of EPs is a difficulty in clinical management. However, as its etiology and mechanism are still unclear, the means to prevent recurrence and the treatment after recurrence need more evidence-based medical evidence. We will design a series of rigorous randomized controlled studies, as well as more translational and basic research, as soon as possible to provide a more convincing theoretical and practical basis for the clinical treatment of EPs.

Data Availability

The labeled datasets used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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Retraction

Retracted: Elevated Plasma Interleukin-35 as a Prognostic Indicator in Localized Clear Cell Renal Cell Carcinoma

Evidence-Based Complementary and Alternative Medicine

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This article has been retracted by Hindawi following an investigation undertaken by the publisher [1]. This investigation has uncovered evidence of one or more of the following indicators of systematic manipulation of the publication process:

(1) Discrepancies in scope
(2) Discrepancies in the description of the research reported
(3) Discrepancies between the availability of data and the research described
(4) Inappropriate citations
(5) Incoherent, meaningless and/or irrelevant content included in the article
(6) Peer-review manipulation

The presence of these indicators undermines our confidence in the integrity of the article's content and we cannot, therefore, vouch for its reliability. Please note that this notice is intended solely to alert readers that the content of this article is unreliable. We have not investigated whether authors were aware of or involved in the systematic manipulation of the publication process.

In addition, our investigation has also shown that one or more of the following human-subject reporting requirements has not been met in this article: ethical approval by an Institutional Review Board (IRB) committee or equivalent, patient/participant consent to participate, and/or agreement to publish patient/participant details (where relevant).

Wiley and Hindawi regrets that the usual quality checks did not identify these issues before publication and have since put additional measures in place to safeguard research integrity.

We wish to credit our own Research Integrity and Research Publishing teams and anonymous and named external researchers and research integrity experts for contributing to this investigation.

The corresponding author, as the representative of all authors, has been given the opportunity to register their agreement or disagreement to this retraction. We have kept a record of any response received.

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[1] J. Zhang, X. Xu, Z. Chen, Z. Zhu, and J. Hou, "Elevated Plasma Interleukin-35 as a Prognostic Indicator in Localized Clear Cell Renal Cell Carcinoma," *Evidence-Based Complementary and Alternative Medicine*, vol. 2022, Article ID 6886590, 9 pages, 2022.

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Research Article

Elevated Plasma Interleukin-35 as a Prognostic Indicator in Localized Clear Cell Renal Cell Carcinoma

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Purpose. The aim of the study is to investigate the prognostic value of plasma interleukin-35 in the surgical treatment of patients with clear cell renal cell carcinoma (ccRCC). *Material and Methods*. Plasma IL-35 levels were measured in patients with ccRCC. The cut-off value of IL-35 was determined by the receiver operating characteristic (ROC) analysis and the area under the curve (AUC). The effects of the IL-35 and other clinicopathological characteristics on overall survival (OS) and progression-free survival (PFS) were evaluated using the univariate and multivariate logistic regression analysis. *Result*. Sixty-four ccRCC patients admitted to the urology department at the First Affiliated Hospital of Soochow University were selected, of whom 50 were diagnosed with localized ccRCC. Plasma interleukin-35 levels were significantly higher in patients with ccRCC than that in healthy controls. The cut-off value of IL-35 was 99.7 pg/mL. Multivariate analysis selected by univariate analyses demonstrated that the preoperative IL-35 was an independent prognostic factor for 5-year OS (OR: 1.02, 95% CI: 1.01 to 1.04, $p < 0.0001$) and 5-year PFS (OR: 1.02, 95% CI: 1.00 to 1.03, $p = 0.011$) in all patients with localized ccRCC. *Conclusion*. Current results indicate that preoperative IL-35 is an independent prognostic marker for OS and RFS in patients with localized ccRCC after surgery.

1. Introduction

With the aging of the population and advances in imaging techniques, the diagnosis of clear cell renal cell carcinoma (ccRCC) is on the rise [1]. About 70% of patients have localized or focal renal cell carcinoma (RCC), and unfortunately, 20–40% of patients develop recurrence and metastasis [2]. Despite advances in RCC treatment, nephrectomy remains the primary treatment [3], with a significant number of patients (20–30%) relapsing and dying after curative resection with RCC [4]. Therefore, accurate risk stratification at diagnosis is key to ensuring optimal treatment strategies for RCC patients.

Over the past decade, several prognostic factors have been proposed for RCC, including inflammatory biomarkers [5]. Growing evidence supports the involvement of systemic nutrition and inflammation in cancer progression. The systemic inflammatory response is strongly associated with nutritional decline, and these are increasingly considered predictors.

Interleukin-35 (IL-35) is a novel immunosuppressive cytokine, a heterodimer protein consisting of an IL-12α chain and an IL-27β chain, encoded by IL-12p35 and Epstein–Barr virus-induced gene 3 (EBI3) genes, respectively [6]. IL-35 signals through a unique heterodimer of receptor chains IL-12Rβ2 and gp130 or the homodimers of each chain in target cells [7]. IL-35 was initially shown to be secreted primarily by CD4 + CD25 + Foxp3 + regulatory T cells (Tregs), essential for Treg mediated immune suppression. IL-35 suppresses immune response by regulating T cell' expansion and inhibiting the development and response of Th1, Th2, and Th17 cells [8, 9].

Recent evidence suggests that interleukin-35 plays an important role in tumor development, pathogenesis, progression, and prognosis, including pancreatic carcinoma, colorectal carcinoma, renal cell carcinoma, and laryngeal squamous cell carcinoma [10–13]. In addition, Wang et al. have reported that tumor-derived IL-35 promotes tumor growth by inducing CD11b + cell accumulation in the tumor

microenvironment [14]. This evidence suggests that interleukin-35 is a novel anti-inflammatory cytokine that contributes to tumor development and metastasis. To the best of our knowledge, the expression patterns and functions of IL-35 in ccRCC have not been extensively studied. Therefore, we examined the expression of plasma IL-35 protein expression levels in the peripheral blood of patients with ccRCC to investigate the possible involvement of IL-35 in the progression.

2. Materials and Methods

2.1. Patients and Follow-Up. Patients admitted to the Department of Urology at the First Affiliated Hospital of Soochow University between March 1st, 2015, and March 30th, 2022, were retrieved. The criteria for study enrollment were as follows: patients with histologically confirmed ccRCC who were newly diagnosed, untreated, without a history of other tumors, and subsequently underwent radical or partial nephrectomy. Clinical stages were determined by computer tomography (CT), magnetic resonance imaging (MRI), ultrasound, and chest-X-ray, and other patient information was preoperatively recorded. Pathological review including Fuhrman nuclear grade as well as the 7th TNM classification of the UICC and AJCC guidelines of renal tumors were examined in all patients [15]. All patients were followed up clinically every 3 months (median 65 months; range 16–72 months), which was calculated from the day of surgery to the day of death or the last visit. This study was approved by the Ethics Committee of the First Affiliated Hospital of Soochow University and was carried out in accordance with the approved guidelines of the committee. Written informed consent has been obtained from all patients. The procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation and with the Helsinki Declaration of 1975.

2.2. Measurement of Plasma IL-35 Levels. Blood samples were collected in EDTA-K2 tubes and processed before surgery. Plasma was isolated from whole blood samples by two-step centrifugation (3000g for 10 mins and 12000g for 5 mins, both at 4°C). All plasma samples were frozen immediately after collection and stored at −80°C until analyzed. A commercial human IL-35 heterodimer ELISA kit (Biolegend, San Diego, CA, USA) was used to quantify levels of IL-35 in accordance with manufacturer agreements. All samples were analyzed in duplicate, and average concentrations were determined for each sample.

2.3. Statistical Analysis. Statistical analysis was performed using SPSS 23.0 (SPSS, Chicago, IL, USA). Numerical data were expressed in mean ± standard deviation (SD). Clinical characteristics of classified variables were examined with the Chi-square test, and continuous variables were examined with t test. For nonparametric data, the two groups were compared using the Mann–Whitney U test or the Kruskal–Wallis test. One-way ANOVA or Student's t test was used for parametric data. Kaplan–Meier survival curves were used to analyze relevant variables. The cut-off value of IL-35 was determined by the receiver operating characteristic (ROC) analysis and the area under the curve (AUC). IL-35 prediction was investigated using single-variable and multivariable logistic regression analysis. The results were then visualized by Hiplot, a science-based information analytics resource (https://hiplot.com.cn/). A p value<0.05 (2-sided) was statistically significant.

3. Results

Sixty-four ccRCC patients were admitted, of whom 50 were diagnosed with localized ccRCC. The clinicopathologic features of all localized patients are shown in Table 1. There were 54 healthy people enrolled in the healthy control (HC) group.

3.1. Plasma IL-35 Was Elevated in ccRCC Patients. A sandwich ELISA was used to examine the levels of IL-35 in the plasma of 54 healthy controls and 64 patients with ccRCC, out of whom 50 patients were diagnosed with localized ccRCC. The plasma IL-35 levels were 123.58 ± 73.51 pg/mL in the 64 ccRCC patients, as shown in Figure 1(a), which were twice as high as that in healthy controls 68.75 ± 31.94 pg/mL ($p < 0.0001$).

3.2. Correlations between the Levels of IL-35 and the Clinicopathologic Factors in Localized ccRCC Patients. The association between plasma IL-35 levels and clinicopathologic features of the patients with ccRCC was evaluated. The plasma IL-35 level among patients was elevated in advanced ccRCC (170.55 ± 52.57 pg/mL) than that in localized ccRCC (110.42 ± 61.96 pg/mL) ($p < 0.05$) (Figure 1(b)), and both were higher than that in the HC group (68.75 ± 31.94 pg/mL) ($p < 0.0001$). As the role of IL-35 has been studied in the detection of patients with RCC, but less so in localized ccRCC, which accounts for an increasing proportion, we investigated the relationship between IL-35 and the clinical features of localized ccRCC. Plasma IL-35 level defers among localized ccRCC patients with different nuclear grades (NG), with higher IL-35 level in NG (3-4) (141.82 ± 79.69 pg/mL) than that in NG (1-2) (92.76 ± 41.12 pg/mL) ($p < 0.05$, Figure 1(c)), suggesting that plasma IL-35 levels may be closely associated with the development and progression of ccRCC.

3.3. Cut-Off Value of the Parameters. Based on the AUC for survival in the ROC analysis (Figure 1(d)), the optimal cut-off value of IL-35 was 99.7 pg/mL. The group based on IL-35 in the study was subsequently defined as follows; patients with elevated IL-35 levels (>99.7 pg/mL) were assigned to group-high IL-35 ($n = 30$); the other patients were assigned to group-low IL-35 ($n = 20$).

3.4. Characteristics in All Localized Patients. Clinicopathological characteristics in all 50 localized patients with 60 months of the median follow-up time from surgery are shown in Table 1. As were shown, the 3-year OS

Table 1: Baseline characteristics of patients with localized RCC ($n = 50$) according to the IL-35.

Characteristics	Patients ($n = 50$)	Group 1 low IL-35 ($n = 30$)	Group 2 high IL-35 ($n = 20$)	p value two-side
OS-time	65.30 ± 12.84	69.10 ± 5.22	59.60 ± 18.06	0.0089*
PFS-time	61.58 ± 16.83	67.90 ± 8.01	52.10 ± 21.79	0.0007*
Age (mean ± SD)	56.82 ± 14.26	59.00 ± 12.19	53.55 ± 16.71	0.1885
≤65	39	23	16	0.78
>65	11	7	4	
Sex (male/female)	31/19	19/11	12/8	0.812
BMI (kg/m^2) (≤24/>24)	32/18	17/13	15/5	0.186
Hypertension (yes/no)	26/24	12/18	14/6	0.038*
Diabetes (yes/no)	6/44	4/26	2/18	0.722
Family history (yes/no)	3/47	1/29	2/18	0.331
Symptom (yes/no)	36/14	26/4	10/10	0.005*
Tumor size (cm) (mean ± SD)	4.21 ± 1.98	3.69 ± 1.22	5.01 ± 2.60	0.0203*
≤4 cm	25	17	8	0.248
>4 cm	25	13	12	
Surgical procedure (PN/RN)	14/36	9/21	5/15	0.700
T classification (I/II)	46/4	30/0	16/4	0.043*
Nuclear grade (1-2/3-4)	32/18	23/7	9/11	0.022*
Necrosis (yes/no)	3/47	0/30	3/17	0.114
IL-35	110.42 ± 61.96	73.28 ± 14.98	166.15 ± 64.12	< 0.0001*
3-year OS rate (%)	94.00% (47/50)	100.00% (30/30)	85.00% (17/20)	0.114
3-year PFS rate (%)	86.00% (43/50)	96.67% (29/30)	70.00% (14/20)	0.025*
5-year OS rate (%)	88.00% (44/50)	100.00% (30/30)	70.00% (14/20)	0.006*
5-year PFS rate (%)	76.00% (38/50)	90.00% (27/30)	55.00% (11/20)	0.012*

OS: overall survival; PFS: progression-free survival. BMI: body mass index. PN: partial nephrectomy; RN: radical nephrectomy. * $p < 0.05$.

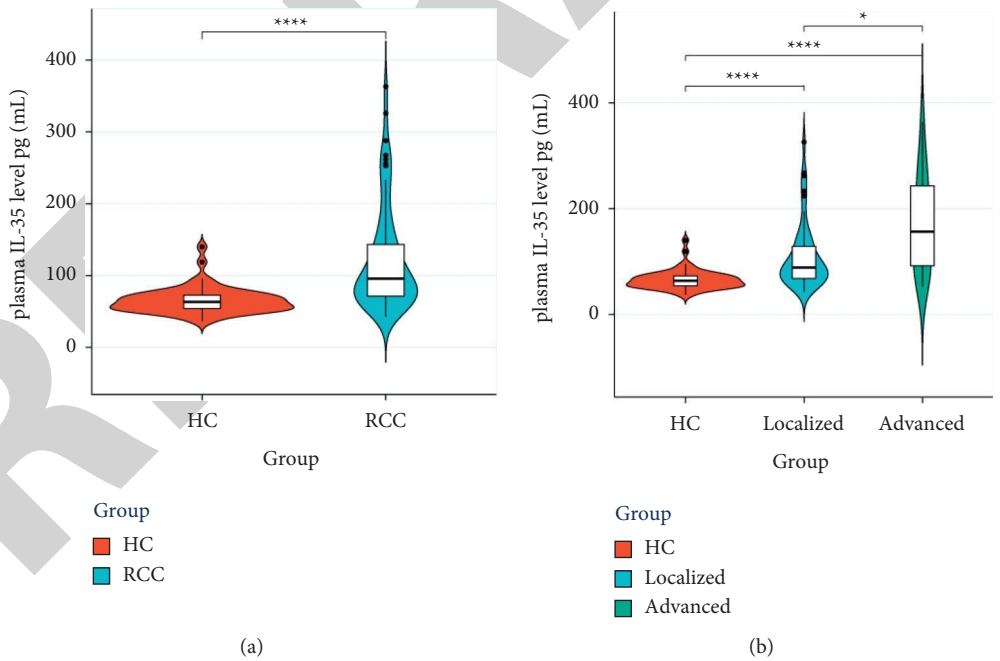

Figure 1: Continued.

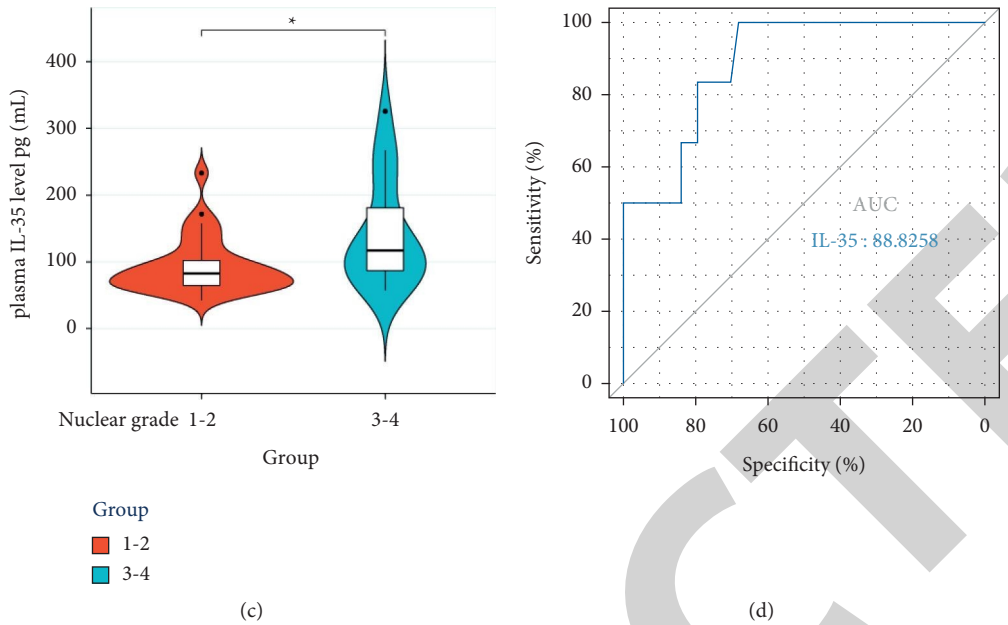

Figure 1: Plasma IL-35 level and clinicopathologic characteristics of patients with ccRCC. (a) Plasma IL-35 level among patients with ccRCC and the HC group. $^{****}P < 0.0001$. (b) Plasma IL-35 level among patients with either advanced ccRCC or localized ccRCC and the HC group. $^{*}P < 0.05$ and $^{****}P < 0.0001$. (c) Plasma IL-35 concentration among of patients with different nuclear grades. $^{*}P < 0.05$. (d) AUC for overall survival in the ROC analysis of plasma IL-35 level, AUC was 88.83%. IL-35, interleukin-35; ccRCC, clear cell renal cell carcinoma; HC; healthy control.

and PFS rates were 94.0% and 86.0% and the 5-year OS and PFS rates were 88.0% and 76.0%, respectively. Grouped according to the level of IL-35, 30 patients were assigned to the low IL-35 group and 20 patients to the high IL-35 group, respectively. The distribution of characteristics was significantly different in hypertension (Yes/No) ($p = 0.038^{*}$), symptom (Yes/No) ($p = 0.005^{*}$), tumor size (cm) (mean ± SD) ($p = 0.0203^{*}$), T classification (I/II) ($p = 0.043^{*}$), and nuclear grade (1-2/3-4) ($p = 0.022^{*}$). The 3-year OS rate was 100% in the low IL-35 group vs. 85.0% in the high IL-35 group, respectively ($p = 0.114$), and the 5-year OS rate was 100% vs. 70.0% ($p = 0.006^{*}$). The 3-year PFS rate was 96.67% vs. 70% ($p = 0.025^{*}$), and the 5-year PFS rate was 90.0% vs. 55.0% ($p = 0.012^{*}$). This led us to a question whether IL-35 is closely associated with ccRCC survival and prognosis.

The Kaplan–Meier estimates demonstrated that the higher IL-35 was significantly associated with shorter 3-year OS (log-rank test: $p = 0.029^{*}$) (Figure 2(a)), and shorter 3-year PFS (log-rank test: $p = 0.0072^{*}$) (Figure 2(b)). During follow-up, a total of 12 patients experienced tumor progression after surgery with a median time of 36 months, and 6 patients died with a median time of 39 months. The Kaplan-Meier estimates demonstrated that the increasing IL-35 was also well-correlated with a shorter 5-year OS (log-rank test: $p = 0.0082^{*}$) (Figure 3(a)), and a shorter 5-year PFS (log-rank test: $p = 0.015^{*}$) (Figure 3(b)).

3.5. Logistics Regression Analysis for OS and PFS. To assess the predictive value of OS and PFS, univariate and multivariate analyses were performed (Table 2). Univariate analysis identified several variables significantly associated with OS including symptom (Yes/No) (OR: 6.80, 95% CI: 1.08–42.73, $p = 0.041^{*}$), tumor size (cm) (OR: 1.55, 95% CI: 1.04–2.31, $p = 0.030^{*}$), nuclear grade (1-2/3-4) (OR: 11.92, 95% CI: 1.26–112.29, $p = 0.030^{*}$), and IL-35 (OR: 1.02, 95% CI: 1.01–1.04, $p = 0.003^{*}$). In multivariate analysis, adjusting for those variables exhibited significant associations with univariate analysis, and tumor size (cm) (OR: 1.59, 95% CI: 1.01–2.50, $p = 0.046^{*}$) and IL-35 (OR: 1.02, 95% CI: 1.01–1.04, $p < 0.0001^{*}$) still remained as significant predictors for OS.

Since the present study was originally designated for patients who had no metastasis at the time of surgery, we then assessed the prognostic value for PFS (Table 3). Univariate analysis identified several variables significantly associated with PFS including symptom (Yes/No) (OR: 10.67, 95% CI: 2.42–47.02, $p = 0.002^{*}$), tumor size (cm) (OR: 1.46, 95% CI: 1.03–2.07, $p = 0.034^{*}$) and IL-35 (OR: 1.02, 95% CI: 1.00–1.03, $p = 0.012^{*}$). In multivariate analysis, adjusting for those variables that exhibited significant associations in univariate analysis, 2 variables, including symptom (Yes/No) (OR: 10.67, 95% CI: 2.42–47.02, $p = 0.002^{*}$) and IL-35 (OR: 1.02, 95% CI: 1.00–1.03, $p = 0.011^{*}$) remained as significant predictors for PFS.

3.6. Predictive Value of IL-35. To evaluate the predictive value of IL-35, prognostic nomograms for 5-year death risk (Figure 4(a)) along with 5-year progression risk (Figure 4(b)) were established. The corresponding score of each variable can be obtained by projecting to the top "points" axis according to the patient's actual situation. In the same way,

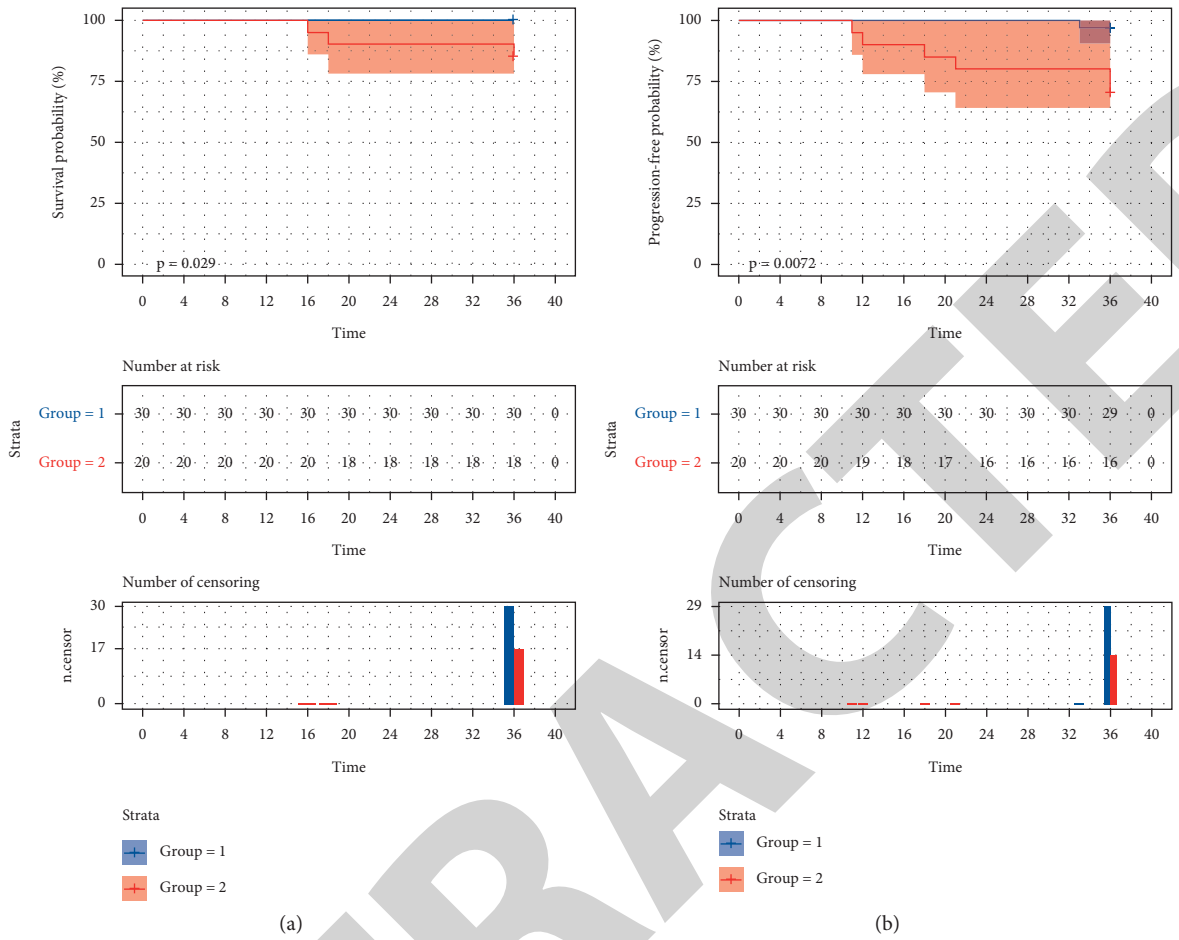

Figure 2: Kaplan–Meier curves of 3-year OS and PFS. (a): Kaplan–Meier curves of 3-year OS according to the IL-35 in localized patients ($p = 0.029^*$). (b): Kaplan–Meier curves of 3-year PFS according to the IL-35 in localized patients ($p = 0.0072^*$). Group 1 (low IL-35), group 2 (high IL-35).

the total points are obtained by adding the corresponding scores of each variable. By projecting the total points to the bottom "5-year dead risk" and "5-year progression" axis, the 5-year dead risk and progression risk can be estimated (Figures 4(a) and 4(b)).

For example, as shown in Figure 4(a), patients diagnosed with localized ccRCC had a symptom (40 points), tumor size = 10 cm (16 points), nuclear grade = 1 (0 points), and plasma IL-35 level 150 pg/mL (45 points), for a total of 101 points, meaning a predicted 5-year dead risk of 20.0%. In Figure 4(b), this patient had a total of 102 points, meaning a predicted 5-year progression risk of 65.0%.

4. Discussion

Interleukins have been mostly used as indicators of inflammation, infections, or hypoxic injuries [16–21]. IL-35 is a newly discovered suppressive cytokine secreted by regulatory T cells (Tregs) and may have therapeutic potential in several inflammatory disorders, including autoimmune diseases and allograft rejection [22, 23]. Recent studies have demonstrated that IL-35 expression is strongly associated with the development, progression, and prognosis of multiple tumors. However, the relationship between IL-35 and the progression of ccRCC is poorly understood, especially in localized ccRCC patients.

In the last decade, an association between preoperative systemic inflammatory response and a poorer postoperative survival has been reported. Accumulated evidence has demonstrated that the systemic inflammatory biomarkers including NLR, dNLR, PLR, CRP, GPS, and mGPS represent independent prognostic factors for various types of cancer, including RCC [24–26]. In addition, the present study indicated that elevated IL-35 was significantly associated with poor prognosis in ccRCC patients, who underwent curative nephrectomy, and showed that the increasing IL-35 was significantly associated with shorter OS and PFS. The results demonstrated that IL-35 is an independent prognostic factor for patients with ccRCC after nephrectomy. By now, several studies have shown a relationship between IL-35 and prognosis in patients with various types of cancers, suggesting its prognostic value [10, 12, 14, 27]. Jin et al. reported that circulating IL-35 was significantly increased in pancreatic ductal adenocarcinoma patients [12]. In colorectal cancer, Zeng et al. reported a high expression of IL-35 in CRC tissues [10]. In addition, Gu et al. showed that, in NSCLC patients,

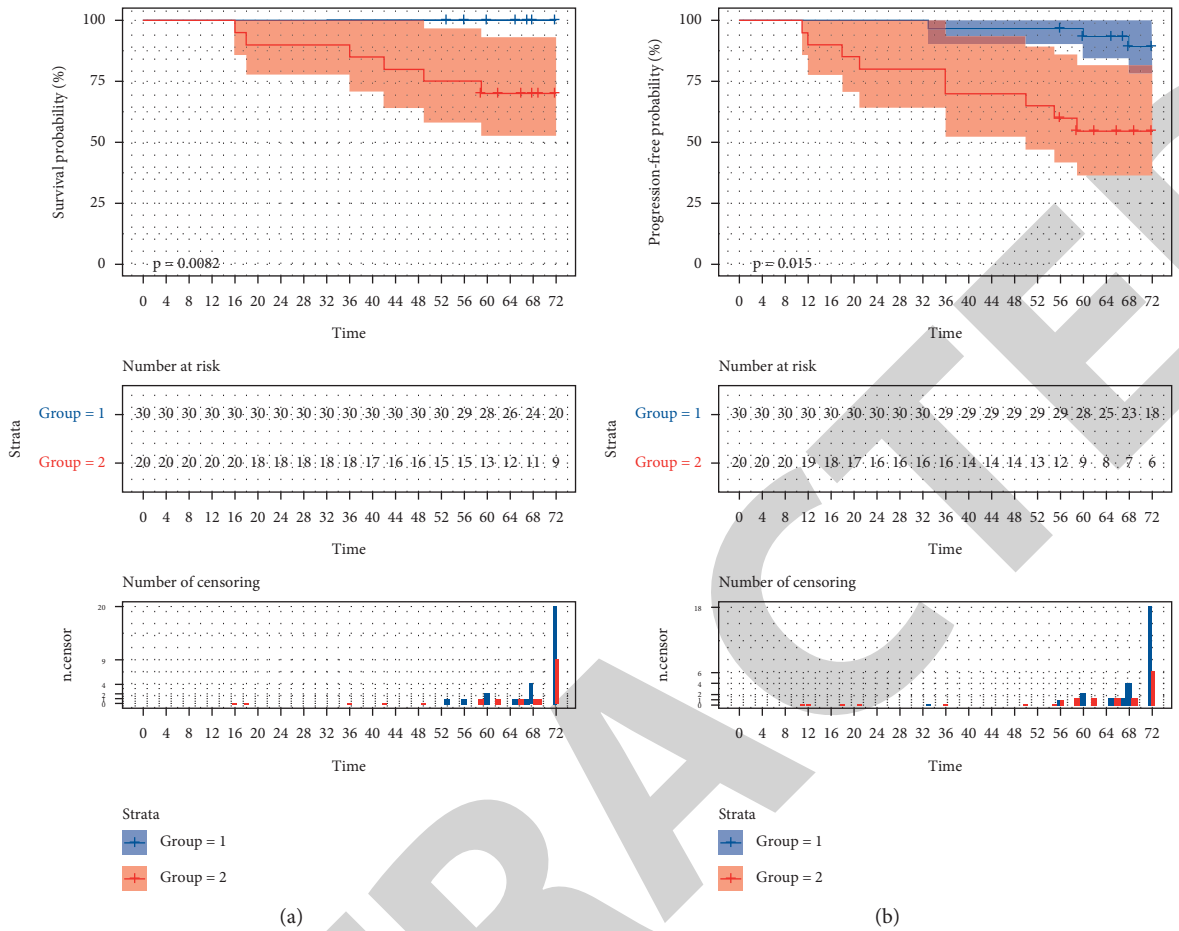

Figure 3: Kaplan–Meier curves of 5-year OS and PFS. (a): Kaplan–Meier curves of 5-year OS according to the IL-35 in localized patients ($p = 0.0082^*$). (b): Kaplan–Meier curves of 5-year PFS according to the IL-35 in localized patients ($p = p = 0.015^*$). Group 1 (low IL-35), group 2 (high IL-35).

Table 2: Univariate and multivariate analysis for OS in localized patients.

Characteristics	*OS*			
	Univariate		*Multivariate*	
	OR (95% CI)	*p* value	OR (95% CI)	*p* value
Age (≤65/>65)	0.99 (0.94–1.06)	0.928		
Sex (male/female)	0.79 (0.13–4.82)	0.902		
BMI (kg/m^2) (≤24/>24)	0.86 (0.14–5.32)	0.885		
Hypertension (yes/no)	2.00 (0.331–12.07)	0.450		
Family history (yes/no)	4.20 (0.32–55.06)	0.274		
Symptom (yes/no)	6.80 (1.08–42.73)	0.041*	2.30 (1.23–7.32)	0.164
Tumor size (cm)	1.55 (1.04–2.31)	0.030*	1.59 (1.01–2.50)	0.046*
Surgical procedure (PN/RN)	0.75 (0.12–4.64)	0.757		
T classification (I/II)	2.73 (0.23–31.56)	0.42		
Nuclear grade (1-2/3-4)	11.92 (1.26–112.29)	0.030*	4.30 (0.81–22.99)	0.088
Necrosis (present/absent)	4.20 (0.32–55.06)	0.274		
IL-35	1.02 (1.01–1.04)	0.003*	1.02 (1.01–1.04)	< 0.0001*

OS: overall survival, OR: odds ratio, CI: confidence interval, BMI: body mass index. PN: partial nephrectomy; RN: radical nephrectomy $^*p < 0.05$.

the plasma levels of IL-35 were significantly higher than in healthy volunteers [27]. In the tumor microenvironment, other than Tregs, tumor-infiltrating dendritic cells (DCs) and tumor cells are also considered to be the primary IL-35 producers [11, 28]. In addition, IL-35 has been found to potently inhibit antitumor T cell responses, and thereby, promotes tumor development [8]. While these findings are important and exciting, the exact impact of IL-35 on human ccRCC progress and metastasis is yet to be fully addressed.

Table 3: Univariate and multivariate analysis for PFS in localized patients.

Characteristics	PFS			
	Univariate		*Multivariate*	
	OR (95% CI)	p value	OR (95% CI)	p value
Age (≤65/>65)	0.99 (0.95–1.04)	0.676		
Sex (male/female)	1.22 (0.33–4.60)	0.764		
BMI (kg/m^2) (≤24/>24)	1.37 (0.36–5.19)	0.640		
Hypertension (yes/no)	1.40 (0.38–5.20)	0.615		
Family history (yes/no)	7.40 (0.61–90.15)	0.117		
Symptom (yes/no)	10.67 (2.42–47.02)	0.002*	10.67 (2.42–47.02)	0.002*
Tumor size (cm)	1.46 (1.03–2.07)	0.034*	2.04 (0.92–4.49)	0.079
Surgical procedure (PN/RN)	1.22 (0.27–5.38)	0.791		
T classification (I/II)	1.06 (0.10–11.26)	0.961		
Nuclear grade (1-2/3-4)	3.44 (0.89–13.19)	0.072		
Necrosis (present/absent)	1.63 (0.14–19.81)	0.699		
IL-35	1.02 (1.00–1.03)	0.012*	1.02 (1.00–1.03)	0.011*

PFS: progression-free survival, OR: odds ratio, CI: confidence interval, BMI: body mass index. PN: partial nephrectomy; RN: radical nephrectomy * $p < 0.05$.

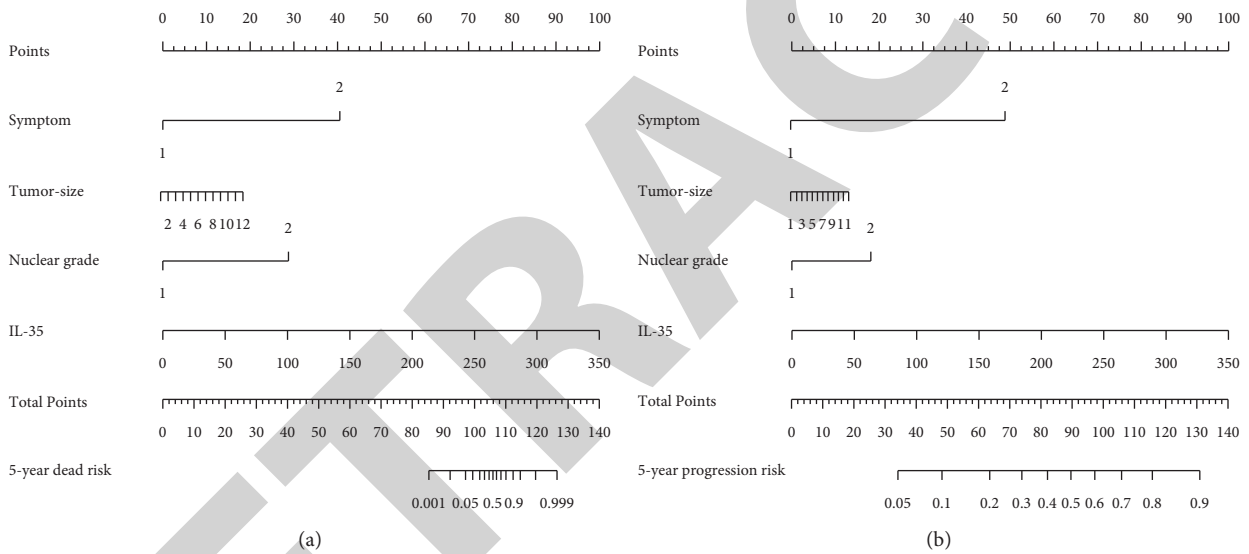

Figure 4: Nomogram for predicting 5-year death risk and progression risk of localized ccRCC patients. (a): Nomogram for predicting 5-year death risk of localized ccRCC patients. (b): Nomogram for predicting 5-year progression risk of localized ccRCC patients.

In the present cohort, the proportion of patients with high IL-35 was 40%, whereas those patients ultimately had a worse prognosis. Kaplan–Meier estimates illustrated significant differences between two groups on OS (5-year OS rate of 100% vs. 70%), and PFS (3-year PFS rate of 96.67% vs. 70%; 5-year PFS rate of 90% vs. 55%). Multivariate analysis revealed that high-plasma-IL-35 was an independent predictor for the lethality and recurrent progression beyond the other major factors, including T classification, tumor size, and nuclear grade. These data suggest that for a multimodal therapeutic approach in addition to conventional curative nephrectomy might be considered in patients with high preoperation plasma IL-35 levels (>99.7 pg/mL). The plasma IL-35 level has the advantage of identifying these patients preoperatively.

Furthermore, the performance of the constructed nomogram was comprehensively evaluated. The results suggested that the established nomogram might be utilized as a powerful and conventional tool to predict survival outcomes for patients with IL-35. To our knowledge, this is the first study to visualize the IL-35 prediction model for localized ccRCC, and our nomogram involved some distinct variables, such as IL-35, tumor size, and symptoms, which were also reported to be important predictors of prognosis.

The limitations of our study include its retrospective, single-institution design, and the small sample size. In addition, other putative patient statuses, such as diabetes mellitus, cardiovascular disease, and smoking [2, 5, 29, 30], which have been shown to be prognostic factors for RCC patients, were not examined in the current study. Larger prospective randomized controlled trials are needed to confirm our preliminary findings.

Data Availability

The data used to support the findings of this study will be available by contacting the corresponding author.

Disclosure

Jun Zhang, Xiaojian Xu, and Zongxin Chen are the co-first authors of this article.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Jun Zhang, Xiao-jian Xu, and Zong-xin Chen conceived and designed the present study. Jun Zhang performed the experiments. Jun Zhang and Xiao-jian Xu wrote the manuscript. Zong-xin Chen and Zheng-yu Zhu had a postoperative follow-up visit. Jian-quan Hou reviewed and edited the manuscript. All authors read and approved the final manuscript. Jun Zhang, Xiaojian Xu, and Zongxin Chen contributed equally to this study.

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Hindawi
Evidence-Based Complementary and Alternative Medicine
Volume 2023, Article ID 9754582, 1 page
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Retraction

Retracted: Feasibility Analysis of 3D Printing-Assisted Pedicle Screw Correction Surgery for Degenerative Scoliosis

Evidence-Based Complementary and Alternative Medicine

Received 20 June 2023; Accepted 20 June 2023; Published 21 June 2023

This article has been retracted by Hindawi following an investigation undertaken by the publisher [1]. This investigation has uncovered evidence of one or more of the following indicators of systematic manipulation of the publication process:

(1) Discrepancies in scope
(2) Discrepancies in the description of the research reported
(3) Discrepancies between the availability of data and the research described
(4) Inappropriate citations
(5) Incoherent, meaningless and/or irrelevant content included in the article
(6) Peer-review manipulation

The presence of these indicators undermines our confidence in the integrity of the article's content and we cannot, therefore, vouch for its reliability. Please note that this notice is intended solely to alert readers that the content of this article is unreliable. We have not investigated whether authors were aware of or involved in the systematic manipulation of the publication process.

In addition, our investigation has also shown that one or more of the following human-subject reporting requirements has not been met in this article: ethical approval by an Institutional Review Board (IRB) committee or equivalent, patient/participant consent to participate, and/or agreement to publish patient/participant details (where relevant).

Wiley and Hindawi regrets that the usual quality checks did not identify these issues before publication and have since put additional measures in place to safeguard research integrity.

We wish to credit our own Research Integrity and Research Publishing teams and anonymous and named external researchers and research integrity experts for contributing to this investigation.

The corresponding author, as the representative of all authors, has been given the opportunity to register their agreement or disagreement to this retraction. We have kept a record of any response received.

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[1] X. Chen, X. Gao, F. Zheng, and H. Lin, "Feasibility Analysis of 3D Printing-Assisted Pedicle Screw Correction Surgery for Degenerative Scoliosis," *Evidence-Based Complementary and Alternative Medicine*, vol. 2022, Article ID 4069778, 5 pages, 2022.

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Research Article

Feasibility Analysis of 3D Printing-Assisted Pedicle Screw Correction Surgery for Degenerative Scoliosis

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Objective. To explore the feasibility of 3D printing-assisted pedicle screw correction surgery for degenerative scoliosis. *Methods*. From January 1, 2015 to January 31, 2020, patients with degenerative scoliosis who received corrective surgery in our hospital were retrospectively analyzed. Patients were grouped based on the fixation methods. Patients in the control group received traditional pedicle screw internal fixation, while those in the study group received 3D printing-assisted pedicle screw fixation. The therapeutic effects were compared. *Results*. There were 78 cases in the control group and 82 cases in the study group. There were no significant differences in scoliotic Cobb's angle, pelvic incidence-lumbar lordosis (PI-LL), VAS score, JOA score, social function, physical function, role function, or cognitive function between the study group and the control group before the surgery, but there were differences in the above parameters between the two groups after surgery. The incidence of postoperative complications in the study group was also significantly lower in the study group. *Conclusion*. 3D printing-assisted pedicle screw correction surgery provides a strong 3D correction force with reliable effect and fewer complications, and is a good treatment choice for degenerative scoliosis.

1. Introduction

In degenerative scoliosis, the deformity of the spine is three-dimensional (3D) and rotational due to the progression of the lesions in the vertebrates. The Cobb angle is characteristically over 10°. The lesions initiate as asymmetric degeneration in the disc and joints, producing asymmetric loading and subsequent deformity. The symptoms include pain with neurological deficits. Nonsurgical treatment includes medication and physical therapy. Alternatively, epidural injections for selective nerve root blockade may be used [1, 2]. In patients with uncontrollable pain or neurological deficits, the purpose of surgery is to decompress neurons by repairing, modifying 3D deformities, and stabilizing vertebrate balance. This study investigated the feasibility of 3D pedicle screw correction surgery for degenerative scoliosis.

2. Materials and Methods

This study included patients who received degenerative scoliosis and correction surgery at our hospital from January 1, 2015, to January 31, 2020. The ethics committee of our hospital approved this study. All included patients and their families were informed of the study and actively signed informed consent. The inclusion criteria were: (1) Clinically confirmed degenerative scoliosis; (2) corrective surgery required; and (3) normal cardiopulmonary function. The exclusion criteria were: malignant disease, mental illness, drug allergy, any previous treatment of degenerative scoliosis with surgeries or medications, chronic diseases that need long-term medication such as hypertension, diabetes, etc., or lost-to-follow-up.

2.1. Methods. All patients received frontal and lateral X-ray photography of the whole spine before surgery for determination of the patient's scoliosis and rotation; left and right refractive photography and suspension traction photography were used to determine the patient's spine elasticity as well as the largest orthopedic angle of the patient during the operation. MRI scans and CT scans of the spine were performed before the operation to determine whether there were lesions with or without neural structures such as spinal

Table 1: Observation Indicators in the present study.

VAS score [4]	0 is the best, the patient has no pain
	1–3 points or less is mild pain that can be tolerated
	4–6 points: The pain is more severe, and the patient's sleep is slightly affected
	7–10 points are unbearable severe pain, the patient cannot sleep, which seriously affects normal life
JOA score [5]	Subjective symptoms
	Clinical signs
	Limitation of daily activities
	The total score is 29 points, a higher score represents a better function
Quality of life score [6]	Cognitive
	Role
	Social
	Physical
	The total score is 100 points. A higher score represents a better life quality

cord cavities and cauda equina and to determine the actual rotation angle of the pedicle of the rotational deformity. The minimum diameter of the pedicle lays the foundation for the determination of intraoperative positioning and screw insertion angle. At the same time, pulmonary function tests, somatosensory evoked potentials, and action evoked potentials were performed to determine whether there was damage to other organs. Before the operation, conventional spinal traction therapy was used to relax the small joints of the cervical spine, and the adaptability of the nerves in each group to stretching was observed. Patients in the control group received traditional pedicle screw internal fixation. Patients in the study group were treated with 3D printing-assisted pedicle screws for 3D fixation [3].

2.2. Observation Indicators. The visual analog scale (VAS) score, Japanese Orthopaedic Association Evaluation Treatment (JOA) score, quality of life score, scoliosis Cobb's angle, pelvic incidence-lumbar lordosis (PI-LL, Table 1), and postoperative complications were compared between the two groups.

2.3. Statistics. The data in this experiment were analyzed by SPSS21.0 (SPSS, Chicago, IL, USA), in which the count data (n, %) were analyzed by $\chi 2$ test, and the measurement data ($\bar{x} \pm$ SD) were analyzed by t-test. $P < 0.05$ (two-sided) represents statistical significance.

3. Results

3.1. Baseline Data of Patients. A total of 160 eligible patients (78 and 82 in the control group and the study group, respectively) were retrieved. The average age of patients in the control group was (56.96 ± 5.02) years old, and that in the study group was (56.51 ± 6.36) years old. There were no differences in gender, age, and past medical history between the two groups ($P > 0.05$ for all comparisons). The clinical characteristics of the patients were shown in Table 2.

3.2. Comparison of Cobb's Angle, Pelvic Projection Angle, and PI-LL before and after Surgery. There was no significant difference in scoliotic Cobb's angle (20.58 ± 2.03 vs 20.47 ± 2.10) or PI-LL (35.51 ± 0.56 vs 35.23 ± 0.49) between the study group and the control group before surgery ($p = 0.564$ and 0.826, respectively). After surgery, Cobb's angle of scoliosis (5.10 ± 0.32) and PI-LL (13.92 ± 0.37) in the study group were lower than those before surgery ($P < 0.05$, Table 3).

Table 2: General information of included patients.

General information	Study	Control	$t/\chi 2$	P
n	82	78		
Age	56.51 ± 6.36	56.96 ± 5.02	1.812	0.074
Gender			0.202	0.653
Male	44	40		
Female	38	38		
Nationality			1.566	0.211
Han	68	70		
Others	14	8		
Congenital spine disease			0.201	0.654
Yes	0	0		
No	82	78		

Table 3: Comparison of scoliotic Cobb's angle and PI-LL between two groups.

Group	Scoliosis Cobb's angle		PI-LL	
	Before	After	Before	After
Study ($n = 82$)	20.58 ± 2.03	5.10 ± 0.32	35.51 ± 0.56	13.92 ± 0.37
Control ($n = 78$)	20.47 ± 2.10	5.91 ± 0.43	35.23 ± 0.49	13.10 ± 0.33
t	1.673	12.274	1.538	8.379
P	0.564	0.005	0.826	<0.001

3.3. Comparison of VAS Score and JOA Score before and after Surgery. No significant differences existed in the VAS score (7.58 ± 2.13 vs 7.47 ± 2.25) or JOA score (12.57 ± 0.38 vs 12.26 ± 0.50) between the study group and the control group ($p = 0.564$ and 0.826, respectively) before surgery. After surgery, there were significant differences in the VAS score (3.10 ± 0.18 vs 4.41 ± 0.56) and JOA score (23.42 ± 0.36 vs 19.50 ± 0.51) between the study group and the control group ($P = 0.005$ and $P < 0.001$, respectively, Table 4).

3.4. Quality of Life Comparison. Before surgery, there were no significant differences in the social function (63.80 ± 3.62 vs 65.61 ± 2.80), physical function (64.23 ± 5.51 vs 64.35 ± 5.08),

Table 4: VAS scores and JOA scores comparisons.

Group	VAS score		JOA score	
	Before	After	Before	After
Study ($n = 82$)	7.58 ± 2.13	3.10 ± 0.18	12.57 ± 0.38	23.42 ± 0.36
Control ($n = 78$)	7.47 ± 2.25	4.41 ± 0.56	12.26 ± 0.50	19.50 ± 0.51
t	1.673	12.274	1.538	8.379
P	0.564	0.005	0.826	<0.001

Table 5: Quality of life comparison.

Group	Social function		Physical function		Role function		Cognitive function	
	Before	After	Before	After	Before	After	Before	After
Control	65.61 ± 2.80	79.13 ± 3.20	64.35 ± 5.08	75.44 ± 4.26	66.53 ± 6.25	76.82 ± 6.34	64.33 ± 3.14	74.35 ± 3.52
Study	63.80 ± 3.62	82.84 ± 1.15	64.23 ± 5.51	84.50 ± 3.80	67.00 ± 6.02	83.30 ± 5.38	62.97 ± 4.28	80.61 ± 4.85
t	2.019	15.943	1.631	12.055	1.461	13.325	2.130	10.142
P	0.245	0.001	0.051	0.005	0.102	0.005	0.067	0.001

Table 6: Comparison of postoperative complications between the two groups of patients (n, %).

Group	Bleeding	Infect	Pulmonary embolism	Total incidence
Study ($n = 82$)	3 (3.66)	2 (2.44)	0 (0)	5 (6.10)
Control ($n = 78$)	4 (5.12)	3 (3.85)	2 (2.56)	9 (11.53)
χ^2				8.310
P				0.001

role function (67.00 ± 6.02 vs 66.53 ± 6.25), or cognitive function (62.97 ± 4.28 vs 64.33 ± 3.14) between the study group and the control group ($P = 0.245, 0.051, 0.102$, and 0.067, respectively). After surgery, there were significant differences in the social function (82.84 ± 1.15 vs 79.13 ± 3.20), physical function (84.50 ± 3.80 vs 75.44 ± 4.26), role function (83.30 ± 5.38 vs 76.82 ± 6.34), and cognitive function (80.61 ± 4.85 vs 74.35 ± 3.52) between the study group and the control group ($P = 0.001, 0.005, 0.005, 0.001$, respectively, Table 5).

3.5. Postoperative Complications Comparison. There was a significant difference in the total incidence of postoperative complications between the study group and the control group ($P = 0.001$, Table 6).

4. Discussion

Degenerative scoliosis occurs through degenerative changes without pre-existing spinal deformity, and it is generally more common in older age groups. It is presented with asymmetric disc space collapse and facet degeneration followed by lateral and/or rotational sliding [7]. This condition, which results in loss of lumbar lordosis as well as sagittal malalignment, will inevitably lead to poor clinical outcomes. Progressive low back pain as well as symptomatic lumbar spinal stenosis are more common in patients with new-onset scoliosis, along with neurogenic claudication or radiculopathy [8, 9]. Generally, symptoms such as progressive clinical deformity and sagittal imbalance will appear later in this group of patients. The treatment of such diseases is complex and based on different pathophysiological and clinical manifestations, with examples of physical therapy and analgesics for many years [10]. Surgical therapies include complex instrumental fusion or simple laminectomy, which are invasive and may have potential complications [11].

About 90% of those patients mainly present with pain, muscle fatigue, and spasticity of scoliosis causing diffuse and axial low back pain in most patients. Judging from the fluoroscopy-guided articular surface injection, all of these people have low back pain due to articular surface degeneration [12, 13]. The disease is characterized by asymmetric disc space and joint degeneration. In this group, there is "hip rib" pain and/or dysfunction due to sagittal imbalance, which is a chronic condition. In the clinical evaluation of such patients, the key is to correctly understand the causes of their pain. Degenerative scoliosis is a complex, multifactorial process that develops over time, so there are many possible etiologies. Pain may be related to major leg joint deformity progression and dysfunction, nerve damage, or degenerative arthritis.

Pedicle screw fixation is aided by 3D printing [14]. The three columns of the spine have a strong control force, and the internal fixation device is tightly mounted to the bone, thereby generating a strong correction force [15, 16]. Suk conducted a retrospective analysis of scoliosis using the pedicle screw technique [17] and found that compared with hook fixation, pedicle screws treated more main thoracic curvature cases (55.8% vs 51.7%). The incidence of postoperative defects was 5.7% and 10.6% in screw therapy and hook therapy, respectively. The postoperative lumbar curvature correction rate was 54.9% and 46.9% in the pedicle screw group and hook fixation group, respectively. In

addition, pedicle screws can increase the control of the apical vertebra with maximum rotational deformity, therefore reducing splaying and compression. Studies have shown that directly acting on the spine can not only achieve correction in the coronal plane and sagittal plane but also reconstruct normal anatomy in the transverse plane [18, 19]. However, the anatomy of the thoracic pedicle limits its widespread use. Rampersaud et al. established a morphological model using normal thoracic vertebrae specimens and showed that the implanted screws on the pedicle of the thoracic vertebra had only 1 mm of parallel displacement and 5° of angular offset. When scoliosis occurs in the spine, the angle and transverse diameter of the pedicle will inevitably change due to the rotation and wedge deformation of the vertebral body itself. Therefore, the preoperative image preparation work is to determine the actual angle of the pedicle, the minimum diameter of the pedicle, the relationship between the pedicle and the transverse process, and the distance between the needle insertion site and the anterior edge of the vertebral body for the correction operation. The selection of positioning, entry angle, and implant provides an important reference to improve the accuracy of screw placement [20]. Simultaneous intraoperative EMG and endoscopy are key to preventing screw penetration of the inner wall of the pedicle. In this study, the patients who underwent pedicle screw 3D correction surgery had better treatment effects and fewer postoperative complications, showing that pedicle screw 3D correction surgery had obvious advantages.

To sum up, 3D printing-assisted pedicle screw 3D correction surgery provides a powerful 3D correction force with reliable effect and few complications, and is effective in dealing with degenerative scoliosis.

Data Availability

The data used in this study is available from the corresponding author upon reasonable request.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

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Research Article

The Prognostic Value of Domain-Specific Cognitive Abilities Assessed by Chinese Version of Oxford Cognitive Screen on Determining ADLs Recovery in Patients with Post-Stroke Cognitive Impairment

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Background. Poststroke cognitive impairment (PSCI) has been increasingly recognized in patients. However, it remains unclear whether ADLs recovery is more susceptible to domain-specific cognitive abilities after a stroke. Therefore, the study was designed to investigate the cognitive functions of patients with PSCI at admission by using the Chinese (Putonghua) Version of the Oxford Cognitive Screen (OCS-P) as well as to identify the prognostic value of domain-specific cognitive abilities on the recovery of ADLs when discharged. *Methods*. A total of 153 hospitalized stroke patients were included in this prospective study. Cognitive function was assessed by OCS-P when participants were admitted to the hospital. The ADLs were measured at admission and discharge, and recovery was estimated by the improvement between admission and discharge. A diagnostic model using logistic regression was constructed to identify the prognostic value of domain-specific cognitive abilities for ADLs. The efficacy and accuracy of the diagnostic model were assessed by receiver operating characteristic (ROC) and Hosmer-Lemeshow's goodness of fit test. The diagnostic model was validated by 10-fold cross-validation and presented as a nomogram. *Results*. The score of OCS-P was 60(49.75, 69). The most frequently impaired cognitive domain was number writing (60.8%), followed by verbal memory (52.9%). Multivariate logistic regression showed executive dysfunction was a risk prognostic factor of ADLs recovery ($P < 0.001$, OR = 3.176 [95% CI, 1.218~8.278]). The ROC curve of the diagnostic model was 0.839, with a good diagnostic efficacy. Hosmer–Lemeshow test showed diagnostic model had good calibration ability ($\chi^2 = 8.939.3$, $P = 0.347 > 0.05$). The average error rate after adjustment of 10-fold cross-validation was 20.93%, within the acceptable range. *Conclusions*. Post-stroke patients generally suffered from multidimensional cognitive impairments. Executive dysfunction screened with OCS-P at clinical admission was a reliable and accessible predictive factor ADLs recovery in patients with PSCI. Early targeted rehabilitation programs are suggested to make them as earlier as possible, especially for those having executive dysfunction while hospitalized.

1. Introduction

Stroke is increasingly recognized as a major leading cause of disability, with high morbidity and mortality rates worldwide [1]. It's also known as a risk factor for cognitive impairment [2]. About 50% of stroke survivors have post-stroke cognitive impairment (PSCI) in the early stage [3], even in those with successful clinical recovery [4]. Up to 10% may develop dementia soon after the first stroke [5]. It directly interferes with patient's ability to perceive and adapt

to the external environment, leading to adverse functional outcomes including but not limited to poor ADLs [6].

It was well established that PSCI can negatively affect ADLs, which now being considered a critical rehabilitation outcome to ensure the quality of life after stroke [7, 8]. A previous study had demonstrated that the global cognitive ability screened by Montreal Cognitive Assessment Scale (MOCA) in the early stage was positively associated with ADLs when they were discharged [9] and can predict long-term neurological recovery, ADLs recovery, even the mortality after stroke [10]. Similarly, Minimal Mental State Examination (MMSE) scores on admission were also identified as a predictor of functional outcomes after stroke [11]. PSCI generally involves impairments in different cognitive domains, concerning cognitive neglect [12], apraxia [13], aphasia [14], abstract reasoning [15], and executive dysfunction [16]. However, it remains unclear whether ADLs recovery is more susceptible to domain-specific cognitive abilities and the results had not been consistent.

Besides, it is important to accurately detect cognitive impairments in stroke rehabilitation. Therefore, a valid measurement specific for the identification of cognitive deficits in post-stroke survivors is critical for effective stroke rehabilitation treatments. Whereas, the most used existing instruments reported in previous studies were MoCA and MMSE [17, 18], which were not specifically targeted for stroke individuals and consequently had certain limitations in evaluating PSCI. Indeed, several studies have reported the flaws of these instruments in assessments of PSCI. Emerging evidence has indicated that MMSE cannot sensitively identify the impairment of abstract reasoning, executive ability, visual perception, and construction ability after stroke [19]. Although MoCA was thought to be more sensitive than MMSE [20–22], it still lacks specificity when patients have common cognitive conflicts after stroke, such as visual impairment, visual neglect, aphasia, or dyslexia [6, 22, 23]. For instance, stroke survivors with aphasia are unable to finish non-verbal tests (like Memory) of MOCA or MMSE. Similarly, those with unilateral spatial neglect usually fail to pass the alternating trail-making test, which may affect the authenticity of the test and its accurate evaluation of functional recovery [21].

Conversely, the Oxford Cognitive Screen (OCS), specially designed according to the characteristics of stroke, is superior to other cognitive screening tools, including MoCA and MMSE [24], for it allows a more precise assessment for common special cognitive impairment domains of stroke, such as aphasia, hemiplegia and spatial neglect and reduce any confounds that may occur because of these often-co-occurring difficulties, whereas the latter cannot. The Chinese (Putonghua) OCS (OCS-P) was the first Chinese version revised in our preliminary study [25]. It was translated in accordance with the requirements of the Chinese language. Moreover, language (semantics/picture pointing, sentence reading, and picture naming subdomains) and memory (free recall and recognition of verbal memory subdomains) domains were modified to accommodate the specificity of Chinese culture, while maintaining their equivalence to the content of the original version. The results revealed that OCS-P has satisfactory content validity, substantive validity, construct validity, inter- and intra-rater reliability, and known group discrimination. It was recommended on the official website of the Oxford Cognitive Screening Scale as a standardized clinical instrument specifically designed for measuring cognitive deficits of Chinese post-stroke patients [26]. In this study, OCS-P was used to comprehensively assess the cognitive function of patients with PSCI.

PSCI has been increasingly recognized in patients and has been proved to be closely related to the recovery of ADLs. However, it's not well explored whether ADLs recovery is more susceptible to domain-specific cognitive abilities after stroke when screening with OCS-P. Furthermore, an early diagnosis of cognitive dysfunction after stroke may have a great significance for the formulation of effective rehabilitation programs on ADLs recovery. Therefore, the study was conducted to identify the prognostic value of domain-specific cognitive abilities assessed by OCS-P in determining the ADLs recovery in patients with PSCI.

2. Materials and Methods

2.1. Study Design and Participants. This was a longitudinal prospective and explorative study. Patients from two tertiary hospitals were consecutively enrolled in the study according to the following inclusion criteria: (1) aged ≥18 years; (2) diagnosed as stroke and confirmed by CT or MRI [27]; (3) within 2 months after the first stroke; (4) MoCA scores <26; (5) volunteer to participate and sign the informed consent. Those with one of the following situations were excluded: (1) having another stroke during a hospital stay; (2) with a history of traumatic brain injury or degenerative brain disease; (3) diagnosed with dementia; (4) being unconscious or having unstable vital signs; (5) unable to complete the evaluation due to severe aphasia or dysarthria. All participants received routine standard rehabilitation treatment during hospitalization and those who could not adhere to rehabilitation treatment were excluded.

2.2. OCS-P Measurement. Cognitive function was assessed by OCS-P. OCS is a first-line, stroke-specific, and domain-specific cognitive screening tool for the identification of PSCI. It was developed by Demeyere at the University of Oxford, following rigorous psychometric and neuropsychological approaches [24]. It is composed of five domains (language, praxis, number, memory, spatial, and controlled attention) and these domains are further subcategorized into ten subscales. The core aim of making these tools available was to improve cognitive screening practices to detect cognitive changes, with a particular focus on vascular cognitive impairments. The modified version of OCS-P in our preliminary work was shown to have good reliability and validity [25].

2.3. ADLs Assessment. The primary outcome was the ADLs at discharge measured by Modified Barthel Index (MBI), ranging from 0 to 100 points, with a higher score

corresponding to a greater ability to complete the ADLs. Participants were categorized into 2 mutually exclusive groups according to MBI scores: good outcome (0~60) or poor outcome (60~100) when they were discharged. The recovery of ADLs was identified by the improvement between admission and discharge.

2.4. Motor Function Assessment. Motor function was assessed by the Fugal–Meyer Assessment scale (FMA). FAM is an instrument commonly administered by physical therapists in both clinical and research fields to evaluate people after stroke. The original scale, which consisted of five domains (motor function, balance, sensation, joint mobility, and pain), has undergone rigorous investigations for reliability, validity, and responsiveness to change [28].

2.5. Neurological Recovery Assessment. The recovery of Neurological function was evaluated by the modified Rankin Scale (mRS). mRS is the most prevalent functional outcome measure in contemporary stroke research [29], with the scores ranging from 0 (asymptomatic), 1 (having a symptom but without obvious disability), 2 (mild disability), 3 (moderate disability), 4 (moderate to severe disability), and 5 (severe disability) to 6 (death).

2.6. Depression and Rehabilitation Participation. In addition, Hamilton Depression Rating Scale (HAMD) and Pittsburgh Rehabilitation Participation Scale (PRPS) were used to evaluate depression and rehabilitation participation respectively.

2.7. Data Collection and Procedure. The demographic information, clinical data, and functional status of participants were collected after hospital admission. Among these measurements, ADLs were assessed both after hospital admission and at discharge. Informed consent was obtained from all individual participants included in the study.

2.8. Statistical Analysis. The data was analyzed by SPSS 22.0 and R software. Two-sided P values of 0.05 were considered statistically significant. Measurement data were described by mean (standard deviation) or median and quartile rang [M (P25, P75)] for normal distribution or skewness distribution respectively. Enumeration data were presented as frequencies and percentages. We compared the recovery of ADLs among patients with different baseline demographic characteristics by using independent samples t tests or Mann-Whitney test for 2-level variables and the one-way ANOVA or Kruskal–Wallis test for variables with 3 or more levels. Pearson's or Spearman rank correlation analyses were used to demonstrating the correlations between clinical variables, cognitive function, and ADLs recovery. Multivariate logistic regression analysis was conducted to determine the prognostic value of domain-specific cognitive abilities for ADLs, with MBI score at discharge as the dichotomous outcome (0 = 60~100, 1 = 0~60) while significant variables from univariate and correlation analysis above as independent variables. The receiver operating characteristic curve (ROC curves) was used to measure the diagnostic effectiveness of the prediction models. The Hosmer Lemeshow goodness-of-fit test was used to evaluate the calibration capability of the prediction models. Prediction models were validated by K-fold cross-validation ($K = 10$). The nomogram was made to provide a more simple and convenient method for estimating the ADLs outcome.

3. Results

3.1. Sample characteristics. A total of 303 stroke patients were recruited from two tertiary hospitals and 148 of them were excluded for several reasons: did not meet the inclusion criteria ($n = 86$); eligible for exclusion criteria ($n = 41$); unwilling to participate ($n = 21$); dropped out because of weakness and fatigue ($n = 2$). Finally, 153 patients with PSCI were enrolled in our study and completed all the evaluations. Sample characteristics including demographic data, clinical data, and functional status were presented in Tables 1 and 2.

3.2. The Recovery of ADLs and Its Influencing Factors. The MBI scores of patients at admission and discharge were 30 (20, 47.5) and 60 (36.5, 80), respectively. The improvement of MBI was 20 (10, 35.5). Stroke subtype, hospital stays, ADLs at admission and neurological function were the influencing factors of ADLs recovery identified by using univariate and correlation analysis in Tables 1 and 2.

3.3. Cognitive Function of Patients with PSCI and Its Correlation with ADLs. The score of OCS-P was 60 (49.75, 69), with 94.12% of participants having at least one cognitive domain impairment. The highest frequency of the number of impaired cognitive domains was 4 (19.61%), while the lowest was 9 (1.31%). The most impaired cognitive domain was number writing (60.8%), followed by verbal memory (52.9%), the least impaired domain was the visual field (13.7%). The scores of cognitive domains of OCS-P and its correlations with ADLs were shown in Table 3. As a domain-specific cognitive function screened by OCS-P, executive function was shown to be a significant correlation with ADLs recovery.

3.4. Prognostic Value of Domain-Specific Cognitive Abilities for ADLs. The significant variables in Tables 1 and 2 and being reported as important factors (education years, PRPS, FMA) which can affect ADLs [30] were introduced into the multivariate logistics regression equation in sequence to identify the prognostic value of domain-specific cognitive ability (executive function in Table 3) on determining the ADLs recovery in patients with PSCI, generating five models in total. The forest plots of five models were presented in Figure 1. The area under curves of ROC curves of five models was presented in Figure 2, with the Model 5 having the best diagnostic performance (area under curves = 0.839). The multivariate logistic regression analysis of Model 5 showed

Table 1: Sample characteristics and the univariate analysis of ADLs recovery.

Variables	N (%)/M (P25, P75)	Improvement of MBI mean (SD)	P value
Gender[#]			0.888
Male	111 (72.5%)	24.54 (19.92)	
Female	42 (27.5%)	22.81 (15.72)	
Age (years)[a*]	63 (53.5, 70)		0.325
≤63	47 (54, 59)	25.51 (20.01)	
>63	66 (70, 75)	22.44 (17.38)	
Smoking history[b]			0.362
Yes	38 (24.8%)	26.63 (19.23)	
No	115 (75.2%)	23.22 (18.69)	
Drinking history[#]			0.738
Yes	38 (24.8%)	24.16 (18.68)	
No	115 (75.2%)	23.79 (19.50)	
Stroke subtype[#]			0.026
Ischemia	103 (67.3%)	21.62 (17.39)	
Hemorrhage	50 (32.7%)	29.10 (20.77)	
Brain injured area[#]			0.713
Right	79 (51.6%)	23.46 (17.07)	
Left	52 (34.0%)	24.96 (18.97)	
Both	22 (14.4%)	24.14 (24.58)	
Atrial fibrillation[#]			0.756
Yes	13 (8.5%)	21.46 (16.57)	
No	140 (91.5%)	24.31 (19.05)	
Hypertension[#]			0.486
Yes	51 (33.3%)	22.61 (18.06)	
No	102 (66.7%)	24.79 (19.24)	

[a]Subjects were divided into younger age group and older age group according to the median age (63 years old).[b]Quit smoking and still smoking were considered to have a history of smoking while never smoking was considered as no smoking history. Like smoking history, quit drinking and still drinking were considered to have a history of drinking while never drinking was considered as no smoking history. [#]Two independent sample t tests or analysis of variance (ANOVA). *Nonparametric rank sum Z test (Mann-Whitney test).

Table 2: Sample characteristics and the correlation analysis of ADLs recovery.

Variables	Mean (SD)/M (P25, P75)	r	P value
Education years	7 (4, 10)	0.099	0.222
BMI	22.49 (20.76, 24.8)	0.081	0.341
Course of disease	22 (11.75, 30)	0.112	0.173
Hospital stays	37 (26, 59.5)	0.197	0.015
Fasting plasma glucose	5.35 (4.65, 6.25)	0.064	0.429
Triglycerides	1.47 (1.06, 1.81)	−0.131	0.106
Total cholesterol	3.7 (0.99)	−0.008	0.920
FMA	18 (10, 43)	−0.075	0.356
HAMD	5 (3, 7)	−0.046	0.568
PRPS	4.75 (4, 5.25)	0.047	0.563
MRS	4 (4, 4)	0.242	0.003
MBI at admission	30 (20, 47.5)	−0.235	0.003

BMI, body mass index; MBI, modified barthel index; FMA, fugal-meyer assessment scale; HAMD, hamilton depression rating scale; PRPS, pittsburgh rehabilitation participation scale; MRS, modified rankin scale.

executive dysfunction was a risk prognostic factor of ADLs recovery ($P < 0.001$, OR = 3.176 ([95% CI, 1.218~8.278]) in Table 4. No statistical significance was found between predictive value and actual observed value of Model 5 (Hosmer-Lemeshow $\chi^2 = 8.939.3$, $P = 0.347 > 0.05$), indicating the model has good calibration ability. The calibration plot and nomogram of Model 5 were shown in Figures 3 and 4. Besides, the model 5 was validated by K-fold cross-validation. The data was randomly and equally divided into 10 groups, 9 of which were used for modeling, and the others were used for verification. After 10 times of modeling and verification in sequence, a relatively stable model was achieved. The average error rate after the correction was 20.93%, within the acceptable range.

4. Discussion

Cognitive impairment is a so common dysfunction existing in post-stroke patients which cannot be overlooked. In this study, we primarily used OCS-P, a more targeted measurement we specially revised for stroke patients in our preliminary study, to identify the prognostic value of domain-specific cognitive abilities on the recovery of ADLs in patients with PSCI. The result showed that post-stroke patients generally suffered from multidimensional cognitive impairments and executive function screened with OCS-P at clinical admission was a reliable and accessible predictive factor of ADLs. Our findings emphasized it's important for medical staff and rehabilitation therapists to focus on the executive dysfunction of stroke inpatients as early as possible.

The original OCS has been designed as a cognitive screening tool that acts as a pointer for further, more detailed domain-specific assessment should impairments in any

Table 3: The cognitive function of participants and its correlation with ADLs recovery.

Domains	Tasks	M (P25, P75)	Impaired percentage (%)	r	P value
Number of cognitive impairment tasks		4 (2, 6)		0.022	0.786
Attention	Executive task	3 (−1, 5)	35.3	−0.22	0.006
	Executive task (mixed)	6 (4, 11)	50.3	0.049	0.546
	Visual field test	4 (4, 4)	13.7	−0.004	0.961
Language	Semantics/picture pointing	3 (3, 3)	18.3	0.061	0.456
	Sentence reading	18 (11.75, 19)	28.7	−0.13	0.113
	Picture naming	3 (2, 4)	36.6	−0.058	0.479
Memory	Orientation	4 (3, 4)	19	0.013	0.876
	Verbal memory: free recall	2 (1, 4)	52.9	0.108	0.186
	Verbal memory: recognition	3 (2, 4)	35.9	0.022	0.789
Number	Number writing	2 (1, 3)	60.8	0.042	0.603
	Calculations	4 (3, 4)	16.3	−0.021	0.801
Spatial neglect	Broken hearts test (gap)	0 (0, 1)	41.4	−0.011	0.892
	Broken hearts test (complete)	0 (−1, 2)	45.1	0.029	0.724
Praxis	Meaningless gesture imitation	9 (7, 11)	29.4	0.121	0.137

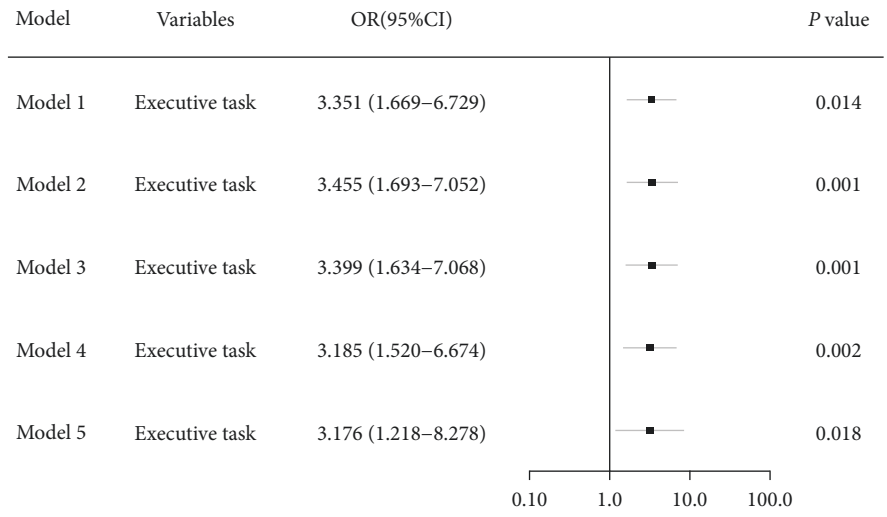

Figure 1: Forest plots of the prognostic value of executive function on ADLs (Model 1~5). Model 1 was unadjusted; Model 2 was adjusted for demographic data (education years); On the basis of model 2, model 3 was adjusted for disease characteristics (type of stroke, hospital stays); Model 4 was mainly considered about the level of participation and adjusted for PRPS based on model 3; Model 5 considered the functional level at admission, so the MBI, MRS, and FMA at admission were adjusted based on Model 4.

cognitive domain be revealed. The common clinical instruments used by clinicians in neurology, rehabilitation medicine, and therapy mostly quantify general cognitive function of patients. Test constructs of these instruments such as MMSE and MOCA cannot capture the cognitive challenges unique to post-stroke patients. Unlike these current screening tools, OCS allows assessment of dysplasia patients, even can be delivered at the bedside in acute stroke. Moreover, OCS provides measures of neglect (both allocentric and egocentric), praxis, and numerical cognition. The test items were presented both visually and verbally, inclusive for the possibility of selecting a correct answer from a multiple-choice array.

Currently, OCS has been translated into several versions, including Hong Kong (Cantonese speaking) [31], Italian [32], and Russian versions [33]. The modified Chinese (Putonghua) version of OCS-P was considered to have good psychometric properties in our previous research. It was validated for use by clinicians in China and among other cultural groups (or individuals) living outside of China. Clinicians and researchers can administer OCS-P to patients who speak Chinese (Putonghua) at admission and during subsequent follow-up assessments [26]. The OCS-P subscale scores can be used for guiding treatment plan, monitoring treatment effect, and tracking rehabilitation outcomes. In this current study, it had been proved to have a good prognostic value for ADLs of stroke survivors with PSCI.

Early findings including our own have demonstrated that cognitive impairment was highly prevalent in patients after stroke. According to our study, 94.12% of the patients had at least one cognitive impairment, and 85.62% of them had at least two. It was basically confirmed in previous studies, which demonstrated that nearly 86% [21] and 91.6% of the post-stroke patients have at least one

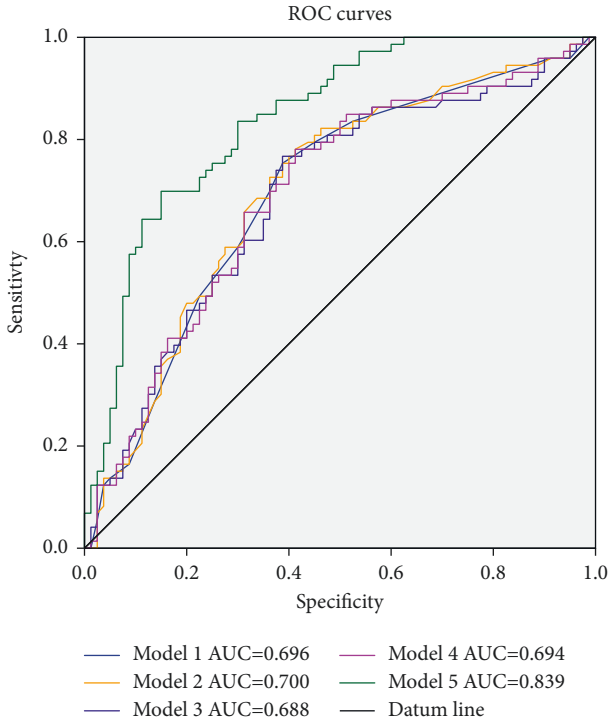

Figure 2: The area under ROC curves of five models. AUC: area under curves. The 95% CI of 5 models were model 1 (0.611~0.780), model 2 (0.616~0.784), model 3 (0.603~0.773), model 4 (0.609~0.778), model 5 (0.777~0.902), with all P value <0.001.

Table 4: Multivariate logistic regression analysis of model 5.

Variables	B	S.E	Or (95% CI)	P value
Executive function	1.156	0.489	3.176 (1.218~8.278)	0.018
Education	0.001	0.056	1.001 (0.897~1.116)	0.990
Hospital stays	−0.010	0.010	0.990 (0.970~1.011)	0.349
Stroke type	0.589	0.531	1.802 (0.637~5.100)	0.267
PRPS	−0.353	0.285	0.703 (0.402~1.227)	0.215
FMA	0.012	0.014	1.012 (0.984~1.040)	0.411
MRS	0.585	0.668	1.794 (0.485~6.642)	0.381
MBI at admission	−0.107	0.020	0.898 (0.864~0.935)	<0.001

MBI, modified barthel index; FMA, fugal-meyer assessment scale; MRS, modified rankin scale; PRPS, pittsburgh rehabilitation participation scale.

impaired task in the cognitive field after stroke by using the OCS scale and more than 80% of them had two or more [34]. The percentage of impaired cognitive domains exceeding 50% among ten cognitive domains of OCS-P were: number writing (60.8%), verbal memory (free recall) (52.9%), and executive function [executive task (mixed)] (50.3%). Demeyere used the OCS scale to assess patients with acute stroke and revealed that executive dysfunction (48.9%), neglect (39.8%), and number writing (31.1%) were mainly impaired cognitive domains [21]. In Mauro's study, cognitive impairment mainly occurred in calculation (50.7%), sentence reading (49.8%), number writing (36%), executive function (32.3%) and neglect (31.3%) [34]. Patients after stroke generally have multiple cognitive domains impairment. Although the impaired cognitive domains were inconsistent in early findings, most of them were mainly concentrated in executive dysfunction, neglect, and number writing, illustrating those domains were highly susceptible to stroke-related impairments.

PSCI has a negative impact on early activity limitations and participation restrictions [35]. Our study verified patients with executive dysfunction after the stroke had a 3.176 times risk of poor ADLs than those with normal executive function. Thus, the executive function of stroke survivors at admission should be considered a powerful independent predictor of ADLs when discharged. Similar results were demonstrated in recent works [9, 16, 36, 37], which collectively identified executive dysfunction as a significant and independent predictor of functional outcome. According to previous studies, executive function subtests of the OCS were reported to predict the long-term functional capabilities of post-stroke patients [37], higher initial executive function scores of MoCA were associated with better ADLs in the subacute stroke phase [9], and trail making test-A scores, as a measurement of executive function, can also highly predict the MBI score at discharge [16]. Even in follow-up, the inhibition of executive function was strongly associated with earlier permanent institutionalization and its prognostic value was also recommended after stroke [36]. Moreover, a prospective study with 7717 individuals revealed that compared to those without executive dysfunction, those with poor baseline executive function had significantly worse ADLs and instrumental activities of daily livings (IADLs) function cross-sectionally over 6 years and had an increased risk of mortality [38]. Another meta-analysis suggested a consistent moderate association between ADLs and executive function [39], supporting the growing evidence for a link between ADLs and executive dysfunction in early cognitive decline.

Executive function refers to a multidimensional goal-directed system, monitoring one's behavior and self-regulating functions [40, 41]. These processes empower us to effectively prioritize goals, weigh benefits and respond adaptively. As cognitive deficits progress, executive dysfunction becomes more prominent and its negative effect on instrument ADLs had also been shown to be an important contributor to the cognitive deterioration [42]. In our study, executive function as an important prognostic factor of ADLs may due to the following reasons: First, executive dysfunction did affect the individual's ability to effectively participate in rehabilitation programs, manifested by the inability to maintain a series of behavioral consistency, initiate actions, suppress impulsive behaviors, and follow the rehabilitation instructions [15]. Second, for stroke patients, executive dysfunction has been found to be related to an increased tendency to adopt avoidant coping strategies [43], which were positively associated with adverse outcomes. Third, executive dysfunction was associated with a lower level of participation [44]. Patients with more worse executive function appear to have a low quality of participation, leading to poorer functional recovery and increased hospitalization time. These results collectively suggest that screening of

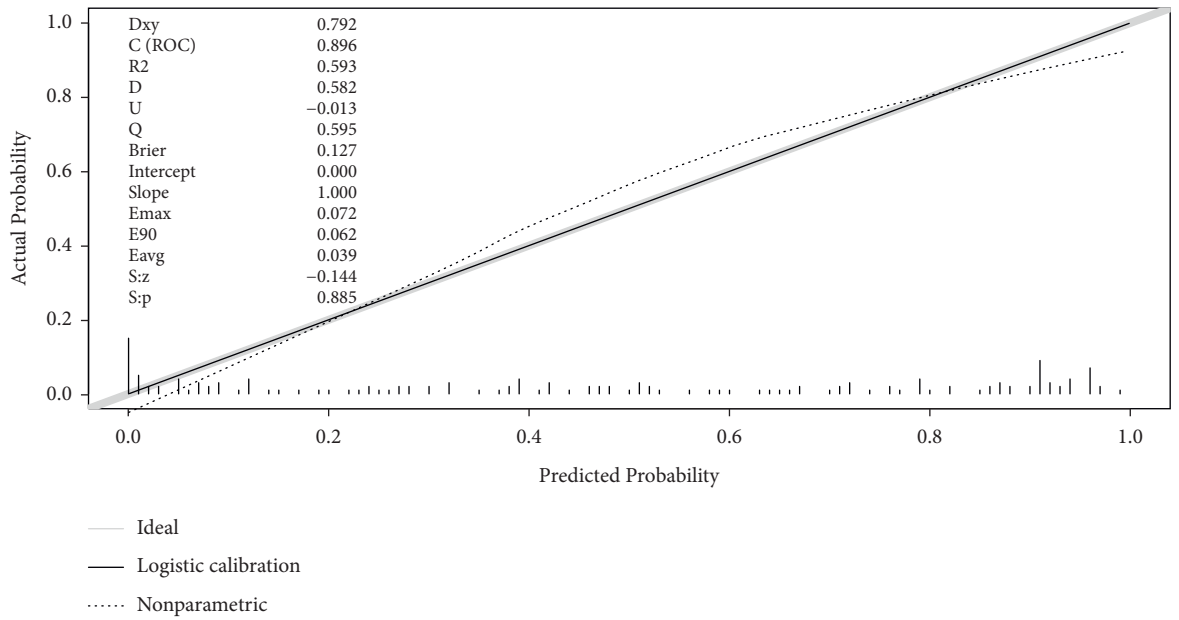

Figure 3: The calibration curve of model 5. The black line is the calibration curve. The gray line is the standard curve ($y = x$), indicating that the predicted number is the same as the actual observation number. The closer the two curves are, the better the calibration capability of the model.

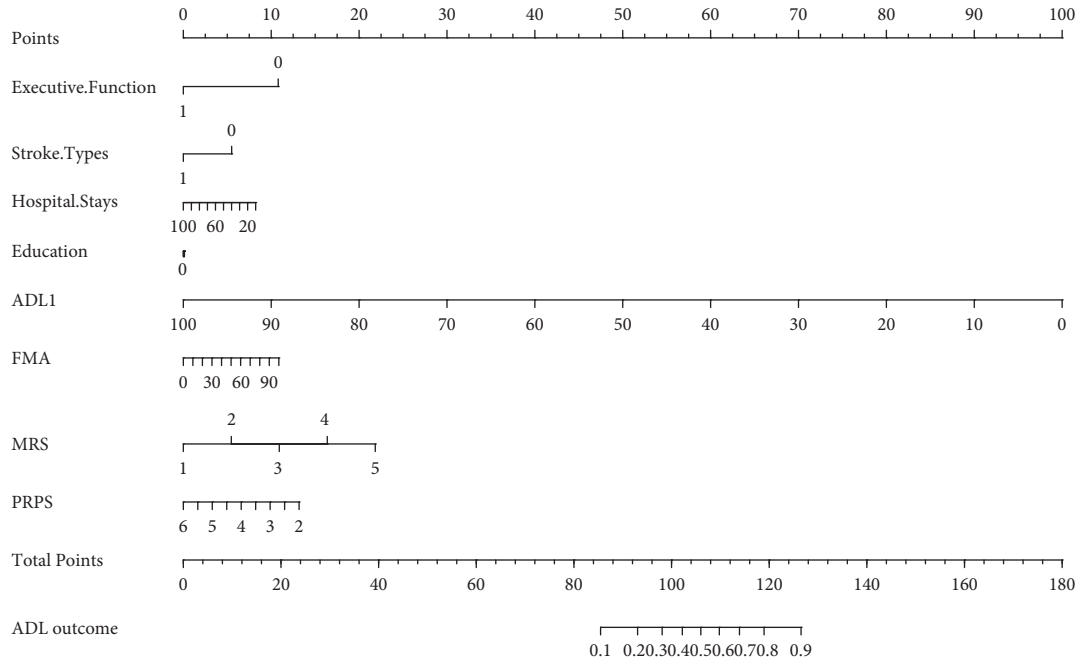

Figure 4: Nomogram of Model 5 to predict the ADLs for patient s with PSCI. ADLs1, the activity of daily living at the admission. FMA, Fugal–Meyer assessment scale. MRS, modified RNAKIN scale. PRPS, the Pittsburgh rehabilitation participation scale. Executive function: 1 = normal, 0 = abnormal. Stroke types: 0 = hemorrhage, 1 = ischemia. ADLs outcome: 0 = good outcome (0~60), 1 = poor outcome (60~100). Each clinical relative factors corresponds to a specific point by drawing a line straight upward to the points axis. After the sum of the points is located on the total points axis, the sum represents the probability of ADLs by drawing straight down to the ADLs outcome axis.

executive dysfunction by OCS-P can help to identify those at risk for loss of ADLs ability.

Except for executive function, the study did not find other cognitive domains of OCS-P that can be served as a predictor of ADLs, which was inconsistent with some previous studies [9, 45]. This may be due to the study design factors, such as sample size, participant's age, or follow-up period [35]. It needs to be further confirmed in longitudinal studies based on a large population. Besides, our results did not support the previous findings that overall cognitive

function has a good predictive value for the recovery of ADLs [10,46]. In our study, we used the number of task impairments of the OCS-P scale as overall cognitive function [24] and no correlation was found with ADLs outcome. However, this finding needs to be further explored with a larger sample size.

The study has several limitations. First, most patients in our study were transferred from general hospital to rehabilitation hospital. They were generally thought to be more seriously ill and this selection bias may lead to an overestimation of the incidence of cognitive impairment after stroke. Moreover, due to the small sample size and single-center study design, which included only Chinese hospitalized post-stroke patients, it should be cautious when we generalized our findings. Finally, the observation time was only limited to the rehabilitation period of stroke patients from admission to discharge. There may be biases in assessing the frequency of task impairments. Future research with larger sample sizes from multiple centers and longer observation time exploring the diagnostic value of cognitive impairment on short-term and long-term ADLs recovery of stroke patients are expected.

5. Conclusion

Executive dysfunction screened with OCS-P at clinical admission was a reliable and accessible predictive factor of ADLs recovery in patients with PSCI. Early targeted rehabilitation programs are recommended to take especially for those who have executive dysfunction while hospitalized.

Data Availability

The data that support the findings of this study are available upon reasonable request to the corresponding author.

Ethical Approval

The studies involving human participants were reviewed with the approval of the Ethics Board of the Rehabilitation Hospital affiliated to Fujian University of Traditional Chinese Medicine (2018KY-011-01).

Consent

All participants provided signed, informed written consent forms before participation in this study.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

Authors' Contributions

ZZ L contributed to the study conceptualization and design. JH, JS W, JT, and LD C were responsible for supervision. Data curation and formal analysis were performed by MR L and JX R. The first draft of the manuscript was written by MR L and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Retraction

Retracted: Low-Dose Apatinib Improves the Prognosis of Patients with Recurrent High-Grade Gliomas

Evidence-Based Complementary and Alternative Medicine

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This article has been retracted by Hindawi following an investigation undertaken by the publisher [1]. This investigation has uncovered evidence of one or more of the following indicators of systematic manipulation of the publication process:

(1) Discrepancies in scope
(2) Discrepancies in the description of the research reported
(3) Discrepancies between the availability of data and the research described
(4) Inappropriate citations
(5) Incoherent, meaningless and/or irrelevant content included in the article
(6) Peer-review manipulation

The presence of these indicators undermines our confidence in the integrity of the article's content and we cannot, therefore, vouch for its reliability. Please note that this notice is intended solely to alert readers that the content of this article is unreliable. We have not investigated whether authors were aware of or involved in the systematic manipulation of the publication process.

In addition, our investigation has also shown that one or more of the following human-subject reporting requirements has not been met in this article: ethical approval by an Institutional Review Board (IRB) committee or equivalent, patient/participant consent to participate, and/or agreement to publish patient/participant details (where relevant).

Wiley and Hindawi regrets that the usual quality checks did not identify these issues before publication and have since put additional measures in place to safeguard research integrity.

We wish to credit our own Research Integrity and Research Publishing teams and anonymous and named external researchers and research integrity experts for contributing to this investigation.

The corresponding author, as the representative of all authors, has been given the opportunity to register their agreement or disagreement to this retraction. We have kept a record of any response received.

References

[1] M. Zhang, L. Gao, X. Liu et al., "Low-Dose Apatinib Improves the Prognosis of Patients with Recurrent High-Grade Gliomas," *Evidence-Based Complementary and Alternative Medicine*, vol. 2022, Article ID 3181133, 6 pages, 2022.

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Research Article

Low-Dose Apatinib Improves the Prognosis of Patients with Recurrent High-Grade Gliomas

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Objective. To evaluate the efficacy, safety, and prognostic value of low-dose apatinib in combination with temozolomide in the treatment of primary or recurrent high-grade gliomas (HGGs). *Methods*. A retrospective analysis of patients with postoperative and recurrent HGGs treated in our hospital from April 1, 2018, to April 30, 2020. Patients should be treated by combination therapy (surgery + radiotherapy + chemotherapy). Patients who received apatinib combined with temozolomide chemotherapy were allocated to the research group (RG), while patients who received temozolomide chemotherapy alone were allocated to the control group (CG). The efficacy and toxic side effects were compared between the two groups. *Results*. There were 67 qualified patients retrieved, including 37 cases in the RG and 30 cases in the CG. There were no significant differences in objective remission rate (ORR) or disease control rate (DCR) between the control group and the study group ($P > 0.05$). However, the overall improvement of clinical efficacy in the observation group was better than that in the control group ($P < 0.05$). There was no significant difference in the incidence of adverse effects between the two groups ($P > 0.05$). There were no significant differences in overall survival (OS) or progression-free survival (PFS) between the two groups ($P > 0.05$). *Conclusion*. Low-dose apatinib combined with temozolomide and radiotherapy for HGGs is effective in improving efficacy, relieving brain edema, reducing the use of glucocorticoid drugs, and improving patients' quality of life. It has mild adverse effects and is well tolerated by patients.

1. Introduction

Even with the development of medicine and new drugs, the clinical treatment of high-grade gliomas (HGGs) has never achieved satisfactory results, and the average survival time of patients is only about 1 year [1, 2]. Currently, the low survival rate and the local susceptibility to recurrence are challenges for treatment [3]. With the advances in imaging technology, molecular pathology, and biology, translational medicine on HGGs has also developed rapidly, especially in the last decade in terms of individualized therapy predicted by biological targets [4]. Molecular targeted therapies for glioma are increasingly favored.

Angiogenesis is one of the typical diagnostic features of HGGs, especially glioblastomas [5]. The neovascularization of HGGs is usually more distorted, forming vascular spheres that apparently lack tight junctions and complete pericyte coverage [6]. Since angiogenesis is a biologically important feature of HGGs, targeted treatment strategies have their advantages [7]. There are guidelines and several studies using the antiangiogenic drug bevacizumab in the treatment of both primary and recurrent HGGs, all of which have shown good efficacy, further affirming the value of anti-angiogenic therapy [8]. However, bevacizumab treatment is relatively expensive, and no oral dosage is available at this time [9]. Clinical studies have shown that apatinib has a strong inhibitory effect on tumor growth, including sarcoma, colorectal cancer, nonsmall cell lung cancer, gastric cancer, and hepatocellular carcinoma, and it is a broad-spectrum antitumor vascular targeting drug [10–12]. It was found that apatinib can cross the blood-brain barrier and inhibit VEGFR-2, which is highly expressed in various

tumor tissues. High expression of VEGFR-2 is a key factor in tumor angiogenesis, leading to speculation that apatinib may be effective in the treatment of HGGs [10, 12]. Therefore, it is necessary to explore the effectiveness and safety of apatinib in HGGs to provide a new option for clinical treatment. We also found that low-dose apatinib is easier to obtain, and it is safe and effective in clinical applications.

We conducted a retrospective study of apatinib treatment in WHO grade III/IV gliomas after primary surgical resection or in those gliomas that have recurred after previous surgery and/or radiotherapy or failed to respond to other regimens, to explore the efficacy and safety of apatinib in primary or recurrent HGGs, and to lay the foundation for later randomized controlled studies.

2. Methods and Materials

2.1. Clinical Data. Patients with postoperative and recurrent HGGs treated in our hospital from April 1, 2018, to April 30, 2020, were screened, and all of them should be treated by combination therapy (surgery + radiotherapy + chemotherapy). Patients who received apatinib combined with temozolomide chemotherapy were allocated to the research group, while those who received temozolomide chemotherapy alone were regarded as the control group. The study was conducted with the approval of our medical ethics committee.

2.2. Inclusion and Exclusion Criteria. Inclusion criteria were as follows: patients with pathologically confirmed glioma and residual or recurrent glioma diagnosed by pathology or imaging (CT, MRI, and PET-CT examination) after treatment with standard protocols (surgery, radiotherapy, and chemotherapy), with complete case records of residual or recurrent glioma before and after; no other antivascular agents or targeted drugs were used during the present treatment; discontinuation of other antivascular drugs or targeted drugs for ≥6 months; and intracranial with ≥1 measurable lesion according to the Response Assessment of Neuro-Oncology (RANO) criteria.

Exclusion criteria were as follows: presence of comorbid tumors; or presence of other serious diseases, such as hypertension, heart disease, liver disease, and kidney failure; or expected survival of less than 3 months; or intolerant to the treatment plans.

2.3. Treatment Options. Patients in the CG were treated with temozolomide capsules (Beijing SL Pharmaceutical Co., Ltd., SFDA Approval No. H20110153), 150 mg/m^2/day once daily for the 1st cycle, with 5 consecutive days of oral administration followed by 23 days of discontinuation. If no adverse effects occurred in cycle 1, the dose was increased to 200 mg/m^2/day in cycle 2. If intolerable adverse effects occur during administration, the dose was adjusted or discontinued. Dose adjustments included doses of 100 mg/m^2, 150 mg/m^2, and 200 mg/m^2.

The RG patients were treated with the addition of apatinib (Jiangsu Hengrui Medicine Co., Ltd., SFDA Approval No. H20140105) based on the CG treatment. Patients took apatinib 500 mg daily. The dose was reduced to 250 mg daily if toxic side effects become intolerable in the medication process. The chemotherapy drug was discontinued when the disease progressed or intolerable adverse symptoms appeared, and the maintenance treatment with apatinib was continued.

2.4. Review and Follow-Up during Treatment. Patients received weekly routine blood tests and monthly biochemical tests, as well as thyroid function and full coagulation tests during radiotherapy. During maintenance treatment, patients received a routine blood test, biochemical test, thyroid function test, and full coagulation test on days 21 and 28 of each cycle, respectively. MRI of the head was reviewed at cycles 2, 4, and 6 of temozolomide chemotherapy during primary maintenance therapy. The hematological examination and imaging efficacy evaluation for relapsed patients given combination temozolomide therapy were consistent with the follow-up of the maintenance phase of primary patients. The follow-up period ended on January 31, 2022.

2.5. Outcome Measures. Main outcome measurements: the clinical efficacy was evaluated after treatment. Complete remission (CR): after treatment, the tumor lesions disappeared completely and the physical signs return to normal. Partial remission (PR): the tumor lesion shrinks by >75% after treatment, clinical symptoms improve dramatically, and signs are basically normal. Disease stabilization (SD): >50% shrinkage of tumor lesions after treatment and slight remission of symptoms. Progression of disease (PD): tumor lesions do not shrink or even increase after treatment or new lesions appear and symptoms worsen. The objective remission rate (ORR), disease control rate (DCR), and overall survival (OS) of patients were recorded. Objective response rate (ORR): number of (CR + PR) patients/total number of patients × 100%. Disease control rate (DCR): (CR + PR + SD) number of patients/total number of patients × 100%. OS: the time from the start of enrollment in apatinib until death from any cause.

Secondary outcome measures: patients were observed for progression-free survival (PFS) and adverse events (AE). Progression-free survival (PFS): the time from the onset of remission after initiation of apatinib in a subgroup until objective tumor progression or death. AE refers to adverse events that occur during drug treatment.

2.6. Statistical Analysis. The data collected were analyzed using SPSS 20.0 (IBM Corp., Armonk, NY., USA) and visualized using GraphPad Prism 8. The count data, expressed as percentages (%), were assessed through the chi-square test (x^2); the rank data were expressed as Z using the Mann–Whitney test. The overall survival of patients was plotted through the Kaplan–Meier survival curves and assessed via the log-rank test, and the independent prognostic factors for patient OS were evaluated via Cox regression. A two-sided $P < 0.05$ indicated a statistically significant difference.

3. Results

3.1. Comparison of Clinical Data. There were 67 qualified patients retrieved. There was no statistical difference in age, gender, BMI, KPS score, WHO pathological classification, and medical history between patients ($P > 0.05$, Table 1).

3.2. Comparison of Clinical Efficacy. The clinical efficacy was evaluated before and after treatment. It was found that there was no statistical difference between ORR and DCR (Table 2, $P > 0.05$). While the rank sum test revealed that the overall clinical outcomes in the RG were better than those in the CG ($P < 0.05$).

3.3. Comparison of Adverse Effects. The adverse events were recorded based on different grades of the events (Table 3). There was no statistical difference in the incidence of adverse events during treatment (Table 4, $P > 0.05$).

3.4. Comparison of Patient Survival. As of January 31, 2022, we recorded the follow-up data of 67 patients. There were no statistical differences in OS or PFS between groups (Figure 1, $P > 0.05$).

Table 1: Baseline data.

Factor	RG ($n = 37$)	CG ($n = 30$)	P value
Age			
≥45 years old	22	14	0.296
<45 years old	15	16	
Gender			
Male	25	25	0.223
Female	12	6	
BMI			
≥23 kg/m^2	20	12	0.252
<23 kg/m^2	17	18	
KPS			
≥80 points	35	30	0.196
<80 points	2	0	
WHO pathological grade			
Grade III	21	16	0.779
Grade IV	16	14	
Medical history			
Hypertension	8	10	0.282
Diabetes	6	8	0.295

4. Discussion

HGGs have a high degree of malignancy, a high incidence of postoperative disease recurrence risk, low long-term survival, and poor quality of life [13]. The standardized treatment process for primary HGG patients involves surgery not only to remove as many tumor lesions as possible but also to protect the neurological function of the brain and postoperative radiotherapy to enhance the efficacy of the treatment, but the long-term survival remains unsatisfactory [14, 15]. It has been shown that the use of bevacizumab for new-onset advanced gliomas did not result in a statistically significant overall survival OS but a markedly prolonged PFS [16]. It also has good therapeutic effects on both recurrent HGGs and metastases, while dramatically reducing the effects of brain edema. Apatinib, a multitargeted antiangiogenic drug, crosses the blood-brain barrier after radiotherapy and has a high blood concentration [17]. Zhang et al. [18] confirmed that the sequential temozolomide and apatinib combination regimen in HGGs patients had a better disease control rate, objective efficiency, disease-free survival, and OS in the RG than those in the CG.

Nitta et al. showed [19] that the combination of apatinib and temozolomide dramatically enhanced temozolomide-mediated inhibition of cell proliferation compared to cells treated with temozolomide alone. It turned out that apatinib increased the antitumor activity of temozolomide in gliomas. Apatinib improves the sensitivity of gliomas to temozolomide by downregulating vascular endothelial growth factor receptor-2 (VEGFR-2). Apatinib is a highly selective VEGFR-2 inhibitor that may increase glioma chemotherapy sensitivity [20]. Zhang et al. [21] reported the success of apatinib in treating 2 cases of refractory recurrent malignant gliomas, where both patients received oral apatinib (500 mg/d) during a recent relapse and experienced rapid relief of central nervous system symptoms. Female patients were with almost complete remission as assessed by MRI after 20 weeks of medication with an OS of 27 weeks. Male patients achieved partial remission and a PFS of 12 months as assessed by MRI after 12 weeks of drug administration. Liu et al. [22] found that the temozolomide intensive regimen combined with apatinib for the treatment of recurrent malignant gliomas resulted in obvious improvement in MMSE and KPS in patients in the short term, marked reduction in peritumoral edema, and prolongation of PFS and OS compared with the application of the temozolomide intensive regimen alone. In this research, we observed that after combined low-dose apatinib and bevacizumab treatment, patients had better OS and PFS than those reported in the literature with bevacizumab, and the overall clinical outcomes of relapsed patients were improved, and the use of dehydrating drugs and hormones was reduced. Hence, patients had stable blood levels after daily oral low-dose apatinib with better efficacy than bevacizumab used alone once every 3 weeks. Apatinib not only inhibits tumor neovascularization [23] and suppresses glioma cell invasion but also may activate the GBM immune response to exert antitumor effects [24–29]. Common adverse effects of apatinib include hypertension, proteinuria, and hand-foot syndrome, most of which can be tolerated and some of which are controlled by adjusting antihypertensive medications. Although low-dose apatinib has demonstrated a good safety profile in clinical studies, attention should be paid to its adverse effects in practice, and grades 3-4 adverse effects should be actively treated symptomatically and, if necessary, discontinued for observation.

Nevertheless, the current study still has some limitations. First, it is a retrospective study, the conclusion of which is less convincing than RCTs. Second, we collected data for a short period of time, which did not allow for long-term

Table 2: Comparison of clinical efficacy.

Group	CR	PR	SD	PD	ORR	DCR
RG ($n = 37$)	0 (0.00)	5 (13.51)	24 (64.86)	8 (21.63)	5 (13.51)	29 (78.37)
CG ($n = 30$)	0 (0.00)	1 (3.33)	16 (53.33)	13 (43.33)	1 (3.33)	17 (56.66)
χ^2/Z value			−2.154		2.106	3.629
P value			0.031		0.146	0.056

Table 3: Comparison of adverse events.

Group	Grade 1	Grade 2	Grade 3
CG ($n = 30$)			
Blood routine	5	3	0
Hemorrhage	2	0	2
Elevated transaminase	3	0	0
Bilirubin elevation	2	0	0
Blood pressure	3	1	0
Thyroid dysfunction	1	0	0
Proteinuria	0	0	0
Abdominal pain	4	2	0
Hand-foot syndrome	3	2	0
Rash	0	0	1
RG ($n = 37$)			
Blood routine	5	4	0
Hemorrhage	3	0	1
Elevated transaminase	3	1	0
Bilirubin elevation	2	0	0
Blood pressure	2	2	0
Thyroid dysfunction	1	0	0
Proteinuria	0	0	0
Abdominal pain	3	2	0
Hand-foot syndrome	4	3	0
Rash	0	0	2

Table 4: Adverse events.

Group	Blood routine	Hemorrhage	Elevated transaminase	Bilirubin elevation	Blood pressure	Thyroid dysfunction	Proteinuria	Abdominal pain	Hand-foot syndrome	Rash
Research group ($n = 37$)	9	4	4	2	4	1	0	5	7	2
Control group ($n = 30$)	8	4	3	2	4	1	0	6	5	1
χ^2 value	0.048	0.103	0.011	0.046	0.103	0.022	—	0.508	0.057	0.166
P value	0.826	0.751	0.914	0.828	0.751	0.880	—	0.476	0.811	0.683

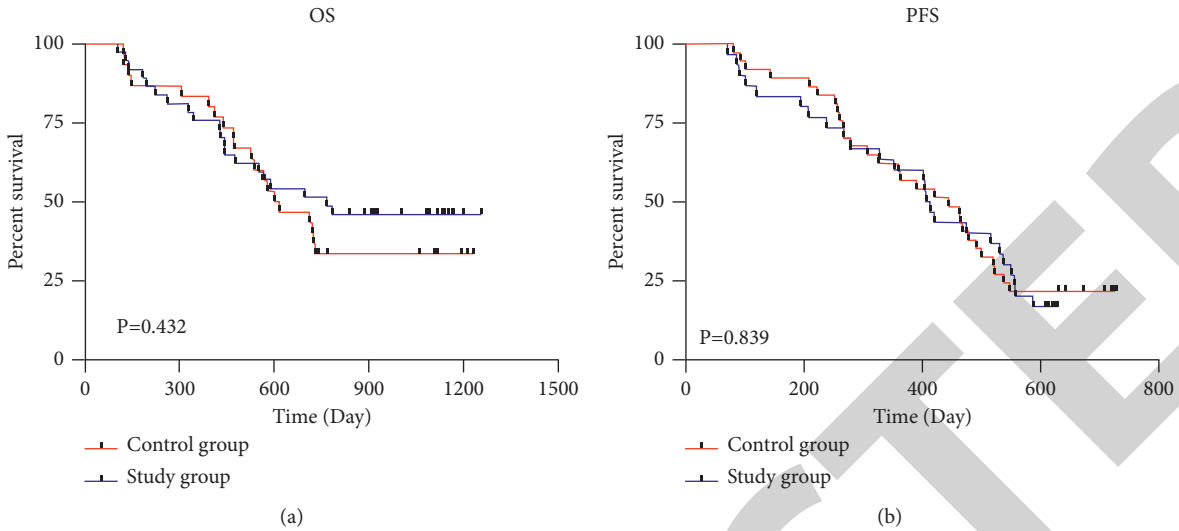

Figure 1: OS and PFS of patients. (a) Comparison of OS after treatment between RG and CG. (b) Comparison of PFS after treatment between RG and CG.

follow-up of patients. Thus, we hope to conduct more clinical trials in subsequent studies to reinforce our results.

In conclusion, low-dose apatinib combined with temozolomide and radiotherapy for HGGs is effective in improving efficacy, relieving brain edema, reducing the use of glucocorticoid drugs, and improving patients' quality of life. It has mild adverse effects and is well tolerated by patients.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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Research Article

Transcriptomic Profiling of Electroacupuncture Regulating the Molecular Network in Hippocampus of Rats with Cerebral Ischemia-Reperfusion Injury

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Objective. To explore the mechanism of electroacupuncture stimulation of the hand-taiyin meridian in regulating the molecular network of rats with cerebral ischemia-reperfusion injury based on transcriptomics. *Methods*. Male SD rats were randomly divided into sham operation group, model group, and electroacupuncture (EA) group. Middle cerebral artery embolization/reperfusion injury (MCAO/R) was used to establish the model group and EA group. The sham operation group only performed sham operation without modeling and any intervention, and the model group was bound daily. The EA group received electroacupuncture to stimulate the acupoints of hand-taiyin meridian for 14 days. Then, neurological scores, pathomorphological observations, and Tunel staining were performed. Finally, the affected hippocampus of the rat was used for transcriptome sequencing and RT-PCR detection. *Results*. After electroacupuncture intervention in rats, neurological function scores were improved, and neuronal apoptosis was reduced. The results of transcriptomics showed that a total of 1097 differentially expressed genes were obtained, of which 422 were upregulated and 675 were downregulated. The bioinformatics analysis showed that those differentially expressed genes were related to axon development, neuron projection development, neuron projection morphogenesis, plasma membrane cell projection morphogenesis, cell part morphogenesis, notch signaling pathway, long-term potentiation, MAPK signaling pathway, Hedgehog signaling pathway, and so on. The results of RT-PCR showed that Caspase 9 mRNA increased and BDNF, Grin2a, and PlexinD1 mRNA decreased after electroacupuncture intervention ($P < 0.05$). *Conclusion*. Electroacupuncture intervention on hand-taiyin meridian may reduce neurological function scores, inhibit neuron apoptosis, and enhance neuronal repair neuroreparation in MCAO/R rats, which may be related to the regulation of genes such as Caspase 9, BDNF, Grin2a, and PlexinD1.

1. Introduction

Stroke is the second leading cause of death in the world and the leading cause of death in China [1]. With the increase of aging population, it brings a great burden to society [2, 3]. The cerebral infarct area can be divided into the infarct core area and the ischemic penumbra, and the ischemic penumbra evolves into the irreversible infarct core area over time. Early opening of occluded cerebral blood vessels to restore cerebral blood flow and save neurons in the ischemic penumbra is the key to the treatment of ischemic stroke [4, 5]. At present, the main clinical treatment strategies for acute ischemic stroke are tissue plasminogen activator thrombolysis and mechanical thrombectomy [6, 7]. However, the prognosis of acute ischemic stroke is still unsatisfactory due to the limitation of the treatment time window,

the possible reperfusion injury caused by the restoration of blood perfusion, and the lag of cerebral protection therapy [8]. Therefore, it is necessary to pay attention to brain protection therapy to provide the possibility to save more damaged neurons.

The basic pathophysiological feature of ischemic stroke is neuronal death, which is not only due to ischemia directly but also secondary to ischemia/reperfusion injury, such as: oxidative stress, excitatory amino acid toxicity, calcium overload, inflammatory cascade, etc. [2, 9]. Among them, excessive inflammatory response is the main culprit leading to secondary damage to the ischemic penumbra. After interruption of cerebral blood flow, the supply of oxygen and glucose decreases, leading to an imbalance in cellular ion homeostasis and depolarization of neurons and glial cells [10]. Voltage-dependent calcium ion channels are activated, causing a large influx of calcium ions into cells and promoting the increased expression of reactive oxygen species (ROS) [11]. Neuronal depolarization promotes the release of the excitatory neurotransmitter glutamate and aggravates intracellular calcium overload. When occluded vessels are reperfused, ROS are produced explosively with increased oxygen content and infiltration of inflammatory cells [12]. This stimulates ischemic cells including ischemic neurons to secrete pro-inflammatory factors and chemokines; further activates microglia, astrocytes, peripheral blood leukocytes, endothelial cells, etc.; and promotes the migration of these inflammatory cells, aggregates, secretes a large number of pro-inflammatory factors, and produces an inflammatory amplification effect, which promotes inflammation in damaged areas of brain tissue [9, 13, 14]. Focal neuroinflammation disrupts the blood–brain barrier and aggravates brain injury by enhancing excitotoxicity, cytolysis, oxidative stress, and thrombotic inflammatory responses [15]. The clinical manifestations are cerebral hemorrhagic transformation, cerebral edema, and neurological deterioration. It can be seen that the excessively activated inflammatory response is an important pathological link that causes the secondary injury of brain tissue after reperfusion. In vitro and in vivo models have demonstrated that inhibition of inflammatory responses can reduce infarct volume and improve neurological deficits. However, no satisfactory efficacy has been achieved in clinical trials [16].

Acupuncture as a form of complementary medicine has been widely used around the world [17]. Electro-acupuncture is the product of modern scientific and technological progress. It is a combination of traditional acupuncture and electrical stimulation. It not only inherits the advantages of traditional acupuncture but also has the physiological effect of electrical stimulation. Electroacupuncture has the advantages of less side effects and controllable treatment intensity, frequency, and duration, which is beneficial to clinical treatment and research [18]. At present, electroacupuncture has been used to treat various diseases, such as stroke, arthritis, pain, depression, inflammatory bowel disease, etc. [19] More and more evidences support the role of electroacupuncture in maintaining body homeostasis and immune regulation, which may be the basis for electroacupuncture in the treatment of various inflammatory-related diseases [19–21]. Electroacupuncture with neuroprotective effect has achieved good therapeutic effect in the treatment of ischemic stroke patients and animal models, and the effect of electroacupuncture is closely related to its regulation of various pathological processes of cerebral infarction [22–24]. For example, in a rat model of cerebral ischemia/reperfusion, electroacupuncture can inhibit inflammation and oxidative stress, reduce the activity of microglia, and promote nerve regeneration, thereby promoting the recovery of motor function [25–27]. Our previous studies have shown that acupuncture can effectively improve the Cerebral Blood Flow (CBF) in the infarcted area, para-infarcted area, and mirror area, which provides a partial visual basis for clinical selection of acupoints on the heart meridian to treat ischemic cerebrovascular disease [28,29]. Therefore, this study would establish a middle cerebral artery embolism/reperfusion (MCAO/R) rat model and explore the molecular network regulation mechanism of acupuncture-intervention in MCAO/R rats through transcriptomics.

2. Materials and Methods

2.1. Experimental Materials

2.1.1. Reagents and Instruments. mRNA reverse transcription kit (Beijing Kangwei Century Biotechnology Co., Ltd., China, CW2569). Huatuo brand filiform needle 0.30 mm × 15 mm, Huatuo electroacupuncture therapeutic apparatus (SDZ-II) (Suzhou Medical Products Factory Co., Ltd., SDZ-II). Automatic enzyme labeling plate washer (PW-812), multi-function enzyme labeling analyzer (MB-530) (Shenzhen Huisong Technology Development Co., Ltd.). Fluorescence quantitative RCP instrument (Thermo, PIKOREAL96, USA). Electrophoresis apparatus (Beijing Liuyi Company, China, DYY-6C).

2.1.2. Experimental Animal. 12-week-old SPF-grade healthy male SD rats, weighing 280–300 g, were purchased from Hunan Slike Jingda Laboratory Animal Co., Ltd. (animal license number: SYXK (Xiang) 2018–0003, animal quality certificate number: 0017970). The experimental protocol was approved by the Animal Ethics Committee of Hunan University of Chinese Medicine; animal experiments were in accordance with the National Institute of Health's Guide for the Care and Use of Laboratory Animals. The rats were reared in an environment with constant temperature (22°C), constant humidity (60–70%), and a 12-h day cycle.

2.2. Experimental Methods

2.2.1. Establishment of Focal Cerebral Ischemia/Reperfusion Injury Model (MCAO/R). The MCAO/R model is constructed according to reference [30]. The rats were anesthetized by intraperitoneal injection of sodium pentobarbital (30 mg/kg) and fixed on the operating table. The neck of the rat was sterilized with 75% alcohol, the neck was opened in the middle, and the right common carotid artery, internal

carotid artery, and external carotid artery were isolated. A nylon surgical thread (18–22 mm) was then inserted into the left internal carotid artery for 120 min. Then, the insertion wire is pulled out to complete the blood reperfusion process in the ischemic area. In the sham operation group, the operation was the same as above except that the left internal carotid artery was not temporarily occluded. After the operation, the rat was placed on an electric blanket to keep warm and resuscitated, and the Langa score was performed after the rat was fully awake [30]. Scores 1–3 represent successful modeling and are included in subsequent experimental studies.

2.2.2. Grouping and Electroacupuncture. In this study, grouping was performed before modeling and intervention. The rats were randomly divided into a sham operation group (Sham group) ($n = 26$) and modeling group with reference to the random number table method after digital marking on the tail of the rat. After the MCAO/R model of the modeling group was successful, the modeling group was randomly divided into model group ($n = 28$) and electroacupuncture group (EA group) ($n = 28$) according to the random number table method. In the electroacupuncture group, stainless needles were used for electroacupuncture intervention, and all needles were inserted into the depth of 2–3 mm at Tianfu acupoint, Chize acupoint, Kongzui acupoint, and Taiyuan acupoint (The representative of the Hand Tai-yin meridian). After the rats were bundled, 15 mm, 36-gauge filigree needles were used to pierce the acupoints on the affected side, and then the electroacupuncture therapeutic apparatus was connected. After connecting the needle, the polarity is fixed, the distal end is negative, and the proximal end is positive. The parameters of electroacupuncture stimulation were: continuous wave, 20 Hz, voltage 2–4 V, current 4–6 mA, stimulation time 30 minutes. Rats in the model group and the sham-operated group were only bundled with the same degree and duration during the intervention, without electroacupuncture intervention. Electroacupuncture was performed 1 day after MCAO/R for 14 consecutive days, once a day. The experimental procedure is shown in Figure 1.

2.2.3. Neurological Score. Neurological function of the rat model was assessed using the Bederson score on days 1, 7, and 14 of electroacupuncture treatment. The evaluation criteria of the Bederson method are: 0 = no detectable defects; 1 = Rotation of trunk and contralateral forelimb when lifted by tail; 2 = Rat turns in circles to the contralateral side when the rat is fixed with the tail on a flat surface, but the posture is normal at rest; 3 = Reduced resistance to push on the opposite side, leaning to the opposite side at rest, turning significantly to the left; 4 = No spontaneous walking and low level of consciousness [31].

2.2.4. Pathological Observation. The brain tissue was fixed with 4% paraformaldehyde, dehydrated with gradient ethanol under vacuum in xylene, and then embedded in paraffin. The brain tissue was then sectioned coronally (5 μm), followed by Nissl staining and HE staining. Six different fields of view were randomly selected from each group of slices under an optical microscope (×400), and the images were processed by Image-Pro 6.2 software to observe the number of intact neurons in the ischemic cerebral cortex.

2.2.5. Terminal Deoxynucleotidyl Transferase-Mediated Nick End Labeling (TUNEL) Staining. Apoptotic cells in affected cerebral hemisphere were detected according to the method of TUNEL kit. Brain tissue sections deparaffinized in xylene were soaked in graded ethanol (100, 95, 90, 80, 70%) and then treated with proteinase K solution at 37°C for 15–30 min. After washing with phosphate buffered saline (PBS) buffer for 5 min, the sections were incubated with 50 μl terminal transferase (Tdt) and 450 μl horseradish peroxidase (HRP)-dUTP in a box for 2 hours at 37°C. After 3 washes with PBS, 50–100 μl of diaminobenzidine (DAB) substrate was added and left at room temperature for 10 minutes. Tissues were then washed and stained with hematoxylin. After dehydration with graded alcohol, after clearing with xylene, the brain tissue was sealed with neutral glue and imaged with a microscope at ×400. Image J was used to perform cell counting and to calculate apoptotic cell rate. Formula: Apoptotic cell rate (%) = number of positive cells/total number of cells.

2.3. Transcriptomic Methods. Total RNA was extracted from the CA1 region of the affected hippocampus of 3 rats in sham operation group, 4 rats in MCAO group, and 4 rats in electroacupuncture group by TRIZOL method. Nanodrop 2000 detects the concentration and purity of samples, evaluates RNA integrity by agarose gel electrophoresis, and determines RNA integrity number (RIN). The total amount of RNA is 1 μg, the concentration is greater than or equal to 50 ng/uL, and the 0D260/280 value is between 1.8 and 2.2; then the library was constructed. Samples with RIN score >8 were used for sequencing. Eukaryotic mRNA sequencing is based on the HiSeq sequencing platform to sequence all mRNAs transcribed from a sample. The Illumnina TnuseqTM RNA sample prep Kit method was used to construct the IlluminaPE library for 2×150 bp sequencing. After the QuantiFluor dsDNA System is quantified, it is mixed according to the data ratio, and then the bridge PCR amplification was performed on the cBot to generate groups. Transcriptome data were analyzed after quality control. After the data quality control was completed, statistics and quality assessment were performed again. The DESeq2 package in R software was used for differential expression analysis of transcriptomic data. P-adjust <0.05, Log2FC >1 or <−1 were considered differential genes.

2.4. Bioinformatics Analysis. The protein–protein interaction (PPI) data were collected from String (https://cn.string-db.org/) with medium confidence >0.4 and the "Organism" was limited to "*Rattus norvegicus*" [32]. Differentially

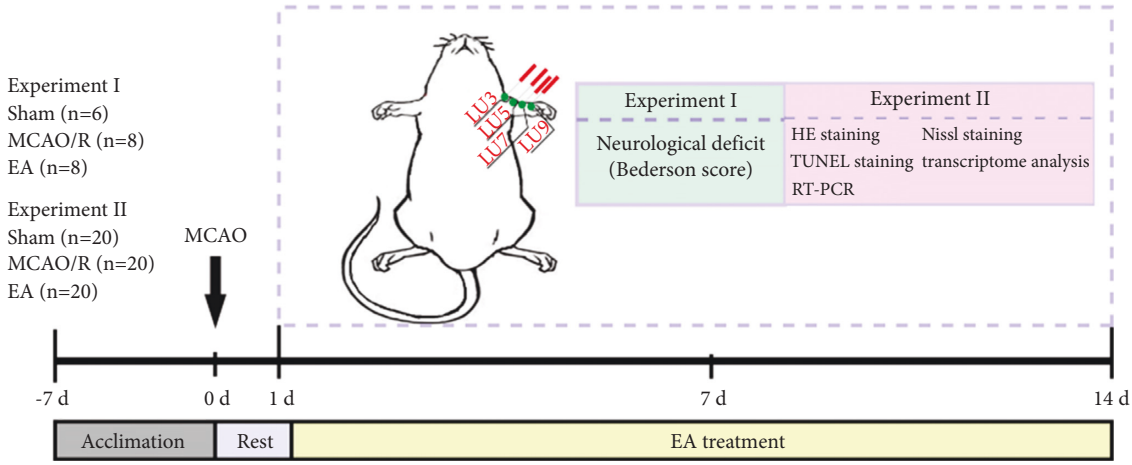


Figure 1: The experimental procedure.

expressed genes were imported into Metascape (https://metascape.org/gp/index.html#/main/step1) for enrichment analysis to obtain Gene Ontology (GO) enrichment results, Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway, and Reactome pathway [33]. Gene Set Enrichment Analysis (GSEA) was performed by GSEA 4.3.0 software (https://www.gsea-msigdb.org/gsea/index.jsp) [34].

2.5. Detection of BDNF, Grin2a, Plexin D1, and Caspase 9 mRNA Expression by Real-Time PCR. The RNA extraction of CA1 region of the affected hippocampus was using the TRIzol Reagent following the instructions. First-strand cDNA was generated from MMLV transcriptase using random primers. Real-time RT-PCR was performed on a CFX96 real-time PCR detection system and gene detection was performed using the Roche SYBR FAST Universal qPCR kit. All primers were purchased from GeneCopoeia. The thermocycling conditions consisted of an initial denaturation for 2 min at 95°C, followed by 40 cycles of 20 sec at 95°C, 30 sec at 60°C, and 40 sec at 72°C. All reactions were performed in an ABI 7500 System. Actin was used as the reference gene. The relative gene expression was calculated using $2^{-\Delta\Delta Ct}$ method.

2.6. Statistical Analysis. SPSS version 19.0 was used for statistical analysis and data are presented as mean ± standard deviation. Analyses were performed using one-way ANOVA followed by Bonferroni correction or unpaired Student's *t*-test. $P < 0.05$ was considered to indicate a statistically significant difference.

3. Results

3.1. Efficacy of Electroacupuncture

3.1.1. Neurological Score. Compared with the sham operation group, the neurological deficit score in the MCAO/R group was increased ($P < 0.05$), indicating that the modeling was successful. Compared with the sham operation group, the neurological function score in the electroacupuncture group was decreased ($P < 0.05$), suggesting that electroacupuncture treatment may improve the neurological function score (Figure 2).

3.1.2. Pathological Changes. HE staining showed that compared with the Sham group, the brain tissue of the model group was obviously loose and the number of nerve cells was significantly reduced. Its structure and shape are irregular, the arrangement is unclear, the surrounding swelling is loose, the cytoplasm is lightly stained, and the nucleus is dissolved. Compared with the model group, the pathological improvement of the brain tissue of the EA group was slightly improved on the whole, the number of nerve cells around the infarct was slightly increased, and the structure and shape were more regular. The results of Nissl staining showed that compared with the sham operation group, the Nissl bodies in the model group were significantly reduced. Compared with the model group, the Nissl body in the EA group and the acupuncture group increased significantly (Figure 3).

3.1.3. Neuronal Apoptosis. Under light microscopy, the nuclei of apoptotic cells appeared brown (Figure 2). Compared with the sham operation group, the percentage of neuronal apoptosis increased in the MCAO/R group ($P < 0.05$). Compared with the MCAO/R group, the percentage of neuronal apoptosis in the electroacupuncture group was significantly reduced ($P < 0.05$). This suggests that electroacupuncture stimulation can alleviate neurological dysfunction by reducing neuronal apoptosis (Figure 4).

3.2. Differentially Expressed Genes. Comparing the sham operation group with the model group (Model/Sham group), a total of 1128 differentially expressed genes were obtained, of which 713 were upregulated and 415 were downregulated (Figure 5) (see Table S1). The results of preliminary enrichment showed that the biological processes involved after MCAO/R include regulation of ion transport, cell morphogenesis, regulation of ion transmembrane transport, regulation of transmembrane transport, cell junction organization, synaptic signaling, neuron projection

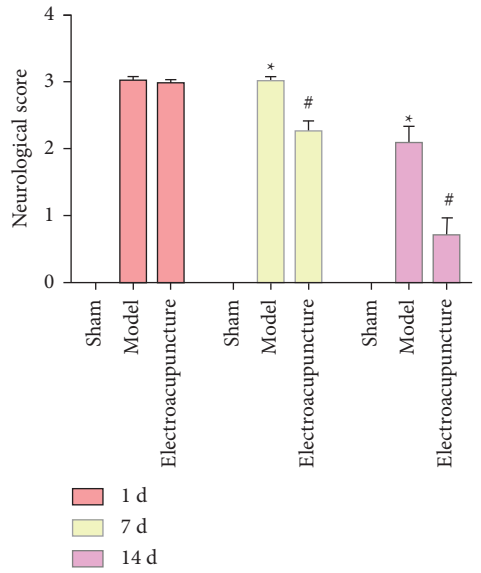


FIGURE 2: Neurological score (Data are presented as the mean ± SD. *Compared with control group, $P < 0.05$; #compared with model group, $P < 0.05$, t-test).

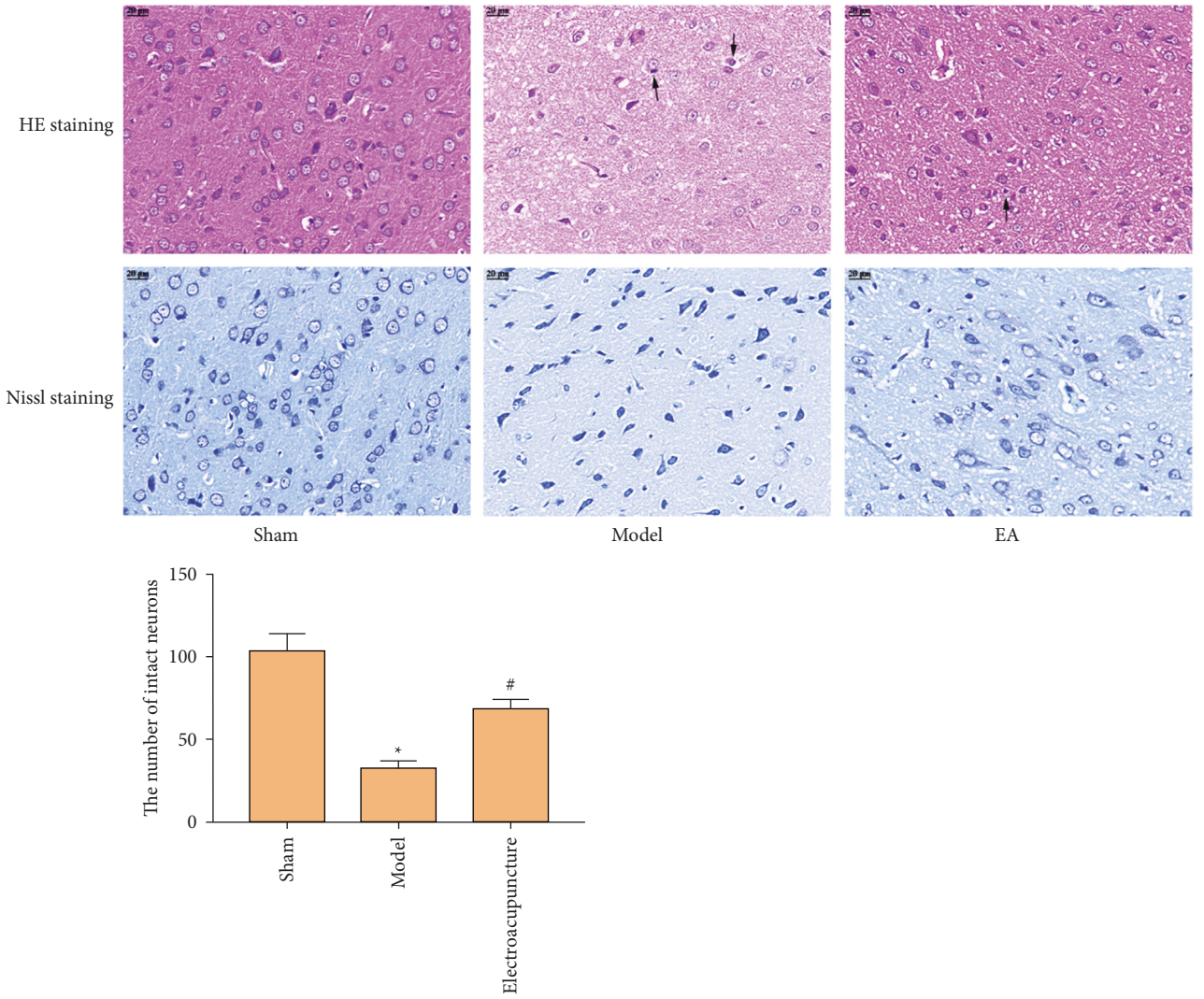


FIGURE 3: Pathological changes (400×; pathological cells are indicated by black arrows. Data are presented as the mean ± SD. *Compared with control group, $P < 0.05$; #compared with model group, $P < 0.05$, t-test).

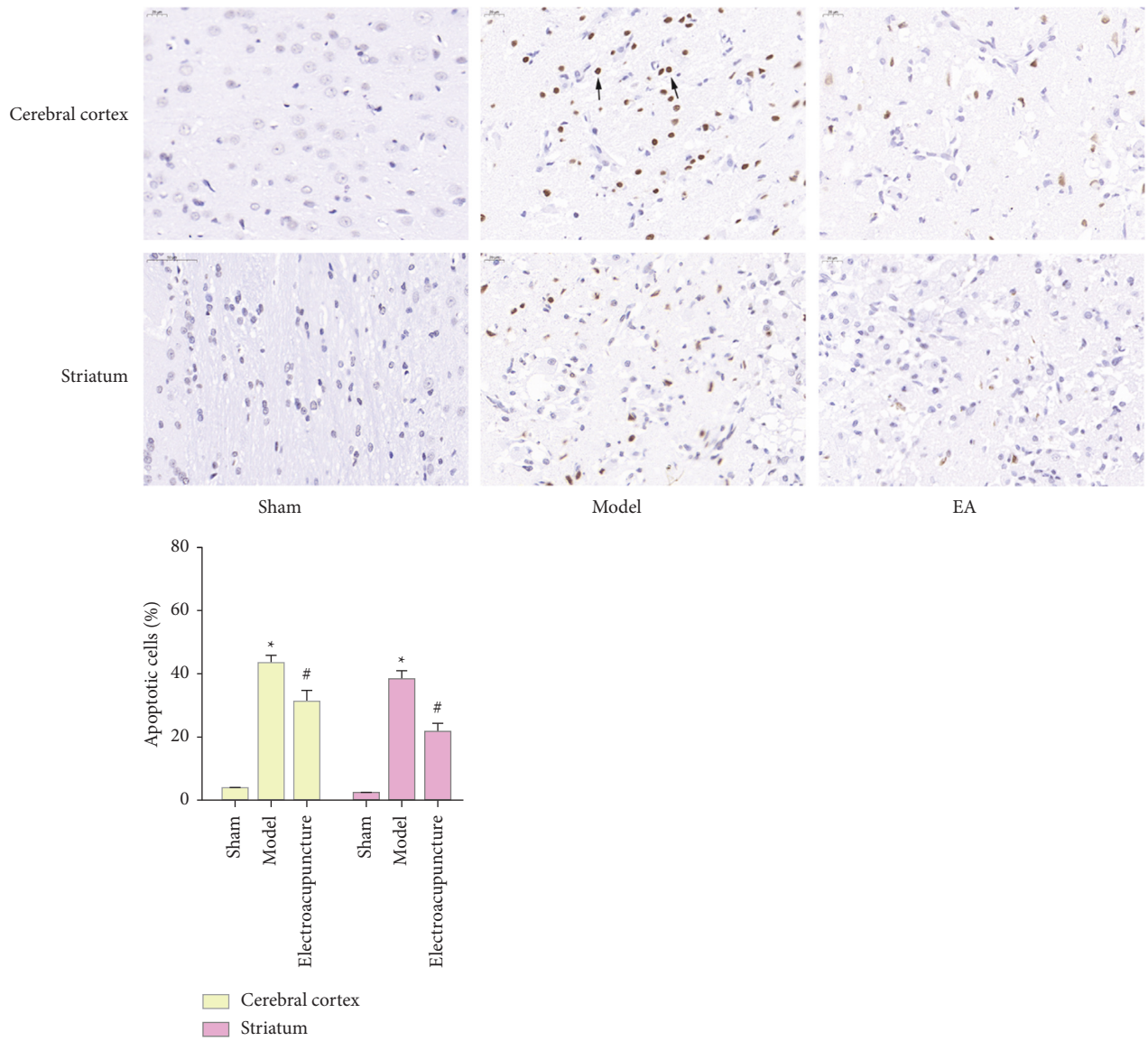


Figure 4: Neuronal apoptosis (400×, Tunel staining. Data are presented as the mean ± SD. ∗ Compared with control group, $P < 0.05$; #compared with model group, $P < 0.05$, t-test; pathological cells are indicated by black arrows).

development, cellular component morphogenesis, neuron projection morphogenesis, plasma membrane bounded cell projection morphogenesis, trans-synaptic signaling, cell projection morphogenesis, regulation of system process, and cell morphogenesis involved in differentiation. The pathways involved Neuroactive ligand-receptor interaction, Calcium signaling pathway, Axon guidance, Circadian entrainment, Aldosterone synthesis and secretion, cAMP signaling pathway, Amphetamine addiction, Inflammatory mediator regulation of TRP channels, ECM-receptor interaction, Long-term potentiation, Transcriptional misregulation in cancer, Glutamatergic synapse, Long-term depression, Adrenergic signaling in cardiomyocytes, and Wnt signaling pathway (Figure 6 and Table S2).

Comparing the model group with the EA group (EA/Model group), a total of 1097 differentially expressed genes were obtained, of which 422 were upregulated and 675 were downregulated (Figure 5) (see Table S3). The upregulated and downregulated genes were input into Cytoscape to construct network (Figure 7). The top 10 upregulated genes were Ptk2b (47 edges), Wnt2 (46 edges), Crebbp (40 edges), Erbb3 (36 edges), Gnb4 (34 edges), Grin2a (31 edges), Gria1 (30 edges), Kit (29 edges), Ldb3 (26 edges), and Fa2h (26 edges); the top 10 downregulated genes were Alb (63 edges), Igf1 (46 edges), Gnb3 (37 edges), Mapk11 (37 edges), Stat4 (32 edges), Plcg2 (31 edges), Mapk13 (27 edges), Mef2c (27 edges), Mapk12 (27 edges), and Cd40 (25 edges).

3.3. Bioinformatics Analysis for Differentially Expressed Genes of EA/Model Group

3.3.1. Upregulated Gene Analysis. The upregulated genes were input into Metascape for enrichment analysis. The biological processes include axon development, cellular component morphogenesis, cell junction organization,

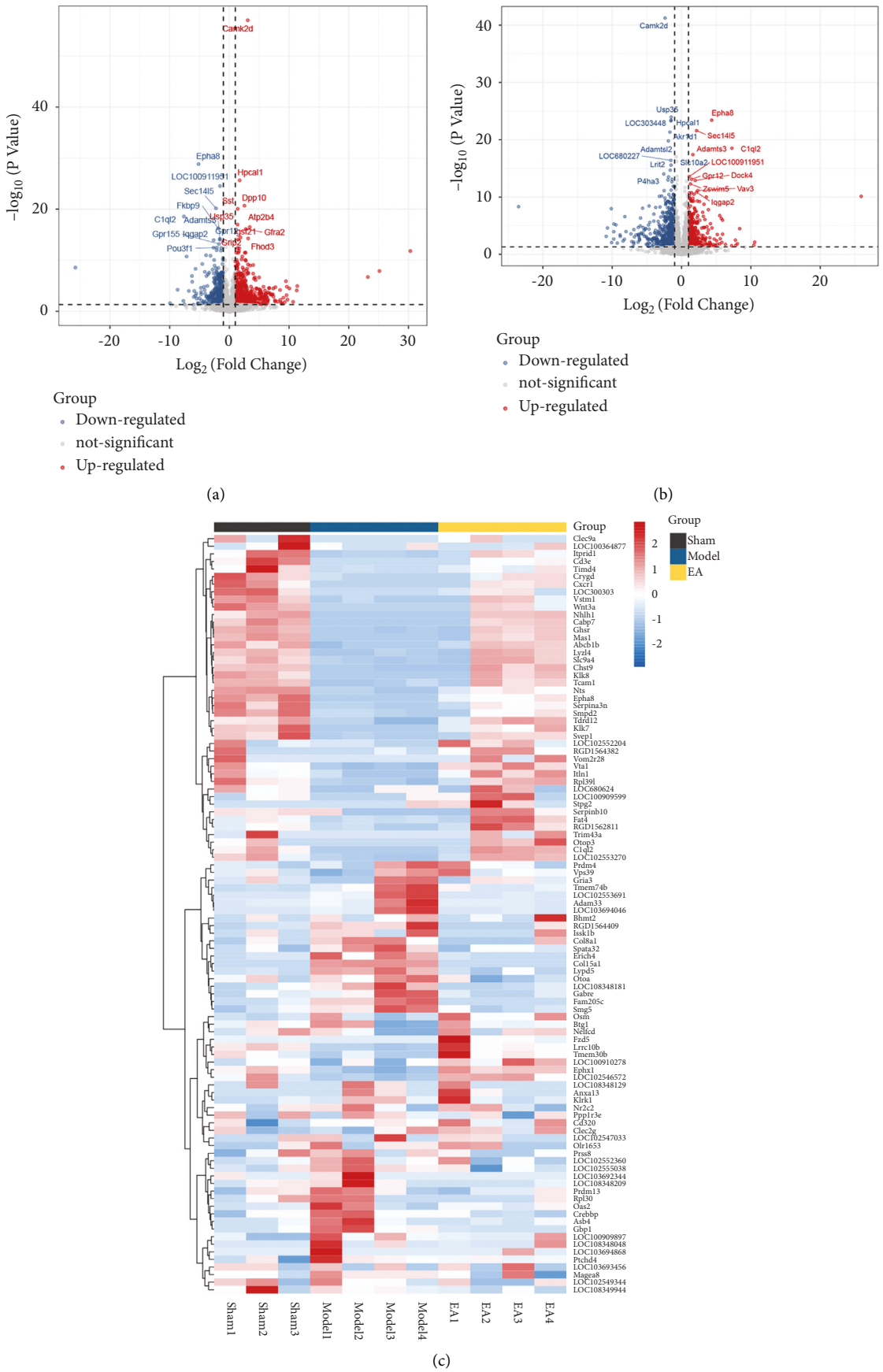


FIGURE 5: Differentially expressed genes. (a) Volcano plot of model/sham group. (b) Volcano plot of EA/model group. (c) Heatmap of top 50 genes.

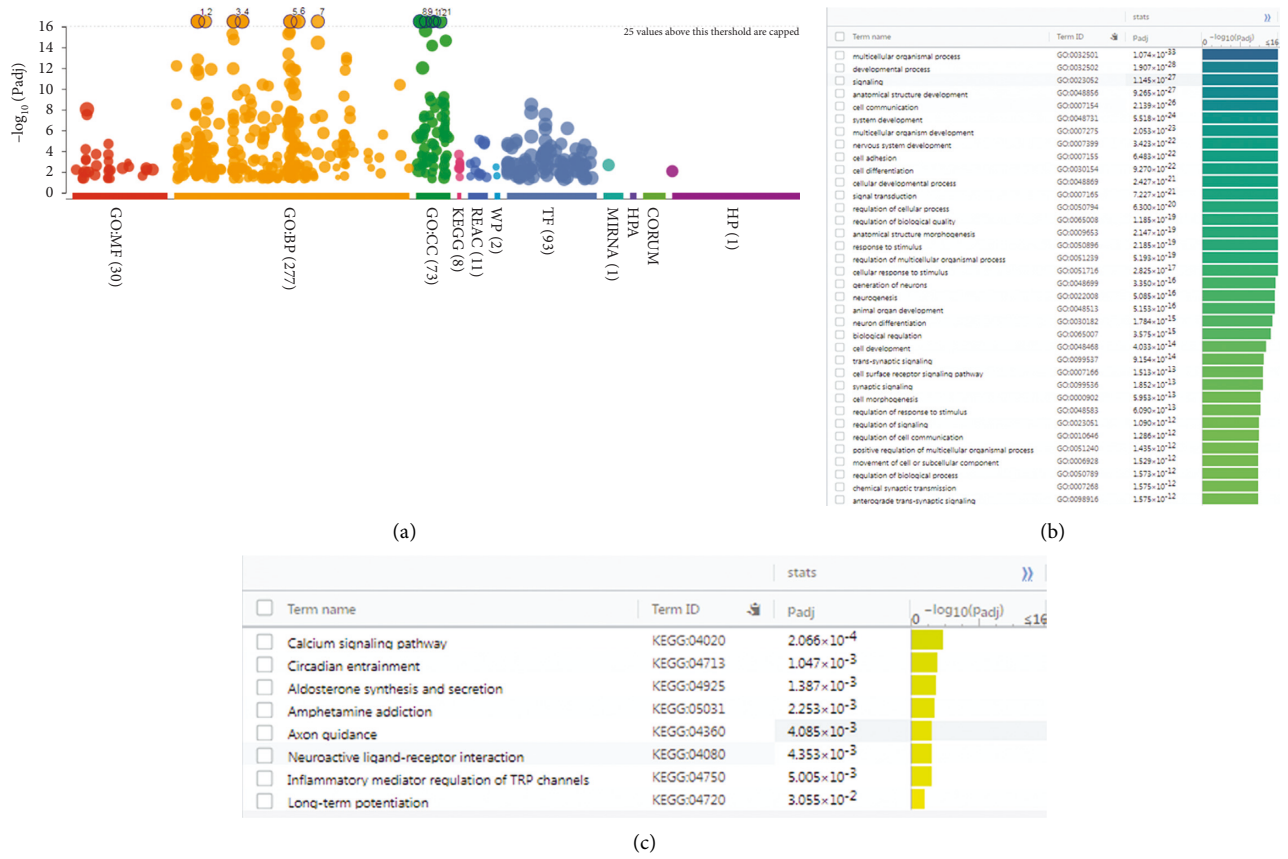


Figure 6: The results of preliminary enrichment of model/sham group. (a) Overview map. (b) Biological processes. (c) Signaling pathways.

neuron projection development, neuron projection morphogenesis, plasma membrane bounded cell projection morphogenesis, cell projection morphogenesis, cell part morphogenesis, axonogenesis, cell adhesion, axon guidance, neuron projection guidance, chemotaxis, and so on. The signaling pathway include Axon guidance, Basal cell carcinoma, Glutamatergic synapse, Nicotine addiction, Notch signaling pathway, Long-term potentiation, MAPK signaling pathway, Hedgehog signaling pathway, Circadian entrainment, and Wnt signaling pathway. The Reactome pathway include Neuronal System, Protein–protein interactions at synapses, Transmission across Chemical Synapses, Neurotransmitter receptors and postsynaptic signal transmission, Unblocking of NMDA receptors, glutamate binding and activation, SLC-mediated transmembrane transport, RHOG GTPase cycle, Receptor-type tyrosine-protein phosphatases, EPH-ephrin-mediated repulsion of cells, Activation of NMDA receptors and postsynaptic events, Signaling by Receptor Tyrosine Kinases, Synaptic adhesion-like molecules, RAF-independent MAPK1/3 activation, and Glutamate Neurotransmitter Release Cycle (Figure 8 and Table S4).

3.3.2. Downregulated Gene Analysis. The downregulated genes were input into Metascape for enrichment analysis. The biological processes include regulation of system process, negative regulation of G protein-coupled receptor signaling pathway, regulation of cytokine production, regulation of cell communication by electrical coupling, phospholipase C-activating G protein-coupled receptor signaling pathway, positive regulation of cytokine production, synaptic signaling, regulation of blood circulation, muscle contraction, trans-synaptic signaling, negative regulation of ion transport, anion transport, modulation of excitatory postsynaptic potential, regulation of membrane potential, chemical synaptic transmission, and so on. The signaling pathway includes Inflammatory mediator regulation of TRP channels, Neuroactive ligand-receptor interaction, NOD-like receptor signaling pathway, Steroid hormone biosynthesis, Prolactin signaling pathway, Leukocyte transendothelial migration, and Protein digestion and absorption. The Reactome pathway includes Class B/2 (Secretin family receptors), GPCR ligand binding, Bile acid and bile salt metabolism, Recycling of bile acids and salts, Nucleotide-binding domain, Leucine-rich repeat containing receptor (NLR) signaling pathways, Signaling by GPCR, G alpha (z) signaling events, G alpha (q) signaling events, Synthesis of bile acids and bile salts via 27-hydroxycholesterol, Ion channel transport, Synthesis, secretion, and inactivation of Glucagon-like Peptide-1 (GLP-1), Myogenesis, Immunoregulatory interactions between a

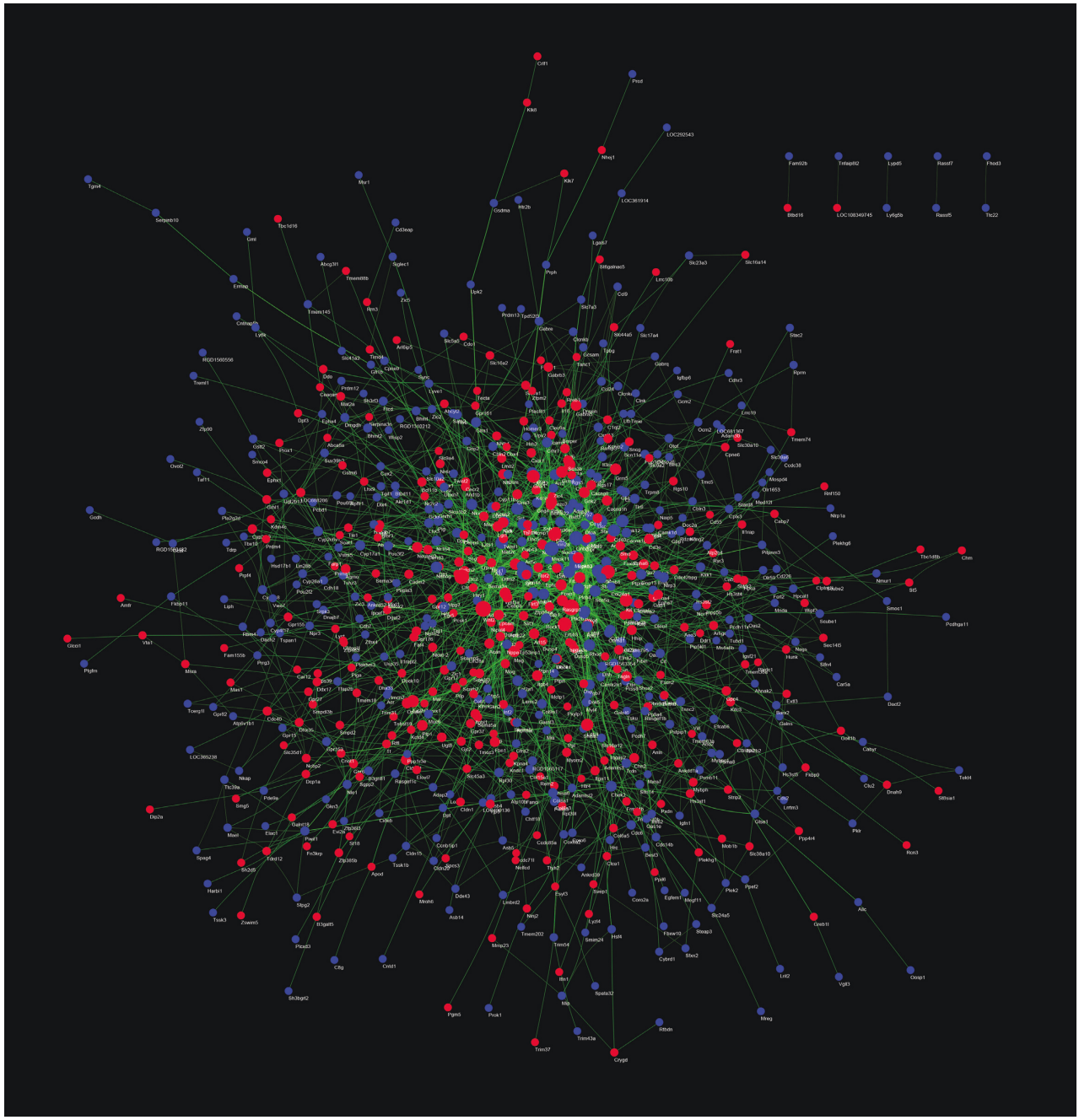
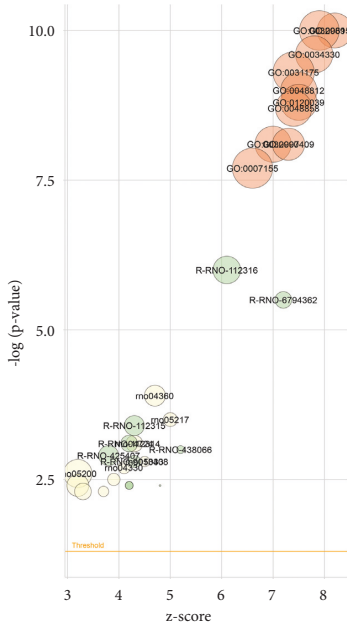


Figure 7: PPI network of differentially expressed genes (red circles stand for upregulated genes; blue circles stand for downregulated genes).

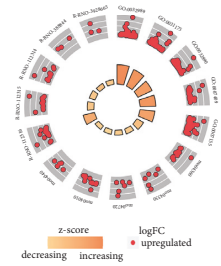
Lymphoid and a nonlymphoid cell, Adrenaline, noradrenaline inhibits insulin secretion, Incretin synthesis, secretion, inactivation, and so on (Figure 9, Table S5).

3.3.3. All Gene Analysis. The up- and down-regulated genes were input into Metascape for enrichment analysis. The biological processes include chemotaxis, taxis, cell junction organization, regulation of system process, neuron projection morphogenesis, cell adhesion, axon development, plasma membrane bounded cell projection morphogenesis, cell projection morphogenesis, neuron projection development, axon guidance, cell morphogenesis, neuron projection guidance, cellular component morphogenesis, and so on. The signaling pathway include Neuroactive ligand-receptor interaction, Inflammatory mediator regulation of TRP channels, Axon guidance, Nicotine addiction, Calcium signaling pathway, Circadian entrainment, Retrograde endocannabinoid signaling, Glutamatergic synapse, Leukocyte transendothelial migration, Dopaminergic synapse. The Reactome pathway includes Neuronal System, Neurotransmitter receptors and postsynaptic signal transmission, Transmission across Chemical Synapses, Protein–protein interactions at synapses, Class B/2 (Secretin family



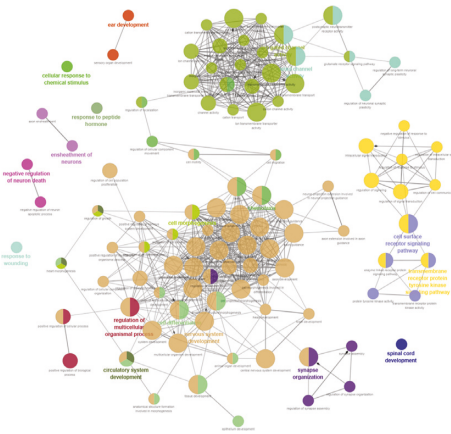
ID	Description
GO:0061564	axon development
GO:0032989	cellular component morphogenesis
GO:0034330	cell junction organization
GO:0031175	neuron projection development
GO:0048812	neuron projection morphogenesis
GO:0120039	plasma membrane bounded cell projection morphogenesis
GO:0048858	cell projection morphogenesis
GO:0032990	cell part morphogenesis
GO:0007409	axonogenesis
GO:0007155	cell adhesion
rno04360	Axon guidance
rno05217	Basal cell carcinoma
rno04724	Glutamatergic synapse
rno05033	Nicotine addiction
rno04330	Notch signaling pathway
rno05200	Pathways in cancer
R-RNO-112316	Neuronal System
R-RNO-6794362	Protein-protein interactions at synapses
R-RNO-112315	Transmission across Chemical Synapses
R-RNO-112314	Neurotransmitter receptors and postsynaptic signal transmission
R-RNO-438066	Unblocking of NMDA receptors, glutamate binding and activation
R-RNO-425407	SLC-mediated transmembrane transport
R-RNO-9013408	RHOG GTPase cycle

(a)

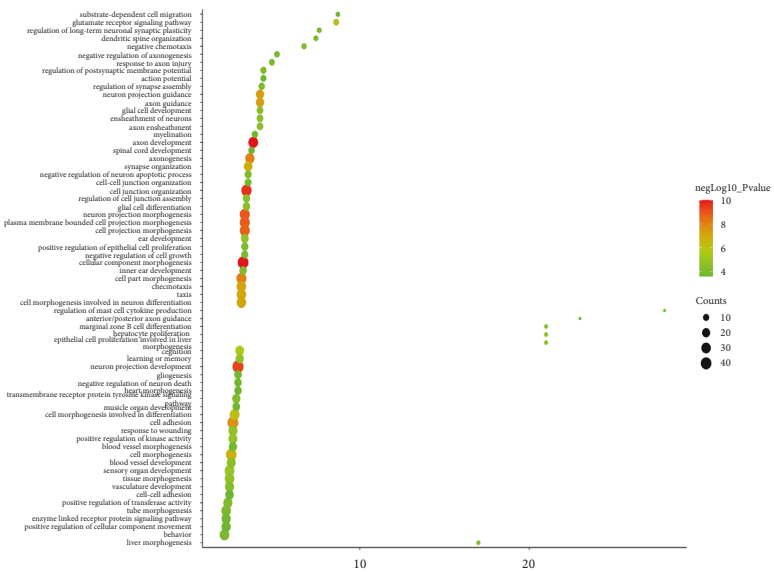


ID	Description
GO:0032989	cellular component morphogenesis
GO:0031175	neuron projection development
GO:0032990	cell part morphogenesis
GO:0007409	axonogenesis
GO:0007155	cell adhesion
rno04360	Axon guidance
rno04330	Notch signaling pathway
rno04720	Long-term potentiation
rno04010	MAPK signaling pathway
rno04340	Hedgehog signaling pathway
R-RNO-112316	Neuronal System
R-RNO-112315	Transmission across Chemical Synapses
R-RNO-112314	Neurotransmitter receptors and postsynaptic signal transmission
R-RNO-388844	Receptor-type tyrosine-protein phosphatases
R-RNO-3928665	EPH-ephrin mediated repulsion of cells

(b)



(c)



(d)

Figure 8: Continued.

(e) (f)

Figure 8: Upregulated gene analysis. (a) Top 10 results of each category. (b) Gene expression profiles of the top 5 enrichment results; (c) enrichment results profile. (d) bubble chart of biological processes; (e) bubble chart of signaling pathways; (f) bubble chat of reactome pathways; *X*-axis stands for enrichment value.

receptors), Transport of small molecules, G alpha (*q*) signaling events, GPCR ligand binding, G alpha (*z*) signaling events, Highly calcium permeable nicotinic acetylcholine receptors, Adrenaline, noradrenaline inhibits insulin secretion, Unblocking of NMDA receptors, glutamate binding and activation, Signaling by GPCR, Phase 0–rapid depolarization, Receptor-type tyrosine-protein phosphatases, and so on (Figure 10, Table S6).

3.4. Gene GSEA Results for Differentially Expressed Genes of EA/Model Group. The GSEA results showed that the upregulated genes were mainly concentrated in GO results such as Schwann Cell Development, Cellular Response To Prostaglandin E Stimulus, Intracellular Sterol Transport, Midbrain Development, CopII Vesicle Coat, Inclusion Body Assembly, Multivesicular Body Sorting Pathway, Neurotransmitter Receptor Internalization, Negative Regulation Of Endocytosis, Autophagosome Organization, and so on; and the downregulated genes were mainly concentrated in Anchored Component Of External Side Of Plasma Membrane, Oxygen Binding, Intrinsic Component Of External Side Of Plasma Membrane, Cgmp-Mediated Signaling, Chemokine Activity, Nitric Oxide–Mediated Signal Transduction, Neuropeptide Hormone Activity, CCR Chemokine Receptor Binding, Chemokine Receptor Binding, Synaptic Transmission Cholinergic, and so on. The upregulated genes were mainly concentrated in signaling pathways such as Basal Transcription Factors, Peroxisome, Alzheimer's Disease, Steroid Biosynthesis, Mapk Signaling Pathway, ERBB Signaling Pathway, Valine Leucine And Isoleucine Degradation, Regulation Of Autophagy, Wnt Signaling Pathway, Adipocytokine Signaling Pathway and so on; and the downregulated genes were mainly concentrated in signaling pathways such as Taste Transduction, Intestinal Immune Network For Iga Production, Steroid Hormone Biosynthesis, Retinol Metabolism, Tyrosine Metabolism, Neuroactive Ligand Receptor Interaction, Cytokine Cytokine Receptor Interaction, Nod-Like Receptor Signaling Pathway, Pentose And Glucuronate Interconversions, Complement And Coagulation Cascades. The top 5 results were shown in Figures 11 and 12.

3.5. Hub Genes Expression Validation. According to the results of bioinformatics analysis and GSEA, four genes (Caspase9, BDNF, Grin2a, and plexinD1) were selected to validate in ischemic hippocampus. The results of RT-PCR showed compared with the sham operation group, the expression of Caspase 9 mRNA increased, and the expression of BDNF, plexinD1, and Grin2a mRNA decreased in MCAO/R groups. Compared with MCAO/R group, the expression of Caspase9 mRNA decreased, and the expression of BDNF and PlexinD1 mRNA increased in the EA group (Figure 13).

4. Discussion

Stroke is a frequently occurring disease among adults in modern society, and it is one of the most common diseases with high disability and high fatality, with a disability rate as high as 33.4%–44.6% [35, 36]. Among them, ischemic stroke is the most important type of stroke in clinical practice, and its incidence accounts for more than 50% of all cerebrovascular diseases [37]. In terms of clinical treatment strategies, reperfusion therapy can improve the clinical symptoms of patients to a certain extent. However, even with the standard therapy of intravenous thrombolytic drugs combined with endovascular thrombectomy, there are still a large number of patients with severe disability, which may be accompanied by complications such as intracranial hemorrhage after thrombolysis. Evidence-based medical studies on the successful endovascular treatment of stroke found that only 18.5%–32.5% of stroke patients could achieve successful reperfusion in time within 3–8 hours after the onset of stroke. Even if patients receive standard medical treatment at an early stage, 50% to 60% of patients still have neuromotor dysfunction of varying degrees [38, 39]. Therefore, it is very important to explore more effective therapies, especially for the improvement of neurological function in the recovery period of cerebral ischemia. Clinical practice and experimental studies have confirmed that acupuncture is safe and effective in treating cerebral ischemic sequelae, and it has been more and more widely used worldwide [40, 41]. Another meta-analysis showed that acupuncture exerted a potential neuroprotective effect in

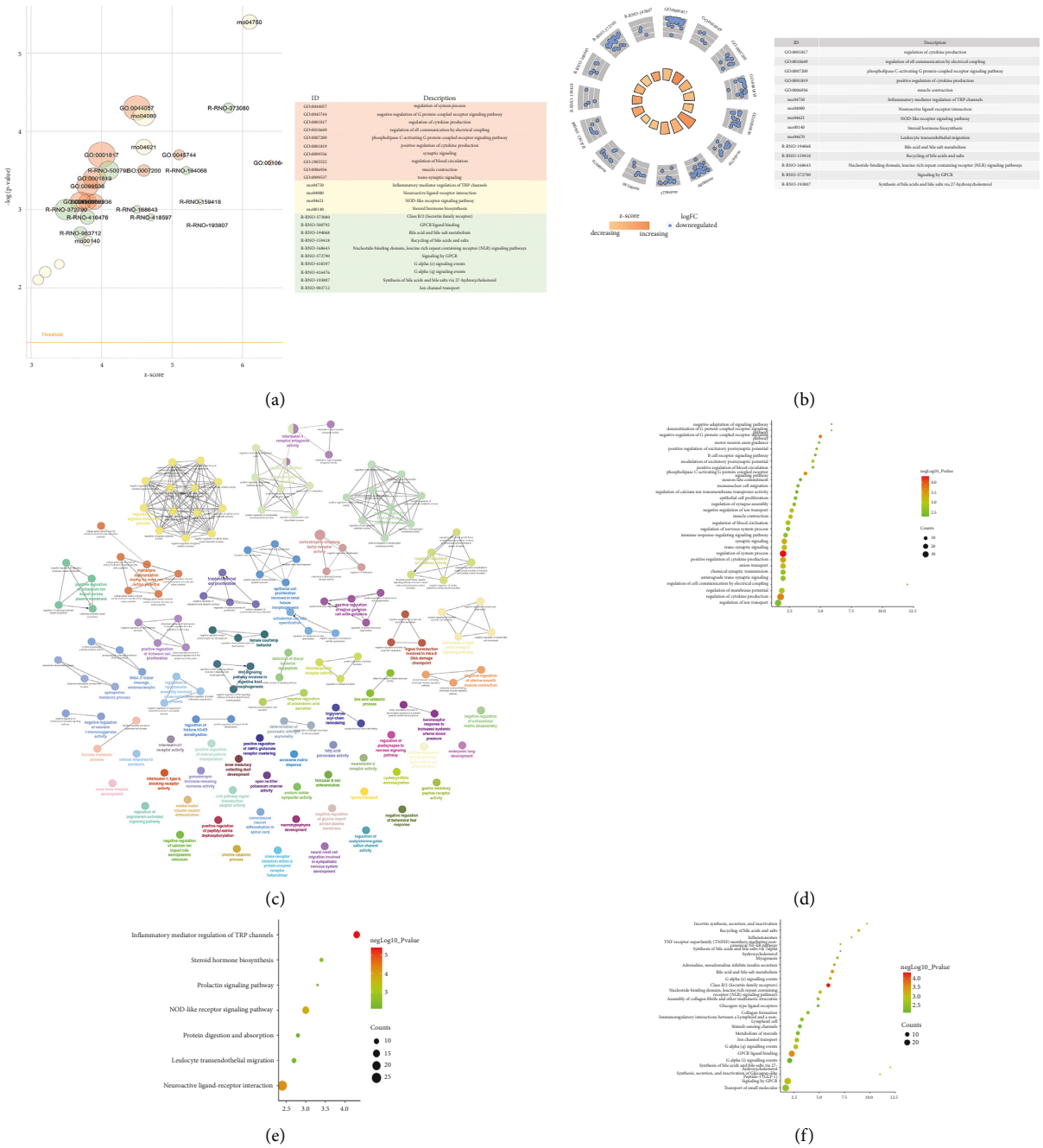


Figure 9: Downregulated gene analysis. (a) Top 10 results of each category. (b) Gene expression profiles of the top 5 enrichment results. (c) Enrichment results profile. (d) Bubble chart of biological processes. (e) Bubble chart of signaling pathways; (f) Bubble chat of reactome pathways; *X*-axis stands for enrichment value.

ischemic stroke, reducing infarct volume and improving neurological function scores [42]. Acupuncture on human acupoints, such as Quchi [43] and Baihui [44], can improve neurological function after cerebral ischemia. Traditional Chinese medicine believes that acupuncture at Neiguan acupoint can calm and dredge the meridians. Acupuncture at Neiguan and Dazhui points can significantly reduce the expression of TNF-α and NF-κB-p65 in the ischemic hippocampal CA1 region [45], thereby reducing cerebral ischemia injury. Our previous study also found that acupuncture at Neiguan acupoint could improve the recovery of neurological function in rats with middle cerebral artery occlusion [46]. This study found that electroacupuncture could improve neurological function and neuronal apoptosis in MCAO/R model rats.

Cerebral infarction is most likely to cause a large number of free radical formation, calcium overload, excitatory amino acid toxicity, inflammatory mediators, and other related changes in the brain tissue in the blood flow supply area after the interruption of blood flow [47], which further leads to

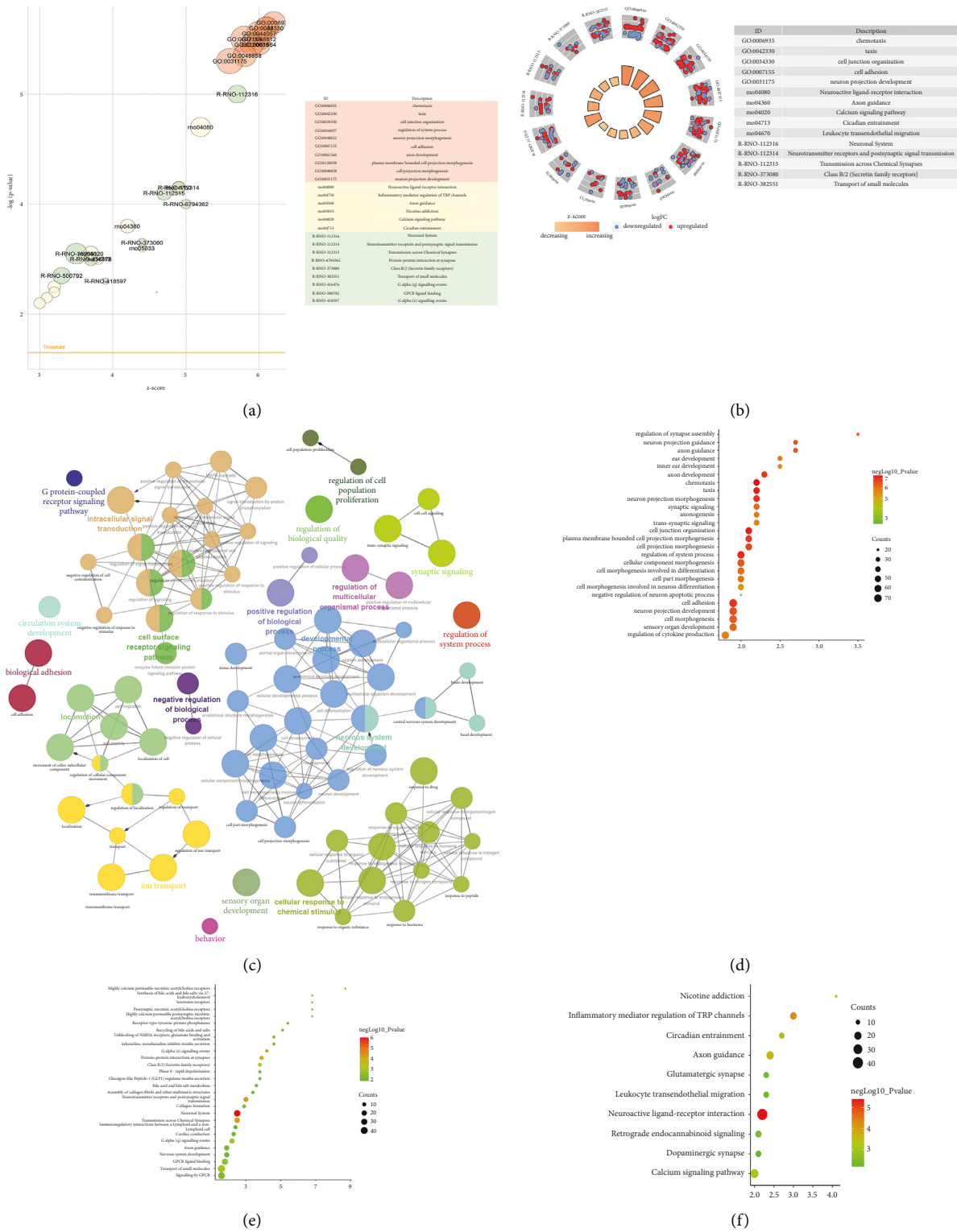


FIGURE 10: All gene analysis. (a) Top 10 results of each category. (b) Gene expression profiles of the top 5 enrichment results; (c) enrichment results profile. (d) Bubble chart of biological processes; (e) bubble chart of signaling pathways; (f) bubble chat of reactome pathways; *X*-axis stands for enrichment value.

the breakdown of the blood–brain barrier in the brain tissue, resulting in increased blood–brain barrier (BBB) permeability, edema in the brain tissue, and then neuronal cell death [48]. Neurovascular unit (NVU) refers to the basic functional unit composed of neurons, BBB, astrocytes, extracellular matrix, and microvessels [49]. When studying cerebral infarction, taking NVU as a whole can help better understand the pathological mechanism of brain injury

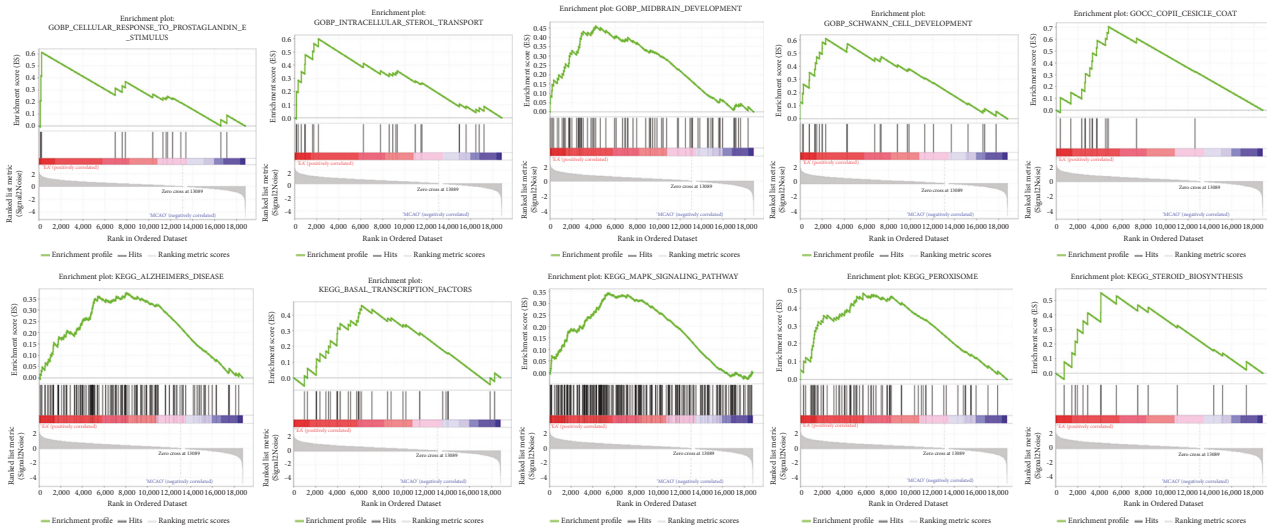


Figure 11: The top 5 GO results and signaling pathways in GSEA (upregulated genes).

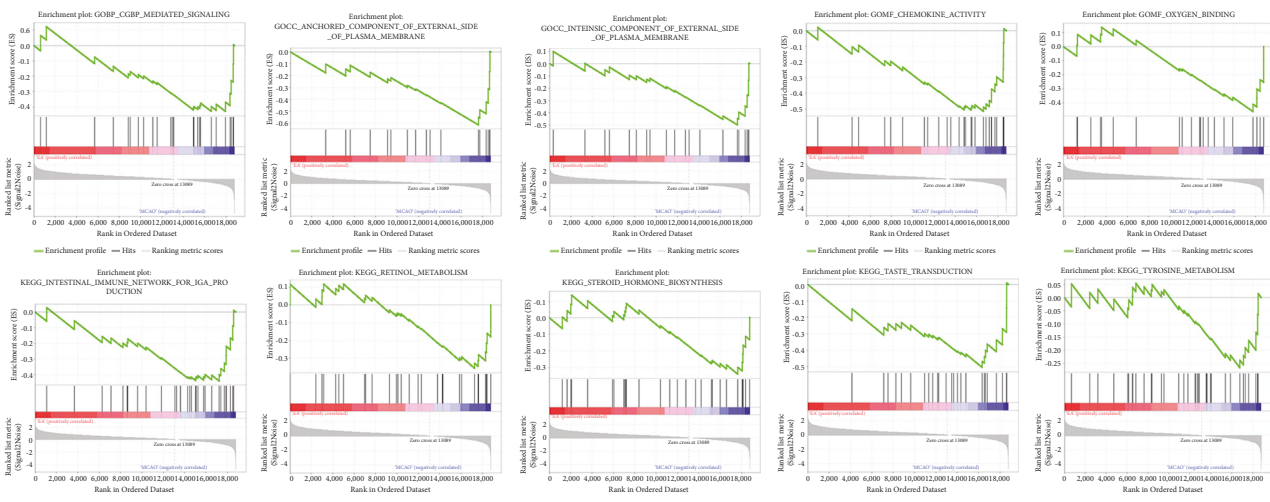


Figure 12: The top 5 GO results and signaling pathways in GSEA (downregulated genes).

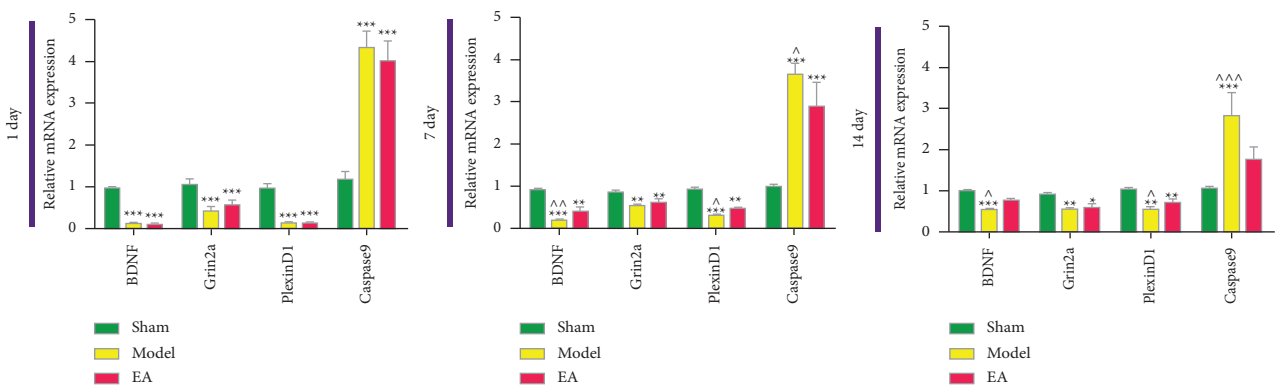


Figure 13: Validation of the expression patterns of four hub genes by RT-qPCR (data are presented as the mean ± SD. Compared with the sham operation group, $^{***}P < 0.001$, $^{**}P < 0.01$ and $^{*}P < 0.05$; compared with EA group, $^{\wedge\wedge\wedge}P < 0.001$, $^{\wedge\wedge}P < 0.01$ and $^{\wedge}P < 0.05$ in the corresponding time, *t*-test).

process, which is helpful for the treatment of cerebral infarction. In the NVU, microvascular endothelial cells, glial cells, basement membrane, etc., together constitute the BBB [50], and microvessels play an important role in the energy supply of brain cells in brain tissue. BBB plays an important role in maintaining the stability of the internal environment of the nervous system, controlling the normal exchange of ions and water, and maintaining the balance of cerebrospinal fluid [51]. Protecting the BBB in the early stage, ensuring the energy supply of microvessels to brain cells, and reducing the death of neuronal cells play an important role in the treatment of cerebral infarction.

This study explored the molecular network mechanism of electroacupuncture stimulation of hand Tai-yin meridian in the treatment of cerebral ischemia-reperfusion by mRNA high-throughput sequencing analysis. Compared with MCAO/R group, 485 upregulated mRNA and 611 downregulated mRNA were obtained. In the network, the top 10 upregulated genes were Wnt2 (48 edges), Ptk2b (43 edges), Crebbp (42 edges), Kdr (37 edges), Fgfr1 (35 edges), Gria1 (32 edges), Ntrk1 (32 edges), Kit (31 edges), Ldb3 (27 edges), and Pdgfb (26 edges); the top 10 downregulated genes were Alb (58 edges), Igf1 (51 edges), Stat4 (30 edges), Pax6 (29 edges), Mef2c (26 edges), Rhod (25 edges), Sst (25 edges), Mapk13 (23 edges), Rnd1 (23 edges), and Nanog (22 edges). The enrichment analysis results and GSEA results showed that those differentially expressed genes were related to Chemotaxis, Taxis, Cell junction organization, Neuron projection morphogenesis, Cell adhesion, Axon development, Neuroactive ligand–receptor interaction, Inflammatory mediator regulation of TRP channels, Axon guidance, Calcium signaling pathway, Circadian entrainment, Retrograde endocannabinoid signaling, Glutamatergic synapse, Leukocyte transendothelial migration, Dopaminergic synapse, Neuronal System, Neurotransmitter receptors and postsynaptic signal transmission, Transmission across Chemical Synapses, Protein–protein interactions at synapses, and so on. In addition, some biological processes and signaling pathways (such as synaptic Signaling, Neuron projection development, Cellular component morphogenesis, Neuron projection morphogenesis, Neuroactive ligand-receptor interaction, Calcium signaling pathway, Axon guidance, Circadian entrainment, Glutamatergic synapse) in the Model/Sham group can also be found in the EA/Model group, suggesting that these pathways may be the core pathways for EA to treat cerebral ischemia-reperfusion injury.

The results of high-throughput sequencing transcriptomics were further validated by qRTPCR. Among them, brain-derived neurotrophic factor (BDNF) is widely distributed in the central system and is one of the important neurotrophic factors that maintain the survival of neurons in the brain [52]. It is secreted by the pyramidal cells of the cerebral cortex and transported anterogradely to the nerve endings through the neuron cell body, thereby nourishing the distal tissues [53]. When the cortex is damaged, the anterograde transported BDNF decreases and cannot continue to maintain the survival of neurons. It may be one of the main factors of motor sensory pathway damage [53]. However, the study found that the expression of BDNF mRNA in the bilateral cerebral cortex in the early stage of stroke was upregulated, and the mRNA expression in the contralateral cortex was higher than that in the affected side. This suggests that the early changes of BDNF on the contralateral side may be involved in the repair and regeneration of damaged cortical nerves [54, 55]. The present study demonstrates that reactivation of Sema3E-Plexin-D1 signaling after ischemic stroke is critical for the re-establishment of healthy vasculature through modulation of VEGF signaling during vascular remodeling. Among them, the expressions of Sema3E and PlexinD1 in the nervous and vascular system changed significantly after birth [56]. In the developing brain, PlexinD1 is widely detected in capillary endothelial cells. Furthermore, the findings suggest that it is different from the developing brain or peripheral vasculature. In the developing mouse retina, PlexinD1 expression is regulated by VEGF signaling, a major hypoxia-inducible pathway, during retinal angiogenesis [57]. Furthermore, Sema3E/PlexinD1 signaling inhibits postischemic angiogenesis by regulating endothelial DLL4 and filopodia formation in a rat model of ischemic stroke [58]. Yu et al. showed that ischemic injury rapidly induced Sema3e expression in neurons in the peri-infarct region, followed by PlexinD1 upregulation in remodeling vessels [59]. Interestingly, the re-emergence of PlexinD1 coincides with the entry of cerebral blood vessels into an active angiogenic process. Consistent with this, Plxnd1 ablation worsened neurological deficit, infarct volume, neuronal survival, and blood flow recovery. Furthermore, decreased and abnormal vascular morphogenesis results from abnormally increased VEGF signaling. Significant extravasation of intravenous tracers in the brain parenchyma, downregulation of connexins, and mislocalization in regenerated vessels were observed in Plxnd1 knockout mice. This suggests that loss of Sema3E-Plexin-D1 signaling is associated with BBB damage. Inhibition of VEGF signaling during vascular remodeling restores abnormal behavioral manifestations, abnormal vascular phenotypes, and defects in BBB disassembly in Plxnd1 knockout mice. These findings suggest that Sema3E-Plexin-D1 signaling can promote functional recovery by downregulating VEGF signaling in the injured adult brain [59]. Thus, the current study shows that PlexinD1 expression is essential in critical situations, such as ischemia-induced vascular remodeling, in which newly sprouted vessels require vascular guidance in response to VEGF signaling [60].

Studies of acupuncture in the treatment of cerebral infarction have also shown that it has an important effect in inhibiting inflammation. Song et al. performed acupuncture at Baihui acupoint and Zusanli acupoint on the ipsilateral side of cerebral ischemia-reperfusion model rats, and found that both IL-1β and ICAM-1 in the brain region of the healthy side of the rats showed a trend of increasing, which was statistically different from that of the model group [61]. It can be seen that acupuncture can upregulate the expression of related inflammatory factors in the brain region, inhibit the inflammatory response, and play a role in brain protection. Wang et al. found that the local blood flow of the healthy side of the rat showed a short-term increase trend

after electroacupuncture at the Renzhong point of the middle cerebral artery embolism model rat, which shows that electroacupuncture treatment can increase the blood flow compensation of the healthy side to the injured side [62]. Huang et al. used the giant needling method to acupuncture the contralateral limbs of rats with focal ischemia, and found that the giant needling method could improve the neurological function of MCAO rats and reduce the infarct size [63]. He treated the patients with acute ischemic cerebral infarction with giant needling, the balance of the affected limbs of the patients was improved, and the neurological damage was alleviated. Therefore, acupuncture has a good effect on cerebral infarction at present [64].

5. Conclusion

In this study, the biological mechanisms of neuroplasticity and angiogenesis of electroacupuncture in the treatment of cerebral ischemia-reperfusion injury were preliminarily explored based on the transcriptomic strategy. In the future, we will further explore the signaling pathways and molecular mechanisms related to neuroplasticity in combination with other omics. Based on current evidence, electroacupuncture intervention on hand-taiyin meridian can improve neurological function scores, neuron apoptosis, and neuroreparation in MCAO/R rats, which may be related to the regulation of genes such as Caspase 9, BDNF, Grin2a, and PlexinD1.

Data Availability

All data generated or analyzed during this study are included in this published article.

Conflicts of Interest

The authors declare that they have no competing interests.

Acknowledgments

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Supplementary Materials

Table S1: differentially expressed genes of Model/Sham group; Table S2: preliminary enrichment results of Model/Sham group; Table S3: differentially expressed genes of EA/Model group; Table S4: upregulated gene analysis; Table S5: downregulated gene analysis; Table S6: all gene analysis. (*Supplementary Materials*)

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Research Article

Effect of Evidence-Based Diet Nursing on Intestinal Flora and Maternal and Infant Prognosis in Patients with Gestational Diabetes

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Background. Gestational diabetes mellitus (GDM) refers to the diabetes first discovered or occurring during pregnancy. The incidence of gestational diabetes in China is about 1%–5%, with an increasing trend in recent years. *Objective*. To observe the effect of evidence-based diet nursing on intestinal flora and maternal and infant prognosis in patients with gestational diabetes. *Methods*. One hundred and thirty patients with GDM admitted to our hospital from January 2020 to January 2022 were selected and divided into two groups according to the intervention method, with 65 cases in each group. The control group was given routine nursing plus diet nursing, while the observation group was given evidence-based nursing plus diet nursing. The changes of blood glucose index and intestinal flora before and after intervention in the two groups were detected, and the compliance behavior, pregnancy outcome, and perinatal outcome in the two groups were statistically analyzed. *Results*. After the intervention, the fasting blood glucose, 2 h postprandial blood glucose, and HbA1c in the two groups gradually decreased ($P < 0.05$). Further comparison between the groups showed that the fasting blood glucose, 2 h postprandial blood glucose, and HbA1c in the observation group were lower than those in the control group ($P < 0.05$). After intervention, the ratios of *Bifidobacterium*, *Lactobacillus*, and *Bifidobacterium* to *E. coli* in the two groups gradually increased ($P < 0.05$). Furthermore, comparison between the groups showed that the ratios of *Bifidobacterium*, *Lactobacillus*, and *Bifidobacterium* to *E. coli* in the observation group were higher than those in the control group ($P < 0.05$). The blood glucose rate, regular prenatal examination rate, and diet control rate of the observation group were 100.00%, 100.00%, and 95.38%, respectively, which were higher than 89.23%, 92.31%, and 84.62% of the control group, and the difference was significant ($P < 0.05$). The pregnancy infection rate and cesarean section rate in the observation group were 0.00% and 33.85%, respectively, which were lower than 6.15% and 60.00% in the control group, and the difference was significant ($P < 0.05$).The premature delivery rate and polyhydramnios rate in the observation group were 3.08% and 1.54%, respectively, which were not significantly different from 6.15% to 7.69% in the control group ($P > 0.05$). The rates of macrosomia, neonatal hypoglycemia, and neonatal hyperbilirubinemia in the observation group were 1.54%, 3.08%, and 9.23%, respectively, which were lower than those in the control group (10.77%, 13.85%, and 23.08%), and the differences were significant ($P < 0.05$). The fetal malformation rate and neonatal asphyxia rate in the observation group were 0.00% and 1.54%, respectively, which were not significantly different from 1.54% to 7.69% in the control group ($P > 0.05$). *Conclusion*. The application of evidence-based care combined with dietary care in GDM patients can improve intestinal flora, control blood glucose, improve patient compliance behavior, and improve maternal and infant outcomes.

1. Introduction

Gestational diabetes can cause polyhydramnios, intrauterine infection, ketoacidosis, macrosomia, abortion, premature delivery, malformation, stillbirth, and other maternal and infant complications, which attracted clinical attention [1–3]. Reasonable dietary control and exercise therapy can restore blood glucose to the normal range in most patients, which needs to be combined with glucose-lowering medication if necessary. Quality nursing intervention plays an

important role in the management of gestational diabetes [4, 5].

Evidence-based nursing is an emerging nursing model that combines nursing experience and patients' desires by finding evidence-based evidence from the previous high-quality literature to develop appropriate nursing measures, including three stages: raising questions, obtaining evidence-based support, and evidence-based nursing practice [6]. At present, evidence-based nursing has been applied in various clinical fields and has certain effects in promoting disease rehabilitation and improving prognosis [6, 7]. In this study, we observed the effects of evidence-based care combined with dietary care on intestinal flora and maternal and infant outcomes in patients with GDM for reference, which are reported as follows.

1.1. Core Tips. Effective management of gestational diabetes helps to improve pregnancy outcomes, but the effect of conventional intervention mode is not ideal. In this study, we implemented evidence-based care for patients with gestational diabetes and found that evidence-based care combined with dietary care could improve gut flora, control blood glucose, increase patient compliance behavior, and improve maternal and infant outcomes in patients with gestational diabetes.

2. Data and Methods

2.1. General Information. A total of 130 patients with gestational diabetes admitted to our hospital (January 2020–January 2022) were selected, aged 22–35 years, with an average of (28.12 ± 3.02) years. The gestational age was 25–33 weeks, with an average of (29.86 ± 2.55) weeks. They were divided into two groups using simple randomization, and there were 65 cases in each group.

2.2. Inclusion and Exclusion Criteria. Inclusion criteria include the following: (1) those diagnosed with gestational diabetes by the OGTT test [8]; (2) those aged 18–35 years; (3) all were singleton pregnancies with spontaneous conception; (4) those without other pregnancy complications and history of high-risk pregnancy; (5) those who complete clinical data.

Exclusion criteria include the following: (1) those with abnormal blood glucose before pregnancy; (2) those with communication and impairment; (3) those with comorbid severe psychiatric disorders; (4) those with growth hormone deficiency or other significant endocrine disorders; (5) those with known congenital malformations or genetic defects in the fetus; (6) those with gastrointestinal disorders; (7) those with comorbid other severe physical disorders.

2.3. Methods. The control group was given routine nursing plus diet nursing, and the body weight, blood pressure, and blood glucose were measured regularly for diet and exercise guidance. Patients who did not control blood glucose well in diet and exercise were given insulin therapy according to the doctor's advice, and the patients were guided to use insulin pen correctly. After 34 weeks of pregnancy, fetal heart rate and fetal movement were monitored regularly to evaluate the fetal intrauterine situation. Diet nursing: Dietary recipes were formulated according to the patient's blood sugar value, body weight, gestational age, and eating habits. The daily caloric intake of those with BMI less than 18.5 kg/m^2 in the first trimester was 35 kcal/kg, the daily caloric intake of those with BMI of 18.5–23.9 kg/m^2 was 30–35 kcal/kg, the daily caloric intake of those with BMI of 24.0–27.9 kg/m^2 was 25–30 kcal/kg, the daily caloric intake of the BMI >28.0 kg/m^2 was 30–35 kcal/kg, and the daily caloric intake of the second and third trimesters was increased 200–300 kcal on this basis. The proportion of carbohydrate, protein, and fat intake in the diet structure is 50–60%, 15–20%, and 20–30%. Pregnant women eat six times a day, and the calorie allocation for each meal is 10–15% for breakfast, 5–10% for breakfast, 30% for lunch, 5–10% for lunch, 30% for dinner, and 5–10% for dinner%.

The observation group was given evidence-based nursing plus dietary nursing. Dietary nursing was the same as that of the control group. Evidence-based nursing: an evidence-based nursing group was established with the head nurse as the team leader, and evidence-based nursing was implemented in three stages. (1) Asking questions: the following problems are found according to the problems found in daily nursing work, the needs of patients, and changes in the course of the disease: patients with gestational diabetes lack sufficient understanding of the harm of this disease; diet control cannot take into account the nutrition and health of pregnancy and blood sugar control; prone to negative emotions such as anxiety and depression; lack of exercise or improper exercise mode and exercise intensity; prone to gestational hypertension syndrome. (2) Obtain evidence-based support: we searched the CNKI database, Wanfang medical network, Chinese biomedical literature database, and so on according to the above questions. The keywords were GDM and nursing. We found evidence-based evidence and screened evidence according to nursing experience and course of disease through reading literature. (3) Evidence-based nursing practice: formulate an evidence-based nursing plan based on evidence-based evidence combined with nursing professional skills and experience. First, health education was provided to the patients to inform them of the hazards of gestational diabetes to the mother and baby and to instruct them to monitor blood glucose and count fetal movements correctly. Communicate and exchange with patients to understand the causes of negative emotions and use relaxation training and psychological suggestion to ease negative emotions. Instruct patients to exercise appropriately and develop individualized exercise programs, with aerobic exercise as the main focus. Blood pressure was measured regularly to detect and manage hypertensive syndrome during pregnancy in a timely manner. The nursing interventions in both the groups were carried out from the first visit to the delivery of the fetus.

2.4. Observation Indicators. The changes in blood glucose indexes and intestinal flora before and after intervention in the two groups were detected, and the medical compliance

behaviors (adherence to blood glucose measurement, regular obstetric examination, and dietary control), pregnancy outcomes (preterm birth, polyhydramnios, infection during pregnancy, and mode of production), perinatal and neonatal outcomes (macrosomia, fetal malformation, neonatal asphyxia, neonatal hypoglycemia, and neonatal hyperbilirubinemia) of the 2 groups were counted.

2.5. Detection Method. Before and after the intervention, 2 ml of fasting venous blood was drawn from the patients in the morning, and the Beckman DXC800 biochemical analyzer was used to detect fasting blood glucose and HbA1c. Blood was drawn again 2 h after eating, and blood glucose was detected 2 h after meal in the same way.

The intestinal flora was detected before and after the intervention, and 0.5 g of the patient's fresh feces was added to sterile saline. The ATB semiautomatic microbial identification system of French BioMérieux was used to identify *Bifidobacterium*, *Escherichia coli*, and *Lactobacillus* and count the bifidobacteria/*Escherichia coli* ratio (B/E).

2.6. Statistical Methods. SPSS 19.0 was used to process the data, and the measurement indicators such as intestinal flora and dietary structure were first tested for normality, and those in line with the normal distribution were described by ($\overline{x} \pm s$), and the t-test was used for comparison. Those not in line with the normal distribution were described by median and quartiles, and the nonparametric test was used for comparison. The count data such as compliance behavior and pregnancy outcome were described by the number of cases (%) and compared by the χ^2 test, and $P < 0.05$ was statistically significant.

3. Results

3.1. Comparison of General Data of Patients in the Two Groups. There was no significant difference in age, gestational age, pregnancy time, body mass index, maternal type, maternal occupation, total family income, education level, defecation frequency, and dietary structure between the two groups ($P > 0.05$), as given in Table 1.

3.2. Comparison of Blood Glucose Indexes between the Two Groups of Patients. The comparison between the groups before the intervention showed that the difference was not significant ($P > 0.05$). After intervention, the fasting blood glucose, 2 h postprandial blood glucose, and HbA1c of the two groups gradually decreased in the two groups ($P < 0.05$), and further comparison between the groups revealed that fasting blood glucose, 2 h postprandial blood glucose, and HbA1c in the observation group were lower than those in the control group ($P < 0.05$), as given in Table 2.

3.3. Comparison of Intestinal Flora between the Two Groups of Patients. The comparison between the groups before the intervention showed that the difference was not significant ($P > 0.05$). After intervention, the ratios of bifidobacteria, lactobacilli, and bifidobacteria to *Escherichia coli* in the two groups gradually increased in the two groups ($P < 0.05$), and further comparison between the groups revealed that the ratios of bifidobacteria, lactobacilli, and bifidobacteria to *Escherichia coli* were higher than those in the control group ($P < 0.05$), as given in Table 3.

3.4. Comparison of Medical Compliance Behavior between the Two Groups of Patients. The rate of adherence to blood sugar measurement, regular obstetric examination rate, and diet control rate in the observation group were 100.00%, 100.00%, and 95.38%, respectively, which were higher than 89.23%, 92.31%, and 84.62% in the control group, with significant differences ($P < 0.05$), as given in Table 4.

3.5. Comparison of Pregnancy Outcomes between the Two Groups of Patients. The infection rate during pregnancy and the cesarean section rate in the observation group were 0.00% and 33.85%, respectively, which were lower than 6.15% and 60.00% in the control group, with a significant difference ($P < 0.05$). The premature birth rate and polyhydramnios rate of the observation group were 3.08% and 1.54%, respectively, compared with 6.15% and 7.69% of the control group, and the difference was not significant ($P > 0.05$), as given in Table 5.

3.6. Comparison of Perinatal Outcomes between the Two Groups of Patients. The macrosomia rate, neonatal hypoglycemia rate, and neonatal hyperbilirubinemia rate in the observation group were 1.54%, 3.08%, and 9.23%, respectively, which were lower than 10.77%, 13.85%, and 23.08% in the control group, with significant differences ($P < 0.05$). The fetal malformation rate and neonatal asphyxia rate in the observation group were 0.00% and 1.54%, respectively, compared with 1.54% and 7.69% in the control group, and the difference was not significant ($P > 0.05$), as given in Table 6.

4. Discussion

GDM belongs to the category of high-risk pregnancy. It is a metabolic disorder caused by absolute or relative insulin deficiency and hyperglycemic secretion, the etiology of which is not completely clear. The existing research is based on multigene genetic defects, which is caused by pregnancy, obesity, stress, and other incentives [9–11]. GDM is more harmful and can increase the incidence of perinatal complications and mortality. Reasonable diet control can not only reduce insulin load and correct metabolic disorders but also prevent the failure of blood glucose control and hypoglycemia [12, 13]. Therefore, diet nursing is the basis and focus of nursing work for patients with gestational diabetes. The ideal gestational diabetes diet should not only meet the nutritional needs of pregnant women but also control fasting blood glucose, postprandial 2 h blood glucose, and nocturnal blood glucose in the normal range [14, 15]. However, in

Table 1: Comparison of general data of the two groups of patients.

Project		The control group ($n = 65$)	The observation group ($n = 65$)
Age (y)		28.56 ± 3.65	28.49 ± 3.25
Gestational week (week)		29.74 ± 2.15	29.83 ± 2.31
Pregnancy (second rate)		2.12 ± 0.53	2.18 ± 0.48
Body mass index (kg/m^2)		28.45 ± 2.36	28.51 ± 2.42
Maternity type	Primipara	48 (73.85)	44 (67.69)
	Multiparous	17 (26.15)	21 (32.31)
Maternity occupation	Light stamina	40 (61.54)	41 (63.08)
	Medium stamina	25 (38.46)	24 (36.92)
Total household income (moon)	< 3000 yuan	11 (16.92)	8 (12.31)
	3000–6000 yuan	26 (40.00)	25 (38.46)
	> 6000 yuan	28 (43.08)	32 (49.23)
Educational level	Junior high school and below	7 (10.77)	6 (9.23)
	High school and college	38 (58.46)	37 (56.92)
	Undergraduate and above	20 (30.77)	22 (33.85)
Frequency of bowel movements (week)	≤2 second rate	0 (0.00)	0 (0.00)
	3–5 second rate	17 (26.15)	20 (30.77)
	6–7 second rate	42 (64.62)	39 (60.00)
	≥8 second rate	6 (9.23)	6 (9.23)
Diet	Dietary calorie intake (× 106 J/d)	4.45 ± 2.26	4.50 ± 2.31
	Percentage of protein intake (%)	19.02 ± 2.85	18.95 ± 3.01
	Carbohydrate intake percentage (%)	44.22 ± 8.23	43.98 ± 8.96
	Percentage of fat intake (%)	36.85 ± 5.23	37.02 ± 5.11
	Vegetable intake (g/d)	362.55 ± 75.26	354.11 ± 81.65

Compared with the control group, $^{\#}P < 0.05$.

Table 2: Comparison of blood glucose indexes in the two groups of patients ($\overline{x} \pm s$).

Group	Number of cases	Fasting blood sugar (mmol/L)		2 h postprandial blood glucose (mmol/L)		HbA1c (%)	
		Before intervention	After intervention	Before intervention	After intervention	Before intervention	After intervention
The control group	65	9.25 ± 2.14	7.20 ± 1.45*	11.85 ± 2.65	8.86 ± 2.11*	12.25 ± 2.02	8.52 ± 1.45*
The observation group	65	9.08 ± 2.36	6.65 ± 1.24$^{*\#}$	11.81 ± 2.72	7.23 ± 1.54$^{*\#}$	12.18 ± 2.25	7.02 ± 1.36$^{*\#}$

Compared with the group before intervention, $^{*}P < 0.05$; compared with the control group, $^{\#}P < 0.05$.

Table 3: Comparison of intestinal flora between the two groups of patients ($\overline{x} \pm s$).

Group	Number of cases	*Bifidobacterium* (lgCFU/g)		*Lactobacillus* (lgCFU/g)		*Bifidobacterium* to *Escherichia coli* ratio	
		Before intervention	After intervention	Before intervention	After intervention	Before intervention	After intervention
The control group	65	6.25 ± 1.25	8.12 ± 1.36*	5.36 ± 1.25	5.96 ± 1.28*	0.72 ± 0.31	1.17 ± 0.34*
The observation group	65	6.31 ± 1.21	8.86 ± 1.41$^{*\#}$	5.30 ± 1.36	6.43 ± 1.08$^{*\#}$	0.70 ± 0.33	1.31 ± 0.28$^{*\#}$

Compared with the group before intervention, $^{*}P < 0.05$; compared with the control group, $^{\#}P < 0.05$.

Table 4: Comparison of medical compliance behavior of the two groups of patients (n, %).

Group	Number of cases	Keep checking blood sugar	Regular obstetric inspection	Diet control
The control group	65	58 (89.23)	60 (92.31)	55 (84.62)
The observation group	65	65 (100.00)$^{\#}$	65 (100.00)$^{\#}$	62 (95.38)$^{\#}$

Compared with the control group, $^{\#}P < 0.05$.

Table 5: Comparison of pregnancy outcomes between the two groups of patients (*n*, %).

Group	Number of cases	Premature birth	Polyhydramnios	Infection during pregnancy	Ways to produce	
					Cesarean section	Vaginal birth
The control group	65	4 (6.15)	5 (7.69)	4 (6.15)	39 (60.00)	26 (40.00)
The observation group	65	2 (3.08)	1 (1.54)	0 (0.00)$^{\#}$	22 (33.85)$^{\#}$	43 (66.15)$^{\#}$

Compared with the control group, $^{\#}P < 0.05$.

Table 6: Comparison of perinatal outcomes between the two groups of patients (*n*, %).

Group	Number of cases	Huge	Infant deformity	Neonatal asphyxia	Neonatal hypoglycemia	Neonatal hyperbilirubinemia
The control group	65	7 (10.77)	1 (1.54)	5 (7.69)	9 (13.85)	15 (23.08)
The observation group	65	1 (1.54)$^{\#}$	0 (0.00)	1 (1.54)	2 (3.08)$^{\#}$	6 (9.23)$^{\#}$

Compared with the control group, $^{\#}P < 0.05$.

practical work, it is found that diet nursing alone often fails to achieve satisfactory nursing intervention effect [16, 17].

Evidence-based nursing is a more scientific nursing model and an important part of evidence-based medicine. In the process of planned nursing, the scientific research conclusions are combined with clinical experience and patient willingness to obtain evidence-based evidence as the basis for clinical nursing decision-making [18, 19]. In this study, evidence-based nursing was applied to the nursing of gestational diabetes, and it was found that the fasting blood glucose, postprandial 2 h blood glucose, and HbA1c of patients after intervention were lower than those of patients receiving routine nursing intervention ($P < 0.05$). The blood glucose rate, regular antenatal examination rate, and diet control rate of patients were higher than those of patients receiving routine nursing combined with diet nursing intervention ($P < 0.05$), suggesting that evidence-based nursing combined with diet nursing can improve the blood glucose control of patients with GDM and improve their compliance behavior. This is due to the fact that evidence-based care is guided by the problems found in the nursing work, the needs of patients, and the changes in the course of disease. It consults the literature, seeks, and filters evidence-based evidence and enables patients to realize the harm of gestational diabetes and the importance of a reasonable diet through health education, so that patients are more proactive in adhering to blood glucose measurement, regular antenatal examination, and dietary control [20, 21]. Through communication and exchange with patients, we understand that the causes of negative emotions and implement interventions to make patients cooperate with treatment in a more positive state of mind. Thus, patients are instructed to exercise appropriately, which is more helpful for blood glucose control [22, 23].

Intestinal flora is closely related to energy metabolism and abnormal of it can affect weight control and immune function [24, 25]. High glucose status in patients with gestational diabetes can alter the intestinal environment and cause changes in intestinal pH [26–28]. Probiotics such as *Bifidobacterium* and *Lactobacillus* are sensitive to changes in the internal environment, which can be significantly reduced once the intestinal environment changes [29–31]. While *Bifidobacterium* can regulate intestinal dysfunction, *Lactobacillus* can maintain intestinal health and regulate immune function, *Escherichia coli* is parasitic in the large intestine, and invasion of the body can cause infection. The ratio of *Bifidobacterium* to *Escherichia coli* can reflect the colonization of intestinal flora to some extent [32–34]. This study found that the ratio of intestinal *Bifidobacterium*, *Lactobacillus*, and *Bifidobacterium* to *Escherichia coli* in the patients receiving evidence-based nursing combined with diet nursing intervention was higher than that in the patients receiving routine nursing combined with diet nursing intervention, suggesting that evidence-based nursing combined with diet nursing can improve the intestinal flora of patients with gestational diabetes, which indirectly proves that evidence-based nursing combined with diet nursing is more conducive to blood glucose control.

Follow-up showed that the pregnancy infection rate, cesarean section rate, macrosomia rate, neonatal hypoglycemia rate, and neonatal hyperbilirubinemia rate of patients receiving evidence-based nursing combined with diet nursing intervention were lower than those receiving routine nursing combined with diet nursing intervention ($P < 0.05$). There was no significant difference in premature delivery rate, polyhydramnios rate, fetal malformation rate, and neonatal asphyxia rate between the two groups ($P > 0.05$), suggesting that evidence-based nursing combined with diet nursing can improve the maternal and infant outcomes of patients with GDM, which is related to the more stable blood glucose control of patients receiving evidence-based nursing intervention.

GDM can endanger maternal and infant health. Reasonable diet, exercise, medication, maintaining emotional stability, and preventing complications are the key points of intervention, but routine nursing and diet nursing cannot achieve satisfactory intervention effect. In this study, evidence-based nursing was used to intervene GDM on the basis of diet nursing. It was found that evidence-based nursing combined with diet nursing had greater advantages in controlling blood glucose, improving patient compliance behavior, and improving maternal and infant outcomes.

This study further confirmed that evidence-based nursing combined with diet nursing can better improve the blood glucose level of patients with GDM by detecting the changes of intestinal flora before and after intervention.

In conclusion, the use of evidence-based care combined with dietary care in GDM patients can improve their intestinal flora, control their blood glucose, improve their compliance behavior, and improve their maternal and infant outcomes.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

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Review Article

Inhibition of Delayed Cerebral Ischemia by Magnesium Is Insufficient for Subarachnoid Hemorrhage Patients: A Network Meta-Analysis

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Objective. After subarachnoid hemorrhage, magnesium could reduce the incidence of delayed cerebral ischemia; however, it is still controversial. This study updated the results of recently published magnesium-related studies and conducted an exploratory analysis of the impact of application strategies and intervention factors on the results. *Methods*. Public databases were searched from the date of their inception to May 10, 2021. Randomized controlled trials on magnesium agent-related regimens for subarachnoid hemorrhage patients were included. *Results*. In total, 28 articles were included in the meta-analysis. For delayed cerebral ischemia, magnesium-related interventions significantly reduced the risk of delayed cerebral ischemia compared with nonmagnesium interventions (odds ratios: 0.40; 95% confidence interval: 0.28–0.56; $p < 0.01$). For cerebral vasospasm, a random effects model showed that magnesium significantly reduced the risk of cerebral vasospasm (odds ratios: 0.46; 95% confidence interval: 0.33–0.63; $p < 0.01$). In the subgroup analysis, intracranial magnesium (odds ratios: 6.67; 95% confidence interval: 1.14–38.83; $p = 0.03$) and magnesium plus hydrogen (odds ratios: 10; 95% confidence interval: 1.59–62.73; $p = 0.01$) produced significant results in improving the good recovery rate compared to the control. In the network meta-analysis, magnesium plus nimodipine and simvastatin even showed an effective trend in death/persistent vegetative status improvement. *Conclusion*. This study supports the beneficial effect of magnesium in reducing the risk of delayed cerebral ischemia. Based on a single randomized controlled trial, immediate intracranial magnesium therapy with intravenous hydrogen after subarachnoid hemorrhage can increase the good recovery rate. Therefore, more high-quality studies are needed to confirm this finding.

1. Introduction

Subarachnoid hemorrhage (SAH) accounts for approximately 5% of all types of stroke incidence, however, this prevalence is higher among the young and middle-aged population and has an extremely poor prognosis [1, 2]. SAH occurs in approximately 9 per 100,000 people every year, and half of SAH patients are younger than 55 years of age and have an extremely poor prognosis [3]. One-third of people die within three months after hemorrhage, and one-fifth of people with SAH need to rely on others for daily activities [4].

The current evidence-based treatment for SAH is neurosurgical clipping or endovascular coiling and administration of nimodipine [5]. However, patients still have a higher incidence of cerebral vasospasm (CVS) and delayed cerebral ischemia (DCI) [6, 7]. It is currently believed that DCI is the main cause of death and neurological deficits in SAH patients [8].

Magnesium is a low-cost neuroprotective agent that has been successfully applied in eclampsia treatment. Eclampsia has the same pathophysiological characteristics as DCI after SAH [9]. A recent observational study supports that magnesium influences hemorrhage severity in patients with SAH, potentially through a hemostatic mechanism [10]. The effect of magnesium on SAH is still controversial. In an individual patient data meta-analysis of magnesium for SAH, it was believed that magnesium intervention in an earlier time window did not bring more beneficial DCI results [11]. Two meta-analyses reported that magnesium

can reduce the risk of DCI [12, 13], and one meta-analysis indicated that magnesium can significantly reduce the incidence of CVS [14]. However, magnesium application did not show benefits with respect to neurological recovery results and mortality.

The incongruence between phase 2 and phase 3 clinical studies of magnesium for SAH was reviewed recently. However, it neglected the impact of combination drugs and infusion routes on the therapeutic effect of magnesium [15]. One of the major concerns arising from the magnesium for aneurysmal subarachnoid hemorrhage (MASH)-2 trial is that magnesium does not cross the blood-brain barrier well. The intracisternal (but not intravenous) magnesium infusion strategy reinspired enthusiasm for its clinical application [16]. In addition, the use of concomitant drugs in Japan, China (fasudil), Europe, and North America (nimodipine) will also affect magnesium treatment for SAH [16]. Another review indicated that the immediate intracisternal infusion of magnesium with intravenous hydrogen may be effective for treating early brain injury after SAH [17]. However, it is qualitative and did not analyze the impact of concomitant drugs. Finally, the review still believes that even if magnesium is not routinely used, it is still reasonable to maintain magnesium levels in the normal range because hypomagnesemia is associated with DCI and poor prognosis on SAH [17].

The current study updated the results of recently published magnesium-related randomized controlled trials (RCTs) and further evaluated the effects of magnesium application regimens on DCI, CVS, the modified Rankin score (mRS), the Glasgow outcome scale (GOS) scores, and mortality through a network meta-analysis. It also tried to analyze the impact of important factors among the studies on the above outcomes by meta-regression.

2. Methods

2.1. Search Strategy. We searched all RCTs on the magnesium-related treatment of SAH published up to 24 May 2021. The searched public databases included PubMed, Embase, the Cochrane Library, Scopus, EBSCO, and the Chinese databases of China National Knowledge Infrastructure (CNKI), Wanfang, Chongqing VIP, and SinoMed. The keywords included subarachnoid hemorrhage, magnesium, and random*. To avoid omission, manual searches of references in related reviews were also performed.

2.2. Inclusion and Exclusion Criteria. Two authors checked the literature according to the established inclusion and exclusion criteria. If there was a dispute, it was discussed with the third author to make a concordant decision. The inclusion criteria were as follows: (1) study researched SAH patients, (2) RCT design, (3) the intervention group used magnesium-related treatment, and the control group did not use magnesium in treatment or a different magnesium-related treatment from intervention group, (4) the study reported one of the following outcomes: frequency of DCI, CVS, good recovery(GR) patients according to mRS or GOS/the Glasgow outcome scale extended (GOSE) assessment, death, or persistent vegetative status (PVS). The exclusion criteria were as follows: (1) the study included SAH patients who were younger than 18 years old, (2) the study researched magnesium intervention that included other stroke patients and did not report SAH patients' results separately, (3) post hoc research, (4) protocol, (5) non-RCTs, and (6) the study did not report the outcomes of interest. Although the search had no language restrictions, the included studies needed to at least have English abstracts. In addition, reviews, comments, and conference abstracts were also excluded.

2.3. Data Extraction. The extracted information included the first author's name, publication year, research location, sample size, magnesium intervention time window, neurosurgical treatment, magnesium intervention, injection dosage and route, magnesium treatment duration, control treatment, and follow-up. The outcomes included the frequency of DCI, CVS, GR according to mRS or GOS/GOSE assessment, and death or PVS. The Cochrane bias risk assessment tool was used to evaluate the methodological quality of the included RCTs [18].

2.4. Statistical Methods. Odds ratios (ORs) and 95% confidence intervals (CIs) were pooled for dichotomous outcomes, and the prediction interval (PI) was also reported. We evaluated heterogeneity by the chi^2 test and calculated I^2. Significant heterogeneity was defined as an I^2 greater than 50%. The Mantel-Haenszel and Peto methods were used for the fixed effects model, and the Mantel-Haenszel method was used for the random effects model [19]. Funnel plots, Begg's test, and Egger's test were performed to identify potential publication bias. If the results had potential publication bias, the trim-and-fill method was used for correction.

The pooled results were further analyzed by a subgroup analysis based on treatment strategies, and frequentist random effect network meta-analysis was also used to rank the effect of strategies based on mixed multiple treatment comparisons [20]. The methods for assessing the extent of the heterogeneity and inconsistency based on generalized Cochran's Q statistic were used for network meta-analysis. The P score was calculated to rank the intervention strategies, and k-means cluster analysis of multiple outcomes was performed [21]. Other important factors that potentially affect outcomes, including publication time, time window, magnesium dose, intervention duration, and follow-up time, were analyzed by metaregression. The software used for analysis included the "meta," "netmeta," and "pheatmap" packages in R language (version 4.0.5) and RevMan (version 5.3).

3. Results

After searching public databases, a total of 611 English publications and 597 Chinese publications were obtained. After removing duplicate publications, 216 English and 195 Chinese publications remained. After screening the titles

and abstracts, 70 English publications and 25 Chinese publications remained. After full-text screening, 67 publications were excluded for the following reasons: 13 publications were reviews, 12 publications were post hoc studies, 3 publications did not include SAH patients or report SAH patients separately, one publication was a protocol, 8 publications did not report the desired outcomes, 2 publications were not RCTs, 11 publications were conference abstracts, 16 publications were non-English articles without English abstracts, and one publication included SAH patients younger than 18 years. Finally, 28 papers were included in this meta-analysis [22–49] (Figure 1).

The publication time ranged from 2002 to 2021. One study clearly excluded patients who needed neurosurgical surgery within 72 hours [24], eight studies did not mention surgery [23, 28, 37–40, 42, 45], and others performed neurosurgery for patients based on actual conditions. Two of the magnesium applied routes are intracranial [22, 27]. The follow-up period ranged from 2 weeks to 1 year (Table 1).

Since all included studies were RCTs, the level of overall evidence was acceptable (Figure 2). However, with the exception of several large-scale phase III clinical studies, the sample sizes in the remaining studies were relatively small. The assessment of DCI, CVS, and GR still suffers from subjective bias, which may cause the results to be more positive. In addition, several studies, including those with small sample sizes, may also impact the robustness of the results.

For DCI, the fixed effects model showed that magnesium-related interventions significantly reduced the risk of DCI compared with nonmagnesium interventions (OR: 0.40; 95% CI: 0.28–0.56; $p < 0.01$). In the subgroup analysis, the fixed effect model showed that magnesium plus nimodipine can significantly reduce the risk of DCI compared to nimodipine (OR: 0.41; 95% CI: 0.25–0.65; $p < 0.01$), and magnesium alone can also reduce the risk of DCI (OR: 0.23; 95% CI: 0.11–0.50; $p < 0.01$) compared to conventional treatment without clear combination drugs, such as nimodipine (Figure 3). There was no significant result in other subgroups. Publication bias analysis showed that there was potential bias (Egger's test, $p = 0.0165$) (Supplementary Figure 1(a)). After correction, the results were still considered stable (OR: 0.49; 95% CI: 0.31–0.77; $p < 0.01$).

For CVS, the random effects model (OR: 0.46; 95% CI: 0.33–0.63; $p < 0.01$) and fixed effects model (OR: 0.52; 95% CI: 0.43–0.64; $p < 0.01$) showed that magnesium can significantly reduce the risk of CVS. In the subgroup analysis, the fixed effects model (OR: 0.64; 95% CI: 0.50–0.83; $p < 0.01$) and the random effects model (OR: 0.57; 95% CI: 0.39–0.83; $p < 0.01$) showed that magnesium plus nimodipine can significantly reduce the risk of CVS compared to nimodipine alone. Magnesium also significantly reduced the risk of CVS compared to conventional treatment (OR: 0.25; 95% CI: 0.12–0.49; $p < 0.01$). Other subgroups also showed significant results, however, they were based on the results of single studies (Figure 4). Egger's test showed potential publication bias ($p = 0.005$) (Supplementary Figure 1(b)). After correction, the random effect models did not support the positive results (OR: 0.73; 95% CI: 0.52–1.03; $p = 0.07$).

For death or PVS assessment, the fixed effect model did not support that magnesium can significantly reduce the risk of death or PVS in SAH patients (OR: 0.72; 95% CI: 0.72–1.09; $p = 0.27$). In the subgroup analysis, only magnesium plus simvastatin and nimodipine had a tendency to reduce the risk, however, the difference was not significant (OR: 0.20; 95% CI: 0.04–1.02; $p = 0.05$) (Figure 5). Potential publication bias was revealed by Egger's test ($p = 0.029$) (Supplementary Figure 1(c)), and the negative results were not changed after correction (OR: 0.97; 95% CI: 0.79–1.19; $p = 0.79$).

For the GR results based on the mRS evaluation, the random effects model (OR: 1.26; 95% CI: 0.90–1.77; $p = 0.17$) did not show a significant effect of magnesium application in improving GR. In the subgroup analysis, magnesium (OR: 6.67; 95% CI: 1.14–38.83; $p = 0.03$) and magnesium plus hydrogen (OR: 10; 95% CI: 1.59–62.73; $p = 0.01$) produced significant results compared to the control. However, these positive results were based on one study [22]. In this study, magnesium was used intracranially, and hydrogen was intravenously used in the magnesium plus hydrogen group. Because of the small number of studies, no publication bias analysis was performed. Based on the GOS/GOSE assessment, a fixed effects model showed that magnesium did not significantly increase the frequency of GR persons (OR: 1.13; 95% CI: 0.87–1.46; $p = 0.34$). The subgroup analysis also did not show the advantages of magnesium application (Figure 6).

For the network meta-analysis of DCI, no significant heterogeneity ($Q = 4.07$; $\mathrm{df} = 4$; $p = 0.396$) or inconsistency ($Q = 1.00$; $\mathrm{df} = 1$; $p = 0.316$) was found. Pairwise comparisons showed that magnesium plus nimodipine was significantly better than nimodipine (OR: 0.45; 95% CI: 0.27–0.74). Magnesium (OR: 4.23; 95% CI: 1.89–9.44), magnesium plus nimodipine (OR: 8.15; 95% CI: 2.21–30.03), and nimodipine (OR: 3.63; 95% CI: 1.09–12.09) were significantly better than the control. In the p-score ranking results, magnesium plus cinepazide (0.94) and magnesium plus nimodipine (0.75) have advantages. Other comparisons and P-score results were shown in Supplementary Table 1. In the CVS results, no significant heterogeneity ($Q = 23.66$; $\mathrm{df} = 15$; $p = 0.07$) or inconsistency ($Q = 2.55$; $\mathrm{df} = 3$; $p = 0.465$) was found. Magnesium plus nimodipine was significantly better than nimodipine (OR: 0.59; 95% CI: 0.41–0.84). Magnesium (OR: 4.11; 95% CI: 1.77–9.53), magnesium plus nimodipine (OR: 5.66; 95% CI: 1.82–17.65), and nimodipine (OR: 3.32; 95% CI: 1.11–9.99) were significantly better than the control. In the p-score ranking results, magnesium plus cinepazide (0.88) and magnesium plus nimodipine and simvastatin (0.84) have relative advantages. Other comparisons and P-score results are shown in Supplementary Table 2. For death or PVS results, there was also no significant heterogeneity ($Q = 5.69$; $\mathrm{df} = 12$; $p = 0.93$) or inconsistency ($Q = 0.10$; $df = 1$; $p = 0.75$). Pairwise comparisons showed that only magnesium plus nimodipine and simvastatin had a significant advantage compared to the control (OR: 11.49; 95% CI: 1.35–98.04). The P-score ranking results show that magnesium plus nimodipine and simvastatin (0.95) has relative advantages (Figure 7). Other comparisons and P-score results are shown

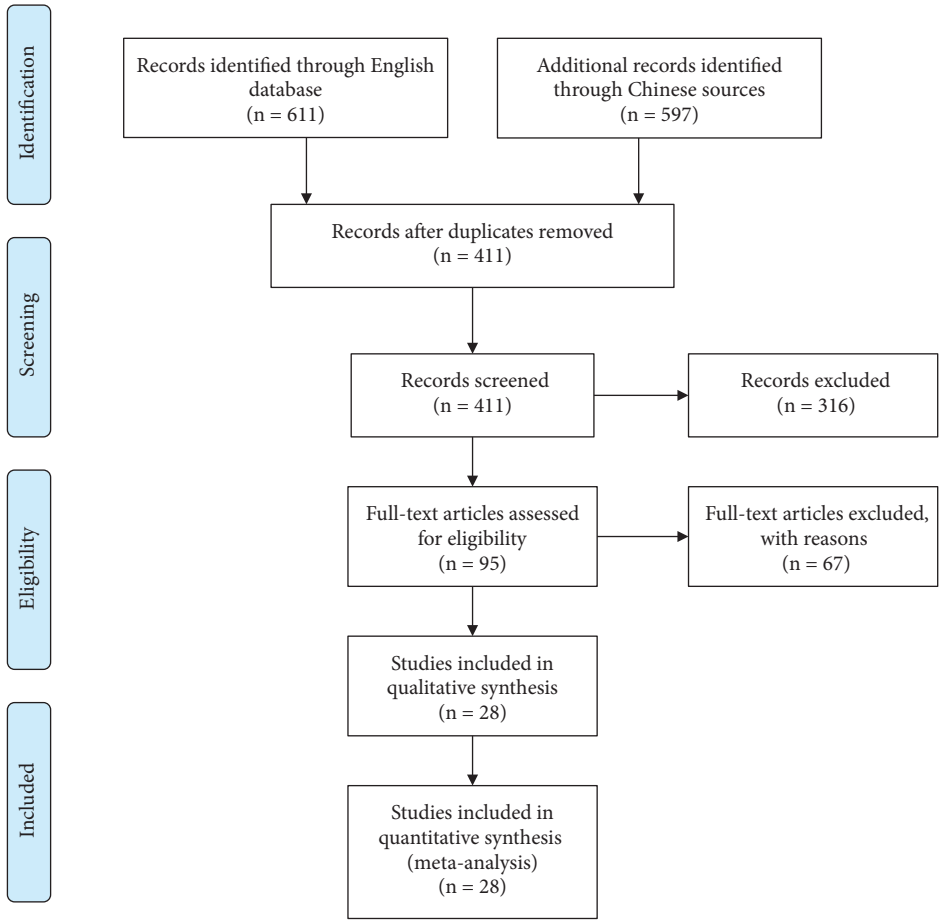


Figure 1: Flowchart of the study identification process.

in Supplementary Table 3. Network meta-analysis was not performed on GR results because of fewer interventions. Therefore, the p-score ranking results of DCI, CVS, and death/PVS were clustered. In general, magnesium plus nimodipine, magnesium plus cinepazide, magnesium plus nimodipine and simvastatin, and magnesium plus flunarizine were categories that had relative advantages (Figure 8).

Metaregression analysis was performed to compare magnesium plus nimodipine and nimodipine alone. However, the factors were not found to have a significant impact on the effect size in all analyzed results. The multivariate analysis was not performed further (Supplementary Table 4).

4. Discussion

In this study, we analyzed the effects of the magnesium application strategy on reducing the risk of DCI, CVS, PVS, and death, as well as on GR and GOSE for SAH patients by conventional meta-analysis with subgroup analysis. Furthermore, network meta-analysis was performed to compare the effects of different magnesium application strategies. This work explained the reasons for the controversy about the effect of magnesium on SAH from the perspectives of different magnesium application strategies. This study provides evidence for improving the magnesium application strategy in the treatment of SAH in the clinic.

This study supported that magnesium can significantly reduce the DCI risk. At the same time, magnesium can also reduce the CVS risk, however, this positive result may be because of potential publication bias. In the subgroup analysis, intracranial magnesium and magnesium plus hydrogen produced significant results in improving the GR rate compared to the control. In the network meta-analysis, magnesium plus nimodipine and simvastatin showed an effective trend in death/PVS outcome. In the comparisons of magnesium plus nimodipine and nimodipine alone, the metaregression analysis did not identify significant factors related to the outcome.

In the exploratory analysis, the advanced results of magnesium plus cinepazide are based on a Chinese study. Cinepazide maleate, a calcium antagonist, also has the ability to inhibit platelet aggregation and inflammatory factor formation. In clinical studies, there is still a lack of well-designed studies on cinepazide for SAH. For ischemic stroke, an RCT showed that cinepazide maleate can improve the neurological function recovery and the activities of daily living in ischemic stroke patients who are better than those in the placebo group [50]. However, the therapeutic effectiveness of magnesium plus cinepazide in SAH still needs to be confirmed by more authoritative clinical studies.

Simvastatin application on the basis of magnesium plus nimodipine can further improve the results of DCI and CVS,

Table 1: Characteristics of included studies.

Studies	Location	Sample size	Average age	Onset window(hour)	Fisher grade	Adopt neurosurgery	Interventions	Dosage of mg#	Intervention ime (day)	Control	Follow-up (month)
Takeuchi S [22]	Japan	37	60.8 ± 11.6	72	3–4	Yes	Mg; Mg plus hydrogen	1.2 mmol/day	14	Control	12
Guosheng R [23]	China	62	59.2 ± 6.7	72	NA	NA	Mg plus cinepazide	20 mmol/day	14	Mg	0.5
Zhang C [24]	China	120	43.51 ± 12.25	24	NA	No	Mg plus nimodipine	27.5 mmol/day	14	Nimodipine	0.75
Soliman R [25]	Egypt	90	51.1 ± 8.31	24	2–3	Yes	Mg plus nimodipine	2mmol/day	21	Milrinone plus nimodipine	0.75
Kunze E [26]	Germany	107	52 ± 13	96	NA	Yes	Mg plus nimodipine	192 mmol/day	10	Nimidipine	NA
Yamamoto T [27]	Japan	73	59.5(NA)	72	2–3	Yes	Mg plus fasudil	2.4 mmol/day	14	Fasudil	3
Songwei W [28]	China	50	50.2 ± 17.9	68	NA	NA	Mg plus nimodipine	30 mmol/day	21	Nimodipine	0.75
Bradford CM [29]	Australia	162	56.6 ± 14.4	72	NA	Yes	Mg plus nimodipine	NA	12	Nimodipine	3
Mees SMD [30]	Europe; South America	1204	57 ± 13	96	NA	Yes	Mg plus nimodipine	64 mmol/day	20	Nimodipine	3
Hassan T [31]	Egypt	30	50(23–80)	96	NA	Yes	Mg plus nimodipine	65 mmol/day	14	Nimodipine	3
Aidaros M [32]	Egypt	20	52.3 ± 11.4	72	NA	Yes	Mg plus nimodipine	64 mmol/day	10	Nimodipine	12
Wong GKC [33]	China	12	56(NA)	NA	3	Yes	Mg	NA	14	Control	0.5
Wong GKC [34]	China; Southeast asia; Australia	327	55(19–90)	48	1–4	Yes	Mg plus nimodipine	80 mmol/day	14	Nimodipine	6
Westermaier T [35]	Germany	110	52 ± 13	96	1–4	Yes	Mg	192 mmol/day	12	Placebo	6
Akdemir H[36]	Turkey	83	53.9(34–74)	72	1–4	Yes	Mg plus nimodipine	64 mmol/day	10	Nimodipine	3
Xiaoyang X [37]	China	43	58 ± 4.27	72	NA	NA	Mg	20 mmol/day	14	Nimodipine	0.5
Fan Y [38]	China	60	53(30–75)	24	NA	NA	Mg plus nimodipine and simvastatin	15 mmol/day	14	Nimodipine	3
Fountas KN [39]	USA	74	62.8(42–76)	NA	1–4	NA	Mg; Mg plus nimodipine	0.016 mmol/day	NA	Nimodipine	NA
Zhitao W [40]	China	88	59 ± 14	NA	NA	NA	Mg plus flunarizine	60 mmol/day	14	Control	1
Muroi C [41]	Switzerland	58	53.6 ± 14.4	72	2–4	Yes	Mg plus nimodipine	64 mmol/day	12	Nimodipine	12
Yu L [42]	China	54	46(22–69)	48	NA	NA	Mg	30 mmol/day	20	Control	0.75
Elsaesser RS [43]	Germany	104	54 ± 18	96	1–4	Yes	Mg	72 mmol/day	14	Nimodipine	12

Table 1: Continued.

Studies	Location	Sample size	Average age	Onset window(hour)	Fisher grade	Adopt neurosurgery	Interventions	Dosage of mg#	Intervention ime (day)	Control	Follow-up (month)
Wong GKC [44]	China	60	60(25–78)	48	2–4	Yes	Mg plus nimodipine	80 mmol/day	14	Nimodipine	6
Baiocchi M [45]	Italy	17	NA	NA	NA	NA	Mg plus nimodipine	NA	15	Nimodipine	6
Yiming S [46]	China	39	59.3 ± 11.94	48	2–4	Yes	Mg plus nimodipine	40 mmol/day; 80 mmol/day	14	Nimodipine	6
Boet R [47]	China	45	57(NA)	48	NA	Yes	Mg plus nimodipine	80 mmol/day	14	Nimodipine	3
van den bergh WM [48]	Netherlands	283	54.6(NA)	96	NA	Yes	Mg plus nimodipine	64 mmol/day	14	Nimodipine	3
Veyna RS [49]	US	40	48(NA)	72	NA	Yes	Mg plus nimodipine	NA	10	Nimodipine	3

#: estimated according to various reporting units from each included study. Abbreviations: Mg: magnesium; NA: not available.

Figure 2: Risk of bias graph for each included study.

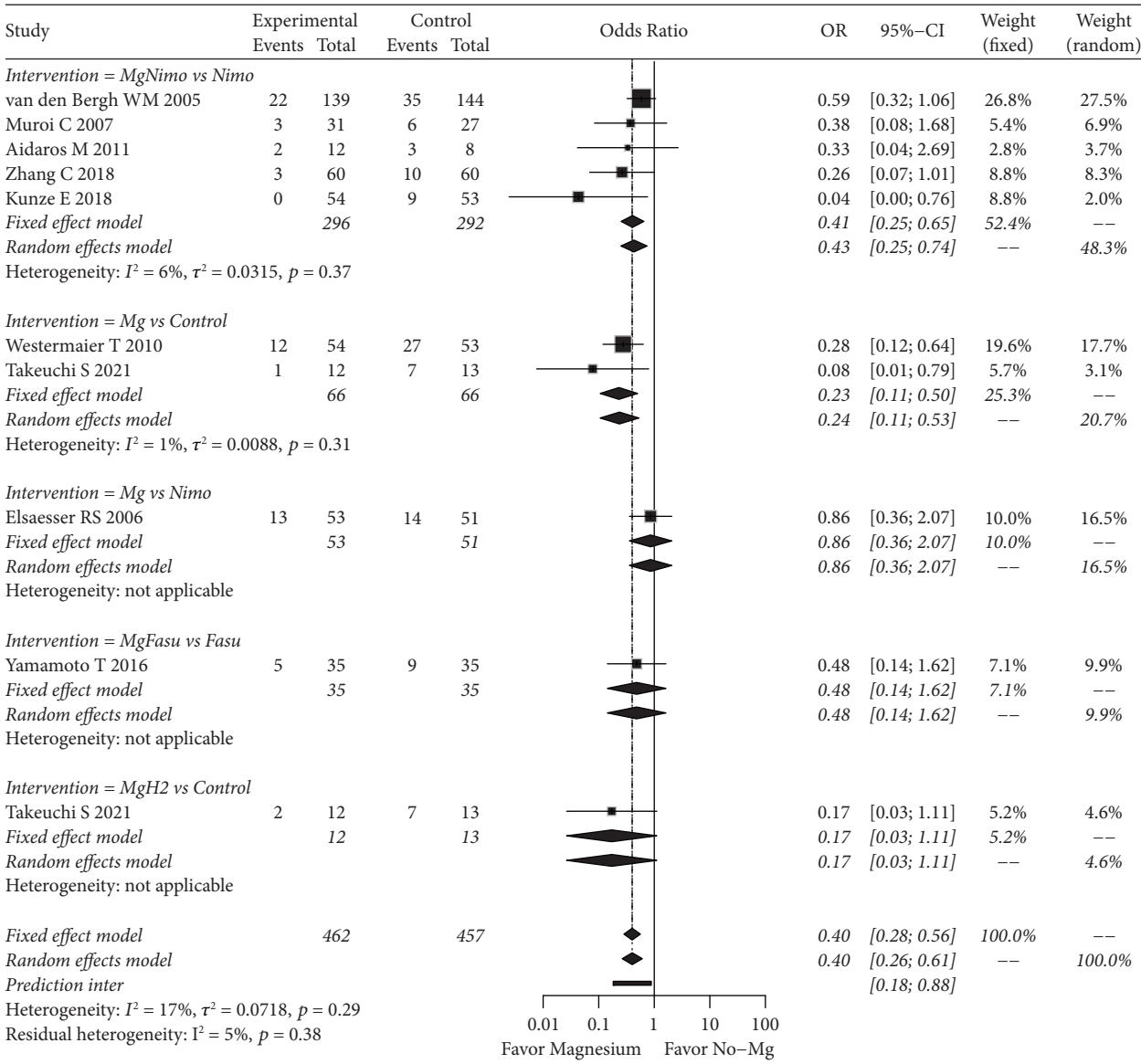


Figure 3: Forest plot of magnesium-related strategies on DCI results in the meta-analysis. Fasu: fasudil; H_2: hydrogen; Mg: magnesium; Nimo: nimodipine.

and it even has a trend of reducing the risk of death/PVS compared to nimodipine. A review showed that the low-dose statin therapy may have a beneficial effect in reducing the hemorrhagic transformation induced by thrombolysis [51]. Therefore, whether the application of simvastatin on the basis of magnesium plus nimodipine inhibits hemorrhagic transformation after a residual secondary cerebral infarction to exert a neuroprotective effect and reduce the risk of death still needs to be confirmed by clinical studies.

In the GR results, an analysis based on a single study suggested that the intracranial application of magnesium with or without the antioxidative stress therapy may be able to improve the patient's neurological outcome. The characteristic of this study is that it significantly increases the level of magnesium in the brain but not in the peripheral circulation. Theoretically, it acts more directly on intracranial blood vessels and exerts neuroprotective effects. An intravenous magnesium injection has a limited effect on increasing its level in cerebrospinal fluid and may also affect other organs, causing bradycardia and hypotension. In addition, the study focused on Fisher grade 3–4 SAH patients and did not apply nimodipine [22]. Therefore, the study suggests that for poor-grade SAH patients, increasing the intracranial magnesium concentration can help reduce DCI and CVS rates and improve neurological function recovery.

Observational studies suggest that hypomagnesemia is independently associated with hemorrhagic transformation, poor functional recovery, and DCI in SAH patients [10, 17]. Therefore, it is still reasonable to maintain magnesium levels in the normal range after SAH [17]. Magnesium showed improvement in neurological function in a phase 2 study [48] and was negative in a phase 3 study [30, 34]. The

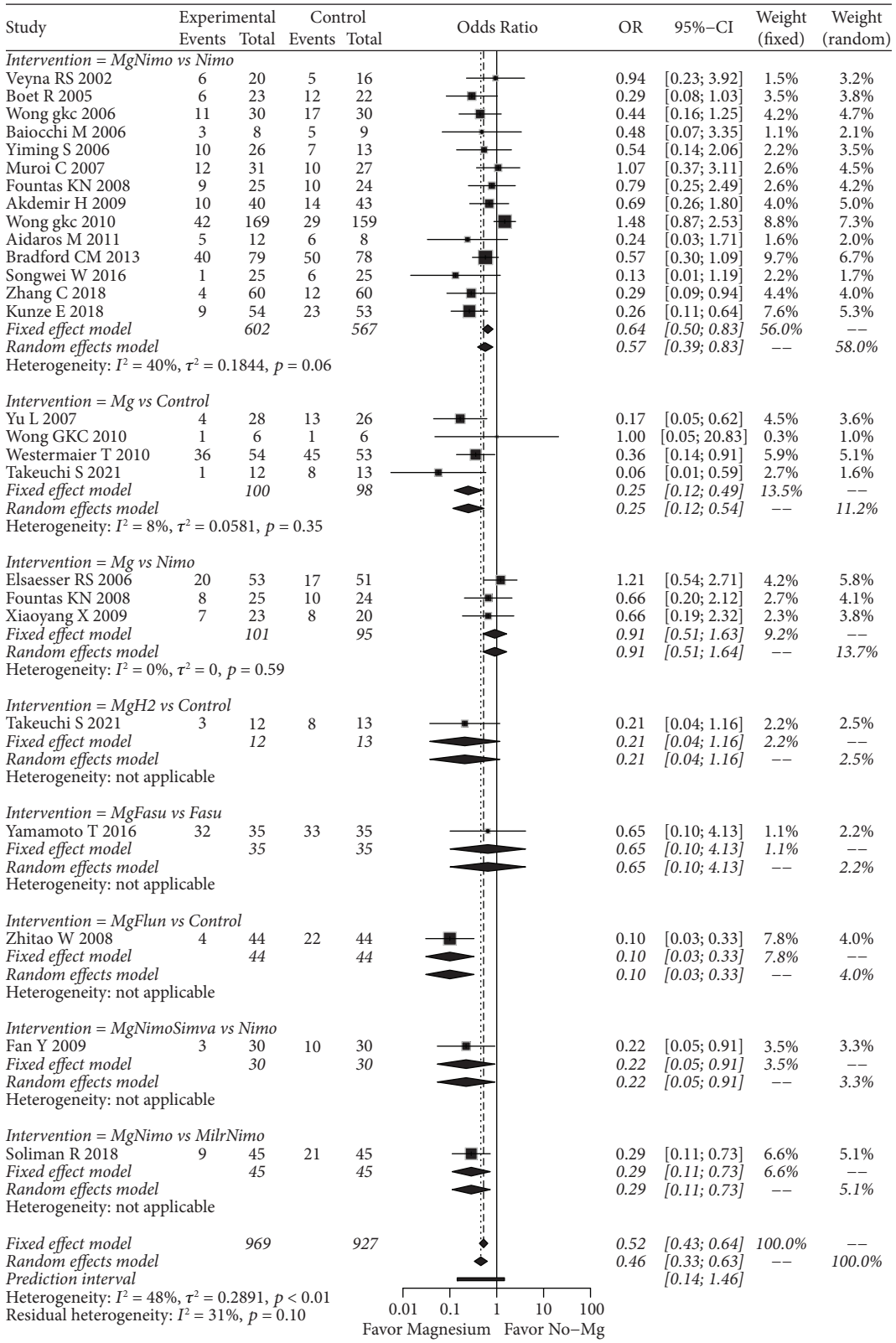


Figure 4: Forest plot of magnesium-related strategies on CVS results in the meta-analysis. Fasu: fasudil; Flun: flunarizine; H_2: hydrogen; Mg: magnesium; Nimo: nimodipine.

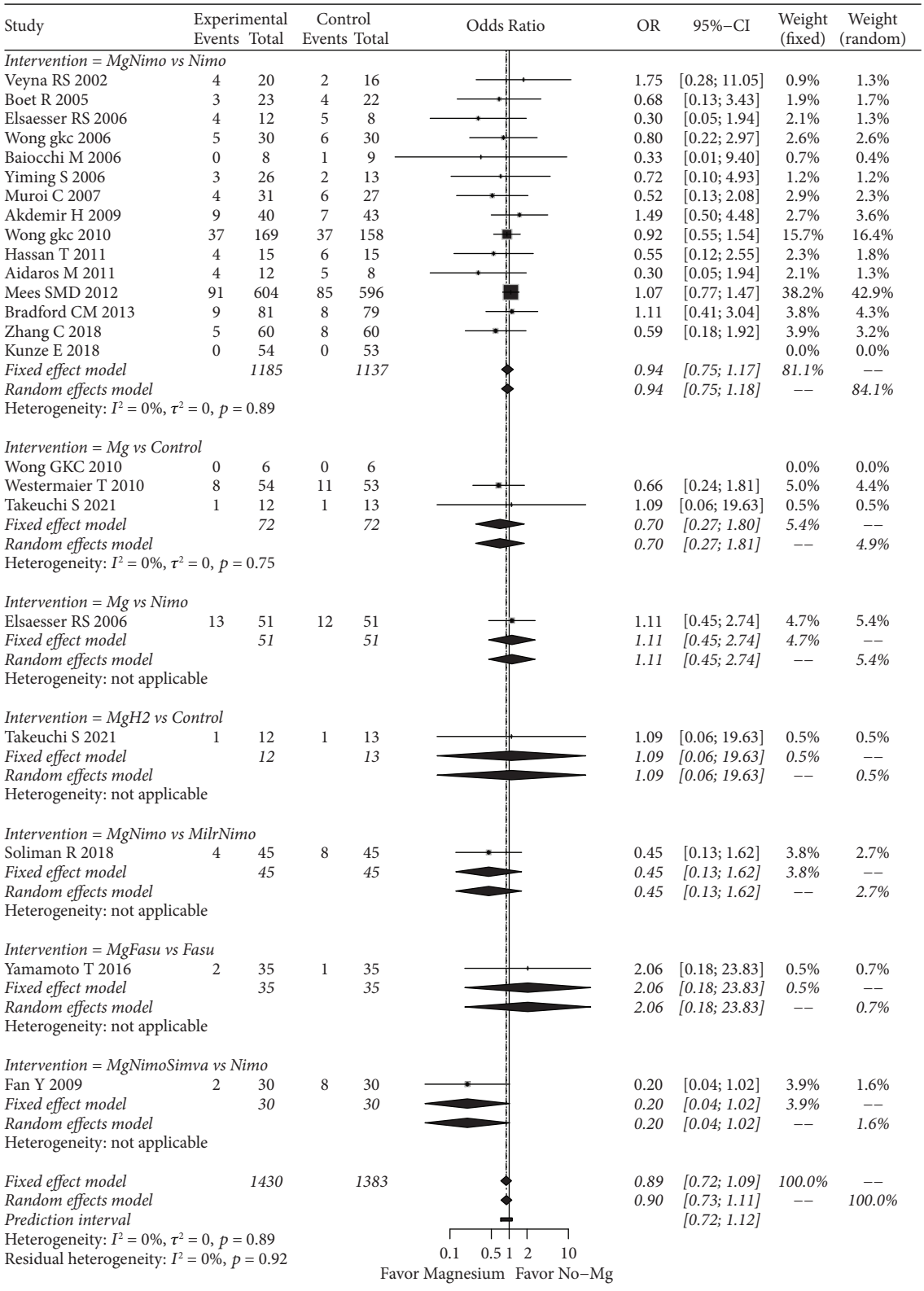
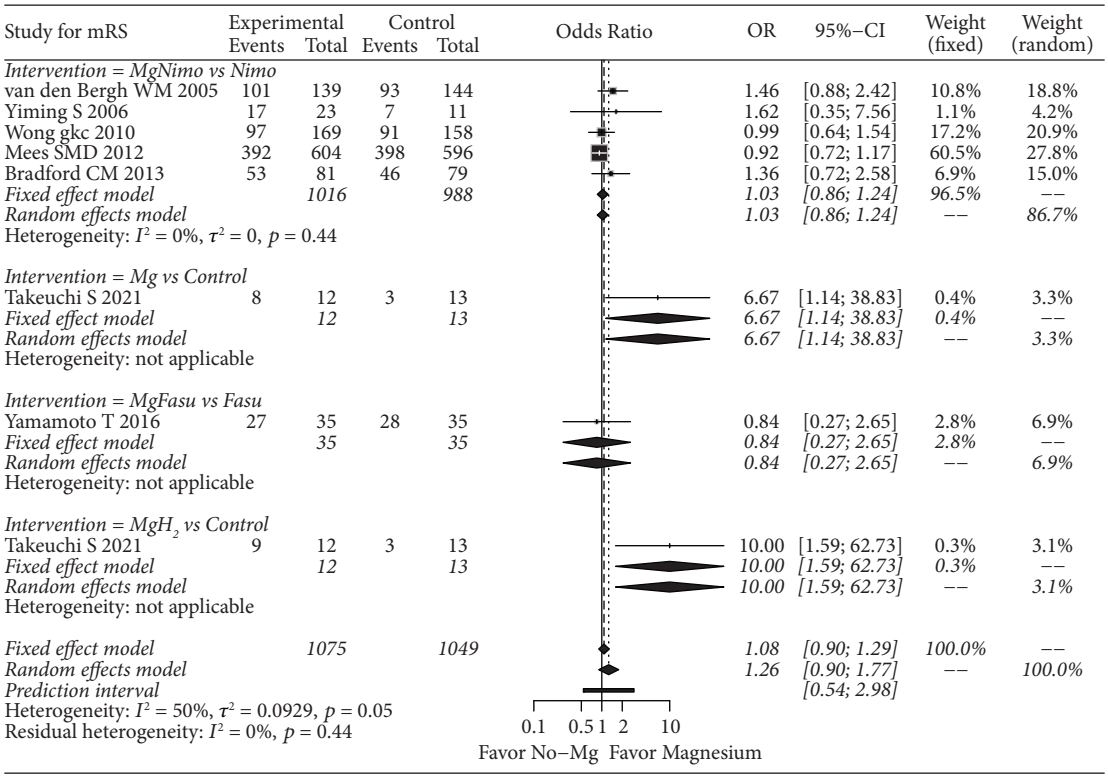
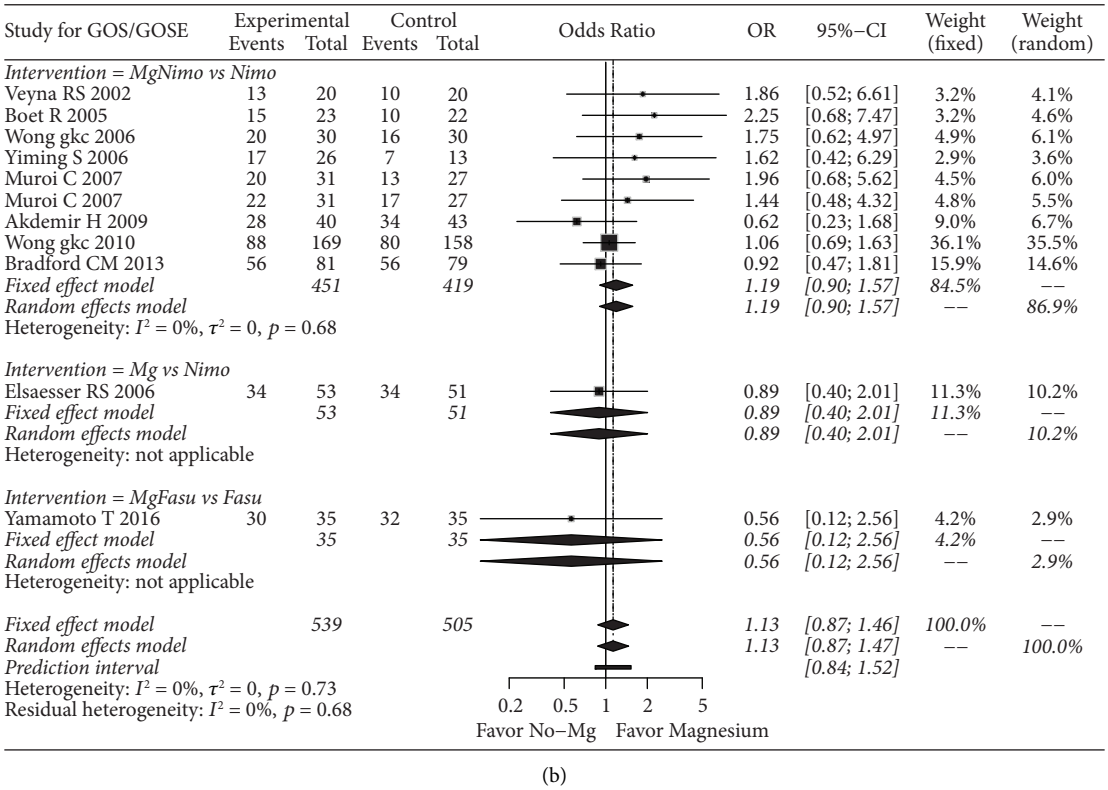


Figure 5: Forest plot of magnesium-related strategies on death/PVS results in the meta-analysis. Fasu: fasudil; H_2: hydrogen; Milr: milrinone; Mg: magnesium; Nimo: nimodipine; Simva: simvastatin.

negative results in the phase 3 study may be because of the long time it takes to increase magnesium levels in the cerebrospinal fluid after the initiation of the intravenous administration of magnesium and the differences in the concomitant drugs with magnesium. In this study, the intracranial application of magnesium combined with the



(a)



(b)

Figure 6: Forest plot of magnesium-related strategies on GR results in the meta-analysis. Fasu: fasudil; H_2: hydrogen; Mg: magnesium; Nimo: nimodipine.

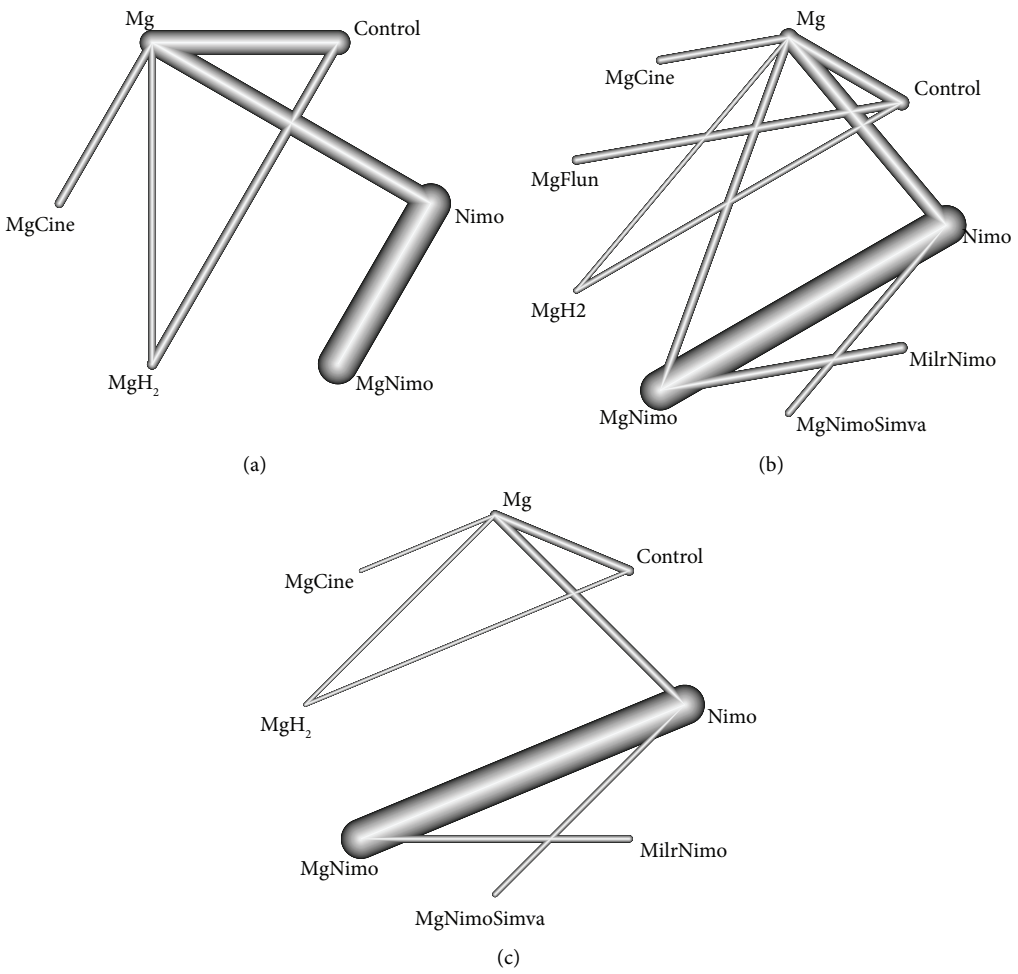


Figure 7: Network comparisons for the strategies included in the analyses. Cine: cinepazide; Flun: flunarizine; H_2: hydrogen; Milr: milrinone; Mg: magnesium; Nimo: nimodipine; Simva: simvastatin.

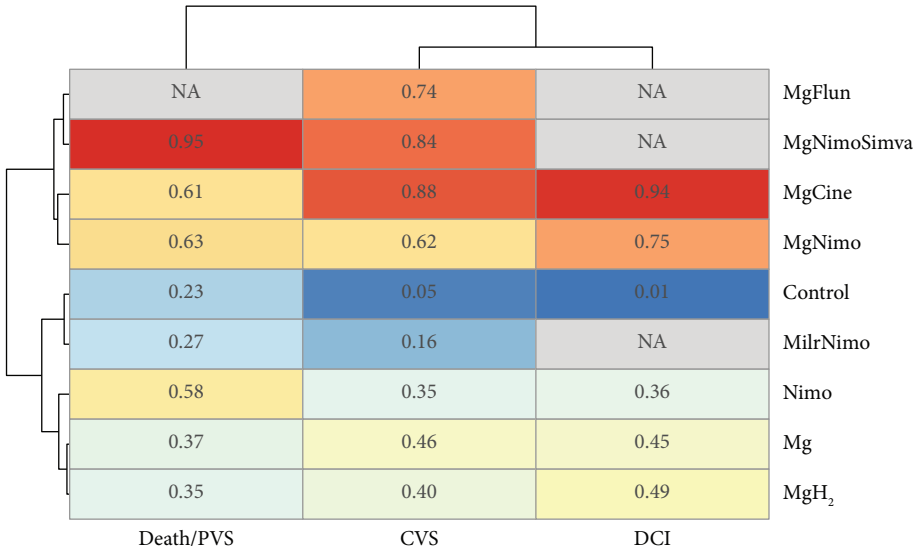


Figure 8: Cluster analysis of outcomes and interventions based on network meta-analysis ranking results. Cine: cinepazide; Flun: flunarizine; H_2: hydrogen; Milr: milrinone; Mg: magnesium; Nimo: nimodipine; Simva: simvastatin.

hydrogen antioxidant significantly promoted the GR rate of SAH patients immediately after surgery. The strategy of the simultaneous application of nimodipine and simvastatin with magnesium showed a trend of reducing the risk of mortality and PVS. These two points may provide new information to modify the existing clinical application strategy of magnesium.

Based on the included clinical studies, magnesium supplementation has been shown to be beneficial in reducing the DCI risk. Modifying the magnesium clinical application strategy may further improve the effect on improving the GR rate and survival prognosis. These studies indicated that immediate intracranial magnesium therapy combined with hydrogen is beneficial to improve the GR rate for SAH patients, and the combination of magnesium plus nimodipine and simvastatin may have a tendency to improve survival outcome. Therefore, two implications may impact future research. Firstly, the immediate intracranial application of magnesium after SAH. On the other hand, it is used in combination with other drugs, such as antioxidants, nimodipine, and simvastatin, to improve the effectiveness of interventions. These directions deserve to be validated by further well-designed studies.

In conclusion, this study supports the beneficial effect of magnesium in reducing the risk of DCI. Based on a single RCT, immediate intracranial magnesium therapy with intravenous hydrogen after SAH can increase the GR rate. Therefore, more high-quality RCTs are needed to confirm this finding.

4.1. Limitations. There were still several limitations in this analysis. First, this analysis is based on the study level but not on the individual level. Second, although this study analyzed the effect of magnesium dose on each outcome by meta-regression, the dependent variable used was the total dose of magnesium application, and it is still not possible to analyze whether differences in magnesium levels in peripheral blood and in the cerebrospinal fluid have an effect on SAH treatment outcome. Third, in pooling results, publication bias was detected. It indicates that there are still some potential negative results that have not been published. It will cause the results of this study to tend to be positive. Fourth, the definition of CVS is inconsistent in the included studies, and if assessor blinding is not performed at the same time, it may also make the result more positive.

4.2. Future Directions. Hypomagnesemia occurs in more than 50% of patients with SAH and is independently associated with DCI, poor neurological prognosis, and hemorrhage severity. Therefore, it is still necessary to maintain magnesium at a reasonable level. However, the results of phase 2 and phase 3 studies on magnesium in the treatment of SAH are incongruent, which may be because of the use of intravenous infusion and the difference in the combination of drugs.

The results of this study support the view that magnesium can reduce the risk of DCI. Based on a single study, the intracisternal infusion of magnesium immediately after SAH with intravenous hydrogen can increase the rate of GR, and the combination of nimodipine and simvastatin with magnesium has a tendency to improve survival/PVS prognosis. Future research can focus on the intracisternal infusion of magnesium immediately after SAH and the combination of magnesium and other drugs, such as antioxidants, nimodipine, and simvastatin, to further explore the application value of magnesium on SAH.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Supplementary Materials

Supplementary 1. Supplementary Figure 1. Funnel plots of the meta-analysis on DCI (A), CVS (B), and death/PVS (C) outcomes showed potential publication bias. Supplementary 2. Supplementary Table 1. The league table for DCI result estimates magnesium treatment strategies according to their relative effects (odds ratio with 95% confidence intervals). Supplementary 3. Supplementary Table 2. The league table for CVS result estimates magnesium treatment strategies according to their relative effects (odds ratio with 95% confidence intervals). Supplementary 4. Supplementary Table 3. The league table for death or PVS result estimates magnesium treatment strategies according to their relative effects (odds ratio with 95% confidence intervals). Supplementary 5. Supplementary Table 4. Meta-regression analysis of the correlation between magnesium intervention-related factors and estimated effects. (*Supplementary Materials*)

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Research Article

Coaching-Based Teleoccupational Guidance for Home-Based Stroke Survivors and Their Family Caregivers: Study Protocol for a Superior Randomized Controlled Trial

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Background. Home-based rehabilitation has been shown to be useful for stroke survivors to participate in daily life activities and return to their families. However, many home-based stroke survivors face challenges in the lack of professional guidance, rational training plans, and insufficient motivation, which will affect their rehabilitation outcomes to varying degrees. Though occupational therapy and coaching are widely recommended for stroke rehabilitation, studies that combine these two interventions via telerehabilitation in home-based rehabilitation are limited. Hence, this study will explore whether coaching-based teleoccupational guidance (CTG) will help stroke survivors and caregivers obtain satisfactory outcomes. *Methods*. This single-blind (assessor), two-arm parallel superior randomised controlled trial will be conducted in the Hebei General Hospital, Shijiazhuang, China. Ninety-two participant dyads in home-based rehabilitation will be recruited and randomised to either CTG (intervention group) or a standard telerehabilitation group (control group). Participant dyads in the intervention group will follow a 6-step circle procedure and receive 12 teleoccupational coaching sessions over 3 months via WeChat. Data will be collected at baseline, after the intervention (3 months), and follow-up (6 months). The Reintegration to Normal Living Index will be the primary outcome to assess the participation of stroke survivors. Secondary outcomes will not only involve an observation of changes in activities of daily living, intrinsic motivation, motor function, and quality of life of stroke survivors but also will focus on the caregivers' perceived benefit and care burden. *Discussion*. This trial will assess the effects of CTG compared with standard telerehabilitation. We believe that the results of this study will add to the understanding of occupational therapy for stroke survivors in home-based rehabilitation and provide a reference for developing health policy and facilitating other chronic management. *Trial Registration Number*. The Chinese Clinical Trial Registry ChiCTR2200061107.

1. Introduction

Stroke has become the leading cause of mortality and morbidity in adults of China [1, 2]. Approximately 70%–80% of stroke survivors experience varying degrees of impairment of their motor, sensory, cognitive, and other functions [3] that might reduce their participation, decrease their quality of life, and increase the caregivers' burden [4]. Home-based rehabilitation, an effective complement to in-hospital rehabilitation, is essential for realizing the stroke survivors' full potential to live better [5]. As one of the core pillars of home-based rehabilitation, occupational therapy is an important intervention to help stroke patients return to their families and society. However, owing to the lack of professional guidance, reasonable training plans, insufficient motivation, poor compliance, and so on, the quality of usual home-based rehabilitation is unsatisfactory [4, 6, 7]. Therefore, the active exploration of efficient home-based rehabilitation strategies has great practical significance.

Coaching is a multidimensional, cognitive behavioural intervention that promotes client-centred, goal-oriented development through various engagements, such as interviews, listening, questioning, and the inculcation of other skills to enhance self-monitoring behaviours and a sense of responsibility, cultivation of internal motivation, skill acquisition, and to improve the health status and quality of life [8, 9]. In 2007, the Canadian occupational therapists association recommended coaching as a practical skill in the occupational therapy practice framework. Although considerable evidence supports the effectiveness of coaching on the self-management quality of life in chronic disease [10, 11], few studies have investigated coaching combined with occupational therapy in home-based stroke survivors. In addition, some previous related studies only added simple coaching strategies to intervention processes, which might not reflect the true effect of the coaching [12, 13].

The aim of this study is to investigate the effectiveness of coaching-based teleoccupational guidance (CTG) for home-based stroke survivors and their caregivers. It is anticipated that, compared to routine telerehabilitation, CTG would lead to better rehabilitation outcomes for stroke survivors and their caregivers.

2. Materials and Methods

2.1. Study Design. This single-blind (assessor), two-arm parallel superior randomised controlled trial of a CTG program in comparison with standard telerehabilitation for stroke survivors and their caregivers will be conducted at the Hebei General Hospital in Shijiazhuang, China (Figure 1). The Hebei General Hospital is one of the earliest large polyclinics that set up a Rehabilitation Medicine Department on the Chinese mainland and is equipped with sufficient, high-quality rehabilitation resources. There will be sufficient patient supply in this research setting. The duration of the intervention and follow-up in this study will be 3 and 6 months, respectively. The Ethics Committee of Hebei General Hospital approved the study protocol, which follows the Standard Protocol Items: Recommendations for Interventional Trials (CONSORT Additional File [1]. Informed consent from all participants will be obtained prior to their enrolment in the study.

2.2. Participants

2.2.1. Inclusion and Exclusion Criteria for Stroke Survivors and Caregivers. In this trial, we will recruit stroke survivors and their primary caregivers as participant dyads subject to their provision of written informed consent. Participants will be included based on the following inclusion criteria: (1) men or women aged 18 to 70 years; (2) diagnosed with ischaemic or haemorrhagic stroke either by computerized tomography scanning or magnetic resonance imaging; (3) being more than 6 months after the onset of stroke; (4) modified Rankin scale (MRS) score of 2 to 4 points; (5) undergoing home rehabilitation and no admission plan within 3 months; and (6) residing within or around the city of Shijiazhuang. Patients will be excluded if they (1) have a Glasgow coma scale (GCS) score of less than 15; (2) have cognitive and (or) psychotic disorders; (3) have severe comorbidities, including circulatory, digestive, immune, and haematological disorders; (4) have other diseases of the locomotor system, such as fracture, severe osteoporosis, and osteoarthritis, that will influence the stroke survivors' motor function; (5) have bilateral brain lesions; and (6) have severe sensory dysfunction.

Caregivers will be included if they meet the following inclusion criteria: (1) men or women who are at least 18 years old; (2) are the primary caregivers and can ensure the time and support of participating in the patient's home rehabilitation; and (3) can operate WeChat.

2.2.2. Inclusion Criteria for Occupational Therapists. The selection of qualified occupational therapists is crucial for ensuring trial quality. We will select occupational therapists who have the following qualifiication: (1) an educational background in occupational therapy, (2) engaged in occupational therapy for at least 5 years, and (3) strong interpersonal skills.

2.3. Recruitment Strategy. Using the medical record checklist of the Rehabilitation Medicine Department, a research assistant will screen the stroke survivors who meet the prespecified inclusion criteria and do not meet the exclusion criteria. If stroke survivors and their primary caregivers meet the study's eligibility requirements, the researcher will contact them via telephone to share relevant information about the study. Participant dyads who are interested to participate in the study will be invited by the principal researcher (YNY) for a detailed face-to-face interview to assess their eligibility to participate in the study.

2.4. Randomisation and Blinding. Participant dyads will be allocated to the CTG or the standard telerehabilitation group by using 1 : 1 randomisation sequences that are generated by IBM SPSS Statistics version 25. The random number sequence generation will be undertaken by a research assistant who is not involved in the assessment and intervention and will be placed in sequentially numbered, opaque, sealed envelopes for blinded group allocation.

Stroke survivors, primary caregivers, and occupational therapists will not be blinded due to the nature of the intervention. However, the assessor will be blinded to collect each outcome measure and undertake data entry. Furthermore, all participant dyads will meet with the same trained and blinded study assessor at all-time points to ensure consistency of outcome measures.

2.5. Description of the Interventions

2.5.1. Coaching-Based Teleoccupational Guidance. We will conduct the intervention according to a 6-step cycle (Figure 2).

(1) Step I: *Building Coaching Relationships.* The treating therapists will establish equal, friendly, and mutual trust

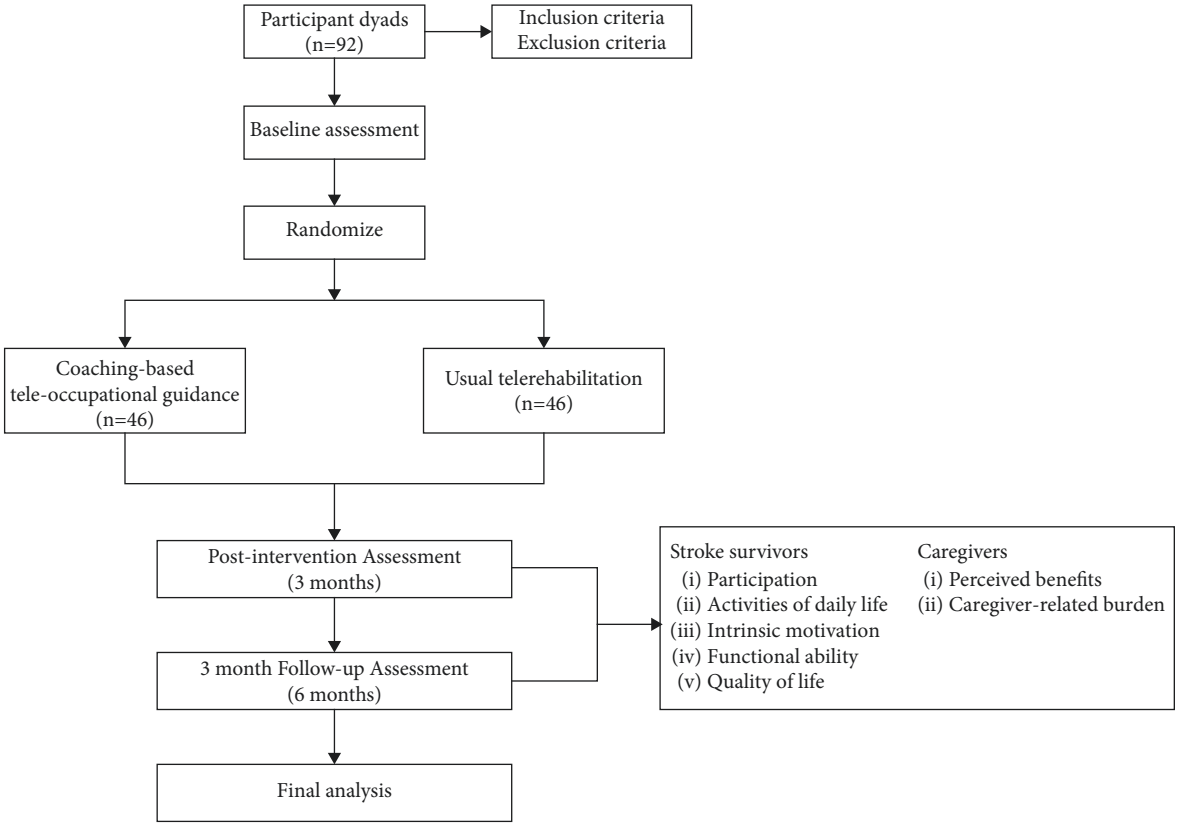

Figure 1: Study flow chart.

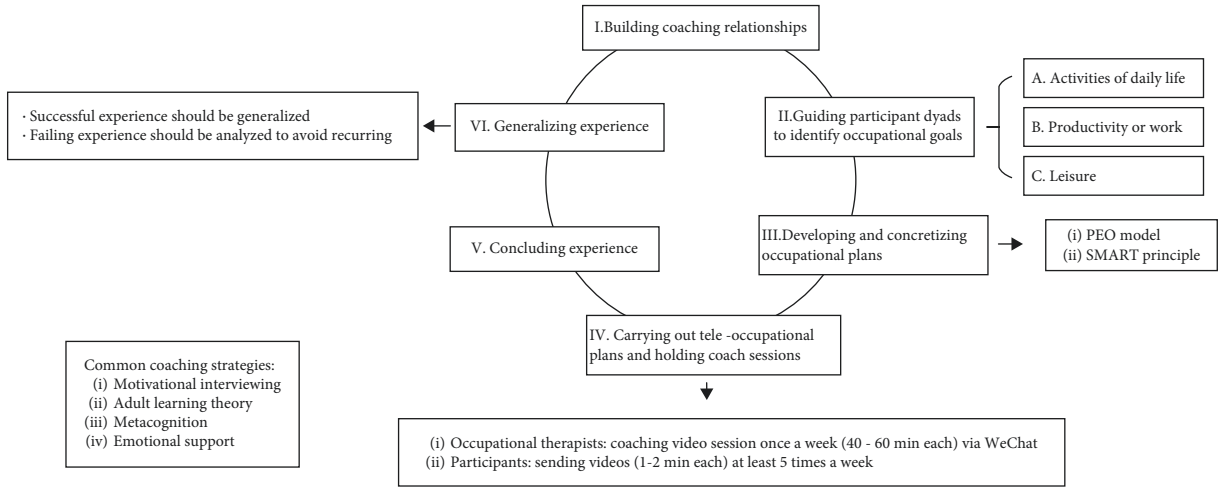

Figure 2: The general cycle procedure of the CTG intervention.

coaching relationships with the participant dyads. A private WeChat group that includes only the occupational therapist, the patient, and the caregiver will be provided to ensure timely guidance and communication in home-based rehabilitation. Occupational therapists will train participants to use WeChat's functions, such as dialling videophones, sending videos, and the methods of clicking photos or capturing videos. If WeChat has technical difficulties due to network problems or other factors, the telephone call will be used as a backup communication method.

(2) Step II*: Guiding Participant Dyads to Identify Occupational Goals.* The treating therapist will use the Canadian occupational performance measure (COPM) and coaching techniques to guide participant dyads to identify up to 5 occupational goals that reflect a wide range of ADLs (e.g., eating a meal with chopsticks and spoon; dressing, and grooming); productivity (e.g., resuming paid employment); community engagement (e.g., going to the weekly market; resuming driving), domestic activities (e.g., cooking a family meal); and leisure activities (e.g., walking the dog; restoring

furniture) [14]. Moreover, the therapist will use the motivational interview of coaching strategies flexibly (e.g., emotional support, expressing empathy, "looking forward," "looking back," and so on) to guide the patients to think and describe the reasons for making changes and to clarify the gap between their current situation and goals, which can help participants to gain intrinsic motivation for change.

(3) Step III*: Developing and Concretizing Occupational Plans.* The person–environment–occupation (PEO) model will be used to help occupational therapists work with participant dyads to identify a structured and individualised problem-solving plan. In conjunction with the use of the PEO model, plans should involve the following 4 aspects: (a) occupational therapists will provide therapeutic occupational activities, ADLs, and social skills training to improve personal functioning based on the stroke survivor's career goals and performance, cultural background, hobbies, and family environment; (b) according to their needs, participants will be provided professional suggestions for environmental adjustment to gain safety and a convenient home environment (e.g., installing toilets and handrails), whereas importantly focussing on support from caregivers (e.g., creating a positive family environment, training care skills); (c) we can adjust occupational activities if necessary (e.g., converting offline shopping to online shopping); (d) participant dyads will receive individualised content education where appropriate, including ergonomic, skills remediation, safe management, compensatory strategies, and alternative methods. To standardise the plan, we will comply with the principles of SMART (Specific, Measurable, Attainable, Relevant, and Time-bound).

(4) Step IV*: Carrying Out Teleoccupational Plans and Holding Coaching Sessions.* Treatment plans will be posted on WeChat as scheduled. Occupational therapists will provide participants with a weekly coaching video session (40–60 min each) via WeChat for 3 months. We will follow the "ORAG" (Open-ended questions, Reflective listening, Affirmation, and Guide) program (Table 1) to structure the sessions.

In addition, participants will be asked to send videos (at least 5 times a week) to the WeChat group. The content of these videos should include the practice of occupational exercises, participating in ADLs, and so on. If the researchers have any questions or detect potentially unsafe behaviours in the videos, they can give suggestions or adjust timely occupational activities by sending voice messages and pictures or conducting video calls on WeChat. Furthermore, if participants have doubts about the study, they can contact the researchers at any time through the WeChat group.

(5) Step V*: Concluding Experience.* An occupational therapist will evaluate the achievement of goals and encourage participant dyads to summarise their experience to help them build up the ability to independently deal with problems.

(6) Step VI*: Generalising Experience and Entering the Next Cycle.* Finally, the successful experience should be affirmed and generalised, whereas the failure experience should be analysed to avoid recurrent failures. Participants enter the next cycle to achieve other goals when a goal has been completed. Though the CTG process is depicted as a cyclic progression (Figure 2), each step may be revisited at any time.

2.6. Standard Telerehabilitation (Control Group). Participants in the standard telerehabilitation group will receive home-based rehabilitation plans according to the selected occupational goals of the stroke survivors and have a weekly video session (40–60 min each) with the occupational therapist via WeChat for 3 months, which aims to help participant dyads to adjust plans and solve problems. Participants should send videos (1-2 min each) of home-based rehabilitation to the WeChat group at least 5 times a week for 3 months. Researchers will ensure timely follow-up and adjust the participants' training via WeChat.

2.7. Outcomes Measures. The participant dyads will visit our outpatient department for outcome evaluation at baseline, immediately after the intervention, and at the 6-month follow-up. Sex, age, type of stroke, hemisphere, dominant side, profession, and other social information will be collected after enrolling participants in this trial. Based on the purpose of the trial, we choose the following parameters as the study's outcomes. Moreover, most of the items are simple questions-and-answers and included patients who are in relatively good health condition, stroke patients, and their caregivers take only approximately 30 minutes to complete the assessment. But if patients' cannot complete all measures at once, the researcher who is in charge of the assessment can use WeChat videos to finish the remaining part the next day. Figure 3 shows the study's timeline.

2.8. Outcomes of Stroke Survivors

2.8.1. Primary Outcomes. Participation: The Reintegration to Normal Living Index (RNLI) is an 11-item self-report scale to test participation in 2 dimensions: physical activities and social events. Scores on the self-report scale range from 1 to 110, with higher scores indicating better participation. The RNLI has been translated into Chinese and has been proved to be reliable and valid for the Chinese population (Cronbach's α 0.92; retest reliability coefficient 0.87) [15].

2.8.2. Secondary Outcomes. ADL: We will measure this outcome using the Modified Barthel Index (MBI) and Lawton Instructive Activities of Daily Life (Lawton IADL). The MBI consists of a 10-item scale to test basic ADLs. Scores range from 0 to 100, with higher scores indicating better independence [16]. The Lawton IADL has 8 items of activities that test instructive ADLs, with each scored from 0 to 2 [17]. The MBI and Lawton IADL have been widely used in stroke rehabilitation studies.

Intrinsic motivation: We will measure this outcome using the Intrinsic Motivation Inventory (IMI). The IMI consists of 54 items of self-report questionnaires to test motivational structures for targeted activities in 6

Table 1: The "ORAG" program for CTG.

Items	Topics
Open-ended questions	Occupational therapists will guide participants to think about whether the occupational performance of this week was moving towards their goals by asking open-ended and enlightening questions.
Reflective listening	Occupational therapists should listen to the feedback information of the participants and analyse the relevant emotional changes and thoughts.
Affirmation	Occupational therapists should praise the progress made by the participants and encourage them to analyse and summarise their successful experiences.
Guide	If participants have barriers to achieving goals, occupational therapists will help them independently analyse possible and potential reasons. Next, suggestions and resources will be provided to identify bridges to success by combining the adult learning model and occupational strategies.

Study period					
	Enrolment		Allocation	Post-allocation	
TIME POINT	Before intervention	Baseline data collection	Start of intervention	Post-intervention (3 months)	Follow-up (6 months)
Enrolment					
Eligibility screening	×				
Informed consent	×				
Allocation			×		
Intervention					
CTG			←	→	
ST			←	→	
Assessment					
Stroke survivors					
1. Participation		×		×	×
2. ADL		×		×	×
3. IM		×		×	×
4. MF		×		×	×
5. QOL		×		×	×
Caregivers					
1. PB		×		×	×
2. CB		×		×	×

Figure 3: The figure shows the enrolment, interventions, and assessment time points. CTG, coaching-based teleoccupational guidance; ST, standard telerehabilitation; ADL, activities of daily living; IM, intrinsic motivation; MF, motor function; QOL, quality of life; PB, perceived benefits; CB, caregiver-related burden.

dimensions (score range: 0–7): interest/enjoyment, perceived competence, pressure/tension, and value/usefulness [18].

Motor function: We will measure this outcome using the Fugl–Meyer Assessment-Upper Extremity (FMA-UE), Motor Activity Log, and 6-minute walking test (6MWT). (1) The FMA-UE is a 33-item scale that is used to assess the movement, coordination, and reflex actions of the shoulder, elbow, forearm, wrist, and hand. The total score ranges from 0 to 66 points, with higher scores indicating better motor function [19]. (2) The Motor Activity Log will be used to test the extent of natural use of the affected upper limb in the real world (outside the rehabilitation setting) in persons who have suffered a stroke. The scale has 2 subscales: quality of movement (QOM) and amount of use (AOU). Overall, it consists of 30 items for assessing the quality and quantity of the use of the affected limb for daily tasks [20]. (3) The 6MWT is a simple test to evaluate the walking function; it is a familiar form of exercise for patients and is more relevant to their everyday lives, reflecting participation and walking capacity [21].

Quality of life: We will measure this outcome using the stroke-specific quality of life scale (SS-QOL). The SS-QOL is a 49-item self-report scale to test the quality of life in 12 dimensions: energy, family roles, language, mobility, mood, personality, self-care, social roles, thinking, upper extremity function, vision, work/productivity, each scored from 1–5, with a higher score indicating well-being [22].

2.9. Outcomes of Caregivers. Perceived benefits: We will measure this outcome using the Caregiver Benefit Finding Scale (CBFS). The CBFS was designed by Chinese scholars with a Cronbach's α range of 0.885 to 0.953 for the subscales. It contains 26 items in 4 dimensions: individual growth, health promotion, family growth, and self-sublimation. The total score ranges from 26 to 130 points, with better scores indicating more perceived benefits [23].

Caregiver-related burden: We will measure this outcome using the Chinese version of the Zarit Caregiver Burden Interview (ZBI-c). The ZBI-c contains 22 items in 2 dimensions: personal strain and role strain. The total score ranges from 0 to 88 points, with better scores indicating a more significant caregiver burden, and the scale was validated in the Chinese population with a Cronbach's alpha of 0.87 [24].

2.10. Sample Size. The RNLI, commonly used as a reliable outcome measure in occupational therapy research, will be employed for sample-size estimation. According to our preliminary test and previous studies, the minimum significant difference between the 2 groups in the T2–T1 interval for the RNLI in the survey is 14 points [25]. The standard deviation of the measure was reported as 21 points in previous studies [25]. Setting the α at 0.05 (two-tailed) and the power at 80% and assuming a drop-out rate of 20%, the minimum sample size for each arm is 46. Based on this estimation, we will recruit at least 92 participant dyads (92 participants and 92 caregivers in each group–intervention versus standard telerehabilitation) for the study.

2.11. Statistical Analysis. An intention-to-treat (ITT) analysis will be used for data analysis, as specified by CONSORT guidelines [26]. Statistical analysis will be performed using IBM SPSS Statistics version 25. Descriptive analysis will be used to analyse the demographic data in each participant group. The Shapiro–Wilk test will be used to assess the normality, and the data will be presented as mean ± standard deviation. In contrast, skewed data will be reported in the median and interquartile range. The categorical data will be expressed as a proportion (percentages). Comparisons of demographic and clinical characteristics at the baseline of both the study groups will be tested by independent sample *t*-tests and the chi-square or nonparametric Mann–Whitney *U*-test, respectively.

Repeated-measures analysis of variance (ANOVA) will be used to analyse the primary and second outcomes with regard to time (baseline, 3-month, and 6-month follow-up) as the within-group factor and interventions (standard telerehabilitation versus coaching-based teleoccupational guidance) as the between-group factor. We will evaluate the main effects of time, group, and time-group interaction effects. The Mauchly test will be performed to check the sphericity of the data; if significant, the Greenhouse–Geisser statistic will be used to adjust the degrees of freedom [27]. The level of significance will be set at 0.05.

2.12. Data Management. The medical data recording sheets, signed informed consent forms, and the data of the WeChat group (photos, videos) from participant dyads will be stored securely. The principal researcher (YNY) will be responsible for monitoring data in this study. Only authorised research assistants will have access to the trial data. In addition, as this study is an early, exploratory, short-term study without severe safety concerns, the Independent Data Monitoring Committee will not be set up.

2.13. Quality Control. Before commencing the study, the principal researcher (YNY), a senior occupational therapist who is adept at using coaching in home-based rehabilitation, will provide 3 workshops (1 hour each) for 2 occupational therapists (ZXS, XJG) and 2 occupational therapist assistants (XLQ and LZ) who will be involved in the intervention to introduce and train them on the related information about this study. The first workshop will inform occupational therapists of the trial flow, division of research, and associated issues of quality control. The second workshop will enable them to master the commonly used coaching strategies and home-based occupational therapy for stroke survivors. The team will recruit 4–6 inpatient stroke patients for the final workshop, which undertakes the role of pre-experiment to help occupational therapists practice the acquired knowledge and get feedback from patients, peers, and the principal researcher. Through the pre-experiment, researchers can discover the problems existing in the trial and solve them in time. In addition, occupational therapists will be divided into 2 groups, each group consisting of 1 occupational therapist who is the primary implementer of the study and 1 occupational therapist assistant who cooperates with the occupational therapist to complete daily work and follow the process of study. Specifically, XLQ and ZXS as a group will be in charge of 23 participant dyads in CTG and 23 pairs in the control group (46 participants and 46 caregivers in total) using a random number sequence, as will the other group of LZ and XJG. And the principal researcher (YNY) will pay close attention to their work and provide help if necessary. To ensure the accuracy of the trial data, two postgraduates who will not participate in this study will enter data independently.

2.14. Intervention Fidelity. The occupational therapist will document the content of the coaching home-based rehabilitation by taking notes and session video recordings to monitor intervention fidelity. The principal researcher will

test fidelity by rating 3 randomly selected video recordings of sessions per participant.

2.15. Dealing with Contingencies. The first possible contingency in the study is the difficulty in recruiting an adequate number of stroke survivors to meet the sample-size requirement. If necessary, expanding the scope of recruitment to the departments of neurology, neurosurgery, or other hospitals in Shijiazhuang should be considered. The second possible unexpected problem is the high drop-out rate of participants. To prevent this possibility, researchers will improve patients' correct perception of clinical trials and establish appropriate expectations. In addition, if the participants feel uncomfortable during the intervention period, researchers will pause the intervention or provide them with appropriate treatment.

3. Discussion

Coaching, which emerged from motivational interviewing, is the practice of health education and health promotion within a coaching context to enhance the well-being of individuals and facilitate the achievement of their health-related goals [28, 29]. Motivational interviewing, a core component of coaching strategies, focuses on motivating the patient to achieve goals that enhance the quality of life and improve the outcome of rehabilitation [28]. In our study, motivational interviewing methods will be adopted for stroke survivors and caregivers, such as emotional support, expressing empathy, supporting self-efficacy, looking forward, looking back, and so on. These strategies will equip them with knowledge and motivation to change behaviours or to identify advantages and barriers for participant dyads to achieve goals in the current situation [30, 31]. Furthermore, coaching sessions are crucial to ensure positive health outcomes, which can help the occupational therapist find and solve timely problems that happen to patients and their caregivers. Coaching sessions will be carried out by structured procedures, including open-ended questions, reflective listening, affirmation, guiding, and summary, which are easier to conduct for occupational therapists who, by their vocation, are nonprofessional coaches [32]. Most importantly, this process will enable the participant dyads to learn to independently solve relevant problems in future home-based rehabilitation.

Many fundamental coaching principles are congruent with occupational therapy values and commitment to client-centred practice [33]. In our study, the occupational therapist will use the COPM model, which is widely used in stroke rehabilitation research, to help participants identify goals. Goal setting should be individualised and realistic to motivate participants to achieve their goals. However, some previous home-based rehabilitation programs paid more attention to physical functions but ignored the demands, and psychological and social functions of stroke survivors, which could not bring a long-term effect [34]. In contrast, researchers focus on the demands of patients and make activities of daily living integrate into occupational plans so that they can quickly return to home life and maintain a long-term effect. According to recent reports, approximately 80% of stroke survivors only participate in a few meaningful activities, which decreases their quality of life and increases the caregivers' burden to different degrees [35]. Participation is the ability to engage in meaningful activities, which is considered a significant outcome measure, especially for home-based stroke survivors [36]. Therefore, enhancing the participation of stroke survivors is prioritised by occupational therapists. Furthermore, researchers choose the RNLI as the primary outcome in this study because it has advantages in assessing overall participation of stroke from the perspective of daily functioning and perception of self and considering the person's satisfaction with the present situation, which is the best fit with the purposes of our study. However, there is relatively little research about participation in stroke. It is anticipated that the results of this intervention will provide related evidence to inform peers in home-based rehabilitation.

In addition, caregivers play an important role in home-based rehabilitation, which are not only in charge of taking care of stroke patients and assisting and monitoring patients participating in rehabilitation training but also bear substantial mental pressure and psychological burden [37, 38]. In this study, we focus on the role and burden of caregivers and listen to their demands. Moreover, the occupational therapist will provide emotional support and stroke-related knowledge for caregivers by CTG in order to improve quality of life and decrease burdens. In terms of perceived benefits, CBFS is chosen to measure this outcome, which takes individual growth, health promotion, family growth, and self-sublimation into consideration and supports caregivers to realize their growth and find personal meaning during the caregiving experience.

Occupational plans for stroke survivors are crucial to enable them to gain well-being. However, making occupational plans is challenging for occupational therapists. Therefore, we combined the PEO model with the SMART principle to optimise both. First, the PEO model will guide occupational therapists to analyse residual function, support available, and degree of challenge and clear the key points of plans from different aspects of the person, the environment, and the demands of the occupation [39]. Second, it is a complex but essential requirement for researchers to set plans. Some of the characteristics of programmes that effectively alter behaviours should be considered. The SMART principle will satisfy this requirement and play a vital role in structuring and quantifying plans to ensure that participants achieve their goals [40].

The delivery of an intervention needs to be safe, private, and easy to use. WeChat is a free software created by the Tencent Technology Company to provide instant messaging services for clients, which supports the quick sending of free voice messages, videos, and pictures as well as voice and video calls. The app is highly popular in China and has the advantages of easy operability in real-time while being convenient, fast, and economical. In the home-based rehabilitation period, stroke survivors may experience various confusing problems. Therefore, timely communication is

essential for both occupational therapists and participants. At least 5 videos each week, video call sessions once a week, and needs-based WeChat contact will allow occupational therapists and participants to solve problems or adjust plans without delay.

Several vital steps affect study fidelity. First, researchers have developed standardised and logical intervention procedures based on coaching theory, occupational therapy strategies, and behavioural change techniques. Second, training interveners and documenting the content of the intervention will improve adherence to the study design. Third, stroke survivors will be asked to send pictures and videos regularly to monitor the completion of the plan mutually. In addition, we will offer a questionnaire survey to participants to assess their general satisfaction across various aspects of the intervention and collect data on the number of participants who drop out of the study to analyse reasons for quitting.

The protocol has some limitations. First, because of the nature of the intervention, it is impossible to blind the occupational therapists and participants. Moreover, emotional support is a crucial aspect of CTG. However, occupational therapists have to provide this support due to professional ethics when stroke survivors in the control group ask for help. Therefore, both possibilities may increase the risk of bias. Second, successful home-based rehabilitation is a sustainable long-term process whereas our observation period is for 6 months. It is expected that we can explore the efficacy of a longer duration of the intervention and follow-up. Finally, future studies should take economic effects into account.

Abbreviations

CTG:	Coaching-based teleoccupational guidance
MRS:	Modified Rankin scale
GCS:	Glasgow coma scale
COPM:	Canadian occupational performance measure
ADL:	Activities of daily living
PEO:	Person–environment–occupation
RNLI:	Reintegration to normal living index
MBI:	Modified Barthel index
Lawton IADL:	Lawton's instructive activities of daily life
IMI:	Intrinsic motivation inventory
FMA-UE:	Fugl–Meyer assessment-upper extremity
6MWT:	6-Minute walking test
QOM:	Quality of movement
AOU:	Amount of use
SS-QOL:	Stroke-specific quality of life scale
CBF:	Caregiver benefit finding scale
ZBI-c:	Chinese version of the Zarit caregiver burden interview
ITT:	Intention-to-treat
ANOVA:	Analysis of variance.

Data Availability

Not applicable.

Ethical Approval

The study protocol has been approved by the Ethics Committee of Hebei General Hospital (approval number: 2022 Scientific Research LunShen, No. 125).

Consent

Written informed consent will be obtained from all participants prior to their enrolment in the study.

Disclosure

The funding source has no role in the study design, nor will undertake any role in the execution, analysis, interpretation of the data, or decision to publish the results of this research.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

LZ conceived and designed the study and wrote the trial protocol. YNY conceived and designed the study and revised the trial protocol. ZXS, XJG, XLQ, and KCL supervised the research and revised the manuscript. All authors read and approved the final manuscript.

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Supplementary Materials

Additional file 1: CONSORT 2010 checklist of information to include when reporting a randomised trial. (*Supplementary Materials*)

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Research Article

Effects of Multimodal Analgesia Combined with Auricular Point Therapy on Physical and Mental Stress and Rehabilitation Quality of Patients with Meniscus Injury during the Perioperative Period

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Objective. To investigate the effect of multimodal analgesia combined with auricular point therapy on physical and mental stress and rehabilitation quality of patients with meniscus injury during the perioperative period. *Methods*. 148 patients in our hospital from October 2019 to October 2021 who were scheduled to undergo meniscus surgery were selected and grouped according to the order of file establishment, with 74 cases in each. The control group was given routine analgesia, and the observation group was given multimodal analgesia combined with auricular point therapy. The pain level (visual analogue scale (VAS)), physical and mental stress (heart rate (HR), mean arterial pressure (MAP), depression scale (PHQ-9), and anxiety scale (GAD-7)), complications, rehabilitation quality, and analgesia satisfaction were observed. *Results*. The VAS scores of pain in the observation group were lower than those in the control group at 6 hours before operation and at 6 hours, 24 hours, and 72 hours after operation ($P < 0.05$). The MAP, HR, PHQ-9, and GAD-7 scores of the observation group were lower than those of the control group 6 hours before operation ($P < 0.05$). There was no significant difference in MAP, HR, PHQ-9, and GAD-7 scores between the two groups at 6 hours and 24 hours after operation ($P > 0.05$). The analgesic satisfaction of the observation group was better than that of the control group ($P < 0.05$). The incidence of complications in the observation group was 8.11% compared with 12.16% in the control group, which was not statistically significant ($P > 0.05$). The first exhaust, getting out of bed, and hospital stay in the observation group were shorter than those in the control group ($P < 0.05$). *Conclusion*. Multimodal analgesia combined with auricular acupuncture therapy is effective in perioperative patients with meniscus injury. It can reduce perioperative pain, reduce physical and mental stress, and promote early postoperative recovery through a variety of analgesic mechanisms.

1. Introduction

Meniscus injury is a common injury disease of the knee joint. At present, surgery is an important way to restore the integrity and stability of the meniscus, which can effectively reduce the symptoms of pain, swelling, and joint locking [1]. However, perioperative and early postoperative rehabilitation exercise pain is an important factor that leads to severe physical and mental stress and affects the compliance and rehabilitation quality of postoperative rehabilitation training. Therefore, it is very important to strengthen perioperative analgesia and management for postoperative joint function recovery.

Multimode analgesia is a kind of analgesic program that combines the application of drugs with different methods or mechanisms to make them have synergistic effects, so as to reduce the dosage of analgesics and improve the analgesic effect, and has achieved remarkable effects in clinical application [2]. In recent years, in addition to drug analgesia, it has been reported that auricular point pressing bean as a nondrug intervention can reduce the dosage of early postoperative analgesics by stimulating the auricle acupoints, without vomiting, nausea, and other adverse reactions [3]. Therefore, this study combined multimode analgesia and auricular acupoint therapy for perioperative patients with meniscus injury surgery, comprehensively analyzed the

physical and mental stress, rehabilitation quality, and pain degree, in order to provide new ideas for improving the rehabilitation quality of patients.

2. Materials and Methods

2.1. Patient Information. From October 2019 to October 2021, 148 patients undergoing meniscus surgery in our hospital were selected. There were no differences between the two groups in gender, age, inducing factors, course of disease, scope of surgical resection, and history of knee joint ($P > 0.05$), as shown in Table 1. This study was approved by the hospital ethics committee.

2.2. Patient Selection Criteria. Inclusion criteria include the following: arthroscopy, confirmed meniscus injury combined with clinical symptoms and signs; treated with arthroscopic surgery; clear language expression and consciousness; and signed the informed consent. Exclusion criteria include the following: cognitive dysfunction; people allergic to analgesic drugs; and patients with acute or chronic serious diseases.

2.3. Intervention Method. The control group took routine analgesia:

(1) Preoperative: no analgesic drugs were used.

(2) Postoperative: parecoxib 40 mg was given orally, once every 12 h, for 3 d. On the 1st postoperative day, 200 mg celecoxib was given orally, twice a day, and tramadol 1co was given orally, 3 times a day for 7 d. If the patient still felt unbearable pain, 100 mg tramadol was injected intramuscular depending on the situation.

The observation group received multimode analgesia combined with auricular acupoint therapy:

(1) Multimode analgesia:

(1.1) 48 hours before surgery: 200 mg celecoxib capsules were taken orally, once every 12 hours, and physical analgesia therapy such as relaxation therapy was informed; night 20 : 00, take a comfortable lying position, close the eyes, focus on the body, relax, and contract head-trunk-upper extremity-buttocks-lower extremity-feet successively. After the muscles of the whole body are completely relaxed, imagine a beautiful, calm, natural scene, feel happy time with your family, have hope for the future, and achieve the purpose of relaxation. Or music therapy, according to the patient's personal characteristics and personality, provide personalized music repertoire, extroverts, soft, and soothing music, and introvert, mainly to positive music. The volume should be tolerated by patients.

(1.2) Before skin excision: 40 mg parecoxib injection intravenously

(1.3) Immediately after the surgery: intravenous controlled analgesia was taken, 2 μg/kg sufentanil injection and 50 mg flurbiprofen ester injection were dissolved in 150 mL normal saline at 2 mL/h, the pressure was 0.5 mL, the shortest interval was 15 min, and ice bag cold compress was continued around the incision for 24 hours.

(1.4) 10 hours after surgery: before homotomy

(1.5) 2–7 d after surgery: same as 48 hours before surgery

(2) Auricular point therapy: since the start to the end of the 7 d after admission, concrete steps as follows: lateral auricular portal nerve, large nerve points, subcortical, small pillow nerve, and sympathetic, such as acupuncture and alcohol disinfection. The adhesive tape (0.6 cm × 0.6 cm) with the seeds of cowherb seed was applied to the auricular acupoint, and then, a little pressure was applied until the patient appeared sore and numb, 3–5 times per day.

2.4. Observation and Evaluation.

(1) Pain degree: visual analogue scale (VAS) [4] was used to evaluate the changes of pain at admission, 6 hours before surgery, 6 hours, 24 hours, and 72 hours after surgery. The score ranged from 0 to 10. The higher the score, the more severe the pain. The scale had good reliability and validity in pain assessment of patients with meniscus injury, and the consistency Cronbach's α coefficient was 0.865.

(2) Physical and mental stress: heart rate (HR) and mean arterial pressure (MAP) were recorded at admission, 6 hours before surgery, and 6 hours and 24 hours after surgery, respectively, and psychological stress state was assessed by patient health questionnaire depression scale (PHQ-9) [5] and generalized anxiety scale (GAD-7) [6]. The total score of the former was 0–27, and the higher the score, the more severe the depression. The latter score was 0–20, and the higher the score was, the more serious the anxiety was. Cronbach's α coefficient of consistency of the above scale was 0.812 and 0.869, respectively.

(3) Analgesia satisfaction: based on expert opinions and previous literature [7], the satisfaction was divided into 3 grades according to VAS score: satisfactory VAS score <3, general VAS score 3–5, and unsatisfactory VAS score >5.

(4) Complications: these include joint popping, sensation of falling apart, and joint effusion.

(5) Rehabilitation quality: the first exhaust, out of bed activity, and hospital stay in the two groups were counted.

2.5. Statistical Analysis. SPSS 23.0 software was used and measurement data ($\pm s$) were expressed. The homogenous t-test of variance was performed for data conforming to

Table 1: Comparison of general data.

Project	Observation group ($n = 74$)	Control group ($n = 74$)	t/χ^2	P
Male/female	40/34	38/36	0.108	0.742
Age (year)	38.96 ± 9.10	41.02 ± 10.36	1.285	0.201
Disease duration (months)	3.25 ± 1.12	2.98 ± 1.03	1.526	0.129
Predisposing factors			0.449	0.799
Long-term weight squat	36 (48.65)	32 (43.24)		
Knee sprain	24 (32.43)	26 (35.14)		
Knee osteoarthritis	14 (18.92)	16 (21.62)		
Surgical resection			0.668	0.414
Half cut	68 (91.89)	65 (87.84)		
Full cut	6 (8.11)	9 (12.16)		
History of the knee			0.363	0.547
Yes	7 (9.46)	5 (6.76)		
No	67 (90.54)	69 (93.24)		

normal distribution, while the t-test was used for heterogeneity of variance. The χ^2 test was used for counting data n (%). Ridit analysis and U test were used for grade data. $P < 0.05$ meant the difference was statistically significant.

3. Results

3.1. Comparison of the Pain Degree of the Two Groups. On admission, there was no significant difference in VAS score between the two groups ($P > 0.05$). VAS scores of the observation group were lower than those of the control group at 6 hours before surgery as well as 6 hours, 24 hours, and 72 hours after surgery, and the differences were statistically significant ($P < 0.05$). The results are shown in Table 2 and Figure 1.

3.2. Comparison of the Physical and Mental Stress Indicators in Two Groups. There were no significant differences in MAP, HR, PHQ-9, and GAD-7 scores between the two groups on admission ($P > 0.05$). MAP, HR, PHQ-9, and GAD-7 scores in the observation group were lower than those in the control group at 6 hours before operation ($P < 0.05$). Besides, there were no significant differences in MAP, HR, PHQ-9, and GAD-7 scores between the two groups at 6 hours and 24 hours after surgery ($P > 0.05$). The results are shown in Table 3 and Figure 2.

3.3. Comparison of the Analgesic Satisfaction in Two Groups. After evaluation, the analgesic satisfaction in the observation group was better than that in the control group, and the difference was statistically significant ($P < 0.05$), as shown in Table 4.

3.4. Comparison of the Complications in Two Groups. The complication rate of 8.11% in the observation group was not significantly different from 12.16% in the control group ($P > 0.05$), as shown in Table 5.

3.5. Comparison of Rehabilitation Quality in the Two Groups. The first exhaust, get out of bed time, and hospital stay in the observation group had shorter than the control group, and the differences were statistically significant ($P < 0.05$), as shown in Table 6.

4. Discussion

At present, arthroscopy is the main method to treat meniscus injury. However, studies have reported that perioperative pain is closely related to the rehabilitation process and prognosis for patients with limb injury [8]. At the same time, studies have confirmed that after arthroscopic surgery, active rehabilitation training is one of the important determinants of the final outcome [9]. Therefore, strengthening perioperative analgesia management, reducing pain degree, alleviating patients' physical and mental stress response, and promoting early postoperative functional exercise are of great importance for postoperative rehabilitation and prognosis.

Multimode analgesia is the combination of drugs or methods with different analgesic mechanisms to complement each other in order to enhance analgesia synergism and reduce the dosage of analgesic drugs. Studies have found that multimode analgesia can not only improve the analgesic effect but also reduce the side effects of anesthesia to maximize the effect/side effects ratio, so as to achieve balanced analgesia [10]. In recent years, multimode analgesia has been clinically considered as an ideal analgesic method in the perioperative period, which can effectively shorten the length of hospital stay and reduce the adverse reactions such as vomiting and nausea caused by anesthesia, and is favored by the majority of doctors and patients. In view of this, this study applied it to the perioperative analgesic management of patients with meniscus injury. In order to further strengthen the analgesic effect and reduce the physical and mental stress caused by pain and surgery, this study combined with the traditional Chinese medicine treatment of auricular acupoint pressing beans, sticking the medicine

Table 2: Comparison of pain degree between two groups (±*s*, points).

Group	*n*	On admission	6 hours before surgery	6 hours after surgery	24 hours after surgery	72 hours after surgery
Observation group	74	5.20 ± 2.33	5.12 ± 2.10	2.18 ± 0.56 act	2.74 ± 0.58	2.10 ± 0.54
Control group	74	5.48 ± 1.97	3.24 ± 1.56	2.46 ± 0.63	3.10 ± 0.47	2.68 ± 0.48
t		0.789	6.182	2.858	4.148	6.906
P		0.431	<0.001	0.005	<0.001	<0.001

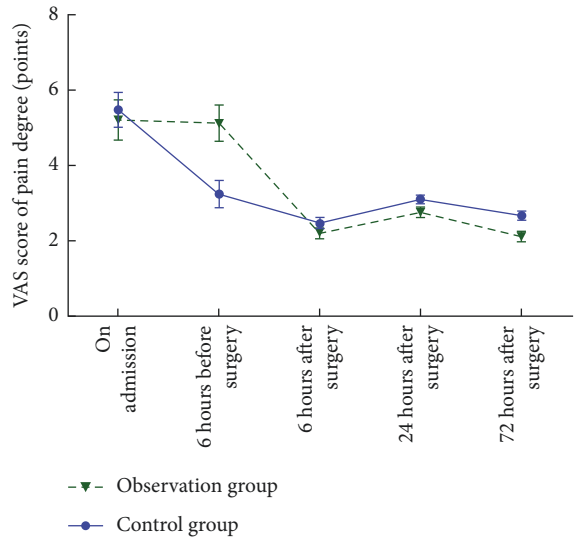

Figure 1: Changes in pain trends.

Table 3: Comparison of psychological stress between two groups (±*s*, points).

Time	Group	*n*	MAP	HR	PHQ-9	GAD-7
On admission	Observation group	74	118.24 ± 10.87	124.25 ± 12.47	7.74 ± 3.01	9.22 ± 1.28
	Control group	74	121.36 ± 13.58	122.48 ± 11.20	8.18 ± 2.69	8.78 ± 1.20
	t		1.543	0.125	0.938	1.102
	P		0.125	0.246	0.350	0.315
6 hours before surgery	Observation group	74	92.36 ± 8.10	96.47 ± 10.35	5.10 ± 2.11	6.24 ± 1.98
	Control group	74	116.78 ± 10.47	114.74 ± 9.54	7.25 ± 1.85	8.35 ± 2.01
	t		15.869	11.165	6.591	10.857
	P		<0.001	<0.001	<0.001	<0.001
6 hours after surgery	Observation group	74	89.58 ± 5.69	82.36 ± 6.75	3.89 ± 1.65	4.01 ± 1.47
	Control group	74	91.01 ± 7.54	83.58 ± 5.98	4.24 ± 1.78	4.21 ± 1.69
	t		0.896	1.164	1.241	0.768
	P		0.412	0.246	0.217	0.444
24 hours after surgery	Observation group	74	84.74 ± 6.10	81.44 ± 5.63	2.01 ± 0.85	2.78 ± 0.62
	Control group	74	86.55 ± 7.54	83.10 ± 6.85	2.13 ± 1.02	2.98 ± 0.96
	t		1.605	1.611	1.234	1.506
	P		0.111	0.109	0.324	0.134

beans on the auricular acupoint through adhesive tape for moderate stimulation, so as to achieve the purpose of external treatment. Traditional Chinese medicine believes that auricular points are related to the physiological functions corresponding to human meridians. A large number of studies have confirmed that auricular acupoint stimulation can produce double stimulation to the cerebral cortex, such as excitation and inhibition, and then exert analgesic and immune regulation effects [11, 12]. In addition, studies have reported that auricular acupoint therapy can reduce the dosage of postoperative analgesic drugs without occurrence of anesthesia-related adverse reactions [13].

This study combined multimode analgesia with auricular acupoint therapy. The results showed that the VAS score of the observation group was lower than that of the control group at 6 hours before surgery and at different time points after surgery, and the analgesic satisfaction was better than that of the control group

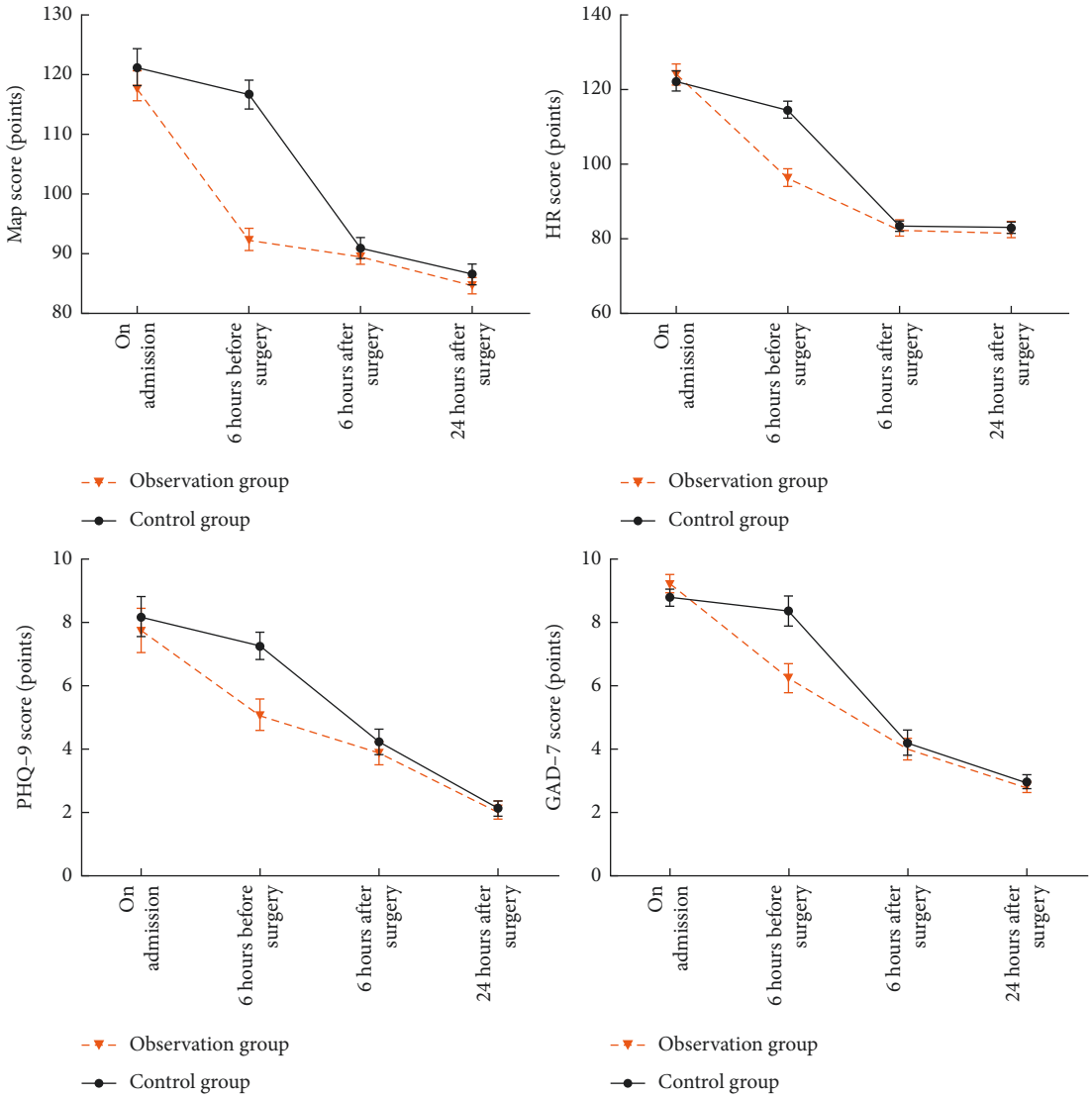

Figure 2: Changes in physical and mental stress.

Table 4: Comparison of analgesic satisfaction between two groups (%).

Group	n	Satisfied	General	Dissatisfied
Observation group	74	57 (77.03)	16 (21.62)	1 (1.35)
Control group	74	43 (58.11)	18 (24.32)	13 (17.57)
u		2.359		
P		0.018		

($P < 0.05$). Therefore, the combined plan adopted in this study had a significant analgesic effect and was widely recognized and satisfied by patients. In addition, from the perspective of stress, stress refers to the nonspecific stress state generated by stress factors in the body when the body is subjected to intense damage stimulus including physiological and psychological parts, namely, physical and mental stress [14]. Psychological stress is an important factor that affects patients' attitude towards care and causes intense physical stress, while abnormal physiological changes can reduce immunity and aggravate inflammatory response, which is not conducive to postoperative recovery [15]. Therefore, this study based on patients with physical and mental stress monitoring found that MAP, HR, PHQ-9, and GAD-7 scores in the observation group at 6 hours before surgery were lower than that of the control group ($P < 0.05$). These indicated that multimodal analgesia combined with auricular application therapy could decrease the degree of physical and mental stress through reducing the preoperative pain, showing that the blood pressure, HR, and anxiety depression were decreased. However, this study showed that there were no significant differences in MAP, HR, PHQ-9, and GAD-7 scores between the two groups at 6 hours and 24 hours after surgery ($P > 0.05$). The reason is that analgesia management was adopted in both groups after surgery. Although the observation group had better analgesia, the pain degree in both the groups was lower, and the effect on physical and mental stress was relatively small.

Studies have reported that the pain caused by surgical injurious stimulation and the disease itself can cause neuroendocrine stress response, resulting in excessive

Table 5: Comparison of complications between two groups (%).

Group	n	Knuckle snapping	Joint loss	Joint effusion	Incidence
Observation group	74	2 (2.70)	3 (4.05)	1 (1.35)	6 (8.11)
Control group	74	3 (4.05)	4 (5.41)	2 (2.70)	9 (12.16)
χ^2					0.668
P					0.414

Table 6: Comparison of rehabilitation quality between two groups (±s, d).

Group	n	First exhaust time	Get out of bed time	Hospital stay
Observation group	74	0.89 ± 0.25	1.48 ± 0.56	6.75 ± 2.11
Control group	74	1.36 ± 0.35	1.97 ± 0.74	8.97 ± 2.89
t		9.400	4.542	5.337
P		<0.001	<0.001	<0.001

metabolism such as elevated blood glucose and water and sodium retention, which hinder postoperative recovery of patients [16]. Affected by pain stimulation, most patients reject early postoperative ambulation, which affects rehabilitation training compliance and is not conducive to prognosis [17]. This study for the first time found that the first exhaust, get out of bed time, and hospital stay in the observation group were shorter than that of the control group ($P < 0.05$), indicating that multimodal analgesia combined with auricular application therapy could achieve good analgesia effect through a variety of analgesic effect mechanism and reduce the adverse effects of traumatic stimulation on patients' body and mind. In addition, patients with pain relief can get out of bed as early as possible, which plays an irreplaceable role in promoting early postoperative recovery and shortening hospital stay [2]. However, this study showed that there was no statistical significance in the incidence of postoperative complications between the two groups ($P > 0.05$), suggesting that in addition to strengthening the analgesic effect, complications prevention and management should be done to further reduce the risk of complications in clinical.

In conclusion, multimode analgesia combined with auricular acupoint therapy has a significant effect on perioperative patients with meniscus injury, which can relieve perioperative pain, reduce physical and mental stress, and promote early postoperative recovery through various analgesic mechanisms. However, there are some shortcomings in this study such as limited sample size and single-center study, so multicenter and large-sample study should be adopted in the future to explore the application effect of this research scheme in a more comprehensive and systematic way.

Data Availability

The labeled datasets used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

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Research Article

Exosomal miR-221-3p Derived from Bone Marrow Mesenchymal Stem Cells Alleviates Asthma Progression by Targeting FGF2 and Inhibiting the ERK1/2 Signaling Pathway

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Exosomes derived from human bone marrow mesenchymal stem cells (BMSCs) play potential protective roles in asthma. However, the underlying mechanisms remain not fully elucidated. Herein, exosomes were isolated from BMSCs, and the morphology, particle size, and exosome marker proteins were identified by transmission electron microscopy (TEM), nanoparticle tracking analysis (NTA), and Western blot, respectively. Then airway smooth muscle cells (ASMCs) were treated with transforming growth factor-β1 (TGF-β1) to construct a proliferation model and then incubated with BMSCs-derived exosomes. We found that exosome incubation increased miR-221-3p expression and inhibited proliferation, migration, and the levels of extracellular matrix (ECM) proteins including fibronectin and collagen III. Moreover, FGF2 was identified as a target gene of miR-221-3p. FGF2 overexpression reversed the inhibitory effects of exosomal miR-221-3p on ASMC progression. Besides, the phosphorylation of extracellular signal-regulated kinase 1/2 (ERK1/2) is inhibited by exosomal miR-221-3p, which was reversed by FGF2 overexpression. And ERK1/2 signaling activator reversed the effects of exosomal miR-221-3p on ASMC progression. Additionally, an ovalbumin (OVA)-induced asthmatic mice model was established, and exosome treatment alleviated airway hyper-responsiveness (AHR), histopathological damage, and ECM deposition in asthmatic mice. Taken together, our findings indicated that exosomal miR-221-3p derived from BMSCs inhibited FGF2 expression and the ERK1/2 signaling, thus attenuating proliferation, migration, and ECM deposition in ASMCs and alleviating asthma progression in OVA-induced asthmatic mice. Our findings may provide a novel therapeutic strategy for asthma.

1. Introduction

Asthma is a common chronic respiratory disease with high morbidity and mortality among children worldwide [1]. It is a complex syndrome characterized by airway hyper-responsiveness (AHR), airway inflammation, and airway remodeling [2]. Airway remodeling is involved in asthma progression through multifaceted processes including basement membrane thickening, extracellular matrix (ECM) deposition and abnormal airway smooth muscle cell (ASMC) growth [3, 4]. It has been emphasized that the abnormal proliferation and migration of AMSCs leads to airway wall thickening and airway narrowing and obstruction, which contributes to airway remodeling and severely affects lung function in patients with asthma [5]. Transforming growth factor-β1 (TGF-β1) was found to be elevated in the airway of patients with asthma, which facilitated airway remodeling by inducing ECM protein production and promoting ASMC proliferation and migration [6]. Although asthma symptoms can be relieved in children with asthma through several therapeutic methods, there is no available complete cure for childhood asthma up to now. Thus, novel effective therapeutic strategies based on a better understanding of molecular mechanisms of childhood asthma progression are urgently needed.

Bone marrow mesenchymal stem cells (BMSCs) are self-replicating multipotent stromal cells extracted from bone marrow mesenchymal tissues. Recently, accumulating

evidence showed that BMSCs played a critical role in the treatment of autoimmune diseases including asthma due to their potent anti-inflammatory and immunomodulatory properties [7]. Importantly, it was found that the therapeutic function of BMSCs mainly depended on cell-to-cell communication through the secretion and transfer of exosomes [8]. Exosomes are small enclosed vesicles with a diameter of 50 to 150 nm, which are secreted by various types of cells and engaged in intercellular communication through transportation of functional signaling factors such as proteins, lipids, mRNAs, and microRNAs (miRNAs) [9]. The regulative role of BMSCs-derived exosomes in asthma has been identified in several types of research. For instance, BMSCs-derived exosomes promoted immunosuppression of regulatory T cells in asthma, indicating the therapeutic potential of BMSCs-derived exosomes for asthma [10]. BMSCs-derived exosomes inhibited airway remodeling and epithelial-mesenchymal transition of the airway epithelium in the lungs of asthmatic rats [11]. However, the regulatory mechanisms of BMSCs-derived exosomes in asthma progression have not been explored clearly.

MiRNAs are a class of small non-coding RNAs that regulate the post-transcriptional expression of target genes to mediate multiple biological and pathological processes. Increasing studies have demonstrated that exosomal miRNAs derived from BMSCs are involved in asthma development. A study suggested that there were different miRNA and mRNA profiles after BMSCs treatment in an asthma mouse model, and the miR-21/activin A receptor IIA axis is an important mechanism for the induction of asthmatic inflammation [12]. Moreover, BMSCs exosomal miR-1470 exerted an immunomodulatory effect by promoting the differentiation of $CD4^+CD25^+FOXP^{3+}$ Tregs in asthmatic patients [13]. Interestingly, it was found that epithelial, sputum, and plasma miR-221-3p expression was significantly decreased in patients with asthma. The decreased expression of miR-221-3p might protect against airway eosinophilic inflammation by upregulating anti-inflammatory chemokine (C-X-C motif) ligand (CXCL) 17 (CXCL17) [14]. Likewise, miR-221-3p was found to have existed in the BMSCs-derived exosomes, and miR-221-3p delivered by BMSCs-derived exosomes was found to be involved in human physiological diseases such as acute myelocytic leukemia and ischemic stroke [15, 16]. Thus, we explored the roles of exosomal miR-221-3p derived from BMSCs in asthma progression.

In the present study, we investigated the effects and regulatory mechanism of exosomal miR-221-3p derived from BMSCs on TGF-β1-induced proliferation, migration, and ECM deposition in ASMCs, and then verified its effect on asthma progression in ovalbumin (OVA)-induced asthmatic mice, aiming to provide a novel therapeutic target for asthma.

2. Materials and Methods

2.1. BMSC Isolation and Culture. The Sprague-Dawley (SD) rats aged 6 weeks old were supplied by the Laboratory Animal Center of Shandong University. BMSCs were isolated according to the previously reported method [17]. The rats were euthanized through intraperitoneally anesthetizing with 1% pentobarbital sodium (100 mg/kg), and bone marrow was obtained from the femur and tibia of each rat under aseptic conditions. The bone marrow was centrifuged at 1000 g for 5 min, and the BMSCs precipitation was collected and suspended with Dulbecco's Modified Eagle Medium (DEME, Gibco, Grand Island, NY) supplemented with 10% fetal bovine serum (FBS; Gibco, Grand Island, NY), 100 U/mL penicillin and 100 μg/mL streptomycin (Invitrogen, Carlsbad, CA, USA). BMSCs were cultured under humidified conditions with 5% CO_2 at 37°C. The medium was changed in half volume after incubation for 24 h and then the whole medium was changed every 2 days. Cells were passaged at a confluence level of 80–90% at a ratio of 1 : 2.

2.2. Extraction and Identification of Exosomes from BMSCs. BMSCs in passage 3 with 80% confluence were selected for exosome extraction. BMSCs-derived exosomes were extracted by ultra-highspeed centrifugation [18]. In brief, the culture medium of BMSCs was successfully centrifuged at 300g for 10 min, 2000g for 10 min, and 10,000g for 35 min, and the supernatant was collected each time. Then the supernatant was filtered by using a 0.22 μm filter membrane (Merck Millipore, Tullagreen, Ireland), followed by ultracentrifugation and centrifugation both 100,000g for 2 h, respectively. Finally, the exacted exosomes were collected, resuspended in phosphate-buffered saline (PBS), and stored at −80°C. The protein quality of exosomes was determined by the BCA Protein Assay Kit (Takara Biotechnology, Dalian, China). The levels of exosomal surface marker proteins including CD9, CD63, CD81, and TSG101 were detected by Western blot assay. The exosome morphology was identified by using Transmission electron microscopy (TEM, Hitachi, Tokyo, Japan), and the particle size of the extracted exosomes was measured by nanoparticle tracking analysis (NTA; Malvern Panalytical, Malvern, UK).

2.3. ASMC Culture and Treatment. ASMCs were obtained from patients without asthma who underwent lung resection surgery ($N = 6$) at the Chengyang District People's hospital of Qingdao City. Informed consent was obtained from each patient, and this study was approved by the Ethics Committee of Chengyang District People's hospital of Qingdao City. ASMCs were obtained according to a reported method [19]. In brief, the smooth muscle layer was isolated from healthy segments of the lobar or main bronchus of subjects through dissecting microscope. The tissues were washed with ice-cold PBS solution containing penicillin-streptomycin three times, and then were cut into small pieces and digested in Hanks' balanced salt solution (HBSS) containing 0.1% collagenase solution (Sigma, St. Louis, MO, USA) at 37°C for 30 min. Then the isolated cells were collected by centrifugation and cultured in DEME supplemented with 10% FBS, 100 U/mL penicillin, and 100 μg/mL streptomycin under humidified conditions with 5% CO_2 at 37°C. Cells cultured from 3 to 5 passages were used for subsequent

experiments. ASMCs were stimulated with TGF-β1 (10 ng/mL; *R* & *D* Systems, Minneapolis, MN, USA) for 24 h to induce the proliferation model, and 100 μg/mL exosomes were used to incubate with ASMCs in each group.

2.4. Cell Transfection. MiR-221-3p mimic (5′-AGC UAC AUU GUC UGC UGG GUU UC-3′), miR-221-3p inhibitor (5′-GAA ACC CAG ACA AUG UAG CU-3′) and their corresponding negative controls (NC mimic, NC inhibitor), and overexpression plasmids of fibroblast growth factor 2 (FGF2) (pcDNA-FGF2) and its negative control (vector) were obtained from RiboBio (Guangzhou, China). AMSCs were seeded in 6-well plates at 37°C in 5% CO_2. When the cells grew to 80% confluence, Lipofectamine 3000 Transfection Reagent (Invitrogen, Carlsbad, CA) was used to transfect NC mimic (50 nM), miR-100-5p mimic (50 nM), NC inhibitor (50 nM), miR-100-5p inhibitor (50 nM), vector (30 nM), and pcDNA-FGF2 (30 nM) into AMSCs according to the manufacturer's instructions. Cells were collected after transfection for 48 h for further experiments.

2.5. Real-Time Quantitative Polymerase Chain Reaction (RT-qPCR). Total RNA was extracted from ASMCs or lung tissues by using the TRIzol reagent (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. The cDNA synthesis was performed by using a PrimeScript RT reagent Kit (Takara Biotechnology, Dalian, China). Real-time quantitative polymerase chain reaction (RT-qPCR) was conducted with the SYBR Premix Ex Taq II (Takara, Dalian, China) on an Applied Biosystems 7500 Real-time PCR System (Applied Biosystems; Thermo Fisher Scientific, Inc.) under the following conditions: 95°C for 1 min, followed by 35 cycles of 95°C for 20 s, then 56°C for 10 s and 72°C for 15 s. The relative expression of miR-221-3p and FGF2 was normalized with U6 and glyceraldehyde 3-phosphate dehydrogenase (GAPDH), respectively. The relative expression levels were calculated by $2^{-\Delta\Delta CT}$ method. The primers used are as follows: miR-221-3p (forward 5′-GAA ATG ATT CCA GGT AGC-3′ and reverse 5′-TGA ACA TCC AGG TCT GGG GCA-3′); U6 (forward, 5′-TGC GGG TGC TCG CTT CGG CAG C-3′, reverse, 5′-CCA GTG CAG GGT CCG AGG T-3′); FGF2 (forward, 5′-GCG ACC CAC ACG TCA AAC TA-3' and reverse, 5′-CTT AGA AGC CAG CAG CCG T-3′), GAPDH (forward: 5′-GGC AAA TTC AAC GGC ACA GT-3′, and reverse: 5′- GGC CTC ACC CCA TTT GAT GT-3′).

2.6. Western Blot Analysis. Proteins were extracted from ASMCs or lung tissues by using RIPA lysis buffer (Beyotime, Shanghai, China), and quantified by using the BCA method (Millipore, Billerica, MA, USA). Then the equal amount of protein (20 μg) was subjected to 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) under the following conditions: 70 V for 30 min, followed by 120 V for 90 min. And then the protein bands were transferred to polyvinylidene difluoride (PVDF) membranes (Millipore, Bedford, MA, USA) at 300 mA for 2 h. The membranes were blocked with 5% nonfat milk for 1 h at room temperature and then incubated overnight at 4°C with the following primary antibodies obtained from Abcam (Cambridge, UK): anti-CD63 antibody (1 : 1000, ab134045), anti-CD9 antibody (1 : 1000, ab236630), anti-CD81 antibody (1 : 1000, ab109201), anti-TSG101 antibody (1 : 1000, ab125011), anti-FGF2 antibody (1 : 1000, ab208687), anti-fibronectin antibody (1 : 1000, ab45688), anti-collagen III antibody (1 : 1000, ab184993), anti-ERK1/2 antibody (1 : 1000, ab17942), anti-ERK1/2 antibody (phospho T202 + Y204) (1 : 1000, ab214362), and anti-GAPDH antibody (1 : 2500, ab9485). Then the membranes were incubated with horseradish peroxidase (HRP)-conjugated goat anti-rabbit IgG (1 : 2000, Abcam, ab6721) for 1 h. The protein bands were visualized with ECL detection reagents and analyzed with ImageJ software (National Institutes of Health, Bethesda, MA, USA).

2.7. CCK-8 Assay. Cell proliferation was measured by using the Cell Counting Kit-8 (CCK-8, Dojindo, Japan) assay. Briefly, ASMCs were seeded into 96-well plates at a density of 1×10^4 cells/well. After incubation for 0, 24, 48, and 72 h at 37°C, 10 μL of CCK-8 solution was added to each well and incubated for 2 h at 37°C. The absorbance at 450 nm of each well was measured by using a microplate reader (Molecular Devices, Shanghai, China).

2.8. Transwell Migration Assay. A Transwell assay was performed to measure ASMC migration. Briefly, HTR8/SVneo cells with the serum-free medium were seeded into the upper chamber of Transwell chambers (8.0 μm pore size; Millipore Corporation, USA). And the DMEM medium was added to the lower chamber. After culturing for 24 h at 37°C, cells on the upper chamber were removed by using a cotton swab, while the cells in the bottom chamber were fixed with 70% ethanol for 10 min and stained with 0.1% crystal violet for 15 min. The migrated cells were quantified by counting five random fields at ×200 magnification under a light microscope (Olympus, Tokyo, Japan).

2.9. Dual-Luciferase Reporter Gene Assay. The potential targets of miR-221-3p were predicted by using the online bioinformatics tool Starbase (https://starbase.sysu.edu.cn/). The binding sites at FGF2 3′-UTR were mutated from AUGUACC to CCCCGAA and then cloned into the pGL3-control luciferase reporter vectors (Promega, Madison, WI) to construct the wild-type (FGF2-WT) and mutant type (FGF2-MUT) reporter vector. Then the reporter plasmids and miR-221-3p mimic or NC-mimic were co-transfected into ASMCs. Following transfection for 48 h, relative luciferase activity was measured by using the Dual Luciferase Reporter Assay System (Promega, Madison, Wisconsin, WI, USA).

2.10. Animal Experimental Protocols. BALB/c mice (6–8 weeks old, 20 ± 2 g) were obtained from the Laboratory Animal Center of Shandong University, which were

maintained in sterile cages under 22–25°C temperature, 55–60% humidity, and 12 h light/dark cycle conditions with free access to food and water. All animal experiments in this study were approved by the Animal Ethics Committee of Laboratory Animal Center of Shandong University.

Mice were randomly divided into three groups ($n = 8$ per group): control group, OVA group, and OVA + Exo group. The Asthma mouse model was established as previously described [20]. The mice in the OVA group were sensitized on days 0, 7, and 14 by intraperitoneal injection of 20 μg of ovalbumin (OVA), emulsified in 1 mg aluminum hydroxide in a total volume of 200 μL. Then the mice were exposed to a 1% OVA aerosol for up to 1 h From day 21 to 23. The mice in the control group were treated with an equal volume of saline instead of OVA. For exosome treatment, 100 μg of exosomes in 100 μL PBS was intratracheally administered every day on days 21 to 23. All mice were euthanized 24 h for the subsequent procedures after the last OVA challenge.

2.11. Measurement of Airway Hyperresponsiveness. Within 24 h after the last OVA challenge, mice were anesthetized with 1% pentobarbital sodium and subjected to mechanical ventilation, and the airway hyperresponsiveness (AHR) of mice was measured by using an animal pulmonary function instrument (Buxco, CT, USA). Subsequently, all mice were exposed to increasing doses of methacholine aerosol (Sigma-Aldrich, USA) at 0, 5, 10, 25, and 50 mg/mL for 3 min, and the enhanced pause (Penh) value of unrestrained mice within 5 min was recorded according to the protocols of the instrument.

2.12. Histopathological Evaluation. The rats were euthanized through intraperitoneally anesthetizing with 1% pentobarbital sodium (100 mg/kg). Lung tissues were collected, fixed with paraformaldehyde, and embedded with paraffin. Then the tissues were cut into 5 μm thick sections and stained with hematoxylin and eosin (HE) using a standard protocol and analyzed by light microscopy (magnification ×200; Olympus, Tokyo, Japan).

Lung tissues were subjected to Masson trichrome staining to determine collagen deposition. Tissue sections were stained with hematoxylin solution for 6 min, ponceau and acid fuchsin solution for 1 min, and phosphomolybdic acid solution for 5 min. And then restained with aniline blue solution for 5 min. The tissue sections were analyzed by light microscopy (magnification ×400; Olympus, Tokyo, Japan).

2.13. BALF Collection and Cell Counting. After mice were anesthetized, the tracheas were cannulated and lavaged with 0.8 mL of cold PBS twice to collect bronchoalveolar lavage fluid (BALF). The BALF samples were immediately centrifuged at 300g for 15 min to collect the supernatant. The number of total cells in BALF was determined with a hemocytometer. The differences in cell number of eosinophils, neutrophils, lymphocytes, and macrophages in BALF were evaluated by using the Kwik-Diff staining set (Thermo, USA) according to the manufacturer's instructions.

2.14. Measurement of the Serum Level of OVA-Specific IgE. On day 24 after the last OVA challenge, the blood samples of mice were collected and centrifuged at 1000g for 10 min to obtain serum samples. The serum level of OVA-Specific immunoglobulin E (IgE) was measured by ELISA kit (Abcam, Cambridge, UK) according to the manufacturer's instructions.

2.15. Statistical Analysis. Statistical analysis was performed by using SPSS version 22.0 software. All data from at least three times independent experiments were presented as mean ± standard deviation (SD). Student's t-test and analysis of variance (ANOVA) followed by Tukey-Kramer correction were performed for the comparison between two groups and comparison among groups, respectively. $P < 0.05$ was considered to be statistically significant.

3. Results

3.1. Identification of Exosomes Isolated from BMSCs. We first identified the isolated exosomes from BMSCs. The expression of exosome-specific markers, including CD9, CD63, CD81, and TSG101 was measured by Western blotting, which showed that all exosome marker levels were significantly higher in the isolated exosomes than that in BMSCs and supernatant (Figures 1(a) and 1(b)). Moreover, the exosome morphology was identified by TEM (Figure 1(c)) and NTA revealed that the mean particle size of exosomes was 134.2 nm (Figure 1(d)). These results indicated that BMSCs-derived exosomes were successfully obtained in our experiments.

3.2. Exosomal miR-221-3p Derived from BMSCs Inhibited Proliferation, Migration, and ECM Deposition in ASMCs. To explore whether exosomal miR-221-3p derived from BMSCs is involved in childhood asthma progression, ASMCs were treated with TGF-β1 to induce a proliferation model and then incubated with BMSCs-derived exosomes alone or together with transfection with miR-221-3p inhibitor. RT-qPCR showed that the level of miR-221-3p was decreased in TGF-β1-treated ASMCs, and exosome incubation upregulated miR-221-3p level, while transfection of miR-221-3p inhibitor reduced miR-221-3p level (Figure 2(a)). Moreover, it was observed that TGF-β1 stimulation promoted cell proliferation (Figure 2(b)) and migration (Figures 2(c) and 2(d)) in ASMCs, and exosome incubation markedly attenuated proliferation and migration, while miR-221-3p inhibition reversed these effects. Additionally, ASMCs play an important role in airway remodeling by producing ECM proteins. We next determined the functional role of exosomal miR-221-3p in TGF-β1-induced ECM deposition. Western blotting demonstrated that TGF-β1 stimulation induced the expression of fibronectin and collagen III in ASMCs, and exosome incubation significantly decreased the expression of fibronectin and collagen III, which were then reversed by miR-221-3p inhibition (Figures 2(e)–2(g)). These results

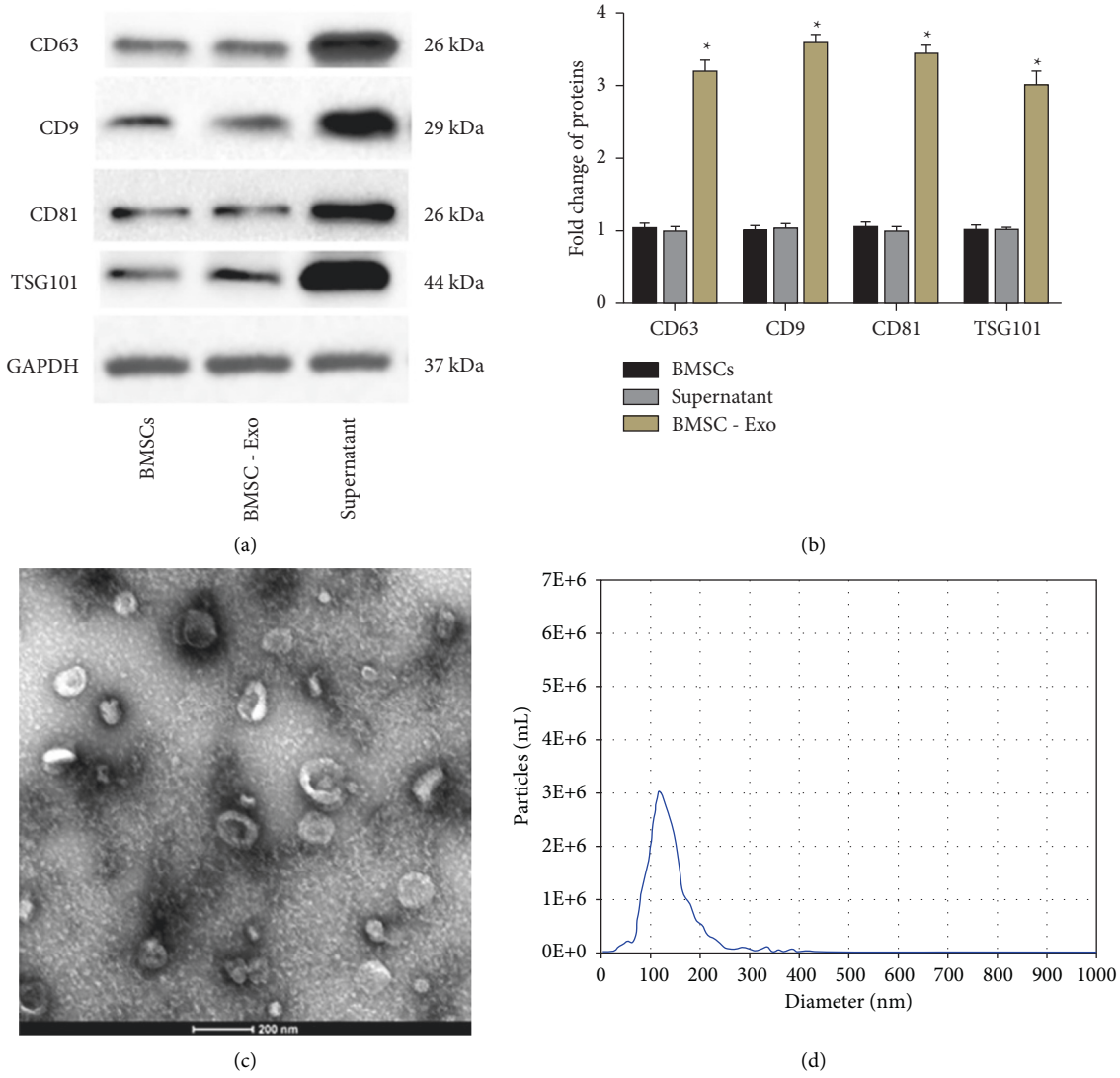
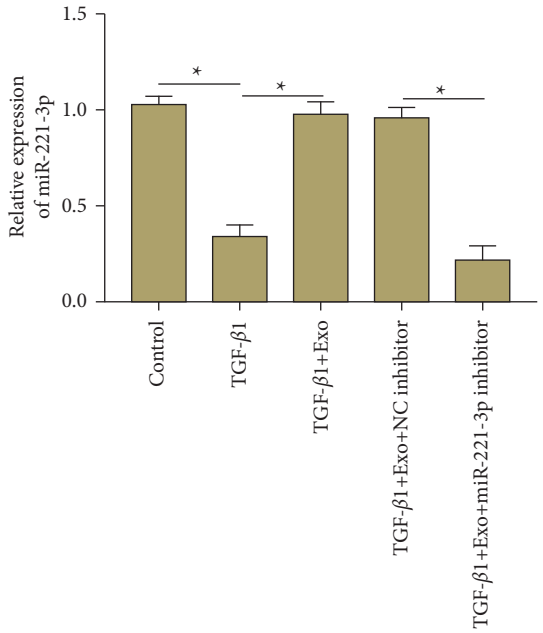


Figure 1: Identification of exosomes isolated from BMSCs. (a)-(b) The levels of exosome-specific markers, including CD9, CD63, CD81, and TSG101 in BMSCs, supernatant, and exosomes were measured by western blot analysis. $^*P < 0.05$. (c) The exosome morphology was determined by transmission electron microscopy (TEM) (scale bar: 100 nm). (d) The particle size of exosomes was detected by nanoparticle tracking analysis (NTA). Data from at least three independent experiments were presented as mean ± SD $^*P < 0.05$.

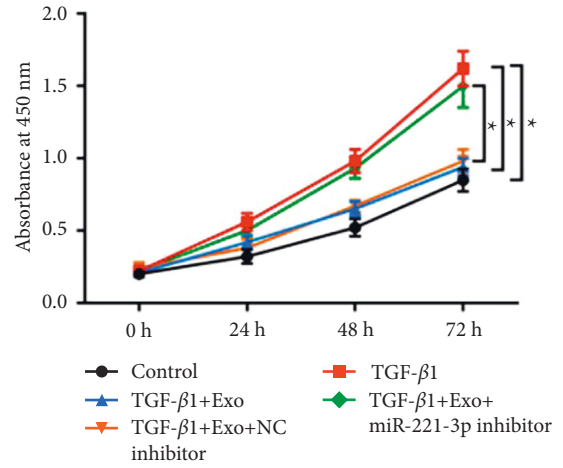
indicated that exosomal miR-221-3p derived from BMSCs inhibited proliferation, migration, and ECM deposition in ASMCs.

3.3. FGF2 is a Target Gene of miR-221-3p. The potential target mRNAs of miR-221-3p were predicted by using the StarBase tool (https://starbase.sysu.edu.cn/), which suggested that there were potential binding sites between miR-221-3p and FGF2 (Figure 3(a)). Dual luciferase reporter gene assay further confirmed that only the relative luciferase activity of FGF2-WT was significantly inhibited after transfection with miR-221-3p mimic, while the relative luciferase activity of FGF2-MUT was not affected by any treatment (Figure 3(b)). Furthermore, miR-221-3p mimic, miR-221-3p inhibitor, and their negative controls were transfected into ASMCs, respectively. As shown in Figure 3(c), the expression of miR-221-3p in ASMCs was increased in the miR-221-3p mimic group and decreased in the miR-221-3p inhibitor group. Moreover, miR-221-3p overexpression apparently inhibited the expression of FGF2 at both mRNA and protein levels, while miR-221-3p inhibition markedly promoted the mRNA and protein expression of FGF2 (Figures 3(d)–3(f)). These findings indicated that FGF2 was a target gene of miR-221-3p.

3.4. Exosomal miR-221-3p Inhibited Proliferation, Migration, and ECM Deposition in ASMCs by Downregulating FGF2. To further investigate whether miR-221-3p exerts its function by regulating FGF2 in ASMCs, TGF-β1-treated ASMCs were incubated with BMSCs-derived exosomes alone or together with transfection with pcDNA-FGF2. We found that TGF-β1 stimulation upregulated the mRNA and protein



(a)



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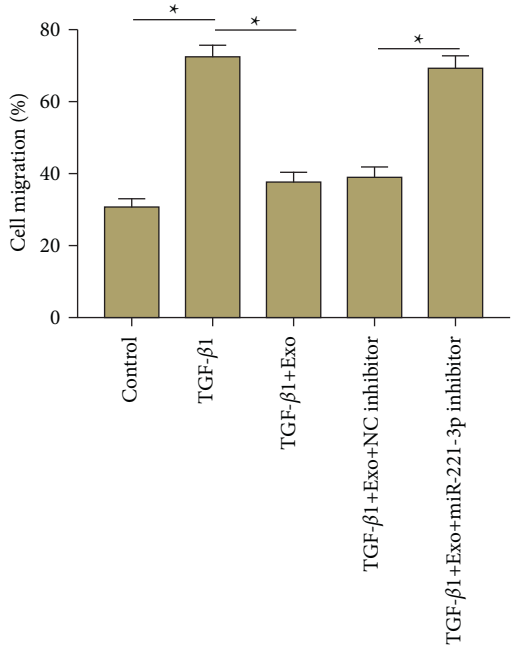
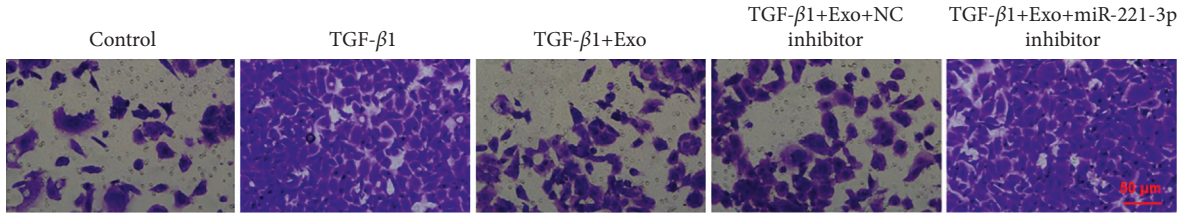


(c)



(d)

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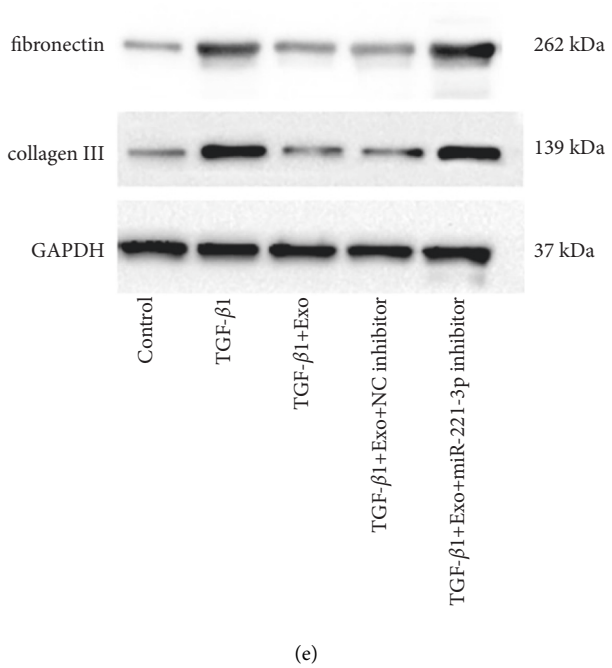
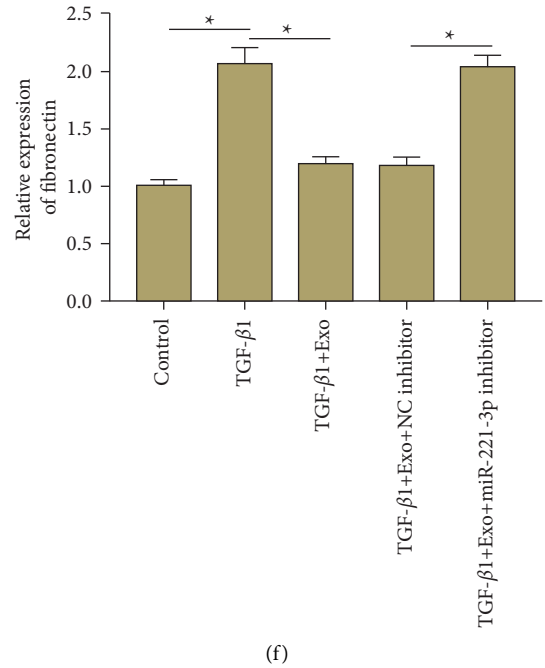


(e)



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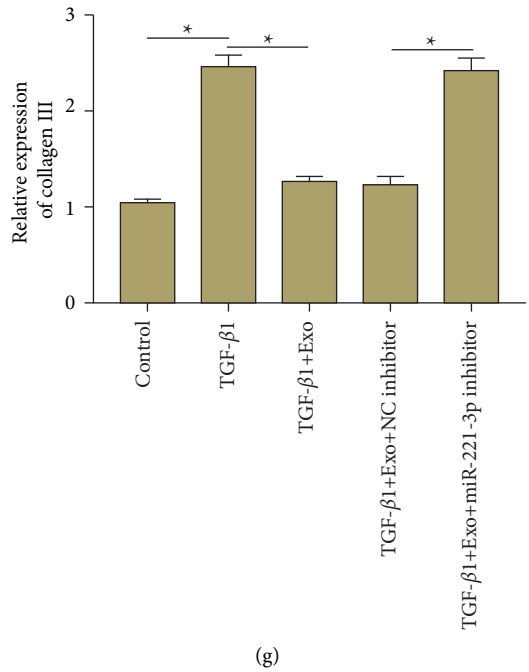


(g)

Figure 2: Exosomal miR-221-3p inhibited proliferation, migration, and ECM deposition in ASMCs. ASMCs were treated with TGF-β1 to induce a proliferation model and then incubated with BMSCs-derived exosomes alone or together with transfection with miR-221-3p inhibitor. (a) RT-qPCR was performed to measure the expression of miR-221-3p. (b) ASMC proliferation was evaluated by using the CCK-8 assay. (c), (d) ASMC migration was measured by using Transwell assay. (e)–(g) The protein expression of fibronectin and collagen III was analyzed by using western blot analysis. Data from at least three independent experiments were presented as mean ± SD $^*P < 0.05$.

levels of FGF2 in ASMCs, and exosome incubation reduced the mRNA and protein levels of FGF2, whereas transfection of pcDNA-FGF2 promoted the mRNA and protein expression of FGF2 (Figures 4(a)–4(c)). Moreover, CCK-8 and Transwell assays illustrated that exosome incubation inhibited TGF-β1-induced proliferation (Figure 4(d)) and migration (Figures 4(e) and 4(f)) in ASMCs, while FGF2 overexpression reversed these effects. Additionally, Western blotting showed that exosome incubation significantly decreased TGF-β1-induced fibronectin and collagen III production in ASMCs, which were abolished by FGF2 overexpression (Figures 4(b), 4(g), and 4(h)). These results

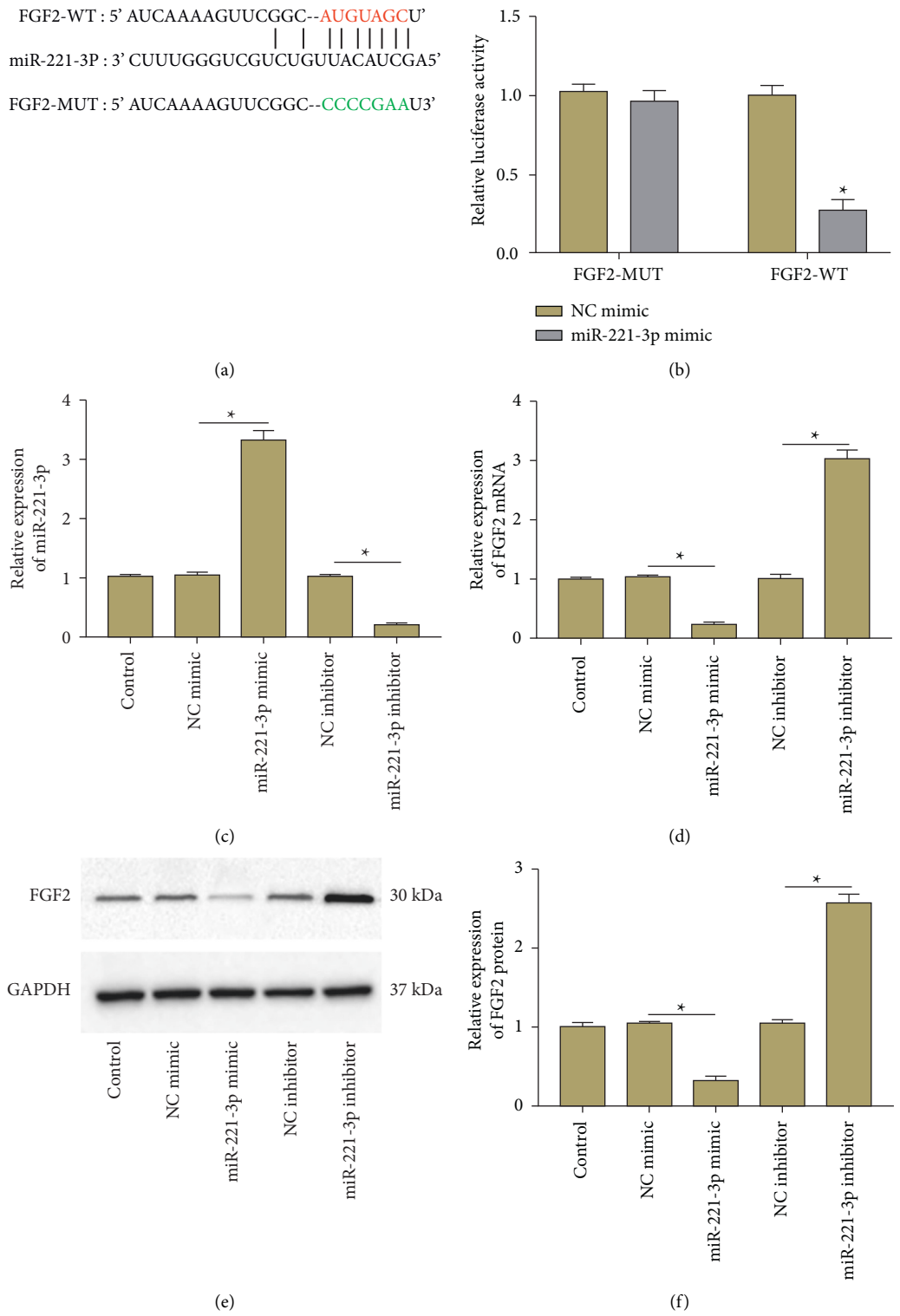
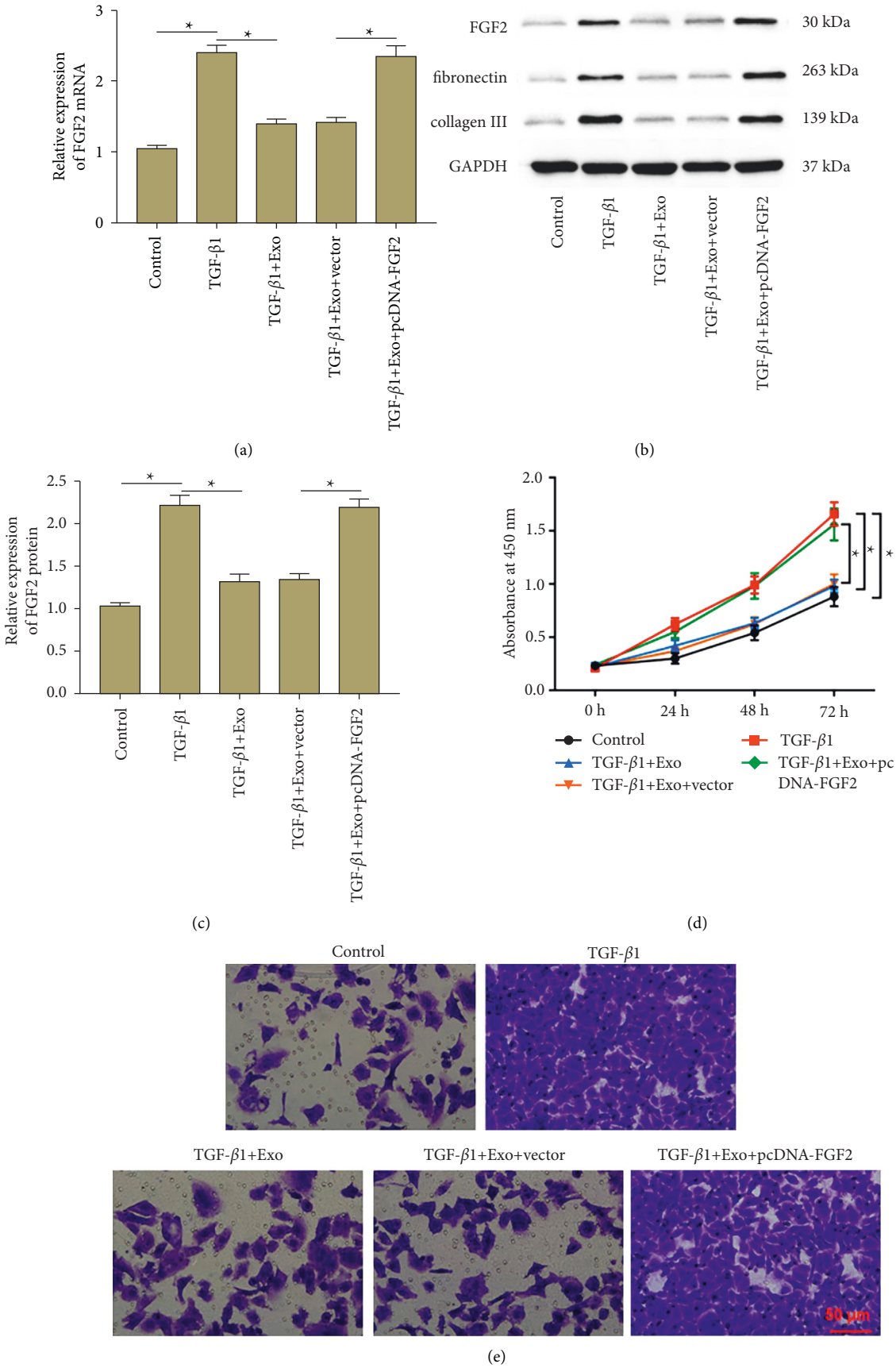


Figure 3: FGF2 is a target gene of miR-221-3p. (a) The binding sites of miR-221-3p were predicted by the starbase tool. (b) Dual-luciferase reporter gene assay verified the binding between miR-221-3p and FGF2. (c) ASMCs were transfected with miR-221-3p mimic, miR-221-3p inhibitor, and their negative controls, respectively. The expression of miR-221-3p was detected by RT-qPCR. (d) The expression of FGF2 mRNA was detected by RT-qPCR (e), (f) The expression of FGF2 protein was measured by using western blot analysis. Data from at least three independent experiments were presented as mean ± SD $^{*}P < 0.05$.



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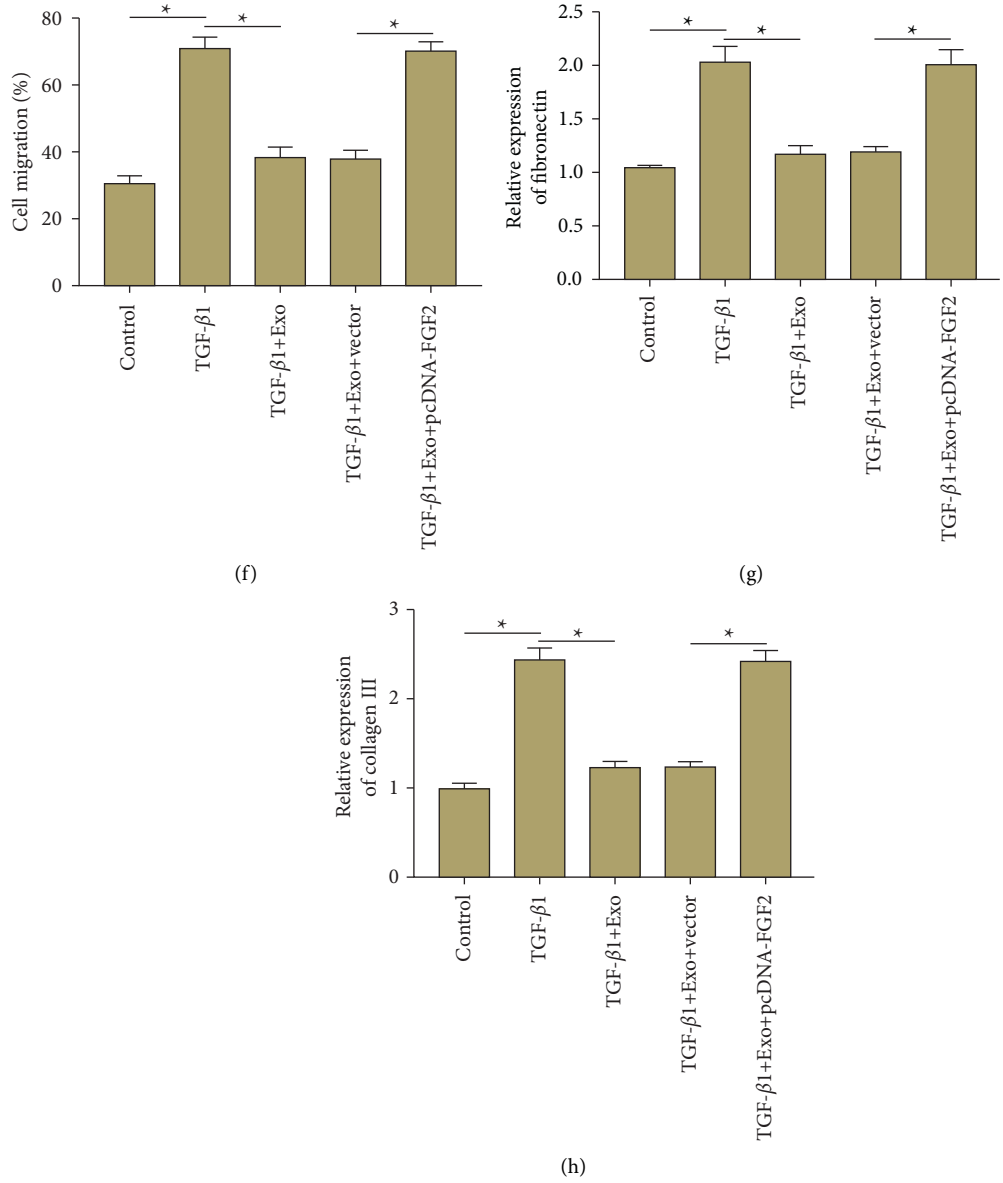

Figure 4: Exosomal miR-221-3p inhibited proliferation, migration, and ECM deposition in ASMCs by downregulating FGF2. TGF-β1-treated ASMCs were incubated with BMSCs-derived exosomes alone or together with transfection with pcDNA-FGF2. (a) The expression of FGF2 mRNA was detected by RT-qPCR. (b), (c) The protein expression of FGF2 was detected by western blot analysis. (d) CCK-8 assay was performed to evaluate ASMC proliferation. (e), (f) Transwell assay was performed to detect ASMC migration. (g)-(h) The protein expression of fibronectin and collagen III was analyzed by using Western blot analysis. Data from at least three independent experiments were presented as mean ± SD $^*P < 0.05$.

implicated that exosomal miR-221-3p inhibited proliferation, migration, and ECM deposition in ASMCs by downregulating FGF2.

3.5. The ERK1/2 Signaling is Involved in the Regulation of Exosomal miR-221-3p/FGF2 Axis on ASMC Progression. We further explored whether the extracellular regulated protein kinases 1/2 (ERK1/2) signaling is perturbed by the exosomal miR-221-3p/FGF2 axis in ASMCs. Western blotting revealed that exosomal miR-221-3p incubation significantly decreased TGF-β1-induced phosphorylated ERK1/2 (p-ERK1/2) expression in ASMCs, while FGF2 overexpression increased p-ERK1/2 expression (Figures 5(a) and 5(b)). To confirm whether the ERK1/2 signaling is involved in the regulation of exosomal miR-221-3p/FGF2 axis on ASMC progression, TGF-β1-treated ASMCs were incubated with BMSCs-derived exosomes in the absence or presence of ERK1/2 signaling activator tert-butylhydroquinone (TBHQ). It was found that exosomal miR-221-3p incubation suppressed p-ERK1/2 expression in ASMCs, while TBHQ treatment enhanced p-ERK1/2 expression (Figures 5(c) and 5(d)). Furthermore, exosomal miR-221-3p incubation attenuated TGF-β1-induced proliferation

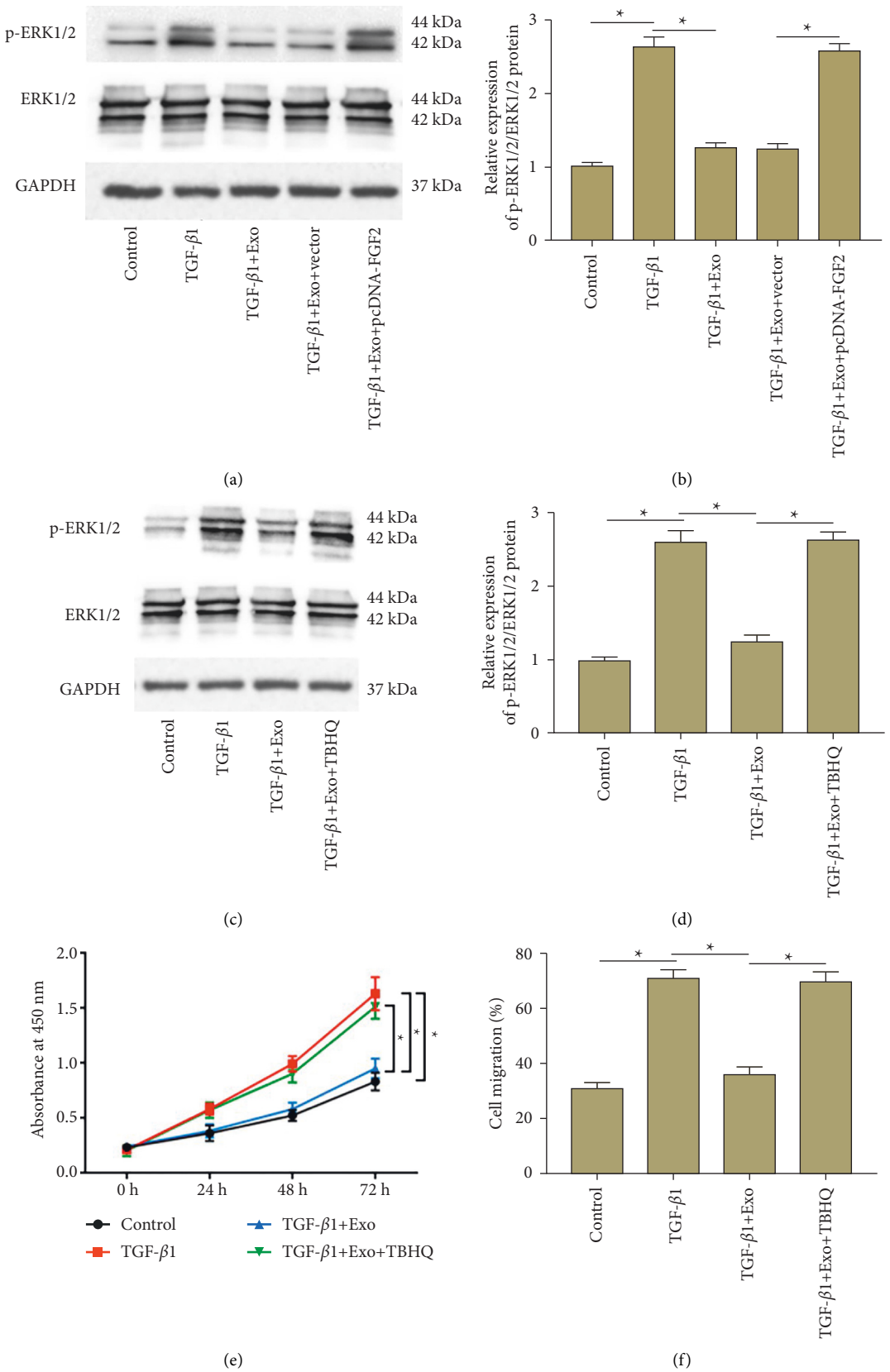

Figure 5: Continued.

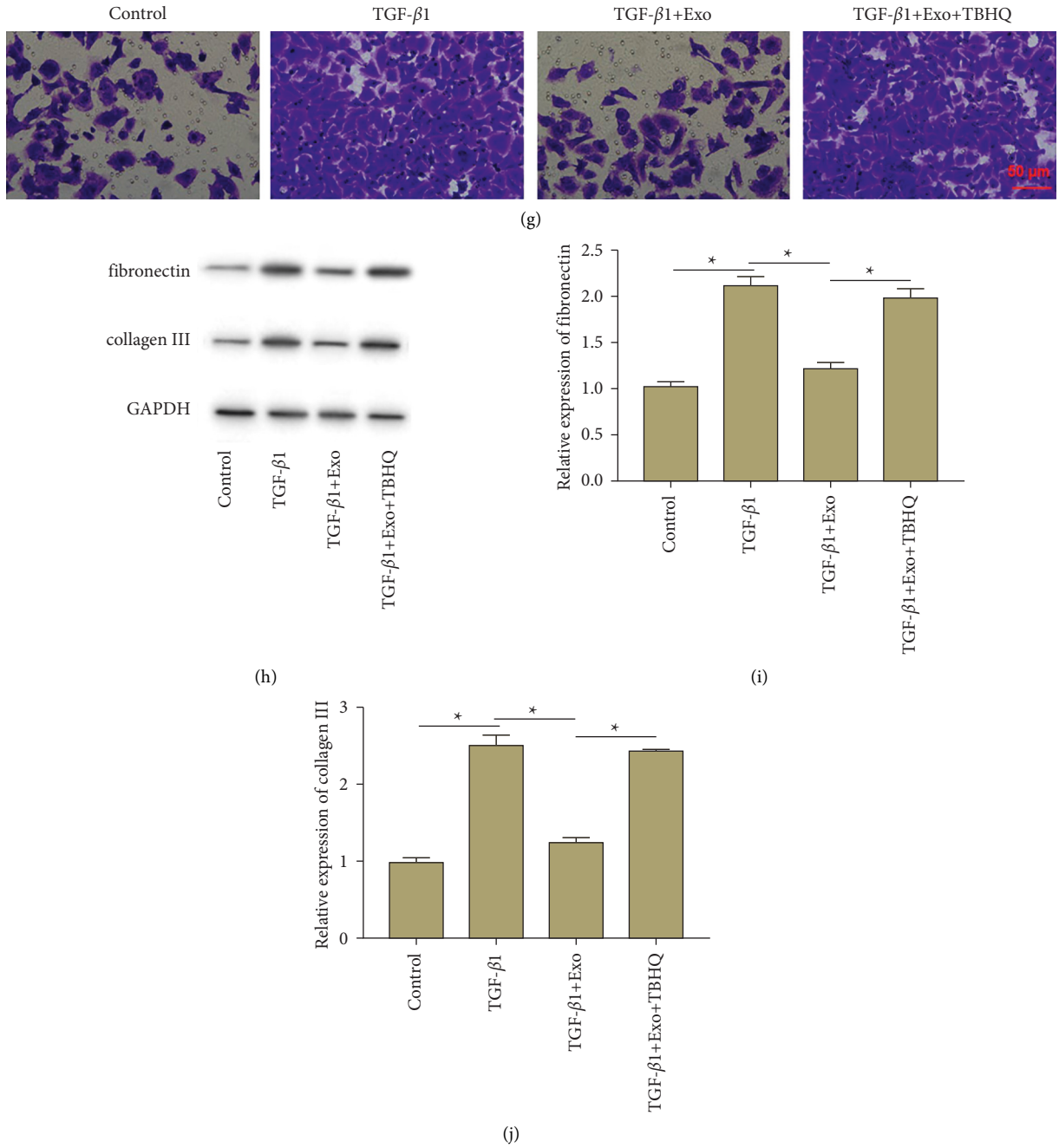

Figure 5: The ERK1/2 signaling is involved in the regulation of the exosomal miR-221-3p/FGF2 axis on ASMC progression. (a), (b) TGF-β1-treated ASMCs were incubated with BMSCs-derived exosomes alone or together with transfection with pcDNA-FGF2. The protein levels of p-ERK1/2 and ERK1/2 were detected by western blot analysis. TGF-β1-treated ASMCs were incubated with BMSCs-derived exosomes in the absence or presence of ERK1/2 signaling activator TBHQ. (c), (d) The protein levels of p-ERK1/2 and ERK1/2 were detected by western blot analysis. (e) ASMC proliferation was measured by using the CCK-8 assay. (f), (g) ASMC migration was measured by transwell assay. (h)–(j) The protein expression of fibronectin and collagen III was analyzed by using Western blot analysis. Data from at least three independent experiments were presented as mean ± SD $^*P < 0.05$.

(Figure 5(e)), migration (Figures 5(f) and 5(g)) and ECM protein production (Figures 5(h)–5(j)) in ASMCs, while TBHQ treatment reversed these effects. These results revealed that the exosomal miR-221-3p/FGF2 axis modulated ASMC progression by regulating the ERK1/2 signaling.

3.6. Exosomal miR-221-3p Derived from BMSCs Alleviated Asthma Progression in Mice. To further explore the role of exosomal miR-221-3p in asthma progression *in vivo*, the OVA-induced asthmatic mice model was constructed, and exosomal miR-221-3p was injected for treatment. We

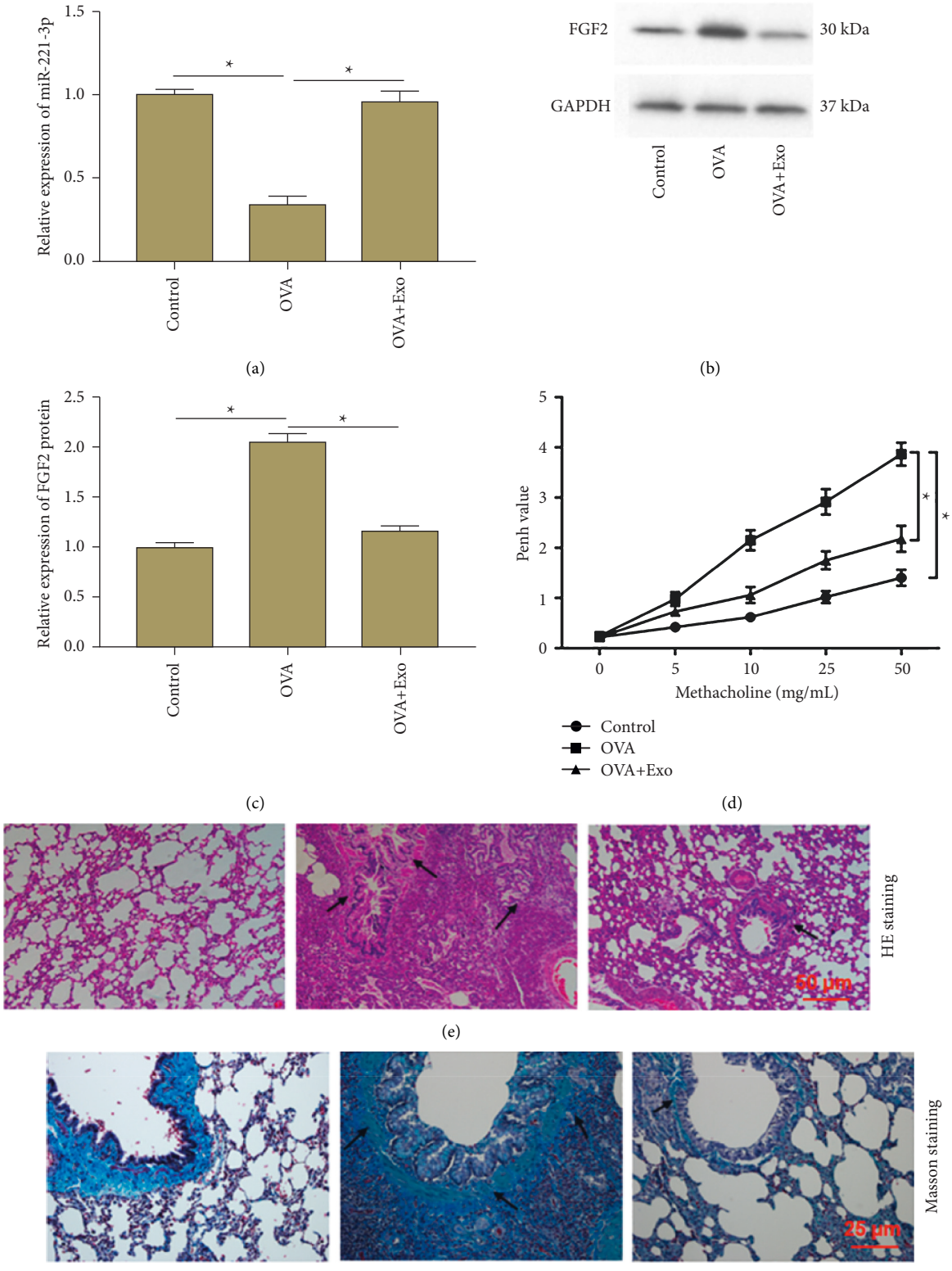

Figure 6: Continued.

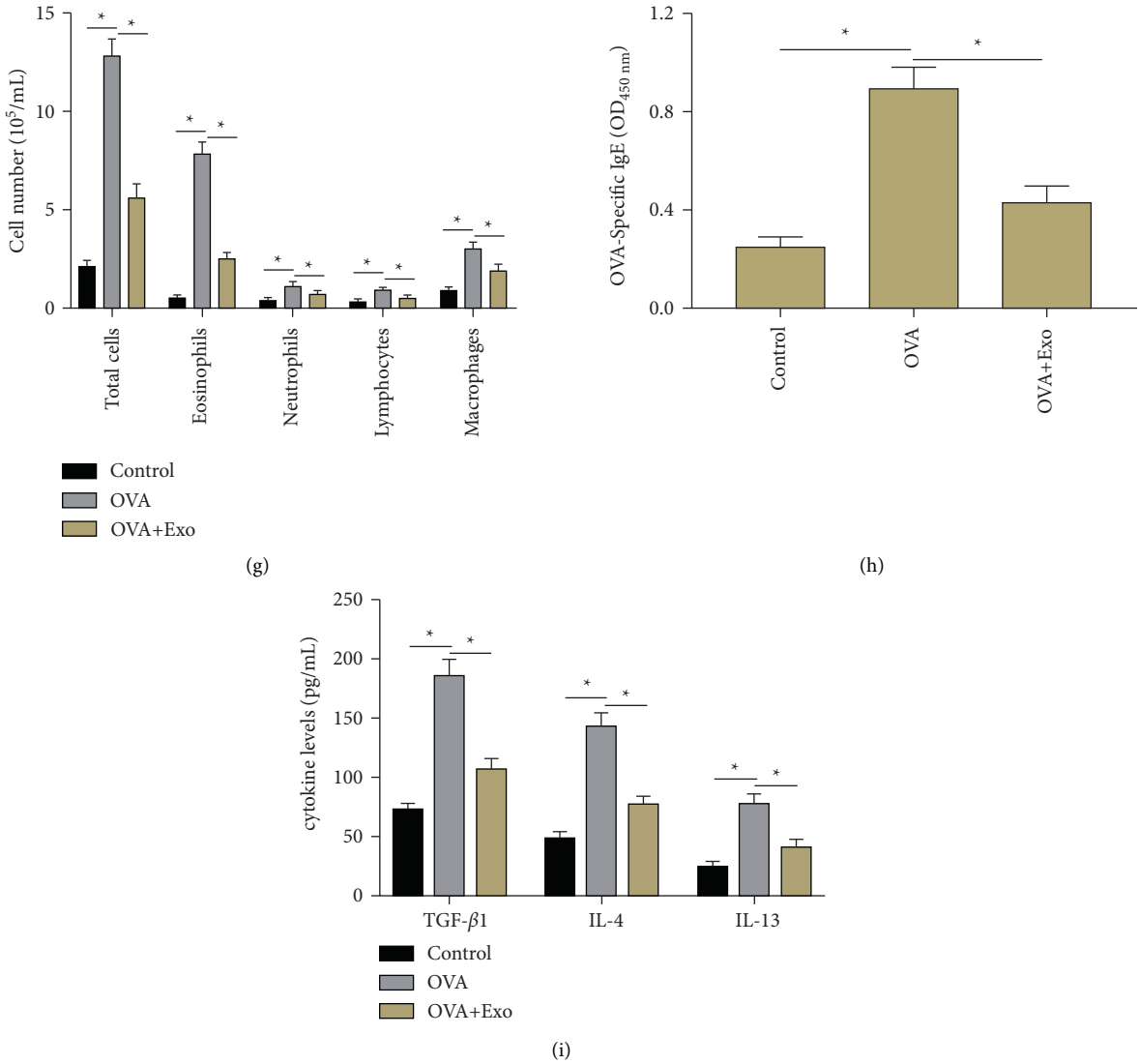

FIGURE 6: The exosomal miR-221-3p derived from BMSCs alleviated asthma progression in mice. BALB/c mice were randomly divided into three groups ($n = 8$ per group): control group, OVA group, and OVA + Exo group. OVA-induced asthmatic mice model was constructed, and exosomal miR-221-3p was injected for treatment. (a) The expression of miR-221-3p in lung tissues of mice was measured by using RT-qPCR. (b), (c) The expression of FGF2 protein in lung tissues of mice was detected by using Western blot analysis. (d) The airway hyperresponsiveness of mice in all groups was detected. (e), (f) Histopathological evaluation of lung tissue sections from each group were detected by HE staining and Masson staining, and significant histopathological changes were marked with an arrow. (g) The number of total and differential inflammatory cells in BALF. (h) The serum OVA-specific IgE levels of mice in all groups were detected by using ELISA. (i) The levels of TGF-β1, IL-4, and IL-13 in BALF were examined by using ELISA. Data from at least three independent experiments were presented as mean ± SD $^*P < 0.05$.

confirmed the decreased miR-221-3p expression (Figure 6(a)) and increased FGF2 expression (Figures 6(b) and 6(c)) in lung tissues of the OVA group compared with the control group, and this expression pattern was reversed by exosomal miR-221-3p treatment. Then the airway hyperresponsiveness was detected by measuring the Penh value, which showed that aerosolized methacholine caused a dose-dependent increase in Penh value in all groups, and the Penh value in the OVA group was higher than that in the control group, while exosomal miR-221-3p treatment significantly reduced the Penh value in OVA-challenged mice (Figure 6(d)). Next, histopathological evaluation of lung tissue sections from each group was detected by HE staining and Masson staining. HE staining showed that OVA-challenged mice showed obvious inflammatory cell infiltration and bronchial wall thickening, while exosomal miR-221-3p treatment attenuated these histopathological changes (Figure 6(e)). Masson staining indicated that a large amount of collagen deposition around the bronchioles in the OVA group, while exosomal miR-221-3p exerted a substantial effect on reducing ECM deposition (Figure 6(f)). Additionally, the number of inflammatory cells including

neutrophils, eosinophils, macrophages, and lymphocytes in BALF was increased significantly in the OVA group compared with that in the control group, while treatment of exosomal miR-221-3p reduced the number of inflammatory cells (Figure 6(g)). ELISA results showed that the levels of serum OVA-specific IgE (Figure 6(h)) and inflammatory cytokines including TGF-β1, IL-3, and IL-14 levels (Figure 6(i)) were significantly elevated in lung tissues of OVA-challenged mice, while exosomal miR-221-3p treatment significantly reversed this effect. Our results revealed that exosomal miR-221-3p derived from BMSCs alleviated asthma progression in mice.

4. Discussion

Emerging evidence has revealed the potential protective role of BMSCs-derived exosomes in asthma progression due to their anti-inflammatory and immunomodulatory properties [7]. However, the possible underlying mechanisms of BMSCs-derived exosomes in the protection of asthma remain not fully understood. A large number of studies have confirmed that BMSCs-derived exosomes play crucial roles in the human pathological process through the transportation of functional miRNAs [7, 21]. A previous study illustrated that miR-221-3p delivered by BMSCs-derived exosomes promoted the development of acute myelocytic leukemia [15]. Moreover, BMSCs-derived extracellular vesicles carrying miR-221-3p exerted a neuroprotective effect in ischemic stroke by targeting activating transcription factor 3 [16]. Interestingly, it was found that epithelial, sputum and plasma miR-221-3p expression was prominently decreased in patients with asthma, and the decreased expression of miR-221-3p might protect against airway eosinophilic inflammation by upregulating anti-inflammatory chemokine CXCL17, indicating that epithelial and sputum miR-221-3p are novel biomarkers for airway eosinophilic inflammation in asthma [14]. Thus, our study investigated the roles and underlying mechanism of exosomal miR-221-3p derived from BMSCs in asthma progression. We found that exosomal miR-221-3p derived from BMSCs inhibited TGF-β1-induced proliferation and migration, and reduced the levels of ECM proteins including fibronectin and collagen III in ASMCs. Additionally, exosomal miR-221-3p alleviated AHR, histopathological damage, and collagen deposition in OVA-induced asthmatic mice. Importantly, we suggested that exosomal miR-221-3p achieved its protective roles by targeting fibroblast growth factor 2 (FGF2) in ASMCs.

FGF2, also known as the basic fibroblast growth factor (bFGF), is a potent mitogenic factor belonging to the FGF family. It was reported that FGF2 was overexpressed in asthma and promoted airway inflammation in airway epithelial cells [22]. Recently, studies have focused on the immunomodulatory function of FGF2 in chronic inflammatory airway diseases including asthma and chronic obstructive pulmonary disease [23]. Notably, FGF2 inhibited TGF-β1-induced differentiation of ASMCs in vitro [24]. Moreover, FGF2 and TGF-β1 synergized in human bronchial smooth muscle cell (BSMC) proliferation, and this synergistic effect might contribute to the hyperplastic phenotype of BSMC in asthmatic airway remodeling [25]. These studies indicated that FGF2 was involved in asthma progression and might be a therapeutic target for asthma diseases. In our study, we showed that FGF2 was a target gene of miR-221-3p, and FGF2 expression was inhibited by miR-221-3p in ASMCs, Furthermore, FGF2 overexpression reversed the inhibitory effect of exosomal miR-221-3p on TGF-β1-induced proliferation, migration, and ECM deposition in ASMCs, indicating that exosomal miR-221-3p exerted its protective role in asthma by inhibiting FGF2 expression.

The ERK1/2 signaling pathway is a common pathway regulated by various proliferative factors, which is identified as a pivotal factor in asthma progression [26]. And the role of ERK1/2 signaling in promoting the proliferation and migration of ASMCs was previously reported. Biased Bitter taste receptor (TAS2R) bronchodilators inhibited ASMC proliferation by downregulating phosphorylated ERK1/2 [27]. Vasoactive intestinal peptides inhibited airway remodeling in asthmatic mice, and inhibited phosphorylation of the ERK1/2 signaling on ASMCs, thus inhibiting the proliferation of ASMCs [26]. More notably, FGF2 is closely related to wound repair and cell proliferation. Recent studies provided us the evidence that FGF2 participated in regulating the ERK1/2 signaling in cell proliferation. For instance, FGF2 significantly enhanced liver cell proliferation by increasing the phosphorylation level of ERK1/2 and c-Jun N-terminal kinase (JNK) [28]. FGF2 promoted proliferation and migration of uterine luminal epithelial cells during early pregnancy, which was reversed by the treatment of inhibitor U0126 [29]. Therefore, we explored whether the ERK1/2 signaling is involved in the regulation of the miR-221-3p/FGF2 axis in ASMC progression. Our results illustrated that exosomal miR-221-3p suppressed FGF2-mediated phosphorylation of the ERK1/2 signaling, and ERK1/2 activator TBHQ reversed the inhibitory effects of exosomal miR-221-3p on proliferation, migration, and ECM deposition in ASMCs, indicating that exosomal miR-221-3p attenuated ASMC progression through inhibiting the ERK1/2 signaling [30].

In conclusion, our findings demonstrated that exosomal miR-221-3p derived from BMSCs inhibited FGF2 expression and the ERK1/2 signaling, thus attenuating proliferation, migration, and ECM deposition in ASMCs. Additionally, exosomal miR-221-3p alleviated asthma progression in OVA-induced asthmatic mice. Our findings may provide a novel therapeutic strategy for asthma.

Data Availability

The datasets used during the present study are available from the corresponding author upon reasonable request.

Ethical Approval

This study was approved by the Ethnic Committee of Chengyang District People's hospital of Qingdao City, and

all subjects had read and signed the informed consent. All animal care and experimental procedures were approved by the Ethics Committee of Laboratory Animal Center of Shandong University.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

Authors' Contributions

Weike Liu and Jieting Wan designed the experiments. Hui Lin and Wuhui Nie performed the experimental work. Qian Jiang provided statistical analysis and figures for the manuscript. Aimei Zhang wrote the manuscript. All authors read and approved the final manuscript.

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Research Article

Clinical Study of Airway Stent Implantation in the Treatment of Patients with Malignant Central Airway Obstruction

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Background. Airway stenting is a therapeutic option for malignant central airway obstructions (MCAO), including both intraluminal and extraluminal obstructions. The objective of this study is to investigate the clinical features and results of long-term improved prognosis for MCAO patients after airway stent implantation. *Methods*. Ninety-eight MCAO patients who underwent stent placement in our hospital from January 2013 to April 2020 were included in this study. The data included baseline data, clinical characteristics, laboratory test data, stent implantation data, and treatment as well as survival after stent implantation. The survival rates among individuals were compared via log-rank tests. Potential prognostic factors were identified using multivariate cox hazard regression models. *Results*. A retrospective analysis of these patients was generated. MCAO was mainly caused by lung cancer (53/98, 54.08%), esophageal cancer (22/98, 22.45%), and thyroid cancer (3/98, 3.06%). The median survival time of participants was 5.5 months. Univariate analysis indicated that the survival rate was related to primary disease, ECOG PS score, stent site, hemoglobin (Hb), albumin (ALB), and serum lactate dehydrogenase (LDH) ($P < 0.05$). The cox risk regression model showed that the survival rate was significantly influenced by ECOG PS score (OR = 3.468, 95%CI = 1.426–8.432, $P = 0.006$) and stent site (OR = 1.544, 95%CI = 1.057–2.255, $P = 0.025$). *Conclusions*. Compared with the site of stent placement, the ECOG PS score is the primary factor in the survival rate of MCAO patients after airway stenting.

1. Introduction

For patients with thoracic malignant tumors, malignant central airway obstruction (MCAO) was mainly caused by intraluminal tumor growth or external tumor compression [1]. Airway stents maintain airway patency by interrupting tumor expansion or preventing malignant tumor compression, which is a palliative treatment for MCAO to improve symptoms, quality of life, or lung function [2, 3]. Stent placement can improve MCAO for patients with malignant tumors. It is a challenge to place an artificial stent in the airway since serious risks exist in stent implantation, including bleeding, intraoperative airway obstruction, and dislocation or migration from the desired site [4]. Therefore, the indications for stent placement in thoracic malignancies should decide on risks and benefits. Although airway stent placement has been mainly used as palliative treatment, the prognostic benefit of stent placement is still controversial [5]. At present, many studies on airway stents have focused on palliative care, lung function, risks, and complications after stent placement, while factors of survival remain confused [6]. For instance, Nagano reported that only good performance status contributed to survival after airway stent placement, of which only 21 patients were analyzed [7].

Herein, factors to determine the clinical features and improve the long-term prognosis for MCAO patients treated with airway stent implantation were performed in this paper.

Malignant central airway stenosis (MCAS) refers to the primary malignancy of the trachea, the left and right main bronchus and the right middle bronchus, or the malignant external pressure of the peritracheal tissues and organs, as well as the airway stenosis and obstruction caused by the metastasis of the peritracheal tissues and organs or tumors in other parts. Obstruction of the airway by tumor tissue will lead to difficulty in ventilation, which is manifested as chest tightness and shortness of breath. In severe cases, it can lead to respiratory failure and even endanger the patient's life. It is a common emergency in clinic. Such patients have severe symptoms, high treatment risk, and poor prognosis, and the key to clinical treatment is the rapid removal of airway obstruction.

There are many malignancies that threaten human health. Lung cancer is one of the most common types. According to statistics, in recent years, lung cancer has the fastest incidence and death rates and has become one of the most harmful malignant tumors to human life and health. In patients with lung cancer, about 30% of patients will progress to develop atmospheric airway stenosis.

Chemotherapy and extracorporeal radiotherapy are an option for cancer patients who cannot undergo a surgery. However, they are slow to start and are not suitable for patients with respiratory distress and even asphyxia. With the continuous development of the airway endoscopic technology, many intraluminal interventional techniques have gradually emerged, such as the internal airway stent implantation, particle implantation, laser, high-frequency electric knife, argon knife, freezing, photodynamic therapy, and other technical means.

2. Methods

2.1. Ethical Statement. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2000), which was approved by the First Affiliated Hospital of Soochow University (No. 2021[336]). Informed consent was obtained from all individual participants involved in the study.

2.2. Study Population and Data Collection. All participants underwent stent placement for MCAO in our hospital from January 2013 to April 2020. Clinical data and follow-up information were obtained from medical records or telephone interviews with family members of patients. The criteria for recruiting patients in this study were as follows: (i) MCAO was determined using bronchoscopy or chest computed tomography (CT); the symptom of cross-sectional area of the lumen decreased by more than 50%, or obvious dyspnea could be observed; (ii) patients received airway stents placement surgery. Patients with incomplete clinical data would be excluded.

The data collected from each patient included baseline data (gender, age, body mass index (BMI), smoking history, and basic comorbidities), clinical characteristics (causes, prestent ECOG PS score), laboratory test data (hemoglobin (Hb), albumin (ALB), and serum lactate dehydrogenase (LDH)), stent placement (location and complications), and treatment and survival rate after stent placement. The evaluation of ECOG PS score before stent implantation was referred to the previous method [8].

2.3. Airway Stent Implantation. All patients underwent chest CT and soft bronchoscopy to assess the severity of MCAO before stent placement. For patients with local anesthesia or general anesthesia, the stent (straight, L-shaped, or Y-shaped metal airway stent) was delivered to the quasi-lesion site under bronchoscope or X-ray guidance. The airway with significant obstruction could be treated with airway intervention, such as balloon dilation, before stent placement. Due to the technical and material limitations, the stents used in this study were all metal airway stents obtained from Nanwei Medical Technology Co., Ltd.

2.4. Postoperative Monitoring. Routine bronchoscopy was performed one week after stent placement to check the status of the stent and refractory secretions. MCAO recurrence would be caused by tumors, granulation tissue formation, or refractory secretions after the initial stent implantation. To maintain airway patency, bronchoscopy intervention would be performed, including argon plasma coagulation (APC), laser, and stent replacement, cryotherapy.

2.5. Statistical Analysis. SPSS 22.0 was used for statistical analysis. Continuous variables were presented as mean ± standard deviation, and categorical variables were frequency (%). The survival time began from the placement of stent to the death of patient or the last follow-up of the living patient, and the last follow-up for this study was in November 2020. The overall survival rate was represented by the Kaplan-Meier curve. The log-rank test was used to analyze the survival rate between each subgroup of potential prognostic factors. Later, the factors ($P < 0.05$) that were based on the log-rank analysis and probably affected the prognosis were analyzed via the multivariate Cox risk regression model based on the forward stepwise regression with maximum likelihood estimation. The results of the potential predictors were presented with hazard ratios (OR) and 95% confidence interval (CI). The level of statistical significance was set at two-tailed $P < 0.05$.

Chronic infectious diseases caused by tuberculosis bacteria are called *tuberculosis*. Respiratory transmission is the main mode of transmission of this disease, and young people are more prone to TB. *Tuberculosis bacillus* can invade any organ in the human body or the whole body. It often invades the lung. Conjunctival disease refers to tuberculosis occurring in the trachea, bronchial mucosa, submucosa, smooth muscle, cartilage, and adventitia, which is called trachea and bronchial tuberculosis (tracheobronchial tuberculosis, TBTB).

Airway stenosis has traditionally been treated by surgery. Surgical resection of tracheal stenosis and suture of broken ends are the classic methods to treat tracheal stenosis. Although the surgical treatment can solve airway stenosis quickly and effectively, it also has the disadvantages of large trauma, many complications, and high cost, which some patients do not easily accept. With the progress of medicine, especially the rapid development of interventional technology, bronchial interventional treatment of airway stenosis has attracted more and more attention because it can make up for the shortcomings of traditional surgical treatment.

3. Results

3.1. Assessment of Clinical Data and Survival Rate. Ninety-eight consecutive patients aged 33 to 83 years were included, with an average age of 65.07 ± 8.89 years, and the ratio of male to female was 74 : 24. The body mass index ranged from 15.94 to 32.03 kg/m^2, the mean of which was 21.72 kg/m^2. Thirty-five patients (35.71%) had a history of smoking. In this study, MCAO was mainly caused by lung cancer (53/98, 54.08%), esophageal cancer (22/98, 22.45%), and thyroid cancer (3/98, 3.06%), with the basic comorbidities of hypertension (46/98, 46.94%), diabetes (11/98, 11.22%), coronary heart disease (4/98, 4.08%), chronic lung disease (4/98, 4.08%), *tuberculosis* (2/98, 2.04%), prostate hyperplasia (2/98, 2.04%), and other diseases (9/98, 9.18%). One or two stents were implanted in each patient, bringing the total of metal stents to 10^8. ECOG PS score indicated that 80 cases (81.63%) had a score of 0–2, while 18.37% had a score of 3–4.

The characteristics of airway stent placement in MCAO were summarized to further analyze the factors of survival (Table 1). Most stent placements were performed under local anesthesia (73.47%). The main stent sites were the trachea (46/98, 46.94%), the left main bronchus (19/98, 19.39%) and the right main bronchus (21/98, 21.43%). Complications after stent placement included neoplasms (36/98, 36.73%), sputum (30/98, 30.61%), mucus secretions (28/98, 28.57%), granulation tissue formation (21/98, 21.43%), necrosis (14/98, 14.29%), tumor endo growth (8/98, 7.14%), fistula (6/98, 6.12%), and stent displacement (3/98, 3.06%). After stent placement, there were 22 cases (22.45%) without any treatment, 21 cases (21.43%) were treated with chemotherapy, 14 cases (14.29%) for radiotherapy, 31 cases (31.63%) received radiotherapy and chemotherapy, and seven cases (7.14%) were given only supportive treatment.

It all depends mainly on the nature and scope of the lesion. It is closely related to the site and degree of lumen stenosis. At the beginning of the disease, when the scope of lesions is relatively small, the patient does not have any clinical symptoms. With the development of the disease, when the scope of exudative lesions expand, or caseous necrosis develops, it is manifested as hyperemia and edema of the tissue, infiltration of neutrophils, lymphocytes and monocytes, exudation of fibrin, and a small number of epithelioid cells and multinucleated giant cells. Acid fast staining can detect *Mycobacterium tuberculosis*.

Table 1: Features of first airway stent placement in 98 MCAO patients.

Variable	Total number of cases ($n = 98$)
Anesthesia	—
Partial	72 (73.47%)
Anesthesia	26 (26.53%)
Stent	–
Trachea	46 (46.94%)
Left main bronchus	19 (19.39%)
Right main bronchus	21 (21.43%)
Right middle bronchus	4 (4.08%)
Left main bronchus + right main bronchus	3 (3.06%)
Right middle bronchus + right distal bronchus	1 (1.02%)

Clinically, the chest *X* tablet is the most basic examination except for lung diseases. The *X* tablets in patients with bronchial *tuberculosis* are mostly complicated with pulmonary *tuberculosis* lesions, which can appear as exudative lesions, caseous lesions, tuberculous nodules, or nodular granuloma. Most of the bronchial *tuberculosis X* tablets cannot show direct signs of airway stenosis and mostly show indirect symptoms, such as localized pulmonary atelectasis and obstructive pneumonia.

Ninety-three patients died, and five patients were still alive till the end of the study. The median survival time was 5.5 months. The survival rates at 1, 3, 6, 9, 12, 24, 36, and 60 months were 79.73%, 63.51%, 43.24%, 37.84%, 29.73%, 15.17%, 7.59%, and 3.79%, respectively. The overall survival curve indicated that the cumulative survival rate decreased with time (Figure 1).

3.2. Univariate Log-Rank Test Analysis of Survival Rate. Univariate analysis of potential prognostic factors in 98 patients showed that the survival rate of participants was related to the primary disease, ECOG PS score, the site of stent, Hb, ALB, and LDH ($P < 0.05$) (Table 2). The survival curve estimated by Kaplan-Meier was shown in Figure 2.

3.3. Multivariate Cox Regression Analysis of Survival Rate. Select factors related to survival of MCAO patients after airway stent placement by univariate analysis ($P < 0.05$) and other factors mentioned in previous studies or considered to have an impact on the prognosis are mentioned in Table 3]. Variable cox risk regression model was used for further analysis. Multivariate analysis showed that ECOG PS score (OR = 3.468, 95%CI = 1.426–8.432, $P = 0.006$) and stent site (OR = 1.544, 95%CI = 1.057–2.255, $P = 0.025$) were significantly related to the survival rate (Table 4).

4. Discussion

MCAO patients were on the low edge of survival rate, and their the median survival time without any treatment was only one to two months [9, 10]. Bronchoscopy interventional techniques have been developed to reduce the

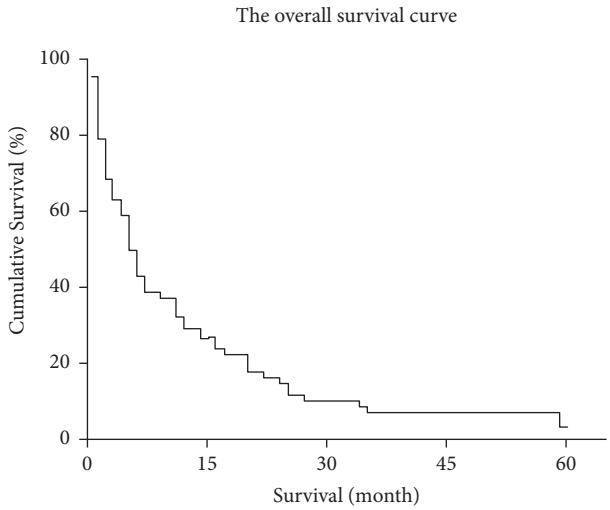

Figure 1: Kaplan-Meier curve showed the overall trend of survival rate with time.

mortality rate of MCAO and prolong the lives of patients by providing more opportunities for further antitumor therapy [11]. The studies on airway stents in the treatment of MCAO are all retrospective because of the difficulty to conduct prospective and large-scale studies as well as the small base of patients.

Chest CT, especially high-resolution CT (high resolution CT, HRCT), can significantly improve the disease diagnosis. The HRCT imaging of bronchial *tuberculosis* usually shows the thickening of the bronchial tube wall, which then leads to lumen stenosis, atelectasis, and obstructive pneumonia. The patient undergoes a tracheal tomography in the anterior and posterior, lateral, and oblique positions, which can show the site and extent of the stenosis and help clinicians understand the length and morphology of the bronchus. HRCT can also show the number and range of the affected bronchi and whether the lymph nodes are involved and complications.

Husain reported that seven of 46 MCAO patients were alive after treatment with Ultraflex metal stents, with a median survival time of 128 days, ranging from 3 to 1859 days [12]. Saji conducted a retrospective study that included 65 patients with advanced lung cancer in MCAO, in which a survival rate of 25.2% in one year and a median survival rate of 6.2 months were obtained [13]. Inchingolo presented a retrospective study of 140 patients with malignant tumors, including 107 non-small cell carcinomas, nine small cell carcinomas, nine lymphomas, eight esophageal cancers, and seven intrabronchial metastases, while the 1-year survival rate was 15% and the median survival time after stent placement was 3.4 months [14]. In this study, the common diseases that mainly resulted in MCAO included lung cancer (54.08%), esophageal cancer (22.45%), and thyroid cancer (3.06%). The median survival time of patients after stent implantation was 5.5 months, which is close to 4.7 months reported by Guibert [15].

The diagnosis of bronchial *tuberculosis* relies on the comprehensive analysis of epidemiology, medical history, clinical manifestations, physical signs, and related auxiliary examinations (such as sputum search for *Mycobacterium tuberculosis*, chest imaging, PPD, T cell spot experiment, and bronchoscopy). Because the clinical manifestations of bronchial *tuberculosis* patients often lack specificity, and the imaging examination has its limitations, the current diagnosis of bronchial *tuberculosis* mainly depend on bronchoscopy, bacteriological, or pathological evidence.

Table 2: Log-rank analysis results of 98 MCAO patients after stent placement.

Feature	*n*	(95% CI) (month)	*P* value
Gender	—	—	0.562
Male	74	9.97 (6.68–13.25)	—
Female	24	13.79 (6.24–21.33)	—
Age	—	—	0.809
>60 years old	72	11.59 (7.72–15.46)	—
≤60 years old	26	9.09 (4.64–13.55)	—
With hypertension	—	—	0.685
No	52	9.67 (5.53–13.81)	—
Yes	46	12.00 (7.49–16.51)	—
With diabetes	—	—	0.632
No	87	11.38 (7.98–14.78)	—
Yes	11	8.09 (1.48–14.70)	—
Primary disease	—	—	0.044
Lung cancer	53	8.28 (5.12–11.44)	—
Esophageal cancer	22	11.33 (4.24–18.43)	—
Other∗	23	20.58 (9.64–31.53)	—
ECOG PS score	—	—	0.021
0–2 points	80	12.35 (8.83–15.86)	—
3–4 points	18	3.85 (0.96–6.73)	—
Treatment after stent placement	—	—	0.371
No treatment	22	11.82 (3.09–20.55)	—
Chemotherapy	21	8.51 (4.01–13.00)	—
Radiotherapy	14	15.33 (3.58–27.09)	—
Chemotherapy	31	11.61 (6.57–16.64)	—
Only receive supportive care	7	5.00 (0.35–9.66)	—
Stent	—	—	<0.001
Trachea	46	15.23 (9.94–20.51)	—
Left main bronchus	19	4.95 (2.13–7.77)	—
Right main bronchus	21	8.63 (5.34–11.91)	—
Other†	8	1.25 (0.45–2.05)	—
Hb (g/L)	—	—	0.007
<110	34	5.48 (3.24–7.72)	—
≥110	64	14.16 (9.37–18.94)	—
ALB (g/L)	—	—	<0.001
<40	60	6.64 (4.53–8.75)	—
≥40	38	23.22 (13.25–33.20)	—
Feature	n	(95% CI) (month)	*P* value
LDH (μg/L)	—	—	0.082
<250	68	12.21 (8.39–16.40)	—
≥250	30	5.51 (1.97–12.99)	—

∗Other diseases included airway stenosis, esophagotracheal fistula, lung infection, thyroid cancer, laryngeal cancer, tracheal space occupying, bronchial adenoid cystic carcinoma, mantle cell lymphoma. †Other diseases included right middle bronchus, left main bronchus + right main Bronchus, and right middle bronchus + right distal bronchi. Hb: hemoglobin; ALB: albumin; LDH: serum lactate dehydrogenase.

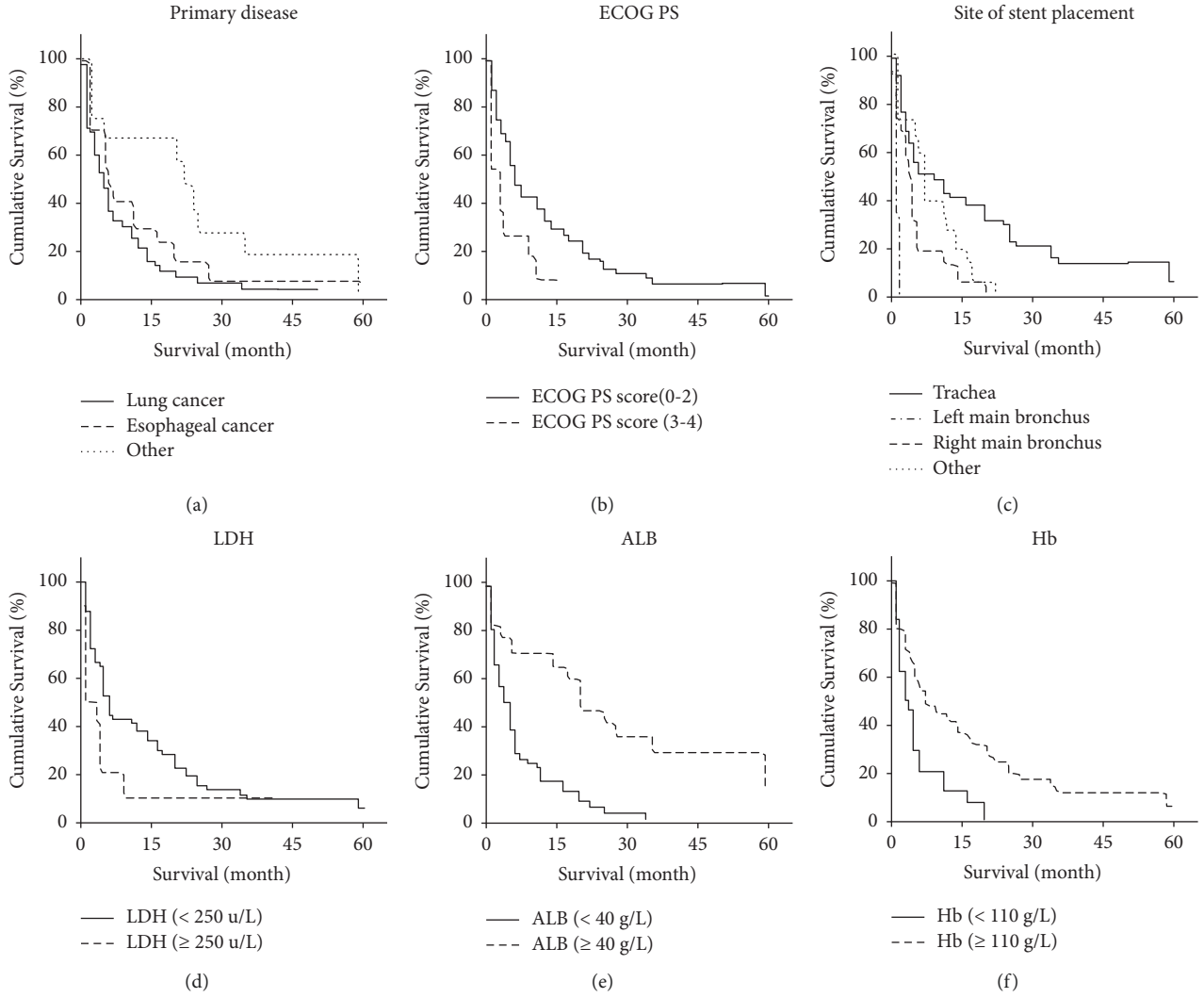

Figure 2: Overall survival curve estimated using Kaplan-Meier. Comparison of survival rates based on (a) primary disease ($P = 0.044$), (b) ECOG PS score ($P = 0.021$), (c) stent placement site ($P < 0.001$), (d) LDH ($P = 0.082$), (e) Hb ($P = 0.007$), and (f) ALB ($P < 0.001$).

Table 3: Survival factors and assignments of MCAO patients after airway stent placement.

Survival factors	Assignment
Gender	Female = 0, male = 1
Age	≤60 years old = 0, >60 years old = 1
Hypertension	No = 0, yes = 1
Diabetes	No = 0, yes = 1
Primary disease	Other* = 0, Lung cancer = 1, esophageal cancer = 2
ECOG PS score	0–2 points = 0, 3–4 points = 1
Treatment after stent placement	Radiotherapy = 0, chemotherapy = 1, Radiotherapy and chemotherapy = 2, Supportive Treatment = 3, No Treatment = 4
Stent	Trachea = 0, left main bronchus = 1, right main bronchus = 2, other† = 3
Hb (g/L)	≥110 = 0, <110 = 1
ALB (g/L)	≥40 = 0, <40 = 1
LDH (μg/L)	<250 = 0, ≥250 = 1

*Other diseases included airway stenosis, esophagotracheal fistula, lung infection, thyroid cancer, laryngeal cancer, tracheal space occupying, bronchial adenoid cystic carcinoma, and mantle cell lymphoma. †Other diseases included right middle bronchus, left main bronchus + right main bronchus, and right middle bronchus + right distal bronchus. Hb: hemoglobin; ALB: albumin; LDH: serum lactate dehydrogenase.

Due to the slow onset of bronchial *tuberculosis*, diverse manifestations, and lack of specificity, grassroots doctors have limited diagnosis and treatment levels, so patients are prone to misdiagnosis. If bronchial *tuberculosis* is not adopted by timely and standardized diagnosis and treatment, once the scar stenosis or tube wall softening occurs,

Table 4: Cox regression analysis results of 98 MCAO patients after stenting.

Factor	B	Se	Wald	P value	OR	95%CI
ECOG PS score	1.243	0.453	7.523	0.006	3.468	1.426–8.432
Stent	0.434	0.193	5.041	0.025	1.544	1.057–2.255

lung ventilation dysfunction can occur. In severe cases, repeated lung infection, lung atresia, and lung damage caused by obstructive pneumonia may even occur, which seriously affects the quality of life of patients.

Bronchoscopy interventional techniques have been seen as one of the positive prognostic factors of MCAO patients. In addition, independent prognostic factors for MCAO patients with interventional bronchoscopy have been reported, such as the etiology, ASA score, obstruction site, external or mixed lesions, preoperative adjuvant therapy, and preoperative ECOG PS score as well as treatment [16, 17]. Compared with the previously mentioned studies, this study focused on MCAO patients undergoing stent implantation and excluded the influence of other interventions. Furthermore, the preoperative ECOG PS score and the stent placement site also influenced the independent prognosis for MCAO patients after airway stent placement.

Some patients with bronchial *tuberculosis* have chest tightness, urgent breath, or dyspnea when diagnosed, and only oral antituberculosis drugs are often effective slowly and take longer than *tuberculosis* treatment, which is easy to delay the disease and increase the sequelae. In order to prevent the formation of airway stenosis, shorten the course of treatment, and significantly improve the quality of life of patients, the bronchoscope airway intervention technology is usually continued to be used for local treatment on the basis of systemic chemotherapy.

The indications are suitable for patients with ulcerative necrotic and granulation proliferative bronchial *tuberculosis* airway stenosis. Some of the disadvantages of the current microwave treatment operation equipment, which limit its use in the previously mentioned cases, are as follows: microwave radiation range is small, larger lesions need repeated operations, it increases the workload of medical staff and causes operation inconvenience, and it can easily cause tube wall perforation, tracheoesophageal fistula, or complications such as cold light source burns.

The ECOG PS score was not only an indicator to determine whether cancer patients should receive chemotherapy but also a prognostic factor for cancer patients [18]. Similar to the study reported by Ong, this study showed that patients with a high ECOG PS score [3, 4] had a worse prognosis than patients with a low ECOG PS score [0–2] (the mean survival time was 3.85 months and 12.35 months, resp., $P = 0.004$), indicating that patients with good PS might survive from stent implantation to prolong survival and improve oxygenation [19]. Stent placement has been proven to prevent acute asphyxia, reduce the patient's fear of sudden death, and provide patients with enough time to receive additional treatments, such as chemotherapy, radiotherapy, and palliative care [20]. Indications of airway stent implantation should be determined carefully for patients with poor PS. Although stent implantation could alleviate severe dyspnea symptoms, patients with high PS scores might have fewer opportunities to receive antitumor therapy, and survival time after surgery was probably still short.

Mohan reported that the location of the lesion not only determined the type (straight, *L* or *Y*) and length of the airway stent but also affected the independent predictors of survival in MCAO patients. This study showed a worse prognosis for patients with both trachea and main bronchus than those who were only placed one stent in the trachea (average survival time was 15.23 months and 1.25 months, resp.), which indicates that compound MCAO (striction of both trachea and main bronchus) was more serious than simple MCAO (striction of trachea or main bronchus only).

Research by Wang reported that the antitumor therapy prolonged the survival time of MCAO patients with the intervention of airway stent implantation ($P < 0.001$), while the effect of different antitumor therapies on the prognosis for these MCAO patients after bronchoscopy intervention was not presented [21]. Our study supplemented this data and showed that the average survival time of the radiotherapy and radiochemotherapy groups after stent placement was 15.33 and 11.61 months, respectively, which exceeded the 5.00 months of the only supportive treatment group. However, the average survival period of the chemotherapy group after stent placement was close to that of the supportive treatment group, probably since most cases were in the terminal stage, and nearly half of the patients received chemotherapy before implanting the stent. As a local treatment method, radiotherapy is more effective than chemotherapy in controlling local airway stenosis. The results in our study indicated that a longer survival time existed in MCAO patients with radiotherapy rather than chemotherapy after stent placement, which meant that the radiotherapy counted for these patients as a local treatment to maintain airway patency, especially to those who received chemotherapy before or after stent placement.

Certain limitations in this study were as follows. (i) This was a single-center, retrospective study, and more multicenter and prospective studies are needed. (ii) Different tumors respond differently to radiotherapy and chemotherapy, which might result in the various data obtained from different participants. Despite these limitations, we obtained the important finding that the ECOG PS score before stent placement and the site of stent placement affected the survival rate of MCAO patients. Therefore, stent implantation was more beneficial to patients with lower ECOG PS scores before stent implantation and simple MCAO patients.

5. Conclusion

This study included 98 MCAO patients after stent placement to investigate the clinical features and results of long-term improved prognosis. The results from this study demonstrate a regular trend regarding the baseline characteristics, clinical figures, and survival time among patients. This will assist clinicians in the treatment of HFM patients. We established that MCAO was mainly caused by lung cancer (53/98, 54.08%), esophageal cancer (22/98, 22.45%), and thyroid cancer (3/98, 3.06%). The median survival time of participants was 5.5 months. The survival rate was related to the primary disease, ECOG PS score, stent site, Hb, ALB, and serum LDH. Compared with the site of stent placement, the ECOG PS score is the primary factor in the survival rate of MCAO patients after airway stenting. However, clinicians should be cautious when applying the results of this study in clinical practice and should consider the medical needs of individual patients.

Data Availability

The labeled dataset used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

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Research Article

Procedural Efficiency, Efficacy, and Safety of High-Power, Short-Duration Radiofrequency Ablation Delivered by STSF Catheter for Paroxysmal Atrial Fibrillation

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Objectives. To investigate the procedural efficiency, efficacy, and safety of high-power, short-term radiofrequency ablation delivered by the SmartTouch Surround Flow (STSF) catheter for paroxysmal atrial fibrillation (AF). *Methods*. We retrospectively analyzed a total of 72 patients who were admitted with paroxysmal AF, and who underwent radiofrequency catheter ablation (RFCA) for the first time. Of these patients, 36 cases underwent low-power, long-duration (LPLD, (30–35 W/20–40 s) pulmonary vein isolation (PVI) delivered by an SmartTouch (ST) catheter (control group), and the other 36 cases underwent high-power, short-duration (HPSD, (45–50 W/10–20 s) PVI delivered by a STSF catheter (study group). The baseline data, duration of PVI, procedural time, fluoroscopy time, the rate of first-pass isolation, irrigation perfusion, eschar and steam pop occurrences, intraoperative complications, and the rate of stable sinus rhythm maintenance following a blanking period of three months were analyzed between the two groups. *Results*. The isolation time of bilateral PVI and procedural time in the study group were markedly less than in controls ($p < 0.01$). The rate of first-pass isolation in the study group was significantly higher than in the control group (95.8% vs. 84.7%, $p = 0.023$), while the fluid perfusion in the study group was approximately 20% less than that in the control group (767 ± 171 vs. 966 ± 227 ml, $p < 0.001$). We observed no severe complications in any patients. The rate of freedom from AF recurrences following a blanking period of three months showed a tendency to be higher than in controls (93.9% vs. 87.1%, $p = 0.348$). *Conclusions*. The HPSD strategy delivered by the STSF catheter was superior to conventional LPLD ablation through the ST catheter with respect to efficiency, acute procedural effectiveness, short-term safety, and the risk of heart failure in patients with paroxysmal AF.

1. Introduction

Radiofrequency catheter ablation (RFCA) is an important therapeutic intervention in the management of sinoatrial rhythm control with atrial fibrillation (AF), and has been applied broadly in recent years [1]. Pulmonary vein isolation (PVI) is the cornerstone of therapy for paroxysmal AF [2], and continuous and transmural tissue damage caused by radiofrequency energy for complete PVI is key to preventing the recurrence of AF. Contact force (CF), energy delivery, and ablation time are critical factors with respect to lesion transmurality and uniformity, and catheter stability and the width of lesions determine linear continuity. Many centers have in recent years adopted a traditional low-power, long-duration (LPLD) ablation strategy, and in previous clinical practice at our center, the effect of using an LPLD strategy guided by the ablation index (AI) for PVI was overwhelmingly positive. However, even for skilled operators, the overall procedural time is generally more than two hours, and the procedural efficiency requires improvement. Recent investigations revealed that a high-power, short-term (HPSD) ablation strategy not only shortened ablation time, expanded lesion width, and improved point-to-point connectivity, but also reduced lesion depth and surrounding

tissue injury. Additionally, HPSD's procedural efficiency, efficacy, and safety have been demonstrated in numerous clinical studies [3, 4].

The development of ablation catheters has focused on optimizing safety and efficiency in the past decade, and the application of a contact force (CF) pressure sensing catheter is a relatively new and important modality. Therefore, a large number of data from the Symptomatic Paroxysmal Atrial Fibrillation (SMART) AF[5] study and the Ablation Catheter Study for Atrial Fibrillation (TOCCASTAR) have confirmed the advantage of pressure sensing using short-and long-term outcomes [5–7]. SmartTouch Surround Flow (STSF) is a novel CF-optimized catheter, where its micropore perfusion is increased from a six-hole version in the SmartTouch (ST) catheter to a 56-hole catheter, which greatly induces a better cooling effect of the catheter tip, better energy delivery, better lesion transmurality, and less eschar. This technology also possesses six symmetrical temperature sensors that accurately monitor the temperatures of the catheter and tissue surface in real time. However, the differences between LPLD ablation strategy delivered by ST catheter and HPSD ablation strategy delivered by STSF catheter are limited. Therefore, we herein employed 45–50 W of power with the STSF catheter or 30–35 W of power with an ST catheter for radiofrequency catheter ablation (RFCA) guided by AI in patients with paroxysmal AF. This allowed us to demonstrate procedural efficiency, efficacy, and safety of HPSD as delivered by an STSF catheter.

2. Methods

2.1. Study Population. In the retrospective single-center study, we analyzed a total of 72 patients who were admitted to the Department of Cardiology of the Second Hospital of Medical University from January 2020 to November 2021 for catheter ablation of paroxysmal AF for the first time, comprising 47 men and 25 women. Of these, 36 patients underwent an LPLD ablation strategy delivered by ST catheter (control group), and the other 36 cases underwent an HPSD ablation strategy delivered by STSF catheter (study group).

Our inclusion criteria were: (1) patients 18–86 years of age with or without symptomatic paroxysmal AF; (2) patients with nonvalvular AF; (3) patients without previous catheter ablation. The exclusion criteria were as follows: (1) patients with valvular or congenital heart disease; (2) a left atrial (LA) anterior posterior diameter >60 mm; (3) a left ventricular ejection fraction (EF) < 40%; (4) an intra-atrial thrombus; (5) acute coronary syndrome; (6) severe renal insufficiency, with an estimated glomerular filtration rate (eGFR) < 30 ml/min/1.73 m^2; and (7) patients with severe infection, recent surgery, or who experienced cerebrovascular accidents. All of the patients met RFCA indications and signed the informed consent form for RFCA. This study was approved by the local of Research Ethics Committee (YX2022-027), and performed according to the Helsinki Declaration as revised in 2013.

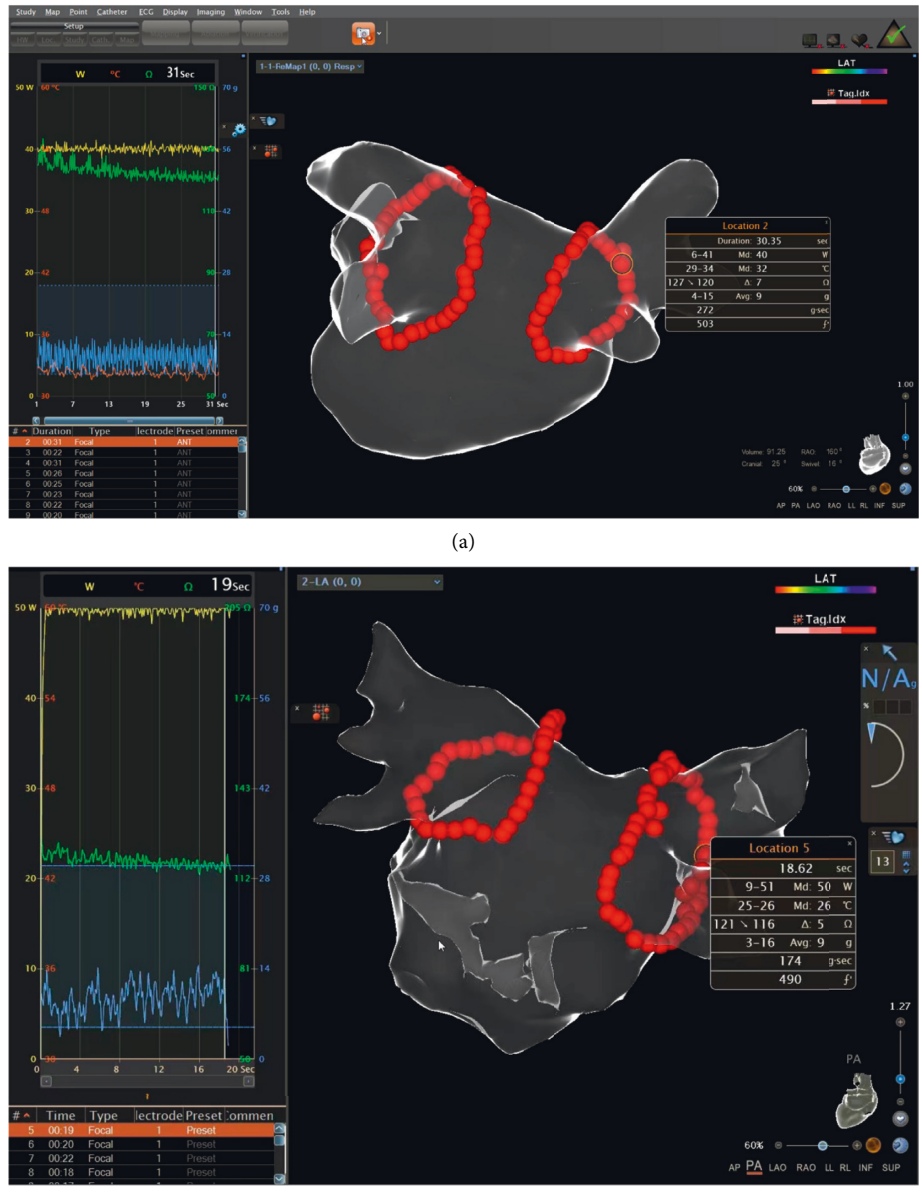
2.2. Study Methods

2.2.1. Baseline Data Collection. We collected data on age, sex, height, and weight, as well as patient history of hypertension, diabetes, coronary heart disease, heart failure, stroke, or transient ischemic attack (TIA), and the course of AF. Routine blood chemistry, biochemistry, coagulation tests, and electrocardiography (EKG) examinations were completed prior to the operation; and we assessed patient CHA_2DS_2-VASc and HAS-BLED scores [8]. After admission, the patients were treated with anticoagulants (low-molecular-weight heparin or rivaroxaban), and the presence of thromboses was determined by transesophageal echocardiography (TEE). Those individuals who could not tolerate TEE underwent three-dimensional (3D) reconstruction of the left atrium and pulmonary veins (PVs) by multi-slice CT.

2.2.2. Catheter Ablation. All of the patients at our center underwent local anesthesia with lidocaine, and a decapolar catheter (Biosense Webster, CA, USA) was placed within the coronary sinus (CS) via their left subclavian vein or a femoral-vein access. After successful puncture of the atrial septum, heparin (Wanbang Biopharmaceuticals, China) (75–100 u/kg) was injected, and anticoagulation was continued during the operation. The dosage of heparin was adjusted according to the Activated Clotting Time of Whole Blood (ACT) to generate a value between 250 and 300 s. A PentaRay NAV eco High-Density Mapping Catheter (Biosense Webster, CA, USA) was inserted in order to construct an electroanatomic map of the left atrium and PV as guided by a Carto 3D electrophysiological mapping system in the FAM model (Carto3, Version 6, Biosense Webster, CA, USA). All of the patients then underwent point-to-point quantitative circumferential ablation of the pulmonary vein guided by AI. Lesion tags were generated through the VisTag model (Carto VISITAG™ Module, Biosense Webster, CA, USA), and our center tag settings were as follows: a maximal range of catheter movement during ablation of 2.5 mm, a minimal time of catheter stability of 3 s, and a lesion-tag display size of 3–5 mm.

In the power-control mode, the control group underwent an LPLD approach (30–35 W for 20–40 s) delivered by ST catheters (Biosense Webster, CA, USA); and the study group experienced a HPSD approach (45–50 W for 10–20 s) delivered by STSF catheters (Biosense Webster, CA, USA) (Figure 1). Different AI thresholds, ablation times, and power settings in various left atrial sites are shown in Table 1, and we targeted a CF range of 5–20 g. The ST or STSF catheter tip was irrigated by saline at a flow rate of 2 mL/min in the nonablation state; in the ablation state the irrigated flow rate of the ST catheter tip was 16 mL/min (25 mL/min if the power was greater than 30 W), and the irrigated flow rate of the STSF catheter tip was 16 ml/min. Upon completion of PVI, the PentaRay NAV eco high-density mapping catheter was used to verify the PVI (first-pass isolation); if there was an absence of isolation, touch-up ablation was performed until complete PV isolation was achieved.

After the completion of PVI, cardiac electrophysiology was implemented to assess whether there were other arrhythmias. During the procedure, fentanyl citrate (YICHANG HUMANWELL PHARMACEUTICAL CO,



(a)

(b)

Figure 1: PVI in paroxysmal AF. (a) LPLD (40 W/31 s, AI 500) ablation delivered by an ST catheter. (b) HPSD (50 W/19 s, AI 490) ablation delivered by an STSF catheter.

Table 1: RF power, AI thresholds, and RF time per lesions in different left atrial sites.

	Group	Anterior	Ridge	Roof	Inferior	Posterior
RF power	Control	35 W	35 W	35 W	35 W	30 W
	Study	50 W	50 W	50 W	50 W	45 W
AI	Control	450–480	480–500	400–450	400–450	380–420
	Study	450–480	480–500	400–450	400–450	380–420
RF time per lesions	Control	30–40 s	35–40 s	25–35 s	25–35 s	20–25 s
	Study	15–20 s	15–20 s	10–20 s	10–20 s	10–15 s

RF, radiofrequency; AI, ablation index.

LTD, China) (5–15 ml/h·kg) was injected intravenously for continuous analgesia and adjusted according to the pain felt by patients; and blood pressure, heart rate, and blood oxygen saturation were continuously monitored. We observed whether there was eschar or audible steam pop and recorded the ablation time of the left and right PVs, first-pass

isolation, fluoroscopy time, total procedural time, and irrigation fluid volume. Postprocedural observations of the puncture site included bleeding, hematoma, pericardial effusion, atrio-esophageal fistula, phrenic nerve injury, and other complications.

2.2.3. Postprocedural Management and Follow-Up. All of the patients were treated with amiodarone (SANOFI, France) or dronedarone (CSPC Pharmaceutical Group Co., Ltd, China) for at least three months postoperatively, with the drugs discontinued if no recurrence was noted or if significant bradycardia developed. The patients were administered warfarin (Shanghai Pharmaceuticals Sine, Shanghai, China) or a new oral anticoagulant (Rivaroxaban, BAYER, Germany, or Dabigatran Etexilate Capsules, Boehringer Ingelheim, Germany) for at least two months, and then evaluated as to whether they were to continue therapy according to the patient's CHA_2DS_2-VASc score; and they were placed on a proton-pump inhibitor for at least one month.

All of the patients were advised to follow up at three months and underwent a 12-lead EKG when palpitation symptoms appeared. A 12-lead EKG or Holter monitoring was executed after a 90-day blanking period to observe whether sinus rhythm was achieved or whether bradycardia arrhythmia remained.

2.2.4. Statistical Analysis. We employed SPSS 20.0 (IBM Corp., Armonk, NY, USA) for all statistical analyses. The measurement data were expressed as mean ± SD (fluoroscopy time was expressed in quartiles), and the counting data were expressed as frequencies and percentages (n (%)). We used a t-test or chi-squared test for comparisons of measurement data or counting data between the two groups, and a Mann–Whitney U test for non-normally distributed measurement data. $p < 0.05$ was considered to be statistically significant.

3. Results

3.1. Baseline Characteristics. A total of 72 patients (61.0 ± 11.1 years of age, 65.3% male) comprised this study, with 36 cases in each of the control and study groups. There were no significant differences between groups with regard to age, sex, or body mass index (BMI); onset time of AF; or patient history of hypertension, diabetes, coronary heart disease, heart failure, or stroke/TIA; nor in eGFR, left ventricular (LV) and LA diameters, EF, or CHA_2DS_2-VASc score, and HAS-BLED score (Table 2).

3.2. Procedural Parameters. Of the 72 patients, one manifested a left pulmonary vein trunk. In the control group, one patient underwent LA posterior wall linear ablation because LA matrix mapping showed a strip-shaped, low-voltage area of the LA posterior wall. Because AF was induced by programmed atrial stimulation in this patient, we executed LA anterior wall linear + mitral isthmus linear + tricuspid isthmus linear ablation, and a double-block was attained in this patient. In one patient in the study group whose AF originated from the superior vena cava (SVC), we defined the function of the sinoatrial node by an activation map under sinus rhythm, and a 3D distribution of the right phrenic nerve was defined by a pacing map; segmental RFCA was then implemented for SVC isolation. In the study group, one patient underwent LA roof linear + mitral isthmus linear ablation due to atrial programmed stimulation, and a double-block was also attained. The other patient in the study group exhibited induced atrioventricular nodal reentrant tachycardia (AVNRT) and underwent modification therapy of the slowed electrical pathway.

Regarding procedural efficiency, the RF times for PVI of left and right veins in the study group were 32.1 ± 7.0 min and 29 ± 7.0 min, respectively, which were significantly less than in the control group (PVI of left veins, 41.3 ± 6.2 min; PVI of right veins, 33.1 ± 4.5 min, $p < 0.01$). The total procedural time in the study group was also significantly less than that in the control group (111.8 ± 36.1 min vs. 145.4 ± 34.3 min, respectively, $p < 0.001$), but the fluoroscopy time showed no difference between groups (Table 3).

Successful PVI was completely achieved in all patients. The rate of first-pass isolation in the study group was 95.8%, while that of the control group was 84.7% ($p = 0.023$). We observed a marked reduction in the study group in the total irrigation perfusion volume by approximately 20% (767 ± 171 ml vs. 966 ± 227 ml, $p < 0.001$, respectively) (Table 3).

3.3. Follow-Up. Clinical follow-up data were available for 33 of 36 (91.7%) patients from the study group and 31 of 36 (86.1%) from the control group at the three-month follow-up. Thirty-one of 33 patients (93.9%) were free of AF recurrence following a blanking period of three months in the study group, whereas the proportion was 27 of 31 (87.1%) in the control group (not significant (NS); $p = 0.348$).

3.4. Complications. There was no eschar generated in either group during the procedure, and three audible steam pops occurred during ablation in the study groups, with two in the control group. Neither steam pop led to pericardial effusion. In the control group, one patient experienced acute heart failure and one patient experienced acute pancreatitis and diabetic ketoacidosis after ablation. In the study group, one patient manifested bradycardia, but no permanent pacemaker was implanted after the procedure; and one patient exhibited a hematoma at the puncture point of the left shoulder that was associated with the puncture. We observed no atrio-esophageal fistula, SVC stenosis, phrenic nerve injury, or sinoatrial nodal injury in the perioperative period in any patient.

4. Discussion

The present study revealed that the HPSD ablation delivered by an STSF catheter not only conveyed distinct advantages in procedural efficiency, acute success, and short-term safety, but also showed a tendency for a higher rate of stable sinus

Table 2: Baseline demographic and patient characteristics.

Characteristics	Control group ($n = 36$)	Study group ($n = 36$)	p value
Sex (male/female)	20/16	27/9	0.083
Age (years)	63.11 ± 9.96	58.92 ± 11.92	0.110
BMI (kg/m^2)	25.38 ± 3.24	24.11 ± 2.98	0.089
Hypertension (n (%))	18 (50.0)	20 (55.6)	0.637
Diabetes (n (%))	7 (19.4)	6 (16.7)	0.759
Coronary heart disease (n (%))	11 (30.6)	6 (16.7)	0.165
Heart failure (n (%))	3 (8.3)	1 (2.8)	0.303
Stroke/TIA (n (%))	4 (11.1)	2 (5.6)	0.394
Mean AF duration (months)	28.31 ± 31.46	35.50 ± 36.38	0.716
CHA_2DS_2-VASC	2.31 ± 1.95	1.69 ± 1.49	0.191
HAS-BLED	0.64 ± 0.59	0.44 ± 0.61	0.130
eGFR (ml/min/1.73 m^2)	89.48 ± 20.71	91.66 ± 26.94	0.165
LA diameter (mm)	38.08 ± 6.40	38.19 ± 6.46	0.942
LV diameter (mm)	45.31 ± 5.06	47.31 ± 4.62	0.084
LV EF (%)	61.53 ± 4.07	63.04 ± 4.40	0.136

Data are expressed as mean ± SD or n (%). BMI, body mass index; TIA, transient ischemic attack; AF, atrial fibrillation; eGFR, estimated glomerular filtration rate; LV, left ventricular; EF, ejection fraction.

Table 3: Procedural characteristics and freedom from AF recurrences following a blanking period of three months.

Characteristics	Control group ($n = 36$)	Study group ($n = 36$)	p value
Left PVI time (min)	41.3 ± 6.2	32.1 ± 7.0	<0.001
Right PVI time (min)	33.1 ± 4.5	29.1 ± 7.0	0.007
Procedural time (min)	145.4 ± 34.3	111.8 ± 36.1	<0.001
Fluoroscopy time (min)	4.5 (4.0–5.0)	4.0 (3.25–5.0)	0.129
First pass isolation n (%)	61/72 (84.7)	69/72 (95.8)	0.023
Irrigation fluid volume (mL)	966 ± 227	767 ± 171	<0.001
Steam pop (n (%))	2 (5.6)	3 (8.3)	0.643
Freedom from AF recurrences (n (%))	31/33 (93.9)	27/31 (87.1)	0.348

Data are expressed as the mean ± SD or n (%) (fluoroscopy time was expressed in quartiles). PVI, pulmonary vein isolation; AF, atrial fibrillation.

rhythm following three months in the treatment of paroxysmal AF. First, compared with LPLD ablation delivered by an ST catheter, the RF and procedural times for HPSD ablation delivered by the STSF catheter were significantly shortened, which greatly enhanced overall procedural efficiency. Second, the rate of first-pass isolation of HPSD ablation delivered by the STSF catheter was 13.1% higher than that with LPLD ablation delivered by the ST catheter, which ameliorated the acute achievement of PVI. Third, the irrigated fluid volume of HPSD ablation delivered by the STSF catheter was diminished by approximately 20%, which reduced the risk of heart failure caused by volume overload, and HPSD did not elevate procedural risk or the incidence of complications.

Although we have observed an annual increase in the prevalence and incidence rates of AF as China's population ages, the efficacy and safety of traditional antiarrhythmic drugs are currently unsatisfactory. Moreover, while numerous guidelines recommend catheter ablation as the first-line therapy for paroxysmal AF [1, 9], how to improve procedural efficiency and safety and promote short- and long-term success rates remain constant concerns. The continuous and transmural nature of ablation lesions exerts a direct influence on recurrence rate, which is the key to circumferential PVI. Catheter stability, RF power and duration, CF, and temperature of the catheter tip are essential parameters of the lesion size and transmurality, and AI modules can thus be exploited to calculate and quantify the RF parameters and be used to accurately evaluate each ablation point so as to improve procedural success rates [10]. Administrators of one multicenter study have suggested that the rate of one-year's relief from AF recurrences in patients who underwent RFCA guided by AI was 91% with respect to paroxysmal AF [11]. Moreover, since 2019, the rates at our center for one-year relief from recurrences of paroxysmal and persistent AF have been 92.7% and 73.9%, respectively (unpublished data). However, in cases of ablation difficulties, it is necessary to explore more efficient ablation strategies.

High-power (HP) ablation comprises one of the most studied clinical modalities. HPSD ablation enhances resistive heating by increasing RF power so as to achieve wider and more transmural lesions, while it also weakens conductive heating by shortening the ablation time and diminishing the depth of the lesions to avoid injury to the surrounding tissues [12]. Some studies conducted *in vivo* have shown that HPSD ablation increased the width of the lesions and led to better continuity between points and because of the shorter ablation time at a single point, the procedural time was commensurately shortened [13–15]. Although complications such as steam pop, eschar at the catheter tip, and perforation of the left atrium may occur

during HP ablation, the STSF catheter, in theory, reduces these complications by optimizing irrigation at the catheter tip. The STSF catheter possesses 56 irrigation holes and allows precise temperature sensing to homogeneously cool the catheter tip, which avoids gasification of water in the tissues and the interruption of ablation caused by overheating. In addition, optimal cooling of the ablation electrode provides a more efficient RF delivery and generates transmural lesions more easily [16].

In the present study, we retrospectively analyzed patients with paroxysmal AF who underwent RFCA for the first time at our center between January of 2020 and November of 2021. The ST catheter was employed for LPLD ablation at a power of 30–35 W, and 30 W was applied for 20–25 s during posterior wall ablation due to possible esophageal injury. The STSF catheter was used for HPSD ablation with a power of 45–50 W, while 45 W was applied for 10–15 s during posterior wall ablation. Our results revealed that the rate of first-pass isolation in the HPSD group with the STSF catheter was 95.8%, which was 13.1% higher than that in the LPLD group with the ST catheter ($p = 0.023$), similar to the findings of Phlips et al. [17]. We hypothesized that the HPSD approach with an STSF catheter improved acute success, and a large number of clinical studies have further confirmed the short-term efficacy outcomes of the HPSD method [18–20]. While numerous recent studies have shown that HPSD ablation resulted in higher first-pass isolation and lower PV reconnection [4, 21], whether the HPSD method reduces the long-term recurrence rate of AF is still controversial. A recent meta-analysis showed that RFCA using HPSD effectively reduced the risk of recurrent AF (RR = 0.72; 95% CI = 0.54 to 0.96; $p = 0.02$), but not of atrial flutter (AFL) or atrial tachyarrhythmia (AT) [4]; and a second meta-analysis revealed that the HPSD strategy did not lessen relief from atrial tachyarrhythmia at 12-month follow-up [22]. Moreover, a retrospective study by Baher et al. depicted a similarity in AF recurrence rates between HPSD and LPLD ablation at a mean follow-up period of 2.5 years (42% vs. 41%, respectively, $p = 0.571$) [23]. It is worth noting that the STSF catheter is superior to the ST catheter in ablation efficacy [24, 25], and that PVI by the STSF catheter reduces the rate of early reconnections of the left PV [26]. Likewise, there was a trend toward improved efficacy of the STSF ablation catheter compared to the ST device in the Kaplan–Meier estimation of 12-month arrhythmia-free survival (NS, $p = 0.18$) [27]. In the present study, the HPSD strategy delivered by the STSF catheter not only improved the rate of first-pass isolation but also showed a tendency for a higher rate of stable sinus rhythm following a blanking period of three months (93.9% vs. 87.1%, $p = 0.348$, respectively, NS). Bunch et al. [28] analyzed the 3-year prognosis of patients undergoing HPSD ablation and those with conventional ablation, and also found no significant difference in the 3-year AF recurrence rate between the two ablation strategies (26.5% vs. 30.7%; $p = 0.23$), which was similar to the prognostic results in this study. Clinical studies with larger sample sizes and longer follow-up times are needed in the future to clarify these potential differences in rates.

Many recent studies have revealed that RFCA using HPSD greatly improves procedural efficiency [18, 23, 29]. In the present study, we established that HPSD ablation delivered by STSF catheter significantly shortened ablation time, thus greatly improving procedural efficiency, which is consistent with the research results of Dhillon et al. [30]. Our results also showed that the PVI times of the left and right PVs with the HPSD method and using the STSF catheter were 32.1 ± 7.0 min and 29 ± 7.0 min, respectively, which were notably shorter than times in the LPLD group (41.3 ± 6.2 min and 33.1 ± 4.5 min, respectively). Importantly, the total procedural time was reduced to less than 120 min. Because the width of the lesions in HPSD ablation was large, lesion continuity was nevertheless assured even when ablation points were few. The ablation time at a single point was greatly shortened, and the total procedural time was also abbreviated accordingly.

The short ablation time at every point was also more conducive to the stability of catheter manipulation. In addition, we found that the fluoroscopy time for HPSD with the STSF catheter was slightly but not significantly reduced; this might portend an association with a clearer display of potential signals during the procedure [31].

It is indisputable that although an HPSD strategy improves overall procedural efficiency and efficacy, it also poses some safety issues. The control of CF and RF duration comprises the critical determinant in complication prevention. As CF increases, the risk of myocardial perforation increases, and excessive CF also affects the flow rate of saline irrigation at the catheter tip, elevating the risk of eschar. Insufficient RF time causes inadequate ablation and results in nontransmural lesions, while excessive ablation may conversely lead to steam pop, pericardial effusion, esophageal and phrenic nerve injury, thrombosis, and stroke. The unique 56-hole irrigated cooling design reduces the occurrence of steam pop and eschar [24], and we observed acute safety with HPSD ablation as delivered by the STSF catheter in the present study. Although we noted no pericardial tamponade, atrio-esophageal fistula, or pulmonary vein stenosis in either the LPLD or HPSD groups we did observe steam pop in three cases of the HPSD group, but this was not significantly different from the LPLD group. Qu et al. [32] showed that the occurrence of steam pop was significantly related to HP under RFCA intervention, which was different from our study. The reason may be related to the improved thermostability of the STSF catheter after optimization in this study.

Continuous high-flow irrigation during procedures may precipitate congestive heart failure (CHF), and thus fluid management is particularly important for patients with pre-existing CHF. In one study, the researchers demonstrated that the irrigation fluid volume using the STSF catheter during PVI was 51.7% less than that with the ST catheter [27]. Furthermore, our study similarly showed that irrigation fluid volume using the STSF catheter was dramatically reduced, and we also noted no incidence of heart failure after the procedure. Thus, patients who have previously experienced CHF may benefit more from the use of the STSF catheter.

Although the effectiveness and safety of RFCA using HPSD have been disclosed, the levels of power that afford the best efficacy and safety—and the upper limit of power—remain ambiguous. Since our center had no prior experience in high-power ablation, we adopted RFCA with a power of 45–50 W and a duration of 10–20 s. There exist very few studies on very-high-power, short-duration (vHPSD) ablation. One center used 70 W for very high-power or 30–40 W for conventional power for PVI in paroxysmal AF, and these authors found that the vHPSD approach exhibited markedly fewer AF recurrences after one year—with a satisfactory safety profile [33]. Moreover, some studies comprising a 90-W ablation mode provided safe and efficient PVI [34, 35]. We will therefore further explore higher-power ablation in the future to observe its effectiveness and safety.

4.1. Limitations. The current study entailed some limitations. First, this was a retrospective study of a single-center experience, with a limited number of patients enrolled. Additional studies of larger sample sizes and that encompass multiple centers are therefore required to confirm these findings. Second, the follow-up period was limited to three months, and a follow-up based upon using a 12-lead EKG or Holter-monitor examination may have underestimated the actual incidence of atrial arrhythmia. Thus, additional studies with long-term follow-up and more robust arrhythmia monitoring are necessary to demonstrate efficacy and safety. Third, since this study was focused on efficacy in first-time AF-ablation patients, we will in the future examine patients undergoing repeated ablations.

5. Conclusions

This study suggests that the HPSD strategy delivered by an STSF catheter in patients with paroxysmal AF is more efficient and that it exhibits a higher rate of first-pass isolation, necessitating a smaller volume of fluid irrigation, and satisfies safety aspects. However, long-term efficacy and safety need to be further evaluated in the future.

Data Availability

The labeled dataset used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

Authors' Contributions

Cheng Cheng and Banglong Xu contributed equally to this work and are the co-first authors. Cheng Cheng and Xiaochen Wang contributed to the conception and design. Jianlong Sheng, Zheng Huang, and Feng Gao contributed to the acquisition of data. Zheng Huang and Fei He contributed to the analysis and interpretation of data. Cheng Cheng contributed to the drafting of the article. Cheng Cheng, Zheng Huang, Jianlong Sheng, Fei He, Feng Gao, and Xiaochen Wang revised the article for intellectual content. Cheng Cheng, Zheng Huang, Jianlong Sheng, Fei He, Feng Gao, and Xiaochen Wang finally approved the completed article.

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Research Article

Ultrasound-Guided Median Nerve Electrical Stimulation to Promote Upper Limb Function Recovery after Stroke

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Peripheral electrical nerve stimulation enhances hand function during stroke rehabilitation. Here, we proposed a percutaneous direct median nerve stimulation guided by ultrasound (ultrasound-guided median nerve electrical stimulation, UG-MNES) and evaluated its feasibility and effectiveness in the treatment of stroke patients with upper limb extremity impairments. Sixty-three stroke patients (2-3 months of onset) were randomly divided into control and UG-MNES groups. Both groups received routine rehabilitation and the UG-MNES group received an additional ultrasound-guided electrical stimulation of the median nerve at 2 Hz, 0.2 ms pulse-width for 20 minutes with gradual intensity enhancement. The Fugl-Meyer Assessment for upper extremity motor function (FMA-UE) was used as the primary outcome. The secondary outcomes were the Functional Test for the Hemiplegic Upper Extremity (FTHUE-HK), Hand Function Rating Scale, Brunnstrom Stages, and Barthel Index scores for motor and daily functions. All the participants completed the trial without any side effects or adverse events during the intervention. After 4 weeks of intervention, the functions of the upper limbs on the hemiplegic side in both groups achieved significant recovery. Compared to the control group, all evaluation indices used in this trial were improved significantly in the UG-MNES group after 2 and 4 weeks of intervention; particularly, the first intervention of UG-MNES immediately improved all the assessment items significantly. In conclusion, the UG-MNES is a safe and feasible treatment for stroke patients with upper limb extremity impairments and could significantly improve the motor function of the affected upper limb, especially in the first intervention. The UG-MNES could be an effective alternative intervention for stroke with upper limb extremity impairments.

1. Introduction

Stroke is the leading cause of death and long-term disability around the world. Although the application of developing medical technology decreases the rates of stroke mortality significantly, most survivors still suffer from neurological deficits such as motor, memory, and cognitive dysfunctions, which results in an immense economic burden on society and families [1, 2]. The upper limb extremity impairments are the most frequent dysfunction following stroke; that is, more than 70% of stroke patients suffer from the paretic arm. Only 5–20% of the patients achieve complete functional recovery after 6 months of onset [3–5]. The impaired upper limb severely limits the independent daily activities of stroke patients. Thus, restoration of upper limb function is vital to the treatment and rehabilitation of stroke. To date, the commonly applied rehabilitation techniques such as classical physiotherapy and impairment-oriented training are limited by efficacy [6–8]. Consequently, it is urgent to establish and explore some efficacious treatments for improving upper limb functional recovery after cerebral ischemia.

Recently, the electrical stimulation applied to the brain and peripheral nervous system has been recognized as a promising treatment for functional recovery after stroke. The transcranial direct current stimulation (tDCS) is a noninvasive brain stimulation technique that can initiate a long-term potentiation or long-term depression and then induce the cortical plasticity and improve the nerve functional restoration of the upper limb motor [9], movement planning and preparation [10], and hemispatial neglect [11]. The clinical effects of tDCS depend on the injured site and the stimulus parameters, and it is difficult to achieve precision function therapy, such as the promotion of upper limb function. The peripheral electrical stimulation has been confirmed as a safe and effective treatment for functional recovery after stroke by stimulating the peripheral neuromuscular system and inducing the cortical plasticity [12, 13]. Currently, the peripheral electrical stimulation includes the functional electrical stimulation (FES), the transcutaneous or neuromuscular electrical stimulation (TENS or NMES), and the transcutaneous electrical acupoint stimulation (TEAS) which combined the meridian theory of traditional Chinese medicine and repetitive sensory stimulation (RSS). These noninvasion peripheral electrical stimulation therapies can stimulate the senses, increase muscle power and movement function, and decrease limb spasticity through various stimulus currents and protocols [14, 15]. Meanwhile, peripheral electrical stimulation-induced brain plasticity contributes to the long-term functional improvement [12, 16].

The median nerve mixed with sensory and motor fibers is a primary important nerve of the hand. It innervates the flexor-pronator muscles in the forearm and most muscle groups in the hand and controls flexion of the wrist, abduction of the thumb, and flexion of the fingers [17]. The electrical stimulation on the area of the median nerve (MNES, usually located on the wrist above the median nerve) increases pinch strength [18] and facilitates the effects of rehabilitation training [19, 20] after stroke. However, these cutaneous MNES stimulate both the median nerve and other tissues. It is difficult to target the median nerve specifically at the same time and determine the optimal stimulus parameters. Implanted electrodes can target specific nerves and reduce the current required to stimulate the nerve. Recently, using a rat model of experimental stroke, the group of Tsai et al. [21] observed that direct median nerve stimulation significantly improved both the motor skills and sensory recovery in the impaired forelimb. Also, the direct MNES promoted the neural plasticity in the cervical spinal cord detected by axonal tracing of biotinylated dextran amine. However, implanted direct nerve stimulation needs an operation and might cause some problems such as long-term biological compatibility and nerve damage. Ultrasound-guided nerve electrical stimulation can target the specific nerve through a percutaneous fine-needle electrode under ultrasound guidance. The ultrasound-guided nerve electrical stimulation has been used for the relief of post-amputation pain [22–24]. However, the feasibility and effectiveness of ultrasound-guided nerve electrical stimulation in the treatment of stroke remain unknown.

The recovery of upper limb function after stroke is a difficult task in rehabilitation. In this study, our team innovatively proposed and applied the musculoskeletal ultrasound intervention technique in clinical practice, combining the nerve electrical stimulation technique with the ultrasound technique. The ultrasound-guided median nerve electrical stimulation (UG-MNES) technique was used to treat the upper limb dysfunction after stroke. The treatment significantly improved the upper limb motor function in the immediate poststroke period which is worthy of clinical promotion and application.

2. Materials and Methods

2.1. Study Design. This study adopted an assessor-blinded, randomized controlled design. All subjects were randomized to control with conventional rehabilitation (control group) and UG-MNES group. A separate investigator who was blind to experiment design was responsible for the functional assessments. The experimental procedure was approved by the Human Ethics Committee of the Second People's Hospital of Kunming (ethical approval no. 2019–01).

2.2. Participants. A total of 63 stroke patients with upper extremity hypotonia were recruited from the Department of Rehabilitation in the Second People's Hospital of Kunming. The inclusion criteria were as follows: (1) meeting the diagnostic criteria of cerebral stroke and confirmed by brain computed tomography (CT) or magnetic resonance imaging (MRI) examination; (2) first-ever ischemic or hemorrhagic stroke and during 2-3 months of onset; (3) Brunnstrom Stages of 1-2 in upper limb; and (4) stable vital signs without severe cognitive impairment. Exclusion criteria were as follows: (1) unstable or uncontrollable systemic diseases; (2) hemiplegic upper limb skin ulcer and inflammation; and (3) severe emotional, visual, and cognitive impairments.

2.3. Procedures. All participants in the study voluntarily signed the written informed consent form. Before the study, basic information, including age, sex, lesion side of the brain, stroke type, and duration after stroke onset, was recorded. The subjects who met the criteria were randomly divided into UG-MNES and control groups. All the participants in both groups received routine rehabilitation, including hemiplegia exercise training therapy, occupational therapy, physical factor therapy, or traditional therapy, according to the actual situation of the patients. The frequency of rehabilitation is 30 to 40 minutes, once a day, 5 to 6 times a week, for a total of 4 weeks. The intensity is appropriate for the patients without any obvious sense of fatigue. In addition to routine rehabilitation, the UG-MNES group received invasive percutaneous electrical stimulation of the median nerve under ultrasound guidance. The electrical stimulation of the median nerve was stimulated once a week for a total of 4 weeks, each time for 20 minutes with a total of 4 times' stimulation. The control group was

evaluated before treatment and 2 and 4 weeks after treatment, while the UG-MNES group was evaluated before and immediately after treatment at 1, 2, 3, and 4 weeks. Assessments were conducted including Fugl-Meyer Assessment for upper extremity (FMA-UE), Functional Test for the Hemiplegic Upper Extremity (FTHUE-HK), Hand Function Rating Scale, Brunnstrom Stages, and Barthel Index scores (Figure 1).

2.4. Ultrasound-Guided Median Nerve Electrical Stimulation. The detailed procedures of median nerve electrical stimulation guided by ultrasound were as follows. The patient's forearm is placed in a posteriorly rotated position on a treatment pillow. A disposable electrical stimulation needle (with 0.5 mm diameter and 50 mm long) was used at 7–10 cm from the forearm of the affected side to the transverse striae of the wrist under the short-axis section. Depending on the length of the patient's arm, the location of the puncture point was selected in which the median nerve penetrates between the deep finger flexors and the superficial finger flexors, where the median nerve is superficial, and the image obtained under ultrasound guidance is the clearest. The short-axial plane of ultrasound was selected to allow the needle to be as perpendicular to the nerve trunk as possible to maximize the intensity of the current field. The needle was inserted in the plane to avoid the blood vessel tendon until the tip was found close to the nerve sheath membrane under ultrasound. The end of the needle core was connected to the peripheral nerve electrical stimulator (model SY-708A) (Figure 2). A bidirectional rectangular wave of an internal model was adopted with a frequency of 2 Hz and a pulse width of 0.2 ms. The stimulus current was adjusted to 1.0 mA for 5 minutes to trigger the thumb and forefinger palm and flexion movement. Then, the amount of stimulation was increased to 1.5 mA for 10 minutes. The amount of stimulation was increased again to 1.8 mA for 5 minutes and the needle was removed. The comprehensive evaluation of hemiplegic upper limb function on the hemiplegic side was performed before and immediately after treatment at 1, 2, 3, and 4 weeks, for a total of 8 assessments.

2.5. The Primary Outcome Assessment. The Fugl-Meyer Assessment of upper extremity (FMA-UE) was used as the primary outcome. The FMA-UE is a well-recognized and recommended observational measure of upper limb impairments. The FMA-UE assessment includes 7 parts, that is, (1) upper limb reflex activity, (2) flexor joint movement, (3) extensor joint movement, (4) isolated movement, (5) normal reflex activity, (6) wrist stability, and (7) finger movement. The maximum score of motor function of FMA-UE is 66.

2.6. The Secondary Outcome Assessments. The assessment of Functional Test for the Hemiplegic Upper Extremity (FTHUE-HK), Hand Function Rating Scale, Brunnstrom Stages, and Barthel Index scores were used as the secondary outcomes.

2.6.1. FTHUE-HK. The assessment of Functional Test for the Hemiplegic Upper Extremity (FTHUE-HK) was as follows: Level 1: no reaction; Level 2: (A) associated reaction, (B) the affected hand was placed on the ipsilateral thigh; Level 3: (C) lift the affected arm while tucking in the affected side clothes into the pants with healthy hands, (D) carry a bag weighing 1 kg (lasts for 15 seconds); Level 4: (E) stabilize the bottle cap (open the bottle cap with the healthy hand and hold the cup with the affected hand), (F) fix one end of the towel with the affected hand and wring the wet towel dry (twist the healthy hand twice); Level 5: (G) pick up and move small pieces of wooden blocks, (H) eat with a spoon; Level 6: (I) lift a box, (J) drink water from a plastic cup; Level 7: (K) use a key to open a lock, (L1) manipulating chopsticks (dominant hand), (L2) manipulating clamps (nondominant hand).

2.6.2. Hand Function Rating Scale. It includes the evaluation of 5 practical prescribed movements: cutting with scissors, taking coins out of wallets, opening umbrellas, cutting fingernails, and fastening buttons on the sleeves with six-level of assessments, that is, lost hand function: cannot complete all the five actions; assistive hand D: five actions can only complete one; assistive hand C: five actions can only complete two; assistive hand B: five actions can complete three; assistive hand A: five actions can complete four; practical hand: complete all five actions.

2.6.3. Brunnstrom Stages. The upper extremity is primarily evaluated in six stages, going from involuntary movement to increase muscle tone to common movement, dissociative movement, and later more dissociative movement, culminating in speed and coordination close to normal movement.

2.6.4. Barthel Index. It consists of ten items: eating, transferring from wheelchair to bed, grooming, going to the bathroom, taking a bath, walking, climbing up and down stairs, wearing and undressing, bowel control, and bladder control.

2.7. Data Analysis. Data analysis was conducted using the Statistical Package for the Social Sciences (SPSS) version 25.0. Shapiro-Wilk test was used to examine the normal distribution of the underlying model assumptions. Categorical variables were expressed as absolute (n) and percentage (%), while continuous variables were expressed as mean ± standard deviation or median (1st–3rd quartiles) [M (P25–P75)], depending on the data distribution. Baseline characteristics for categorical variables between groups (i.e., gender and stroke type) were compared using the chi-square test or Fisher's exact test. Baseline characteristics for continuous variables between groups were examined with the Mann-Whitney U test (i.e., duration of disease and NIHSS score) and t-test (i.e., age). The Wilcoxon signed rank-sum test was used to compare FMA-UE, FTHUE-HK, Hand Function Rating Scale, Brunnstrom Stage, and Barthel Index

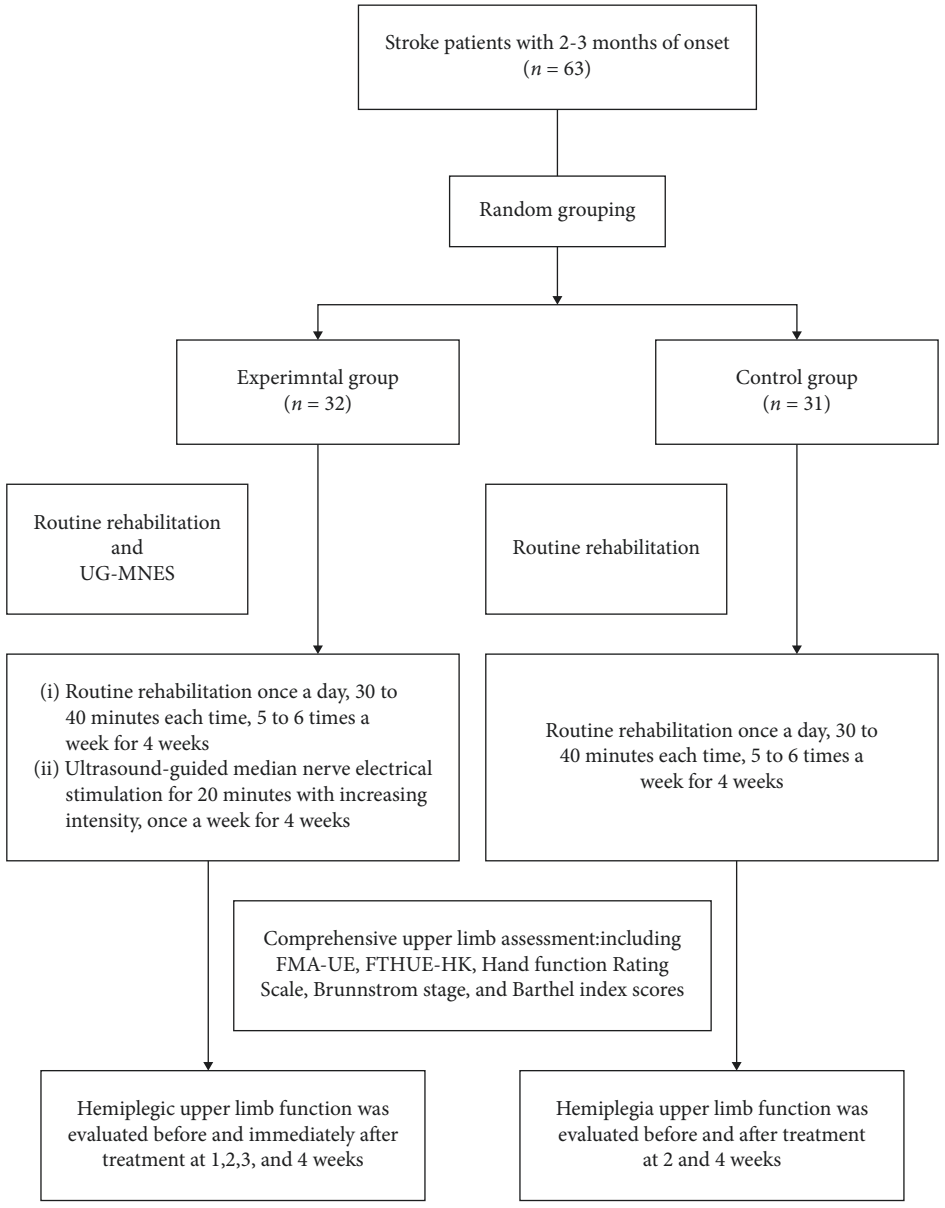


Figure 1: Flowchart of the study.

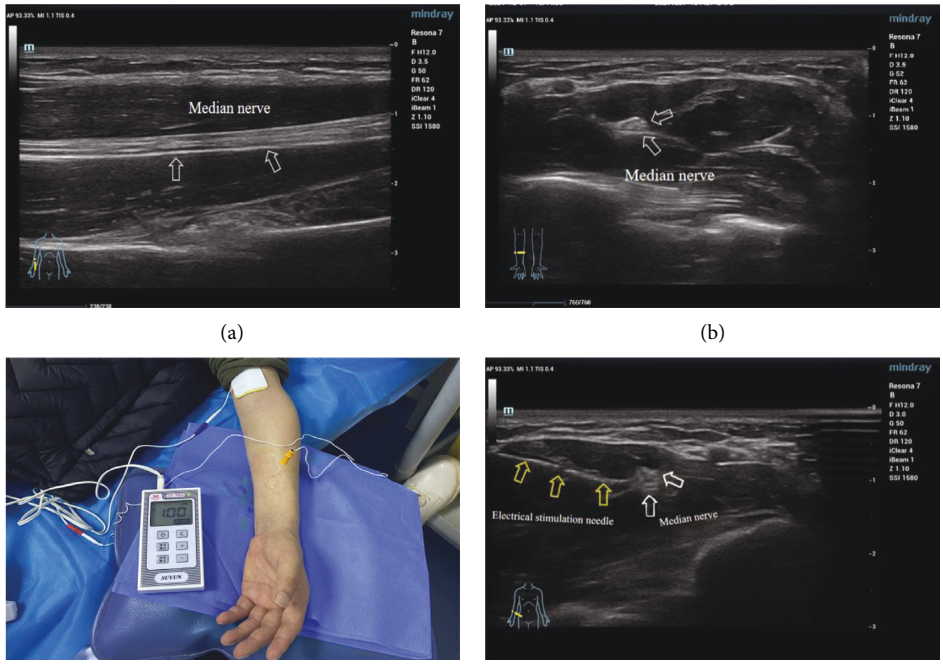
scores between the two groups before and after 2 and 4 weeks of treatment. The Wilcoxon rank-sum test was used to test for differences between before and immediately after intervention in the UG-MNES group. The statistical level of significance was $p < 0.05$.

3. Results

3.1. Comparison of General Data between the Two Groups. The patients who met the inclusion criteria were randomly divided into the control group ($n = 31$) and the UG-MNES group ($n = 32$). The mean age of the control group was 56.39 ± 2.11, and the mean age of the UG-MNES group was 54.5 ± 2.12. There was no significant difference in age and gender between the two groups ($p > 0.05$). There was no difference in time after stroke and stroke type (cerebral hemorrhage and cerebral infarction) between the groups ($p > 0.05$). There was no significant difference in neurological deficit scores between the groups ($p > 0.05$). The general data of all participants are summarized in Table 1.

3.2. The Median Nerve Stimulation Group and the Routine Rehabilitation Group Improve the Function of the Upper Limbs of Hemiplegic Patients with Stroke. Before treatment, there was no significant difference in upper limb motor function, Brunnstrom Stage, and activities of daily living between the control and UG-MNES groups ($p > 0.05$). After 2 and 4 weeks of treatment, the motor function of the upper limbs and activities of daily living of hemiplegic patients with stroke had a significant improvement in both the control and UG-MNES groups ($p < 0.05$).

The Fugl-Meyer motor function score of the upper limbs in the UG-MNES group was higher than that in the control



(a) (b) (c) (d)

Figure 2: Median nerve electrical stimulation under ultrasound: (a) median nerve imaging of longitudinal section, (b) median nerve imaging of transection, (c) the ultrasound-guided median nerve electrical stimulation, and (d) UG-MNES ultrasound image. The white arrow points to the median nerve and the yellow arrow points to the electrical stimulation needle.

Table 1: Demographic and clinical characteristics of subjects.

Team		Control group ($n = 31$)	Experimental group ($n = 32$)	p
Age (years)		56.39 ± 2.11	54.5 ± 2.12	0.531
Gender (n, %)	M F	17 (54.8) 14 (45.2)	17 (53.1) 15 (46.9)	0.892
Time after stroke (months) M (P25, P75)		2 (1, 3)	2 (1, 3)	0.736
Stroke type (n, %)	CI CH	17 (54.8) 14 (45.2)	10 (31.25) 22 (68.75)	0.061
NIHSS score M (P25, P75)		10 (7, 13)	8 (6.25, 9)	0.061

M: male; F: female; CI: cerebral ischemia; CH: cerebral hemorrhage.

group ($p < 0.01$) at 2 weeks of treatment and $p < 0.05$ at 4 weeks of treatment (Table 2 and Figure 3).

Compared to the control group, the median nerve stimulation significantly enhanced the motor impairment of the upper limb assessed by FTHUE-HK and Hand Function Rating Scale at 2 and 4 weeks of treatment. The hand function classification of hemiplegia in the UG-MNES group was mainly Grade 3, while in the control group was mostly Grade 2 ($p < 0.01$) (Tables 3 and 4 and Figures 4 and 5).

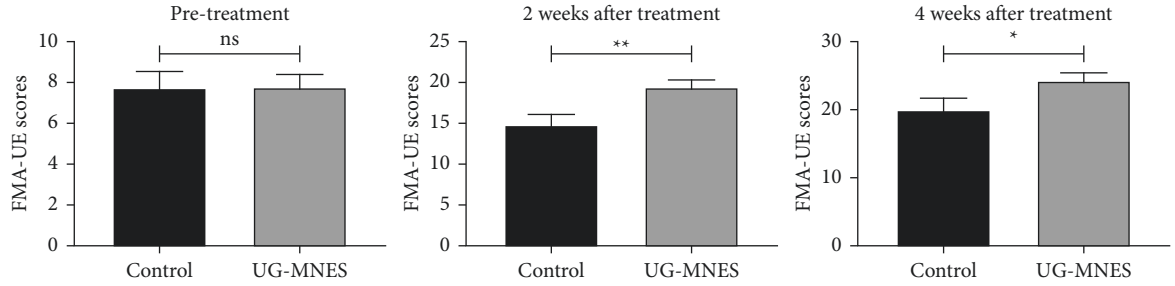
Similarly, after 4 weeks of trial, the Brunnstrom motor stages of the upper limbs on the hemiplegic side in the UG-MNES group had reached Stages 3 and 4, while those in the control group were mostly in Stages 2 and 3. There was a significant difference in Brunnstrom Stages between the two groups ($p < 0.01$) ($P < 0.01$)(Table 5 and Figure 6).

The Barthel Index for activities of daily living in the UG-MNES group was slightly higher than that in the control group at 2 and 4 weeks of treatment ($p < 0.01$) ($P < 0.01$) (Table 6 and Figure 7). This result indicated that improved hand function in the UG-MNES group had contributed to the improved activities of daily living function.

3.3. Ultrasound-Guided Electrical Stimulation of the Median Nerve Immediately Improved the Function of the Upper Extremity on the Hemiplegic Side. In this trial of 4 consecutive weeks, the patients in the UG-MNES group received 4 ultrasound-guided electrical stimulation (once a week). The upper limb functions on the hemiplegic side were assessed before and immediately after each stimulus. The results showed that ultrasound-guided electrical stimulation of the

Table 2: Comparison of FMA-UE scores in the two groups, M (P25, P75).

Team	Before	2 weeks	4 weeks
Control group ($n = 31$)	6 (4, 9)	13 (8, 17)	20 (10, 26)
Experimental group ($n = 32$)	6.5 (4, 9)	19 (16, 22)	26 (19.25, 30)
Z	4.42	3.21	2.225
P value	0.659	0.001	0.026



*: P<0.05, **: P<0.01, ns: P>0.05

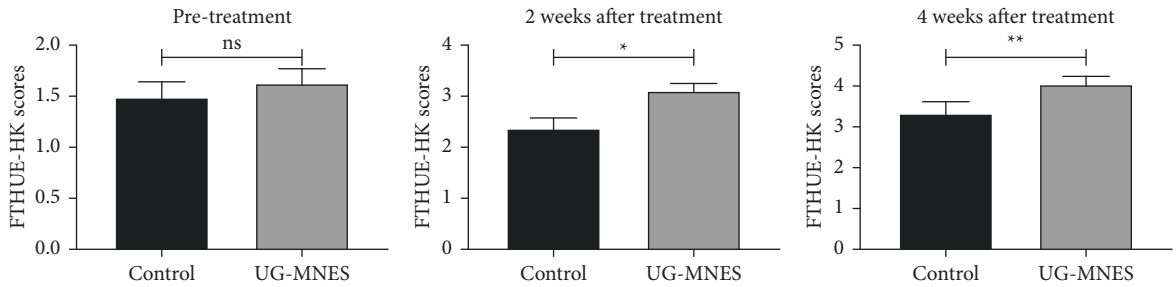
Figure 3: UG-MNES promoted the FMA-UE scores.

Table 3: Comparison of FTHUE-HK in the two groups, M (P25, P755).

Team	Before	2 weeks	4 weeks
Control group ($n = 31$)	1 (1, 2)	3 (2, 3)	3 (3, 4)
Experimental group ($n = 32$)	1 (1, 2)	3 (3, 4)	4 (4, 4.75)
Z	0.99	2.219	2.687
P value	0.32	0.027	0.007

Table 4: Comparison of Hand Function Rating Scale in the two groups, M (P25, P75).

Team	Before	2 weeks	4 weeks
Control group ($n = 31$)	1 (1, 2)	2 (2, 3)	2 (2, 3)
Experimental group ($n = 32$)	1 (1, 2)	3 (2, 3)	3 (3, 3)
Z	0.079	2.818	4.291
P value	0.937	0.005	<0.001



*: P<0.05, **: P<0.01, ns: P>0.05

Figure 4: UG-MNES promoted the FTHUE-HK scores.

median nerve immediately improved the Fugl-Meyer Assessment of the upper extremity motor function on the hemiplegic side on the first, third, and fourth intervention. In particular, the first intervention immediately improved all the assessment items including FMA-UE, FTHUE-HK, Brunnstrom Stages, Hand Function Rating Scale, and Barthel Index scores. However, the immediate improvement effect on motor and daily functions did not last into the second, third, and fourth week. These results indicated that the ultrasound-guided electrical stimulation of the median nerve mainly improved the gross function of the upper extremity (Table 7).

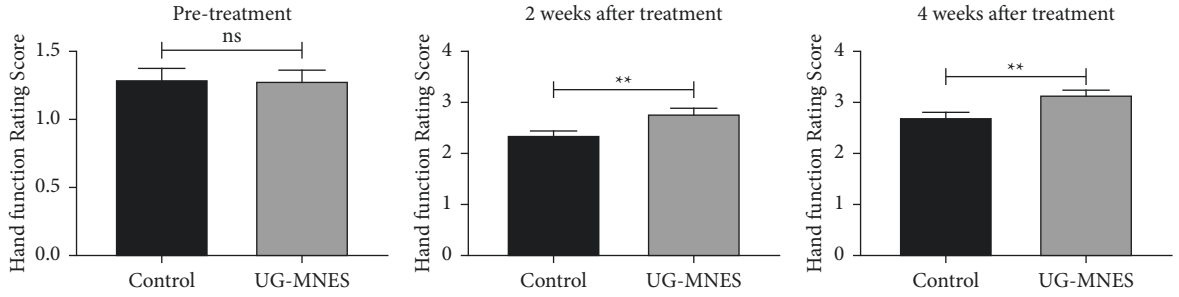


Figure 5: UG-MNES promoted the Hand Function Rating Scale.

Table 5: Comparison of Brunnstrom Stages in the two groups, M (P25, P75).

Team	Before	2 weeks	4 weeks
Control group ($n = 31$)	2 (1, 2)	2 (2, 3)	2 (2, 3)
Experimental group ($n = 32$)	2 (1, 2)	3 (2, 3)	3 (2.25, 4)
Z	0.366	2.099	3.240
P value	0.714	0.036	0.001

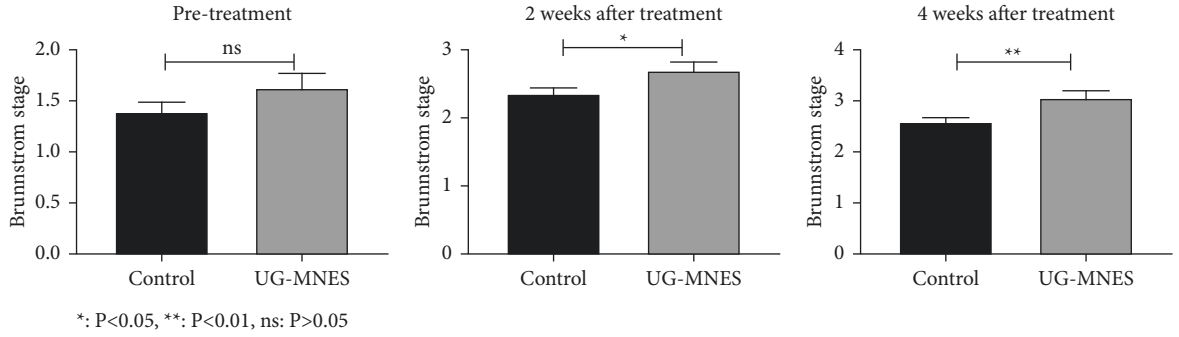


Figure 6: UG-MNES improved the Brunnstrom Stages.

4. Discussion

In this study, we proposed a percutaneous direct median nerve stimulation guided by ultrasound (UG-MNES) and evaluated its feasibility and effectiveness in the treatment of stroke patients with upper limb extremity impairments. The UG-MNES stimulates the median nerve directly without surgical incision through the insertion of a lead under ultrasound guidance. All patients who participated in this study completed the intervention without any adverse events and side effects, proving that UG-MNES was safe and feasible in treatment for stroke patients with upper limb extremity impairments. This study confirmed that UG-MNES could improve the function of the affected upper limb; in particular the first intervention immediately improved all assessment items including FMA-UE, FTHUE-HK, Brunnstrom Stages, Hand Function Scale, and Barthel Index. These results indicated that the UG-MNES could be an effective intervention for stroke patients with upper limb extremity impairments.

Stroke damages the important region in the brain, such as the motor cortex and sensory cortex, destroys the neural network connection, and results in the loss of sensory and motor function in the affected extremity. The structural damage during stroke initially occurs only in the brain and the peripheral nervous system and its target organs such as skeletal muscle are structurally intact. The disconnected network in the brain results in the denervation of skeletal muscle fiber and further leads to various functional disorders. The persistent lack of nerve stimulation leads to the structural and functional abnormality of the neuromuscular junction (NMJ) and the atrophy in denervated muscle. The disconnected network in the brain and the abnormality in NMJ together contribute to various dysfunctions after stroke and increase the difficulty of rehabilitation treatment during the sequela stage of stroke. Thus, the targets for poststroke treatment need to focus on the neural plasticity of the brain and the functional reconstruction between the nervous system and the muscular [25, 26].

The nervous system is complicated and closed, and the peripheral stimulation can promote the plasticity of the center neural system and improve the recovery of neural function, such as repetitive sensory stimulation (RSS), repetitive somatosensory electrical stimulation (SES), electrical stimulation of peripheral nerves, and passive rehabilitation training [27, 28]. RSS facilitates the neuroplastic processes in cortical areas that represent the site of

Table 6: Comparison of Barthel Index in the two groups, M (P25, P75).

Team	Before	2 weeks	4 weeks
Control group ($n = 31$)	20 (15, 25)	35 (30, 40)	40 (35, 45)
Experimental group ($n = 32$)	20 (15, 25)	40 (35, 45)	45 (40, 55)
Z	0.691	2.721	2.690
P value	0.490	0.007	0.007

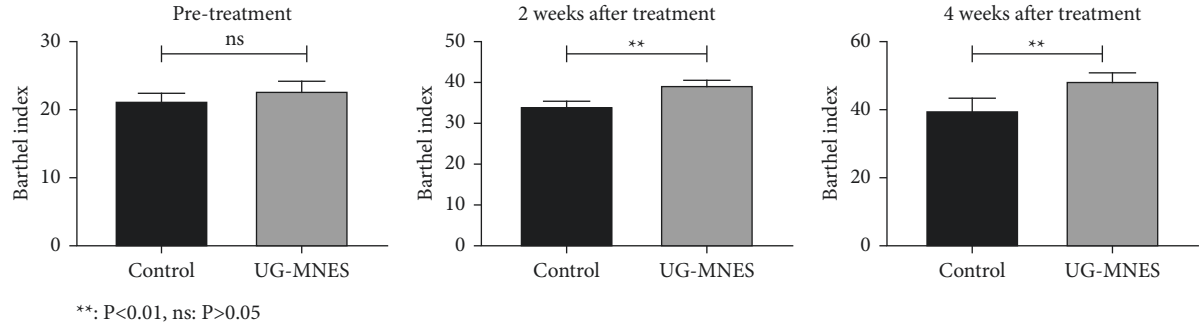


Figure 7: UG-MNES improved the Barthel Index.

Table 7: Comprehensive function assessment of the affected upper limb before and immediately after median nerve electrical stimulation, M (P25, P75).

Outcomes	1 week before immediately	p value$P$	2 weeks before immediately	p value$P$	3 weeks before immediately	Pp value	4 weeks before immediately	p value$P$
FMA-UE	6.5 (4, 9) 12 (12, 14.75)	$p <$ $0.0001P < 0.0001$	11 (8, 13.75) 11 (10, 13.75)	0.243	19 (16, 22) 20 (16, 23.75)	0.039	20 (16, 23.75) 28 (21.25, 32)	0.004
FTHUE-HK	1 (1, 2) 3 (2, 3)	$p <$ $0.0001P < 0.0001$	3 (2, 3) 3 (3, 3)	0.068	3 (3, 4) 4 (3, 4)	0.035	4 (4, 4.75) 4 (4, 5)	0.129
Brunnstrom	2 (1, 2) 3 (3, 3)	$p <$ $0.0001P < 0.0001$	2 (2, 2) 2 (2, 3)	0.140	2 (2, 3) 3 (2, 3)	0.102	3 (2.25, 4) 3 (3, 3)	0.705
Hand Function	1 (1, 2) 3 (2, 3)	$p <$ $0.0001P < 0.0001$	2 (2, 3) 3 (2, 3)	0.138	2 (2, 3) 3 (2, 3)	0.417	3 (3, 3) 3 (3, 4)	0.152
Barthel Index	20 (15, 25) 35 (31.25, 47.5)	$p <$ $0.0001P < 0.0001$	32.5 (25, 40) 35 (30, 40)	0.003	40 (35, 45) 40 (40, 45)	0.252	40 (35, 50) 47.5 (40, 55)	0.104

FMA-UE: Fugl-Meyer Assessment of upper extremity, FTHUE-HK: Functional Test for the Hemiplegic Upper Extremity. *Pp* value: before the intervention *versus* after the intervention immediately.

sensory stimulation and improve sensorimotor performances (light-touch, tactile discrimination, and proprioception) through altering cortical maps and cortical excitability [29, 30]. In a clinical study of SES used for the treatment of acquired brain injury and distal upper limb motor impairments, Adelyn et al. found that SES in median and ulnar nerves induced cortical oscillations detected by electroencephalograph (EEG) and identified typical electrophysiological biomarkers [ipsilesional motor theta (4.8–7.9 Hz) and alpha (8.8–11.7 Hz)] which were significantly correlated with finger fractionation improvements [31]. Using functional near-infrared spectroscopy (fNIRS), Huo et al. [32] observed that median nerve electrical stimulation triggered sensorimotor stimulations of the affected hand and induced functional reorganization of distant cortical areas after stroke. These reports confirmed that transcutaneous peripheral electrical nerve stimulation could induce neuroplasticity in the sensorimotor cortex of the brain. In addition, the functional electrical stimulation (FES) triggered by surface electromyography in the uninjured limb or cortical signals was able to stimulate voluntary muscle activity, decrease spasticity in the affected limb, and improve the restoration of motion function [33–35]. In contrast to transcutaneous peripheral electrical nerve stimulation described above, the FES stimulated the paretic muscles, maintaining and improving their activity [36]. Some studies indicated that FES is more effective than TENS at improving gait speed after stroke [37].

Although the noninvasive peripheral electrical nerve stimulation enhanced hand function during stroke rehabilitation through neural plasticity in the brain and the maintenance of NMJ, The transcutaneous devices also stimulated some nontargeted nerves and muscles and are difficult to target the deeper nerves and muscles. Direct electrical nerve stimulation can target specific nerves and duration while reducing nontargeted nerve activation [21]. Because the median nerve carries both sensory and motor nerve fibers, the direct electrical stimulation of the median nerve could activate NMJ and paretic muscles through the motor nerve fibers and improve the hand function immediately. Meanwhile, the direct electrical

stimulation on median nerve could enhance cortical excitability and induce neural plasticity in the brain, which led to long-term functional improvement [38, 39]. In this study, we observed that the first week of direct electrical stimulation for 20 minutes significantly promoted the hand function, but the improvement tended to decline in the subsequent interventions. This might be due to the limit of the activity of NMJ and paretic muscles. Additionally, the advantage of ultrasound-guided median nerve electrical stimulation is almost noninvasive, easy to operate, and safe.

The study has some limitations. Firstly, although to our knowledge we first proposed the direct median nerve stimulation guided by ultrasound (UG-MNES) and observed the effectiveness for the treatment of stroke, we need to compare the effects with noninvasive peripheral electrical nerve stimulation such as RSS and FES. Secondly, it is necessary to apply some objective evaluation indices such as functional magnetic resonance imaging (fMRI) and near-infrared spectroscopy to all the outcomes from the assessment scale. Thirdly, the optimal intervention plan of UG-MNES needs to be further explored, including intensity, duration, and interval time. Lastly, the undergoing cellular mechanism needs to be investigated.

5. Conclusion

In conclusion, the UG-MNES is safe and feasible in the treatment of stroke patients with upper limb extremity impairments and could improve the function of the affected upper limb; in particular the first intervention immediately improved all assessment items. The clinical application of the UG-MNES technique for the treatment of upper limb dysfunction in stroke was effective, but the effect was maintained for a short period of time, with significant immediate effect and no significant long-term effect, which may be related to the loss of nerve in the upper limb after stroke. Thus, there is a need to investigate its mechanism of action. At present, we adopted the model of gradual intensity enhancement, and the parameter selection is based on the evoked action and patient tolerable intensity. The parameter selection of electrical stimulation needs to be further optimized and explored to finally form a new treatment method for upper limb dysfunction in stroke. Our results indicated that the UG-MNES could be an effective rehabilitation intervention for stroke with upper limb extremity impairments.

Data Availability

The data used to support the findings of this study are available within the article.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

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Review Article

Evidence Quality Assessment of Tai Chi Exercise Intervention in Cognitive Impairment: An Overview of Systematic Review and Meta-Analysis

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Background. Tai Chi (TC) exercise has recently received wide attention for its efficacy in the management of cognitive impairment. The purpose of this overview is to summarize the available evidence on TC treatment of cognitive impairment and assess its quality. *Methods*. We retrieved relevant systematic reviews/meta-analyses (SRs/MAs) from 7 databases from the time they were established to January 2, 2022. Two reviewers independently evaluated the methodological quality, risk of bias, report quality, and evidence quality of the included SRs/MAs on randomized controlled trials (RCTs). The tools used are Assessment System for Evaluating Methodological Quality 2 (AMSTAR-2), the Risk of Bias In Systematic (ROBIS) scale, the list of Preferred Reporting Items for Systematic Reviews And Meta-Analysis (PRISMA), and the Grading of Recommendations Assessment, Development, and Evaluation (GRADE) system. *Results*. This overview finally included 8 SRs/MAs. According to the results of AMSTAR-2, all included SRs/MAs were rated as very low quality. Based on the ROBIS tool, none of the SR/MA had a low risk of bias. In light of PRISMA, all SRs/MAs had reporting deficiencies. According to the GRADE system, there was only 1 high-quality piece of evidence. *Conclusion*. TC is a promising complementary and alternative therapy for cognitive impairment with high safety profile. However, in view of the low quality of the included SRs/MAs supporting this conclusion, high-quality evidence with a more rigorous study design and a larger sample size is needed before making a recommendation for guidance.

1. Introduction

As the aging population continues to grow, global public health is facing the serious problem of age-related cognitive decline. It is noteworthy that more and more people suffer from mild cognitive impairment (MCI) and dementia [1]. MCI occurs on a continuum from normal cognition to dementia, and individuals with MCI have a higher risk of dementia [2]. A recent report showed a 10%–25% incidence of MCI in people over 65 years of age [3], and the risk of dementia in MCI patients (10–15%) is much higher than in healthy older adults (1-2%) [4]. As cognitive performance declines, most individuals develop neuropsychiatric or behavioral [5] abnormalities in activities of daily living [6], ultimately resulting in a decline in quality of life (QoL) and an increased burden for family caregivers [7], and health professionals [8]. However, there is currently no drug treatment approved by the U.S. Food and Drug Administration to treat MCI or slow the long-term progression of MCI to dementia [9]. Therefore, complementary and alternative therapies have become a research hotspot in improving cognitive impairment in recent years [10].

In recent decades, increasing evidence suggests that exercise could be considered as a promising nonpharmacological intervention to improve cognitive performance [11]. As a traditional Chinese martial art, Tai Chi (TC) is a body-mind coordination exercise, and it perfectly integrates traditional philosophy and traditional Chinese medicine theory and

pursues the unity of strength, shape, qi, and consciousness [12]. TC exercise mainly includes the stretching and relaxation of skeletal muscles, as well as various movements such as body coordination, regular breathing, and meditation [13]. TC has widely been accepted as a supplementary form of physical exercise in Western countries such as the United States and Britain [14], and there is now growing evidence that TC may help improve cognitive function and mental health in older adults with mild dementia [15, 16]. TC may be a potential treatment modality for patients with cognitive impairment.

Systematic reviews (SRs)/meta-analyses (MAs) are significant tools to conduct evidence-based clinical work. A growing number of SRs/MAs based on TC intervention for cognitive impairment suggest that TC can improve patients' cognitive function, delay the development of cognitive impairment, and improve the quality of life. However, without objective and comprehensive assessment of their methodological and evidentiary quality, it remains controversial whether these findings provide credible evidence for clinicians [17, 18]. This overview aimed to objectively and comprehensively evaluate the scientificity of TC exercise in the treatment of cognitively impaired SRs/MAs.

2. Methods

2.1. Research Methods and Protocol Registration. The overview of SRs/MAs was based on the guidelines specified in Cochrane Handbook [19], and other overviews with high-quality research methodology [20–22]. This overview protocol has been registered with the INPLASY website (Registration number: INPLASY202240055).

2.2. Eligibility Criteria

2.2.1. Literature Inclusion Criteria

(a) Type of research

This overview includes SRs/MAs of randomized controlled trials (RCTs) of the effects of TC exercise on cognitive impairment.

(b) Type of participants

Subjects were patients diagnosed with MCI or dementia by any international or national standard.

(c) Type of intervention

The intervention for the control group was conventional treatment (CT) or daily life activities, and the intervention for the experimental group was TC exercise or TC combined with the treatments received by the control group. CT includes health education, routine care, attention control, or medication.

(d) Types of outcomes

At least one measure of cognitive domains was reported, such as global cognitive function, memory, executive function, attention, verbal fluency, and visuospatial function. Also, other assessment results obtained from relevant scales were included as well.

2.2.2. Exclusion Criteria. (1) Animal studies and (2) network MAs, research protocols, narrative reviews, overviews, dissertation, and conference abstracts.

2.3. Data Sources and Search Strategy. Two researchers searched seven electronic databases for inception date up to January 2, 2022, including PubMed, Cochrane Library, EMBASE, Wanfang Database, CNKI, China Biomedicine (CBM), and Chongqing VIP, respectively. A literature search was carried out using a combination of key terms and free words, such as "Tai Chi," "Cognitive Impairment," "Systematic Review," and "Meta-Analysis," and the search strategy was finely adjusted according to different databases. The search strategy of PubMed database is shown in Table 1.

2.4. Literature Screening and Data Extraction. The literature screening (WW-L and LJ-C) and information extraction (H-C and MY-X) were independently performed by two researchers. The retrieved documents were imported into Endnote X9 document management software, and then, the duplicates were removed. The literature that potentially met the inclusion and exclusion criteria was then obtained by reading the titles and abstracts of the literature. Finally, we finalized the included MAs by reading the full text. All SRs/MAs were read by two independent researchers, and the following data were extracted from the SRs/MAs: first author, publication year, country, number of RCTs included, interventions for experimental and control groups, included RCT quality assessment tools, and main conclusion. The disagreement between the two researchers was resolved through discussion.

2.5. Quality Assessment for Inclusion in MAs. Two researchers (BQ-Y and D-W) independently assessed the methodological and evidence quality of the included SRs/MAs.

2.5.1. Estimate of Methodological Quality. The methodological quality of the included SRs/MAs was assessed by the Assessment System for Evaluating Methodological Quality 2 (AMSTAR-2) [23]. Seven (2, 4, 7, 9, 11, 13, and 15) of the 16 items in the tool were critical areas.

2.5.2. Estimate of Risk of Bias. The Risk of Bias In Systematic Review (ROBIS) [24] scale was used in this overview to evaluate the risk of bias of the inclusion of SRs/MAs. The scale was divided into three stages to assess the overall risk of bias in the inclusion of SRs/MAs.

2.5.3. Estimate of Reporting Quality. The quality of each SR/MA report was evaluated by the list of Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) [25], which consisted of 27 items focusing on reporting methods and results that were incorporated into SRs/MAs.

Table 1: Search strategy for the PubMed database.

Query	Search term
#1	"Tai Ji" [Mesh]
#2	"Tai-ji" OR "Tai Chi" OR "Chi, Tai" OR "Tai Ji Quan" OR "Ji Quan, Tai" OR "Quan, Tai Ji" OR "Taiji" OR "Taijiquan" OR "T'ai Chi" OR "Tai Chi Chuan" OR "Taiji"
#3	#1 OR #2
#4	"Cognitive Dysfunction" [Mesh]
#5	"Cognitive Dysfunctions" OR "Dysfunction, Cognitive" OR "Dysfunctions, Cognitive" OR "Cognitive Impairments" OR "Cognitive Impairment" OR "Impairment, Cognitive" OR "Impairments, Cognitive" OR "Mild Cognitive Impairment" OR "Cognitive Impairment, Mild" OR "Cognitive Impairments, Mild" OR "Impairment, Mild Cognitive" OR "Impairments, Mild Cognitive" OR "Mild Cognitive Impairment" OR "Mild Neurocognitive Disorder" OR "Disorder, Mild Neurocognitive" OR "Disorders, Mild Neurocognitive" OR "Mild Neurocognitive Disorders" OR "Neurocognitive Disorder, Mild" OR "Neurocognitive Disorders, Mild" OR "Cognitive Decline" OR "Cognitive Dysfunction" OR "Cognitive Declines" OR "Decline, Cognitive" OR "Declines, Cognitive" OR "Mental Deterioration" OR "Deterioration, Mental" OR "Deteriorations, Mental" OR "Mental Deteriorations"
#6	#4 OR #5
#7	Meta-Analysis as Topic [Mesh]
#8	"Systematic review" OR "meta-analysis" OR "meta analysis" OR "meta-analyses" OR "Review, Systematic"
#9	#7 OR #8
#10	#3 AND #6 AND #9

2.5.4. Assessment of Quality of Evidence. The quality of evidence for each SR/MA outcome was evaluated by The Grading of Recommendations Assessment, Development, and Evaluation (GRADE) [26], and five aspects will lead to the degradation of evidence quality, including limitations, inconsistencies, indirectness, imprecision, and publication bias. Evidence with less than one degrading factor (including one) was rated as high-quality evidence, while evidence with two degrading factors was rated as moderate quality, three degrading factors as low quality, and more than three (including three) degrading factors as very low quality.

3. Results

3.1. Results on Literature Search and Selection. Through our search strategy, a total of 146 articles were identified. After removing 43 duplicate articles, the researchers screened the remaining 103 articles by reading titles and abstracts. Subsequently, the 12 articles were obtained. After reading the full text, it was found that two articles were not about SRs/MAs in RCTs, and two SRs/MAs were not about people with cognitive impairment. Finally, a total of 8 SRs/MAs [27–34] were finally included in this overview. The process of study selection is shown in Figure 1.

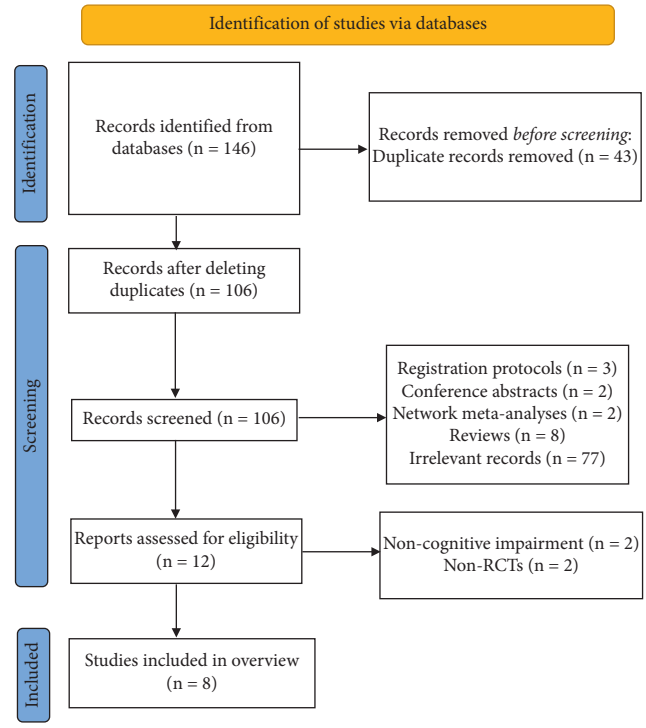

Figure 1: The flowchart of the screening process.

3.2. Description of the Included SRs/MAs. The characteristics included in the overview are shown in Table 2. These SRs/MAs were all published between 2017 and 2021, 5 [27–31] of which were in English, and the remaining 3 [32–34] were in Chinese, and all were written by Chinese authors. The number of RCTs was between 3 and 19, and the sample size was between 378 and 1,970. In 5 SRs/MAs [27–31], the intervention method for the control group was CT or daily life activities, while that for the experimental group was TC or TC combined with the intervention methods for the control group. In 3 SRs/MAs [32–34], the intervention method for the control group was CT or daily life activities, while that for the experimental group was TC exercise. In terms of quality evaluation scales, 6 SRs/MAs [27, 29, 30, 32–34] used the Cochrane risk of bias standard, and 2 SRs/MAs [28, 31] used the Physiotherapy Evidence Database scale.

3.3. Results of the Methodological Quality. By using AMSTAR-2 to assess the methodological quality, all SRs/MAs were considered to be of very low quality because more than one key item was missing from the included SRs/MAs. The restrictions came from the following items: Item 2 (only 2 SRs/MAs [29, 30] have registered protocol), Item 7 (the list of excluded studies was not mentioned by any SR/MA), Item

Table 2: Characteristics of the included SRs/MAs.

Author, year (country)	Trials (subjects)	Intervention group	Control group	Quality assessment	Main results
Liu et al., 2021 (China) [27]	10 (580)	TC, TC + CT	CT and daily life activities	Cochrane criteria	TC may have a positive effect on cognitive function improvement in middle-aged and elderly patients with cognitive impairment
Yang et al., 2020 (China) [28]	11 (1,061)	TC, TC + CT	CT and daily life activities	Physiotherapy Evidence Database scale	TC may be beneficial in improving cognitive function in older adults with MCI. However, good RCTs need to be rigorously designed and reported
Gu et al., 2021 (China) [29]	9 (827)	TC, TC + CT	CT and daily life activities	Cochrane criteria	Evidence that supports the efficacy of TC in older adults with cognitive impairment is limited. Tai Chi appears to be a safe exercise that leads to better changes in cognitive function scores
Lin et al., 2021 (China) [30]	7 (1,265)	TC, TC + CT	CT and daily life activities	Cochrane criteria	This meta-analysis demonstrates that TC has a positive clinical effect on cognitive function (overall cognitive function, memory and learning, and executive function) and physical abilities in older adults with MCI, and provides a feasible approach for MCI management
Cai et al., 2020 (China) [31]	19 (1,970)	TC and TC + CT	CT and daily life activities	Physiotherapy Evidence Database scale	TC is a promising approach to improve overall cognitive function, memory, executive function, attention, and language fluency in older adults with cognitive impairment
Li et al., 2021 (China) [32]	11 (1,234)	TC	CT and daily life activities	Cochrane criteria	TC has a certain positive effect on the cognitive function of MCI patients, but the research on the rehabilitation effect should still be increased
Zhang et al., 2017 (China) [33]	3 (378)	TC	CT and daily life activities	Cochrane criteria	TC exercise has a good effect on improving the cognitive function of the elderly with cognitive impairment
Zhang et al., 2020 (China) [34]	7 (1,068)	TC	CT and daily life activities	Cochrane criteria	TC can improve memory and visuospatial function in the elderly with mild cognitive impairment, but there is no significant improvement in indicators such as overall cognitive function, executive ability, language fluency, and depression

10 (none reported the funding of RCTs included in SRs/MAs), and Item 15 (only one SR/MA [31] conducted publication bias assessment or discussed their impact on SR/MA). The AMSTAR-2 assessment breakdown for each SR/MA is shown in Table 3.

3.4. Risk of Bias of the Included SRs/MAs. By means of ROBIS, we evaluated the relevance of Phase 1 of the research theme, and all SRs/MAs were rated as low risk of bias. In Phase 2, Domain 1, all SRs/MAs were rated as low risk of bias. In Domain 2, 5 SRs/MAs [27, 28, 30, 31, 34] were rated as low risk. In Domain 3, 6 SRs/MAs [27, 28, 31–34] of which were rated as low risk of bias, and none of one SR/MA was rated as low risk of bias in Domain 4. In Phase 3, all SRs/MAs were rated as low risk of bias. The included ROBIS evaluation details of SRs/MAs are shown in Table 4.

3.5. Report Quality of the Included SRs/MAs. Table 5 lists the details of the PRISMA checklist for each SR/MA. Although the title, abstract, introduction, and discussion were reported in full, some reporting flaws were still found in other sections. In the methods section, Item 7 (search strategy), Item 14 (reporting bias assessment), and Item 15 (certainty assessment) were insufficiently reported (<50%). In the results section, Item 16b (study selection), Item 20d (results of syntheses), Item 21 (reporting biases), and Item 22 (certainty of evidence) were reported as less than 50%. In addition to this, the Item 24 a, b, c (registration and protocol) reports for the included SRs/MAs were missing.

3.6. Evidence Quality of the Included SRs/MAs. The 42 outcomes included in the 8 SRs/MAs were assessed using the GRADE system. In the evaluation results based on the outcome indicators, 1 SR/MA was rated high, 8 moderate, 19 low, and 14 very low in terms of the quality of evidence. Publication bias ($n = 36$) was the most common downgrading factor, followed by imprecision ($n = 24$), inconsistency ($n = 21$), risk of bias ($n = 12$), and indirectness ($n = 0$) (Table 6).

3.7. Summary Results of the Included Studies. The result indicators extracted from the included studies are listed in Table 6.

3.7.1. Global Cognitive Function. All the included SRs/MAs reported the effect of TC on the overall cognitive function of the included population, and the results of 7 SRs/MAs

Table 3: Result of the AMSTAR-2 assessments.

Author, year (country)	Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8	Q9	Q10	Q11	Q12	Q13	Q14	Q15	Q16	Overall quality
Liu et al., 2021 (China) [27]	Y	PY	Y	Y	Y	Y	N	Y	Y	N	Y	Y	Y	Y	N	Y	VL
Yang et al., 2020 (China) [28]	Y	PY	Y	Y	Y	Y	N	Y	Y	N	Y	Y	Y	Y	N	Y	VL
Gu et al., 2021 (China) [29]	Y	Y	Y	PY	N	Y	N	Y	Y	N	Y	Y	Y	Y	N	Y	VL
Lin et al., 2021 (China) [30]	Y	Y	Y	Y	N	N	N	Y	Y	N	Y	Y	Y	Y	N	Y	VL
Cai et al., 2020 (China) [31]	Y	PY	Y	Y	Y	Y	N	Y	Y	N	Y	Y	Y	Y	Y	Y	VL
Li et al., 2021 (China) [32]	Y	PY	Y	PY	Y	Y	N	Y	Y	N	Y	Y	Y	Y	N	Y	VL
Zhang et al., 2017 (China) [33]	Y	PY	Y	PY	Y	Y	N	Y	Y	N	Y	Y	N	Y	N	Y	VL
Zhang et al., 2020 (China) [34]	Y	PY	Y	Y	Y	Y	N	Y	Y	N	Y	Y	Y	Y	N	N	VL

Note. Y, yes; PY, partial yes; N, no; VL, very low; L, low; key items are marked in red; Item 1, whether the research question and inclusion criteria include PICO elements; Item 2, whether to report systematic review research methods that were determined prior to implementation, and whether to report inconsistencies with the proposal; Item 3, did the authors explain why the systematic review was chosen for inclusion in the type of study design; Item 4, whether the authors used a comprehensive literature search strategy; Item 5, whether the literature screening was completed by 2 people independently; Item 6, whether the data extraction was completed independently by 2 people; Item 7, whether a list of excluded literature and reasons for exclusion is provided; Item 8, whether the authors describe the essential characteristics of the included studies in sufficient detail; Item 9, whether the authors used reasonable tools to assess the risk of bias of the included studies; Item 10, whether the authors reported funding for the studies included in this systematic review; Item 11, if a meta-analysis was performed, whether the authors used appropriate statistical methods to pool the results; Item 12, if meta-analyses were performed, whether the authors considered the potential impact of the included studies' risk of bias on meta-analyses or other evidence integration; Item 13, whether the authors considered the risk of bias of the included studies when interpreting/discussing the results of the systematic review; Item 14, whether the authors gave a satisfactory explanation or discussion of the heterogeneity in the results of the systematic review; Item 15, if quantitative synthesis was performed, whether the authors adequately investigated publication bias and discussed its possible impact on the findings; Item 16, whether the authors reported any potential conflicts of interest, including any funding received to conduct the systematic review.

Table 4: Results of the ROBIS assessments.

Author, year (country)	Phase 1	Phase 2				Phase 3
	Assessing relevance	Domain 1: study eligibility criteria	Domain 2: identification and selection of studies	Domain 3: collection and study appraisal	Domain 4: synthesis and findings	Risk of bias in the review
Liu et al., 2021 (China) [27]	√	√	√	√	×	√
Yang et al., 2020 (China) [28]	√	√	√	√	×	√
Gu et al., 2021 (China) [29]	√	√	×	×	×	√
Lin et al., 2021 (China) [30]	√	√	√	×	×	√
Cai et al., 2020 (China) [31]	√	√	√	√	×	√
Li et al., 2021 (China) [32]	√	√	×	√	×	√
Zhang et al., 2017 (China) [33]	√	√	×	√	×	×
Zhang et al., 2020 (China) [34]	√	√	√	√	×	√

Note: √, low risk; ×, high risk.

[27–33] indicated that TC could significantly improve the overall cognitive function of the cognitively impaired population.

3.7.2. Memory and Learning. 7 SRs/MAs [28–34] reported the effect of TC on memory and learning, and the results of 6 SRs/MAs [28–32, 34] indicated that TC could significantly improve the memory and learning performance in people with cognitive impairment.

3.7.3. Visuospatial Ability. 4 SRs/MAs [28, 30, 31, 34] reported the effect of TC on visuospatial ability of which 3 SRs/MAs [28, 30, 34] reported that TC could significantly improve the visuospatial ability of patients with cognitive impairment.

3.7.4. Executive Function. 5 SRs/MAs [27, 29–31, 34] reported the effect of TC on executive function of which 3 SRs/MAs [27, 30, 31] reported that TC could significantly improve the executive function of patients with cognitive impairment.

Table 5: Results of the PRISMA checklist.

Section/topic		Items	Liu et al., 2021 (China) [27]	Yang et al., 2020 (China) [28]	Gu et al., 2021 (China) [29]	Lin et al., 2021 (China) [30]	Cai et al., 2020 (China) [31]	Li et al., 2021 (China) [32]	Zhang et al., 2017 (China) [33]	Zhang et al., 2020 (China) [34]	Number of yes or partially yes (%)
Title	Title	Item 1	Y	Y	Y	Y	Y	Y	Y	Y	100
Abstract	Abstract	Item 2	PY	PY	PY	PY	PY	PY	PY	PY	100
Introduction	Rationale	Item 3	Y	Y	Y	Y	Y	Y	Y	Y	100
	Objectives	Item 4	Y	Y	Y	Y	Y	Y	Y	Y	100
Methods	Eligibility criteria	Item 5	Y	Y	Y	Y	Y	Y	Y	Y	100
	Information sources	Item 6	Y	Y	Y	Y	Y	Y	Y	Y	100
	Search strategy	Item 7	PY	N	PY	N	N	N	N	PY	37.50
	Selection process	Item 8	Y	Y	N	N	Y	Y	Y	Y	75
	Data collection process	Item 9	Y	Y	Y	N	Y	Y	Y	Y	87.50
	Data items	Item 10 (a)	Y	Y	Y	Y	Y	Y	Y	Y	100
		Item 10 (b)	PY	PY	PY	PY	PY	PY	PY	PY	100
	Study risk of bias assessment	Item 11	Y	Y	Y	Y	Y	Y	Y	Y	100
	Effect measures	Item 12	Y	Y	Y	Y	Y	Y	Y	Y	100
	Synthesis methods	Item 13 (a)	Y	Y	Y	Y	Y	Y	Y	Y	100
		Item 13 (a)	Y	Y	Y	Y	Y	Y	Y	Y	100
		Item 13 (c)	Y	Y	Y	Y	Y	Y	Y	Y	100
		Item 13 (d)	Y	Y	Y	Y	Y	Y	Y	Y	100
		Item 13 (e)	Y	N	Y	Y	N	N	Y	Y	62.50
		Item 13 (f)	Y	N	N	Y	N	N	Y	Y	50
	Reporting bias assessment	Item 14	N	N	N	N	Y	N	N	N	12.50
	Certainty assessment	Item 15	N	N	N	N	N	N	N	N	0

Table 5: Continued.

Section/topic		Items	Liu et al., 2021 (China) [27]	Yang et al., 2020 (China) [28]	Gu et al., 2021 (China) [29]	Lin et al., 2021 (China) [30]	Cai et al., 2020 (China) [31]	Li et al., 2021 (China) [32]	Zhang et al., 2017 (China) [33]	Zhang et al., 2020 (China) [34]	Number of yes or partially yes (%)
Results	Study selection	Item 16 (a)	Y	Y	Y	Y	Y	Y	Y	Y	100
		Item 16 (b)	N	Y	N	Y	N	N	N	N	25
	Study characteristics	Item 17	Y	Y	Y	Y	Y	Y	Y	Y	100
	Risk of bias in studies	Item 18	Y	Y	Y	Y	Y	Y	Y	Y	100
	Results of individual studies	Item 19 (a)	Y	Y	Y	Y	Y	Y	Y	Y	100
		Item 19 (b)	Y	Y	Y	Y	Y	Y	Y	Y	100
	Results of syntheses	Item 20 (a)	Y	Y	Y	Y	Y	Y	Y	Y	100
		Item 20 (b)	Y	Y	Y	Y	Y	Y	Y	Y	100
		Item 20 (c)	Y	Y	Y	Y	Y	Y	Y	N	87.50
		Item 20 (d)	Y	N	N	N	N	N	N	Y	25
	Reporting biases	Item 21	N	N	N	N	Y	N	N	N	12.50
	Certainty of evidence	Item 22	N	N	N	N	N	N	N	N	0
Discussion	Discussion	Item 23 (a)	Y	Y	Y	Y	Y	Y	Y	Y	100
		Item 23 (b)	Y	Y	Y	Y	Y	Y	Y	Y	100
		Item 23 (c)	Y	Y	Y	Y	Y	Y	Y	Y	100
		Item 23 (d)	Y	Y	Y	Y	Y	Y	Y	Y	100
Other information	Registration and protocol	Item 24(a)	N	N	Y	Y	N	N	N	N	25
		Item 24 (b)	N	N	Y	Y	N	N	N	N	25
		Item 24 (c)	N	N	N	N	N	N	N	N	0
	Support	Item 25	Y	Y	Y	Y	Y	Y	Y	N	87.50
	Competing interests	Item 26	Y	Y	Y	Y	Y	Y	Y	Y	100
	Availability of data, code, and other materials	Item 27	Y	Y	Y	Y	Y	Y	Y	Y	100

Note. Y, yes; N, no; PY, partially yes.

Table 6: Results of evidence quality.

Author, year (country)	Outcomes	Studies (participants)	Limitations	Inconsistency	Indirectness	Imprecision	Publication bias	Relative effect (95% CI)	Heterogeneity (%)	Quality
Liu et al., 2021 (China) [27]	MoCA (global cognitive function)	5 (344)	0 (1)	−1 (2)	0	−1 (3)	−1 (4)	WMD = 3.23, 95% CI (1.88, 4.58)*	I2 = 92	Very low
	MMSE (global cognitive function)	3 (187)	0	−1 (2)	0	−1 (3)	−1 (4)	WMD = 3.69, 95% CI (0.31, 7.08)*	I2 = 83	Very low
	TMT-B (executive function)	2 (147)	0	0	0	−1 (3)	−1 (4)	WMD = −13.69, 95% CI (−21.64, −5.74)*	I2 = 0	Low
Yang et al., 2020 (China) [28]	Global cognitive function	5 (858)	0	−1 (2)	0	0	−1 (4)	SMD = 0.40, 95% CI (0.24, 0.55)*	I2 = 63	Low
	Memory and learning	7 (855)	0	−1 (2)	0	0	−1 (4)	SMD = 0.37, 95% CI (0.24, 0.51)*	I2 = 67	Low
	Mental speed and attention	6 (929)	0	−1 (2)	0	0	−1 (4)	SMD = 0.51, 95% CI (0.31, 0.71)*	I2 = 84	Low
	Ideas, abstraction, figural creations, and mental flexibility	6 (782)	0	0	0	0	−1 (4)	SMD = 0.29, 95% CI (0.16, 0.42)*	I2 = 0	Moderate
	Visuospatial ability	3 (192)	0	0	0	−1 (3)	−1 (4)	SMD = 0.29, 95% CI (0.10, 0.48)*	I2 = 0	Low
Gu et al., 2021 (China) [29]	MMSE (global cognitive function)	6 (673)	−1 (1)	−1 (2)	0	0	−1 (4)	WMD = 1.52, 95% CI (0.90, 2.14)*	I2 = 63	Very low
	MoCA (global cognitive function)	3 (244)	−1 (1)	−1 (2)	0	−1 (3)	−1 (4)	WMD = 3.5, 95% CI (0.76, 6.24)*	I2 = 92	Very low
	CDR (global cognitive function)	2 (285)	−1 (1)	0	0	−1 (3)	−1 (4)	WMD = −0.55, 95% CI (−0.80, −0.29)*	I2 = 0	Very low
	LMD (memory and learning)	3 (435)	−1 (1)	−1 (2)	0	0	−1 (4)	WMD = 1.10, 95% CI (0.04, 2.16)*	I2 = 77	Very low
	DSF (executive function)	2 (287)	−1 (1)	−1 (2)	0	−1 (3)	−1 (4)	WMD = 0.53, 95% CI (−0.65, 1.71)	I2 = 64	Very low
	DSB (executive function)	2 (287)	−1 (1)	0	0	−1 (3)	−1 (4)	WMD = −0.1, 95% CI (−0.38, 0.19)	I2 = 0	Very low
Lin et al., 2021 (China) [30]	Global cognitive function	2 (272)	0	0	0	0	−1 (4)	WMD = -2.24, 95% CI (−3.51, −0.97)*	I2 = 0	Moderate
	Memory and learning	3 (126)	0	−1 (2)	0	−1 (3)	−1 (4)	SMD = 0.83, 95% CI (0.22, 1.45)*	I2 = 57	Very low
	Visuospatial ability	2 (85)	0	0	0	−1 (3)	−1 (4)	WMD = 3.15, 95% CI (0.74, 5.56)*	I2 = 0	Low
	Executive function	3 (376)	0	0	0	−1 (3)	−1 (4)	WMD = 0.32, 95% CI (0.03, 0.61)*	I2 = 0	Low
	Physical activity	2 (53)	0	0	0	−1 (3)	−1 (4)	WMD = 18.78, 95% CI (10.80, 26.76)*	I2 = 0	Low
	Psychological assessment	2 (272)	0	0	0	−1 (3)	−1 (4)	WMD = 0.17, 95% CI (−0.62, 0.96)	I2 = 0	Low

Table 6: Continued.

Author, year (country)	Outcomes	Studies (participants)	Limitations	Inconsistency	Indirectness	Imprecision	Publication bias	Relative effect (95% CI)	Heterogeneity (%)	Quality
Cai et al., 2020 (China) [31]	Global cognitive function	12 (1,738)	0	−1 (2)	0	0	0	SMD = 0.41, 95% CI (0.33, 0.48)*	I2 = 67	Moderate
	Memory function	16 (1,708)	0	−1 (2)	0	0	0	SMD = 0.31, 95% CI (0.22, 0.39)*	I2 = 69	Moderate
	Executive function	9 (1,586)	0	−1 (2)	0	0	0	SMD = 0.33, 95% CI (0.25, 0.42)*	I2 = 77	Moderate
	Verbal fluency	5 (1,325)	0	0	0	0	0	SMD = 0.27, 95% CI (0.13, 0.41)*	I2 = 0	High
	Attention	6 (1,479)	0	−1 (2)	0	0	0	SMD = 0.25, 95% CI (0.17, 0.34)*	I2 = 96	Moderate
	Visual space function	3 (192)	0	−1 (2)	0	−1 (3)	0	SMD = 0.03, 95% CI (−0.28, 0.33)	I2 = 55	Low
Li et al., 2021 (China) [32]	GDS (psychological assessment)	2 (110)	−1 (1)	0	0	−1 (3)	−1 (4)	WMD = −2.81, 95% CI (−3.48, −2.14)*	I2 = 45	Very low
	DSF (executive function)	2 (355)	0	−1 (2)	0	−1 (3)	−1 (4)	WMD = 1.22, 95% CI (−0.68, 3.12)	I2 = 82	Very low
	DSB (executive function)	3 (620)	0	0	0	−1 (3)	−1 (4)	WMD = 0.17, 95% CI (−0.03, 0.36)	I2 = 18	Low
	MoCA (global cognitive function)	2 (136)	−1 (1)	−1 (2)	0	−1 (3)	−1 (4)	WMD = -1.58, 95% CI (−9.79, 6.64)	I2 = 97	Very low
	AVLT (memory and learning)	2 (123)	−1 (1)	−1 (2)	0	−1 (3)	−1 (4)	WMD = 1.27 , 95% CI (0.31, 2.23)*	I2 = 51	Very low
	LMD (memory and learning)	3 (660)	0	−1 (4)	0	0	−1 (4)	WMD = 2.26, 95% CI (0.35, 4.16)*	I2 = 93	Low
	MMSE (global cognitive function)	4 (704)	0	0	0	0	−1 (4)	WMD = 0.93, 95% CI (0.40, 1.47)*	I2 = 0	Moderate
Zhang et al., 2017 (China) [33]	Global cognitive function	3 (678)	−1 (1)	0	0	0	−1 (4)	WMD = 0.91, 95% CI (0.37, 1.46)*	I2 = 0	Low
	Verbal fluency	2 (654)	−1 (1)	0	0	0	−1 (4)	WMD = 2.17, 95% CI (0.88, 3.45)*	I2 = 0	Low
	Memory function	2 (654)	−1 (1)	−1 (2)	0	−1 (3)	−1 (4)	WMD = 0.16, 95% CI (−0.14, 0.45)	I2 = 55	Very low
Zhang et al., 2020 (China) [34]	Global cognitive function	5 (785)	0	0	0	−1 (3)	−1 (4)	WMD = 0.29, 95% CI (−0.16, 0.74)	I2 = 0	Low
	Memory function	4 (726)	0	0	0	0	−1 (4)	WMD = 0.37, 95% CI (0.13, 0.61)*	I2 = 7	Moderate
	Executive function	4 (726)	0	0	0	−1 (3)	−1 (4)	WMD = 0.03, 95% CI (−0.16, 0.22)	I2 = 0	Low
	Verbal fluency	2 (594)	0	0	0	−1 (3)	−1 (4)	WMD = 0.47, 95% CI (−0.76, 1.70)	I2 = 0	Low
	Visual space function	4 (726)	0	−1 (2)	0	0	−1 (4)	SMD = 0.57, 95% CI (0.23, 0.91)*	I2 = 75	Low
	Psychological assessment	4 (730)	0	0	0	−1 (3)	−1 (4)	SMD = 0.00, 95% CI (−0.14, 0.15)	I2 = 0	Low

Note. (1) The included studies had a large bias in methodology such as randomization, allocation concealment, and blinding. (2) The confidence interval overlapped less or the I2 value of the combined results was larger. (3) The sample size from the included studies did not meet the optimal sample size or the 95% confidence interval crossed the invalid line. (4) The funnel chart was asymmetry. *The 95% confidence interval did not cross the invalid line. MoCA, Montreal Cognitive Assessment Scale; MMSE, mini-mental state examination; TMT-B, trail marking test B; CDR, clinical dementia rating; DSF, digit span forward; DSB, digit span backward; LMD, Logical Memory Delayed Recall Score; GDS, Geriatric Depression Scale; AVLT, auditory verbal learning test.

3.7.5. Verbal Fluency. 3 SRs/MAs [28, 30, 34] reported the effect of TC on verbal fluency of which 2 SRs/MAs [28, 34] reported that TC could significantly improve the verbal fluency of patients with cognitive impairment.

3.7.6. Psychological Evaluation. 3 SRs/MAs [30, 32, 34] reported on psychological assessments, and only 1 SR/MA [32] showed that TC could improve the mental activity of patients with cognitive impairment.

3.7.7. Other Outcome Indicators. A SR/MA [28] reported that TC can significantly improve mental speed and attention and ideas, abstraction, figural creations, and mental flexibility in patients with cognitive impairment. A separate SR/MA [30] reported that TC could improve the physical activity and attention [31] of patients.

3.7.8. Adverse Reactions. The narrative descriptions in 5 SRs/MAs [27–31] indicated that TC was a safe treatment modality.

4. Discussion

Currently, drug treatments have limited effectiveness in improving cognition or slowing disease progression [35]. Physical activity is a well-studied behavioral intervention for cognitive function [36], and TC may be a good one. A literature search revealed that although several SRs/MAs on the impact of TC on cognitive impairment have been published, the quality of these publications has not been assessed. Therefore, we carried out this overview to evaluate the multiple SRs/MAs that meet the inclusion criteria in a bid to provide clinicians with higher-quality evidence.

4.1. Summary of the Main Findings. This is the first overview of SRs/MAs on the effects of TC on cognitive impairment, including 8 SRs/MAs on TC for cognitive impairment, published between 2011 and 2021, and 7 of the SRs/MAs (7/8, 87.5%) were published after 2020. This may indicate that TC, as a complementary and alternative therapy for cognitive impairment, has drawn increasing attention from people.

As indicated by the assessment for method quality, report quality, risk of bias, and evidence quality, the included 8 SRs/MAs were not satisfactory. In AMSTAR-2, all the included SRs/MAs are considered to be of very low quality, and the main defects are pointed out as follows: (1) only two SRs/MAs [29, 30] were registered with the study protocol, which may affect its standardization and sophistication, and increase the possibility of selective reporting bias; (2) none of the SR/MA provided a list of excluded literature, which may reduce the transparency of the SRs/MAs and affect the credibility of the results; (3) only one SR/MA [31] assessed publication bias in the included RCTs, which would reduce confidence in the results. In addition, this was also related to the insufficient number of RCTs included in the relevant outcome measures; (4) in addition, no SR/MA reporting was included in the RCT funding resources, which may increase bias in clinical trials since the results of corporate-funded studies may be biased in favor of the funder. All of the above methodological flaws limit the accuracy of SRs/MAs. In the ROBIS assessment, insufficient assessment of publication bias was the main reason for the high risk of final results, which was consistent with the AMSTAR-2 scale. Moreover, the absence of sensitivity analysis was also an important factor leading to high risk of bias, which would affect the stability of the SRs/MAs results. Regarding the results of the PRISMA checklist, lack of protocol registration and publication bias in SRs/MAs was the main cause of underreporting, as shown in AMSTAR-2. However, none of the SRs/MAs provided comprehensive search strategies, which reduced the reproducibility and credibility of the study.

For GRADE, publication bias was the most common downgrading factor included in SRs/MAs. Insufficient assessment of publication bias in the outcome measures was the main downgrading factor, which was also related to the inadequate number of RCTs included in the relevant outcome measures. In addition, the insufficient study population included in a single effect size was also an important reason for the decline in the quality of the evidence. Although almost all SRs/MAs showed that TC had a positive effect on cognitive function in patients with cognitive impairment, the conclusions of SRs/MAs may deviate from the real results due to the inadequate methodological and evidence quality of the included studies. Caution should be exercised in recommending TC as a complementary intervention for cognitive impairment.

4.2. Implications for Future Study. Our overview may have some reference value for future research. Authors should pay attention to the registration of research protocols before proceeding with SRs/MAs to ensure the rigor of their procedures. In terms of literature search and selection, information on excluded literature and complete search strategy for all databases should be listed and elaborated on to ensure transparency. In the quantitative calculation of effect size, care should be taken to exclude the results of a single study one by one to ensure the stability of the results. In addition, a complete assessment of publication bias would also improve the accuracy of the meta-analysis results. TC is not only easy to learn and practice but also has many advantages in physiology and psychology, and it has clinical significance for further research. Although TC originated from traditional Chinese medicine theory, the duration, frequency, and mode of TC movement vary greatly in different studies. Therefore, we propose to use a standardized TC training program, including fixed duration, frequency, and pattern, to better study the impact of TC on cognitive performance. In addition, the assessment of cognitive function should identify areas of cognition specifically improved by TC in patients with cognitive impairment, as indicated by as physiological outcomes, such as circulating biochemical markers and neuroimaging structure and function. With the evolution of evidence-based medicine, it is hoped that researchers will continue to

promote the standardization of relevant individual RCTs in the future. A well-designed, rigorously implemented, and complete reporting RCT with complete reporting can minimize or avoid bias. It is the gold standard for evaluating interventions [37].

4.3. Strength and Limitations. Our overview is the first to use AMSTAR-2, ROBIS, PRISMA, and GRADE to evaluate SRs/MAs regarding the impact of TC on cognitive impairment. Based on the current results, TC may be an effective adjunctive replacement therapy for cognitive impairment. Furthermore, the evaluation process revealed clear limitations of the current relevant SRs/MAs and RCTs, which may help guide high-quality clinical studies in the future. However, this overview has certain limitations because of the subjectivity of the assessment. Although our assessments were reviewed by two independent assessors, different assessors may have their own judgment on each factor, so the results may vary.

5. Conclusion

Based on current evidence, TC appears to have a positive effect on cognitive impairment with a high safety profile. However, the low quality of the SRs/MAs supporting these results is concerning, and we should therefore approach this conclusion with caution. In the future, RCTs with more stringent TC interventions for cognitive impairment should be performed. At the same time, more rigorous, standardized, and comprehensive SRs/MAs in related fields are needed to provide stronger evidence.

Data Availability

The datasets analyzed during the current study are available from the corresponding author on reasonable request.

Disclosure

Hongshuo Shi and Chengda Dong are the co-first authors.

Conflicts of Interest

The authors declare no conflicts of interest.

Authors' Contributions

SHS, YTT, and SGM participated in the research design. SHS, CLJ, LWW, CH, XMY, and DCD conducted a literature search and screened data extraction. SHS and DCD analyzed the data, did a statistical analysis, and wrote the manuscript. DCD, SGM, and SHS participated in the correction of the manuscript. All authors reviewed the manuscript. All authors read and approved the final version of the manuscript.

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