BioMed Research International

Autophagy, Oxidative Stress and their Interactions in Senescence

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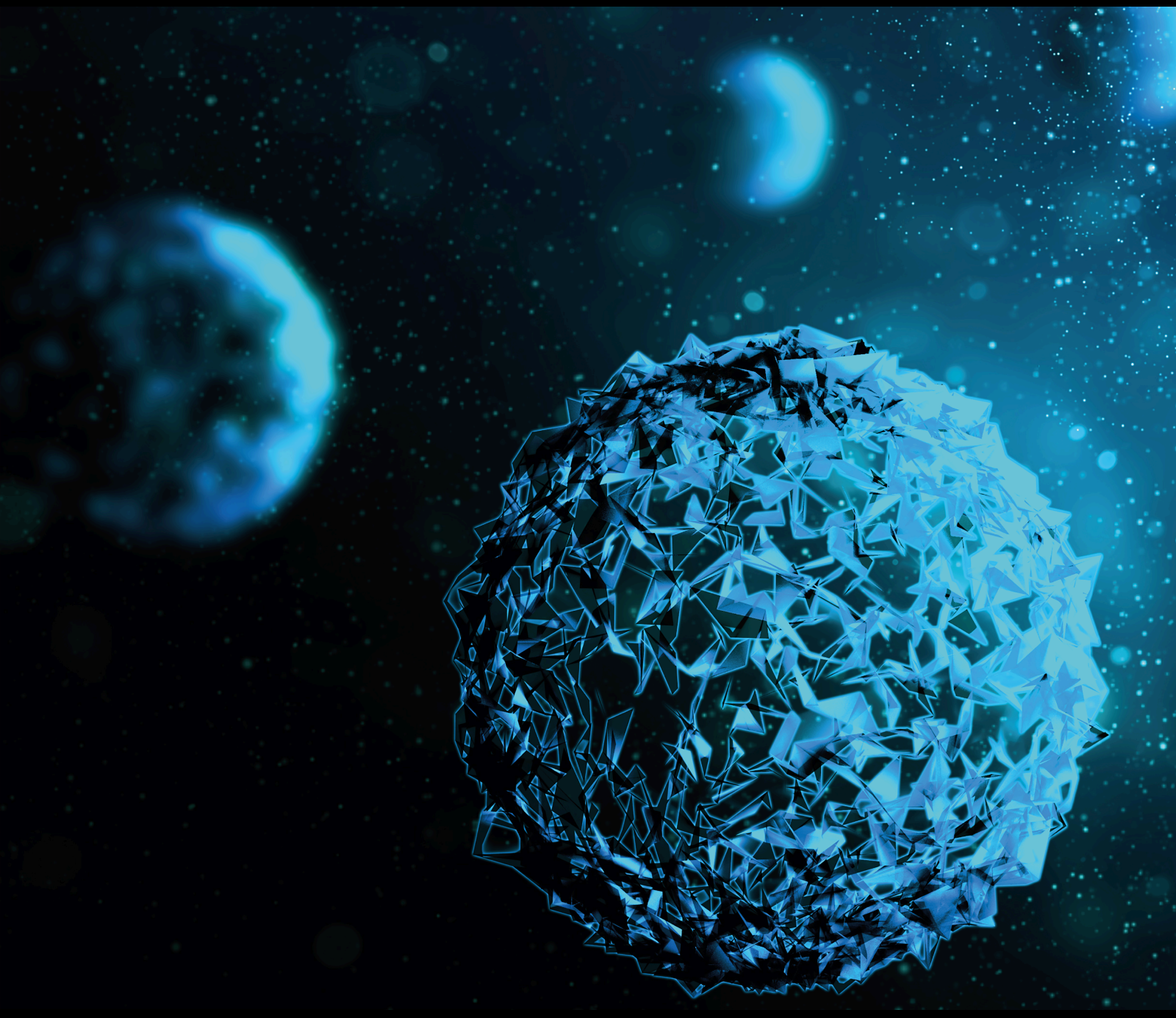

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Retraction

Retracted: The Correlation of PM2.5 Exposure with Acute Attack and Steroid Sensitivity in Asthma

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This article has been retracted by Hindawi following an investigation undertaken by the publisher [1].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) Manipulated or compromised peer review

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.

References

[1] J. Luo, H. Liu, S. Hua, and L. Song, "The Correlation of PM2.5 Exposure with Acute Attack and Steroid Sensitivity in Asthma," *BioMed Research International*, vol. 2022, Article ID 2756147, 8 pages, 2022.

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Volume 2024, Article ID 9842578, 1 page
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Retraction

Retracted: Fetal Congenital Cardiac and Vascular Disorders Associated with Sertraline Treatment during Pregnancy: Analysis of FAERS Data

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[1] F. Hong, J. Qiu, S. Zhang, and L. Zhang, "Fetal Congenital Cardiac and Vascular Disorders Associated with Sertraline Treatment during Pregnancy: Analysis of FAERS Data," *BioMed Research International*, vol. 2022, Article ID 9914931, 11 pages, 2022.

Hindawi
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Retraction

Retracted: Recent Advances in Cell and Functional Biomaterial Treatment for Spinal Cord Injury

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References

[1] T. Liu, W. Zhu, X. Zhang et al., "Recent Advances in Cell and Functional Biomaterial Treatment for Spinal Cord Injury," *BioMed Research International*, vol. 2022, Article ID 5079153, 20 pages, 2022.

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Retraction

Retracted: Knockdown of PVT1 Exerts Neuroprotective Effects against Ischemic Stroke Injury through Regulation of miR-214/Gpx1 Axis

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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:

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(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

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[1] X. Liu, T. Wang, P. Jing, M. Zhang, F. Chang, and W. Xiong, "Knockdown of PVT1 Exerts Neuroprotective Effects against Ischemic Stroke Injury through Regulation of miR-214/Gpx1 Axis," *BioMed Research International*, vol. 2022, Article ID 1393177, 9 pages, 2022.

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Volume 2023, Article ID 4683542, 7 pages
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Research Article

Inflammatory and Oxidative Stress Biomarkers in the Elderly, the Birjand Longitudinal Aging Study

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Cigarette smoking is a significant risk factor for chronic and atherosclerotic vascular disease that causes preventable considerable morbidity and mortality worldwide. This study is aimed at comparing inflammation and the levels of oxidative stress biomarkers in elderly subjects. The authors recruited the participants (1281 older adults) from the Birjand Longitudinal of Aging study. They measured oxidative stress and inflammatory biomarkers serum levels in the 101 cigarettes and 1180 nonsmokers. The mean age of smokers was 69.3 ± 7.95 years, and most were male. The most percentage of male cigarette smokers have lower body mass index (BMI) ($\leq 19\,kg/m^2$). Females have higher BMI categories than males ($P \leq 0.001$). The percentage of diseases and defects was different between cigarette and non-cigarette smoker adults ($P \leq 0.01$ to $P \leq 0.001$). The total white blood cells, neutrophils, and eosinophils were significantly higher in cigarettes compared to non-cigarette smokers ($P \leq 0.001$). Besides, cigarette consumers' percentage of hemoglobin and hematocrit compared to other aged people was significantly different ($P \leq 0.001$). However, biomarkers of oxidative stress and antioxidant levels were not significant differences between the two senior groups. Cigarette smoking in older adults was associated with increased inflammatory biomarkers and cells, but it did not find a significant difference in oxidative stress markers. Longitudinal prospective studies may help illuminate the mechanisms inducing oxidative stress and inflammation due to cigarette smoking in each gender.

1. Introduction

According to free radicals theory, the aging process results from cellular damages caused by metabolism, mainly mitochondrial respiration [1, 2].

Decreasing muscle mass versus increasing fat tissue can be considered chronic low-grade inflammatory conditioning in aging. It can lead to high levels of reactive oxygen species (ROS) and changing inflammatory adipocytokines and antioxidative enzymes [3, 4]. Although normal levels of ROS are essential for various cellular mechanisms [1], insufficiency of the antioxidants system and inability to detoxify the increased ROS would lead to oxidative stress (OS), or a state of imbalance between the production and regulation of ROS [5–7].

Oxidative stress in old age may harm cellular pieces of the membrane leading to loss of function and reduced metabolic efficiency [5, 6]. In this regard, OS might be the primary contributor to the formation of cardiovascular disorders owing to endothelial dysfunction through increased inflammatory and reduced anti-inflammatory cytokines [3]. Hence, inactivating nitric oxide within endothelial cells by OS may impair lipids metabolism, insulin regulation, and blood flow [3].

Genetic and social factors may harm the accumulation of OS, for example, UV light, X-ray, air pollutants, certain drugs, and tobacco smoking [1, 7]. OS is significantly an identified risk factor in most annual mortalities and fatal diseases such as cancer, cardiovascular and cerebral events due to carcinogenic, proinflammatory, and prooxidative effects [8, 9].

Smoking has been recognized as the most significant risk factor for cardiovascular disease due to widespread use in low-middle countries [10]. Existed evidence has demonstrated that tobacco smoking has a higher prevalence rate in the Asian old population, and men consume more (22.5%) compared to women (8.7%) [11]. Another study on smoking prevalence among Iranians has found cigarette consumption of nearly 13.5% for current older smoker men and 16% among past users [12].

High rates of oxidants and toxic substances in cigarettes damage airway epithelial cells, lipids, and DNA, stimulating oxidative stress, systemic inflammation, and increasing leukocytes [13]. In addition, research has found that tobacco smoking is associated with changes in inflammatory mediators, CRP, fibrinogen level, and type or number of lymphocytes identified as predictors of cardiovascular disorders [13, 14]. According to studies findings, the surge of inflammatory markers like interleukin- (IL-) 6 and C-reactive protein (CRP) is related to the number of cigarettes consumed in the life period [15, 16]. Thus, serum concentrations, accumulation, and pathophysiological effects of inflammatory markers should be higher in heavy smokers' older adults than others. Although evidence has demonstrated some atherogenic properties of tobacco smoking, probably owing to OS and inflammation, it is not clear that OS is primarily responsible for vascular dysfunction and causes inflammation or vice versa. Limitations of previous studies, including not considering the underlying characteristics of participants, persuaded researchers to perform a population-based investigation on the aged population [17, 18]. This study aimed at studying oxidative stress and inflammation changes in elderly cigarette and nonsmoker. This data can help understand the effects of cigarette consumption on the reaction of inflammatory/immune systems in old age.

2. Materials and Methods

2.1. Study Design and Participants. The aged population sample ≥ 60 years who were residents in urban and rural regions of Birjand County were invited to participate in the Birjand Longitudinal of Aging study. The participants were selected using multistage stratified cluster sampling. The city's postal areas identified 70 clusters. In each group, 20 subjects (equal numbers of males and females) were assigned [19].

Through a short interview, an expert nurse collected demographic information such as sex, age, and history of cigarette smoking. Current smokers identified with a cigarette smoking index of ≥10 pack-years (calculated by multiplying the number of cigarettes per day by the number of years spent smoking, divided by 20 cigarettes in one pack). The anthropometric measurements were also performed, and blood samples were obtained [19]. Exclusion criteria included confirmed asthma, COPD, and upper/lower respiratory tract infection in the preceding four weeks. Furthermore, the aged were completely bedridden or unable to communicate owing to severe cognitive impairment and Alzheimer's disease, and those with very short life expectancy (less than six months). The Institutional and Science committees ethically confirmed the study, and all participants gave written informed consent.

The serum concentration levels of oxidative stress and inflammatory markers from 101 cigarettes and 1180 non-cigarette smokers included as a reference control group were analyzed.

2.2. Serum Sample. Blood samples were drained from the cubital vein in the morning after fasting for 12 hours and collected into blood collection tubes (ORUM TADJHIZ CO, Iran, ISO 9001& 13438) without an anticoagulant using a clot separator gel. The serum was separated after 30 minutes by centrifugation at 1600g for 10 minutes, aliquoted, and stored at -80°C until use.

2.3. Hematologic Analysis. Hematologic indexes were analyzed by an automated hematology analyzer (SysmexKX-21 (Sysmex Corporation Kobe, Japan)). Those indexes include cell blood count (CBC), hemoglobin (Hb), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC).

2.4. Cytokine Analysis. According to the manufacturer's instructions, serum cytokine (hsCRP) profiling was performed, using Pars Azmun, Prestige 24i analyzer (Tokyo, Japan) using the serum samples without dilution. Serum cytokine levels were expressed as the median (range) in pg/ml.

2.5. Oxidative Stress Measurement. Evaluating oxidant stress was performed by determining the plasma concentration of markers such as porcine plasma protein hydrolysate (PPPH; radical scavenging activity (RSA) based on DPPH assay), thiol (total thiol groups based on Ellman assay), and thiobarbituric acid reactive substances (TBARS; lipid peroxidation based on thiobarbituric acid reactive substances). Furthermore, the researchers measured ferric reducing antioxidant power (FRAP) to investigate the antioxidant potential of serum levels (total antioxidant capacity (TAC) based on FRAP assay) (Table 1).

2.6. Ethical Approval. All the participants signed the informed consent after reading or explaining the content of the informed consent. The biobanking of the blood sample and how the researchers will be able to use it for future research were described in the consent form. The ethical research committee simultaneously approved the protocols of the current study of Endocrinology and Metabolism Research Institute (EMRI) of Tehran University of Medical Sciences (TUMS), (IR.TUMS.EMRI.REC.1396.00158) and the ethical committee of Birjand University of Medical Sciences (IR.BUMS.Rec.1397.282).

Table 1: Oxidant stress measurement.

Test	Method	Type of kite	Company	Apparatus
PPPH	Radical scavenging activity (RSA) based on DPPH assay)	Microplate colorimetry (Zantox)	Kavosh Arian Azma Co.	Microplate reader, Biotek instruments (Epock, USA)
Thiol	Total thiol groups based on Ellman assay	Microplate colorimetry (Zantox)	Kavosh Arian Azma Co.	Microplate reader, Biotek instruments (Epock, USA)
TBARs	Lipid peroxidation based on Thiobarbituric acid reactive substances	Flourometry (Zantox)	Kavosh Arian Azma Co.	Microplate reader, Bioteck Life Science instrumentations, Citation3 imaging reader (Vermont, USA)
FRAP	Total antioxidant capacity (TAC) based on FRAP assay	Microplate colorimetry (Zantox)	Kavosh Arian Azma Co.	Microplate reader, Biotek instruments (Epock, USA)

Table 2: Demographic information of elderly.

Variable			Cigarette smoker	Non-cigarette smoker	P value
Age			69.3 ± 7.95	69.55 ± 7.49	0.691
Gender	Male		92 (16%)	474 (84%)	0.00
	Female		9 (1%)	706 (99%)	
BMI (kg/m^2)	Male	≤19	19 (37%)	32 (63%)	0.00
		19 < 21	15 (21%)	57 (79%)	
		21 < 23	10 (10%)	86 (90%)	
		≥23	48 (10%)	415 (90%)	
	Female	≤19	0	28 (100%)	0.00
		19 < 21	1 (3%)	35 (97%)	
		21 < 23	1 (1%)	73 (99%)	
		≥23	7 (1%)	590 (99%)	

Data are expressed as n (%) or mean ± SD. Body mass index (BMI).

2.7. Statistical Analysis. Data were analyzed by the SPSS (v.22) software using the t-test, Mann–Whitney, and Kolmogorov-Smirnov test. Demographic and clinical data were expressed as the mean (SD), and differences between the study groups were analyzed by t-test (parametric data). Data for cytokine concentration levels were expressed as the median (interquartile range), and differences across the study groups were analyzed using Mann–Whitney and t-test. $P < 0.05$ was considered significant.

3. Results

A total of 1281 elderly were included in the current study. The mean age of cigarette and non-cigarette smokers was 69.3 ± 7.95 and 69.55 ± 7.49 years, respectively. Generally, the frequency of the male gender cigarette smokers was significantly more compared to the non-cigarettes smokers' group ($P \leq 0.001$) (Table 2). The most percentage of male cigarette smokers have lower body mass index (BMI) (≤19 kg/m^2). Also, the most percentage of females have a higher BMI (≥23 kg/m^2) compared to male adults ($P \leq 0.001$).

The history of diseases percentage in the total population and disease and defects history in both two groups of elderly cigarette and non-cigarette smokers are presented in Table 3. The rate of high blood pressure, diabetes, blood lipid profile, and osteoarthritis in non-cigarette smokers was significantly higher than in the smoker group ($P \leq 0.01$ to $P \leq 0.001$). The percentage of prostate diseases in cigarette smokers was significantly higher compared to the non-cigarette group ($P \leq 0.001$) (Table 3). The two study groups did not differ substantially concerning inflammatory and oxidative stress biomarkers (Table 4). Total white blood cells (WBC), neutrophils (Neut), and eosinophils (Eos) in cigarette smokers were significantly higher compared to the non-cigarette smoker's group ($P \leq 0.01$ to $P \leq 0.001$). The lymphocyte percentage (Lymph) was considerably lower in cigarette smokers ($P \leq 0.05$) compared to the non-cigarette smoker group (Table 5).

HCT, MCV, MCH, and MCHC were significantly higher in cigarettes than the non-cigarette smoker's group ($P \leq 0.05$ to $P \leq 0.001$). On the contrary, hemoglobin (Hb) was substantially lower in cigarette smokers ($P \leq 0.001$) compared to the elderly non-cigarette smokers (Table 5).

4. Discussion

This investigation analyzed the inflammatory and oxidative stress biomarkers levels in non-cigarettes smokers and older adults.

The present study showed that the percentage of high blood pressure, diabetes, blood lipid profile, and osteoarthritis

Table 3: Percentage of diseases and defects in elderly.

Diseases	History of diseases (N (%))	Cigarette smoker	Non-cigarette smoker	P value
High blood pressure	551 (43%)	22 (22%)	529 (48%)	0.00
Diabetes	325 (25%)	9 (9%)	316 (27%)	0.00
Heart failure	150 (12%)	11 (11%)	139 (12%)	0.790
Prostate	166 (13%)	26 (26%)	140 (12%)	0.00
Coronary angiogenesis	216 (17%)	14 (14%)	202 (17%)	0.386
Blood lipid	416 (33%)	12 (12%)	404 (34%)	0.00
Fatty liver	152 (12%)	8 (8%)	144 (12%)	0.200
Osteoarthritis	254 (20%)	9 (9%)	245 (21%)	0.004
Osteoporosis	149 (12%)	7 (7%)	142 (12%)	0.120
Visual impairment	567 (44%)	45 (45%)	522 (44%)	0.822
Uses of glasses	330 (26%)	28 (28%)	302 (25%)	0.615
Dysaudia	141 (11%)	9 (9%)	132 (11%)	0.475

Data are expressed as n (%).

Table 4: Inflammatory and oxidative stress biomarkers in elderly cigarette and non-cigarette smokers.

Variables	Cigarette smoker (mean ± SD)	Non-cigarette smoker (mean ± SD)	P value
hsCRP (mg/ml)	22.7 ± 24.3	15.5 ± 13.46	0.1**
FRAP (pg/ml)	796 ± 205.8	805 ± 195.5	0.722**
PPPH (pg/ml)	401.66 ± 97.8	421.53 ± 109.9	0.266**
Thiol (pg/ml)	441 ± 66.15	452.7 ± 80.79	0.341**
TBAR (pg/ml)	0.49 ± 0.1	0.47 ± 0.13	0.125*

Data are expressed as mean ± SD, hsCRP: high-sensitivity C-reactive protein, FRAP: ferric reducing antioxidant power, PPPH: porcine plasma protein hydrolysate, TBARS: thiobarbituric acid reactive substances. *Mann–Whitney, **t-test.

was significantly higher in non-cigarettes smokers than the elderly cigarette smokers. Based on the gender of participants, female is the major of non-cigarette smokers. A previous study showed that healthy females' lipid profile and blood pressure were significantly higher than healthy males [20]. Also, gender affects lipid parameters, independent of age, and menopausal status [21]. Presumably, these differences are due to the different levels of circulating sex hormones, specifically estrogens and androgens, in women versus men. It has been suggested that menopause may potentiate the age-related increase in systolic blood pressure, perhaps due to reduced arterial compliance.

The older age, male sex, diabetes, tobacco use, overweight, and obesity are independent predictors of coronary plaque and high-risk plaque in the population with a mean age of 53 years [22]. The prospective cohort study in Asia showed that secondhand smoke might raise the risk of ischemic heart disease (IHD) and the risk of incident cardiovascular disease (CVD) in middle-aged never-smoking women [23]. The study also showed that coffee consumption was associated with a low risk of all and non-Alzheimer's dementia. In contrast, smoking was associated with a high risk of non-Alzheimer's dementia in the general population with a median age of 58 years [24].

Furthermore, healthy lifestyles, including never vs. current smoking, leisure-time physical activity vs. none, and 7–9 hours vs. >9 hours of sleep, were individually associated with an 11%–25% reduced risk of Alzheimer's disease and related dementias in patients' age > 65 years old [25]. These results suggest that maintaining a healthy lifestyle is associated with a lower risk of diseases among older people.

The results of the current study show the BMI scores of female elderly were higher compared to male cigarette smokers. It has been reported that the prevalence of obesity among older *females was* higher *than among males (42.9% and 38.3%*, respectively) [26]. The prevalence of obesity among the elderly was significantly higher, and females had more predilection for obesity than males [27]. In addition, the previous study indicated that older adult females were more obese than males, and current smokers were less [28], which supports the present study's results.

The current study showed that 92% of smokers are males, and the percentage of prostate diseases was significantly higher in male cigarettes than in non-cigarette smokers. The possible relation between prostate cancer and cigarette smoking has been considered previously [29], which supported the current study results. Antioxidant parameter levels, including ferric reducing antioxidant power (FRAP), PPPH, and thiobarbituric acid reactive substances (TBARS), were decreased. Versus, the high-sensitivity C-reactive protein (hsCRP) was increased in cigarette smokers compared to the non-cigarette smoker subjects. Although these changes observed between the two groups were not significant, it may be due to the gender and small sample size of the cigarette smoker group. Based on the obtained data in the current study, BMI in female elderly was higher compared to male cigarette smokers. It has been reported that the macronutrients in the adipose tissues stimulated the release of inflammatory mediators such as TNF-α and IL-6 and reduced adiponectin production, predisposing to a proinflammatory state and oxidative stress [30]. Moreover, obesity was associated with an increase in

Table 5: Hematological index in elderly cigarette and non-cigarette smokers.

Variables	Cigarette smoker (mean ± SD)	Non-cigarette smoker (mean ± SD)	Reference value	P value
WBC (Tho/μl)	6.89 ± 1.77	6.2 ± 1.58	4-11	0.00*
RBC (Mil/μl)	5 ± 0.00	4 ± 0.00	F: 4.2-5.4	0.00*
	4 ± 0.00	5.01 ± 0.00	M: 4.5-6.3	0.017*
Hemoglobin (g/dL)	13 ± 0.00	13 ± 1	F: 12-16	0.00*
	14 ± 1	14 ± 1	M: 14-18	0.00*
Hematocrit (%)	41 ± 3	40 ± 3	F: 36-46	0.00*
	42 ± 4	43 ± 3	M: 39-52	0.00*
MCV (fL)	87.4 ± 6	85.7 ± 5.2	77-100	0.00*
MCH (pg)	30 ± 2.59	29.2 ± 2.8	26-34	0.00*
MCHC (g/dL)	34.3 ± 1.46	34 ± 1.5	30-37	0.098**
Platelet (Tho/μl)	221.59 ± 75.28	230.6 ± 66.87	150-450	0.225**
Neutrophils (%)	54.48 ± 10.6	52.7 ± 9.63	42.2-75.2	0.095**
Lymphocytes (%)	35.3 ± 9.8	37.85 ± 10.6	20.5-51.1	0.016*
Monocyte (%)	6.9 ± 2.45	6.5 ± 2.7	1.7-9.8	0.177**
Eosinophil (%)	3.29 ± 1.6	2.9 ± 1.4	0.0-3.0	0.013**

Data are expressed as mean ± SD. *Mann–Whitney, **t-test. WBC: white blood cells, RBC: red blood cells, MCV: mean corpuscle (cell) volume, MCH: mean corpuscular hemoglobin, MCHC: mean corpuscular hemoglobin concentration. F: female, M: male.

the production of leptin as a proinflammatory and a reduction in adiponectin as an anti-inflammatory mediator [31]. Also, adipokines (adipocytokines) that are produced by the adipose tissue induce the production of reactive oxygen species (ROS), generating a process known as oxidative stress (OS). Obesity produces OS by mitochondrial and peroxisomal oxidation of fatty acids, which can produce ROS [32]. The results of these studies indicated that upon the increase of adipose tissue, the activity of antioxidant enzymes significantly diminished while OS and inflammatory mediators enhanced. These results indicated that although the levels of antioxidant parameters and inflammatory mediator increase in cigarette smokers, due to gender and obesity in non-cigarette smokers (female), these changes were not significant.

Exposure to tobacco promotes a more rapid decline in lung function raised oxidative stress and persistence of inflammation [33]. It has been reported that oxidative stress is generally higher in men than in premenopausal women. However, the biomarkers of oxidative stress and the risk of experiencing cardiovascular events increased in postmenopausal women [34]. Besides, different serum hydroperoxides (as the oxidative stress index) levels were observed in elderly subjects of both genders with or without coronary artery disease [35].

Our findings showed that cigarette smoking has adverse effects on hematological parameters, including white blood cell (WBC) count, Hb, HCT, MCV, MCH, and MCHC.

In our study, the number of leukocytes, hemoglobin, and hematocrit values was significantly higher in smokers than in nonsmokers. The study results revealed that the hematological parameters were significantly increased in smokers of both genders than in nonsmokers [36]. Lakshmi et al. also showed that the Hct and Hb levels were significantly higher in smokers [37], supporting the current study results. Cigarette smoking is associated with an increase in WBC count and Hb levels in total blood. Also, obesity and aging are inversely related to Hb levels in the blood [38]. An increase in hemoglobin concentration is believed to be mediated by carbon monoxide exposure, which suggests that an increase in hemoglobin level in the blood of smokers could be a compensatory mechanism.

MCV, MCH, and MCHC are the three leading red blood cell indices that help measure the red blood cells' average size and hemoglobin composition. The larger MCV, MCH, and MCHC in smokers elder were observed compared to the nonsmoker subjects, which was confirmed by other studies [39, 40].

As gender (for example, differences in body mass index) could influence inflammatory markers status, our results may be affected. The significant difference in the percentage of inflammatory markers and hematocrit of cigarette consumers follows the evidence and is in line with other research and confirms our hypothesis. Many studies have reported an association between smoking and inflammatory or immune response [41, 42]. The cross-sectional design of the current research and a more significant number of older women than males were some of its limitations. On the other hand, the majority of smokers had male gender.

5. Conclusion

Cigarette smoking increases hematological and inflammatory parameters associated with a greater risk for various diseases. Cigarette smoking in older adults was associated with increased inflammatory biomarkers, but any significant difference was found in biomarkers of oxidative stress. The higher BMI and obesity were associated with increased production of oxidative stress and proinflammatory markers. Longitudinal prospective studies may help determine the causal pathways and mechanisms inducing oxidative stress and inflammation due to cigarette smoking in each gender.

Data Availability

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this article.

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Research Article

CTGF Promotes the Osteoblast Differentiation of Human Periodontal Ligament Stem Cells by Positively Regulating BMP2/Smad Signal Transduction

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Objective. This work is aimed at revealing the role and the molecular mechanism of connective tissue growth factor 2 (CTGF) in the osteoblast differentiation of periodontal ligament stem cells (PDLSCs). *Methods*. The osteogenic differentiation of PDLSCs was induced by osteogenic induction medium (OM), and the expression level of osteogenic related proteins ALP, RUNX2, OCN, and CTGF was estimated using quantitative real-time polymerase chain reaction (qRT-PCR) and Western blotting analysis. We constructed cell lines with CTGF overexpression or knockdown to verify the role of CTGF in the osteoblast differentiation of PDLSCs. Alkaline phosphatase (ALP) staining was introduced to measure the osteoblasts activity, and alizarin red S (ARS) staining was employed to test matrix mineralization. The interaction between CTGF and bone morphogenetic protein-2 (BMP-2) was determined by endogenous coimmunoprecipitation (Co-IP). *Results*. The expression level of CTGF was increased during the osteogenic induction of PDLSCs. Additionally, CTGF overexpression effectively maintained the stemness and facilitated the osteoblast differentiation in PDLSCs, and CTGF knockdown exerted opposite effects. Moreover, at molecular mechanism, CTGF increased the activity of BMP-2/Smad signaling pathway. *Conclusion*. This investigation verified that CTGF promotes the osteoblast differentiation in PDLSCs at least partly by activating BMP-2/Smad cascade signal.

1. Introduction

Periodontitis, a chronic and infectious disease, is mainly caused by the loss of alveolar bone and aberrant vascularization [1]. Previous studies have illustrated that severe periodontitis already affects 9 to 11 percent of adults worldwide [2]. Conventional treatment methods such as root planning and induced bone regeneration have not achieved satisfactory clinical results [3]. The periodontal ligament could be a soft tissue in the alveolar fossa that improves tooth nutrition and promotes alveolar bone remodeling [4, 5]. Periodontal ligament stem cells (PDLSCs) rooted from periodontal ligament tissue are identified as a group of mesenchymal stem cells (MSCs) and have the potential for self-renewal and multidirectional differentiation [6]. Current studies pointed that PDLSCs exhibit different differentiation abilities and serve as the most crucial candidate stem cells for inducing periodontal regeneration [7–9]. It has been reported that PDLSCs ameliorate periodontal intra bone loss. Besides, osteogenic differentiation of PDLSCs is crucial for repairing alveolar bone loss as well as tooth defects [10–13]. Increasing evidence indicates that various factors including mechanical vibration, electromagnetic field, and genes are able to affect the process of osteogenic differentiation of PDLSCs [14–17]. Therefore, exploring novel targets to regulate PDLSCs osteogenic differentiation is an important goal for the treatment of periodontitis.

It is reported that multiple signaling pathways such as mitogen-activated protein kinase (MAPK) pathway [18] and Wnt/β-catenin pathway regulate the osteogenic differentiation progression [19]. Bone morphogenetic proteins (BMPs), the essential components of the transforming growth factor -β (TGF-β) superfamily, have been proved to be closely associated with the osteogenic differentiation of MSCs [20]. BMP-2 was first noted for its ability to induce ectopic bone and chondrogenesis [21]. Recent investigations demonstrated that BMP-2 effectively accelerate the osteoblast differentiation of

PDLSCs [22]. Zhang's team revealed that BMP-2 increases the expression of osteogenic differentiation-related proteins RUNX2, ALP, Colla, and Oc, thereby facilitating bone formation [23]. Mechanistically, activated BMP-2 phosphorylates Smad pathway such as Smad1/5/8 and ultimately activates the expression of downstream target genes including RUNX2 [24, 25]. However, how BMP-2/Smad pathway is regulated in the osteoblast differentiation of PDLSCs needs to be further explored.

Connective tissue growth factor 2 (CTGF, CCN2), a member of the CCN family, is closely related to a variety of biological processes, including angiogenesis and wound healing [26, 27]. Increasing evidence revealed that CTGF accelerates the production of extracellular matrix components and stimulates the differentiation of chondrogenic MSC [28–30]. Wang et al. verified that CTGF overexpression facilitates the osteogenic differentiation of MSCs [31]. Lee et al. revealed that CTGF accelerates the differentiation of human MSCs into fibroblasts and influences connective tissue healing in rodents [32]. Asano et al. discovered that CTGF possess the ability to promote the growth and differentiation of mouse PDL cells [33]. Additionally, Yuda et al. proved that CTGF accelerates the proliferation, cycle, migration, and osteoblast differentiation of a clonal cell line (1-11) by constructing and isolating fibroblast cell lines [34]. However, the effect of CTGF on the differentiation of PDLSCs into osteoblasts remains uncovered. Moreover, increasing findings have indicated that CTGF is able to negatively regulate BMP-2 in mouse osteoblasts but antagonizes and systemizes BMP-2 signaling in chondrocytes [35, 36]. Nevertheless, whether and how CTGF regulates BMP-2/Smad pathway in the osteoblast differentiation of PDLSCs is still unclear.

This work revealed that CTGF facilitates the osteoblast differentiation of PDLSCs at least partly by upregulating BMP-2/Smad cascade signaling.

2. Materials

2.1. Cell Culture and Induction. Primary human PDLSCs were obtained as introduced in previous reporters [20, 37]. Premolars extracted from healthy people after orthodontics are preserved. The periodontal ligament tissue was scraped from 1/3 of the root of the premolars and placed in a petri dish containing fresh α-minimum primary medium (α-MEM, P03-2610, PAN, Germany) in advance. Next, a scalpel was used to separate 1 mm^3 of periodontal tissues, and the tissues were cultured in a culture flask supplemented with complete α-MEM (α-MEM, P03-2610, PAN, Germany) containing 10% fetal bovine serum (FBS, DX101, DENING BIO, China). Place the flask upside down in a CO_2 incubator (S@feGrow 188, BioMatrix Life Science (Qingdao) Co., Ltd., China) at 37° C. After 4 hours, the tissues in the flask were turned over once and then were serial subcultured. Then, PDLSCs were purified using the monoclonal screening approach. After 3-5 generations, PDLSCs were frozen and stored, or further studies were carried out. After the cells reached 70-80% density, the original growth medium (α-MEM, also known as GM) was replaced with osteogenic induction medium (OM), α-MEM medium containing 100 nM dexamethasone (HY-14648, MCE, USA), 200 μM L-ascorbic acid (HY-B0166, MCE, USA), and 2 mM β-glycerophosphate (G9422, Sigma-Aldrich, USA). The old OM was replaced every 2 days. All patients have signed informed consent, and all protocols were permitted by the Ethic Committee of The Second Affiliated Hospital of Shandong University of Chinese Medicine.

2.2. Cell Transfection. The transfected small interfering RNAs (siRNAs) were obtained from Zoonbio Biotechnology (China), and plasmids were obtained from Kingsley Biotechnology Co., Ltd. (China). When the cell density reached 50-60%, PDLSCs were transfected with specific vectors employing Lipofectamine 2000 Transfection Reagent (11668030, Invitrogen, USA) following the instruction of the manufacturer. CTGF overexpression lentivirus and CTGF siRNA lentivirus were obtained by GeneChem (China) and transduced into PDLSCs following the instructions. The sequences of siRNAs are listed in Table 1.

2.3. Measurement of the Activity of Antioxidative Stress Markers. PDLSCs transfected with indicated vectors were divided into 3 groups: control group, lipopolysaccharide (LPS, 10 μM, L8880, Solarbio, China) group, and LPS + pcDNA3.1/CTGF group or LPS + si-CTGF-1# group. After overexpression of 24 h, the content of superoxide dismutase (SOD) or glutathione (GSH) was estimated utilizing superoxide dismutase activity assay kit (BC5165, Solarbio, China), microreduced glutathione (GSH) assay kit (BC1175, Solarbio, China), or malondialdehyde (MDA) content detection kit (BC0020, Solarbio, China) following the kit's instruction, respectively.

2.4. QRT-PCR. The total RNA from PDLSCs was collected at 0, 7, and 14 days after osteogenic induction using TriQuick Reagent (R1100, Solarbio, China) abiding by the kit's protocol. Next, 1 μg total RNA was employed to synthesize complementary DNA (cDNA), and then, the cDNA was used to amplify the targeted gene utilizing a BeyoFast™ SYBR Green One-Step qRT-PCR Kit (D7268S, Beyotime, China) according to the manufacturer's instruction. The relative expression of each RNA was estimated via $2^{-\Delta\Delta CT}$ method, and β-actin was considered as the control gene. The sequences of primers are presented in Table 2.

2.5. Western Blotting. PDLSCs transfected with indicated vectors were harvested utilizing RIPA Lysis Buffer (P0013C, Beyotime, China) following the instruction of the regent. Then, the lysate was centrifuged at 4°C (12000 rpm, 10 min), and total protein was isolated with 12% SDS-PAGE. Next, the blots were shifted onto polyvinylidene fluoride (PVDF) membrane (YA1701-1EA, Solarbio, China), followed by blocking with 5% nonfat powdered milk (D8340, Solarbio, China) dissolving in TBS with Tween-20 (ST671, Beyotime, China) solution for 40 min. The membranes were treated with antibody anti-CTGF antibody (ab6992, Abcam, UK), ANTI-FLAG(R) antibody produced in rabbit (F7425, MilliporeSigma, USA), anti-Nanog antibody (ab80892, Abcam, UK), anti-SOX2 antibody

Table 1: The sequences of siRNAs.

si-RNA	Forward (5′-3′)	Reverse (5′-3′)
si-NC	GTTCTCCGAACGTGTCACGT	ACGTGACACGTTCGGAGAAC
si-CTGF-1#	GGUCAAGCUGCCCGGGAAATT	UUUCCCGGGCAGCUUGACCTT
si-CTGF-2#	GCACCAGCAUGAAGACAUA	UAUGUCUUGAUGCUCCUGC
si-CTGF-3#	CCAGACCCAACUAUGAUUA	UAAUCAUAGUUGGGUVUGG

Table 2: The sequences of qRT-PCR primers.

Gene	Forward	Reverse
RUNX2	5′-ACTACCAGCCACCGAGACCA-3′	5′-ACTGCTTGCAGCCTTAAATGACTCT-3′
OCN	5′-ACCCTGACCCATCTCAGAAGCA-3′	5′-CTTGGAAGGGTCTGTGGGGCTA-3′
ALP	5′-GAACGTGGTCACCTCCATCCT-3′	5′-TCTCGTGGTCACAATGC-3′
CTGF	5′-GGCCTCTTCTGCGATTTCG-3′	5′-GCAGCTTGACCCTTCTCGG-3′
β-Actin	5′-GTGACGTTGACATCCGTAAAGA-3′	5′-GCCGGACTCATCGTACTCC-3′

(ab97959, Abcam, UK), c-Myc Rabbit mAb (A19032, Abclonal, China), anti-RUNX2 antibody (ab23981, Abcam, UK), anti-collagen I antibody (ab34710, Abcam, UK), anti-ALP antibody (ab83259, Abcam, UK), anti-BMP2 antibody (ab14933, Abcam, UK), Phospho-Smad1 (Ser463/465)/Smad5 (Ser463/465)/Smad9 (Ser465/467) (D5B10) Rabbit mAb (13820, Cell signaling Technology, USA), or anti-beta actin antibody (ab8227, Abcam, UK) overnight at 4°C. Finally, HRP-labeled Goat anti-Rabbit IgG(H + L) (A0208, Beyotime, China) and BeyoECL Plus (P0018S, Beyotime, China) were employed to observe the protein bands.

2.6. Alkaline Phosphatase (ALP) Staining. ALP staining analysis was carried out employing an alkaline phosphatase (ALP/AKP) test kit (ml092964, Shanghai Enzyme Linked Biotechnology Co., Ltd.) after 7 days of osteogenic induction according to the manufacturer's instruction.

2.7. ALP Activity. The ALP activity was determined as described in the previous study [37]. In brief, PDLSCs transfected with indicated vectors were washed with precooled phosphate buffer saline (PBS, C0221A, Beyotime, China); then, the cells were permeated with 1% Triton X-100 (ST797, Beyotime, China) and scraped into distilled water using a sterilized cell scraper. Next, a kit (P0012S, Beyotime, China) was used to estimate the protein concentration, and the activity of ALP was calculated according to the absorbance at 405 nm.

2.8. Alizarin Red S (ARS) Staining. ARS staining is utilized to determine the formation of mineralized nodules using an osteoblast-mineralized nodule staining kit (C0148S, Beyotime, China) according to the kit's protocol. Briefly, PDLSCs transfected with indicated vectors were immobilized with stationary liquid for 30 min at day 14 of osteogenic induction. Then, the cells were stained by utilizing the ARS solution for another 20 min. Next, the ARS staining solution was dissolved in 10% cetylpyridine chloride (C9002, Sigma-Aldrich, USA) for 1 hour, and the absorbance of the solution at 570 nm was measured to determine the degree of mineralized nodules.

2.9. Coimmunoprecipitation (Co-IP) Assay. PDLSCs were collected using the cell lysis buffer for Western and IP (P0013, Beyotime, China). The lysates were incubated with protein A + G agarose (P2012, Beyotime, China) combined with Rabbit Control IgG (AC005, Abclonal, China), anti-CTGF antibody (ab6992, Abcam, UK), or anti-BMP2 antibody (ab14933, Abcam, UK) overnight at 4°C. After washing with lysis buffer for 3 times, the solution was centrifuged at 4°C for 5 min (12000 rpm), and the supernatant was removed. Then, the agarose with IgG, CTGF, or BMP-2 was lysed utilizing SDS lysis buffer (P0013G, Beyotime, China), and the proteins were isolated as described in Western blotting section.

2.10. Statistical Analysis. The statistical analysis was conducted by comparing mean ± standard deviation (SD) employing a two-tailed Student's t test (two groups) or one-way analysis of variance (ANOVA) combined with Tukey's test (multiple groups). Additionally, the significance was indicated with $^{****}p < 0.0001$, $^{***}p < 0.001$, and $^{**}p < 0.01$.

3. Results

3.1. CTGF Is Highly Expressed during the Osteoblast Differentiation of PDLSCs. PDLSCs were maintained in osteogenic medium for 14 days, and the osteogenic induction efficiency was verified by qRT-PCR analysis. The data illustrated the expression level of osteogenic related proteins ALP, RUNX2, and OCN was obviously upregulated during the induction (Figures 1(a)–1(c)). In addition, the CTGF expression was increased notably with the process of osteogenic induction (Figure 1(d)). Collectively, these data suggested that CTGF might be correlated to the osteogenic differentiation of PDLSCs.

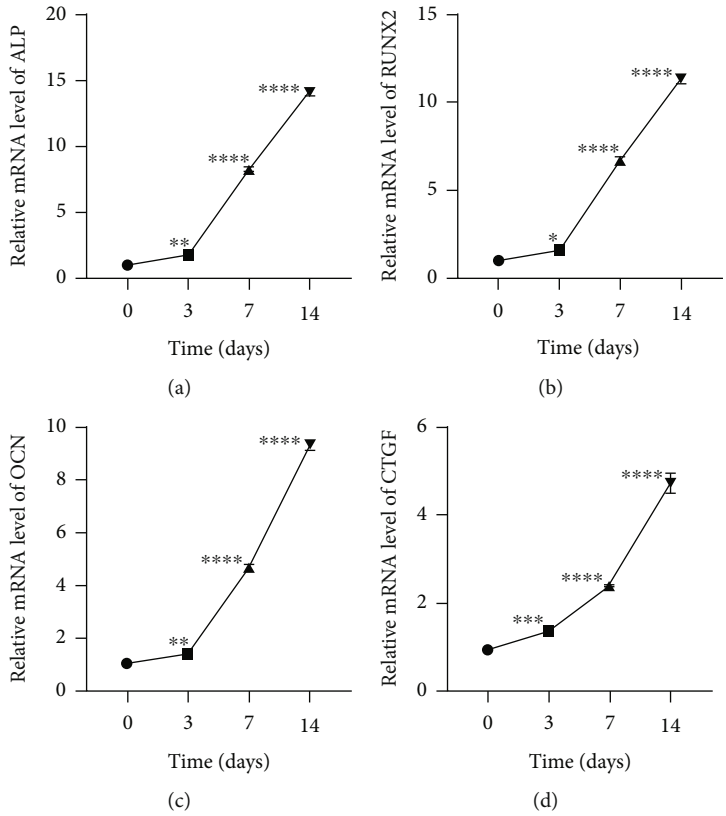

Figure 1: CTGF is highly expressed during the osteoblast differentiation of PDLSCs. (a–c) The content of ALP, RUNX2, and OCN detected via qRT-PCR ($^{****}p < 0.0001$, $^{**}p < 0.01$). (d) The content of CTGF detected via qRT-PCR ($^{****}p < 0.0001$, $^{***}p < 0.001$). $^{****}p < 0.0001$, $^{***}p < 0.001$, and $^{**}p < 0.01$ versus 0 day.

3.2. CTGF Affects the Stemness of PDLSCs and Regulates Oxidative Stress. To explore the role of CTGF in PDLSCs, we constructed PDLSC lines with overexpression (Figure 2(a)) or knockdown (Figure 2(b)) of CTGF. Besides, as exhibited in Figure 2(b), si-CTGF-1# presented the highest efficiency and was chosen to conduct subsequent experiments (Figure 2(b)). Firstly, we determined the effects of CTGF on the stemness of PDLSCs by measuring the expression level of stemness associated genes. Interestingly, overexpression of CTGF dramatically upregulated the content of NANOG, SOX2, and CMYC in PDLSCs (Figure 2(c)). Consistently, CTGF silence notably downregulated the load of these genes (Figure 2(d)). Subsequently, we tested the roles of CTGF in oxidative stress in PDLSCs. With LPS treatment, the production of oxidative stress-associated markers SOD as well as GSH was dramatically decreased, while the level of MDA was increased notably in PDLSCs. Besides, overexpression of CTGF effectively reversed these effects on PDLASCs. S. However, CTGF silence further reduced the secretion of SOD and GSH (Figures 2(e)–2(g)). These findings indicated that CTGF effectively maintains the stemness of PDLSCs and might protect PDLSCs from oxidative stress to some extent.

3.3. CTGF Overexpression Promotes the Osteoblast Differentiation of PDLSCs. To investigate the influence of CTGF on the osteogenic ability of PDLSCs, osteogenesis was induced again, and ALP staining analysis was performed. The data demonstrated that CTGF overexpression dramatically enhanced the ALP staining and ALP activity after osteogenic induction for 7 days (Figure 3(a)). After 14 days of osteogenic induction, ARS staining assay showed that overexpression of CTGF increased the amount of ARS staining remarkably, which implied that CTGF overexpression enhanced the matrix mineralization in PDLSCs (Figure 3(b)). Additionally, the expression level of proteins related to osteoblast differentiation was tested. The data illustrated that CTGF obviously increased the expression of RUNX2, ALP, and COL1 in PDLSCs (Figure 3(c)). These findings demonstrated that overexpression of CTGF facilitates the osteoblast differentiation in PDLSCs.

3.4. CTGF Knockdown Inhibits the Osteoblast Differentiation of PDLSCs. To further prove the role of CTGF in PDLSC osteogenic differentiation, we conducted above-mentioned experiments in PDLSCs with CTGF silence. As expected, CTFG knockdown dramatically reduced the intensity of ALP staining and ALP activity at 7 days after osteogenic induction (Figure 4(a)). After 14 days, decrease of matrix mineralization was observed in the CTGF-knockdown group by performing ARS staining analysis (Figure 4(b)). Additionally, Western blotting analysis revealed that the protein level of RUNX2, ALP, and COL1 was downregulated via CTGF silence obviously. Overall, these results confirmed that knockdown of CTGF suppressed the osteogenic differentiation of PDLSCs.

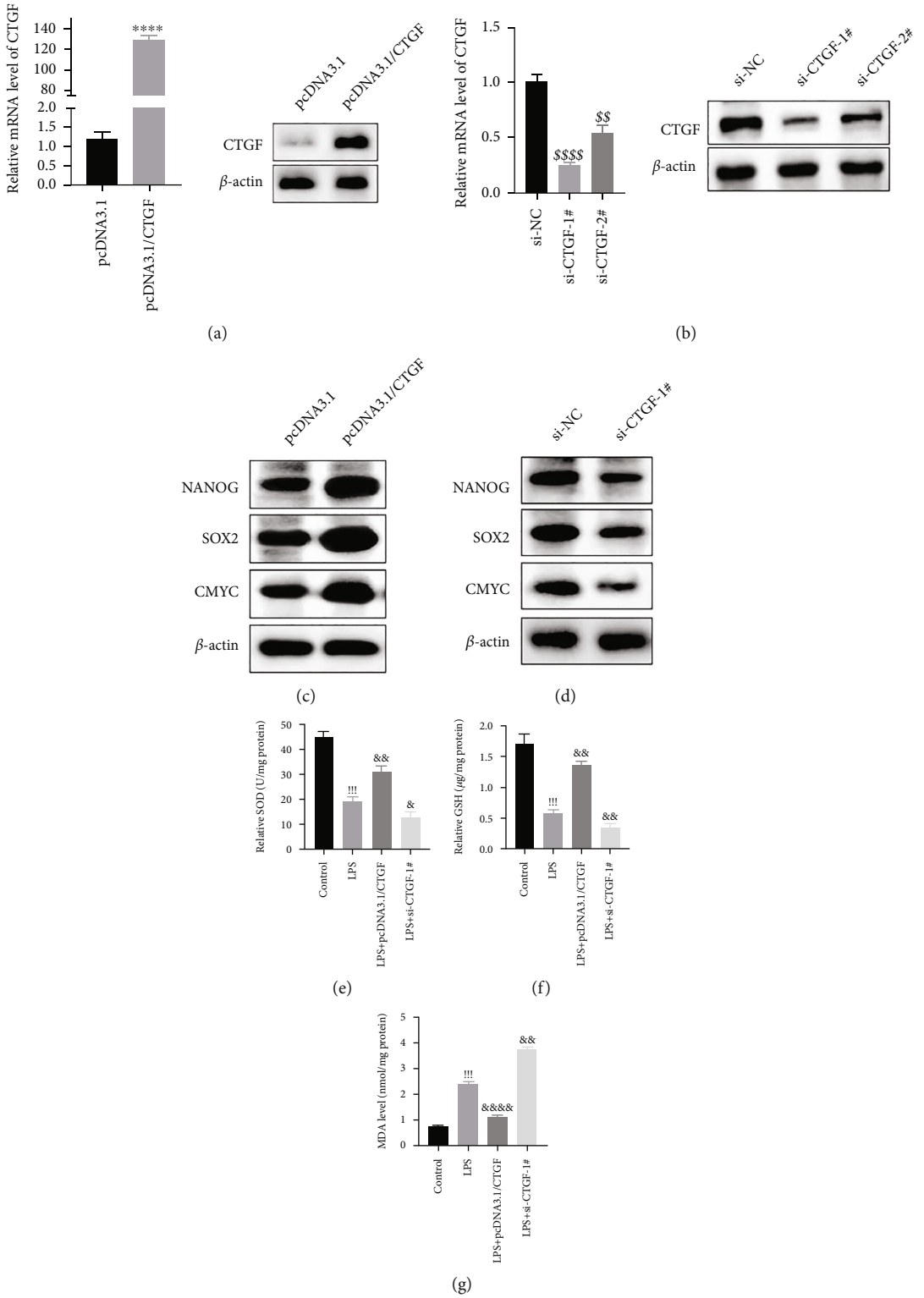

Figure 2: CTGF affects the stemness of PDLSCs. (a) The content of CTGF measured via Western blotting analysis ($^{****}p < 0.0001$). (B) The content of CTGF determined via Western blotting analysis ($^{\$\$\$\$}p < 0.0001$, $^{\$\$}p < 0.01$). (c) The content of NANOG, SOX2, and CMYC detected via Western blotting analysis. (d) The content of NANOG, SOX2, and CMYC determined via Western blotting analysis (e, f) The production of SOD (e), GSH (f), and MDA (g) estimated via specific kit ($^{!!!!}p < 0.0001$, $^{!!!}p < 0.001$, $^{\&\&\&\&}p < 0.0001$, $^{\&\&}p < 0.01$, $^{\&}p < 0.05$). $^{****}p < 0.0001$, $^{***}p < 0.001$ versus pcDNA3.1. $^{\$\$\$\$}p < 0.0001$, $^{\$\$}p < 0.01$ versus si-NC. $^{!!!!}p < 0.0001$, $^{!!!}p < 0.001$ versus control. $^{\&\&\&\&}p < 0.0001$, $^{\&\&}p < 0.01$, $^{\&}p < 0.05$ versus LPS.

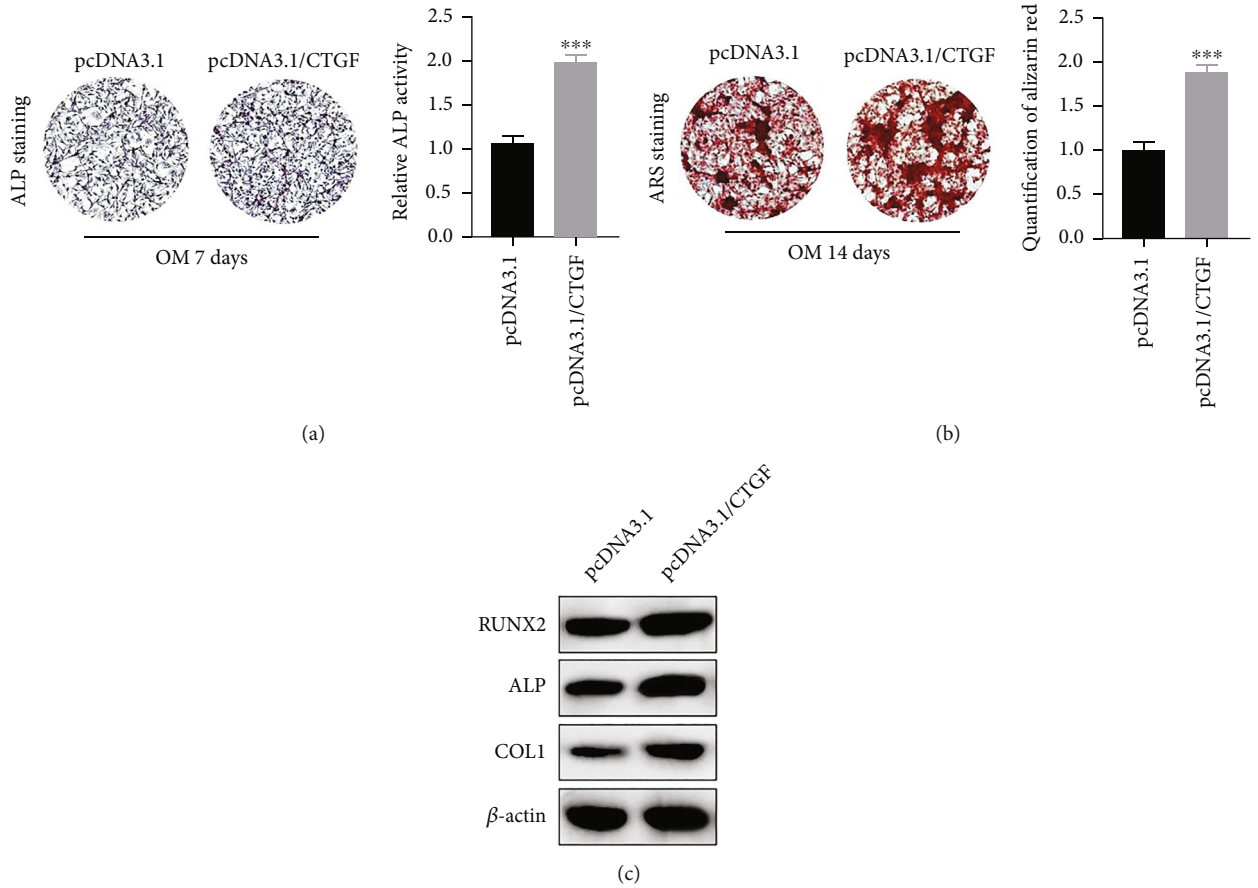

Figure 3: CTGF overexpression promotes the osteoblast differentiation of PDLSCs. (a) ALP staining and ALP activity ($^{***}p < 0.001$). (b) Images of ARS staining and quantification of ARS ($^{***}p < 0.001$). (c) The content of RUNX2, ALP, and COL1 detected via Western blotting analysis. $^{***}p < 0.001$ versus pcDNA3.1.

3.5. CTGF Promotes the Osteoblast Differentiation in PDLSCs via the BMP-2/Smad Pathway. To verify whether CTGF affects the osteogenic differentiation of PDLSCs by mediating BMP-2/Smad signal, we firstly performed endogenous Co-IP analysis to determine the relationship between CTGF and BMP-2 in PDLSCs. As presented in Figures 5(a) and 5(b), endogenous BMP-2 in PDLSCs was immunoprecipitated by CTGF antibody. Besides, the direct interaction was further proved via immunoprecipitation of CTGF with BMP-2 antibody (Figures 5(a) and 5(b)). Moreover, CTGF overexpression notably increased the BMP-2 expression and the phosphorylation of p-Smad1/5/9 after osteogenic induction (Figure 5(c)). In addition, CTGF knockdown reduced the content of BMP-2 and p-Smad1/5/9 after osteogenic induction remarkably (Figure 5(d)). Taken together, these findings revealed that CTGF facilitates the osteoblast differentiation of PDLSCs at least partly by promoting BMP-2/Smad cascade signal.

4. Discussion

In summary, this investigation revealed that the CTGF expression was increased during osteogenic induction. Additionally, CTGF overexpression enhanced the stemness of PDLSCs, while CTGF silence impaired that notably. Moreover, overexpression of CTGF facilitated the osteoblast differentiation in PDLSCs, whereas knockdown of CTGF exerted opposite effects obviously. Furthermore, CTGF effectively upregulated the activities of BMP-2/Smad pathway by interacting with BMP-2 directly.

Recently, the application of MSCs plays a crucial role in the field of regenerative medicine. Besides, bone marrow MSCs and adipogenic stem cells are regarded as two types of MSCs that are widely investigated in clinical practice [38]. It is of great clinical significance to further explore the underlying mechanism of ASCs. In addition, PDLSC, as a superior regenerative cell in periodontal tissue, has been reported to have multidifferentiation abilities [6, 39]. It is identified as an ideal cell type for exploring periodontal tissue as well as bone regeneration [40]. Thus, it is vital to elucidate the molecular mechanism of its multivalent differentiation.

It has been reported that CTGF is tightly associated with the repair of a variety of tissues including bone regeneration [41, 42]. Besides, previous studies have reported that CTGF is able to promote the differentiation of bone marrow MSCs into tendon fibroblasts [43]. Asano et al. discovered that CTGF effectively accelerates the tooth development of mice and also promotes the progression of PDL cells [33, 44]. Recent evidence suggests that the expression of CTGF in

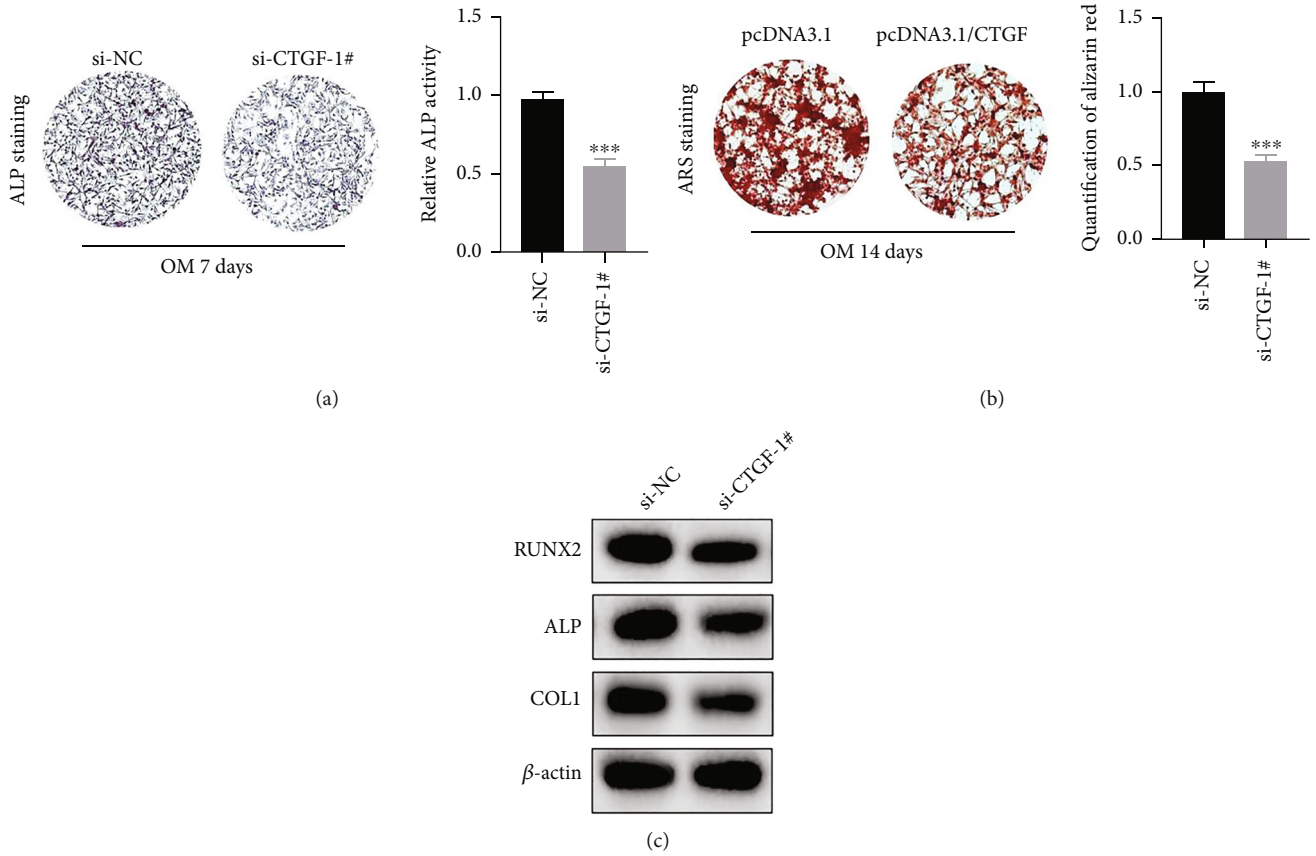

Figure 4: CTGF knockdown inhibits the osteoblast differentiation of PDLSCs. (a) Images of ALP staining and the activity of ALP (***$p < 0.001$). (b) Images of ARS staining and quantification of ARS (***$p < 0.001$). (c) The expression level of RUNX2, ALP, and COL1 detected via Western blotting analysis. ***$p < 0.001$ versus si-NC.

human PDL cells will be dramatically increased under tensile loading [34]. Nevertheless, the role of CTGF in the osteogenic differentiation of PDLSCs has not been expounded now. In this work, we carried out osteoblast induction in PDLSCs and revealed that the expression of CTGF was upregulated obviously with the process of induction. Meanwhile, interestingly, overexpressed CTGF notably increased the content of stemness-associated proteins including NANOG, SOX2, and CMYC but CTGF knockdown reduced the expression of NANOG, SOX2, and CMYC in PDLSCs. These findings implied that CTGF could ensure PDLSCs more differentiational potentials.

Additionally, emerging evidence revealed that the occurrence of oxidative stress is closely associated with PDLSC osteoblast differentiation [45], and CTGF has been proved to participate in oxidative stress in various biological progresses [46]. However, the detailed role of CTGF in PDLSCs remains unclear. LPS, as a cell wall component of Gram-negative bacteria, is a vital factor contributing to oxidative stress and periodontitis [47]. Moreover, SOD, GSH, and MDA is verified the crucial biomarker of oxidative stress [48, 49]. SOD clears away superoxide anion radical and maintain the balance between oxidation and antioxidation [50]. GSH serves as a scavenger to reduce the content of O_2 and H_2O_2. [51]. Besides, the accumulation of MDA also signifies the occurrence of oxidative stress [52]. Consistently, this work proved that LPS treatment decreased the production of SOD and GSH while increased MDA level in PDLSCs. Nevertheless, CTGF overexpression effectively reversed these effects of LPS, whereas CTGF knockdown further aggravated those in PDLSCs. These results indicated that CTGF exerted its role in PDLSCs might by inhibiting LPS-induced oxidative stress to some extent.

Subsequently, this work demonstrated that overexpressed CTGF notably increased the intensity of ALP and ARS staining, which is identified as the significant symbol of osteoblast differentiation [37]. Similar to previous investigation [53], the increased activity of ALP and upregulated expression of osteogenic proteins (RUNX2, COL1, and ALP) was observed after CTGF overexpression. Moreover, knockdown of CTGF exhibited opposite effects in the osteoblast differentiation of PDLSCs. These results preliminarily verified that CTGF promotes the osteoblast differentiation in PDLSCs.

It is well-known that BMPs exerted essential effects on the regulation of cascade signal associated with the progression of bone formation [25], and the role was modulated via Smad pathway [54]. Increasing evidence proved that CTGF regulates diverse biological progressions including cell viability and migration as well as differentiation by interacting with various growth factors or matrix proteins [55], which are vital for osteogenic differentiation. Previous investigation

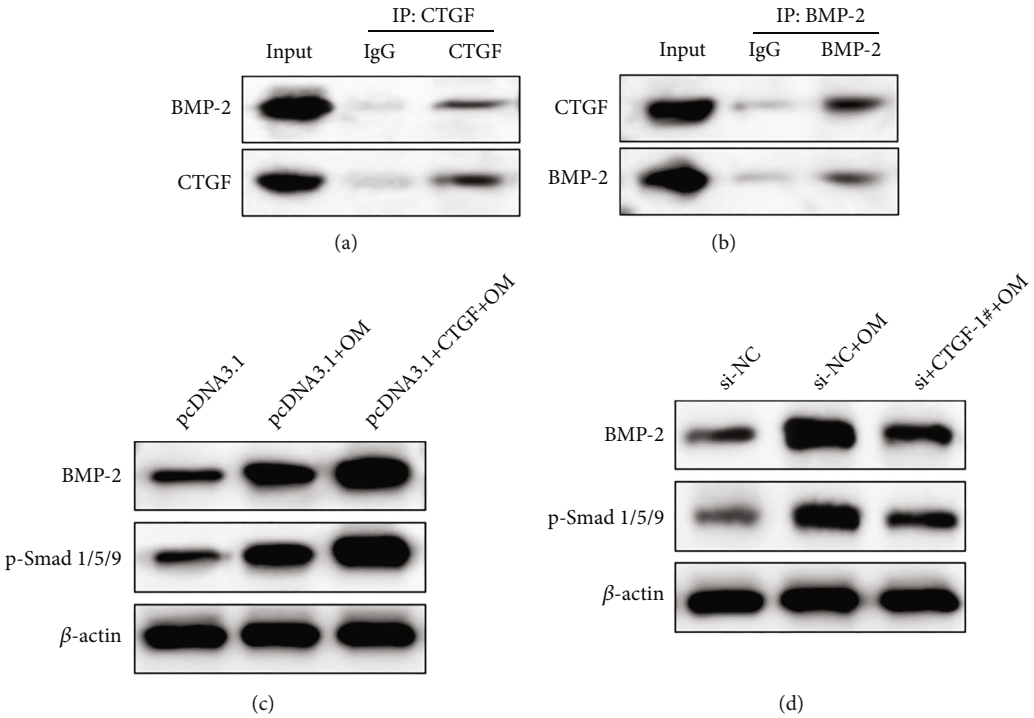

Figure 5: CTGF promotes the osteoblast differentiation in PDLSCs via the BMP-2/Smad pathway. (a, b) The interaction between CTGF and BMP-2 detected via Co-IP analysis. (c) The content of BMP-2 as well as p-Smad1/5/9 detected via Western blotting analysis. (d) The content level of BMP-2 as well as p-Smad1/5/9 detected via Western blotting analysis.

revealed that CTGF negatively regulated BMP-2 pathway in the osteoblast [35]. Maeda et al. found that CTGF exerted both antagonistic role and agonistic role for BMP-2 in chondrocyte [36]. Nevertheless, the effects of CTGF for BMP-2 in the osteogenic differentiation of PDLSCs remain unknown. In this study, we found that CTGF interacted with BMP-2 directly. Additionally, overexpression of CTGF increased the expression of BMP-2 and the phosphorylation of Smad1/5/9, while CTGF knockdown decreased the activity of BMP-2/Smad pathway effectively during the osteogenic induction in PDLSCs. The data suggested that CTGF dramatically promotes BMP-2/Smad signal in the osteoblast differentiation of PDLSCs.

However, there existed some limitations in our present study. For example, whether CTGF facilitates osteogenic differentiation *in vivo* needs to be further verified. Moreover, whether and how CTGF plays its inducible role in the osteoblast differentiation of PDLSCs by regulating oxidative stress needs to be further verified and investigated in the subsequent study. Furthermore, more experiments will be conducted to prove how CTGF regulates BMP-2/Smad signaling pathway in PDLSCs. Additionally, we mean to explore whether CTGF affect the other cascade signal mediated via BMP-2 in the subsequent study.

5. Conclusion

This investigation verified that CTGF facilitates the osteogenic differentiation of PDLSCs at least partly by activating BMP-2/Smad signaling pathway.

Data Availability

The data used to support the findings of this study are included within the article.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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Retraction

Retracted: The Correlation of PM2.5 Exposure with Acute Attack and Steroid Sensitivity in Asthma

BioMed Research International

Received 8 January 2024; Accepted 8 January 2024; Published 9 January 2024

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) Manipulated or compromised peer review

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] J. Luo, H. Liu, S. Hua, and L. Song, "The Correlation of PM2.5 Exposure with Acute Attack and Steroid Sensitivity in Asthma," *BioMed Research International*, vol. 2022, Article ID 2756147, 8 pages, 2022.

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Review Article

The Correlation of PM2.5 Exposure with Acute Attack and Steroid Sensitivity in Asthma

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Bronchial asthma is a common chronic inflammatory disease of the respiratory system. Asthma primarily manifests in reversible airflow limitation and airway inflammation, airway remodeling, and persistent airway hyperresponsiveness. PM2.5, also known as fine particulate matter, is the main component of air pollution and refers to particulate matter with an aerodynamic diameter of ≤2.5 μm. PM2.5 can be suspended in the air for an extensive time and, in addition, can contain or adsorb heavy metals, toxic gases, polycyclic aromatic hydrocarbons, bacterial viruses, and other harmful substances. Epidemiological studies have demonstrated that, in addition to increasing the incidence of asthma, PM2.5 exposure results in a significant increase in the incidence of hospital visits and deaths due to acute asthma attacks. Furthermore, PM2.5 was reported to induce glucocorticoid resistance in asthmatic individuals. Although various countries have implemented strict control measures, due to the wide range of PM2.5 sources, complex components, and unknown pathogenic mechanisms involving the atmosphere, environment, chemistry, and toxicology, PM2.5 damage to human health still cannot be effectively controlled. In this present review, we summarized the current knowledge base regarding the relationship between PM2.5 toxicity and the onset, acute attack prevalence, and steroid sensitivity in asthma.

1. Introduction

With increasing levels of smog in cities worldwide, the impact of air pollution on human health is attracting global attention. PM2.5, also known as fine particulate matter, is the main component of air pollution and refers to particulate matter with an aerodynamic diameter of ≤2.5 μm in the atmosphere [1]. PM2.5 can be suspended in the air for an extensive time and, in addition, can contain or adsorb heavy metals, toxic gases, polycyclic aromatic hydrocarbons, bacterial viruses, and other harmful substances. Moreover, PM2.5 can enter the bronchi and alveoli before finally entering the blood, causing damage to multiple organs, especially those of the respiratory and cardiovascular systems [2–5]. The Global Environment Outlook 5 released by the United Nations Environment Program in 2012 highlighted that the fine particles in air pollution cause more than two million deaths globally every year and cause huge economic losses. With the rapid development of economy and industry, China has become substantially affected by PM2.5 ambient air pollution. According to a study conducted in 2010 by the School of Public Health of Peking University, in only 4 cities (Beijing, Shanghai, Guangzhou, and Xi'an), 7,770 premature deaths were reported to be caused by PM2.5 pollution, associated with an economic loss of 6.17 billion Yuan [6]. Although various countries have implemented strict control measures, due to the wide range of PM2.5 sources, complex components, and unknown pathogenic mechanisms involving the atmosphere, environment, chemistry, and toxicology and medical professions, PM2.5 damage to human health still cannot be effectively controlled. Therefore, it is of great practical significance to prevent and control PM2.5 exposure.

Bronchial asthma is one of the most common chronic lung diseases. It is currently estimated that around 5%-16% of the global population suffers from asthma, and about 250,000 people die from asthma attacks every year. With gradual atmospheric deterioration due to pollution, the

morbidity and mortality rates of asthma are increasing [7]. Epidemiological studies have demonstrated that PM2.5 levels in the atmosphere are positively correlated with the incidence of asthma, the number of hospitalizations, and the number of emergency room admissions, prolonging the number of days patients spend in hospital and increasing hospitalization costs [8–10]. Glucocorticoids (GCs) are the first-line treatment for asthma; they have been shown to significantly reduce inflammation of the respiratory tract and ameliorate impaired lung function. Most patients have satisfactory treatment outcomes with GCs alone, or in combination with β-receptor agonists. However, clinical and scientific studies have demonstrated that exposure to pollutants such as fine particulate matter, ozone, and cigarettes can reduce the sensitivity of asthma patients to GCs and increase the amount of GCs required for asthma symptom control, resulting in increased occurrences of drug side effects and difficulties in the clinical treatment of asthma [11–13].

2. PM2.5 Exposure and Incidence of Asthma

PM2.5 exposure results in a chronic nonspecific inflammatory disease of the airway mediated by a variety of inflammatory cells dominated by mast cells, eosinophils, and T cells and their secreted cytokines, primarily manifesting as reversible airflow limitation, airway inflammation, airway remodeling, and hyperresponsiveness [14]. Studies have reported that inhalable particulate matter such as PM2.5 is capable of carrying a substantial quantity of allergens such as microorganisms, organic compounds, and metals, which have been shown to induce type I hypersensitivity and increase the risk of asthma [15]. A study by Carlsten et al. revealed that an interquartile range (IQR) of PM2.5 concentration at birth year of 4.1 $\mu g/m^3$ significantly increased the risk of developing asthma in children (OR, 3.1; 95% CI: 1.3-7.4) [16]. Moreover, PM2.5 exposure increases the risk of asthma in adults, and further, exposure of pregnant women to high levels of PM2.5 during pregnancy increases the risk of asthma in newborns [17, 18]. Conversely, the biological activity of PM2.5 has been demonstrated to aggravate pathological processes such as airway hyperresponsiveness and remodeling by promoting airway inflammation and oxidative stress. Overall, it is clear that when the atmospheric level of ambient PM2.5 rises sharply, the number of outpatients and hospitalizations for respiratory diseases such as asthma increases significantly [17].

2.1. PM2.5 Exposure Induces Acute Asthma Attacks. Acute asthma attack refers to the exacerbation of airway inflammation and airflow limitation in patients, during infection or on inhalation of allergens or air pollution. Acute asthma attack usually requires systemic glucocorticoid therapy [19]. Although most acute attacks are relieved by bronchodilator combined with hormone therapy, repeated asthma attacks can lead to aggravated and irreversible pathological pulmonary changes such as airway remodeling [20]. The most common cause of acute asthma attack is respiratory infection, but accompanied by the gradual deterioration of human living environments, air pollution has become another risk factor for acute asthma attack that cannot be ignored.

Inhalable particulate matter such as PM2.5 carries a large number of allergens that can induce type I hypersensitivity reactions; these include microorganisms, organic compounds, and metals. Organic matter (OM), black carbon (BC), and $SO4^{2-}$ were demonstrated to contribute more to the risk of asthma in early life when compared to other PM2.5 constituents. Among them, the effects of BC were only identified during pregnancy. Early-life exposures to ambient PM2.5, particularly OM, BC, and $SO4^{2-}$, are associated with an increased risk of childhood asthma [21]. In addition to being associated with asthma, PM2.5 exposure is an independent risk factor for acute asthma attacks. Epidemiological studies have revealed that elevation in ambient PM2.5 levels by 10 $\mu g/m^3$ resulted in increased risk of respiratory symptoms (cough, wheezing, or dyspnea) in asthmatic children by 21% and an increase in the rate of visits for asthma attacks in adults by 13.75% [22]. The potential for PM2.5 to induce asthma attacks is greater than that of PM10 and ozone [22], and its effect during warm seasons (20.09% increase in asthma visitation rate) is significantly higher than during cold seasons (2.39%) [23]. Since children have reduced airway defense mechanisms and a higher level of inhaled gas per kilogram of body weight than adults, the effect of elevated ambient PM2.5 concentrations on childhood asthma attacks appears to be more pronounced, with a higher rate of exacerbations reported in male children than in female children [17, 24–26]. Studies by Hua et al. [27] and Xie et al. [28] demonstrated that elevated PM2.5 concentrations are also closely related to acute asthma attacks in the Chinese population.

Evidence generated by basic research further supports the epidemiological view that PM2.5 exposure induces asthma attacks, in addition to causing phenotypic changes in alveolar macrophage populations (upregulation of CD14, CD11b, and HLA-DR expression) and their increased synthesis [29]. Moreover, PM2.5 has been reported to stimulate the synthesis and release of macrophage and epithelial cells in the lung. Interleukin- (IL-) 6 and IL-8 exert chemotactic effects upon neutrophils [30, 31]. Intraperitoneal injection or intranasal instillation of PM2.5 was reported to induce eosinophilic infiltration in the airways, elevated Th2 cytokines in bronchoalveolar lavage fluid, and airway hyperresponsiveness in mice with allergic asthma phenotypes [32, 33]. Furthermore, He et al. identified that airway instillation of PM2.5 induced neutrophilic alveolar and bronchitis in mice, while combined ovalbumin (OVA) and PM2.5 airway instillation resulted in massive lung eosinophilic granulocyte infiltration and increased expression of Th2 cytokines, such as IL-13 and IL-4, which caused mice to develop symptoms similar to acute asthma attack [34]. In asthma models that have been sensitized by OVA, PM2.5 exposure induced inflammatory cell infiltration and increased the levels of inflammatory factors, goblet cell metaplasia, and changes in lung ultrastructure [35, 36].

2.2. The Role of Oxidative Stress in PM2.5-Induced Asthma Attack. During normal physiological processes, reactive

oxygen species (ROS) and reactive nitrogen species (RNS) produced by the body are absorbed by glutathione (GSH) before, and they are cleared by antioxidant systems such as superoxide dismutase (SOD) [37, 38]. Conversely, during events initiated by harmful stimuli in vitro and in vivo, ROS and RNS are generated in large quantities, exceeding the scavenging ability of antioxidant systems. This process often leads to tissue damage and is known as oxidative stress [37]. Oxidative stress is broadly involved in various pathophysiological processes such as aging, inflammation, and tumorigenesis [39]. A large body of evidence suggests that oxidative stress plays a key role in inducing and exacerbating asthma attacks. As such, it is considered that concentrated ROS can lead to DNA fragmentation and oxidation of cell membrane lipids and proteins, events that directly damage lung epithelial cells and vascular endothelial cells, increasing the permeability of the air-blood barrier and causing the contraction of airway smooth muscle cells to induce asthma attacks [40]. Moreover, oxidative stress mediates the activation of signaling pathways such as nuclear factor kappa B (NF-κB) and PI3K/Akt in alveolar macrophage and lung epithelial cells, which release a large number of inflammatory mediators resulting in increased airway mucus secretion, airway remodeling, and chronic inflammation. Persistent airway inflammatory response activation results in the generation of ROS and the formation of a positive feedback loop, which together promote the recurrence and progression of asthma.

The induction of oxidative stress is the initiating factor and core link between PM2.5 and respiratory toxicity. Polycyclic aromatic hydrocarbons (PAHs), carbon particles, and inorganic metal ions in PM2.5 have been reported to induce the production of reactive oxygen species (ROS) intracellularly. Among the major constituents of PM2.5, organic matter originating indoors contributed primarily to oxidative potential. Reducing the oxidative potential of PM2.5, particularly by reducing the indoor-generated organic matter constituents of PM2.5, may be used as a targeted control strategy in asthma management [41]. Clinical evidence implicates PM2.5 in the induction of acute asthma attacks by mediating oxidative stress. The activity of paraoxonase in the serum of patients with asthma is decreased, while the activity of myeloperoxidase is increased. Moreover, antioxidant capacities are lower in asthma patients than in the normal population, making oxidative stress more likely after PM2.5 exposure, which in turn results in infiltration of neutrophils within the respiratory tract [42]. 8-Isoprostaglandin levels in exhaled breath condensate (EBC) in asthmatic children were positively correlated with PM2.5 levels at home [43], and after 1 hour exposure to PM2.5 in volunteers with mild to moderate asthma, the nitrite content in exhaled condensate was significantly increased compared to when breathing clean air, an effect that lasted for 24 hours. Simultaneously, the patient experienced acute exacerbation symptoms [44]. Furthermore, Weichenthal et al. [45] demonstrated that differences in oxidative capacity were the reason for the distinct effects of PM2.5 on asthma attack sufferers in different cities, while Yang [46] and Bates et al. [47] identified that the occurrence or attack of asthma was more closely related to the oxidative capacity of PM2.5.

2.3. PM2.5 and MicroRNA. MicroRNAs (miRNAs, miRNAs) are a class of endogenous, 18-25 nt, noncoding small RNA molecules that are widespread and highly conserved in eukaryotic cells. miRNA does not have an open reading frame; it induces degradation of target mRNA or inhibits its transcription by binding to the 3′ untranslated region (3′ UTR), playing an important role in gene regulation [48]. miRNAs regulate about 1/3 of human gene expression and have been implicated in pathological and physiological processes such as inflammation, oxidative stress, stem cell development, tumor growth, and metastasis [49–51]. Recently, studies have further confirmed that microRNAs play an important regulatory role in PM2.5-mediated toxicity (Figure 1). For example, PM2.5 exposure caused downregulation of 138 miRNAs, including miR-182 and miR-185, in mouse embryonic NIH3T3 cells. Moreover, PM2.5 resulted in downregulation of miR-182 and miR-185, leading to upregulated expression of targets SLC30A1, SERPINB2, and AKR1C1 and eventually inducing NIH3T3 cell malignant fibrosarcoma in nude mice [52]. Using bronchial brushing, Rider et al. obtained airway epithelial cells from 13 volunteers before and after PM2.5 exposure and compared the changes in their cellular transcriptome. The results revealed that the expression of various miRNAs and mRNAs involved in immune and inflammatory responses was significantly altered [53]. Let-7a was downregulated in airway epithelial cells in response to PM2.5 exposure, leading to an increase in the expression of Arginase 2, which aggravated oxidative stress-induced cellular injury [54]. Furthermore, Song et al. reported that PM2.5 exposure downregulated miR-331 expression in the human airway epithelial cell line Beas 2B, resulting in increased expression of NF-κB kinase beta (IKK-β) and aberrant activation of NF-κB [55]. In addition, some studies have demonstrated that abnormal expression of miRNA is involved in the process wherein PM2.5 promotes the occurrence and development of lung cancer [56–58].

3. PM2.5 Exposure and Asthma Treatment

In addition to being attributable to the occurrence and development of asthma, inhalable particulate matter such as PM2.5 further impacts the treatment of asthma. Both Slaughter et al. [12] and Gent et al. [59] identified that PM2.5 not only increased the risk of asthma but also increased the use of inhaled drugs to rescue illness. von Klot et al. [60] reported that the concentration of inhalable particulate matter of various diameters, including PM2.5, was related to the amount of inhaled GCs used by asthma patients; this was the case whether it was the PM2.5 concentration on the day or the average PM2.5 on the 5th or 14th day. High PM2.5 concentrations were further shown to significantly increase the level of GCs required. Another study revealed that PM2.5 concentration was related to the amount of oral GCs required by asthmatic patients, especially adult patients [61]. Furthermore, some studies have demonstrated that smoking, as an important source of PM2.5, can reduce the activity of histone deacetylase 2 (HDAC2) and mediate hormone resistance, which is an important factor affecting the sensitivity to GCs in respiratory diseases such as asthma and COPD. However, there is

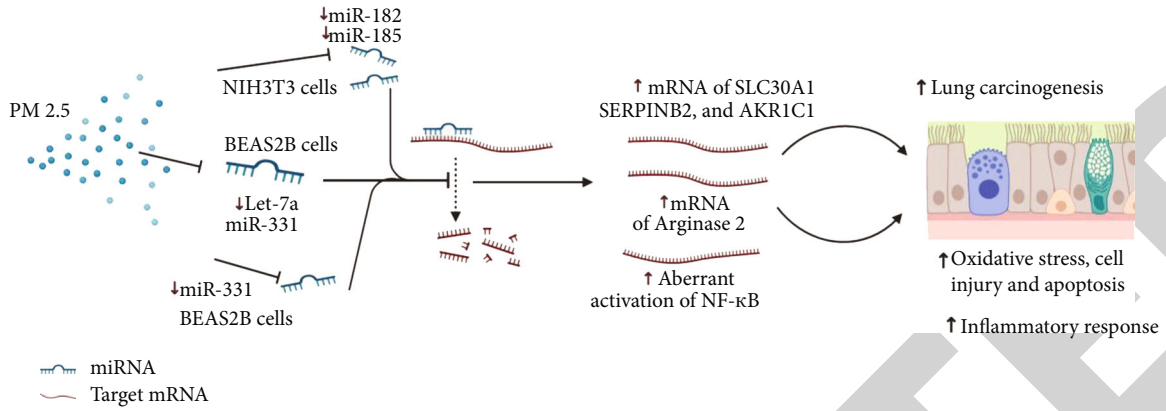

Figure 1: PM2.5 caused abnormal gene expression through the downregulation of miRNAs. PM2.5 exposure caused a lot of downregulation of miRNAs, such as miR-182, miR-185, Let-7a, and miR-331, which usually induce degradation of target mRNA, resulting in inflammation, oxidative stress, cell injury, and carcinogenesis.

no direct clinical and basic research evidence to confirm the effect and mechanism of PM2.5 on the sensitivity of asthmatic GCs. Therefore, there is an urgent requirement to further combine clinical and basic medicine to further verify and interpret the effect and mechanism of PM2.5 on GC sensitivity in animal models and at cellular and molecular levels. Studies of this kind may provide theoretical references for the treatment and intervention of asthma patients under environmental deterioration.

Although PM2.5 is associated with a variety of diseases, the pathological mechanism by which PM2.5 causes tissue damage has not been fully described. Existing evidence demonstrates that the main components of PM2.5, carbon particles, metal ions, and organic aromatic hydrocarbons, not only cause oxidative damage to cell membranes and DNA but also trigger downstream signaling pathways. Such pathways include NF-κB, mitogen-activated protein kinase (MAPK), and PI3K [62–64], which act to potentiate inflammation and apoptosis in tissues.

In recent years, studies have confirmed that the three main subfamilies of the MAPK family, ERK, c-Jun N-terminal kinase (JNK), and p38 MAPK, are involved in PM2.5-mediated inflammatory response and cell damage (Figure 2). For example, in lung epithelial cells, RNA-sequencing identified that MAPK pathway-related genes are activated and are closely related to inflammatory damage in cells. It was reported that PM2.5 exposure increased the levels of phosphorylation of ERK, JNK, and p38 MAPK in cardiomyocyte H9c2 cells, and p38 MAPK promoted inflammation. Meanwhile, ERK protected cells from PM2.5-induced apoptosis. Corsini et al. reported that PM2.5 induced the release of inflammatory factor IL-8 from lung epithelial cells (A549) and macrophage (THP-1). Conversely, inhibition of p38 MAPK attenuated PM2.5-mediated IL-8 release, suggesting that p38 MAPK is an important pathway that mediates inflammatory response to PM2.5. In addition, activation of the MAPK pathway in the myocardium and airway epithelium was observed in mouse and rat models of PM2.5 exposure.

As a steroid hormone, GCs bind to the receptor GRα on the target cell membrane (GC receptors are divided into GRα and GRβ, and GRβ does not regulate gene transcription). GRα is activated, and transfer into the nucleus causes it to bind to DNA. Glucocorticoid response elements (GRE) bind to other transcription factors to form a transcription initiation complex that regulates the expression of downstream target genes [65]. Any abnormality in this link can cause asthma GC resistance. A small number of asthmatic patients are resistant to GC treatment due to the reduced availability of GCs caused by genetic susceptibility [66] or the reduced capacity of GRα in binding GCs [67]. The responses of asthma patients to GC therapy are extremely complex due to the joint participation of acquired factors such as environment, infection, and the multiple signaling pathways mediated by a plethora of cytokines. Among them, MAPK activation-mediated GRα intranuclear transport disorder plays an extremely important role in the resistance of asthmatics to GC treatment.

MAPK can regulate the binding ability and stability of GRα and GCs and the ability of GRα to transfer into the nucleus to form a transcription complex, by specifically phosphorylating some serine residues in GRα. For example, phosphorylation of Ser226 of GRα by JNK leads to enhanced GRα translocation out of the nucleus [68]. Meanwhile, phosphorylation of Ser211 by p38 MAPK inhibits the translocation of GRα into the nucleus [69], causing GC resistance. In peripheral blood monocytes (PBMC) of GC-resistant patients, the abnormal activation of the p38 MAPK signaling pathway is associated with the decreased efficacy of dexamethasone in inhibiting cytokine release. However, administration of the p38 MAPK inhibitor can restore the effect of dexamethasone on lipopolysaccharide-induced inhibition of IL-8 release [70]. Chang et al. [71, 72] demonstrated the occurrence of excessive activation of p38 MAPK in the lung smooth muscle cells of patients with severe asthma, and the inhibition of p38 MAPK enhanced the efficacy of GCs in reducing tumor necrosis factor alpha- (TNF-α-) mediated inflammatory response. In addition, the activation of JNK was also significantly increased in the PBMC and bronchial biopsy tissues of GC-resistant patients, and administration of high-dose oral corticosteroids did not reduce its activation level [73].

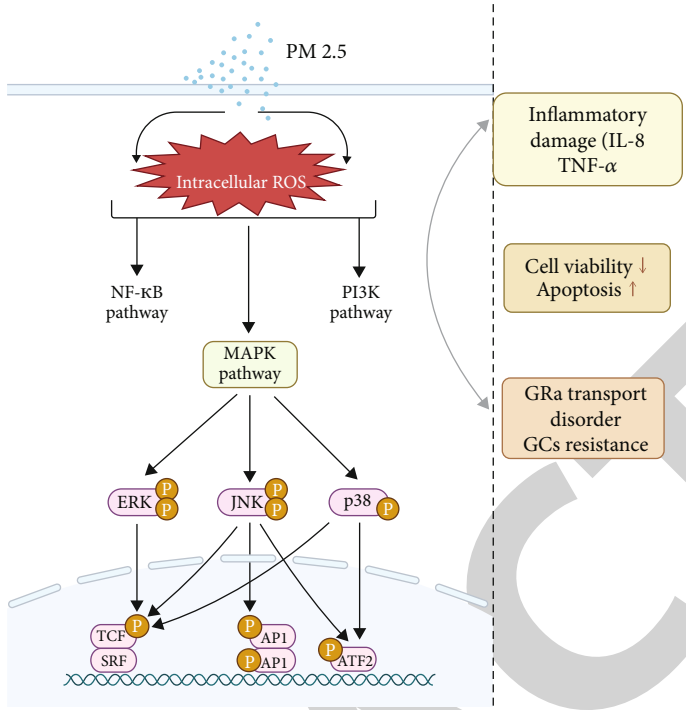

Figure 2: Modeling the mechanism by which PM2.5 exposure caused MAPK pathway activation and GCs resistance. PM2.5 mediates abnormal activation of downstream signaling pathways, including NF-κB, MAPK, and PI3K pathways, leading to inflammatory damage, decreased cell viability, increased cell death, and GC treatment resistance, resulting in increased severity of asthma.

Although PM2.5 has been confirmed to be associated with MAPK activation, due to research on the pathogenesis of PM2.5 being in its early stages, there are relatively few related reports. It will be of clinical importance to determine the role that MAPK plays in PM2.5-related diseases. In particular, the regulatory mechanism of PM2.5-mediated MAPK activation remains to be revealed by further research.

4. Perspective

The studies described herein suggest that PM2.5 exposure aggravates the progression of asthma by mediating oxidative stress. However, the specific regulatory mechanisms and networks by which PM2.5 mediates oxidative stress are not yet fully understood. Research on the effects of the mechanism of PM2.5 toxicity will pave the way for development of targeted drugs. Importantly, therapeutic approaches derived from natural chemicals and novel drug delivery systems shed light on the prevention of PM2.5 toxicity in the respiratory system [74–76]. Coordinating disparate disciplines within the study of PM2.5 toxicity mechanisms will provide comprehensive cross-over advantages and has important practical significance for the formulation of prevention and control intervention strategies for the asthmatic population exposed to PM2.5.

Data Availability

The data used to support the findings of this study are included within the article.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Jingjing Luo and Han Liu contributed equally.

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Research Article

Melatonin Ameliorates the Progression of Alzheimer's Disease by Inducing TFEB Nuclear Translocation, Promoting Mitophagy, and Regulating NLRP3 Inflammasome Activity

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Background. The NLRP3 inflammasome is overactivated in the brains of APP/PS1 transgenic mice and AD patients, and mitophagy has an obvious negative regulatory role on NLRP3 inflammasome activation. The protective effect of melatonin in AD may be related to the regulation of mitophagy and NLRP3 inflammasome activity. TFEB plays a critical role in maintaining autophagy/mitophagy. Studies have found that TFEB plays a protective role in AD. *Methods*. APP/PS1 transgenic mice were given melatonin in their drinking water for 3 months. Compared with mice without melatonin treatment, the mice given melatonin showed changes in the following features: (1) cognitive function, (2) mitophagy-related proteins in the brain, (3) ROS, (4) NLRP3 inflammasome and related proteins and the concentrations of inflammatory cytokines, and (5) Aβ deposition. In *in vitro* experiments, effects of melatonin on mitophagy, NLRP3 inflammasome activity, and TFEB in SH-SY5Y cells with A$\beta_{25\text{-}35}$ were observed. TFEB knockdown was implemented in combination with A$\beta_{25\text{-}35}$ and melatonin treatment, and the expressions of TFEB, Parkin, p62, IL-1β, caspase-1, ROS, and IL-18 were explored. *Results*. Melatonin improved cognitive function in APP/PS1 transgenic mice and decreased ROS and senile plaques. Melatonin promoted mitophagy in SH-SY5Y cells with A$\beta_{25\text{-}35}$ and APP/PS1 transgenic mice. NLRP3 inflammasome activity was inhibited, and the concentrations of IL-18 and IL-1βwere clearly reduced. Compared with C57/BL6J mice, the amount of TFEB in the brain nucleus of APP/PS1 transgenic mice was decreased. Melatonin treatment increased the nuclear translocation of TFEB in SH-SY5Y cells. TFEB knockout was implemented in combination with A$\beta_{25\text{-}35}$ and MT treatment; the expressions of Parkin, p62, caspase-1, IL-1β, IL-18, and ROS were accelerated. *Conclusions*. Melatonin promotes mitophagy by inducing TFEB nuclear translocation, downregulates NLRP3 inflammasome activation, and exerts protective effects in SH-SY5Y cells and APP/PS1 transgenic mice.

1. Background

Alzheimer's disease (AD) is one of the most common diseases closely related to age, is also known as senile dementia, occurs in the elderly and presenile patients, and is characterized by behavioural disorder and progressive cognitive impairment. To date, the pathogenesis and aetiology of AD remain unknown, and there is no effective treatment for AD. Hence, further research on the pathogenesis and aetiology of AD is urgently needed to aid the identification of treatment methods for AD treatment.

A persistent excessive inflammatory response plays a key role in the pathophysiological mechanisms of AD [1]. The microglial-mediated inflammatory response is a focus of AD research. Microglia play a vital role in Aβ clearance.

Pattern-recognition receptors (PRRs) have the capacity to recognize foreign stimuli and sense damage-specific proteins in the body. NLRs (NOD-like receptors) are PRRs that are localized in the cell and can recruit precursors of procaspase-1 directly or through apoptosis-associated speck-like protein containing a CARD (ASC) to form a protein complex of inflammasomes. The assembly of the inflammasome can convert procaspase-1 into caspase-1, which converts pro-IL-18 and IL-1β into mature IL-18 and IL-1β in turn [2]. The best characterized inflammasome is

Table 1: Primers for real-time quantitative PCR.

	Forward	Reverse
Mouse β-actin	GGCTGTATTCCCCTCCATCG	CCAGTTGGTAACAATGCCATGT
Mouse IL-1β	GCAACTGTTCCTGAACTCAACT	ATCTTTTGGGGTCCGTCAACT
Mouse IL-6	CTGCAAGAGACTTCCATCCAG	AGTGGTATAGACAGGTCTGTT
Mouse IL-18	GACTCTTGCGTCAACTTCAAGG	CAGGCTGTCTTTTGTCAACGA
Human β-actin	CATGTACGTTGCTATCCAGGC	CTCCTTAATGTCACGCACGAT
Human IL-1β	GTCGGAGATTCGTAGCTGGAT	CTCGCCAGTGAAATGATGGCT
Human IL-6	TGAGGAGACTTGCCTGGTGAA	CAGCTCTGGCTTGTTCCTCAC
Human IL-18	TCTTCATTGACCAAGGAAATCGG	TCCGGGGTGCATTATCTCTAC

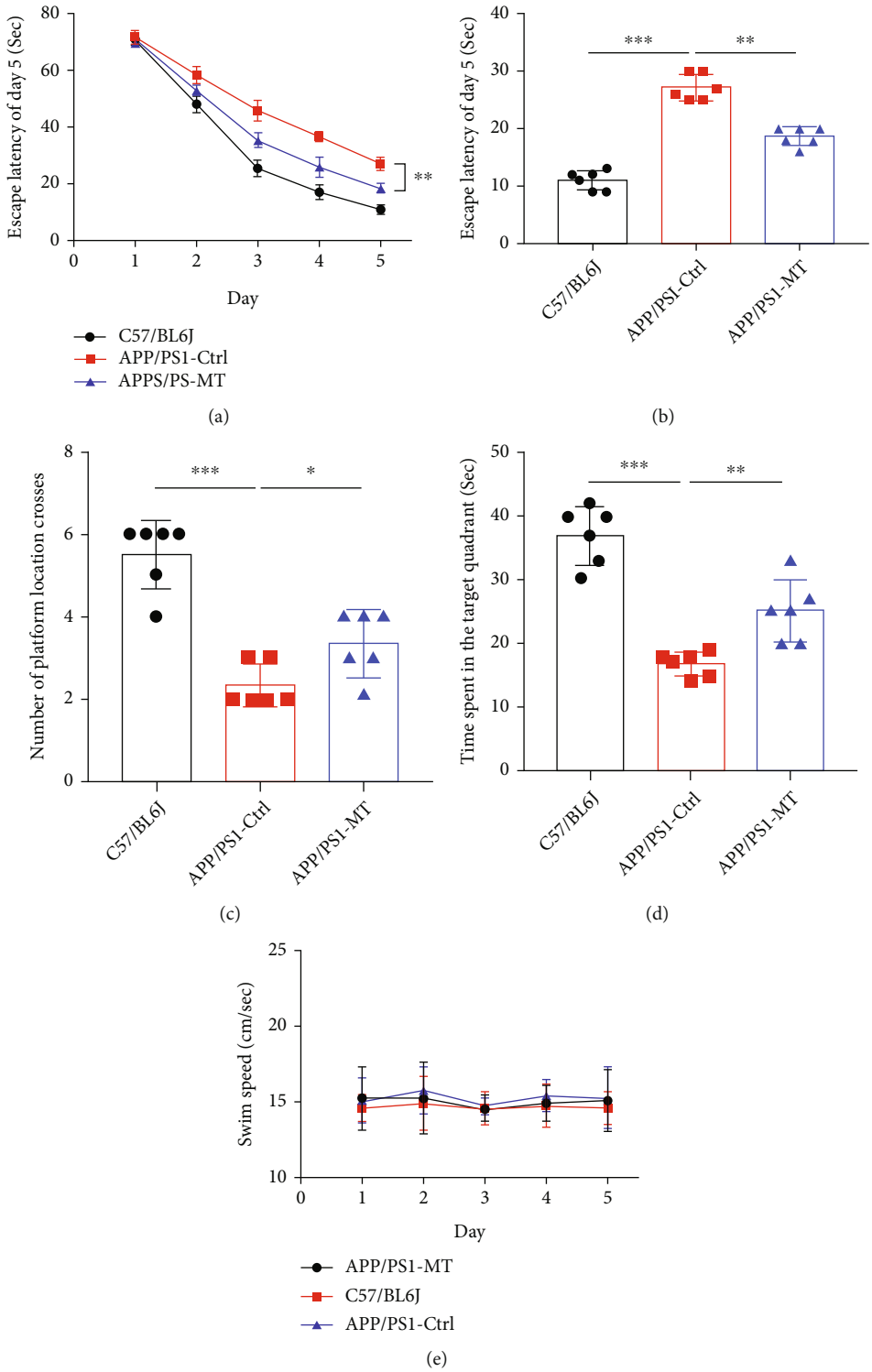
the NLRP3 inflammasome. Mitochondria are essential for NLRP3 inflammasome activation, and signals from abnormal mitochondrial function, such as phospholipid externalization, oxidized mtDNA, and ROS, can intervene in the activation of NLRP3 [3]. Kirchmeyer et al. [4] found that caspase-1, ASC, and NLRP3 were in brain microglia, indicating that the activation of the NLRP3 inflammasome in the brain is mainly in microglia. Aβ can stimulate IL-1β production in microglia [5]. IL-1β in AD patients' cerebrospinal fluid, brain tissue, and peripheral blood is sharply accelerated [6]. Caspase-1 and IL-1β are highly expressed in brain tissues or neurons of AD transgenic mice treated with Aβ [7], especially around Aβ plaques [8]. IL-1β plays a key role in the pathophysiology of AD, and is a main effector of the activation in the NLRP3 inflammasome. Halle et al. [9] found that Aβ promotes microglial lysosome destruction and cathepsin B release, which activates the NLRP3 inflammasome and further leads to microglial secretion of IL-1β, inducing an inflammatory response and continuous overactivation of microglia via downstream signal transduction pathways. These studies suggest that the NLRP3 inflammasome is overactivated and is the core mechanism of inflammation in AD.

Overactivation of the NLRP3 inflammasome is related to the mitochondrial damage and abnormal mitophagy. Mitochondria play an important role in eukaryotic cells by participating in biological oxidation and energy conversion and participate in multiple biological processes, such as intracellular homeostasis, proliferation, ageing, and cell death. Asymmetric division of mitochondria can lead to weakening or disappearance of mitochondrial membrane potential or depolarization of mitochondria; various stimuli both *in vitro* and *in vivo*, such as Aβ oligomers, can also cause pathological depolarization of mitochondria. These depolarized mitochondria can be recycled through mitochondrial fusion or through selective autophagy, which is called mitophagy. When the mitochondrial membrane is damaged, PINK1 recruits Parkin into mitochondria to facilitate mitophagy. This decrease in mitochondrial clearance can lead to the accumulation of damaged mitochondria, causing mitochondrial dysfunction, and eventually leading to neurodegenerative diseases. Mitophagy plays a vital role in the pathogenesis of AD, and the number of normal mitochondria in the hippocampal neurons of AD patients is significantly reduced.

Mitochondrial injury plays a key role in NLRP3 inflammasome activation, and as an important mechanism of clearing damaged mitochondria, mitophagy plays a key regulatory role in NLRP3 inflammasome activation. Zhong et al. discovered that when cells undergo inflammatory damage, the increased P62 content is transferred to damaged mitochondria, where Parkin ubiquitination induces mitochondrial autophagy [10]. Consistent with this finding, Kim et al. also observed an increase in P62 in NLRP3 inflammasome-activated cells and transfer of P62 to impaired mitochondria to induce mitochondrial autophagy [11].

Melatonin (N-acetyl-5-methoxy-tryptamine), a neurohormone from the pineal gland, participates in the regulation of various physiological functions. In disease and ageing, decreased melatonin levels lead to abnormal physiological functions. Melatonin reduction in elderly individuals may itself be an important factor leading to neurodegenerative disease in the elderly and is considered to be one of the main causes of AD [12]. Melatonin can prevent the increase of lipid peroxidation, free radical production, oxidative protein damage, and oxidative DNA damage in AD and decrease ATP production. Melatonin plays a protective role by improving mitophagy, which has been demonstrated in models of many diseases other than AD. Ma et al. [13] found in animal models of atherosclerosis that melatonin prevents atherosclerosis progression by inducing Parkin signalling pathway-mediated mitophagy and attenuating NLRP3 inflammasome activation. Cao et al. [14] found that mitophagy mediated by melatonin protected the early brain injury after subarachnoid haemorrhage by inhibiting NLRP3 inflammasome activation. However, the effect of melatonin on mitophagy in AD has not been reported.

Autophagy/mitophagy is regulated by a series of complex signalling molecules, among which transcription factor EB (TFEB) is the main regulator. TFEB is a member of the MiT transcription factor family that promotes mitophagy via the regulation of autophagosome-lysosomal fusion and autophagosome formation [15]. TFEB, a transcription factor with a BHLH-ZIP structure [16], binds to promoter motifs or CLEAR elements to regulate lysosomal gene expression [17]. Normally, TFEB is located in the cytoplasm. Under



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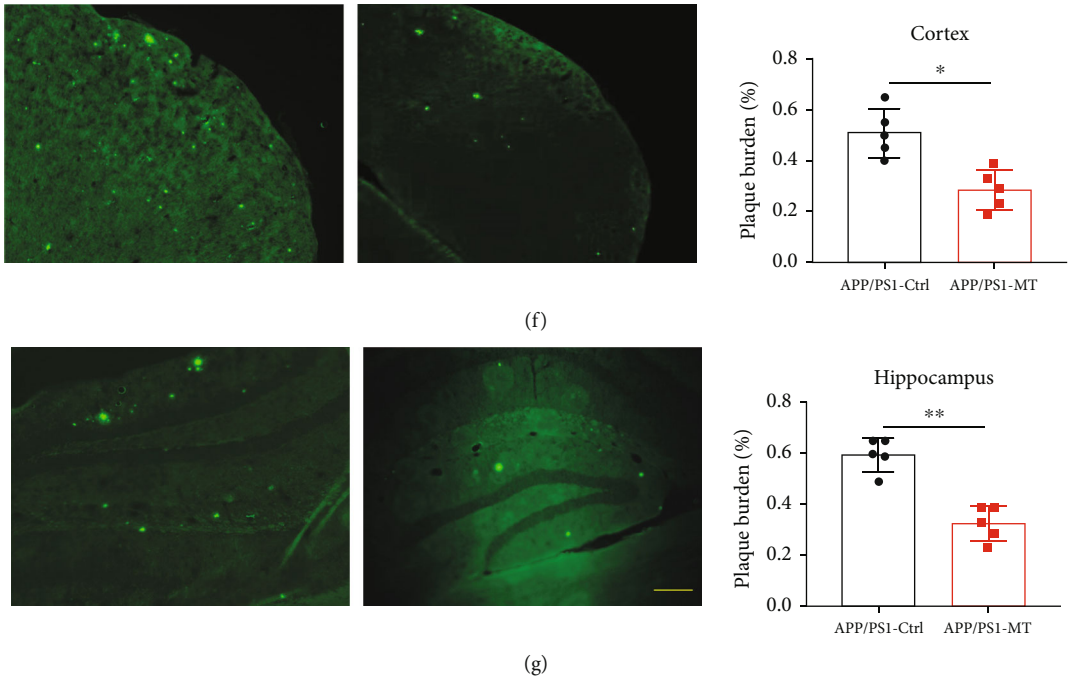

Figure 1: (a) Compared with placebo control APP/PS1 transgenic mice, the escape latency of the melatonin (MT) intervention group was shortened. (b) The escape latency was significantly different on day 5. (c) Compared with placebo control APP/PS1 transgenic mice, the number of platform crossings significantly increased in the MT intervention group. (d) The time in the target quadrant of the MT intervention group was significantly increased. (e) There was no significant statistical difference in swimming speed between the placebo control and MT intervention groups. (f) The number of senile plaques in the cerebral cortex was measured by thioflavin sulfur staining in APP/PS1 transgenic mice with TM treatment. The relative plaque burden ratio is shown on the left. (g) The number of senile plaques in the hippocampus was measured by thioflavin sulfur staining in APP/PS1 transgenic mice with TM treatment. The relative plaque burden ratio is shown on the left. $n = 5$ in each group. Data are presented as mean ± SEM. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

stress conditions, such as starvation and lysosomal dysfunction, TFEB can be translocatesd to the nucleus to facilitate the transcription of target genes. TFEB-mediated mitophagy has been found to improve the pathological changes and cognitive function of AD [18].

In this article, we observed the effects of melatonin on the cognitive function of APP/PS1 transgenic mouse models, mitophagy, the NLRP3 inflammasome and related regulatory proteins, and TFEB.

2. Methods

2.1. Animals and Drug Treatment. The experimental animals were C57/BL6J mice and APP/PS1 transgenic mice with C57/BL6J as the genetic background. All 30 mice were male to eliminate possible gender influence. The experimental animals were obtained from Beijing HFK Bio-Technology Co., Ltd.

The experimental animals were distributed into 3 groups with 10 mice in each group. The mice in each group were fed from 6 months to 9 months of age. C57/BL6J mice were fed double steamed water; APP/PS1 transgenic mice in the placebo group were fed double steamed water. APP/PS1 transgenic mice in the melatonin intervention group were fed double steamed water plus melatonin. The mice drank 5 mL water on average every day, and the estimated daily intake of melatonin was approximately 0.5 mg. All mice were subjected to a water maze test. The mice in each group were randomly distributed into two groups with 5 mice in each group. One group was used for brain harvesting by cardiac perfusion, and the other group was used for fresh brain tissue harvesting.

2.2. Morris Water Maze. The Morris water maze test was carried out to evaluate the spatial memory and learning of mice. The same conditions were used around the water maze to prevent the influence of environmental changes on the memory reference of the mice and to prevent interference from other objects and the video tracking system of the experimenters. Lamps were placed around the water maze to provide light, and the water temperature was controlled at 22 ± 1°C. The first day was the adaptation period for the mice. The mice to be tested were successively put into the water maze without a platform for approximately 1 minute of swimming practice. Days 2-6 of the water maze were the learning period, which was mainly used to evaluate the learning ability of the mice. At this stage, the platform was put into the water maze in the SW (southwest) direction. The camera was set 2 m above the pool, and software was used to record the mouse position, swimming path, and time required to reach the platform position (escape latency). The seventh day was the exploratory period. In the exploration experiment, the platform was moved away, and the mice were introduced into the water in a new direction, NE (northeast). The mice needed to find the original platform

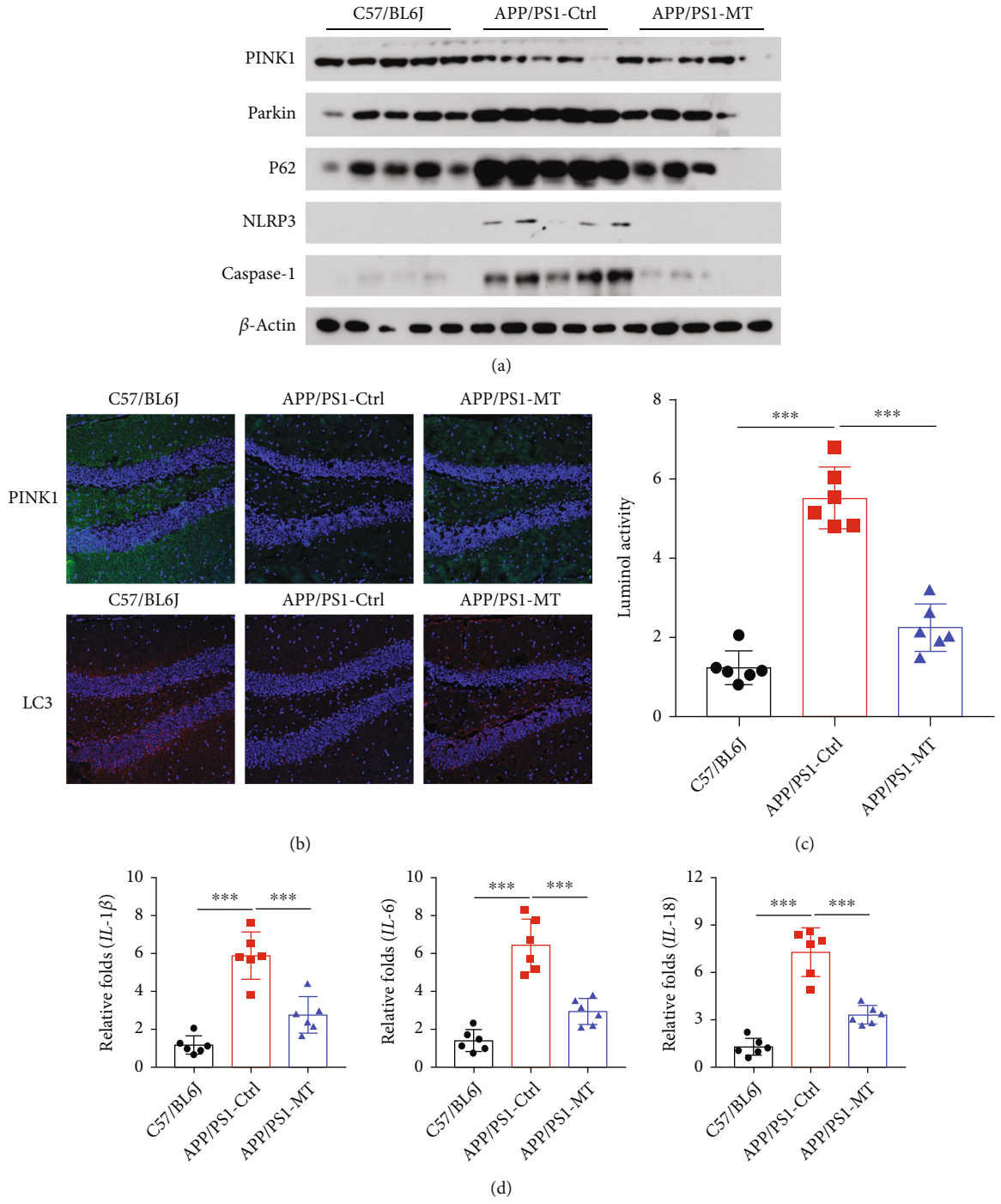

Figure 2: (a) The levels of mitophagy-related proteins PINK1, Parkin, and p62 and the inflammasome-related protein caspase-1 and NLRP3 were detected by western blotting between placebo control and MT intervention groups. (b) Immunofluorescence method was used to detect the mitophagy-related proteins PINK1 and LC3. (c) The oxidative activated oxygen (ROS) of animal tissue was detected by luminol chemiluminescence kit between placebo control and MT intervention groups. (d) Expression of IL-1β, IL-6, and IL-18 mRNA was detected by qRT-PCR between placebo control and MT intervention groups. Data are presented as mean ± SEM. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

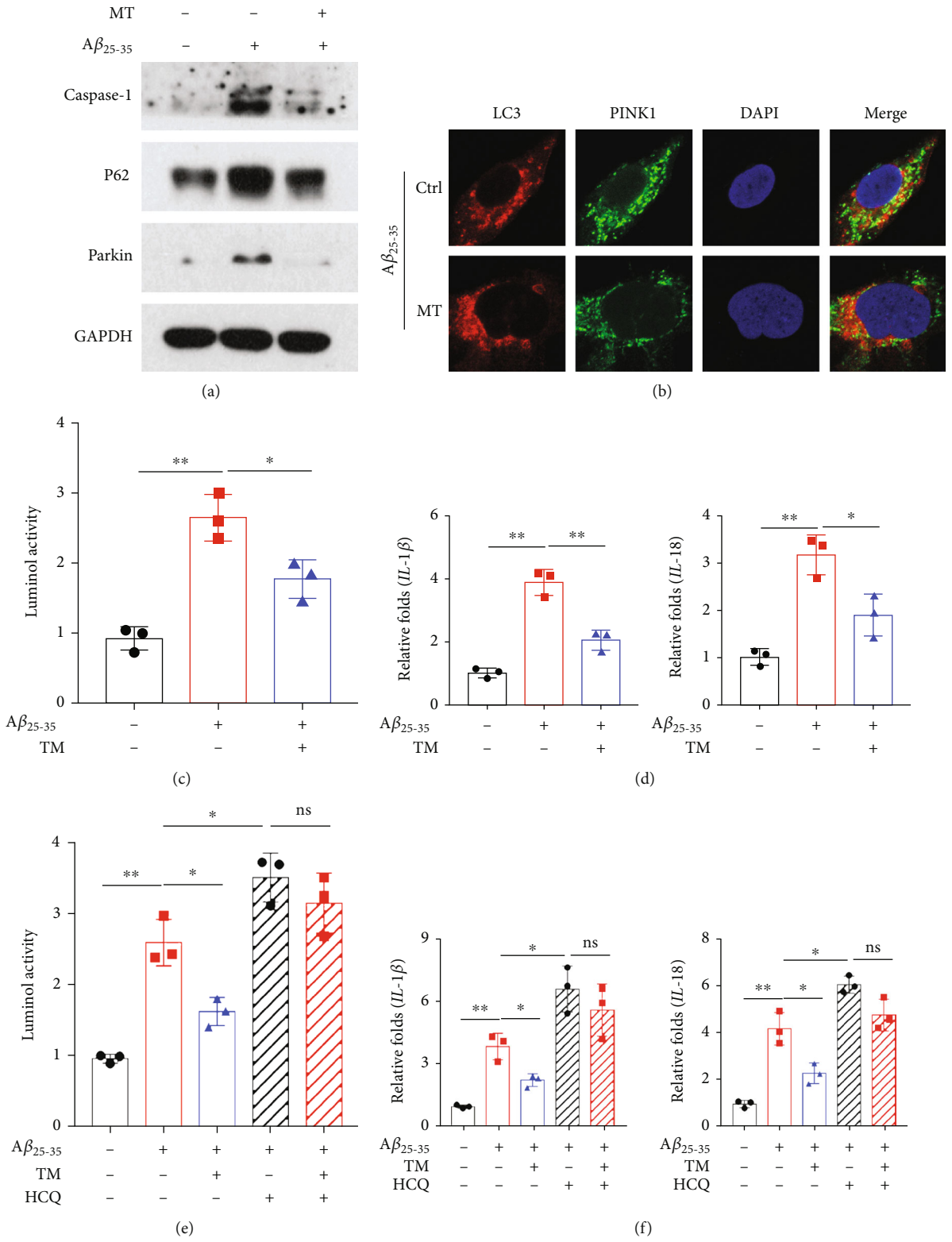

Figure 3: The AD cell model was constructed in SH-SY5Y cells with A$\beta_{25\text{-}35}$ treatment. (a) The levels of mitophagy-related proteins Parkin and p62 and the inflammasome-related protein caspase-1 were detected by western blotting with the MT treatment. (b) The colocalization between LC3 and PINK1 was detected by immunofluorescence with the MT treatment. (c) The lever of ROS was detected by luminol chemiluminescence kit with the MT treatment. (d) Expression of IL-1β and IL-18 mRNA was detected by qRT-PCR with the MT treatment. (e) Combined with MT and hydroxychloroquine (HCQ), the lever of ROS was detected by the luminol chemiluminescence kit. (f) Combined with MT and HCQ, the expression of IL-1β and IL-18 mRNA was detected by qRT-PCR. Data are presented as mean ± SEM. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

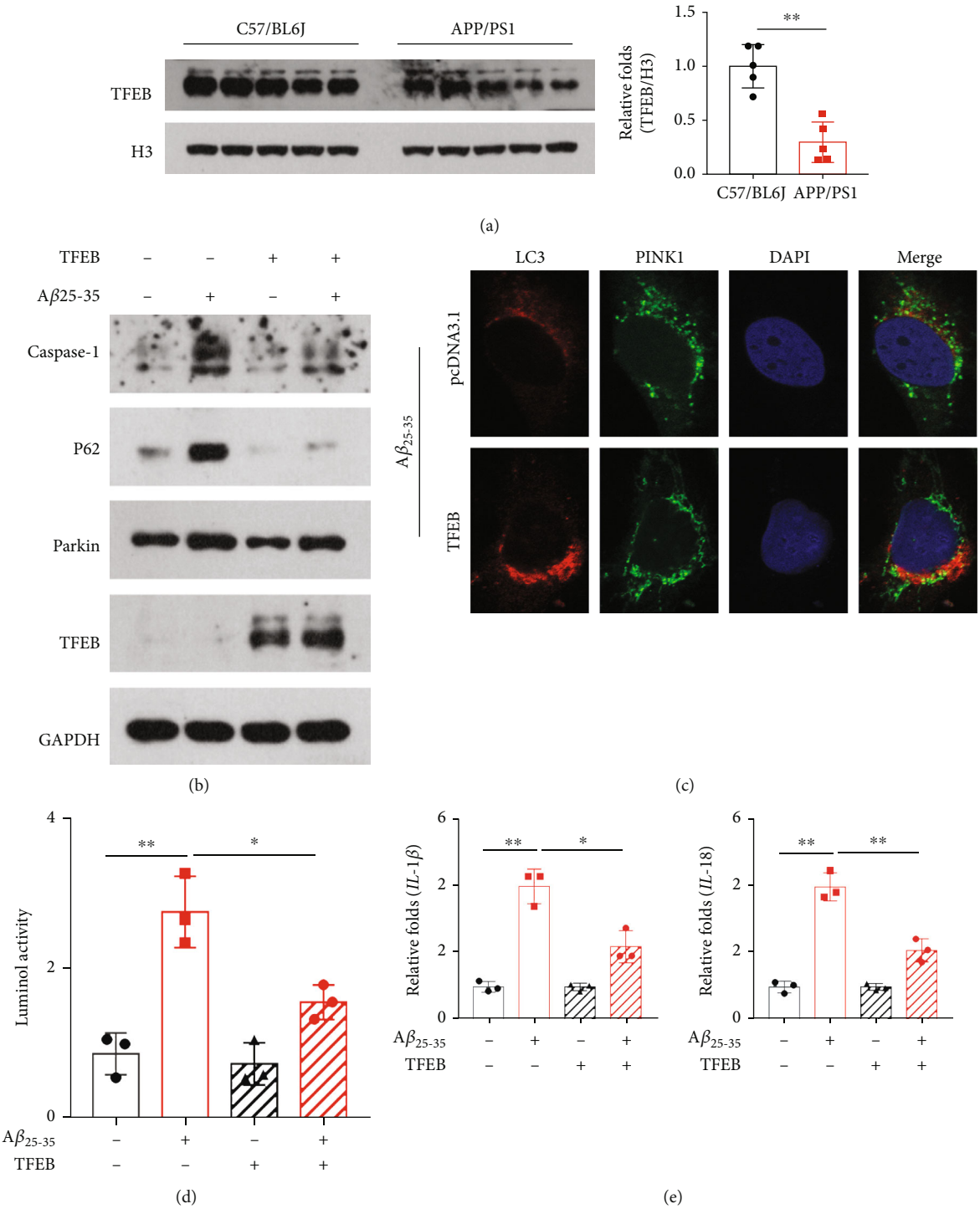

FIGURE 4: (a) Western blotting analysis of the expression of TFEB in nucleus was performed in APP/PS1 transgenic mice. (b) SH-SY5Y cells overexpressing TFEB were treated with a combination of Aβ25-35, and the expressions of TFEB, Parkin, p62, and caspase-1 were detected by western blotting. (c) SH-SY5Y cells overexpressing TFEB were treated with a combination of Aβ25-35, and the colocalization between LC3 and PINK1 was detected by immunofluorescence. (d) SH-SY5Y cells overexpressing TFEB were treated with a combination of Aβ25-35, and the oxidative activated oxygen (ROS) was detected by luminol chemiluminescence kit. (e) SH-SY5Y cells overexpressing TFEB were treated with a combination of Aβ25-35, and the expression of IL-1β and IL-18 was analysed. Data are presented as mean ± SEM. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

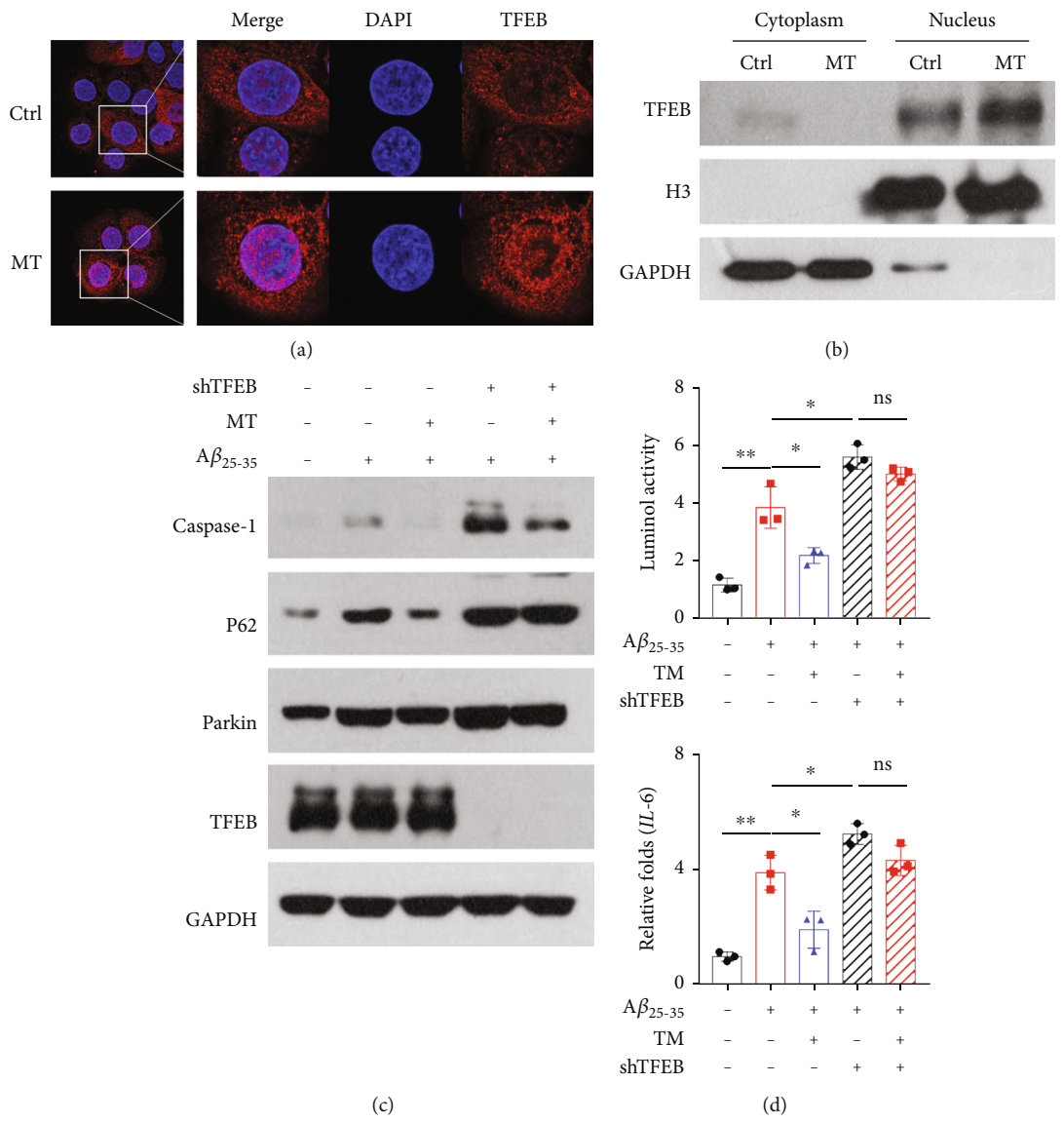

Figure 5: Continued.

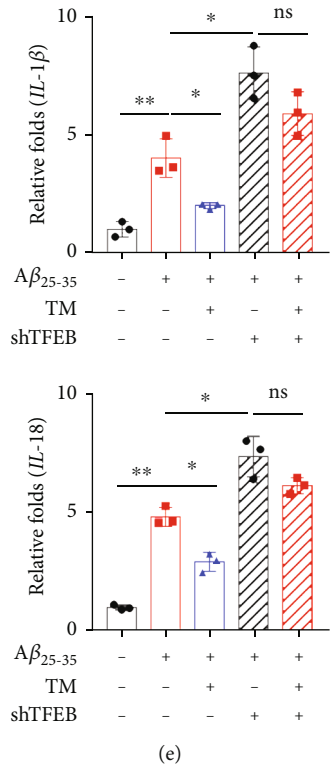

Figure 5: (a) Immunofluorescence analysis of the nuclear translocation of TFEB in SH-SY5Y cells incubated with MT. (b) Western blotting analysis of the nuclear translocation of TFEB in SH-SY5Y cells incubated with MT. (c) shRNA knockdown of TFEB was performed in combination with Aβ25-35 and MT treatment, and the expressions of TFEB, Parkin, p62, and caspase-1 were detected by western blotting. (d) shRNA knockdown of TFEB was performed in combination with Aβ25-35 and MT treatment, and the lever of ROS was detected by luminol chemiluminescence kit. (e) shRNA knockdown of TFEB was performed in combination with Aβ25-35 and MT treatment, and the expression of IL-1β and IL-18 was analysed. Data are presented as mean ± SEM. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

location by memory. The recording time was 60 s, and the time spent in the target quadrant and the number of platform location crosses were recorded.

2.3. Cell Culture and Reagents. The human neuroblastoma cell line SH-SY5Y was from the Cell Bank of the Chinese Academy of Sciences and was cultivated in DMEM with 15% FBS (Gibco, Rockville, USA), 100 U/mL penicillin, and100 g/mL streptomycin. Cells were cultured under the conditions of 5% CO_2, 37°C, and high humidity.

2.4. Extraction of Total Proteins and Cells from Brain Tissues. Brain tissue were quickly frozen and stored at −80°C. Ice-cold RIPA lysis buffer (Beyotime, Shanghai, China) was used for homogenizing brain samples. After centrifugation at 12,000 × g at 4°C for 20 minutes, western blotting was performed on the supernatant.

After digestion and centrifugation, the cultured cells were cultured in 6 mm petri dishes. The cells was prepared into a single cell suspension with 1x PBS (1 mL) and placed on ice. Then, an appropriate amount of protein lysate was added. After trituration and denaturation at 100°C for 5 h, the cells were lysed completely. The protein concentration was quantified by 5 μL aliquot, and the remaining protein was stored at -20°C.

2.5. Western Immunoblotting Analyses. Proteins were obtained by RIPA lysis buffer containing fresh protease and phosphate inhibitor mixture and boiled for denaturation. Protein concentration was determined by BCA protein assay. Cell lysate was then prepared for western blotting using a 10% polyacrylamide gel and 30 μg of protein. After electrical transfer and blocking with 5% BSA, the membrane was incubated with primary antibodies at 4°C overnight. Antibodies against β-actin (A5441) were obtained from Sigma–Aldrich; antibodies against TFEB (ab270604), NLRP3 (ab263899), PINK1 (ab23707), and Parkin (ab77924) were purchased from Abcam; and antibodies against GAPDH (SC-365062), caspase-1 (SC-56036), and P62 (SC-48402) were obtained from Santa Cruz Biotechnology.

2.6. Real-Time Quantitative PCR. Total RNA was obtained via a TRIzol Kit. PrimeScript RT Kit (Takara, Japan) was used for preparing the cDNA. SYBR Green was used for qRT-PCR, and the primers are listed in Table 1.

2.7. Transfection. The pcDNA3.1-TFEB plasmid and pcDNA3.1 (negative control) were transfected into cells by Lipofectamine 3000 (Invitrogen, Carlsbad, USA). Lentiviral shRNA interference vectors targeting TFEB (shTFEB) and

negative control vector (short hairpin RNA [shRNA]) were obtained from Hanbio Biotechnology (Shanghai, China). The TFEB shRNAs were as follows: shTFEB (forward), 5′-CCGGCCCACTTTGGTGCTAATAGCTCTCGAGAGCTATTAGCACCAAAGTGGGTTTTTG-3′; shTFEB (reverse), 5′-AATTCAAAAACCCACTTTGGTGCTAATAGCTCTCGAGAGCTATTAGCACCAAAGTGGG-3′.

2.8. Immunofluorescence. Cells were transfected with the pcDNA3.1-TFEB plasmid or treated with MT and then inoculated on glass slides overnight. The cells were fixed with 4% paraformaldehyde for 5 min and incubated with the primary antibody overnight at 4°C. The next day, the cells were incubated with the secondary antibody for 2 h. After 10 min of DAPI staining, the samples were then imaged by a microscope (LSM800, Carl Zeiss).

2.9. Reactive Oxygen Species Assay. The level of ROS in SH-SY5Y cells were examined by a 2′7′-dichlorofluorescin diacetate (DCFH-DA) assay kit. 1×10^6 cells/well were inoculated in a 6-well plate. The cells were suspended in DCFH-DA (200 μL) at 37°C in the dark for 20 minutes. After washing with PBS twice, the fluorescence intensity of cells was measured by a microplate reader (Cytation 5, America).

2.10. Subcellular Fractionation. Subcellular fractions (cytosolic and nuclear) were isolated from cells as described with modifications [19]. Briefly, cells were lysed in a NP-40 lysis buffer with EDTA (0.5 mM), NaCl (150 mM), Tris-HCl (20 mM, pH 7.9), and NP-40 (0.5%) with phosphatase and protease inhibitors. The lysed cells were placed on ice for 15 min and then centrifuged at 2,000 × g for 5 min. The corresponding pellets representing the nuclear fractions were washed once in an NP-40-containing lysis buffer and sonicated in a nuclear lysis buffer (450 mM NaCl, 20 mM Tris-HCl (pH 7.4), 0.5% Triton X-100, 0.1% SDS, and 0.5 mM EDTA). The lysates were centrifuged at 12,000 × g for 15 min to acquire the nuclear and cytosolic fractions.

2.11. Statistical Analysis. The data were analysed by GraphPad Prism software and presented as the mean ± SD. One-way ANOVA was used for comparisons among different groups. Student's t test was used for comparisons between two groups. All experiments were repeated at least three times. $P < 0.05$ was defined as a statistical significance.

3. Results

3.1. Melatonin Can Improve Cognitive Function and Decline Aβ Deposition in APP/PS1 Transgenic Mice. APP/PS1 transgenic mice were given melatonin through drinking water for 3 months (from 9 to 12 months, 0.5 mg melatonin daily). Compared with the placebo control APP/PS1 transgenic mice, the escape latency of the melatonin intervention group was shortened (Figure 1(a)) and significantly different on Day 5 (Figure 1(b)), with an increased number of platform crossings (Figure 1(c)) and a longer time in the target quadrant (Figure 1(d)). There was no obvious difference in swimming speed (Figure 1(e)). Thioflavin staining was used to detect senile plaques. The results showed that the number of senile plaques in the cerebral cortex and hippocampus of APP/PS1 transgenic mice in the melatonin intervention group was significantly reduced compared with that of APP/PS1 transgenic mice in the placebo control group (Figures 1(f) and 1(g)).

3.2. Melatonin Promoted Mitophagy and Inhibited NLRP3 Inflammasome Activity in APP/PS1 Transgenic Mice. After 3 months of melatonin intervention, western blot analysis showed that compared with the placebo control group APP/PS1 transgenic mice, the levels of the mitophagy-related protein PINK1 were increased in the brains of APP/PS1 transgenic mice in the melatonin intervention group, while the levels of Parkin and P62 were decreased. The levels of caspase-1 and NLRP3 inflammasome components were significantly decreased (Figure 2(a)). The expression of PINK1 and LC3 in frozen brain tissue sections was observed by immunofluorescence. The levels of PINK1 and LC3 were increased in the melatonin intervention group (Figure 2(b)). ROS levels in the APP/PS1 transgenic mice were dramatically reduced in the melatonin intervention group (Figure 2(c)). The levels of IL-18, IL-6, and IL-1β were notably decreased according to real-time quantitative PCR (Figure 2(d)). These results suggest that melatonin can promote mitophagy and inhibit NLRP3 inflammasome activity.

3.3. Melatonin Improved Mitophagy and Inhibited the NLRP3 Inflammasome In Vitro. After pretreatment with melatonin (10 μM), the levels of the mitophagy-related proteins caspase-1, P62, and Parkin were decreased in SH-SY5Y cells treated with $A\beta_{25\text{-}35}$ (Figure 3(a)). Colocalization of PINK1 and LC3 was detected by immunofluorescence. The colocalization of PINK1 and LC3 was increased in the melatonin-pretreated group (Figure 3(b)). ROS levels were clearly reduced in the melatonin intervention group (Figure 3(c)). The levels of IL-1β and IL-18 were significantly decreased, as shown by real-time quantitative PCR (Figure 3(d)). With the addition of the autophagy inhibitor hydroxychloroquine (HCQ-10 μM), ROS levels significantly increased (Figure 3(e)). The levels of IL-18 and IL-1 were notably increased (Figure 3(f)).

3.4. Melatonin Increased the Amount of TFEB in the Brain Nucleus of APP/PS1 Transgenic Mice, and TFEB Promoted Mitophagy and Alleviated the Aβ-Induced Inflammatory Response In Vitro. TFEB levels in the brain nucleus of APP/PS1 transgenic mice were significantly reduced in APP/PS1 transgenic mice (Figure 4(a)). With the addition of TFEB, the levels of the mitophagy-related proteins caspase-1, P62, and Parkin were decreased in SH-SY5Y cells with $A\beta_{25\text{-}35}$ (Figure 4(b)). After overexpressing TFEB in SH-SY5Y cells and treating them with $A\beta_{25\text{-}35}$, the colocalization of LC3 and PINK1 increased, as detected by immunofluorescence, suggesting enhanced mitophagy (Figure 4(c)). With the addition of TFEB, ROS levels significantly decreased (Figure 4(d)). The levels of IL-18 and IL-1 were significantly decreased (Figure 4(e)).

3.5. Melatonin Alleviated the Toxic Effects of Aβ by Promoting TFEB Nuclear Translocation. After melatonin treatment, an increase in the nuclear translocation of TFEB was detected by immunofluorescence in SH-SY5Y cells (Figure 5(a)). The level of TFEB in the nucleus was significantly increased after treatment with melatonin, as detected by western blotting (Figure 5(b)). shRNA knockdown of TFEB was implemented in combination with $A\beta_{25\text{-}35}$ and MT treatment, and the expression of Parkin, p62, and caspase-1 increased (Figure 5(c)). The level of ROS increased (Figure 5(d)). The expression of IL-18 and IL-1β also increased (Figure 5(e)).

4. Discussion

Mitochondrial damage is an early feature of AD and causes pathological changes in Aβ and Tau [20]. Mitophagy is a normal process in which cells detect and remove damaged mitochondria, and it is very important for maintaining a healthy mitochondrial pool and the healthy survival of neurons. Mitophagy disorders lead to the accumulation of dysfunctional mitochondria in neurons, causing inflammation and promoting the progression of AD. The NLRP3 inflammasome is the core factor underlying AD inflammation, and excessive activation of the NLRP3 inflammasome is related to the mitochondrial damage and abnormal mitochondrial autophagy function. After mitochondrial injury, increased ROS production, mitochondrial DNA release, NLRP3 translocation to mitochondria, increased cardiolipin in the mitochondrial intima, etc., can induce the NLRP3 inflammasome and promote the activation and maturation of the interleukins IL-18 and IL-1β, leading to downstream inflammatory reactions and cell damage. Mitophagy can restrain the activation of the NLRP3 inflammasome. Mitophagy can inhibit NLRP3 inflammasome activation by scavenging ROS [21].

Melatonin is widely distributed in organisms and has antioxidative, antiageing, antiapoptotic, and other effects. Many basic and clinical studies have confirmed the protective effect of melatonin on AD. Previous studies have found that melatonin protects against AD by reducing Aβ production, alleviating Aβ toxicity, and regulating the expression of senescence-related genes. Intracellular oxidative damage is mainly caused by reactive oxygen species (ROS) produced in mitochondria [22]. The melatonin concentration in mitochondria is significantly higher than that in other organelles or cells [23]. Mitochondrial membranes contain transporters that help mitochondria rapidly absorb melatonin against a concentration gradient [24]. Mitochondria originate from bacteria that produce melatonin. Mitochondria are thought to produce melatonin [25]. Studies have shown that melatonin can improve mitophagy and inhibit NLRP3 inflammasome activation in animal models of subarachnoid haemorrhage and atherosclerosis. In macrophages cultured in vitro, oxidation-modified LDL stimulation can increase ROS production and lead to NLRP3 inflammasome activation, while melatonin treatment can reduce ROS production and inhibit NLRP3 inflammasome activation. However, there are few studies on the effect of melatonin on mitophagy in AD. In this study, we used oral melatonin to treat APP/PS1 transgenic mice and confirmed the protective role of melatonin in AD by water maze tests and assessment of Aβ deposition and soluble Aβ40 and Aβ42 concentrations. By detecting the levels of mitophagy and NLRP3 inflammasome-related proteins and inflammatory factors, we observed mitophagy disorder and excessive activation of the NLRP3 inflammasome in APP/PS1 transgenic mice, confirming that melatonin can improve mitophagy and inhibit NLRP3 inflammasome activity in AD animal models.

In this study, the specific mechanism by which melatonin promotes mitophagy in AD was further explored. Lysosomes are key in maintaining normal autophagy [26]. The autophagy–lysosome pathway is involved in regulating Aβ metabolism and Tau protein degradation, and there is obvious autophagy–lysosome pathway dysfunction in AD [27]. TFEB, a major regulator discovered in recent years, plays a key role in autophagy/mitophagy [28]. miR-128, an upregulated microRNA, is present in the hippocampus and monocytes of AD patients. miR-128 can downregulate TFEB, suggesting the presence of endogenous TFEB abnormalities in AD [29]. In multiple AD mouse models with Aβ and Tau pathology, the enhancement of the ALP expression by exogenous TFEB has been demonstrated to significantly reduce Aβ and Tau pathological changes, improve cognitive impairment, upregulate PINK1, and enhance autophagy/mitophagy [30]. Based on the above findings, we hypothesized that the mechanism by which melatonin promotes mitophagy in AD may be related to TFEB. In this study, we found that overexpression of TFEB improved mitochondrial autophagy, alleviated mitochondrial damage, and antagonized the cytotoxic effects of Aβ in AD cells, demonstrating that exogenously induced TFEB expression plays a protective role in AD. In animal models, TFEB was found to be reduced in neuron nuclei in AD mouse brain tissue, suggesting inadequate TFEB function and confirming the presence of endogenous TFEB abnormalities in AD. In vitro experiments showed that melatonin can promote TFEB entry into the nucleus and improve mitophagy in AD.

This study confirmed the protective effect of melatonin in AD and found that the protective mechanism was related to the regulation of TFEB and the promotion of mitophagy, providing more evidence for the application of melatonin in the treatment of AD. TFEB intervention may become another potentially promising target for the treatment of AD.

5. Conclusions

Mitophagy disorder, overactivity of the NLRP3 inflammasome, and reduced TFEB content in the nucleus were found in APP/PS1 mouse brain tissue. Melatonin promotes mitophagy by inducing TFEB nuclear translocation, inhibits NLRP3 inflammasome activation, and exerts protective effects in AD.

Data Availability

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Ethical Approval

All procedures described in this study were performed in accordance with Directive 2010/63/EU in Europe and approved by the Ethical Committee for Animal Experiments of Shandong University.

Conflicts of Interest

None of the authors have any competing interests to declare.

Authors' Contributions

Li Fan performed the experiments, analysed the data, and wrote the paper; Lai Chao, Yan Jieke, and Xie Zhaohong designed the study; Wang Xiangxue, Xu Yingying, Zhou Xiaoyan, and Zhang Xiao performed the experiments; and Lai Chao and Wang Yan Jieke analysed the data and wrote the paper.

Acknowledgments

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Retraction

Retracted: Knockdown of PVT1 Exerts Neuroprotective Effects against Ischemic Stroke Injury through Regulation of miR-214/Gpx1 Axis

BioMed Research International

Received 11 July 2023; Accepted 11 July 2023; Published 12 July 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] X. Liu, T. Wang, P. Jing, M. Zhang, F. Chang, and W. Xiong, "Knockdown of PVT1 Exerts Neuroprotective Effects against Ischemic Stroke Injury through Regulation of miR-214/Gpx1 Axis," *BioMed Research International*, vol. 2022, Article ID 1393177, 9 pages, 2022.

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Research Article

Knockdown of PVT1 Exerts Neuroprotective Effects against Ischemic Stroke Injury through Regulation of miR-214/Gpx1 Axis

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Previous studies have reported that lncRNA PVT1 was closely related to ischemic stroke. Here, the role of PVT1 in ischemic stroke and the underlying mechanism were investigated. OGDR-stimulated PC12 cells were used to construct a cell model to mimic ischemic stroke. si-PVT1, miR-214 mimic, inhibitor, or the negative controls were transfected into PC12 cells prior to OGDR treatment. PVT1, miR-214, and Gpx1 expression was measured by qRT-PCR and western blotting assays. Cell proliferation and apoptosis were tested by CCK-8 assay and western blotting. The expression levels of inflammatory factors were determined by ELISA Kit. Results showed that PVT1 was increased significantly in OGDR PC12 cells. PVT1 knockdown significantly enhanced cell viability and attenuated cell apoptosis, ROS generation, and inflammation in OGDR PC12 cells. More importantly, PVT1 or Gpx1 was a target of miR-214. Mechanistically, PVT1 acted as a competing endogenous RNA of miR-214 to regulate the downstream gene Gpx1. In conclusion, PVT1 knockdown attenuated OGDR PC12 cell injury by modulating miR-214/Gpx1 axis. These findings offer a potential novel strategy for ischemic stroke therapy.

1. Introduction

Ischemic stroke is the most common cause of disability and death among adults worldwide. The current clinical effective treatment is to restore blood flow as soon as possible. However, the short treatment time and the high risk of secondary damage limit the applicability. Ischemic stroke involves several pathophysiological mechanisms, such as inflammatory response, cytotoxicity, and oxidative stress-induced necrosis or neuronal apoptosis, which is rather complex. Therefore, finding new therapeutic targets is essential for the treatment of ischemic stroke.

Long noncoding RNA (lncRNA), an emerging regulatory RNA, regulates gene expression at posttranscriptional levels. Previous research has reported that lncRNAs play important regulatory roles in the physiological processes of multiple diseases including stroke, malignancies, chronic lung diseases, and cardiovascular diseases [1–3]. For instance, Wang et al. displayed that lncRNA MALAT1 was upregulated in ischemic stroke and MALAT1 knockdown facilitated cell viability and suppressed cell apoptosis [4]. Knockdown of SNHG15 protected against ischemic stroke injury via inhibiting neuronal apoptosis and suppressing infarct area [5]. Fan et al. discovered that inactivation of H19 inhibited the functional recovery in MCAO rats [6]. lncRNA plasmacytoma variant 1 (PVT1) has been considered a candidate oncogene in various cancers, including breast cancer, glioblastoma, and bladder cancer [7–9]. Liu et al. discovered that PVT1 was obviously increased in acute ischemic stroke patients, suggesting PVT1 might be a potential diagnostic biomarker. However, the role of PVT1 in ischemic stroke remains largely unclear.

MicroRNAs (miRNAs), short single-stranded RNAs, exert diverse functions in various pathological processes via regulating gene expression at posttranscriptional level [10–14]. Previous studies have reported that the dysregulation of miRNAs was related to ischemic stroke, such as miR-143, miR-190, miR-195, and miR-451 [15–17]. Lu et al. displayed that miR-214 was elevated in ischemia stroke patients, indicating the association of miR-214 with ischemic stroke. Thereby, miR-214 was speculated to be involved in the pathogenesis of ischemic stroke.

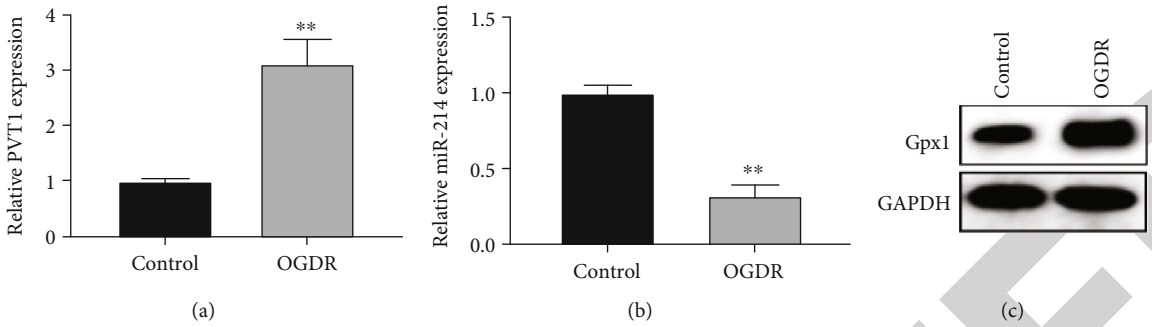

Figure 1: PVT1 was overexpressed in OGDR PC12 cells. (a) qRT-PCR analysis of PVT1 and (b) miR-214 mRNA level in OGDR PC12 cells. (c) Western blotting analysis of Gpx1 level in OGDR PC12 cells. $^{**}p < 0.01$. Data were shown as the mean ± SD based on three independent experiments.

Glutathione peroxidase 1 (Gpx1) is an essential component of the intracellular antioxidant enzyme. Increasing evidence has demonstrated that Gpx1 exhibited a critical role in the progress of brain diseases. For instance, Gpx1 was found to be increased in glioma [18]. Sharma et al. found that Gpx1 silencing promoted the proinflammatory response and activated vascular endothelium [19]. Moreover, Karahalil et al. reported that Gpx1 served as a risk factor for ischemic stroke [20], indicating that Gpx1 might be involved in the development of ischemic stroke.

Long noncoding RNA (lncRNA) was another emerging regulatory RNA, interacting with miRNA through conserved sequences to release mRNAs from RNA-induced silencing complexes. Prior studies have shown that miR-214 served as a target of PVT1 in regulation of hepatocellular carcinoma, ovarian cancer, and diabetic cataract [21–23]. Here, we speculated whether lncRNA PVT1 modulated cerebral ischemic stroke through targeting miR-214. Xiao et al. discovered that Gpx1 was a target of miR-214 in acute lymphoblastic leukemia [2]. In this present study, we performed an OGDR model in PC12 cells to explore the role of PVT1 in ischemic stroke injury and its underlying mechanism involved.

2. Material and Methods

2.1. Cell Culture. PC12 cells were obtained from the Chinese Academy of Sciences (Shanghai, China). They were cultured in DMEM (Gibco, USA) containing with 10% FBS (Life Science, USA) at 37°C in a humidified incubator containing 5% CO_2.

2.2. Establishment of OGDR Model. The model was constructed following the instructions described previously [24]. In brief, PC12 cells, purchased from Shanghai Institutes of Cell Biological Sciences (Shanghai, China), were exposed to OGD for 2 h at 37°C and then returned to a normal environment. PC12 cells were incubated in an anaerobic chamber with 95% N_2 and 5% CO_2 without glucose. Subsequently, the cells were transferred to a normoxic conditions for 24 h to reoxygenation. The sham group was performed with the same treatment except for OGD exposure.

2.3. Cell Transfection. PVT1 small interfering RNA (PVT1 siRNA), pcDNA-PVT1 overexpression vector (pcDNA-PVT1), Gpx1 plasmid vector (Gpx1 vector), and miR-214 mimic/inhibitor were designed and synthesized by GenePharma (Shanghai, China). With the help of Lipofectamine 2000 Reagent (Invitrogen, Carlsbad, USA), the transfections were performed for 24 h, according to the protocol company's instructions.

2.4. Quantitative Reverse Transcription-Polymerase Chain Reaction (qRT-PCR). Total RNA was extracted using TRIzol reagent (Invitrogen; Thermo Fisher Scientific, Inc.). The TaqMan miRNA assays were applied to perform RT-qPCR reactions. The ABI PRISM 7700 sequence detection system (Thermo, Waltham, USA) was used to run all reactions. The relative expression was calculated using the 2-$\Delta\Delta$Ct method and was normalized to U6 or GAPDH.

2.5. MTT Assay. 2×10^4 PC12 cells were cultured for 24 h. MTT solution (5 mg/mL, Sigma) was added and incubated 4 h at 37°C. Afterwards, 150 μL DMSO (Beijing, China) was added and incubated for 1 h. The absorbance at 490 nm was detected using a microplate reader (Bio-Tek, Winooski, USA).

2.6. Western Blotting Assay. 50 μg of samples isolated from PC12 cells was separated by SDS-PAGE, followed by transferred to PVDF membranes (Millipore, Billerica, MA, USA). Afterwards, the membranes were incubated with 5% BSA, the primary antibodies against Bax (Abcam, ab32503, 1 : 1000), Bcl-2 (Abcam, ab182858, 1 : 2000), cleaved-caspase-3 (Abcam, ab32042, 1 : 1000), pro-caspase-3 (Abcam, ab32150, 1 : 1000), Gpx1 (Abcam, ab22604, 1 : 1000), and GAPDH (Cell Signaling Technology, #97166, 1 : 1000) and the secondary HRP antibodies (Abcam, ab205718, ab205719, 1 : 2000). The bands were detected by an ECL detection system (Pierce, Rockford, USA), and the quantification was performed by Image Lab™ Software (Bio-Rad, Shanghai, China).

2.7. ELISA Assay. The ELISA kits (Sigma) purchased from Beyotime Biotechnology (Beijing, China) were applied to detect the activity of ROS and inflammatory cytokines in PC12 cells with different treatment. The microplate reader

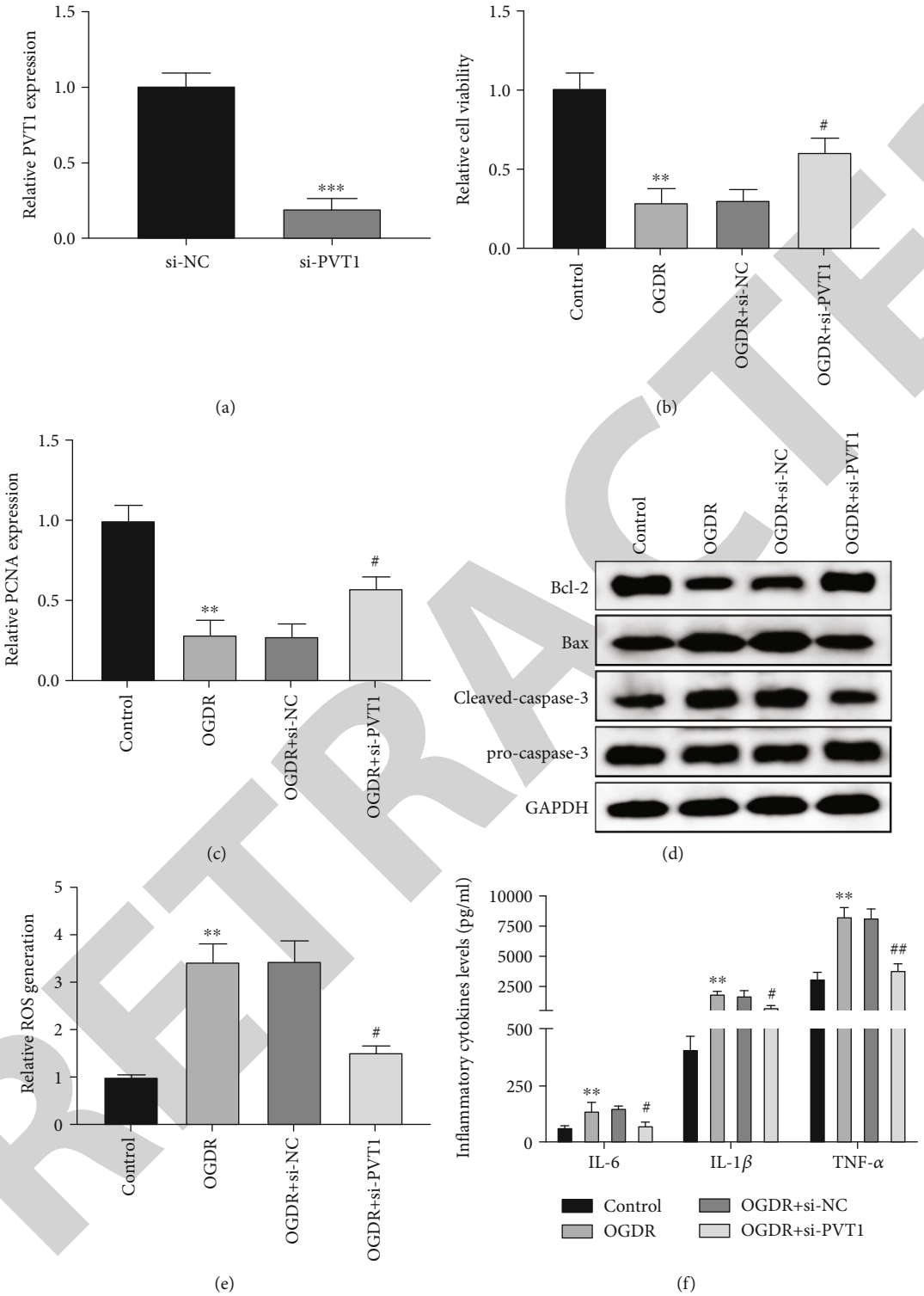

FIGURE 2: PVT1 knockdown attenuated ischemic injury through miR-214/Gpx1 in OGDR PC12 cells. (a) PVT1 expression was measured by qRT-PCR in si-PVT1 PC12 cells. (b) CCK-8 analysis of si-PVT1 effect on PC12 cell viability. (c) qRT-PCR analysis of si-PVT1 role on PCNA expression. (d) Western blotting analysis of si-PVT1 effect on Bcl-2, Bax, and cleaved-caspase-3 levels in PC12 cells. (e) ELISA assay analysis of the effect of si-PVT1 on the productions of ROS and (f) IL-6, IL-1β, and TNF-α in OGDR PC12 cells. $^{**}p < 0.01$ and $^{***}p < 0.001$; $^{\#}p < 0.05$ and $^{\#\#}p < 0.01$. Data were shown as the mean ± SD based on three independent experiments.

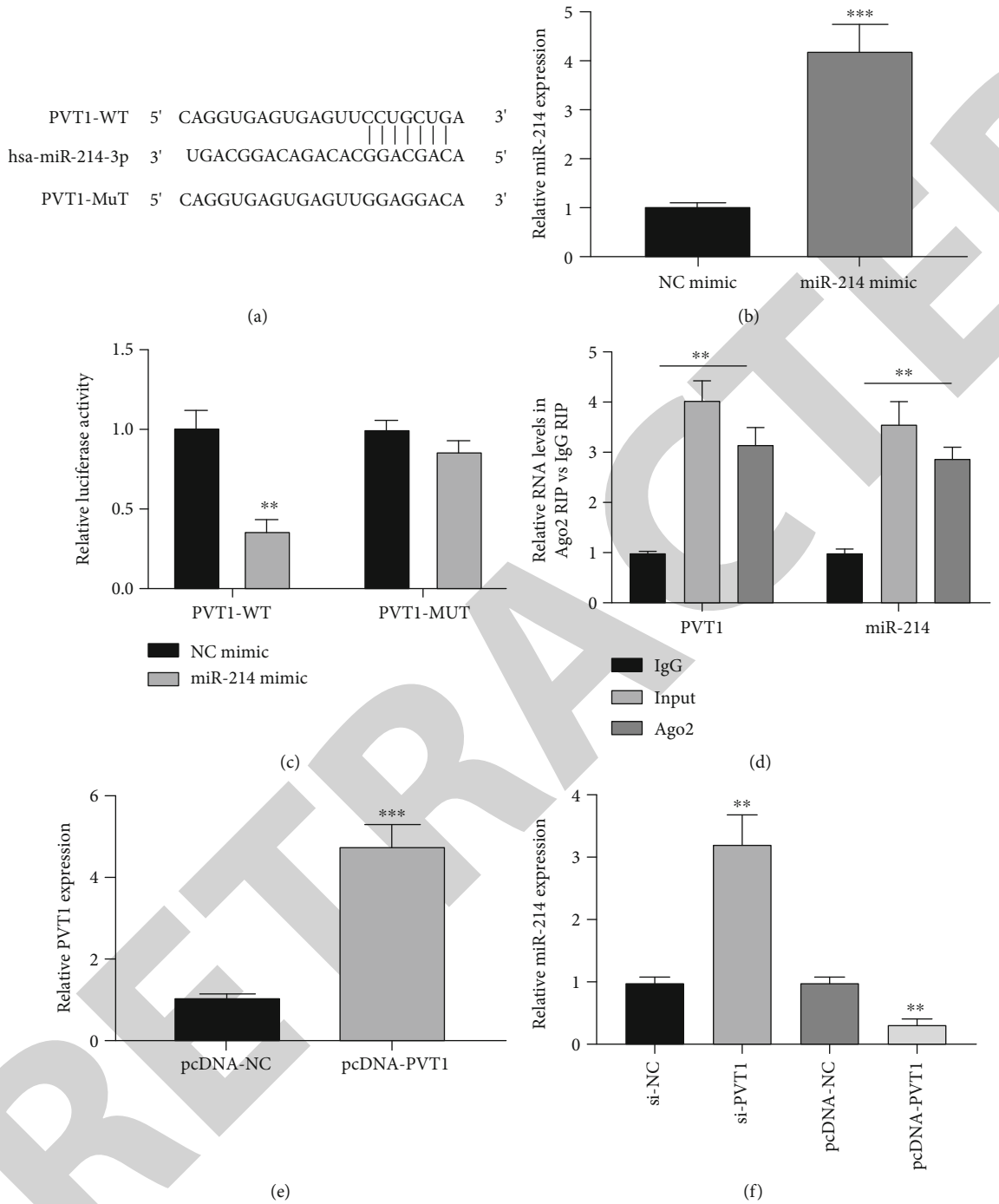

Figure 3: PVT1 functions as a ceRNA of miR-214 in PC12 cells. (a) The binding sites of PVT1 and miR-214, as shown by Starbase. (b) Detection of miR-214 expression in PC12 cells. (c) Measurement of the luciferase activity of PVT1. (d) Measurement of PVT1 or miR-214 expression in Ago2, IgG, and Input groups. (e) Detection of PVT1 expression by qRT-PCR. (f) Observation of PVT1 effect on miR-214 expression by qRT-PCR. $^{**}p < 0.01$ and $^{***}p < 0.001$. Data were shown as the mean ± SD based on three independent experiments.

(Bio-Tek, Winooski, USA) was applied to measure the optical density of each group at 450 nm.

2.8. Luciferase Reporter Assay. PC12 cells were transfected together with miR-214 mimic and constructed pGL3 luciferase vector (Promega, Madison, USA) using Lipofectamine 2000 reagent (Invitrogen). 48 h after transfection, the relative luciferase activity was analyzed by the dual luciferase reporter assay system (Promega, Madison, USA) and normalized to Renilla luciferase activity.

2.9. RIP Assay. PC12 cells were lysed in RIP buffer containing magnetic beads conjugated with anti-Ago2 antibody, negative control IgG, and positive control Input. Cells were

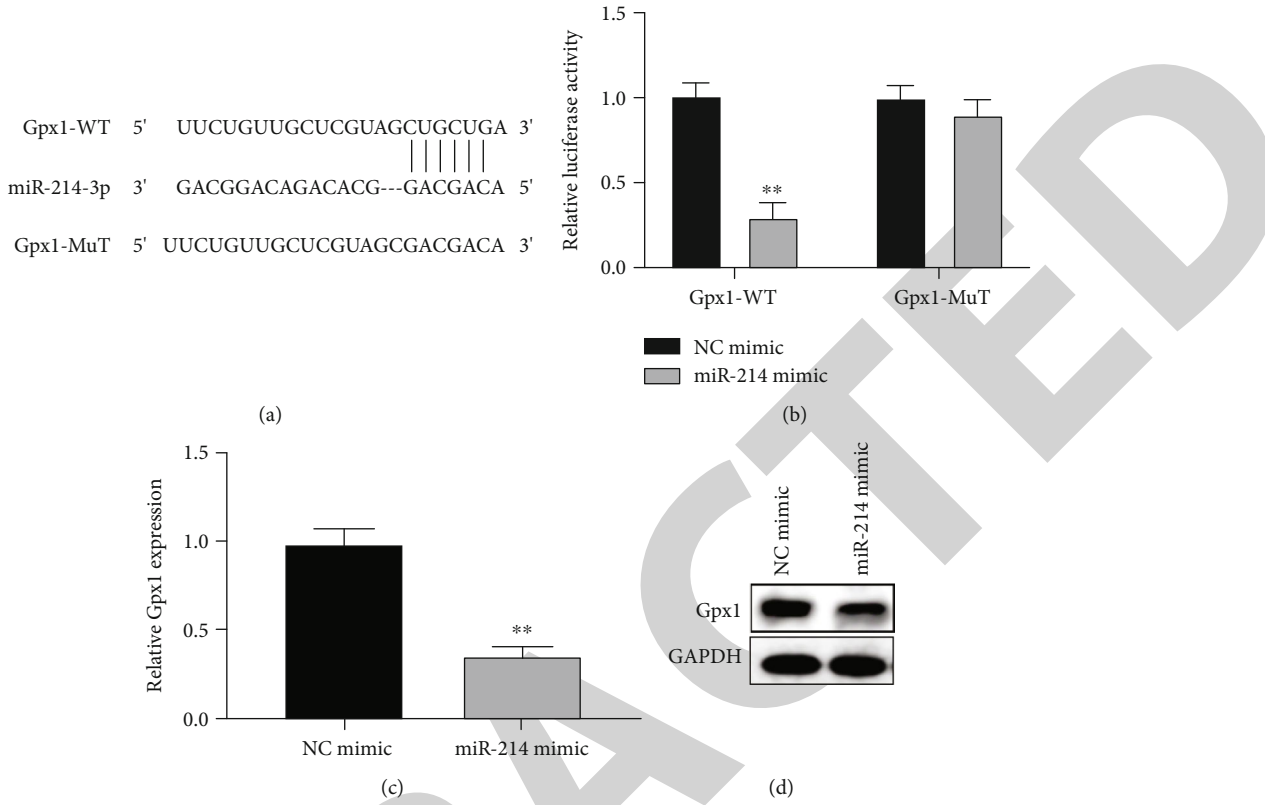

Figure 4: miR-214 directly targeted Gpx1 in PC12 cells. (a) The binding sites of Gpx1 and miR-214, as shown by Starbase. (b) Measurement of the luciferase activity of Gpx1. (c) qRT-PCR analysis of Gpx1 mRNA expression. (d) Detection of Gpx1 protein level by western blotting. $^{**}p < 0.01$. Data were shown as the mean ± SD based on three independent experiments.

incubated with Proteinase K buffer, and the RNA was extracted by TRIzol regent after washed by ice-cold saline water (150 mmol/L NaCl), and the purified RNA expression were analyzed by qRT-PCR.

2.10. Statistical Analysis. The experimental data were expressed as the mean ± standard deviation (SD). The differences between two or more groups were analyzed using two-tailed Student's *t*-tests or one -way ANOVA followed by Turkey's post hoc test, respectively. Statistical analysis was performed using SPSS Statistics 20.0 software (IBM Corp.) and GraphPad Prism version 6.0 software (GraphPad Software Inc.). $p < 0.05$ was considered statistically significant.

3. Results

3.1. PVT1 and Gpx1 Were Increased, and miR-214 Was Decreased in an Ischemic Stroke Cell Model. To evaluate the levels of PVT1, miR-214, and Gpx1 affected by ischemia stroke, the OGDR model in PC12 cells was established. qRT-PCR results showed that PVT1 was overexpressed, while miR-214 was underexpressed in OGDR PC12 cells (Figures 1(a) and 1(b)). Results from western blotting discovered that Gpx1 level was highly expressed as well in OGDR PC12 cells (Figure 1(c)). These data indicated that OGD enhanced PVT1 and Gpx1 level, while inhibiting miR-214 level.

3.2. PVT1 Knockdown Promoted Cell Proliferation and Alleviated Apoptosis, ROS Generation, and Inflammation in Ischemic Stroke Cells. To evaluate the functional role of PVT1 on ischemic stroke, the loss of function experiments in OGDR PC12 cells were performed. As shown in Figure 2(a), PVT1 expression was obviously lower in si-PVT1 group than in the si-NC group. CCK-8 results indicated that PVT1 knockdown promoted cell viability reduced by OGDR operation (Figure 2(b)). Moreover, the PCNA expression was increased by si-PVT1, which was reduced by OGDR operation (Figure 2(c)). These findings indicated that PVT1 inhibition facilitated cell proliferation in ischemic stroke cells. In addition, western blotting results discovered that si-PVT1 inhibited Bcl-2 level, while enhancing Bax and cleaved-caspase-3 levels in OGDR PC12 cells (Figure 2(d)), indicating the inhibitory effect of si-PVT1 on OGDR PC12 cells apoptosis. Furthermore, the ROS generation and inflammatory cytokines in OGDR PC12 cells were increased significantly under OGDR operation, and knockdown of PVT1 obviously reduced these levels (Figures 2(e) and 2(f)). These findings indicated that PVT1 knockdown enhanced cell proliferation and alleviated apoptosis, ROS generation, and inflammation in ischemic stroke cells.

3.3. PVT1 Functions as a ceRNA of miR-214 in PC12 Cells. To explore the molecular mechanism of PVT1 in ischemic stroke, Starbase was first used to identify the miRNAs interacting with PVT1 in PC12 cells. The binding sites for miR-

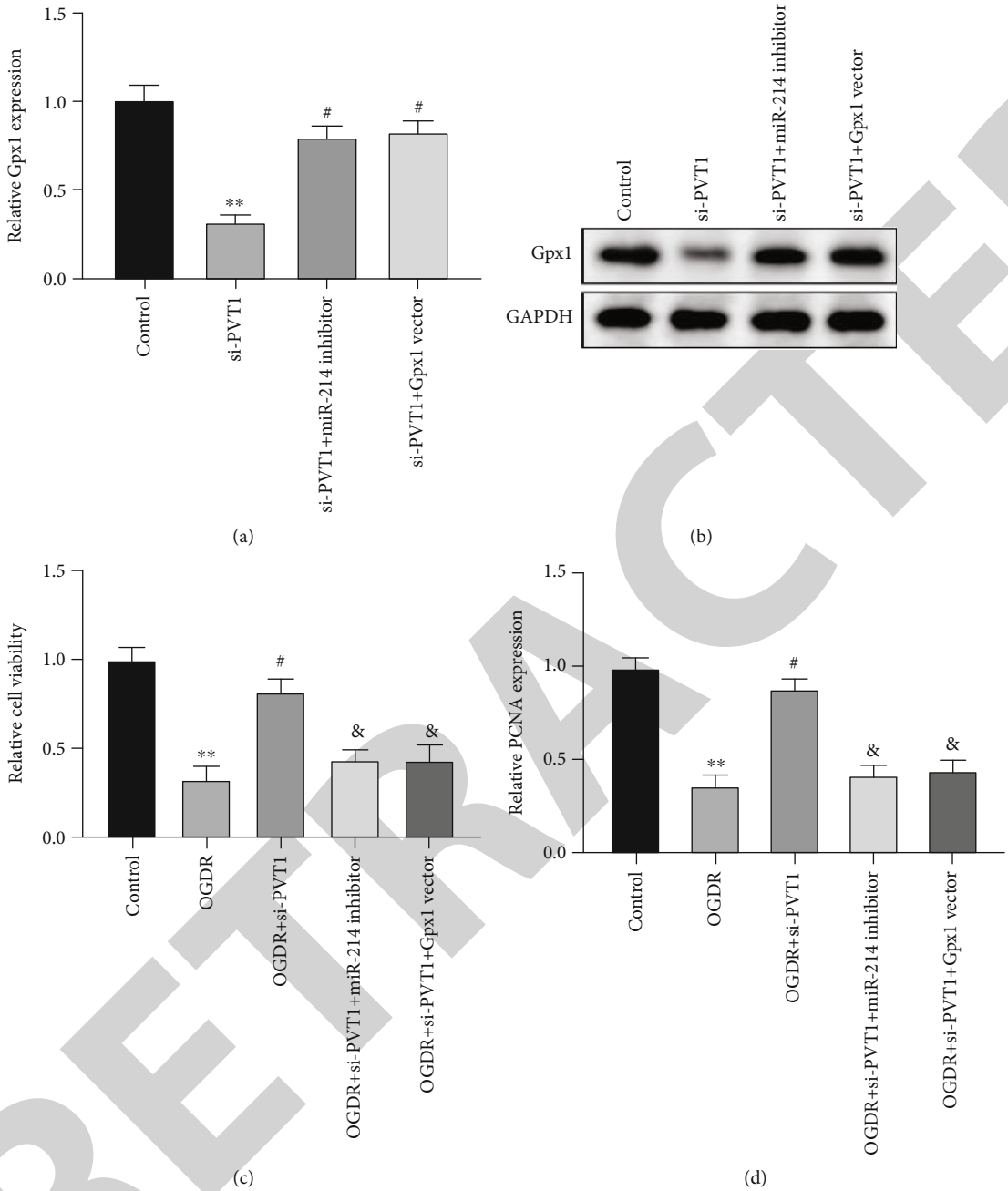

Figure 5: Continued.

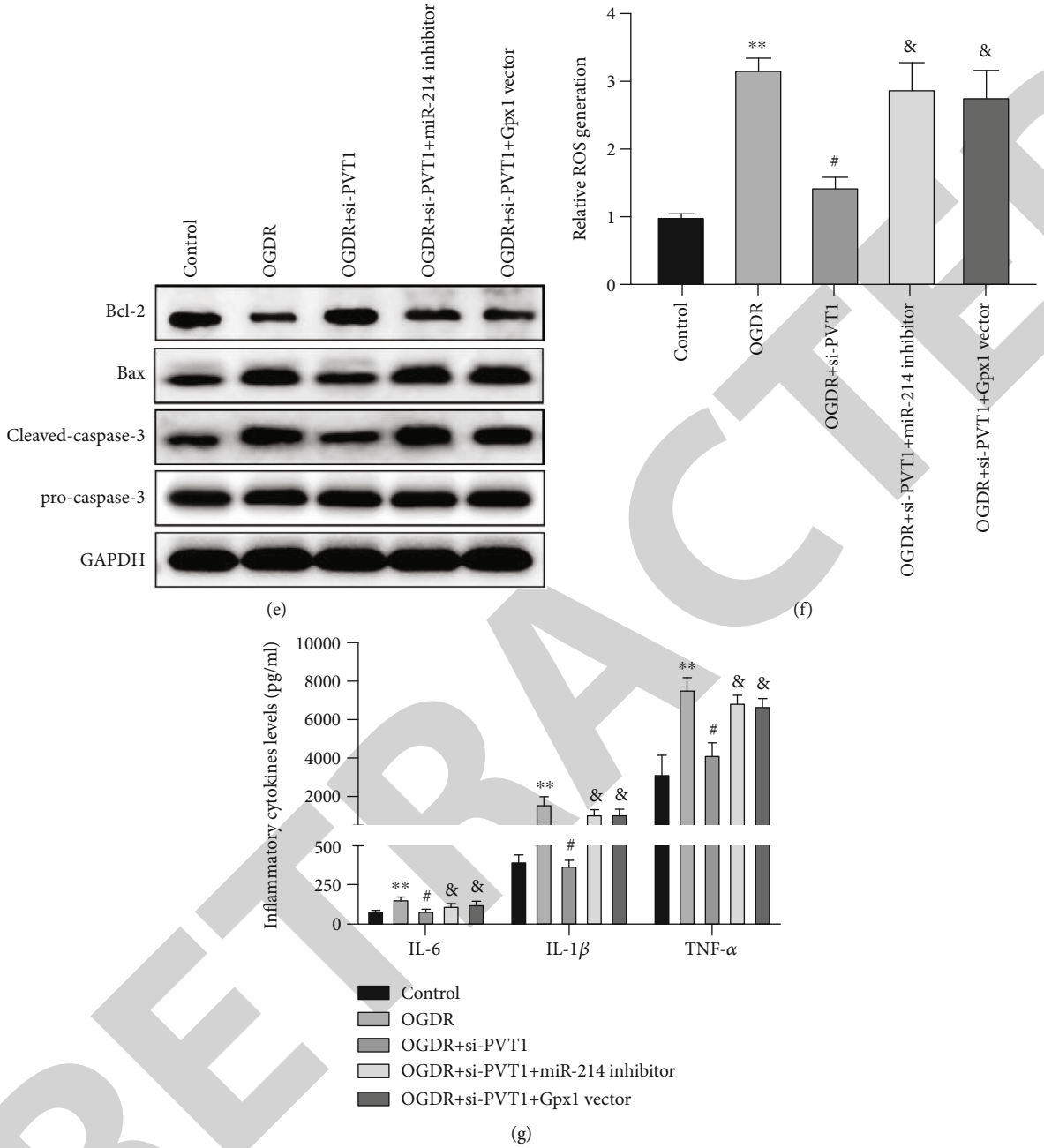

Figure 5: PVT1 knockdown attenuated ischemic injury through miR-214/Gpx1 in PC12 cells. PC12 OGDR cells were treated with si-PVT1, combined with miR-214 inhibitor or pcDNA-Gpx1. (a) Measurement of the mRNA expression or (b) protein level of Gpx1. (c) CCK-8 analysis of the cell viability. (d) QRT-PCR analysis of PCNA expression. (e) Western blotting analysis of Bcl-2, Bax, and cleaved-caspase-3 levels. (f) ELISA assay analysis of the levels of ROS and (g) IL-6, IL-1β, and TNF-α, respectively. $^{**}p < 0.01$; $^{\#}p < 0.05$; $^{\&}p < 0.05$. Data were shown as the mean ± SD based on three independent experiments.

214 in PVT1 are shown in Figure 3(a). To further identify the interaction between miR-214 and PVT1, RIP and dual luciferase reporter assays were applied. Restoration of miR-214 obviously increased miR-214 expression, when compared with the control group (Figure 3(b)). Results from luciferase reporter assays found that PVT1-WT luciferase activity in PC12 cells was significantly decreased by miR-214 mimic; however, there was no change in the PVT1-MuT group (Figure 3(c)). Meanwhile, RIP results manifested that the levels of PVT1 and miR-214 were increased in the Ago2 group compared to the negative control IgG group. Compared with the Input group, the two groups of samples have good parallelism (Figure 3(d)). Moreover, PVT1 was significantly upregulated by treatment with pcDNA-PVT1 (Figure 3(e)). Furthermore, PVT1 knockdown enhanced cell viability and attenuated cell apoptosis, ROS generation, and inflammation in OGDR PC12 cells, while PVT1 overexpression inhibited miR-214 expression in PC12 cells (Figure 3(f)). These results demonstrated that PVT1 was directly binding to miR-214 in PC12 cells.

3.4. miR-214 Directly Targeted Gpx1. To observe the target of miR-214 in PC12 cells, TargetScan and miRDB were applied. As shown in Figure 4(a), miR-214 possessed very high binding affinities with Gpx1. Moreover, the luciferase activities in PC12 cell o-transfection of Gpx1-WT vector and miR-214 mimic were decreased remarkably compared with the Gpx1-MuT vector (Figure 4(b)). In addition, overexpression of miR-214 significantly inhibited Gpx1 expression, which is shown in Figures 4(c) and 4(d) by western blotting and RT-PCR assays. Taken together, Gpx1 was the target of miR-214 in PC12 cells.

3.5. PVT1 Knockdown Attenuated Ischemic Injury through miR-214/Gpx1 in PC12 Cells. To identify whether the PVT1/miR-214/Gpx1 signaling axis was involved in the progression of ischemic injury, rescue experiments in OGDR PC12 cells were performed. PC12 cells were transfected with the si-PVT1 along with or without miR-214 inhibitor or Gpx1 vector prior to OGDR. As shown in Figures 5(a) and 5(b), Gpx1 expression decreased by si-PVT1 was increased by the miR-214 inhibitor or Gpx1 vector. CCK-8 results discovered that PVT1 knockdown decreased PC12 cells viability, while the miR-214 inhibitor or Gpx1 vector increased cell viability (Figure 5(c)). Moreover, the expression of PCNA decreased by PVT1 knockdown was significantly elevated by miR-214 inhibition or Gpx1 promotion (Figure 5(d)). In addition, western blotting results showed that the miR-214 inhibitor or Gpx1 vector overturned the promotion effect of si-PVT1 on OGDR PC12 cell apoptosis (Figure 5(e)). Furthermore, the ROS and inflammatory cytokines in OGDR PC12 cells increased by si-PVT1 were decreased by the miR-214 inhibitor or Gpx1 vector (Figures 5(f) and 5(g)). These data demonstrated that PVT1 knockdown facilitated cell proliferation and alleviated apoptosis, ROS generation, and inflammation in ischemic stroke cells via the miR-214/Gpx1 signaling axis.

4. Discussion

Growing evidence has shown that lncRNAs are considered a new type of diagnostic biomarker and a promising therapeutic target for ischemic stroke. Lu et al. found that PVT1 level was upregulated in the plasma of acute ischemic stroke patients [25]. However, the functional role of PVT1 in cerebral ischemia has not been confirmed. As far as we know, PVT1 has been discovered as a tumor promoter that facilitates cell proliferation, invasion, and migration in various tumors [26–28]. This present study revealed that PVT1 was increased in OGDR-treated PC12 cells. PVT1 inhibition promoted cell proliferation and alleviated apoptosis and inflammation in OGDR PC12 cells. These findings demonstrated that PVT1 knockdown might have neuroprotective effect against OGDR-induced injury.

PVT1 was reported to be involved in several central neuronal system diseases [29, 30]. For instance, silence of PVT1 decreased the loss of neurons and inhibited the activation of astrocytes in hippocampus tissues of epileptic rats [31]. Jin et al. discovered that PVT1 promoted glioblastoma multiforme progression [8]. Moreover, it has been reported that PVT1 is involved in Parkinson's disease and Alzheimer's disease development [3]. In this current study, we displayed that PVT1 was increased in ischemic stroke, which was in line with the previous study which reported that PVT1 was highly expressed in the plasma of acute ischemic stroke [25]. Also, PVT1 knockdown attenuated cell apoptosis and inflammation in OGDR PC12 cells.

It has been reported that miRNAs modulate mRNA translation via targeting their 3′-UTR. Increasing evidence has shown the dysregulation of miRNAs in ischemic stroke, indicating the association of miRNAs with ischemic stroke. This present study demonstrated that miR-214 was validated to bind to PVT1 and was negatively modulated by PVT1. Previous research has found that reexpression of miR-214 relieves cerebral ischemic injury [32]. In line with the above studies, we found that miR-214 knockdown reversed PVT1 inhibition effect on OGDR PC12 cells. Furthermore, Gpx1 was a direct target of miR-214. Previous research has reported that Gpx1 participates in the modulation of ischemia related diseases. Similarly, we illustrated that Gpx1 is increased in ischemic stroke *in vitro*, and overexpression of Gpx1 enhanced cell apoptosis, ROS generation, and inflammation reduced by si-PVT1.

5. Conclusions

Here, we found that Gpx1 restoration attenuated si-PVT1 effect on OGDR induced cell apoptosis and inflammation injury, suggesting that PVT1 knockdown inhibits ischemic stroke injury via regulation of miR-214/Gpx1 axis.

Data Availability

The datasets used and/or analyzed during the present 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

Xiaozhou Liu and Tian Wang contributed equally to this work.

Acknowledgments

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Retraction

Retracted: Recent Advances in Cell and Functional Biomaterial Treatment for Spinal Cord Injury

BioMed Research International

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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) Manipulated or compromised peer review

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] T. Liu, W. Zhu, X. Zhang et al., "Recent Advances in Cell and Functional Biomaterial Treatment for Spinal Cord Injury," *BioMed Research International*, vol. 2022, Article ID 5079153, 20 pages, 2022.

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Review Article

Recent Advances in Cell and Functional Biomaterial Treatment for Spinal Cord Injury

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Spinal cord injury (SCI) is a devastating central nervous system disease caused by accidental events, resulting in loss of sensory and motor function. Considering the multiple effects of primary and secondary injuries after spinal cord injury, including oxidative stress, tissue apoptosis, inflammatory response, and neuronal autophagy, it is crucial to understand the underlying pathophysiological mechanisms, local microenvironment changes, and neural tissue functional recovery for preparing novel treatment strategies. Treatment based on cell transplantation has become the forefront of spinal cord injury therapy. The transplanted cells provide physical and nutritional support for the damaged tissue. At the same time, the implantation of biomaterials with specific biological functions at the site of the SCI has also been proved to improve the local inhibitory microenvironment and promote axonal regeneration, etc. The combined transplantation of cells and functional biomaterials for SCI treatment can result in greater neuroprotective and regenerative effects by regulating cell differentiation, enhancing cell survival, and providing physical and directional support for axon regeneration and neural circuit remodeling. This article reviews the pathophysiology of the spinal cord, changes in the microenvironment after injury, and the mechanisms and strategies for spinal cord regeneration and repair. The article will focus on summarizing and discussing the latest intervention models based on cell and functional biomaterial transplantation and the latest progress in combinational therapies in SCI repair. Finally, we propose the future prospects and challenges of current treatment regimens for SCI repair, to provide references for scientists and clinicians to seek better SCI repair strategies in the future.

1. Introduction

Spinal cord injury (SCI) is a complex and challenging destructive disease of the central nervous system, resulting in permanent motor and sensory dysfunction due to disruption of neural circuits composed of descending motor neurons and ascending sensory neurons. In addition, because SCI is a serious injury caused by multiple primary and secondary mechanisms simultaneously or sequentially, it often results in chronic consequences such as respiratory dysfunction, cardiovascular complications, neuropathic pain, spasticity, bladder and bowel dysfunction, and mental illness [1, 2]. The most common causes of SCI include traffic accidents, falls, and violence but can also be caused by inappropriate sports and recreational activities [3]. According to 2020 data from the National Center for SCI, about 294,000 people in the United States suffer from spinal cord injuries, with about 17,810 new cases each year. According to statistics, the incidence rate of SCI in China is about 25 to 60 cases per million people, and the age of onset is mostly 40 to 60 years old. The incidence rate is significantly higher in males than in females. Globally, there are 3.6 to 195.4 cases of SCI per million people, of which male patients can reach 78% [4, 5]. SCI has an enormous impact on the patient's personal life, social life, and professional development, followed by a tremendous psychological and financial burden, which brings great pressure to both family and society [6, 7]. Although the current clinical treatment regimens can improve the prognosis of patients with SCI to a certain extent, due to its complex pathophysiological mechanism,

there is currently no effective treatment. To improve the recovery effect of SCI patients, scholars have conducted extensive and in-depth basic research on the pathophysiological mechanism and treatment strategies of SCI. Some spinal nerve regeneration methods have shown good results in animal models, and some of the findings have entered the clinical trial stage. This study started from the physiological anatomy of the spinal cord and the pathophysiological change mechanism of SCI and discussed the regeneration and repair mechanism and strategies of spinal cord nerves. We focused on the latest methods and progress in the treatment of SCI based on cell and functional biomaterials and their combination therapy. We also collated and analyzed preclinical and clinical studies of SCI.

2. Anatomy and Physiology Function of the Spinal Cord

2.1. Anatomy of the Spinal Cord. The spinal cord originates from the end of the neural tube during the embryonic period, and the lumen of the primitive neural tube forms the central canal of the spinal cord. The spinal cord in the lower part of the central nervous system retains segmental structure and connects with 31 pairs of spinal nerves distributed in the trunk and extremities. The spinal cord is located in the spinal canal and is surrounded by 3 layers of membranes (dura mater, arachnoid, and pia mater), which are consistent with the curvature of the spine. The spine itself consists of cervical, thoracic, lumbar, and caudal segments [8]. The upper end is connected to the medulla oblongata at the foramen magnum, and the lower end is tapered, called the conus medullaris, with a total length of about 42-45 cm, a transverse diameter of 1-1.2 cm at the widest point, and a weight of about 20-25 g. The conus medullaris continues downward as a filament of connective tissue called filum terminal, which ends at the back of the coccyx and serves to fix the spinal cord. The spinal cord consists of gray matter surrounding the central canal and white matter located peripherally. In the transverse section of the spinal cord, a small central canal can be seen in the center, surrounded by an "H"-shaped gray matter, and the gray matter is surrounded by white matter. Spinal cord gray matter is a complex composed of the cell bodies of motor neurons and interneurons, glial cells, and blood vessels. Spinal white matter is composed of glial cells and nerve fibers in ascending and descending tracts. Among them, oligodendrocytes, astrocytes, and microglia are present in the spinal cord [9, 10]. Astrocytes are related to the migration of neurons in the embryonic development stage, and when the nervous system matures, they constitute the structural basis of other cells. Astrocyte foot processes are involved in the formation of the blood-brain barrier and play an important role in protection to the central nervous system. Oligodendrocytes are involved in myelin formation, and microglia are associated with immune function in the nervous system [8]. Between cells in the spinal cord, there is an extracellular matrix, which is mainly composed of growth factors, hyaluronic, laminin, thrombospondin, fibronectin, proteoglycans (such-like chondroitin sulfate proteoglycan and heparan sulfate proteoglycan), Tenascin-C/-R, and other proteins. Interactions of neurons, glial cells, and the extracellular matrix form the structural framework of the spinal cord. In fact, cell differentiation, migration, proliferation, survival, synapse formation, and axonal growth also mainly depend on the interaction between glial cells and the extracellular matrix. The spinal cord has three main arteries supplying blood, and the blood distribution between different segments is not identical, and the blood-spinal cord barrier (BSCB) plays a protective role in the spinal cord [11] .

2.2. Physiology Function of the Spinal Cord. The function of the spinal cord is as follows. First, it receives somatic and visceral sensory information in most areas of the body, and this information is relayed in the spinal cord for preliminary integration and analysis. Part of the relayed information is transmitted upward to the higher center, and part is transmitted to motor neurons and other spinal cord neurons. Second, an ascending conduction pathway is sent to upload the relayed sensory information and the information of the spinal cord itself to the higher center. Third, it sends out motor fibers, manages body movement and visceral activity, and is the lower center of body and visceral movement. Fourth is the center of various basic reflexes. Last, through the descending conduction pathway, it relays the information transmitted by the superior center, accepts the control and regulation of the superior center, and completes the functions of the superior center.

3. Pathophysiological Changes of SCI

3.1. Primary and Secondary Injuries. The pathological process of SCI includes primary and secondary injuries [3] (Figure 1). The former refers to mechanical damage to the spinal cord caused by trauma, including instantaneous or sustained mechanical compression, contusion, stretch, laceration, or even transection, with or without spinal fracture or dislocation [12, 13]. Primary injury can cause mechanical and physical damage to neural tissues such as neurons and oligodendrocytes and at the same time cause damage to the vascular structure of the spinal cord, resulting in intramedullary hemorrhage and damage to the blood-spinal cord barrier [14]. This leads to various pathological reactions such as spinal cord edema, hemorrhage, or ischemia and ultimately leads to tissue destruction and cell death through mechanisms such as inflammatory response, lipid peroxidation, oxygen free radical formation, oxidative stress, nerve excitotoxicity, and ion imbalance [3, 15–17]. Secondary injury refers to a pathological process that starts within a few minutes after the primary injury and can last for a long time. It induces a variety of molecular biochemical cascade reactions in tissues, cells, etc., eventually leading to aggravation of SCI and hindrance of regeneration and repair of nerve tissue. In recent years, studies have also shown that autophagy is a cytoprotective mechanism for neurological diseases and injuries. After spinal cord injury, regulating autophagy can promote neuroprotection and functional recovery while reducing neuronal apoptosis and inhibiting inflammatory responses. However, excessive autophagy

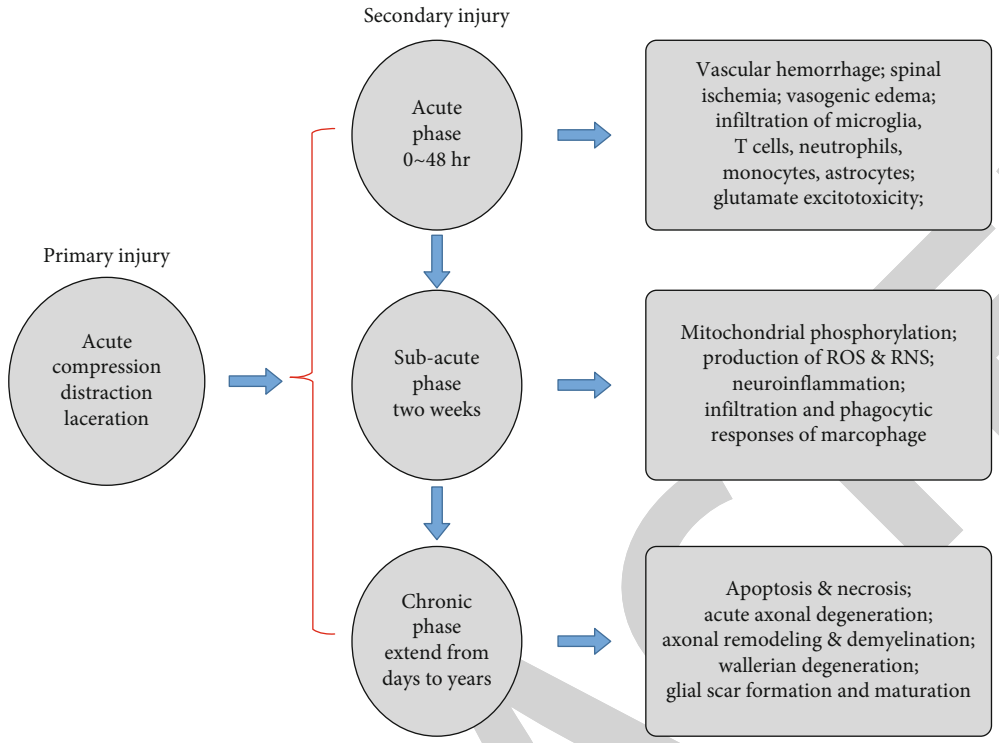

Figure 1: The mechanism of pathophysiological changes after SCI.

may activate apoptosis or other cell death mechanisms and lead to the secretion of proinflammatory factors, thereby affecting the recovery of nerve regeneration [18–20].

Secondary injuries are usually divided into the following stages: acute phase (<48 h), subacute phase (48 h-14 days), intermediate phase (14 days-3 months), and chronic phase (>3 months) [13, 21]. The acute phase is the first stage of secondary injury, which may include inflammation, lipid peroxidation, death of necrotic cells, spinal cord edema, free radical formation, calcium influx, ion disturbance, and excitotoxicity caused by neurotransmitter accumulation [3, 13, 22]. If combined with vascular injury, which leads to the enhanced permeability of the blood-spinal cord barrier, the damage of vascular endothelial cells, and the local exudation of immune cells and proteins, the situation will be further aggravated [23, 24]. In the subacute phase, astrocytes at the injury site will proliferate and transform into reactive astrocytes. The infiltration and phagocytic responses of macrophage can also be observed. Over time, the glial scars gradually develop [13, 24, 25]. The formation of glial scars is beneficial in rebuilding the damaged blood-spinal cord barrier, thereby reducing cell exudation, producing antioxidants, reducing edema, restoring ion balance, and reducing excitotoxic effects. However, it also has a certain inhibitory effect on the regeneration and repair of nerve tissue in the late stage [25]. In the intermediate phase, the scar formed by astrocytes matures, followed by axonal sprouting heralding the onset of nerve regeneration [26]. The chronic phase is typically characterized by cyst formation and Waller's degeneration, as well as inflammation, apoptosis, and nerve demyelination [27, 28] (Figure 2).

According to the location and degree of spinal cord injury and clinical manifestations, we often divide it into different syndromes. These include central cord syndrome, anterior cord syndrome, conus medullaris syndrome, cauda equina syndrome, and Brown-Sequard syndrome. The central spinal cord syndrome mostly occurs in the injured area of the cervical spine, and the conus medullaris syndrome and the cauda equina syndrome are mostly related to the injury of the conus medullaris and lumbosacral nerve roots. Anterior cord syndrome and Brown-Sequard syndrome are often accompanied by varying degrees of motor function loss and changes in pain and temperature sensation.

3.2. Effects of SCI on Resident Cells. After SCI, changes in the local microenvironment can cause dynamic changes in resident cells such as neurons, astrocytes, oligodendrocytes, and microglia, including cell phenotype, number, and distribution. Understanding the dynamic changes of cell behavior could help develop better strategies for neural regeneration.

3.2.1. Neurons and Neural Progenitor Cells (NPCs). The neurons of the spinal cord are divided into different categories, including motor neurons, sensory neurons, excitatory neurons, inhibitory neurons, and relay neurons of the spinal cord itself. After SCI, the damage and apoptosis time and degree of damage of various types of neurons are different. In the transection injury model, neurons undergo apoptosis 1 hour after injury and are localized to the injury site [29]. In the compression injury model, neuronal apoptosis can persist from 1 hour to 2 weeks after injury [30]. In addition, injury disrupts the continuity of ascending or descending

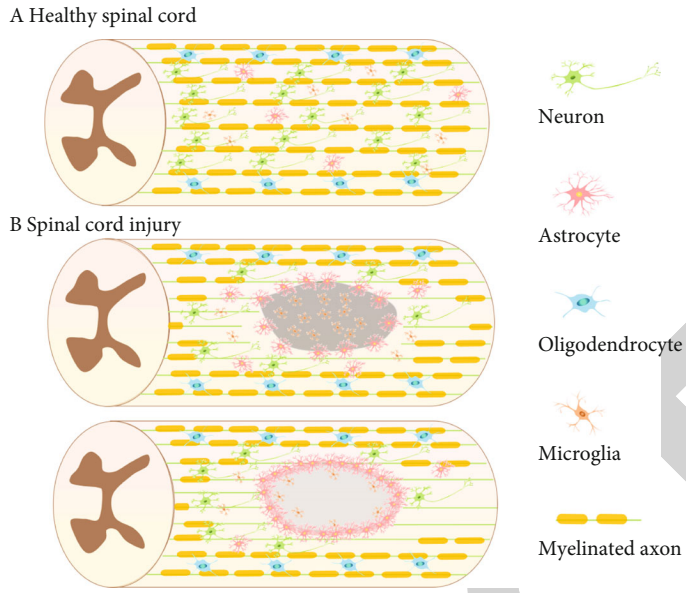

Figure 2: Structural changes in healthy and injured spinal cords: (a) healthy spinal cord and (b) SCI. The acute phase is the first stage of secondary injury, which may include inflammation, lipid peroxidation, spinal cord edema, free radical formation, and excitotoxicity caused by neurotransmitter accumulation. In the subacute phase, astrocytes at the injury site will proliferate and transform into reactive astrocytes. The infiltration and phagocytic responses of macrophage can also be observed. The glial scars gradually develop which is beneficial to rebuild the damaged blood-spinal cord barrier. The chronic phase is typically characterized by cyst formation and Waller's degeneration, as well as inflammation, apoptosis, and nerve demyelination.

nerve fibers, leading to the Wallerian degeneration [31]. Following SCI, many endogenous NPCs migrate to the injured area and proliferate and differentiate into neural cells to rebuild damaged neural network circuits [32]. NPCs can differentiate in different directions under different stimulation conditions [33]. Strengthening the research on the plasticity of NPCs will help us formulate neural regeneration strategies by regulating the proliferation, differentiation, and migration of endogenous NPCs and improve the ability of neural regeneration [34].

3.2.2. Astrocytes. Astrocytes are the most abundant cells in the nervous system. They can provide a variety of neurotrophic factors and energy substances, regulate the metabolism of glutamate and potassium ions and the water content in the spinal cord, and participate in various physiological behaviors of neurons. They also play a role in the resistance to oxidative stress and excitotoxicity [35, 36]. Naive astrocytes are typically activated within hours of SCI and undergo a transition to reactive astrocytes, which play an important role in wound healing in the acute phase but ultimately form dense glial scars, which hinder the regeneration and repair of neural network circuits [37]. Recently, it has been found that Nrf2 is a major transcriptional regulator of cellular antioxidant stress response in astrocytes; its regulation of antioxidant system plays a key role in the early stage after spinal cord injury. Increased Nrf2 activity in astrocytes can improve the inflammatory response after spinal cord injury and promote the recovery of motor function [38].

3.2.3. Oligodendrocytes and Oligodendrocyte Precursor Cells. Oligodendrocytes are involved in the formation of myelin, and primary injury of the spinal cord and secondary excitotoxicity and reactive oxygen species can have a serious impact on them. Damage to oligodendrocytes and myelin inhibits the propagation of action potentials, decreases effective conduction of nerve signals, and will lead to functional impairment [39]. Studies have reported that oligodendrocytes were significantly reduced within 15 minutes after SCI in rats and peaked within hours [40]. Oligodendrocytes are produced from their precursor cells. Oligodendrocyte precursor cells are the most proliferative cells in the nervous system. They can proliferate and differentiate into oligodendrocytes and Schwann cells after SCI and thereby promote axonal remyelination [41]. Some scholars regulate the proliferation and differentiation of oligodendrocyte precursor cells through various pathways, such as regulating BMP signaling and Nrg1 signaling, indicating that it is also an important factor and therapeutic target for SCI repair [42].

3.2.4. Microglia. Microglia are immune cells with self-renewal ability and are an important part of the immune function of the central nervous system [43, 44]. After SCI, microglia are rapidly activated and persist for longer periods of time. Activated microglia can polarize and secrete inflammation-related factors with multiple effects on neuronal survival and the local microenvironment [45]. Different microglia phenotypes were found in injured spinal cord by a single nuclear RNA sequencing technique, suggesting that in the future, people can use multiple potential immunotherapy

targets to regulate microglia-induced inflammation-related pathological changes [46].

3.3. Factors Affecting Nerve Regeneration

3.3.1. Syringomyelia. Following SCI, early massive cell death and degeneration of spinal cord structure lead to a loss of parenchymal tissue in the central area of the injury, forming cystic cavities with extracellular fluid, connective tissue bands, and infiltrating macrophages [47, 48]. The presence of CSF pressure within the cystic lumen may cause the smaller lumen to fuse with each other and expand, further impeding axonal regeneration and cell migration [49, 50]. Researchers need to adopt more effective regeneration strategies to overcome the hindrance of syringomyelia on nerve regeneration to reshape the connection circuits of neural networks.

3.3.2. Inflammatory Response. The immune cells of the central nervous system mainly include microglia and macrophages around blood vessels, meninges, choroid plexus, and periventricular. Microglia, as resident cells of the central nervous system, are rapidly activated after SCI, transform into phagocytic microglia, and migrate and aggregate to the site of injury at the same time [51, 52]. A strong local inflammatory response also promotes infiltration of macrophages into lesions [53]. Microglia can secrete anti- or proinflammatory cytokines, neurotrophic factors, chemokines, growth factors, etc. while removing toxic substances and cellular debris through phagocytosis [51]. After injury, microglia activate, proliferate, and migrate to the injury site, which can help maintain local cellular homeostasis, but a large number of proinflammatory factors released by them can also induce neuronal death, and the pathological mechanism may involve oxidative stress, ionic imbalance, etc. [54–56]. Studies have reported that M2-activated subset of microglia has a lower proinflammatory response than M1-activated subset of microglia and are more conducive to axonal growth [57, 58]. Therefore, researchers are also trying to promote the regeneration and recovery of neural tissue after SCI by regulating the phenotype of microglia and macrophages.

3.3.3. Glial Scar. After SCI, astrocytes, the most abundant resident cells in the central nervous system, begin to proliferate and transform into reactive astrocytes, which interweave to isolate the injured area from the normal area [59]. Over time, reactive astrocytes around the lesion begin to surround fibroblast-like pericytes, eventually forming an astrocyte scar [60, 61]. Glial scar is often considered to be the main obstacle to nerve regeneration because it hinders the growth of neurons, but some studies have also pointed out that it helps to repair the damaged blood-brain barrier, effectively preventing damage from spreading while improving inflammation response [59, 62–65]. Research into regulating the formation and development of scars has potential therapeutic implications for promoting SCI recovery.

3.3.4. Chondroitin Sulfate Proteoglycan. Chondroitin sulfate glycoproteins are widely expressed in the central nervous system and are closely associated with cell migration and axonal growth [66, 67]. CSPGs include neurocan, versican, brevican, phosphacan, and NG2 [68]. After SCI, the inflammatory response greatly increases the secretion of CSPGs from cells, and the deposited CSPGs promote the formation of glial scars and hinder axonal regeneration [69]. Furthermore, CSPGs have inhibitory effects on the migration and differentiation of oligodendrocyte precursor cells, thereby inhibiting remyelination [70]. Studies have shown that chondroitinase ABC (ChABC) is a potential therapeutic strategy against the inhibition of CSPGs, which promotes axonal growth and functional improvement after SCI by degrading CSPGs [71].

3.3.5. Other Relevant Factors. SCI leads to oligodendrocyte death and myelin damage, which affects nerve signaling. At the same time, myelin-related components such as myelin-associated protein (MAG) and oligodendrocyte myelin glycoprotein (OMgp) are also the inhibited factors of axonal growth [72]. In addition, neurite outgrowth inhibitor (NOGO), Repulsive Guidance Molecule A, etc. also inhibit axon regeneration and repair through their respective signaling pathways [73, 74].

4. Neural Repair Mechanisms in SCI

After SCI, the final repair effect is often unsatisfactory due to its limited ability of nerve regeneration. The injury-induced syringomyelia, glial scarring, inflammatory responses, and the local inhibitory microenvironment caused by release and accumulation of axonal growth-inhibiting substances also play an important role. At present, the recognized nerve repair mechanism of SCI includes two aspects. On the one hand, the intrinsic motor nerves of the spinal cord regenerate through their axons, cross the area of injury, establish connections with distant neurons, and remodel neural circuits [75]. Some scholars have constructed functional biomaterials by combining natural or synthetic biomaterials with growth factors, anti-inflammatory cytokines, drugs, antibodies, nanoparticles, etc., through their specific therapeutic targets, which can effectively promote the disconnected nerve axon regeneration [76–78]. On the other hand, the transplanted exogenous NPCs or the endogenous NPCs migrated to the injured area formed relay neurons through differentiation, which played a bridging role and promoted new synaptic connections and neural circuit formation [79–82]. It is worth mentioning that when using functional biomaterials, the activated endogenous neural stem cells showed stronger neuronal differentiation ability and neural stem cell recruitment ability; in addition, exogenous neural stem cells cotransplanted with functional biomaterials are able to survive longer at injury site and enhance differentiation into neurons [33, 83, 84].

5. Treatment Strategies for SCI

5.1. Current Clinical Treatment of SCI. The current treatments after SCI include maintaining the stability of the spinal cord after injury, early surgical decompression, and

corresponding treatment measures from the aspects of neuroprotection and nerve regeneration. The goal of postinjury spinal cord fixation is to avoid additional trauma, while early surgical decompression relieves persistent compression and avoids further expansion of ischemia and nerve tissue damage [13, 85, 86].

Neuroprotective strategies include drug therapy such as glucocorticoid methylprednisolone, sodium channel blocker riluzole, and nondrug therapy such as cerebrospinal fluid drainage, blood pressure augmentation, and therapeutic hypothermia. Methylprednisolone can enhance neuron survival after injury by regulating the release of anti-inflammatory cytokines and attenuating oxidative stress, and riluzole reduces excitotoxicity influence to cells by preventing sodium influx and regulating glutamine release. Combined treatment of cerebrospinal fluid drainage and blood pressure augmentation can increase the blood supply and perfusion pressure in the injured area and prevent ischemic injury. Therapeutic hypothermia can reduce the basal metabolic rate of the central nervous system and improve the inflammatory response at the site of injury, while also reducing oxidative stress and excitotoxicity [15, 87–90].

In recent years, research in the field of nerve regeneration has gradually become a hot spot in the field of SCI repair, especially treatment strategies based on cell transplantation and functional biomaterials, which have given people better expectations for the prognosis of SCI patients. In addition, treatment regimens for Rho-ROCK inhibitors and anti-NOGO antibodies have also shown certain therapeutic effects in animal models and clinical trials [91, 92].

5.2. Cell Therapy for SCI. Cell-based regenerative therapy is a very promising direction for the treatment of SCI, including both exogenous cell transplantation and enhancement of endogenous stem cell function [93–95]. We can achieve better regenerative treatment effects by selecting different types and states of cells, as well as different treatment methods and intervention timings [96, 97]. Transplanted cells can replace damaged and lost neural tissue, secrete essential neurotrophic factors, modulate the local microenvironment and immune responses, and provide the substrate and support needed for regeneration of axonal, remyelination, and neural tissue repair [98–101]. Of course, cell transplantation therapy also faces some problems, such as local tumor formation, poor cell differentiation, enhanced immune response of transplanted cells, and low cell survival rate, which need to be further solved.

5.2.1. Selection of Transplanted Cells

(1) Neural Stem Cells. Neural stem cells are self-renewing cells with multiple differentiation potentials that can differentiate into neurons, astrocytes, and oligodendrocytes under different conditions [102, 103]. The theoretical basis for the transplantation of neural stem cells for the treatment of SCI is that, on the one hand, neurons differentiated from neural stem cells can act as relay neurons to integrate into the broken neural circuit; on the other hand, oligodendrocytes differentiated from neural stem cells can participate in the formation of myelin sheaths and promote the regeneration and repair of axons and signal transduction [81, 104, 105]. According to literature reports, transplanted neural stem cells can survive and differentiate in multiple directions at the site of injury and at the same time play a role in regulating local immune function by promoting the infiltration of anti-inflammatory M2 macrophages [106, 107]. Scholars are trying to use neural stem cell transplantation for clinical research; considering the variety of the type and function of neurons in the spinal cord tissue, whether the neural stem cells transplanted can successfully differentiate into subtypes of neurons that we need, especially the spinal interneurons and spinal motor neurons whose functions are seriously affected because of the damage, is still worth our further discussion.

(2) Mesenchymal Stem Cells. Mesenchymal stem cells are self-renewing cells with multiple differentiation potentials [108]. It can perform tissue regeneration repair by differentiation into osteoblasts, chondrocytes, myocytes, and adipocytes [109–112]. Studies have shown that mesenchymal stem cells can enhance tissue protection and promote neural tissue repair and neovascularization by secreting neurotrophic factors and regulating immune function [113, 114]. Significant tissue repair and reduction of peripheral inflammatory cell infiltration can be observed in animal models of SCI treated with MSCs [115]. Reviewing recent studies on MSCs in the treatment of SCI, the possible mechanisms of MSC transplantation to promote tissue regeneration include regulating immune responses (regulation of macrophage phenotype M1 to M2 and reducing inflammatory cell infiltration), inhibiting apoptosis (inhibiting inflammatory corpuscles), promoting angiogenesis, and promoting regeneration of axons and myelin [116–121]. In addition, MSCs are important candidates for regenerative medicine because of their ease of acquisition, ease of storage, low immunogenicity, and strong proliferative capacity [108, 122–124]. In preclinical studies, the use of MSCs for the treatment of SCI has yielded promising results. However, in the clinical studies that have been performed, the therapeutic effect of MSC transplantation for SCI varies. In a clinical study of 277 patients, 43.3% showed improvement in clinical function [124]. In another study of 44 patients, there was no significant recovery of neurological function after MSC treatment and the treatment effect was poor [125].

(3) Induced Pluripotent Stem Cells. Induced pluripotent stem cells are cells with self-renewal and multidifferentiation potential, which are generated by genetic modification and reprogramming of differentiated somatic cells, avoiding ethical and immune rejection, and bringing more possibilities to regenerative medicine [126–129]. Studies have shown that IPSC-derived neural stem cells can be used for the repairing treatment of SCI, acting as relay neurons at the injury site to form a neural circuit, and promoting neovascularization and remyelination, ultimately improving functional recovery [130, 131]. Another study also pointed out that IPSC-derived NSCs have the tumorigenicity. In this study, neural cells derived from IPSCs were transplanted for treatment.

Although these treatments were initially effective and motor function was improved, but during follow-up, tumor formation at the site of cell transplantation also resulted in a poor prognosis [132].

(4) Astrocytes. Naive astrocytes are activated after SCI to form reactive astrocytes that prevent the expansion of injury and inflammatory responses, and the formation of astrocyte scars is often thought to inhibit axon regeneration [59, 63, 65, 133]. However, it has been reported that inhibiting the growth of reactive astrocytes by genetic modification does not promote tissue regeneration but rather reduces the regenerative capacity of axons [65]. Studies have shown that, as the most abundant glial cells in the central nervous system, astrocytes form the basis of the structural framework between cells, provide a variety of neurotrophic factors, and provide support for the stability of the internal environment [134, 135]. After SCI, activated astrocytes interweave into networks that can provide physical support for axonal regeneration. Considering the above roles, astrocytes have been used to explore the treatment of SCI, and studies have shown that they can survive, integrate, and migrate at the injury site and show some potential for neuroprotection and functional improvement [136]. How to better exert the beneficial effects of astrocytes still needs more in-depth research.

(5) Oligodendrocyte Precursor Cells. Oligodendrocyte precursor cells can be rapidly activated after SCI, proliferate massively, and differentiate into oligodendrocytes and Schwann cells to promote remyelination [137]. Animal experiments have shown that the transplantation of oligodendrocyte precursor cells in the treatment of SCI has a certain recovery effect. It can be used as a substrate for remyelination, regulate local immune function, and secrete a variety of nutritional factors, cytokines, chemokines, etc. [129, 138, 139]. Further evidence is needed to support how oligodendrocyte precursor cells exert their role in the treatment of SCI.

(6) Olfactory Ensheathing Cells. Olfactory ensheathing cells are specialized types of glial cells present in the peripheral and central nervous systems and are found in the olfactory mucosa and olfactory bulb [140, 141]. It secretes neurotrophic factors, promotes angiogenesis, has phagocytic functions, and regulates local immune responses [142–144]. Some scholars have transplanted olfactory ensheathing cells to treat SCI. The results of animal models show that olfactory ensheathing cells produce extracellular matrix, which supports and guides the growth of axons and improves motor function [2, 145]. It has also been reported that olfactory ensheathing cells at different culture stages have different nerve repair effects and that cells cultured for less than three weeks have better therapeutic effects than cells cultured for seven weeks [146]. At present, olfactory ensheathing cells have been used in clinical trials for the treatment of SCI, and the safety and efficacy need to be confirmed by a larger number of clinical samples.

(7) Schwann Cells. Schwann cells are the most numerous glial cells in the peripheral nervous system and are an important part of nerve regeneration in the peripheral nervous system [147]. In animal models of SCI, Schwann cells can promote remyelination, provide growth factors and extracellular matrix components, reduce cyst formation, enhance axon regeneration in the central nervous system, and ultimately improve motor and sensory function [148–152]. In clinical studies that have been performed, autologous Schwann cell transplantation and continuous follow-up have shown that patients' motor and sensory functions have improved, and autonomic function has also recovered to some extent [153].

(8) Genetically Modified Cells. With the development of gene technology and cell culture technology, scholars are also trying to use gene-modified cells to treat SCI. It can more efficiently express the required protective nerve growth factor, enhance the differentiation of neural stem cells or progenitor cells into neurons, and improve the survival rate of transplanted cells, thereby improving the effect of cell therapy and promoting the regeneration and repair of SCI [154–156]. According to literature reports, human umbilical cord blood mononuclear cells genetically modified with VEGF, GDNF, and FGF2 significantly improve the therapeutic effect compared with the nontransduced cells when transplanted into the site of SCI [157, 158]. Some scholars have used Wnt4-modified neural stem cells to stimulate nerve regeneration while enhancing the differentiation of cells into neurons, which ultimately significantly improves neural repair and functional recovery [159]. There are also studies reported that the chondroitinase ABC (ChABC) gene and tumor suppressor gene (PTEN) of adipose-derived mesenchymal stem cells were modified by gene modification technology. By promoting the expression of chondroitinase ABC (ChABC) and downregulating the expression of tumor suppressor gene (PTEN), it can enhance cell survival and function and reduce the inhibitory effect of glial scar [160]. In the future, gene-modified cell therapy also has great application potential.

5.2.2. Enhancing the Survival and Function of Transplanted Cells. With the increasing number of attempts to treat SCI with cell transplantation, some problems have also been identified, for example, the survival rate of transplanted cells is low, the extravasation of transplanted cells at the injury site, and how to make the transplanted cells function better at the injury site. Researchers have also made many attempts in this regard. Intrathecal injection of neurotrophic factors (e.g., BDNF), growth factors (e.g., IGF-1), etc. has been reported to enhance the survival of transplanted cells [161]. Using genetic modification techniques to modify cells to express more factors required for cell survival has also been used in multiple preclinical studies [157, 162, 163]. The combined transplantation of cells and functional biomaterials with specific therapeutic targets not only facilitates cell adhesion and prevents cell extravasation but also enhances the survival rate of transplanted cells and promotes stem cells to differentiate in beneficial directions. Functional biomaterials themselves can also act as a support for axonal growth and promote regeneration and repair of

neural tissue [164, 165]. In addition, application of an external electric field promotes cell migration and differentiation; alleviates the barrier inhibition of glial scars to increase the integration of transplanted cells into the host; and genetically modifies the major histocompatibility complex and CD47 of transplanted cells to reduce central nervous system immune rejection has all been shown to be effective [61, 166–169].

5.2.3. Utilization of Endogenous Stem Cells. Another direction of cell therapy is to fully exploit the regenerative potential of endogenous stem cells [170]. Following SCI, endogenous stem cells in the spinal cord proliferate, migrate to the injury site, and further differentiate into neurons, astrocytes, and oligodendrocytes to promote tissue regeneration [171]. Improving the understanding and regulation of these cells will help us promote our ability to use endogenous stem cells for tissue regeneration and increase therapeutic targets for SCI. It has been reported that intrathecal injection of the growth factors EGF and FGF2 increases the proliferative capacity of endogenous stem cells and facilitates tissue repair [161, 172]. Some scholars have also found that the small molecule drug metformin can activate endogenous stem cells and promote their differentiation into neurons and oligodendrocytes [173]. The transplantation of exogenous stem cells also has a certain stimulating effect on endogenous stem cells and makes them develop and differentiate in the direction that is beneficial to nerve regeneration [174]. The related signaling pathways and therapeutic targets that regulate the proliferation, migration, and differentiation of endogenous stem cells still require further study.

5.3. Functional Biomaterials for the Treatment of SCI. After SCI, the huge gap formed by the loss of neural tissue is the main obstacle to axon regeneration and neural circuit repair. At the same time, the therapeutic effect of simple cell therapy is limited by local severe inflammatory response, extravasation of transplanted cells, and poor cell survival rate. In order to achieve a more ideal therapeutic effect, people try to transplant and integrate biomaterials into the injured spinal cord tissue. Initially, people only tried to find suitable biomaterials, which, through certain processing, were structurally suitable for implantation into the injured area and acted as a "bridge" to support and guide axon regeneration [175]. With the development of medicine, biology, tissue engineering, chemistry, and other technologies and the deepening of people's understanding of the pathophysiological changes of SCI, scholars began to modify biomaterials to have specific biological functions [176–179]. The emergence of functional biomaterials has brought new hope for the repair of spinal cord injuries. Through specific therapeutic targets, it can better improve the local inhibitory microenvironment, promote axonal and angiogenesis, reduce scarring, regulate immune response, and ultimately contribute to neural tissue repair [178–183].

5.3.1. Characteristics of Functional Biomaterials. Essential elements of functional biomaterials include good biocompatibility, suitable biodegradability, and low immunogenicity [179, 184]. Most of them have directional channels or fibers or three-dimensional porous structure, which not only conducive to cell migration and adhesion and directional growth of axons but also have certain regulatory effects on cell differentiation [185, 186]. In addition, functional biomaterials can carry a variety of biomolecules such as growth factors, drugs, antibodies, genes, enzymes, and exosomes through physical, chemical, and biological modifications and help tissue repair through different therapeutic targets, and at the same time, by adjusting the electrical conductivity, mechanical properties, structural morphology, etc. of the material, the optimal therapeutic effect can be achieved [176–179, 185, 187, 188].

5.3.2. Types of Functional Biomaterials. We divide functional biomaterials into the following four categories according to their main components and preparation methods: natural materials, synthetic materials, composite materials, and micro-/nanomaterials [189–191]. Natural materials have similar biological properties to tissues, are less toxic, and can be degraded, but some materials may also cause severe local inflammatory reactions. People try to find a balance between the mechanical strength and degradation rate of materials suitable for tissue repair, so that they can degrade at a suitable rate while meeting the requirements of certain tissue mechanical strength, so as to achieve matching with tissue repair [184, 192–194]. Commonly used natural materials include agarose, collagen, gelatin, chitosan, alginate, fibrin, hyaluronic acid, and extracellular matrix. Synthetic materials have many advantages which combine the required mechanical properties and degradability for tissue engineering design while also satisfying their economics, reliability, toxicity, and biocompatibility [193, 195–197]. Synthetic materials mainly include degradable polymers (PGA, PCL, PLA, PLGA, and PEG), nondegradable polymers (PHEMA and PHPMA), synthetic polypeptide molecules, and conductive polymers. In order to realize the complementary advantages of two or more biomaterials, composite materials emerge as the times require, which may have better tissue engineering properties and bioremediation effects [190, 198–200]. Nanomaterials also have promising applications in tissue damage repair, including nanofibrous scaffolds as well as nanoparticles [188, 201]. Micro-/nanofibrous scaffolds prepared by electrospinning techniques have been used in preclinical studies [186, 202–204]. In addition, 3D printing technology for the treatment of SCI is also one of the research hotspots in the field of organ and tissue regeneration in recent years. Tissue construction is based on self-assembly and bionics. Using a variety of biomaterials and biomolecules provides targeted and individualized treatment of tissue damage [203, 205, 206].

5.3.3. Therapeutic Targets of Functional Biomaterials. After SCI, due to the complex and dynamic changes in the pathophysiological mechanisms affecting nerve repair, people have developed and prepared a variety of functional biomaterials based on injury factors and nerve repair mechanisms, which have shown gratifying effects in animal models. Clinical trials have been carried out. From the perspective of

promoting axonal growth and reformation of neural circuits, some scholars have combined PLGA microspheres loaded with FGF2 with a biopolymer mixture hydrogel containing hyaluronic acid and methylcellulose, which can repair SCI through local delivery and sustained release effects [207]. Some scholars also combine collagen with neurotrophic factor 3 (NT-3)/brain-derived nerve growth factor (BDNF) through the collagen binding domain and use the slow-release characteristics of recombinant collagen and the biological properties of growth factors to construct bioactive scaffolds [208, 209]. Zhang et al. used hydrogel scaffolds encapsulated with a variety of microRNAs and neurotrophic factors for animal model research, by regulating the expression of proinflammatory genes and extracellular matrix deposition-related genes and promoting local protein synthesis in growth cones that play an important role in axonal growth and development to improve nerve damage [210]. Histological, behavioral, and electrophysiological analysis showed that the above bioactive scaffolds could effectively promote the growth of axons and ultimately promote tissue repair. Some scholars have also studied immunomodulation as a therapeutic target. Fan et al. combined collagen with Fab fragments of EGFR antibodies to construct bioactive scaffolds and improved the microenvironment of axon regeneration through the regulation of myelin-related inhibitors by EFGR antibodies [84]. Some scholars have combined carriers with drugs, growth factors, anti-inflammatory cytokines, etc. to construct anti-inflammatory functional biomaterials, which can regulate the immune response and microenvironment after injury by regulating the phenotype and number of macrophages and microglia at the injury site and ultimately promote functional recovery [211–214]. From the perspective of promoting angiogenesis, Wang et al. designed a collagen scaffold loaded with vascular endothelial growth factor (VEGF), which not only promotes neovascularization but also contributes to functional recovery [215]. On this basis, a functional collagen scaffold containing both stromal cell-derived factors and paclitaxel liposomes was further developed. Histological analysis showed that the scaffold had a synergistic effect on promoting axonal and angiogenesis in the lesion area [216]. Some scholars have regarded scar tissue as a therapeutic target. In order to reduce the hindrance of scar tissue with high expression of chondroitin sulfate caused by injury, methylcellulose hydrogel containing stromal cell-derived factor-1α was combined with chondroitinase. The functional bioactive scaffolds can maintain ChABC activity with long-term slow release and enhance the recruitment of endogenous neural precursor cells, ultimately promoting tissue regeneration [217–219]. Some scholars have also used exosomes for the treatment of spinal cord injury. The exosomes derived from human mesenchymal stem cells were immobilized on the peptide-modified hydrogel with adhesion and transplanted into the spinal cord injury. Unlike the systemic delivery of exosomes, this treatment modality provides an extracellular matrix containing exosomes to nerve tissue at the site of injury, reducing neuronal inflammation and oxidative stress. At the same time, the exosomes maintain better activity and sustained release effect [220]. Considering the mechanism of nerve repair, how to promote the formation of relay neurons at the injury site through functional biomaterials is also the focus of research. According to reports, collagen is used as the main component of the bioactive scaffold, through the preparation process and method to make it have a certain appearance characteristics and then modify it with the stromal cell-derived factors, neurotrophic factors (NT-3 and BFGF), genes, antibodies (cetuximab), and drugs (paclitaxel) which can effectively promote the migration and survival of endogenous neural stem cells and induce them to differentiate into neurons, eventually forming complete neural circuits [76, 221, 222]. Nanomaterials also have their own unique functional properties, and studies have shown that the structure and morphology of nanofibers have an impact on the therapeutic effect [185, 186, 188]. Fibers with a diameter of 400 nanometers were more effective in promoting cell migration and growth of protrusions than nanofibers with diameters of 800 nanometers and 1200 nanometers [223]. Furthermore, oriented nanofibers promote the growth of nascent axons, which may be involved in glutamate transport [224]. It has also been reported that oriented nanofibers can promote the recruitment and migration of endogenous neural stem cells and guide them and exogenous neural stem cells to differentiate into neurons, expressing higher levels of neuron-related proteins [225–227]. Nanoparticles are widely used in drug delivery systems due to their unique advantages, which can successfully reach and stay at the injury site for a long time, and achieve the purpose of promoting injury repair by continuously releasing growth factors, drugs, etc. [228]. Li et al. designed a nanoparticle containing the polypeptide CAQK (CAQK-MET-NPs) for targeted delivery of metformin. While exerting anti-inflammatory, antioxidant, and neuroprotective effects, it overcomes the disadvantages of poor water solubility and low bioavailability of drugs and ultimately promotes spinal cord repair and motor function improvement [229].

5.4. Combination Therapy of Cells and Functional Biomaterials. SCI leads to disruption of neural circuits, tissue loss, and cyst formation. Both cell transplantation and functional biomaterial transplantation have been proven to effectively promote the repair of nerve tissue after SCI. In order to improve the therapeutic effect and repair efficiency, people have tried to combine cells with functional biomaterials to treat SCI.

5.4.1. Methods of Combining Cells with Functional Biomaterials. The methods of combining cells and functional biomaterials to treat SCI mainly include the following: the first mixes cells with functional biomaterials in vitro to form a tissue-like matrix, which is then implanted at the injury site. The second is the simultaneous injection of self-assembled biomaterials and cells into the injury site to form tissue scaffolds containing cells in vivo. The first two are mostly used for gel materials. The third type is to plant cells on the prepared bioactive scaffolds in vitro and then implant the scaffolds with cells into the damaged area. Most of the scaffolds have a fixed shape. The fourth type is to implant prefabricated scaffolds into the injury and then inject cells around the material to promote the integration

of the biomaterial with the injured tissue. The latter two categories are mostly used for solid-state biomaterials [230].

5.4.2. The Role of Cells Combined with Functional Biomaterials. Functional biomaterials can not only fill the lesion cavity and provide physical support for axonal regeneration, but their unique biological functions can also effectively promote axonal growth and angiogenesis, regulate immune responses, alleviate scar inhibition, and help transplant cell survival and differentiation. Transplanted cells can secrete trophic factors necessary for nerve repair and promote axon regeneration and endogenous stem cell migration, and neural stem/progenitor cells can also differentiate into relay neurons to integrate damaged neural circuits. In addition, biomolecules released from functional biomaterials and transplanted cells can also downregulate the concentration of growth inhibitory components (cells, myelin debris, inflammatory cytokines, etc.) caused by nerve injury and improve the local microenvironment (Figure 3). It has been reported in the literature that mouse neural stem cells were planted on a PLGA scaffold with a special morphology and transplanted into the SCI. Animal models had the highest behavioral scores and the best treatment outcomes and significantly improved transplant cell survival compared to scaffold or cell therapy alone [231–233]. In other studies, NPCs from the neonatal rat telencephalon were combined with a collagen scaffold modified with cetuximab for SCI repair, regulating cell differentiation behavior and promoting axon regeneration by inhibiting downstream signaling pathways activated by myelin-related inhibitors. The results showed that in the experimental group, the differentiation of neural precursor cells into neurons was increased, the regeneration of axons was enhanced, and the functional recovery effect was better [234–237]. Other scholars have planted human umbilical cord blood mesenchymal stem cells on silk fibroin/alginate scaffolds carrying glial cell-derived nerve growth factors, increasing neuronal survival at the injury site, and promoting tissue repair by slow-release effect of functional scaffolds and biological effects of growth factors and transplanted cells. The combination of cells and functional bioscaffolds showed higher therapeutic efficiency and better repair results compared to the cell-free transplantation group and the group using only the scaffold without biological modification alone [238]. In another study, some scholars modified a hydrogel scaffold composed of hyaluronic acid and methylcellulose with platelet-derived growth factors and then cotransplanted neural precursor cells with bioactive scaffolds for the treatment of SCI. The bioactive scaffold significantly improved the survival rate of transplanted cells and induced the differentiation of neural precursor cells into oligodendrocytes. The experimental group showed better tissue repair and functional recovery compared to the simple transplanted cell group [164]. Lu et al. reported the synergistic effect of neural stem cells and functional bioscaffolds containing multiple growth factors. In an animal model of severe SCI, GFP-expressing neural stem cells were transplanted at the injury site in combination with a fibrin matrix containing a cocktail of growth factors (brain-derived neurotrophic factor, neurotrophic factor-3, glial cell-derived neurotrophic factor, epidermal growth factor, basic fibroblast growth factor, acidic fibroblast growth factor, hepatocyte growth factor, insulin-like growth factor, platelet-derived growth factor, vascular endothelial growth factor, and calpain inhibitors). The results indicate that transplanted stem cells can differentiate into neurons, and a large number of axons can be seen growing through the damaged area, forming abundant synapses with host cells, and ultimately improving functional recovery [82]. With the maturity of cell culture technology and the innovation and development of functional biomaterials, the combination of the two in the treatment of SCI may bring better prognosis for SCI patients in the future.

6. Prospects and Challenges

The regeneration and repair process of SCI is complex and affected by many factors, and its pathophysiological mechanism still needs further research by scholars. In recent years, many preclinical studies have been carried out based on cell transplantation, functional biomaterials, and their combination therapy, and some studies have been carried out to the clinical trial stage. Although evidence shows that most of the research is beneficial to the repair of SCI and the improvement of motor function, there are still many problems before it can be used in clinical practice and for the benefit of patients. Different types of SCI, patient conditions including age and underlying diseases, severity of injury, and different lesion volumes are suitable for different treatment modalities. When using cell transplantation to treat SCI, the selection of cell types, the number of cells to be transplanted, and the exact location and method of transplantation will affect the therapeutic effect. Inappropriate treatment may result in poor cellular viability, poor cell differentiation, and tumor formation, all of which need to be further addressed and standardized. In addition, due to the complexity of organisms, the biological functions of some cells have two sides, and it is necessary to explore the balance point of treatment. For example, naive astrocytes are activated after SCI to form reactive astrocytes, which prevent the expansion of the injury and inflammatory response, and their eventual formation of astrocyte scars is often considered to inhibit the regeneration of axon. However, some scholars inhibited the growth of reactive astrocytes by gene editing but did not help tissue regeneration. On the contrary, studies showed that the regeneration ability of axons was reduced. Oligodendrocyte precursor cells can differentiate into oligodendrocytes and Schwann cells, which act as substrates for remyelination, regulate local immune function, and secrete a variety of trophic factors, cytokines, chemokines, etc. They aid in myelination and tissue repair. However, they can also hinder axonal regeneration by promoting CSPG deposition to form inhibitory glial scars. Researchers need to use a variety of research methods such as omics sequencing to reveal the dynamic changes and interactions of cells in the process of growth and differentiation, so as to achieve better therapeutic effects. When using functional biomaterials to treat SCI, the key point is whether the tissue structure and biological function can be simulated

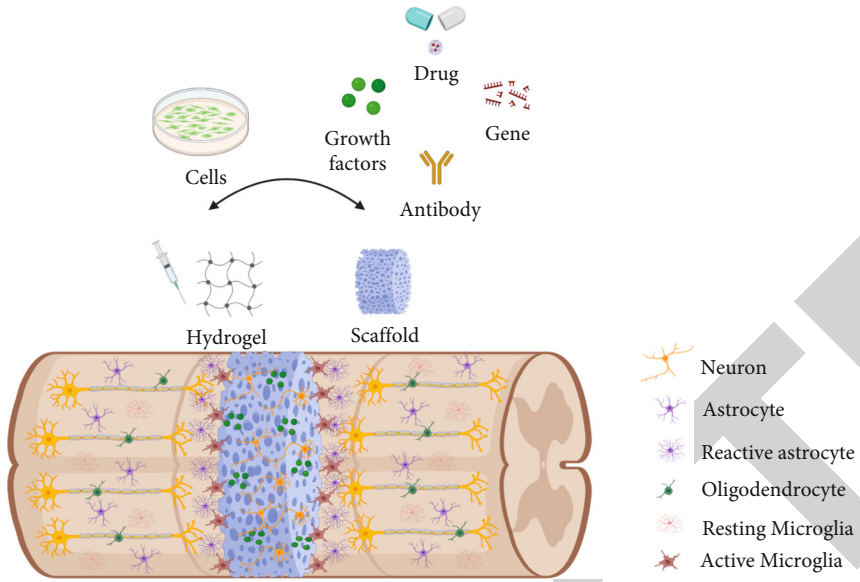

Figure 3: Combination therapy of cells and functional biomaterials. Functional biomaterials can not only fill the lesion cavity and provide physical support for axonal regeneration, but their unique biological functions can also effectively promote axonal growth and angiogenesis, regulate immune responses, alleviate scar inhibition, and help transplant cell survival and differentiation. Transplanted cells can secrete trophic factors necessary for nerve repair and promote axon regeneration and endogenous stem cell migration, and neural stem/progenitor cells can also differentiate into relay neurons to integrate damaged neural circuits. In addition, biomolecules released from functional biomaterials and transplanted cells can also downregulate the concentration of growth inhibitory components (cells, myelin debris, inflammatory cytokines, etc.) caused by nerve injury and improve the local microenvironment.

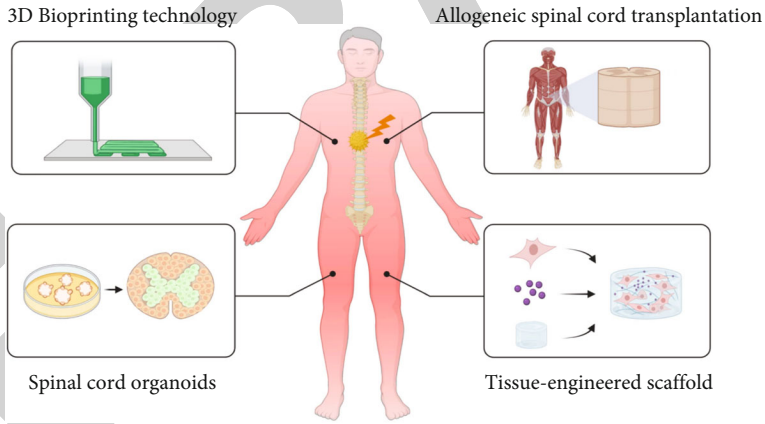

Figure 4: Advanced approaches to treating SCI.

to the greatest extent, whether the functional biomaterials can release biomolecules continuously and provide nerve cells, axonal structures, and extracellular matrix similar to natural tissues at the same time, and whether it can repair injury through specific therapeutic targets while having the degradation properties and degradation products that match the tissue recovery. Combining functional biomaterials with cell transplantation for the treatment of SCI is a promising treatment modality. This combined strategy provides the transplanted cells with a physical matrix for adhesion, proliferation, and differentiation and improves the survival rate of cell transplantation. Functional biomaterials play a regulatory role through specific therapeutic targets and are more conducive to the formation and continuous growth of new axons, forming tissue bridges, integrating the materials into the host, and finally forming a complete neural circuit. In addition to the treatment modalities highlighted in this article, other single or combined strategies (cells and growth factors, cells and pharmacological agents, etc.) have also been used to treat SCI. Considering the complexity and diversity of the pathophysiological mechanisms of SCI, combined strategies often show better therapeutic effect. In addition, technologies such as allogeneic spinal cord tissue transplantation, spinal cord organoid research, and bioengineered spinal cord-like tissue construction have also been tried to be applied to the treatment of SCI (Figure 4). How to assess the effect of treatment after spinal cord injury is also critical. In addition to behavioral improvements,

scholars have also used neural tracers to evaluate the repair effect of neural networks, including biotinylated dextran amine (BDA) and horseradish peroxidase (HRP). Tracers can be injected into specific areas of neural tissue, transported anterograde or retrograde, and analyzed in tissue sections after arriving at a distance. In addition, neurotropic viruses, electromyography, magnetic resonance imaging (MRI), and diffusion tensor imaging (DTI) have also been used to evaluate neural networks [239–242].

In this paper, we started from the anatomical structure of the spinal cord, the pathophysiological changes after injury, and the regeneration and repair mechanism of spinal cord nerves, and then, we discussed treatment methods based on functional biomaterials and cell transplantation. All in all, considering the complex pathophysiological mechanisms and dynamic changes of SCI, the combined therapy of the two has great potential for clinical translation in the future and is expected to provide new hope for patients suffering from SCI. Of course, the combination therapy strategy also requires the cooperation of scholars from different disciplines such as medicine, chemistry, biology, and tissue engineering to provide more effective and safer treatment options for SCI.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

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Retraction

Retracted: Fetal Congenital Cardiac and Vascular Disorders Associated with Sertraline Treatment during Pregnancy: Analysis of FAERS Data

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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

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(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.

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

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Research Article

Fetal Congenital Cardiac and Vascular Disorders Associated with Sertraline Treatment during Pregnancy: Analysis of FAERS Data

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Objective. Sertraline is one of the most commonly used antidepressants worldwide and is one of the first-choice treatments for depression during pregnancy. This study is aimed at testing the possible association between sertraline intrauterine exposure and congenital cardiac and vascular disorder occurrences by assessing the publicly available US Food and Drug Administration Adverse Event Reporting System (FAERS). *Methods*. Disproportionality analysis and Bayesian analysis were used to mine FAERS for suspected congenital cardiac and vascular disorder data for sertraline intrauterine exposure from the first quarter of 2004 to the second quarter of 2021. *Results*. Among the 914 cases of sertraline used with congenital cardiovascular disease in the FAERS database, the reporting areas were mainly in the United States and Europe. The number of adverse events reported every year since 2004 has no many differences. Congenital anomalies are the most frequently reported serious clinical outcome. Among the 69 positive signals detected from 914 cases, 31 were invalid signals, and 38 were valid signals according to criteria. The most common ones are heart disease congenital, atrial septal defect, ventricular septal defect, patent ductus arteriosus, and persistent fetal circulation. *Conclusions*. Mining FAERS data can analyze and study the adverse reactions of sertraline in a more comprehensive and in-depth manner, thereby effectively reducing the risk of clinical medication.

1. Introduction

The prevalence rate of depression during pregnancy is high, ranging from 7% to 20% [1]. Depression during pregnancy is associated with smoking, maternal malnutrition, alcohol, insufficient weight gain and other substance intake, and increased risk of postpartum depression [2]. Nonpharmacological interventions are more suitable for mild-to-moderate depression, while antidepressant prescribing is preferable for severe depression or when other treatments are unavailable or ineffective [3]. Deciding whether to stop, change, or start antidepressants during pregnancy requires weighing the treatment-related maternal and infant risks with untreated depression-related risks. The widespread use of antidepressants during pregnancy makes it essential to understand the safety and the risk of adverse outcomes in the fetus.

Selective serotonin reuptake inhibitors (SSRIs) are considered as the most prescribed antidepressants during pregnancy because of its wealth and reassuring of data [4]. Approximately 63% to 85% of pregnant women who exposed to antidepressants are treated with SSRI [5]. Among the SSRIs, sertraline is one of the most commonly used antidepressants worldwide and is one of the first-choice treatments for depression during pregnancy [6, 7]. SSRIs are believed to be effective in treating psychiatric disorders by increasing the synaptic bioavailability of the neurotransmitter serotonin (5-HT) [8]. 5-HT easily crosses the placenta and plays a role in cardiac morphogenesis during endocardial cushion formation, which may cause heart malformations [9]. In addition, Sari and Zhou suggested that serotonin can promote the proliferation of fetal heart cells, and abnormal levels of serotonin or abuse of serotonin uptake blockers may alter heart development [10]. Several studies [11, 12] have shown that sertraline increases the risk of cardiovascular-related malformations in infants, while other studies [13, 14] found no correlation at all. Because

studies of antidepressants in pregnancy are usually not random, it is often difficult to determine whether the adverse results associated with antidepressants are related to the drug itself and other confounding exposures (such as alcohol, smoking, drug abuse, nutrition, and other drugs). Considering that the scope of application of sertraline has been expanded to special populations such as pregnant women, it is necessary to dig deeper into its adverse reaction signals to avoid drug risks and ensure drug safety.

Some countries or organizations have established pharmacovigilance systems, such as the US Food and Drug Administration (FDA), European Medicines Agency (EMA), and the World Health Organization (WHO), for adverse drug reaction (ADR) monitoring. The FDA's Adverse Event Reporting System (FAERS) is the largest repository of passively reported adverse drug events worldwide [15]. Given the inconsistency of previous results, as well as to provide the best estimates of the effect of sertraline usage during pregnancy, we performed a real-world analysis of FAERS to investigate the association between sertraline treatment and congenital cardiac and vascular disorders, with a view to revealing the regularity of sertraline adverse events, and for the control and management of sertraline safety risks, providing reference for clinical rational use of sertraline.

2. Material and Methods

2.1. Data Source. A retrospective pharmacovigilance study was performed using data from FAERS database covering period from the first quarter of 2004 to the second quarter of 2021. FAERS is a spontaneous reporting system (SRS) that contains adverse event reports, medication error reports, and product quality complaints resulting in adverse events submitted to FDA by healthcare professionals, consumers, manufacturers, and patients. The database contains demographic information, drug information, and reaction information. Each report has a primary suspected drug with one or more adverse drug reactions (ADR) and may include other drugs taken by the patient [16].

The ASCII data files of the FAERS contain demographic and administrative information (DEMO), drug information (DRUG), adverse events (REAC), patient outcomes (OUTC), report sources (RPSR), therapy start dates and end dates for reported drugs (THER), and indications for use (INDI).

A total of 15881123 reports were retrieved from the FAERS. A deduplication procedure was performed according to the FDA's recommendations to select the latest FDA_DT with the same CASEID and select the higher PRIMARYID when CASEID and FDA_DT are both the same, resulting in a reduction in the number of reports to 13327865. On the basis of the PRIMARYID of the deduplicated DEMO, DRUG and REAC were both deduplicated as well.

2.2. Data Mining. FAERS data requires substantial curated cleaning and normalizing before they can be used appropriately; otherwise, data can have material impact on analysis results. We used Python (version 3.8) and PostgreSQL (version 12) to deal with cleaning and normalizing process which includes merging data, deduplicating records, applying standardized vocabularies with drug names mapped to RxNorm concepts and indications and outcomes mapped to SNOMED-CT concepts, and normalizing reaction to MedDRA (version 24.0) concepts and used the R software (version 4.1.0) to statistical compute drug-reaction signals. Figure 1 presents main steps.

2.3. Statistical Analysis. "Sertraline" was chosen as the drug name with a reported role code "PS" (primary suspect drug) [17] and reactions with the MedDRA SOC term "congenital, familial, and genetic disorders" (MedDRA code: 10010331) from DRUG_REACTION PAIRS to be evaluated. In total, 127 drug-reaction pairs were retrieved.

Both disproportionality analysis and Bayesian analysis applied with the use of the reporting odds ratio (ROR), proportional reporting ratio (PRR), Bayesian confidence propagation neural network (BCPNN), and multi-item gamma Poisson shrinker (MGPS) algorithms were used to investigate the potential signals between the drug and the specific adverse event of interest. The equations and criteria for the four algorithms above [18–25] are demonstrated in Table 1. One of the four algorithms meet the criteria should be considered as a positive signal.

2.4. Compliance with Ethics Guidelines. All procedures performed in this study involving human participants were in accordance with the 1964 Helsinki declaration. This study was exempted from approval by the ethics committee of the Second Hospital of Shandong University.

3. Results

In total, 914 cases of intrauterine sertraline exposure with fetal congenital cardiovascular disease were identified in the FAERS database. Of these events, 43% occurred in male offspring, 42.23% occurred in female offspring, and 14.77% of the events were gender unknown (Table 2). It noted that a case may have multiple clinical outcomes. The proportion of males and females in offspring of 914 cases is balanced, consistent with the results of previous studies, and there is no obvious gender difference in offspring.

The number of adverse events reported every year since 2004 has no many differences, which demonstrates the stable safety of sertraline for a long period (Table 2). The majority of adverse events came from the Americas (724 (79.3%)) (Table 2). In most reports, congenital anomalies (774, 41.21%) are the most frequently reported serious clinical outcome, and other serious events (major medical events) or hospitalizations (initial or long-term) are 664 (35.36%) and 220 (11.71%), respectively. Since the adverse reactions in this study are all associated with the congenital diseases of offspring after intrauterine sertraline exposure, the patient's outcome is described from the babies born in the population identified in this study and related to congenital disease characteristics.

Among the 69 positive signals detected from 914 cases, 31 were invalid signals, and 38 were valid signals according to criteria. The most common ones are heart disease

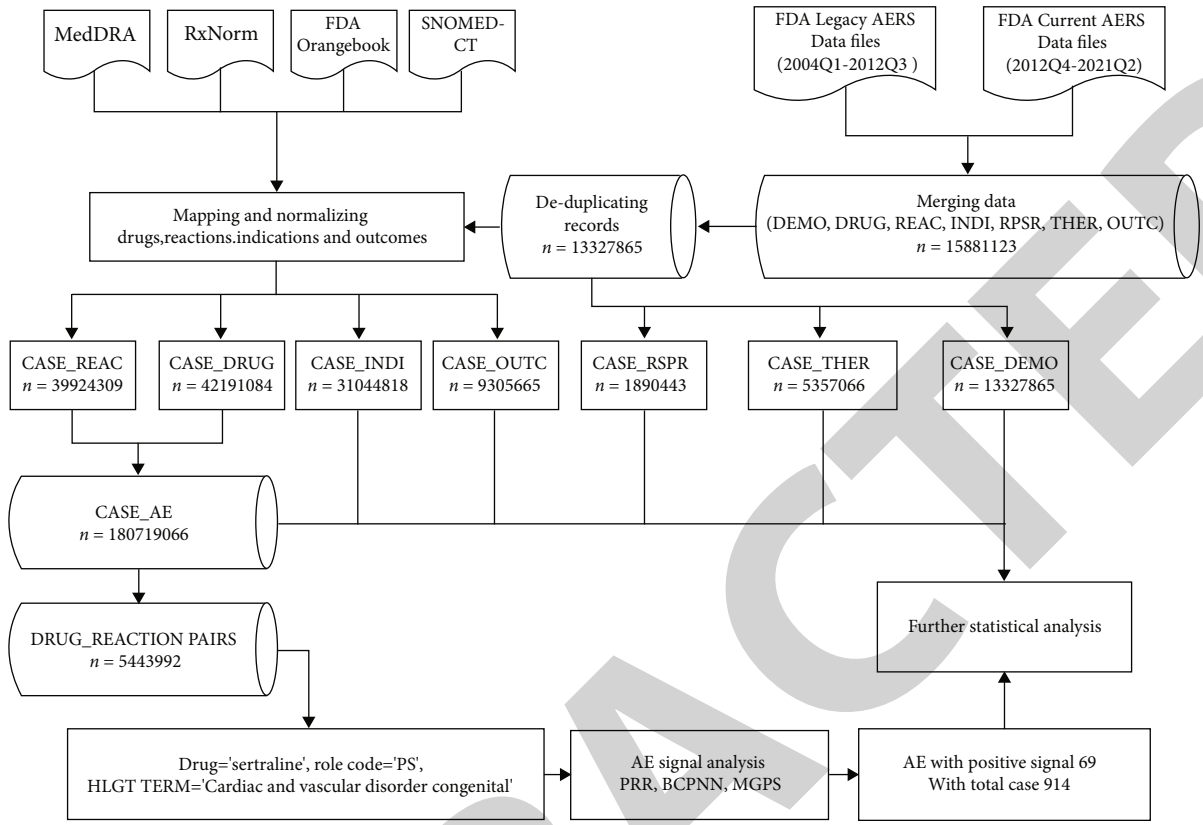

Figure 1: Main steps of process.

Table 1: Summary of major algorithms and criteria used for signal detection.

Algorithms	Equation	Criteria
ROR	$\mathrm{ROR} = \frac{ad}{bc}$, $95\%\mathrm{CI} = e^{\ln(\mathrm{ROR}) \pm 1.96\sqrt{\frac{1}{a}+\frac{1}{b}+\frac{1}{c}+\frac{1}{d}}}$	ROR ≥ 1; CI025 ≥ 1
PRR	$\mathrm{PRR} = \frac{a(c+d)}{c(a+b)}$, $95\%\mathrm{CI} = e^{\ln(\mathrm{PRR}) \pm 1.96\sqrt{\frac{1}{a}-\frac{1}{a+b}+\frac{1}{c}-\frac{1}{c+d}}}$, $\chi^2 = \sum \frac{(O-E)^2}{E}$; $\left(O = a, E = \frac{(a+b)(a+c)}{a+b+c+d}\right)$	PRR ≥ 2; $\chi^2 \geq 4$; $a \geq 3$
BCPNN	$\mathrm{IC} = \log_2 a(a+b+c+d)(a+c)(a+b)$, $95\%\mathrm{CI} = e^{\ln(\mathrm{IC}) \pm 1.96\sqrt{\frac{1}{a}+\frac{1}{b}+\frac{1}{c}+\frac{1}{d}}}$	IC25 − 2sd > 0
MGPS	$\mathrm{EBGM} = \frac{a(a+b+c+d)(a+b)}{a+c}$, $95\%\mathrm{CI} = e^{\ln(\mathrm{EBGM}) \pm 1.96\sqrt{\frac{1}{a}+\frac{1}{b}+\frac{1}{c}+\frac{1}{d}}}$	EBGM05 ≥ 2

ROR: reporting odds ratio; PRR: proportional reporting ratio; BCPNN: Bayesian confidence propagation neural network; MGPS: multi-item gamma Poisson shrinker.

congenital (PT: 10019273), atrial septal defect (PT: 10003664), ventricular septal defect (PT: 10047298), patent ductus arteriosus (PT: 10034130), and persistent fetal circulation (PT: 10034708) (Table 3).

The most common clinical indications for sertraline in the report were depression 355 (51.52%) and anxiety 136 (19.74%), which are consistent with the indications of sertraline (Table 4). Since the disease in this study is a congenital disease, all reports occurred in infants and young children (<36 M) (Table 5). The five most common adverse events of sertraline were atrial septal defect (PT: 10003664), heart disease congenital (PT: 10019273), ventricular septal defect

Table 2: Characteristics of reports associated with sertraline.

	Reports no.	%
Reporting region		
Europe	145	15.86%
Oceania	2	0.22%
Americas	729	79.76%
Asia	37	4.05%
Africa	1	0.11%
Reported year		
2004 and before	224	24.42%
2005	47	5.15%
2006	58	6.35%
2007	58	6.35%
2008	60	6.57%
2009	55	6.02%
2010	78	8.54%
2011	68	7.45%
2012	44	4.82%
2013	11	1.2%
2014	35	3.83%
2015	33	3.61%
2016	49	5.37%
2017	25	2.74%
2018	10	1.1%
2019	31	3.4%
2020	20	2.19%
2021Q1Q2	8	0.88%
Gender		
Female	386	42.23%
Male	393	43%
Unknown	135	14.77%
Outcome		
Congenital anomaly	774	41.21%
Death	67	3.57%
Disability	21	1.12%
Hospitalization—initial or prolonged	220	11.71%
Life-threatening	124	6.6%
Other serious (important medical event)	664	35.36%
Required intervention to prevent permanent impairment/damage	8	0.43%

(PT: 10047298), patent ductus arteriosus (PT: 10034130), and persistent fetal circulation (PT: 10034708).

4. Discussion

SSRI may be associated with an increased risk for birth defects or congenital heart defects overall [1, 12, 26, 27], although findings are occasionally mixed. In this study, we focused on the risks of sertraline medication to the fetus related to the fetal congenital heart diseases during pregnancy. The adverse reaction signals detected based on the FAERS database are quite different from the common adverse reactions of fetus in sertraline's instructions. The very common adverse reactions associated with fetus exposed to sertraline during pregnancy in the instructions dyspnea, cyanosis, apnea, seizures, unstable body temperature, difficulty feeding, vomiting, hypoglycemia, hypotonia, increased muscle tone, tendon hyperreflexia, tremor, nervousness, irritability, and constant crying noise. The side effects of strong sertraline signal related to fetal safety are congenital heart and vascular disorders, including corrected transposition of great vessels, newborn persistent pulmonary hypertension, shone complex, congenital mitral valve stenosis, congenital tricuspid valve stenosis, congenital coronary artery malformation, and congenital aortic valve stenosis, from the results of this study. These are different from the common adverse reactions of sertraline given in the instructions, indicating that the discovery of adverse reactions related to sertraline therapy during pregnancy and fetal congenital diseases is worthy of additional attention in clinical prescription and adequate measurement of the benefits and risks of medication during pregnancy. Due to ethical restrictions, there is currently no high-quality prospective randomized double-blind controlled study on the relationship between intrauterine exposure to sertraline and abnormal development of offspring in pregnant women. Previous population-based cohort studies have shown that utero exposure of sertraline in the first trimester is significantly associated with fetal atrial or ventricular defects [28], and a meta-analysis showed that the intrauterine exposure of sertraline in the first trimester was significantly related to the offspring's atrial septal defect and/or ventricular septal defect [12]. Our finding is also consistent with above researches. In our study, there are totally 914 cases with fetal congenital heart diseases reported in the database which are related to sertraline intrauterine exposure. Among these cases, 127 cases are reported as atrial septal defect, and 99 cases are reported as ventricular septal defect, which are all strongly related with utero exposure of sertraline during pregnancy.

Embryonic development is a complex process; it is commonly accepted that the first trimester of pregnancy is a key period in fetal development (i.e., sensitive period to teratogenic agent). Therefore, studies in the literature have explored the effects of intrauterine exposure of sertraline in the first trimester on the fetus. However, in these studies, the number of malformations after exposure is small, which is related to the lower incidence of fetal malformations. The number of positive cases may cause deviations in the results. FAERS database collects the global adverse drug reaction reporting data, which has a higher population base. We analyzed the FAERS database and obtained 914 cases of fetal heart development related adverse reactions after sertraline exposure and obtained 69 adverse reaction entries through statistical analysis, which obtained more positive results compared with the previous literature. The vast majority of the 69 adverse reactions significantly related to sertraline intrauterine exposure were structural abnormalities of fetal cardiovascular system, of which 38 were strongly significantly associated with, except that "trisomy 21" was not related to cardiovascular system; the other 31 were adverse reactions of cardiovascular system, which were structural

Table 3: Signal detection for sertraline-associated congenital cardiovascular disease in the FAERS database.

Reaction (PT code)	Case	ROR (95% two-sided CI)	PRR (95% two-sided CI, χ^2)	BCPNN (IC-2SD)	EBGM (90% two-sided CI)	
Heart disease congenital (10019273)	128	22.6 (18.88, 27.06)	22.56 (22.38, 22.74, 2429.61)	4.19 (4.12)	21.02 (20.87, 21.17)	P^*
Atrial septal defect (10003664)	127	15.63 (13.07, 18.69)	15.6 (15.42, 15.78, 1634.23)	3.74 (3.69)	14.86 (14.71, 15.01)	P^*
Ventricular septal defect (10047298)	99	16.12 (13.17, 19.74)	16.1 (15.90, 16.30, 1315.07)	3.74 (3.68)	15.32 (15.15, 15.49)	P^*
Patent ductus arteriosus (10034130)	72	19.15 (15.09, 24.31)	19.13 (18.89, 19.37, 1144.72)	3.87 (3.78)	18.02 (17.82, 18.22)	P^*
Persistent fetal circulation (10034708)	34	37.82 (26.46, 54.06)	37.8 (37.45, 38.16, 1047.24)	4.12 (3.95)	33.62 (33.32, 33.92)	P^*
Fallot's tetralogy (10016193)	30	20.42 (14.11, 29.57)	20.41 (20.04, 20.78, 499.97)	3.59 (3.46)	19.16 (18.85, 19.47)	P^*
Transposition of the great vessels (10044443)	27	24.12 (16.29, 35.71)	24.11 (23.72, 24.50, 531.58)	3.66 (3.51)	22.36 (22.03, 22.69)	P^*
Pulmonary valve stenosis congenital (10037451)	21	35.17 (22.37, 55.30)	35.16 (34.70, 35.61, 592.37)	3.72 (3.51)	31.52 (31.14, 31.89)	P^*
Cardiac septal defect (10064021)	21	29.2 (18.65, 45.72)	29.19 (28.74, 29.64, 494.71)	3.62 (3.43)	26.65 (26.27, 27.02)	P^*
Pulmonary artery stenosis congenital (10037339)	20	21.05 (13.37, 33.14)	21.05 (20.59, 21.50, 337.90)	3.38 (3.21)	19.71 (19.33, 20.09)	P^*
Coarctation of the aorta (10009807)	19	14.11 (8.90, 22.35)	14.1 (13.64, 14.56, 208.35)	3.05 (2.91)	13.5 (13.12, 13.89)	P^*
Hypoplastic left heart syndrome (10021076)	18	19.58 (12.15, 31.55)	19.57 (19.09, 20.05, 280.22)	3.26 (3.09)	18.41 (18.01, 18.81)	P^*
Bicuspid aortic valve (10004552)	17	22.27 (13.60, 36.46)	22.26 (21.77, 22.76, 301.45)	3.3 (3.12)	20.77 (20.36, 21.18)	P^*
Newborn persistent pulmonary hypertension (10053592)	15	176.83 (93.23, 335.42)	176.79 (176.15, 177.43, 1530.31)	3.81 (3.36)	110.87 (110.33, 111.40)	P^*
Haemangioma congenital (10018818)	14	40.85 (23.36, 71.45)	40.84 (40.28, 41.40, 443.43)	3.43 (3.16)	35.99 (35.52, 36.46)	P^*
Congenital cardiovascular anomaly (10061054)	14	8.72 (5.13, 14.84)	8.72 (8.19, 9.25, 85.58)	2.5 (2.37)	8.5 (8.05, 8.94)	P^*
Congenital heart valve disorder (10064086)	12	35.36 (19.43, 64.36)	35.36 (34.76, 35.96, 327.59)	3.23 (2.96)	31.68 (31.18, 32.18)	P^*
Congenital aortic valve stenosis (10010371)	9	57.66 (28.22, 117.80)	57.65 (56.93, 58.36, 372.82)	3.07 (2.69)	48.38 (47.78, 48.98)	P^*
Hypertrophic cardiomyopathy (10020871)	9	7.78 (4.01, 15.08)	7.78 (7.11, 8.44, 45.37)	2.19 (2.04)	7.6 (7.05, 8.16)	P^*
Congenital coronary artery malformation (10061060)	8	58.94 (27.59, 125.92)	58.93 (58.17, 59.69, 332.75)	2.95 (2.54)	49.27 (48.64, 49.91)	P^*
Trisomy 21 (10044688)	8	5.12 (2.55, 10.31)	5.12 (4.43, 5.82, 22.19)	1.8 (1.67)	5.05 (4.47, 5.64)	P^*
Pulmonary artery atresia (10037337)	8	47.15 (22.35, 99.46)	47.14 (46.40, 47.89, 272.85)	2.91 (2.53)	40.78 (40.15, 41.40)	P^*
Double outlet right ventricle (10013611)	7	18.42 (8.58, 39.53)	18.42 (17.65, 19.18, 92.68)	2.51 (2.24)	17.39 (16.75, 18.03)	P^*
Shone complex (10066802)	6	104 (41.00, 263.80)	103.99 (103.06, 104.92, 379.20)	2.69 (2.11)	77.13 (76.35, 77.90)	P^*
Aorta hypoplasia (10049209)	5	16.01 (6.51, 39.39)	16.01 (15.11, 16.91, 53.23)	2.17 (1.88)	15.24 (14.49, 15.99)	P^*
Congenital mitral valve stenosis (10010548)	5	73.67 (27.65, 196.29)	73.66 (72.68, 74.64, 231.34)	2.46 (1.90)	59.13 (58.31, 59.95)	P^*
Multiple cardiac defects (10028178)	5	22.32 (8.99, 55.41)	22.32 (21.41, 23.23, 75.82)	2.27 (1.93)	20.82 (20.06, 21.58)	P^*
Anomalous pulmonary venous connection (10058079)	4	12.41 (4.56, 33.74)	12.41 (11.41, 13.41, 30.02)	1.9 (1.61)	11.95 (11.11, 12.78)	P^*
Atrioventricular septal defect (10063836)	4	7.96 (2.95, 21.50)	7.96 (6.97, 8.96, 17.40)	1.72 (1.49)	7.78 (6.95, 8.61)	P^*
Congenital aortic stenosis (10010369)	4	13.87 (5.09, 37.80)	13.87 (12.86, 14.87, 34.11)	1.94 (1.63)	13.29 (12.45, 14.13)	P^*
Corrected transposition of great vessels (10011120)	4	589.33 (107.94, 3217.69)	589.29 (587.60, 590.99, 598.66)	2.29 (1.20)	197.1 (195.68, 198.52)	P^*
Ventricular hypoplasia (10047296)	3	10.16 (3.21, 32.12)	10.16 (9.01, 11.31, 15.89)	1.61 (1.31)	9.85 (8.89, 10.82)	P^*

Table 3: Continued.

Reaction (PT code)	Case	ROR (95% two-sided CI)	PRR (95% two-sided CI, χ^2)	BCPNN (IC-2SD)	EBGM (90% two-sided CI)	
Univentricular heart (10045545)	3	18.81 (5.85, 60.43)	18.81 (17.64, 19.97, 32.23)	1.77 (1.37)	17.74 (16.76, 18.72)	P^*
Truncus arteriosus persistent (10044703)	3	23.89 (7.37, 77.49)	23.89 (22.71, 25.07, 41.47)	1.81 (1.36)	22.17 (21.19, 23.16)	P^*
Congenital pulmonary artery anomaly (10061074)	3	19.22 (5.98, 61.79)	19.22 (18.05, 20.38, 32.99)	1.77 (1.37)	18.1 (17.12, 19.08)	P^*
Congenital tricuspid valve stenosis (10010656)	3	73.67 (20.79, 261.06)	73.66 (72.40, 74.93, 118.64)	1.92 (1.20)	59.13 (58.07, 60.19)	P^*
Congenital pulmonary valve atresia (10052644)	3	8.26 (2.62, 26.02)	8.26 (7.11, 9.41, 12.21)	1.54 (1.27)	8.06 (7.10, 9.02)	P^*
Dextrocardia (10012592)	3	15.24 (4.78, 48.64)	15.24 (14.08, 16.40, 25.58)	1.73 (1.36)	14.54 (13.57, 15.51)	P^*
Congenital pulmonary valve disorder (10061075)	2	15.93 (3.84, 66.09)	15.93 (14.50, 17.35, 14.24)	1.4 (0.95)	15.16 (13.97, 16.35)	P
Trisomy 18 (10053884)	2	5.12 (1.27, 20.74)	5.12 (3.73, 6.52, 3.09)	1.1 (0.83)	5.05 (3.88, 6.22)	P
Hypoplastic right heart syndrome (10064962)	2	8.93 (2.19, 36.46)	8.93 (7.52, 10.34, 7.04)	1.28 (0.93)	8.7 (7.52, 9.87)	P
Noonan syndrome (10029748)	2	39.29 (8.98, 171.81)	39.29 (37.81, 40.76, 36.31)	1.5 (0.82)	34.78 (33.55, 36.02)	P
Congenital arterial malformation (10062325)	2	11.56 (2.81, 47.47)	11.55 (10.14, 12.97, 9.76)	1.34 (0.95)	11.16 (9.97, 12.34)	P
Vascular malformation (10074979)	2	8.54 (2.09, 34.84)	8.54 (7.13, 9.95, 6.63)	1.27 (0.93)	8.33 (7.15, 9.50)	P
Right ventricle outflow tract obstruction (10064195)	2	14.03 (3.40, 57.97)	14.03 (12.61, 15.45, 12.31)	1.38 (0.95)	13.44 (12.25, 14.63)	P
Congenital aortic anomaly (10061052)	2	8.67 (2.12, 35.36)	8.67 (7.26, 10.07, 6.76)	1.27 (0.93)	8.45 (7.27, 9.62)	P
Congenital mitral valve incompetence (10010547)	2	13.7 (3.32, 56.58)	13.7 (12.29, 15.12, 11.98)	1.38 (0.95)	13.14 (11.95, 14.33)	P
Interruption of aortic arch (10022599)	2	22.67 (5.38, 95.50)	22.67 (21.23, 24.10, 20.92)	1.45 (0.91)	21.12 (19.91, 22.32)	P
Cor triatriatum (10010972)	1	49.11 (5.91, 407.93)	49.11 (46.99, 51.22, 9.62)	0.96 (-0.06)	42.24 (40.46, 44.01)	P
Digeorge's syndrome (10012979)	1	7.96 (1.09, 58.04)	7.96 (5.98, 9.95, 1.08)	0.82 (0.36)	7.78 (6.12, 9.44)	P
Heart block congenital (10019263)	1	10.91 (1.48, 80.31)	10.91 (8.92, 12.91, 1.74)	0.87 (0.33)	10.56 (8.89, 12.23)	P
Trisomy 13 (10044686)	1	5.56 (0.77, 40.20)	5.56 (3.58, 7.54, 0.55)	0.75 (0.36)	5.48 (3.82, 7.13)	P
Congenital aortic dilatation (10058150)	1	32.74 (4.15, 258.43)	32.74 (30.67, 34.80, 6.45)	0.95 (0.08)	29.56 (27.84, 31.29)	P
Persistent left superior vena cava (10064193)	1	8.42 (1.15, 61.45)	8.42 (6.43, 10.41, 1.18)	0.83 (0.36)	8.21 (6.55, 9.88)	P
Aorticopulmonary septal defect (10063732)	1	32.74 (4.15, 258.43)	32.74 (30.67, 34.80, 6.45)	0.95 (0.08)	29.56 (27.84, 31.29)	P
Malformation venous (10025532)	1	10.16 (1.38, 74.59)	10.16 (8.17, 12.15, 1.57)	0.86 (0.34)	9.85 (8.19, 11.52)	P
Ebstein's anomaly (10014075)	1	5.17 (0.72, 37.33)	5.17 (3.19, 7.15, 0.47)	0.74 (0.36)	5.1 (3.44, 6.75)	P
Fallot's pentalogy (10059205)	1	147.33 (13.36, 1624.84)	147.32 (144.92, 149.72, 23.73)	0.98 (-0.46)	98.55 (96.54, 100.56)	P
Ductus arteriosus stenosis fetal (10013808)	1	1.94 (0.27, 13.85)	1.94 (-0.03, 3.90, 0.00)	0.4 (0.16)	1.93 (0.29, 3.58)	P
Ductus arteriosus premature closure (10049996)	1	1.89 (0.26, 13.49)	1.89 (-0.08, 3.86, 0.00)	0.38 (0.15)	1.88 (0.24, 3.53)	P
Vacterl syndrome (10066022)	1	5.36 (0.74, 38.71)	5.36 (3.38, 7.33, 0.51)	0.75 (0.36)	5.28 (3.62, 6.93)	P
Congenital aortic atresia (10010368)	1	24.55 (3.19, 188.85)	24.55 (22.51, 26.59, 4.75)	0.93 (0.16)	22.74 (21.04, 24.45)	P
Ectopia cordis (10014144)	1	58.93 (6.88, 504.44)	58.93 (56.78, 61.08, 11.38)	0.97 (-0.12)	49.27 (47.48, 51.07)	P
Cardiac malposition (10007585)	1	36.83 (4.61, 294.50)	36.83 (34.75, 38.91, 7.27)	0.95 (0.04)	32.85 (31.11, 34.59)	P

Table 3: Continued.

Reaction (PT code)	Case	ROR (95% two-sided CI)	PRR (95% two-sided CI, χ^2)	BCPNN (IC-2SD)	EBGM (90% two-sided CI)	
Mitral valve atresia (10066800)	1	15.51 (2.08, 115.85)	15.51 (13.50, 17.52, 2.77)	0.9 (0.27)	14.78 (13.10, 16.47)	*P*
Congenital tricuspid valve atresia (10049767)	1	5.17 (0.72, 37.33)	5.17 (3.19, 7.15, 0.47)	0.74 (0.36)	5.1 (3.44, 6.75)	*P*
Charge syndrome (10064063)	1	32.74 (4.15, 258.43)	32.74 (30.67, 34.80, 6.45)	0.95 (0.08)	29.56 (27.84, 31.29)	*P*
Right aortic arch (10067407)	1	4.68 (0.65, 33.72)	4.68 (2.70, 6.65, 0.37)	0.71 (0.36)	4.62 (2.97, 6.27)	*P*
Congenital aortic valve incompetence (10010370)	1	4.53 (0.63, 32.67)	4.53 (2.56, 6.51, 0.34)	0.71 (0.35)	4.48 (2.83, 6.13)	*P*

Table 4: Clinical indication of sertraline.

Characteristics	Reports, no. *n* (%)	
Indication	689	
Depression (10012378)	355	(51.52%)
Anxiety (10002855)	136	(19.74%)
Affective disorder (10001443)	71	(10.3%)
Product used for unknown indication (10070592)	24	(3.48%)
Perinatal depression (10078366)	15	(2.18%)
Ill-defined disorder (10061520)	11	(1.6%)
Panic disorder (10033666)	10	(1.45%)
Prophylaxis of neural tube defect (10054930)	7	(1.02%)
Adrenal disorder (10001347)	6	(0.87%)
Anxiety disorder (10057666)	6	(0.87%)
Morning sickness (10027975)	6	(0.87%)
Depressive symptom (10054089)	5	(0.73%)
Obsessive-compulsive disorder (10029898)	5	(0.73%)
Prophylaxis (10036898)	4	(0.58%)
Bacterial test positive (10059421)	3	(0.44%)
Generalised anxiety disorder (10018075)	3	(0.44%)
Major depression (10057840)	3	(0.44%)
Posttraumatic stress disorder (10036316)	3	(0.44%)
Psychiatric symptom (10061472)	3	(0.44%)
Bronchitis (10006451)	2	(0.29%)
Maternal drugs affecting fetus (10026923)	2	(0.29%)
Panic attack (10033664)	2	(0.29%)
Antidepressant therapy (10054976)	1	(0.15%)
Depressed mood (10012374)	1	(0.15%)
Disability (10013050)	1	(0.15%)
Maternal exposure timing unspecified (10071415)	1	(0.15%)
Mood swings (10027951)	1	(0.15%)
Nervous system disorder (10029202)	1	(0.15%)

malformations. The above results from the real-world analysis of FAERS have also been more accurately verified by echocardiography in the studies performed by Ansah et al. [29] and Kolding et al. [30]. The cardiac function of fetuses without cardiac structural malformation exposed to sertraline was evaluated; the results of both studies showed that sertraline intrauterine exposure did not cause changes in fetal cardiac function under the premise of no structural malformations. However, the ventricular size of the sertraline intrauterine exposure group was significantly smaller than that of the control group, which suggested that sertraline has a specific effect on the structural development of fetal heart. Animal experiments also support the effect of sertraline exposure on cardiac development of offspring [31]. In the results of this study, we mined the types of fetal malformations related to sertraline intrauterine exposure based on larger population data, which added new evidence for specific fetal cardiovascular abnormalities caused by sertraline intrauterine exposure.

In sertraline's instructions, persistent pulmonary hypertension (PPHN) is the only clearly stated description of adverse reactions of fetal malformations; the relationship between it and SSRIs has been discussed in related studies [32]. Masarwa et al. [33] conducted a systematic review, estimating the risks of maternal exposure to SSRI, SNRI, and PPHN, and the results indicated that exposure to SSRI and/or SNRI at any stage of pregnancy significantly increased the risk of PPHN (OR = 1.82; 95% CI: 1.31~2.54). Sertraline's instruction also mentions that exposure of infants to SSRIs in late pregnancy may increase the risk of persistent pulmonary hypertension (PPHN) in newborns. In addition, pregnant women using SSRIs tend to be older, more likely to smoke, higher body mass index, and higher frequency of caesarean section [34], which are potential risk factors for PPHN. Although there is no conclusive evidence for the risk of PPHN after exposure to SSRIs during pregnancy, this study, based on the evidence from the FAERS database, confirms the connection between sertraline and PPHN from another perspective, providing evidence-based on the real world. However, the number of reported cases of PPHN in the adverse reactions we analyzed only ranked 14th; the results of this study show that maternal sertraline application during pregnancy has more potential risk of cardiovascular malformations in offspring. Moreover, there is no obvious downward trend in the number of adverse reaction cases reported every year, suggesting that sertraline still has a relatively stable clinical application in pregnant women. Testing institutions and clinical practice should be vigilant, and there should be more perfect drug instructions to remind doctors and patients to fully evaluate the risks and benefits before medication.

In the results of this study, one of the items of abnormal offspring development caused by sertraline intrauterine exposure is corrected transposition of great vessels. Corrected transposition of great vessels is a rare complex congenital heart disease often accompanied by ventricular septal defect, pulmonary artery stenosis or atresia, Ebstein-like malformation of the tricuspid valve, and median heart, and is worth noting because it has the highest reporting odds ratio of ROR, PRR, and EBGM. Moreover, corrected transposition of great vessels has not been noticed in previous sertraline-related studies. Moreover, due to the highest reporting odds ratio of the corrected transposition of great vessels but the low frequency, there may be statistical deviations. The pathogenesis of corrected transposition of great vessels is still unclear; the specific mechanism of sertraline interfering with fetal programming in uterus requires further experimental research.

Among the fetal adverse reactions significantly related to sertraline exposure analyzed in this study, three terms are more interesting, namely, trisomy 21, trisomy 18, and trisomy 13, of which trisomy 21 is strongly related. At present, no reports and studies have been retrieved about the intrauterine exposure of sertraline during pregnancy and the chromosomal abnormalities of the offspring. However, with the existence of 5-HT and its receptor in female reproductive system and subsequent functional researches, 5-HT was believed to play an important role in egg maturation and early embryonic development [35]. In animal models, SSRI drugs can cause abnormal follicular development by acting on ovaries [36]. Therefore, the effect of sertraline on

Table 5: Age and gender of top 20 adverse reactions associated with using sertraline.

	n	Gender					
		Average (month)	Male (n, %)		Female (n, %)	Unknown (n, %)	
Atrial septal defect (10003664)	146	5.43	61	(41.78%)	78	(53.42%)	7	(4.79%)
Heart disease congenital (10019273)	128	9.20	45	(35.16%)	43	(33.59%)	40	(31.25%)
Ventricular septal defect (10047298)	111	9.28	36	(32.43%)	63	(56.76%)	12	(10.81%)
Patent ductus arteriosus (10034130)	83	3.44	34	(40.96%)	44	(53.01%)	5	(6.02%)
Persistent fetal circulation (10034708)	34	1.00	18	(52.94%)	12	(35.29%)	4	(11.76%)
Fallot's tetralogy (10016193)	32	1.67	11	(34.38%)	16	(50%)	5	(15.63%)
Transposition of the great vessels (10044443)	31	3.75	14	(45.16%)	12	(38.71%)	5	(16.13%)
Pulmonary valve stenosis congenital (10037451)	21	0.00	6	(28.57%)	10	(47.62%)	5	(23.81%)
Cardiac septal defect (10064021)	21	0.00	3	(14.29%)	3	(14.29%)	15	(71.43%)
Pulmonary artery stenosis congenital (10037339)	21	2.50	8	(38.1%)	12	(57.14%)	1	(4.76%)
Coarctation of the aorta (10009807)	20	11.50	12	(60%)	6	(30%)	2	(10%)
Hypoplastic left heart syndrome (10021076)	19	6.33	14	(73.68%)	5	(26.32%)	0	(0%)
Bicuspid aortic valve (10004552)	17	9.33	11	(64.71%)	6	(35.29%)	0	(0%)
Congenital cardiovascular anomaly (10061054)	17	4.00	2	(11.76%)	4	(23.53%)	11	(64.71%)
Newborn persistent pulmonary hypertension (10053592)	15	1.00	9	(60%)	6	(40%)	0	(0%)
Haemangioma congenital (10018818)	14	1.00	11	(78.57%)	1	(7.14%)	2	(14.29%)
Congenital heart valve disorder (10064086)	12	10.50	9	(75%)	3	(25%)	0	(0%)
Pulmonary artery atresia (10037337)	10	21.00	6	(60%)	2	(20%)	2	(20%)
Congenital coronary artery malformation (10061060)	9	4.00	5	(55.56%)	4	(44.44%)	0	(0%)
Congenital aortic valve stenosis (10010371)	9	2.00	6	(66.67%)	2	(22.22%)	1	(11.11%)
Hypertrophic cardiomyopathy (10020871)	9	0.00	6	(66.67%)	2	(22.22%)	1	(11.11%)
Trisomy 21 (10044688)	8	1.00	3	(37.5%)	3	(37.5%)	2	(25%)
Double outlet right ventricle (10013611)	8	20.00	6	(75%)	0	(0%)	2	(25%)
Shone complex (10066802)	6	0.00	0	(0%)	6	(100%)	0	(0%)
Aorta hypoplasia (10049209)	6	4.00	3	(50%)	1	(16.67%)	2	(33.33%)
Atrioventricular septal defect (10063836)	5	2.00	1	(20%)	4	(80%)	0	(0%)
Ventricular hypoplasia (10047296)	5	1.00	4	(80%)	1	(20%)	0	(0%)
Congenital pulmonary artery anomaly (10061074)	5	5.33	2	(40%)	3	(60%)	0	(0%)
Multiple cardiac defects (10028178)	5	19.00	2	(40%)	2	(40%)	1	(20%)
Congenital mitral valve stenosis (10010548)	5	12.00	2	(40%)	2	(40%)	1	(20%)

chromosome abnormalities in offspring may be related to this. In the correlation analysis between chromosomal abnormalities and sertraline intrauterine exposure, ROR, PRR, BCPNN, and EBGM showed statistical differences; however, we note that the reported number of trisomy 21 cases is 8, trisomy 18 cases are 2, and trisomy 13 cases are 1; a small number of cases may also affect the analysis results. In brief, this study found that there was a significant correlation between intrauterine sertraline exposure during pregnancy and the triploidy of offspring trisomy 21, trisomy 18, and trisomy 13, but more rigorous prospective cohort studies were needed.

The specific mechanism of fetal cardiovascular malformation caused by sertraline intrauterine exposure is not clear. In this study and previous literature, the gender of offspring did not show a significant correlation with cardiovascular malformations in sertraline exposed cases, suggesting that gender has nothing to do with the incidence [28]. Animal experiments showed that sertraline intrauterine exposure resulted in a significant decrease in Akt phosphorylation, proliferation, and cardiocyte cross-sectional area of offspring cardiomyocytes, resulting in a dose-dependent effect on cardiac structure and function [31]. Current studies have found that 5-HT is not only a neurotransmitter but also an important regulator in the biological process of embryonic heart development and participates in the regulation of key events in heart development [9]. Previous studies have confirmed that the concentration of sertraline in amniotic fluid is significantly correlated with the maternal intake dose, suggesting that the maternal intake of sertraline may spread to the fetal circulation through the placenta and then interfere with the embryonic heart development process through the above mechanisms [37]. Currently, the mechanism of 5-HT in the development of human embryonic

cardiovascular system has not been clarified; an in-depth mechanism study should be carried out to provide more evidence for the safety of drugs during pregnancy.

Further excavation of the FAERS database can expand new ideas for evaluating the safety of drugs used during pregnancy and is a research method that is worthy of further in-depth development. But this method also has limitations. First, because FAERS data is a voluntary report, including public reports, there are problems such as underreporting, selective reporting, and lack of information. In addition, although the analysis shows that there is a statistical correlation between the drug and the adverse event, the actual causality still needs further verification. In addition, most of the FAERS data come from European and American populations and relatively few Asian populations.

5. Conclusion

In this study, ADR signal detection is carried out through the FAERS database; a comprehensive and in-depth analysis indicated that the maternal application of sertraline during pregnancy has a significant association with cardiac abnormalities of offspring. This result provides new supplements for the related adverse reactions in the instructions and also provides an objective basis for clinical safe medication. At the same time, the detection of adverse reaction signals should also rely on large, well-organized epidemiologic studies to obtain relatively sufficient evidence.

Data Availability

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Conflicts of Interest

The authors declare that they have no competing interests.

Authors' Contributions

Fanzhen Hong and Jianqing Qiu contributed equally to this work.

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Research Article

Maackiain Prevents Amyloid-Beta–Induced Cellular Injury via Priming PKC-Nrf2 Pathway

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Amyloid-beta (Aβ) peptide induces neurotoxicity through oxidative stress and inflammatory response. Brain deposition of a large amount of amyloid-beta (Aβ), in particular Aβ_{42}, promotes the development of Alzheimer's disease (AD). Maackiain is extracted from traditional Chinese medicine peony root and possesses antioxidative, antiosteoporosis, antitumor, and immunoregulatory effects. Whether Maackiain can reduce neurotoxicity caused by Aβ accumulation remains elusive. Herein, we found that Maackiain downregulated Aβ_{42}-induced cell injury and apoptosis in PC12 cells. Moreover, Maackiain prevented Aβ_{42} stimulation-induced generation of oxidative stress and reduced Aβ_{42}-caused impairment of mitochondrial membrane potential in PC12 cells. Maackiain increased the superoxide dismutase activity and decreased malondialdehyde content that was induced by Aβ_{42}. Mechanistic studies showed that Maackiain increased intranuclear Nrf2 expression. Consistently, Nrf2 silencing by RNA interference weakened the protective role of Maackiain against Aβ exposure. In addition, calphostin C, a specific antagonist of protein kinase C, attenuated the promoting effects of Maackiain on Nrf2 nuclear translocation. Moreover, calphostin C attenuated the antioxidant and anti-inflammatory capabilities of Maackiain in PC12 cells. Collectively, Maackiain promoted Nrf2 activation through the PKC signaling pathway, thus preventing PC12 cells from Aβ-induced oxidative stress and cell injury, suggesting that Maackiain is a potential drug for AD treatment.

1. Introduction

Alzheimer's disease (AD) is the most common neurodegenerative disease and is characterized by senile plaques, neurofibrillary tangles, and loss of nerve cells and synapses [1]. Amyloid-beta (Aβ) especially Aβ_{42} is the main component of senile plaques and promotes the formation of neurofibrillary tangles and loss of synapses during the progression of AD leading to neuronal apoptosis [2, 3]. Oxidative stress and inflammatory response have been shown to contribute to Aβ_{42}-induced neurotoxicity. Deposition of Aβ_{42} in the brain decreases mitochondrial redox activity and induces the generation of a mass of reactive oxygen species (ROS), leading to the occurrence of oxidative stress in the nervous system [4, 5]. Excessive oxidative stress reaction induces neural inflammatory response through multiple signaling pathways such as nuclear factor kappa B (NF-κB) pathways, thereby further worsening nervous system injury [4, 6, 7]. Therefore, prevention of oxidative stress and neuroinflammatory response is a potential approach for the development of AD neuroprotective drugs.

NF-E2-related factor 2 (Nrf2) functions as a pivotal transcription factor that modulates oxidative stress reaction. Nrf2 is sequestered in cytoplasm under physiological condition by direct binding to Kelch-like ECH-associated protein 1 (Keap1), which prevents the translocation and activity of Nrf2 [8]. In response to internal and external environment stress, such as increase of free oxygen radicals, Nrf2 is

liberated from Keap1-Nrf2 complex and translocated into the nucleus, where it promotes the transcription of antioxidant genes [8]. Nrf2 plays an essential role in maintenance of the physiological states of the brain. Nrf2 knockout mice show proteasomal dysfunction and apoptosis in neuron, as well as age-related atrophy of the basal forebrain and neurobehavioral impairment [9, 10]. Nrf2 deregulation is strongly linked to the pathophysiology of AD. Nrf2 expression as well as its inactivation is decreased in the brain of AD patients [11]. In Aβ deposition-related APP/PS1 mice hippocampal Nrf2 expression is decreased [12]; however, injection of lentiviral vectors overexpressing Nrf2 into hippocampus remarkably increases the cognitive and learning abilities of the APP/PS1 mice [13]. In addition, the therapeutic effects of some antioxidants on APP/PS1 mice associated with Nrf2 activation [14–16]. Importantly Nrf2 has been demonstrated to prevent against Aβ-induced oxidative stress reaction and reduce inflammation during the pathological progression of AD [17]. Therefore, Nrf2-targeting drugs are promising in the clinical treatment of AD.

Maackiain, a typical isoflavonoid, is extracted from traditional Chinese medicine peony root. Maackiain possesses antioxidative [18], antiseptic [19], antitumor [20], and immunoregulatory properties [21]. Maackiain has recently been reported to exhibit beneficial effects on preventing and improving diabetes mellitus-related metabolic disturbance [18]. Moreover, Maackiain can reduce dopaminergic neuron damage and improve neurological deficits of *Caenorhabditis elegans* with Parkinson's disease [22]. However, it remains to be explored whether Maackiain can alleviate Aβ-induced neurotoxicity. In the study, we investigated the therapeutic properties of Maackiain in treatment of oxidative stress and inflammation in PC12 cells exposed to Aβ and the underlying mechanisms. Our results demonstrate that Maackiain protects PC12 cells against Aβ exposure through Nrf2 activation in a PKC signaling pathway-dependent manner. Our findings highlight that Maackiain can provide a potential avenue for clinical treatment of AD.

2. Materials and Methods

2.1. Cell Culture. PC12 cells derived from rat pheochromocytoma were purchased from ATCC and cultured in RMPI 1640 culture medium (Gibco, Carlsbad, CA, USA) containing 10% fetal bovine serum at 37°C in a 5% CO_2 incubator. Culture medium was refreshed once every 3 days. Aβ_{42} (Abcam, cat # ab120301, USA) was dissolved in dimethyl sulfoxide (DMSO) to a final concentration of 1 mM and then incubated at 37°C for 4 days. Maackiain (Sigma-Aldrich) was dissolved into dimethyl sulfoxide to a concentration of 10 mM. Fresh PC12 cell culture medium was added 6 h prior to Aβ_{42} stimulation.

2.2. siRNA Transfection. Nrf2 and control siRNAs were transfected into PC12 cells in the presence of Lipofectamine 3000 (Invitrogen) according to the protocol provided by the manufacturer. Briefly, PC12 cells were inoculated into a 6-well plate and transfected with 50 nM siRNA when reaching 70-80% confluency.

2.3. Cell Counting Kit-8 (CCK-8) Assay. Cell viability of the PC12 cells was determined by CCK-8 assay as previously described [23]. Briefly, the cells were inoculated into a 96-well plate (2000 cells/well). After Maackiain treatment with or without Aβ exposure, CCK-8 solution (Sigma-Aldrich) was added into the medium, and the cells were incubated for additional 2 h. Absorbance at 450 nm was determined using a microplate reader (CANY, Shanghai, China).

2.4. Western Blot Analysis. Total cell protein was extracted by a Tris lysis buffer (50 mM Tris-base, 150 mM sodium chloride) with 1% Triton. The protein levels were determined by immunoblotting using specific antibodies according to a previous standard protocol [24]. Briefly, the samples were loaded by SDS polyacrylamide gel electrophoresis (PAGE), and then the protein was transferred onto a polyvinylidene fluoride membrane using a wet transfer method. Membranes were blocked using 5% nonfat dry milk in PBS for 1 hour. After wash with TBST, the membrane was incubated with primary rabbit anti-Nrf2, rabbit anti-p65, or anti-GAPDH polyclonal antibody (1: 1000; Abcam) at 4°C overnight. After washing with TBST, the membrane was incubated with horseradish peroxidase conjugate secondary antibody (Abcam) at room temperature for 2 hours. The protein bands were visualized using an ECL chemiluminescence detection kit (Abcam).

2.5. DCFH-DA Assay. Dichlorodihydrofluorescein diacetate (DCFH-DA) assay was performed to detect intracellular ROS level as previously described [25]. Briefly, PC12 cells were inoculated into a 6-well plate and added with 20 nM DCFH-DA. Following incubation in a 37°C incubator for 24 hours, the cells was observed and photographed under an Olympus IX73 fluorescence microscope.

2.6. Mitochondrial Membrane Potential Assay. PC12 cells were inoculated into a 6-well plate at 1×10^5 cells/mL with 2 mL cell suspension per well and incubated with 5 μM rhodamine 123 (dissolved in dimethyl sulfoxide; Sigma-Aldrich) in 37°C for 45 minutes [26]. After washing, cells were collected by centrifugation at 1500 r/min for 5 minutes. Mean fluorescence intensity (MFI) was calculated using a flow cytometry.

2.7. SOD Activity and MDA Levels Measurement. Cells were lysed, and the supernatant was collected after centrifuged at 12000 *g* for 10 minutes. The intracellular SOD activity and MDA content were measured in strict accordance with the kit instructions (Jiancheng, Nanjing, China) [27].

2.8. Detection of Lactate Dehydrogenase (LDH) Activity. Aβ-induced cell injury was assessed using LDH activity assay according to manufacturer's instruction (Beyotime, China). Briefly, cell supernatant was incubated with reaction buffer and coenzyme I at 37°C for 15 minutes, followed by addition of 2,4-dinitrophenylhydrazine and incubation at 37°C for 15 minutes. After addition of 0.4 M NaOH and incubation for 5 minutes at room temperature, absorbance of cell supernatant at 450 nm was measured.

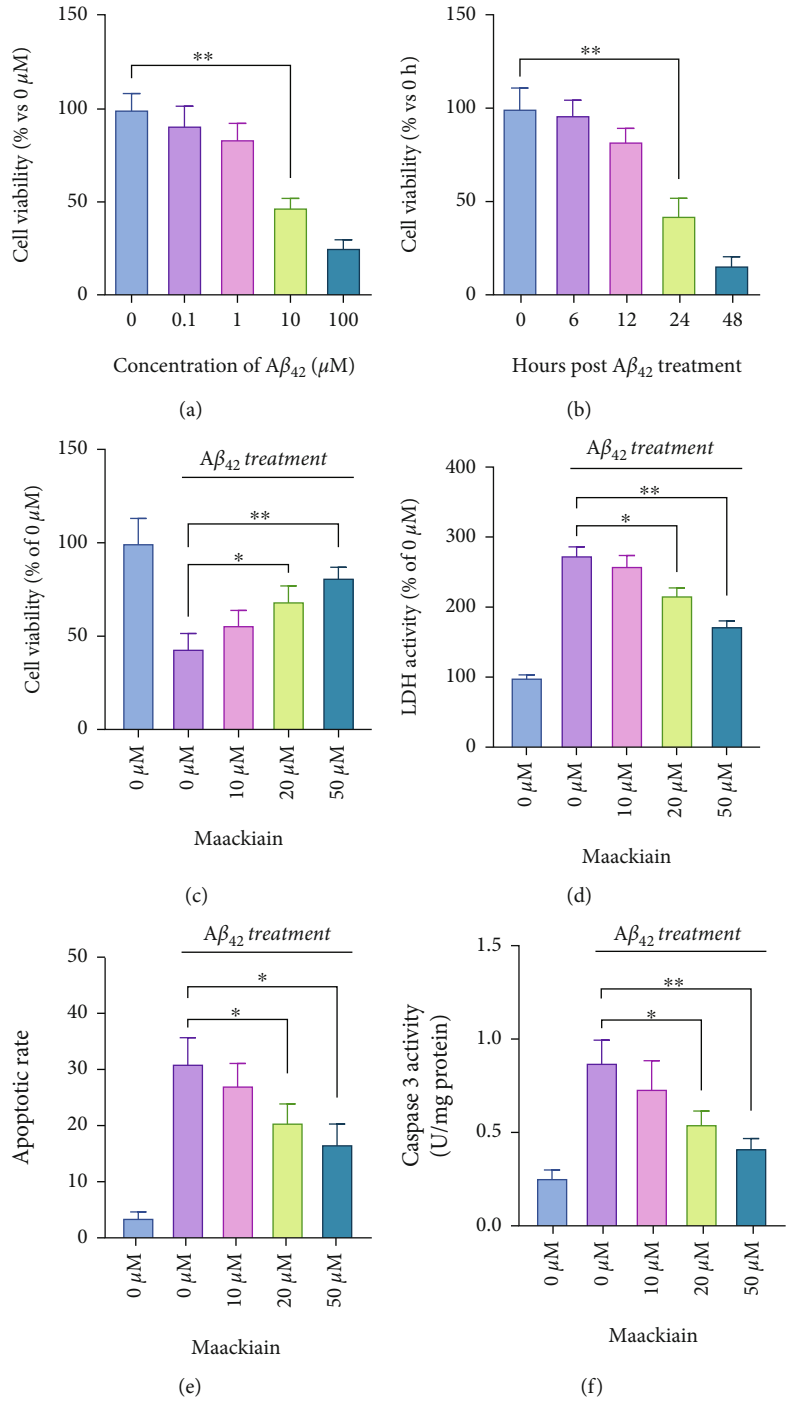

Figure 1: Maackiain prevented Aβ_{42}-induced PC12 cell injury. (a) PC12 cells were stimulated by different doses of Aβ_{42} (0, 0.1, 10, and 100 μM) for 24 hour or 10 μM Aβ_{42} at different timepoints (0, 6, 12, 24, and 48 hours). CCK-8 assay was conducted to determine the cell viability in PC12 cells. (b) PC12 cells were stimulated by different doses of Maackiain (10, 20, and 50 μM) for 6 hours and then exposed to Aβ_{42} (10 μM) for additional 24 hours. CCK-8 (b) and LDH activity (c) assay were performed to detect the viability and injury severity in PC12 cells. TUNEL staining (d) and caspase-3 activity assay (e) were performed to assess the cell apoptosis. $*P < 0.05$ and $**P < 0.01$.

2.9. TUNEL Staining. A TUNEL apoptosis detection kit purchase from Beyotime was used to assess the cell apoptosis. PC12 cells were fixed using 4% paraformaldehyde at room temperature for 20 minutes followed by three times of wash with PBS for 5 minutes each time. After permeabilized with 1% Triton X-100, the cells were treated with 3% H_2O_2 for 10 minutes. After washing with PBS for three times, cells were incubated with TdT enzyme reaction solution containing

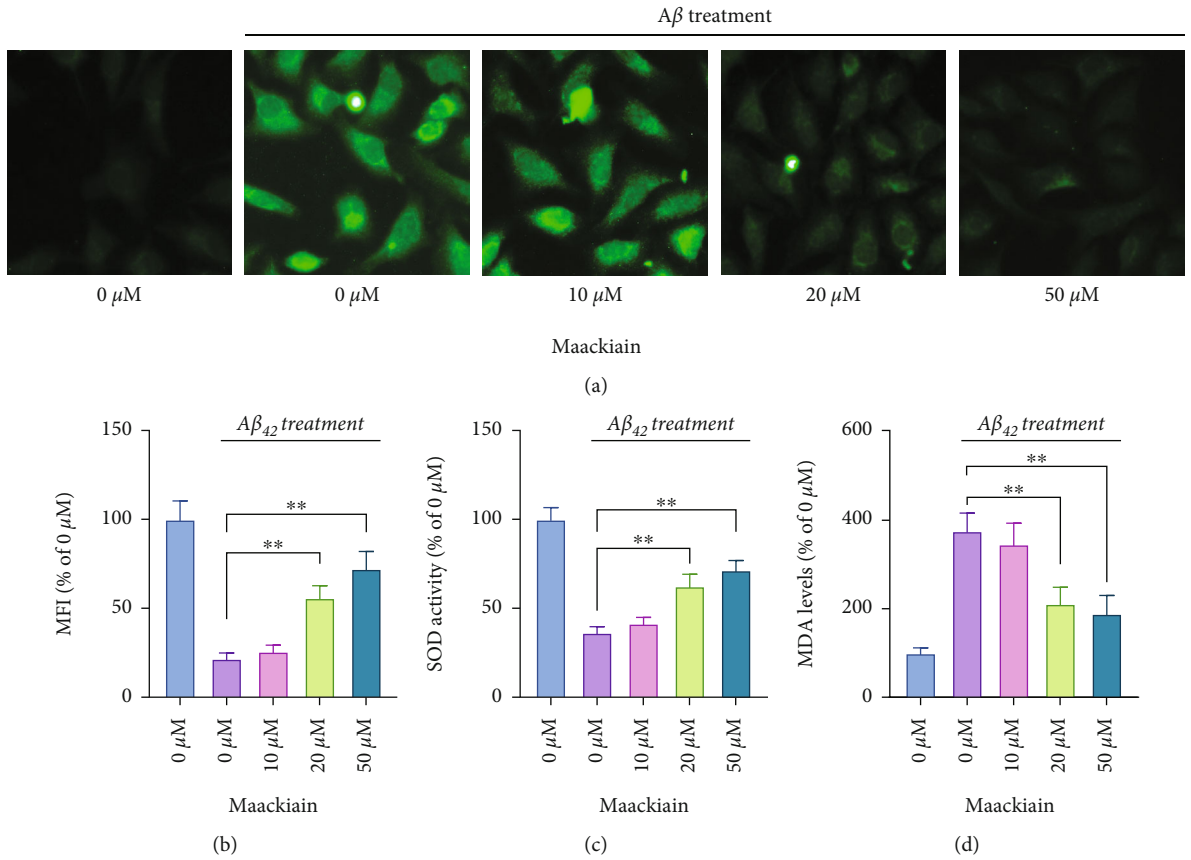

FIGURE 2: Maackiain abolished oxidative stress in PC12 cells stimulated with Aβ_{42}. PC12 cells were stimulated by different doses of Maackiain (10, 20, and 50 μM) for 6 hours and then exposed to 10 μM Aβ_{42} for additional 24 hours, followed by determination of intracellular ROS level by DCFH-DA assay (a), mitochondrial membrane potential with rhodamine 123 coupled with flow cytometry (b), SOD activity (c), and MDA levels (d). $*P < 0.05$ and $**P < 0.01$.

TRITC-5-dUTP in the dark for 60 minutes. The nuclei were stained using 4′,6-diamidino-2-phenylindole (DAPI), and the fluorescence signal was visualized using a fluorescence microscope.

2.10. Statistical Analysis. All data are expressed as the mean ± SD. Analysis of variance and q test were used for comparison between groups. $P < 0.05$ was considered statistically significance.

3. Results

3.1. Maackiain Reduces Aβ_{42}-Induced Cell Injury. In order to determine the toxicity, different concentrations of Aβ_{42} were tested on PC12 cells using the CCK-8 assay. The results showed that a dose-dependent toxic effects of Aβ_{42} on PC12 cells and treatment with 10 μM Aβ_{42} lead to reduction of cell viability to 50% of normal cells (Figure 1(a)). Further analysis showed 10 μM Aβ_{42}-induced toxic effects on PC12 cells time dependently with a significant effect for 24 hours (Figure 1(b)). Therefore, 10 μM Aβ_{42} stimulation for 24 hours was selected as a condition to induce injury to PC12 cells for the following experiments. To determine the protective effects of Maackiain on cell injury induced by Aβ_{42}, PC12 cells were pretreated with different doses of Maackiain (10, 20, and 50 μM) six hours before 10 μM Aβ_{42} stimulation. The results of CCk-8 and LDH activity assay showed that Maackiain prevented Aβ_{42}-induced cell injury in a dose-dependent way (Figures 1(c) and 1(d)). The results of TUNEL staining demonstrated that Aβ_{42} induced apparent apoptosis in PC12 cells, which was remarkably reduced by treatment of Maackiain (Figure 1(e)). Moreover, Maackiain significantly inhibited Aβ_{42}-induced caspase-3 activation in a concentration-dependent way (Figure 1(f)).

3.2. Maackiain Inhibits Aβ_{42}-Induced Oxidative Stress in PC12 Cells. To clarify mechanisms underlying the amelioration of Aβ_{42}-induced cell injury by Maackiain in PC12 cells, DCFH-DA assay was performed to determine intracellular ROS level. Aβ_{42}-induced ROS accumulation in PC12 cells as indicated by green fluorescence. Maackiain significantly reduced the generation of intracellular ROS by Aβ_{42} in PC12 cells dose dependently (Figure 2(a)). Flow cytometry analysis showed that Aβ_{42} decreased MMP in PC12 cells, and Maackiain restored intracellular MMP (Figure 2(b)). In addition, detection of intracellular SOD activity and MDA level revealed that Maackiain significantly abolished the decrease of SOD activity (Figure 2(c)) and increase of MDA levels induced by Aβ_{42} (Figure 2(d)).

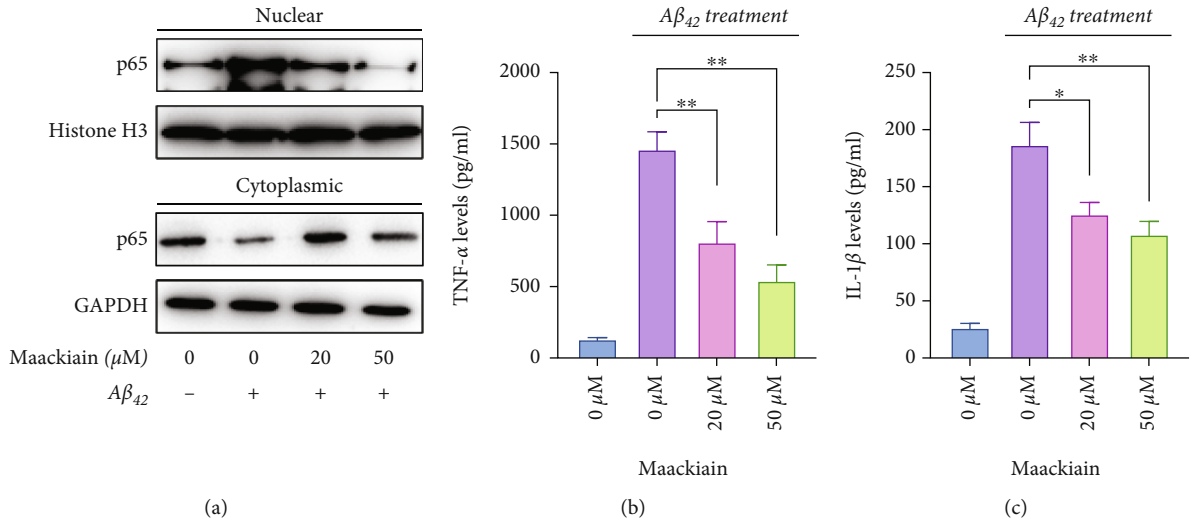

Figure 3: Inhibition of Aβ_{42}-induced inflammatory response by Maackiain. PC12 cells were stimulated by different doses of Maackiain (10, 20, and 50 μM) for 6 hours and then exposed to 10 μM Aβ_{42} for additional 24 hours, followed by immunoblotting of the p65 levels in nuclear and cytoplasm (a). TNF-α (b) and IL-1β (c) levels in the supernatant were determined by ELISA. $*P < 0.05$ and $**P < 0.01$.

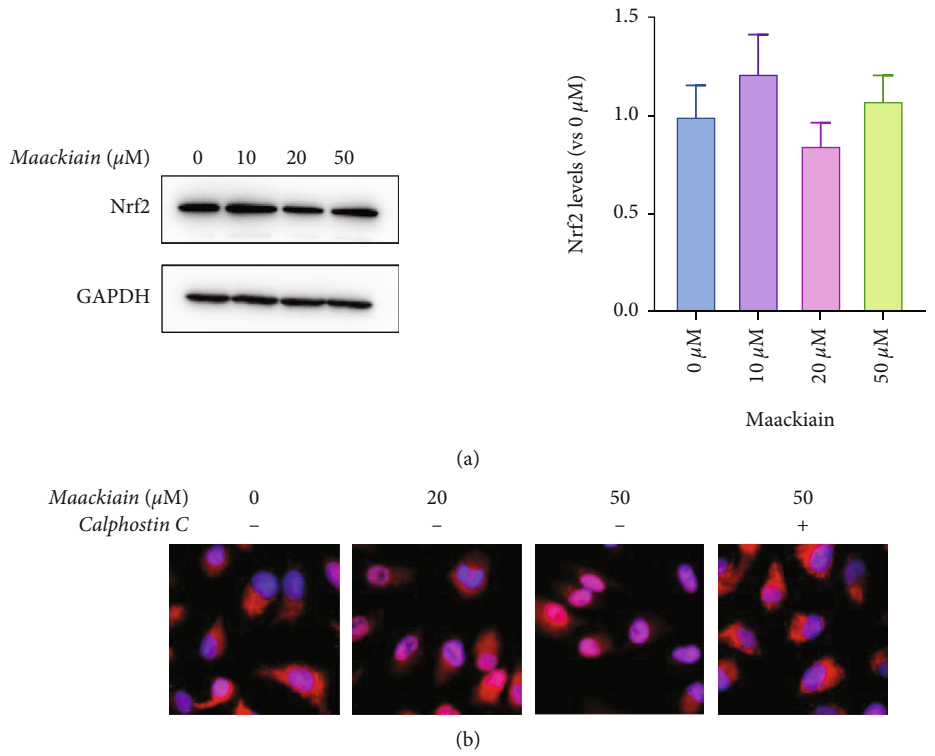

Figure 4: Maackiain promoted Nrf2 translocation dependent on PKC signaling. (a) PC12 cells were treated with different concentrations of Maackiain (0, 10, 20, and 50 μM) for 12 hours. The levels of Nrf2 were determined using western blot analysis with GAPDH as loading control. (b) PC12 cells were treated with different concentrations of Maackiain (0, 10, 20, and 50 μM) or calphostin C (100 nM) 1 hour before 50 μM Maackiain stimulation. Immunofluorescence staining was performed to detect intracellular Nrf2 expression and location. Red fluorescence indicates Nrf2, and the nuclei were stained with DAPI (Blue).

3.3. Maackiain Prevents Aβ_{42}-Induced Inflammatory Response. To investigate whether Maackiain affects Aβ_{42}-induced inflammatory response, we detected NF-κB activation in Aβ_{42}-treated PC cells with or without Maackiain. The results showed that Aβ_{42} significantly promoted the translocation of p65, the key component of NF-κB complex

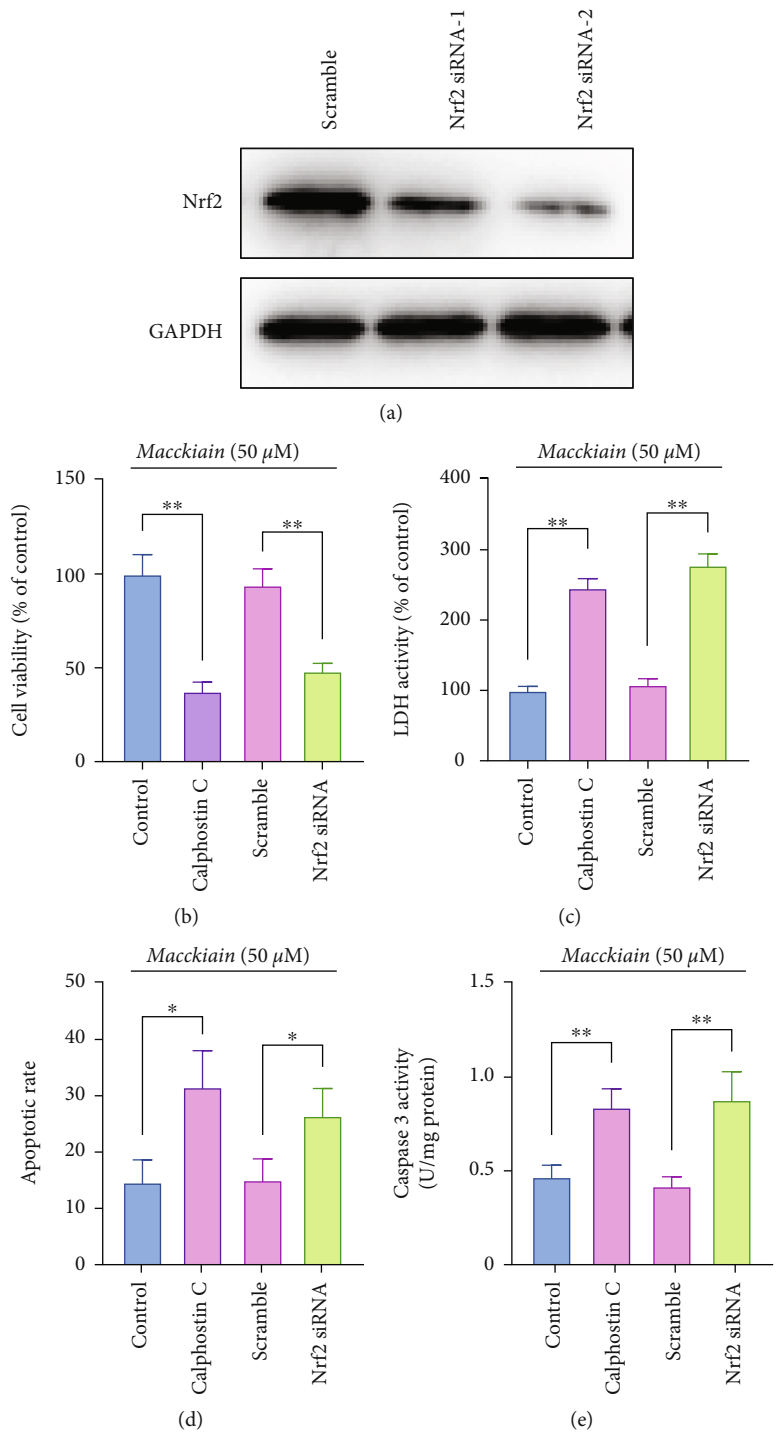

Figure 5: Effects of Nrf2 siRNA and calphostin C on PC12 cell injury. (a) Nrf2 and control siRNAs were transfected into PC12 cells. At 24 hours after transfection, the Nrf2 levels in PC12 cells were determined by immunoblotting. Prior to Maackiain (100 μM) treatment, PC12 cells were transfected with Nrf2 siRNA or incubated with calphostin C for 1 hour and then exposed to Aβ_{42}. CCK-8 (b) and LDH activity (c) assay were performed to detect cell viability and injury severity. Cell apoptosis was assessed by TUNEL staining (d) and caspase-3 activity assay (e). $*P < 0.05$ and $**P < 0.01$.

in PC12 cells, which was obviously prevented by Maackiain (Figure 3(a)). Consistently, ELISA detection of TNF-α and IL-1β protein level in the supernatant of PC12 cells indicated that Maackiain reduced the TNF-α (Figure 3(b)) and IL-1β (Figure 3(c)) levels that was increased by Aβ_{42}.

3.4. Maackiain Promotes Nrf2 Nuclear Translocation via the PKC Signaling Pathway. We further determined Nrf2 expression in PC12 cells treated with Maackiain. The results of western blot analysis revealed that Maackiain did not affect Nrf2 expression (Figure 4(a)). The results

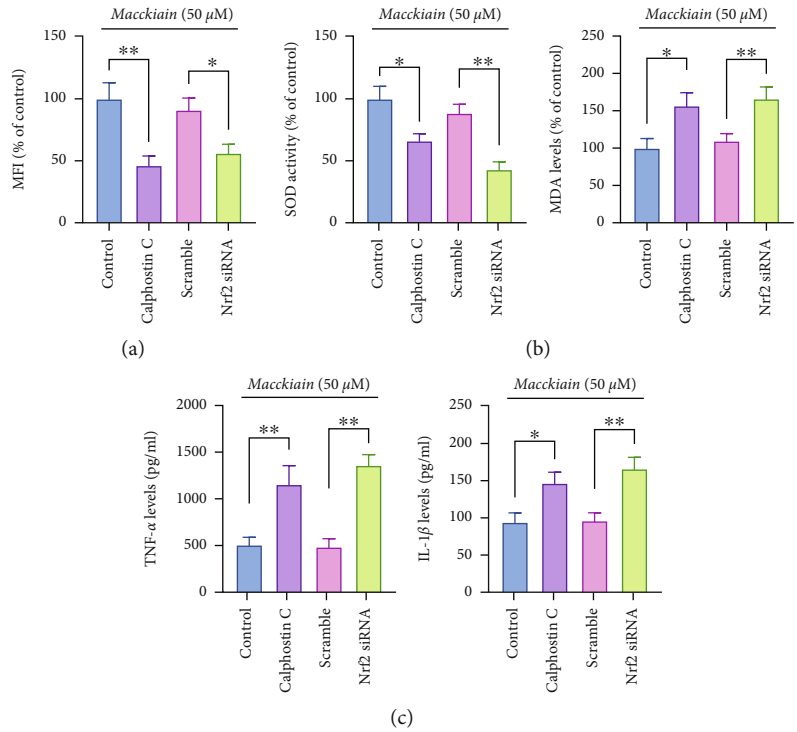

FIGURE 6: Effects of Nrf2 siRNA and calphostin C on oxidative stress. (a) Prior to treatment with 100 μM Maackiain, PC12 cells were transfected with Nrf2 siRNA or incubated with calphostin C for 1 hour and then exposed to Aβ_{42}. (a) MMP value was evaluated by rhodamine 123 staining. SOD activity and MDA levels (b) as well as TNF-α and IL-1β (c) were also assessed. $*P < 0.05$ and $**P < 0.01$.

of immunostaining showed that Nrf2 was present in the cytoplasm of PC12 cells, while Maackiain stimulation increased the intranuclear Nrf2 levels dose-dependently (Figure 4(b)), suggesting that Maackiain promotes the activation of Nrf2. After administration of PKC inhibitor calphostin C, the ability of Maackiain to promote intranuclear translocation of Nrf2 was weakened (Figure 4(b)).

3.5. Nrf2 Silencing or PKC Inhibition Attenuates the Neuroprotective Effects of Maackiain. To validate the involvement of Nrf2 and PKC in the cytoprotective effects of Maackiain, prior to Maackiain treatment, PC12 cells were transfected with Nrf2 siRNA or treated with calphostin C, followed by exposure to Aβ_{42}. As shown in Figure 5(a), transfected with Nrf2 siRNA significantly decreased the protein levels of Nrf2 in PC12 cells. CCk-8 assay showed that Nrf2 siRNA and calphostin C decreased the cell viability compared to that treated with Maackiain plus Aβ_{42} (Figure 5(b)), while LDH activity was increased compared to the control group (Figure 5(c)). TUNEL staining found that Nrf2 siRNA and calphostin C treatment increased PC12 cell apoptosis (Figure 5(d)) and promoted the caspase-3 activities in PC12 cells (Figure 5(e)).

3.6. Nrf2 Silencing or PKC Inhibition Attenuates the Antioxidant and Anti-inflammatory Effects of Maackiain. To investigate the role of Nrf2 and PKC in Maackiain preventing against oxidative stress, prior to Maackiain treatment, PC12 cells were transfected with Nrf2 siRNA or incubated with calphostin C, followed by exposure to Aβ_{42}. DCFH-DA assay was performed to detect intracellular ROS. After Nrf2 siRNA and calphostin C treatment, MFI value in the PC12 cells was weaker than that in the scramble and control group (Figure 6(a)), respectively. Nrf2 siRNA and calphostin C pretreatment decreased SOD activity and increased MDA levels (Figure 6(b)), as well as upregulated TNF-α and IL-1β (Figure 6(c)) in Aβ_{42}-stimulated PC12 cells.

4. Discussion

Peony root is a perennial herb that is widely used in traditional Chinese medicine. Various active ingredients, flavonoids, saponins, and polysaccharides, have been identified from the extracts of peony root. Maackiain is an important flavonoid of peony root. Maackiain has been shown to alleviate adipogenic activity [28] and improve metabolic disturbance rats with diabetes mellitus [18]. Moreover, Maackiain shows a neuroprotective role in *Caenorhabditis elegans* with Parkinson's disease [22]. However, whether Maackiain can reduce Aβ_{42}-induced neurotoxicity is unclarified. In this study, we showed that Maackiain reduced Aβ_{42}-induced cell injury and apoptosis in PC12 cells. These findings suggest that Maackiain prevents Aβ_{42}-induced neurotoxicity.

Aβ-induced oxidative stress plays an important role in the pathogenesis and development of AD [29]. Our results showed that Maackiain reduced ROS level as well as $\Delta\Psi m$ in PC12 cells. Importantly, we found that Maackiain prevented Aβ_{42}-induced decrease of $\Delta\Psi m$ and SOD activity and increase of MDA content. The chronic inflammatory

response of the nervous system is another important pathological feature of AD and plays a key role in promoting AD progression. In vivo and in vitro studies have demonstrated that abnormal deposition of Aβ in the brain is an initiation factor of neuroinflammatory response in AD [30]. Aβ promotes the release of inflammatory factors by binding to receptors on the surface of microglia and other neuronal cells. Moreover, Aβ-induced oxidative stress indirectly enhances the activation of inflammatory pathways. In this study, we found that Aβ_{42} significantly increased the mRNA levels and secretion of TNF-α and IL-1β in PC12 cells, which was however reduced by Maackiain pretreatment. These results demonstrate that Maackiain protects PC12 cells from Aβ_{42} through reducing Aβ_{42}-induced oxidative stress and neuroinflammatory response.

Guo et al. [18] and Bai et al. [19] found that Maackiain exhibits antioxidative effect through promoting Nrf2 activation. Results from this study demonstrated that Maackiain did not affect Nrf2 expression but significantly increased intranuclear Nrf2 expression. These results indicate that Maackiain promotes intranuclear translocation of Nrf2, which is consistent with the findings of previous studies [18, 19]. In this study, we further investigated whether Nrf2 is involved in the antioxidative and anti-inflammatory effects of Maackiain. Our results showed that Nrf2 silencing using RNA interference technology, the antioxidative and anti-inflammatory effects of Maackiain were significantly weakened, and the protective effects of Maackiain on PC12 cells exposed to Aβ were also reduced. These findings suggest that promotion of Nrf2 nuclear translocation contributes to the antioxidative stress and anti-inflammatory effects of Maackiain.

Some protein kinases phosphorylate Nrf2 to alter its conformation and facilitate its separation from Keap1 [31]. PKC is a multifunctional serine/threonine kinase downstream of G protein-coupled receptor and is involved in various biochemical processes including the regulation of transcription factors [32, 33]. It has been reported that PKC can phosphorylate Nrf2 at Ser40 leading to Nrf2 dissociation from Keap1, entrance into the nucleus and promotion of antioxidant gene transcription [34, 35]. In this study, we showed that PKC-specific inhibitor calphostin C inhibited Maackiain in the intranuclear translocation of Nrf2. Moreover, calphostin C inhibited the effects of Maackiain on Aβ_{42}-induced alteration of PC12 cell membrane potential, MDA content, and TNF-α and IL-1β levels and weakened the protective effects of Maackiain on cell viability.

Taken all together, our results demonstrated that Maackiain reduced Aβ_{42}-induced oxidative stress, inflammatory responses, cell injury, and apoptosis in PC12 cells in a dose-dependent manner. Our results also revealed that Maackiain promoted Nrf2 intranuclear translocation through the PKC signaling pathway, and inhibiting PKC signaling pathway or knocking down Nrf2 weakened the antioxidative stress and anti-inflammatory effects of Maackiain. Our results suggest that Maackiain can prevent against Aβ_{42}-induced neurotoxicity and holds promise to be used as a potential drug for AD treatment in the clinic.

Data Availability

All data used to support the findings of this study are included within the article.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

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