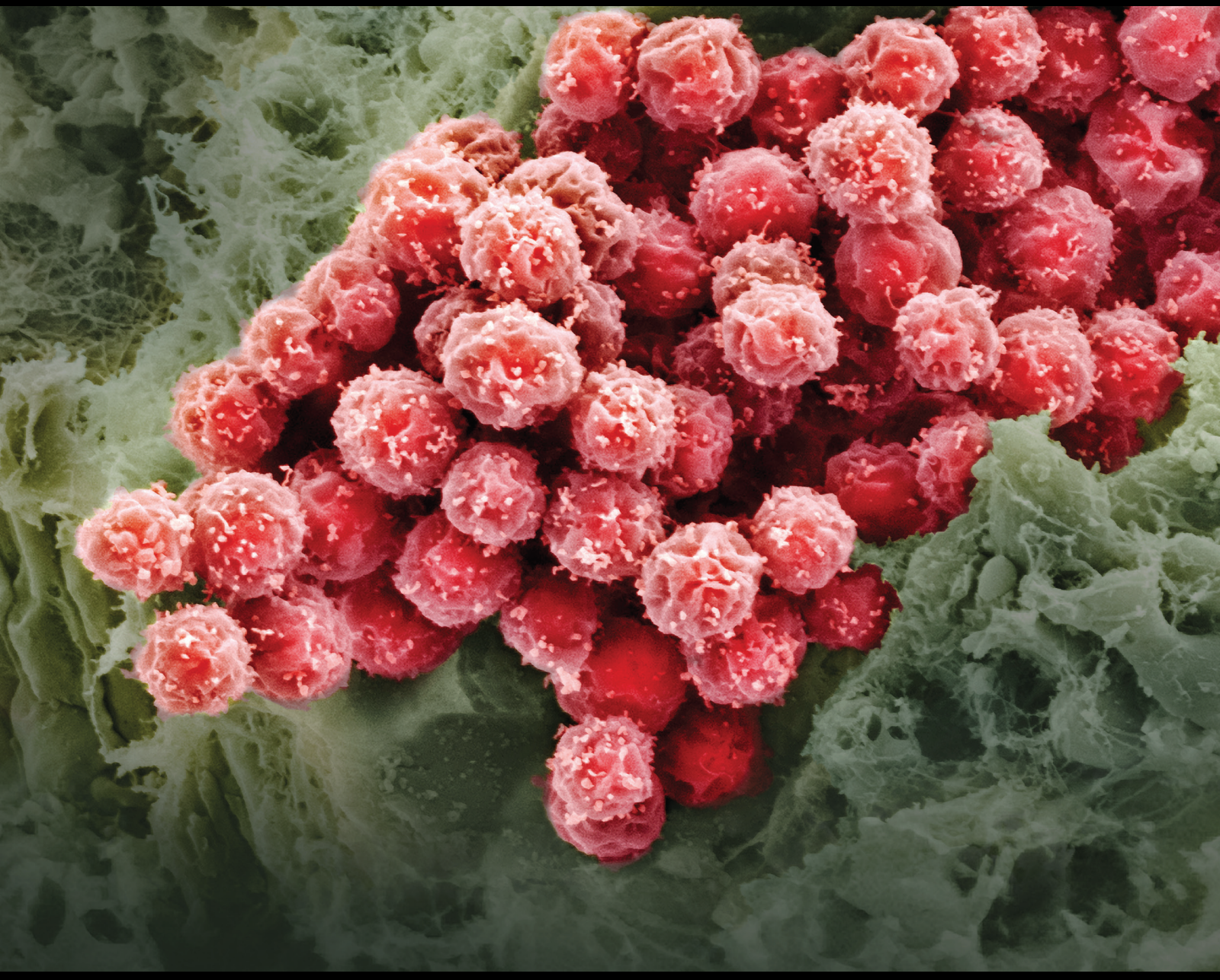
Stem Cells International

Regenerative Medicine and Rehabilitation in Musculoskeletal Diseases

Lead Guest Editor: Jasmin Nurkovic
Guest Editors: Radiša Vojinović and Džana Dervović



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Contents

The Effectiveness and Safety of Mesenchymal Stem Cells in the Treatment of Osteoarthritis: A Systematic Review and Meta-analysis of 28 Randomized Controlled Trials
Zhiyong Long, Mingsheng Zhang, Tianqing Zhang, Liuting Zeng, Kailin Yang, Tiejun Yang, Ganpeng Yu, Jun Li, Yang Wu, and Hua Chen
Research Article (22 pages), Article ID 6151866, Volume 2022 (2022)

Wharton's Jelly-Derived Mesenchymal Stem Cells with High Aurora Kinase A Expression Show Improved Proliferation, Migration, and Therapeutic Potential
Sun Jeong Kim, Sang Eon Park, Jang Bin Jeong, Shin Ji Oh, Alee Choi, Yun Hee Kim, Suk-joo Choi, Soo-young Oh, Gyu Ha Ryu, Hong Bae Jeon, and Jong Wook Chang
Research Article (15 pages), Article ID 4711499, Volume 2022 (2022)

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Research Article

The Effectiveness and Safety of Mesenchymal Stem Cells in the Treatment of Osteoarthritis: A Systematic Review and Meta-analysis of 28 Randomized Controlled Trials

Zhiyong Long,[1] Mingsheng Zhang,[2,3] Tianqing Zhang,[4] Liuting Zeng,[4] Kailin Yang,[4] Tiejun Yang,[4] Ganpeng Yu,[4] Jun Li,[4] Yang Wu,[4] and Hua Chen[4]

[1]*Department of Rehabilitation Medicine, Guangzhou Panyu Central Hospital, Guangzhou, China*
[2]*Department of Rehabilitation Medicine, South China Hospital of Shenzhen University, Shenzhen, China*
[3]*Department of Rehabilitation Medicine, Guangdong Provincial People's Hospital, Guangdong Academy of Medical Sciences, Guangdong Provincial Institute of Geriatrics, Guangzhou, China*
[4]*Hunan University of Chinese Medicine, Changsha, Hunan, China*

Correspondence should be addressed to Mingsheng Zhang; ymsheng321@126.com

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Objective. To evaluate the effectiveness and safety of mesenchymal stem cells (MSCs) in the treatment of osteoarthritis (OA). *Methods*. Chinese databases (such as CNKI and SinoMed) and English databases (such as PubMed and Embase) were searched to collect randomized controlled trials (RCTs) of MSCs in the treatment of OA. The retrieval time is from inception to October 10, 2021. The literature was strictly selected according to the inclusion and exclusion criteria, data was extracted, and the quality was evaluated. RevMan 5.3 software was used for meta-analysis. STATA was used to evaluate publication bias. The registration number of this systematic review and meta-analysis is CRD42021277145. *Results*. A total of 28 RCTs involving 1494 participants were included. The primary outcomes showed that MSCs may reduce WOMAC pain and VAS at the 3rd-month follow-up [WOMAC pain: -3.81 (-6.95, -0.68), $P = 0.02$. VAS: -1.11 (-1.53, -0.68), $P < 0.00001$], and the effect lasts for at least 12 months [WOMAC pain: -4.29 (-7.12, -1.47), $P = 0.003$. VAS: -1.77 (-2.43, -1.12), $P < 0.00001$]. MSCs may also reduce WOMAC stiffness and physical function at the 6th-month follow-up [WOMAC stiffness: -1.12 (-2.09, -0.14), $P = 0.03$. WOMAC physical function: -4.40 (-6.84, -1.96), $P = 0.0004$], and the effect lasts for at least 12 months [WOMAC stiffness: -0.99 (-1.95, -0.03), $P = 0.04$. WOMAC physical function: -3.26 (-5.91, -0.61), $P = 0.02$]. The improvement of WOMAC pain, VAS, WOMAC stiffness, and WOMAC physical function may be clinically significant. Meanwhile, after the MSC injection, Lequesne had been reduced compared with the control group [-4.49 (-8.21, -0.77), $P = 0.002$]. For adverse events, there is no significant difference in the safety of MSC injection and the control group [1.20 (0.97, 1.48), $P = 0.09$]. The quality of WOMAC physical function and adverse events were moderate. *Conclusion*. Based on current evidence, MSCs may be a safety therapy that have a good curative effect in the treatment of OA, the onset time is no later than 3 months, and the time to maintain the curative effect is no less than 12 months. However, these results should be generalized with caution due to the generally low quality of evidence and RCTs.

1. Introduction

Osteoarthritis (OA) is a disease involving movable joints characterized by cellular stress and extracellular matrix degradation triggered by microscopic and macroscopic lesions that activate maladaptive repair responses, including proinflammatory pathways of innate immunity [1, 2]. It is estimated that by 2032, 30% of people over the age of 45 will have OA [3, 4]. At present, there is no clinical cure for OA. The main goal of treatment is to control the pain caused by OA, delay the progression of the disease, correct deformity, improve or restore joint function, and strive to

improve the quality of life of patients [5, 6]. For example, conventional treatment methods are oral medications to control the condition, local joint injections, physical therapy, or direct joint replacement surgery. Especially for advanced OA, artificial joint prosthesis replacement is the gold standard for the treatment of advanced OA, but the life of the prosthesis is limited, and many complications will occur after the operation [7]. The ideal treatment plan is to improve the patient's clinical symptoms and promote cartilage regeneration [6, 8]. At present, symptomatic treatments such as physiotherapy, auxiliary braces, anti-inflammatory drugs, analgesics, hyaluronic acid (HA), glucocorticoids, arthroscopic debridement, and osteotomy cannot promote cartilage repair and cannot substantially improve OA [6]. Therefore, there is an urgent clinical need for new clinical therapeutic approaches to reduce the development of arthritis and relieve pain.

Mesenchymal stem cells (MSCs) are a type of pluripotent adult stem cells with stem cell characteristics isolated and cultured from the mesoderm and ectoderm of various tissues and organs. They are an important type of stem cell family and the most representative adult stem cells. [9], indicating that MSCs may be of great significance in the treatment of OA and cartilage defects [10]. In recent years, a large number of clinical trials of MSCs in the treatment of OA have been conducted in many countries, accumulating a large amount of conclusive clinical evidence, and a number of meta-analyses and systematic reviews have been published [11–18]. These systematic reviews and meta-analysis showed that compared with the control group, after treatment in the MSCs group, the Western Ontario and McMaster University (WOMAC) score decreased significantly, the visual analogue scale (VAS) decreased significantly, and the knee Lequesne index score decreased significantly. There was no statistically significant difference in the incidence of adverse events between the two groups. However, the above meta-analysis has certain shortcomings. For example, the number of RCTs included is not large, mainly retrospective studies and non-RCT clinical trials. The efficacy and safety of MSCs in the treatment of OA are still unclear. Therefore, in order to explore the therapeutic effect and safety of MSCs in the treatment of OA, this study conducted a systematic review and meta-analysis of the randomized controlled trials (RCT) of MSCs in the treatment of OA, so as to provide evidence support for the application of MSCs in the treatment of OA.

2. Materials and Methods

2.1. Protocol. This systematic review and meta-analysis were conducted strictly in accordance with the protocol registered in PROSPERO (CRD42021277145) and PRISMA-guidelines (see supplementary materials (available here)) [19].

2.2. Literature Search Strategy. The researcher searched VIP Database, SinoMed, Wanfang Database, CNKI, PubMed, Embase, Medline Complete, and Web of Science. The retrieval time is from inception to October 10, 2021. The researchers also searched the Cochrane Library and ClinicalTrials.gov. The search strategy of PubMed and Embase is shown in Table S1 as an example.

2.3. Inclusion and Exclusion Criteria. (1) Study design: RCTs without any restrictions on the language, year of publication, and so on. (2) Participants: adult patients diagnosed with OA by recognized standards. (3) Intervention: the experimental group is treated with MSCs, which can be combined with other therapies, and there are no restrictions on cell types and administration methods. The control group used the therapy without MSCs. (4) Outcomes: pain [WOMAC pain (0-20) and VAS (0-10 cm)], stiffness [WOMAC stiffness (0-8)], physical function [WOMAC physical function (0-68)], Lequesne index, and adverse events. The value of minimal clinically important difference (MCID) for VAS for pain was 1.02, WOMAC pain score as 1.79, WOMAC physical function score as 5.13, and WOMAC stiffness score as 0.65 [16]. (5) Exclusion criteria: animal experimental research and basic research, case report, comments, reviews or systematic reviews, and research that has been withdrawn

2.4. Literature Screening and Data Extraction. Two reviewers screened the literature separately, extracted the data, and cross-checked [20]. When two researchers have a disagreement, they discuss a joint decision with the third researcher. In the literature screening, first read the title and abstract of the literature, exclude irrelevant literature, and then read the full text of the selected literature to further screen out the final candidate literature. The content of the materials to be finally included in the literature includes (1) title, author, publication time, country, and other information; (2) the characteristics of the research object; and (3) follow-up time, intervention measures, outcome indicators, etc. If some important data are missing from the RCTs, we will try to contact the original authors to obtain the data or to estimate the missing standard deviation according to Cochrane Handbook 6.1.0 [21].

2.5. Risk of Bias Assessment. Risk of bias was assessed using the Cochrane Risk of Bias 2.0 tool, which assessed randomization process, deviation from included interventions, missing outcome data, outcome measures, and selective reporting [20]. The evaluation results of each module are obtained according to the module decision road map, and the overall bias evaluation is finally summarized and evaluated, including three levels: "low risk of bias," "some concerns," or "high risk of bias." The evaluation was conducted independently by 2 researchers and then cross-checked. Any differences would be discussed and resolved with the third researcher.

2.6. Statistical Analysis. The RevMan 5.3 software provided by the Cochrane Collaboration was used for statistical analysis [22]. Enumeration data were expressed using relative risk (RR) and 95% confidence interval (CI). Measurement data were expressed using the weighted mean difference (WMD) and 95% CI. For meta-analysis, the postintervention data (endpoint data) and change data (difference between endpoint and baseline) of WOMAC and VAS were mixed, and WMD was used for pooled effects according to

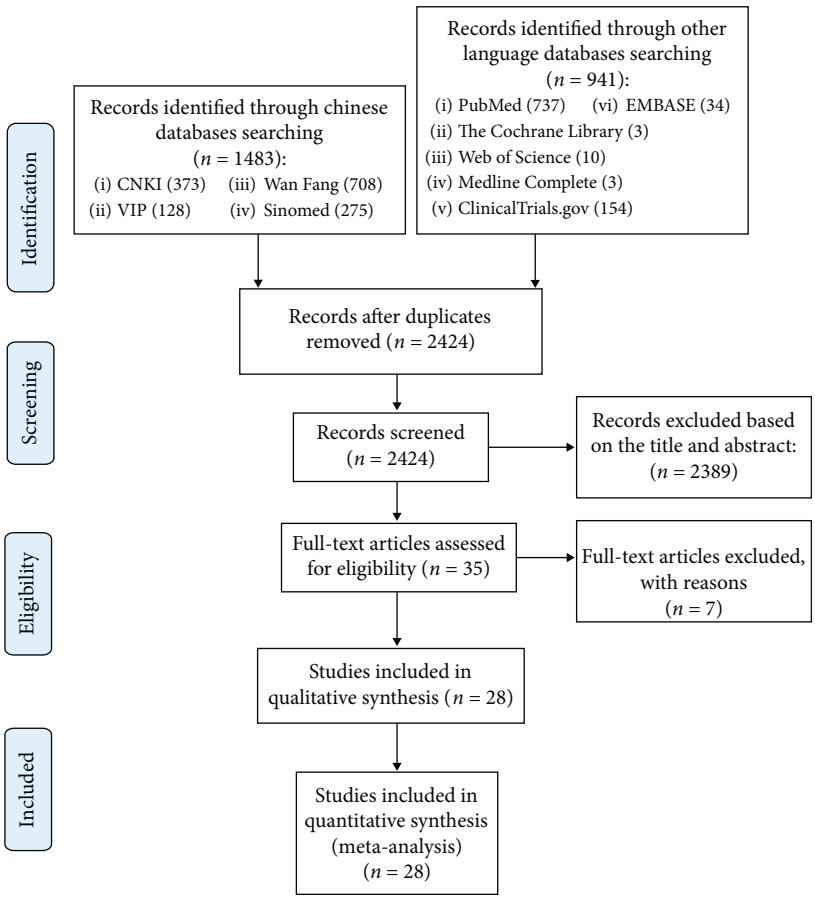

Figure 1: Flow diagram.

the Cochrane Handbook 6.1.0 [21]. Q-test was used for analysis (test level is $a = 0.1$), combined with I^2 to quantitatively evaluate the size of heterogeneity. If I^2 < 50% and $P > 0.1$, it can be considered that there is homogeneity among multiple similar studies, and the fixed effects model was used for analysis. If I^>50% and $P < 0.1$, the random effects model was used. Subgroup analysis would be carried out based on follow-up time and cell source. STATA was used for publication bias analysis (Harbord method for enumeration data and Egger method for measurement data).

2.7. Evidence Quality Assessment. GRADE is currently the most widely used grading system, especially in guidelines. The quality of each outcome measure was assessed by the GRADEprofiler software, which generally began to default to high-quality evidence for RCTs, but there were five factors that downgraded high quality and three factors that upgraded [23]. Downgrading factors included inconsistency, risk of bias (limitation), precision, indirectness, and publication bias. Upgrading factors were mainly large effect sizes, dose-response, and confounding factors. If there is no downgrading factor, it is still high-quality evidence, if there is one downgrading factor, it is moderate-quality evidence, two-level downgrade becomes low-quality evidence, and three-level or more downgrade is very low-quality evidence. Finally, the quality of evidence for each outcome was graded as very low, low, moderate, and high.

3. Results

3.1. Results of the Search. A total of 2424 documents were retrieved. After reading the title and abstract, articles that did not fit the topic and duplicates were excluded. Further screening was carried out according to the inclusion and exclusion criteria, and finally 28 RCTs were obtained [24–51], and 7 were excluded [52–58] (Figure 1).

3.2. Description of Included Trials. A total of 28 RCTs involving 1494 participants were included. Some RCTs consist of 2 experimental groups, so the control group is divided into 2 equal parts (each containing half the population) to match the two experimental groups, and the matched groups are labeled a and b (as in Kuah et al. [24]). The study characteristics are shown in Table 1.

3.3. Risk of Bias Assessment. The risk of bias is assessed and shown in Figures 2 and 3.

3.4. Primary Outcomes

3.4.1. Pain. Pain indicators are reflected by VAS and WOMAC pain. Ten RCTs reported WOMAC pain with exact values. The analysis of heterogeneity showed that I^2 > 50% and $P < 0.1$ in each subgroup, so the random effects model was adopted. The results showed that after the MSC injection, WOMAC pain had been reduced compared with

Table 1: The characteristics of the included studies.

Study	Trial registration number	Country	Blind	Sample size (female/male)		Intervention		Origin	Dosage	Route of medication	Relevant outcomes	Mean age (years)		Follow-up
				Trial group	Control group	Trial group	Control group					Trial group	Control group	
Kuah et al. [24]	ACTRN12615000439549	Australia	Double-blind	16 (5/11)	4 (3/1)	MSC+placebo	Placebo (cell culture media and cryopreservative)	Human adipose tissue	a: 3.9 ∗ 10^6 cells; b: 6.7 ∗ 10^6 cells	Intra-articular injection	WOMAC, VAS, adverse events	Low: 50.8 ± 7.29; high: 55.0 ± 5.15	55.0 ± 10.42	12 months
Wang et al. [25]	—	China	Not known	18 (8/10)	18 (7/11)	MSC+sodium hyaluronate	Sodium hyaluronate	Human umbilical cord	2 – 3 ∗ 10^7 cells	Intra-articular injection	WOMAC	45-63	42-69	6 months
Gupta et al. [26]	NCT01453738	India	Double-blind	20 (15/5)	10 (10/0)	MSC+placebo	Placebo (Plasmalyte A)	Human bone marrow	a: 2.5 ∗ 10^7 cells; b: 5 ∗ 10^7 cells	Intra-articular injection	VAS, adverse events	a: 58.10 ± 8.23; b: 57.30 ± 9.45	54.90 ± 8.27	12 months
Yang et al. [27]	—	China	Not known	28 (22/6)	16 (11/5)	MSC+sodium hyaluronate	Sodium hyaluronate	Human umbilical cord	a: 3 ∗ 10^7 cells; b: 6 ∗ 10^7 cells	Intra-articular injection	VAS, adverse events	a: 70.6 ± 20.1; b: 71.5 ± 16.3	72.2 ± 17.8	12 months
Vega et al. [28]	NCT01586312	Spain	Blind for participants	15 (9/6)	15 (10/5)	MSC+sodium hyaluronate	Sodium hyaluronate	Human bone marrow	4 ∗ 10^7 cells	Intra-articular injection	VAS, Lequesne, adverse events	57 ± 9		12 months
Hernigou et al. [29]	—	France	Not known	30 (18/12)	30 (18/12)	MSC+total knee arthroplasty	Total knee arthroplasty	Human bone marrow	2.6 ∗ 10^5 cells	Intra-articular injection	Adverse events, other clinical indicators	18-41		8-16 years
Hou et al. [30]	—	China	Not known	92 (52/40)	88 (50/38)	MSC+sodium hyaluronate	Sodium hyaluronate	Human bone marrow	Not known	Intra-articular injection	WOMAC	57 ± 8.3	55 ± 9.2	6 months
Lamo-Espinosa et al. [31, 32]	NCT02123368	Spain	Not known	20 (8/12)	10 (3/7)	MSC+sodium hyaluronate	Sodium hyaluronate	Human bone marrow	a: 1 ∗ 10^7 cells b: 1 ∗ 10^8 cells	Intra-articular injection	WOMAC, VAS, adverse events	a: 65.9 (59.5, 70.6); b:57.8 (55.0, 60.8)	60.3 (55.1, 61.1)∗	4 years
Lu et al. [33]	—	China	Not known	40 (26/14)	40 (27/13)	MSC+sodium hyaluronate	Sodium hyaluronate	Human bone marrow	Not known	Intra-articular injection	WOMAC, adverse events	55.9 ± 8.1	55.1 ± 6.8	12 months
Gan et al. [34]	—	China	Not known	6 (5/1)	6 (4/2)	MSC+sodium hyaluronate	Sodium hyaluronate	Human umbilical cord	Not known	Intra-articular injection	WOMAC, Lequesne	56.27 ± 7.52	55.96 ± 6.93	12 months
Vangsness et al. [35]	NCT00225095	The US	Double-blind	36 (not kwon)	19 (not kwon)	MSC+sodium hyaluronate	Sodium hyaluronate	Human bone marrow	a: 5 ∗ 10^7 cells; b: 1.5 ∗ 10^8 cells	Intra-articular injection	VAS, adverse events	—	—	2 years
Tan et al. [34]	—	China	Not known	36 (26/10)	36 (27/9)	MSC+arthroscopy cleanup	Arthroscopy cleanup	Human bone marrow	2 – 3 ∗ 10^7 cells	Intra-articular injection	Lequesne	53.37 ± 6.94	53.76 ± 5.68	12 months
Wong et al. [36]	—	The US	Not known	28 (13/15)	28 (14/14)	MSC+sodium hyaluronate	Sodium hyaluronate	Human bone marrow	1.46 ± 0.29 ∗ 10^7 cells	Intra-articular injection	Other clinical indicators	36-54	24-54	2 years
Xu et al. [37]	—	China	Not known	20 (not kwon)	20 (not kwon)	MSC+arthroscopic surgery+sodium hyaluronate	Arthroscopic surgery +sodium hyaluronate	Human bone marrow	Not known	Intra-articular injection	WOMAC	44-74		3 years
Matas et al. [38]	NCT02580695	Chile	Double-blind	18 (11/7)	8 (5/3)	MSC+sodium hyaluronate	Sodium hyaluronate	Human umbilical cord	a: 2 ∗ 10^7 cells; b: 4 ∗ 10^7 cells	Intra-articular injection	WOMAC, VAS, adverse events	a: 56.1 ± 6.8 ; b: 56.7 ± 4.1	54.8±4.5	12 months
Ha et al. [39]	—	China	Not known	89 (60/29)	86 (63/23)	a: MSC+platelet-rich plasma v.s. platelet-rich plasma; b: MSC v.s. sodium hyaluronate +triamcinolone acetonide		Human umbilical cord	1 ∗ 10^7 cells	Intra-articular injection	VAS, adverse events	a: 56.8 ± 6.1 v.s. 55.6 ± 3.6; b: 57.0 ± 3.2 v.s. 56.2 ± 6.7		12 months
Lu et al. [40]	NCT02162693	China	Double-blind	26 (23/3)	26 (23/3)	MSC+sodium hyaluronate	Sodium hyaluronate	Human adipose tissue	5 ∗ 10^7 cells	Intra-articular injection	WOMAC, VAS, adverse events	55.03 ± 9.19	59.64 ± 5.97	12 months

Table 1: Continued.

Study	Trial registration number	Country	Blind	Sample size (female/male)		Intervention		Origin	Dosage	Route of medication	Relevant outcomes	Mean age (years)		Follow-up
				Trial group	Control group	Trial group	Control group					Trial group	Control group	
Zhang et al. [41]	NCT03955497	China	Not known	14 (9/5)	14 (8/6)	MSC+high tibial osteotomy	High tibial osteotomy	Human adipose tissue	Not known	Intra-articular injection	WOMAC, VAS	61.51 ± 8.80	64.64 ± 9.11	12 months
Liang et al. [42]	—	China	Not known	26 (not known)		MSC	Placebo	Human bone marrow	2 ∗ 10^7 cells	Intra-articular injection	WOMAC, adverse events	40-65		4 weeks
Zhou [43]	—	China	Not known	54 (not known)	54 (not known)	MSC+sodium hyaluronate	Conventional therapy	Not known	Not known	Intra-articular injection	WOMAC, VAS	56.05 ± 2.39	55.65 ± 3.56	3 months
Freitag et al. [44]	ACTRN12614000814673	Australia	Not known	20 (9/11)	10 (5/5)	MSC	Conventional therapy	Human adipose tissue	a: 1 ∗ 10^8 cells (single injection); b: 1 ∗ 10^8 cells (two injection)	Intra-articular injection	WOMAC, adverse events	a: 54.6 ± 6.3; b: 54.7 ± 10.2	51.5 ± 6.1	6 months
Lee et al. [45]	—	South Korea	Double-blind	12 (9/3)	12 (9/3)	MSC	Normal saline	Human adipose tissue	1 ∗ 10^8 cells	Intra-articular injection	WOMAC, VAS, adverse events	62.2 ± 6.5	63.2 ± 4.2	6 months
Garza et al. [46]	NCT02726945	The US	Double-blind	26 (11/15)	13 (7/6)	MSC	Placebo	Human stromal vascular fraction	a: 1.5 ∗ 10^7 cells; b: 3 ∗ 10^7 cells	Intra-articular injection	WOMAC, adverse events	a: 60.5 ± 7.9; b: 59.5 ± 11.7	57.1 ± 9.1	12 months
Lamo-Espinosa et al. [47]	NCT02365142	Spain	Not known	24 (7/17)	26 (10/16)	MSC+platelet-rich plasma	Platelet-rich plasma	Human bone marrow	1 ∗ 10^8 cells	Intra-articular injection	WOMAC, VAS, adverse events	40-62	33-70	12 months
Bastos et al. [48]	—	Multi-center	Double-blind	30 (15/15)	17 (8/9)	a: MSC+platelet rich plasma; b: MSC	Corticosteroid	Human bone marrow	Not known	Intra-articular injection	Other clinical indicators	a: 60.8 ± 9.9; b: 55.7 ± 7.8	55.9 ± 13.4	12 months
Hong et al. [49]	ChiCTR1800015125	China	Double-blind, but does not describe the implementation	MSC in left: 8 (7/1); MSC in right 8 (6/2)		MSC+sodium hyaluronate	Sodium hyaluronate	Human adipose tissue	Not known	Intra-articular injection	WOMAC, VAS, adverse events	MSC in left: 53 ± 10.97; MSC in right 51 ± 5.95		12 months
Khalifeh Soltani et al. [50]	IRCT2015101823298N	Iran	Double-blind	10 (not known)	10 (not known)	MSC	Normal saline	Human placenta	0.5 – 0.6 ∗ 10^8 cells	Intra-articular injection	VAS, adverse events	35-75		24 weeks
Zhang et al. [51]	—	China	Blind for outcome assessment	72 (56/16)	36 (8/28)	MSC+sodium hyaluronate or MSC only	Sodium hyaluronate	Human adipose tissue	Not known	Intra-articular injection	WOMAC, VAS	a: 57.56 ± 14.06; b: 53.39 ± 12.66	56.89 ± 14.53	3 years

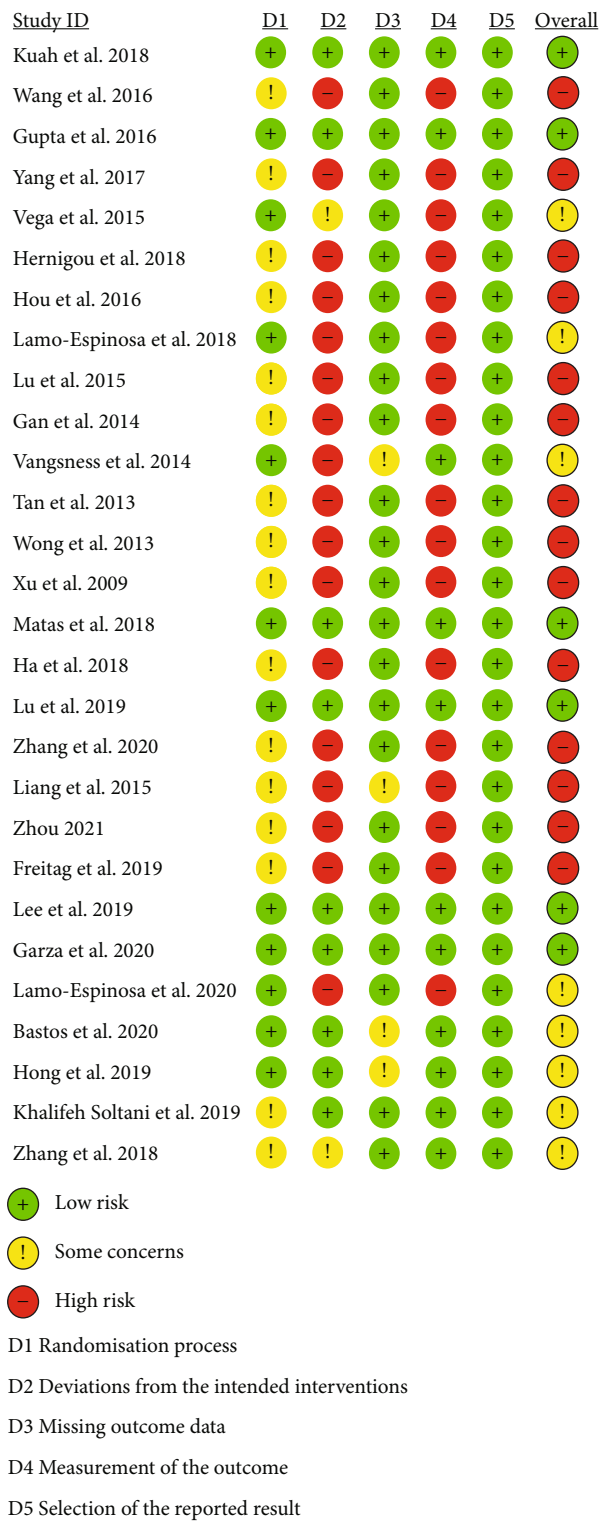

Figure 2: Risk of bias graph.

the control group at the 3rd-month follow-up [-3.81 (-6.95, -0.68), $P = 0.02$], and the effect lasts for at least 12 months [-4.29 (-7.12, -1.47), $P = 0.003$] (Figure 4). The improvement of WOMAC-pain may be clinically significant.

Thirteen RCTs reported VAS with exact values. The analysis of heterogeneity showed that $I^2 > 50\%$ and $P < 0.1$ in each subgroup, so the random effects model was adopted. The results also showed that after the MSC injection, VAS had been reduced compared with the control group at the 3rd-month follow-up [-1.11 (-1.53, -0.68), $P < 0.00001$], and the effect lasts for at least 12 months [-1.77 (-2.43, -1.12), $P < 0.00001$] (Figure 5). The improvement of VAS may be clinically significant.

3.4.2. Stiffness. Stiffness indicators are reflected by WOMAC stiffness. Seven RCTs reported WOMAC stiffness with exact values. The analysis of heterogeneity showed that $I^2 > 50\%$ and $P \leq 0.1$ in each subgroup, so the random effects model was adopted. The results showed that after the MSC injection, WOMAC stiffness had been reduced compared with the control group at the 6th-month follow-up [-1.12 (-2.09, -0.14), $P = 0.03$], and the effect lasts for at least 12 months [-0.99 (-1.95, -0.03), $P = 0.04$] (Figure 6). The improvement of WOMAC stiffness may be clinically significant.

3.4.3. Physical Function. Physical function indicators are reflected by WOMAC physical function. Six RCTs reported WOMAC physical function with exact values. The analysis of heterogeneity showed that $I^2 < 50\%$ and $P > 0.1$ in each subgroup, so the fixed effects model was adopted. The results showed that after the MSC injection, WOMAC physical function had been reduced compared with the control group at the 6th-month follow-up [-4.40 (-6.84, -1.96), $P = 0.0004$], and the effect lasts for at least 12 months [-3.26 (-5.91, -0.61), $P = 0.02$] (Figure 7). The improvement of WOMAC physical function may be clinically significant.

3.4.4. Lequesne. Three RCTs reported Lequesne with exact values. The analysis of heterogeneity showed that $I^2 = 96\%$ and $P < 0.00001$, so the random effects model was adopted. The results showed that after the MSC injection, Lequesne had been reduced compared with the control group [-4.49 (-8.21, -0.77), $P = 0.002$] (Figure 8).

3.5. Secondary Outcomes

3.5.1. Efficacy of Different Cell Sources on Pain. The WOMAC pain and VAS data were divided into subgroups according to follow-up time and cell source. For WOMAC pain, the analysis of heterogeneity showed that $I^2 > 50\%$ and $P < 0.1$ in almost subgroups, so the random effects model was adopted. (1) For bone marrow derived, only 1 study was involved, and there were no positive findings in this study ($P > 0.05$). (2) For umbilical cord derived, only 1 study was involved, and there were no positive findings in this study ($P > 0.05$). (3) For adipose derived, the results showed that after the MSC injection, WOMAC pain had been reduced compared with the control group at the 3rd-month follow-up [-4.95 (-7.44, -2.46), $P < 0.0001$], and the effect lasts for at least 12 months [-5.83 (-10.21, -1.45), $P = 0.009$] (Figure 9).

For VAS, the analysis of heterogeneity showed that $I^2 > 50\%$ and $P < 0.1$ in almost subgroups, so the random effects model was adopted. (1) For bone marrow derived, the results showed that after the MSC injection, VAS had been reduced compared with the control group at the 6th-

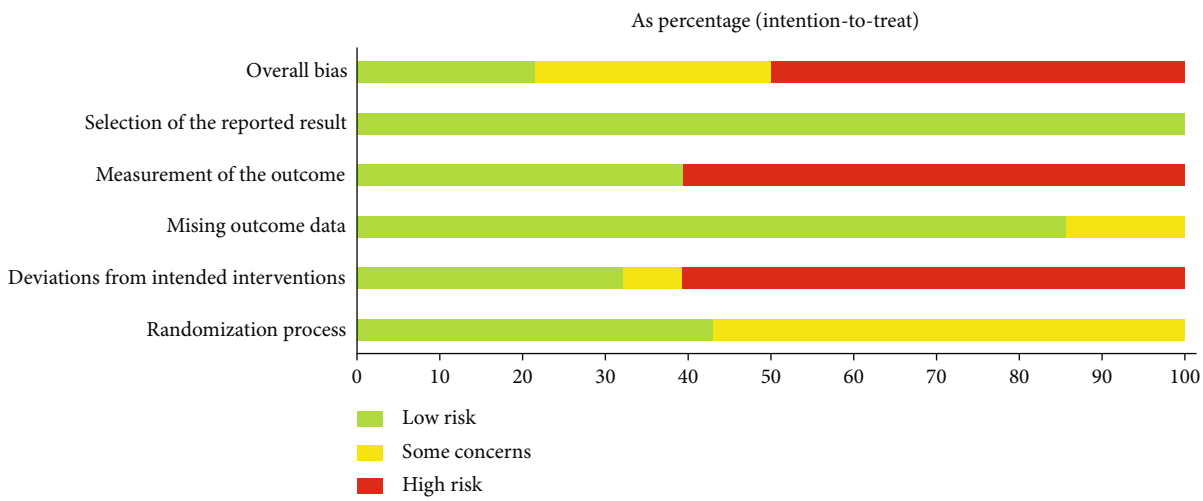

Figure 3: Risk of bias summary.

Study or subgroup	Experimental Mean	Experimental SD	Experimental Total	Control Mean	Control SD	Control Total	Weight	Mean difference IV, random, 95% CI
1.1.1 3 months								
Kuah et al. 2018a	−1.5	2.5	8	−0.4	3.2	2	14.3%	−1.10 [−5.86, 3.66]
Kuah et al. 2018b	−3.5	2.5	8	−0.4	3.2	2	14.3%	−3.10 [−7.86, 1.66]
Lamo-Espinosa et al. 2020	4.6	3.3	24	4.3	3.3	26	20.0%	0.30 [−1.53, 2.13]
Hong et al. 2019	−7.31	3.52	16	2.06	6.84	16	16.4%	−9.37 [−13.14, −5.60]
Zhang et al. 2018a	6.81	4.76	36	11.5	5.94	18	17.6%	−4.69 [−7.84, −1.54]
Zhang et al. 2018b	6.25	5.28	36	11.5	5.94	18	17.4%	−5.25 [−8.49, −2.01]
Subtotal (95% CI)			**128**			**82**	**100.0%**	**−3.81 [−6.95, −0.68]**
Heterogeneity: $\tau^2 = 11.94$; $\delta^2 = 26.70$, df = 5 ($P < 0.0001$); $I^2 = 81\%$								
Test for overall effect: $Z = 2.39$ ($P = 0.02$)								
1.1.2 6 months								
Kuah et al. 2018a	−1.8	1.8	8	−1	2.5	2	10.5%	−0.80 [−4.48, 2.88]
Kuah et al. 2018b	−3.4	1.7	8	−1	2.5	2	10.5%	−2.40 [−6.06, 1.26]
Matas et al. 2018a	2.1	1.4	9	4.2	3.8	4	10.3%	−2.10 [−5.93, 1.73]
Matas et al. 2018b	2.4	1.7	9	4.2	3.8	4	10.2%	−1.80 [−5.69, 2.09]
Lu et al. 2019	−2.62	2.21	26	−1.48	1.53	24	13.6%	−1.14 [−2.19, −0.09]
Lamo-Espinosa et al. 2020	4.3	3.6	24	4.4	3	26	12.9%	−0.10 [−1.95, 1.75]
Hong et al. 2019	−7.94	3.84	16	3.38	5.73	16	10.9%	−11.32 [−14.70, −7.94]
Zhang et al. 2018a	4.17	5.55	36	12.36	6.92	18	10.5%	−8.19 [−11.87, −4.51]
Zhang et al. 2018b	4.78	5.91	36	12.36	6.92	18	10.4%	−7.58 [−11.31, −3.85]
Subtotal (95% CI)			**172**			**114**	**100.0%**	**−3.80 [−6.19, −1.40]**
Heterogeneity: $\tau^2 = 10.64$; $\delta^2 = 55.66$, df = 8 ($P < 0.00001$); $I^2 = 86\%$								
Test for overall effect: $Z = 3.11$ ($P = 0.002$)								
1.1.3 12 months								
Kuah at al. 2018a	−3.5	2	8	−0.4	2.4	2	10.8%	−3.10 [−6.70, 0.50]
Kuah et al. 2018b	−1.2	1.7	8	−0.4	2.4	2	10.8%	−0.80 [−4.33, 2.73]
Matas et al. 2018a	3.7	2.6	9	4.3	3.5	4	10.5%	−0.60 [−4.43, 3.23]
Matas et al. 2018b	1.1	1.3	9	4.3	3.5	4	10.8%	−3.20 [−6.73, 0.33]
Lu et al. 2019	−2.63	2.36	26	−1.44	1.85	24	12.7%	−1.19 [−2.36, −0.02]
Lamo-Espinosa et al. 2020	4.1	3.6	24	4.5	3.2	26	12.3%	−0.40 [−2.29, 1.49]
Hong et al. 2019	−8	4.77	16	5.69	4.29	16	11.2%	−13.69 [−16.83, −10.55]
Zhang et al. 2018a	5.08	6.02	36	13.61	7.3	18	10.4%	−8.53 [−12.43, −4.63]
Zhang et al. 2018b	5.53	6.34	36	13.61	7.3	18	10.4%	−8.08 [−12.04, −4.12]
Subtotal (95% CI)			**172**			**114**	**100.0%**	**−4.29 [−7.12, −1.47]**
Heterogeneity: $\tau^2 = 16.01$; $\delta^2 = 77.03$, df = 8 ($P < 0.00001$); $I^2 = 90\%$								
Test for overall effect: $Z = 2.97$ ($P = 0.003$)								
Test for subgroup differences: $\delta^2 = 0.08$, df = 2 ($P = 0.96$). $I^2 = 0\%$								

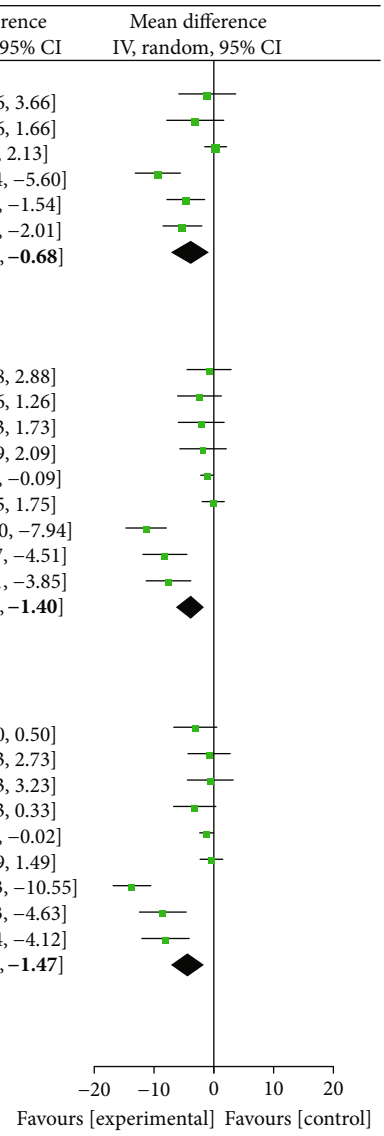

Figure 4: WOMAC pain.

Study or subgroup	Experimental Mean	Experimental SD	Experimental Total	Control Mean	Control SD	Control Total	Weight	Mean difference IV, random, 95% CI
1.4.1 3 months								
Kuah et al. 2018a	−1.6	1.9	8	−0.4	2.7	2	1.0%	−1.20 [−5.17, 2.77]
Kuah et al. 2018b	−2.6	1.9	8	−0.4	2.7	2	1.0%	−2.20 [−6.17, 1.77]
Yang et al. 2017a	7.43	0.5	15	8	0.45	8	10.9%	−0.57 [−0.97, −0.17]
Yang et al. 2017b	6.08	0.76	13	8	0.45	8	10.2%	−1.92 [−2.44, −1.40]
Ha et al. 2018a	2.2	1.2	45	2.6	0.7	44	10.9%	−0.40 [−0.81, 0.01]
Ha et al. 2018b	2.6	0.7	43	3.1	1	43	11.1%	−0.50 [−0.86, −0.14]
Zhang et al. 2020	2.14	0.94	26	3.57	2.1	24	7.7%	−1.43 [−2.34, −0.52]
Zhou 2021	1.17	0.13	54	2.62	0.42	54	12.0%	−1.45 [−1.57, −1.33]
Lamo-Espinosa et al. 2020	3.8	2	24	3.8	1.6	26	7.2%	0.00 [−1.01, 1.01]
Hong et al. 2019	−3.38	1.09	16	−0.69	0.7	16	9.5%	−2.69 [−3.32, −2.06]
Zhang et al. 2018a	1.75	1.2	36	3	1.15	18	9.3%	−1.25 [−1.91, −0.59]
Zhang et al. 2018b	2.39	1.38	36	3	1.15	18	9.1%	−0.61 [−1.31, 0.09]
Subtotal (95% CI)			324			263	100.0%	−1.11 [−1.53, -0.68]
Heterogeneity: $\tau^2 = 0.39$; $\delta^2 = 90.47$, df = 11 ($P < 0.00001$); $I^2 = 88\%$								
Test for overall effect: $Z = 5.12$ ($P < 0.00001$)								
1.4.2 6 months								
Kuah et al. 2018a	−2.9	1.5	8	−1.1	2.1	2	2.4%	−1.80 [−4.89, 1.29]
Kuah et al. 2018b	−2.5	1.5	8	−1.1	2.1	2	2.4%	−1.40 [−4.49, 1.69]
Yang et al. 2017a	7.67	0.62	15	7.72	0.41	8	7.5%	−0.05 [−0.47, 0.37]
Yang et al. 2017b	4.77	1.09	13	7.72	0.41	8	7.1%	−2.95 [−3.61, −2.29]
Vangsness et al. 2014a	−3.5	0.9	17	−2.2	0.9	10	7.0%	−1.30 [−2.00, −0.60]
Vangsness et al. 2014b	−3.9	0.9	18	−2.2	0.9	9	6.9%	−1.70 [−2.42, −0.98]
Matas et al. 2018a	1.2	0.75	9	2.8	0.87	4	6.3%	−1.60 [−2.58, −0.62]
Matas et al. 2018b	1.08	0.78	9	2.8	0.87	4	6.3%	−1.72 [−2.71, −0.73]
Ha et al. 2018a	2.2	1.1	45	2.6	0.6	44	7.5%	−0.40 [−0.77, −0.03]
Ha et al. 2018b	2.6	0.6	43	3.5	1	43	7.6%	−0.90 [−1.25, −0.55]
Lu et al. 2019	3	2.62	26	4.5	2.71	24	5.2%	−1.50 [−2.98, −0.02]
Zhang et al. 2020	1.21	0.57	26	1.21	0.89	24	7.5%	0.00 [−0.42, 0.42]
Lamo-Espinosa et al. 2020	3.3	2.2	24	3.5	2	26	5.9%	−0.20 [−1.37, 0.97]
Hong et al. 2019	−3.69	1.01	16	0.06	0.93	16	7.0%	−3.75 [−4.42, −3.08]
Zhang et al. 2018a	1.06	1.39	36	3.25	1.5	18	6.7%	−2.19 [−3.02, −1.36]
Zhang et al. 2018b	1.31	1.39	36	3.25	1.5	18	6.7%	−1.94 [−2.77, −1.11]
Subtotal (95% CI)			349			260	100.0%	−1.44 [−2.01, −0.86]
Heterogeneity: $\tau^2 = 1.12$; $\delta^2 = 173.12$, df = 15 ($P < 0.00001$); $I^2 = 91\%$								
Test for overall effect: $Z = 4.87$ ($P < 0.00001$)								
1.4.3 12 months								
Kuah et al. 2018a	−3.3	1.5	8	−0.9	2.2	2	2.6%	−2.40 [−5.62, 0.82]
Kuah et al. 2018b	−0.8	1.3	8	−0.9	2.2	2	2.6%	0.10 [−3.08, 3.28]
Yang et al. 2017a	4.77	1.09	15	7.69	0.31	8	6.4%	−2.92 [−3.51, −2.33]
Yang et al. 2017b	3.04	1.3	13	7.69	0.31	8	6.2%	−4.65 [−5.39, −3.91]
Vangsness et al. 2014a	−3.7	0.9	17	−2.5	0.9	10	6.2%	−1.20 [−1.90, −0.50]
Vangsness et al. 2014b	−4.7	0.8	18	−2.5	0.9	9	6.2%	−2.20 [−2.89, −1.51]
Matas et al. 2018a	1.33	0.84	9	2.21	0.98	4	5.6%	−0.88 [−1.99, 0.23]
Matas et al. 2018b	0.24	0.21	9	2.21	0.98	4	5.9%	−1.97 [−2.94, −1.00]
Ha et al. 2018a	2.2	1.1	45	2.7	0.6	44	6.6%	−0.50 [−0.87, −0.13]
Ha et al. 2018b	2.6	0.8	43	3.9	1	43	6.6%	−1.30 [−1.68, −0.92]
Lu et al. 2019	2.78	2.58	26	4.4	2.43	24	5.2%	−1.62 [−3.01, −0.23]
Zhang et al. 2020	0.71	0.61	26	0.64	0.49	24	6.6%	0.07 [−0.24, 0.38]
Lamo-Espinosa et al. 2020	3.5	2.5	24	4.5	2.2	26	5.3%	−1.00 [−2.31, 0.31]
Hong et al. 2019	−3.19	0.98	16	0.06	1.18	16	6.2%	−3.25 [−4.00, −2.50]
Zhang et al. 2018a	1.47	1.56	36	3.72	1.47	18	6.0%	−2.25 [−3.10, −1.40]
Zhang et al. 2018b	1.75	1.83	36	3.72	1.47	18	6.0%	−1.97 [−2.87, −1.07]
Gupta et al. 2016a	−4.03	1.73	10	−2.13	2.83	5	3.1%	−1.90 [−4.60, 0.80]
Gupta et al. 2016b	−3.03	3.1	10	−2.13	2.83	5	2.6%	−0.90 [−4.04, 2.24]
Vega et al. 2015	3.3	2.324	15	5.1	3.098	15	4.2%	−1.80 [−3.76, 0.16]
Subtotal (95% CI)			384			285	100.0%	−1.77 [−2.43, −1.12]
Heterogeneity: $\tau^2 = 1.66$; $\delta^2 = 246.62$, df = 18 ($P < 0.00001$); $I^2 = 93\%$								
Test for overall effect: $Z = 5.31$ ($P < 0.00001$)								
Test for subgroup differences: $\delta^2 = 2.96$. df = 2 ($P = 0.23$); $I^2 = 32.5\%$								

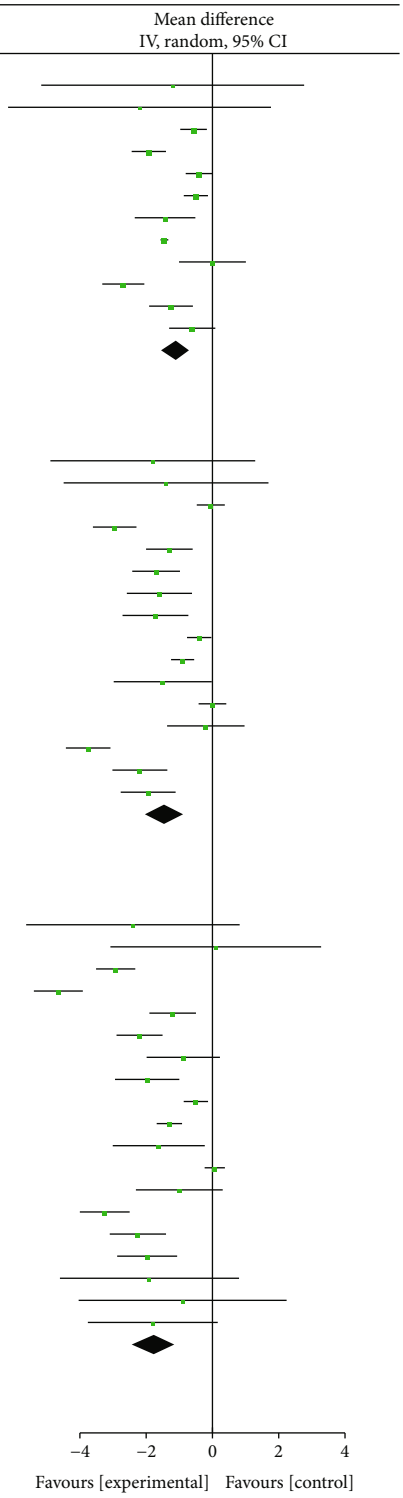

Figure 5: VAS.

month follow-up [-1.19 (-1.92, -0.46), $P = 0.001$], and the effect lasts for at least 12 months [-1.62 (-2.07, -1.16), $P < 0.00001$]. (2) For umbilical cord derived, the results showed that after the MSC injection, VAS had been reduced compared with the control group at the 3rd-month follow-up [-0.83 (-1.43, -0.23), $P = 0.007$], and the effect lasts for at least 12 months [-2.04 (-3.21, -0.86), $P = 0.0007$]. (3) For adipose derived, the results showed that after the MSC injection, VAS had been reduced compared with the control group at the 3rd-month follow-up [-1.50 (-2.01, -0.99), $P < 0.00001$], and the effect lasts for at least 12 months [-1.67 (-3.04, -0.31), $P = 0.02$] (Figure 10).

Study or subgroup	Experimental Mean	SD	Total	Control Mean	SD	Total	Weight	Mean difference IV, random, 95% CI
1.2.1 3 months								
Kuah et al. 2018a	−0.7	2.5	8	−0.2	1.2	2	13.4%	−0.50 [−2.90, 1.90]
Kuah et al. 2018b	−1.1	0.9	8	−0.2	1.2	2	16.1%	−0.90 [−2.68, 0.88]
Lamo-Espinosa et al. 2020	2.3	2.2	24	1.9	1.4	26	19.4%	0.40 [−0.63, 1.43]
Hong et al. 2019	−2.19	1.8	16	1.94	2.49	16	17.4%	−4.13 [−5.64, −2.62]
Zhang et al. 2018a	1.08	1.7	36	2.22	3.18	18	17.1%	−1.14 [−2. 71, 0.43]
Zhang et al. 2018b	1.97	2.38	36	2.22	3.18	18	16.7%	−0.25 [−1.91, 1.41]
Subtotal (95% CI)			128			82	100.0%	−1.09 [−2.52, 0.34]
Heterogeneity: $\tau^2 = 2.46$; $\delta^2 = 24.57$, df = 5 ($P = 0.0002$); $I^2 = 80\%$								
Test for overall effect: $Z = 1.50$ ($P = 0.13$)								
1.2.2 6 months								
Kuah et al. 2018a	−1	0.8	8	−0.2	0.9	2	11.5%	−0.80 [−2.16, 0.56]
Kuah et al. 2018b	−1.2	0.7	8	−0.2	0.9	2	11.6%	−1.00 [−2.34, 0.34]
Matas et al. 2018a	1.4	1.2	9	1.1	1.8	4	9.4%	0.30 [−1.63, 2.23]
Matas et al. 2018b	0.9	0.9	9	1.1	1.8	4	9.7%	−0.20 [−2.06, 1.66]
Lu et al. 2019	−0.69	1.49	26	−0.52	1.26	24	13.6%	−0.17 [−0.93, 0.59]
Lamo-Espinosa et al. 2020	2	1.9	24	2.2	1.6	26	12.9%	−0.20 [−1.18, 0. 78]
Hong et al. 2019	−2.5	1.59	16	2.44	2.56	16	11.1%	−4.94 [−6.42, −3.46]
Zhang et al. 2018a	0.78	1.68	36	2.56	3.56	18	10.1%	−1.78 [−3.51, −0.05]
Zhang et al. 2018b	1.17	2.02	36	2.56	3.56	18	10.0%	−1.39 [−3.16, 0.38]
Subtotal (95% CI)			172			114	100.0%	−1.12 [−2.09, −0.14]
Heterogeneity: $\tau^2 = 1.68$; $\delta^2 = 37.57$, df = 8 ($P < 0.00001$); $I^2 = 79\%$								
Test for overall effect: $Z = 2.23$ ($P = 0.03$)								
1.2.3 12 months								
Kuah at al. 2018a	−0.3	0.5	8	−0.7	0.7	2	12.9%	0.40 [−0.63, 1.43]
Kuah et al. 2018b	−1.2	1.8	8	−0.7	0.7	2	10.7%	−0.50 [−2.08, 1.08]
Matas et al. 2018a	1.7	2.1	9	1.7	1.4	4	9.3%	0.00 [−1.94, 1.94]
Matas et al. 2018b	0.6	0.8	9	1.7	1.4	4	11.2%	−1.10 [−2.57, 0.37]
Lu at al. 2019	−0.67	1.61	26	−0.44	1.26	24	13.7%	−0.23 [−1.03, 0.57]
Lamo-Espinosa et al. 2020	2.1	1.9	24	2.1	1.6	26	13.0%	0.00 [−0.98, 0.98]
Hong et al. 2019	−2.25	2.11	16	2.69	2.57	16	10.5%	−4.94 [−6.57, −3.31]
Zhang et al. 2018a	0.92	2.14	36	2.89	3.77	18	9.6%	−1.97 [−3.85, −0.09]
Zhang et al. 2018b	1.56	2.95	36	2.89	3.77	18	9.2%	−1.33 [−3.32, 0.66]
Subtotal (95% CI)			172			114	100.0%	−0.99 [−1.95, −0.03]
Heterogeneity: $\tau^2 = 1.59$; $\delta^2 = 36.53$, df = 8 ($P < 0.0001$); $I^2 = 78\%$								
Test for overall effect: $Z = 2.01$ ($P = 0.04$)								
Test for subgroup differences: $\delta^2 = 0.04$, df = 2 ($P = 0.98$); $I^2 = 0\%$								

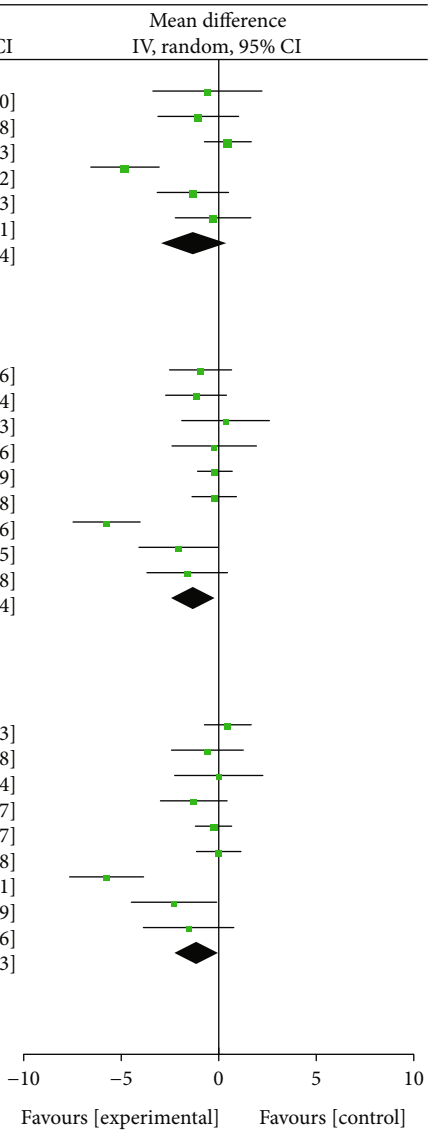

Figure 6: WOMAC stiffness.

3.5.2. Efficacy of Different Cell Sources on Stiffness. The WOMAC stiffness was divided into subgroups according to follow-up time and cell source. The analysis of heterogeneity showed that I^2 > 50% and $P < 0.1$ in almost subgroups, so the random effects model was adopted. (1) For bone marrow derived, only 1 study was involved, and there were no positive findings in this study ($P > 0.05$). (2) For umbilical cord derived, only 1 study was involved, and there were no positive findings in this study ($P > 0.05$). (3) For adipose derived, the results showed that after the MSC injection, WOMAC stiffness had been reduced compared with the control group at the 6th-month follow-up [-1.64 (-3.02, -0.25), $P = 0.02$], while the effect was weakened at the 12th-month follow-up [-1.34 (-2.79, 0.10), $P = 0.07$] (Figure 11).

3.5.3. Efficacy of Different Cell Sources on Physical Function. The WOMAC physical function was divided into subgroups according to follow-up time and cell source. The analysis of heterogeneity showed that I^2 < 50% and $P > 0.1$ in almost subgroups, so the fixed effects model was adopted. (1) For bone marrow derived, the results showed that the results of WOMAC physical function did not improve significantly ($P > 0.05$). (2) For umbilical cord derived, the results showed that after the MSC injection, WOMAC physical function had been reduced compared with the control group at the 6th-month follow-up [-6.06 (-11.42, -0.70), $P = 0.03$], while the effect was weakened at the 12th-month follow-up [-3.52 (-10.47, 3.42), $P = 0.32$]. (3) For adipose derived, the results showed that after the MSC injection, WOMAC stiffness had been reduced compared with the control group at the 6th-month follow-up [-4.55 (-7.59, -1.51), $P = 0.003$], and the effect lasts for at least 12 months [-4.27 (-7.46, -1.08), $P = 0.009$] (Figure 12).

3.6. Adverse Events. Ten RCTs reported the number or frequency of adverse events. The heterogeneity test showed that the heterogeneity was low (I^2 = 15%, $P = 0.28$); hence, the fixed effects model is used for analysis. The results showed

Study or subgroup	Experimental Mean	Experimental SD	Experimental Total	Control Mean	Control SD	Control Total	Weight	Mean difference IV, fixed, 95% CI
1.3.1 3 months								
Kuah et al. 2018a	−4	1	8	−6	10	2	8.6%	2.00 [−11.88, 15.88]
Kuah et al. 2018b	−10	6	8	−6	10	2	7.9%	−4.00 [−18.47, 10.47]
Lamo-Espinosa et al. 2020	17.6	12.6	24	15.5	12.6	26	33.8%	2.10 [−4.89, 9.09]
Zhang et al. 2018a	12.67	12.27	36	19.08	15.7	18	24.1%	−6.41 [−14.70, 1.88]
Zhang et al. 2018b	14.83	10.51	36	19.08	15.7	18	25.7%	−4.25 [−12.27, 3.77]
Subtotal (95% CI)			112			66	100.0%	−2.07 [−6.13, 2.00]
Heterogeneity: $\delta^2 = 3.10$, df = 4 ($P = 0.54$); $I^2 = 0\%$								
Test for overall effect: $Z = 1.00$ ($P = 0.32$)								
1.3.2 6 months								
Kuah et al. 2018a	−5	5	8	−6	7	2	5.6%	1.00 [−9.30, 11.30]
Kuah et al. 2018b	−11	5	8	−6	7	2	5.6%	−5.00 [−15.30, 5.30]
Matas et al. 2018a	10.3	7.2	9	13.3	6.9	4	8.8%	−3.00 [−11.24, 5.24]
Matas et al. 2018b	5	3.1	9	13.3	6.9	4	12.0%	−8.30 [−15.36, −1.24]
Lu et al. 2019	−6.65	7.11	26	−4.32	7.24	24	37.6%	−2.33 [−6.31, 1.65]
Lamo-Espinosa et al. 2020	14.9	11.8	24	16.3	11.1	26	14.7%	−1.40 [−7.76, 4.96]
Zhang et al. 2018a	7.14	8.57	36	21.67	17.17	18	8.4%	−14.53 [−22.94, −6.12]
Zhang et al. 2018b	13.25	13.42	36	21.67	17.17	18	7.3%	−8.42 [−17.48, 0.64]
Subtotal (95% CI)			156			98	100.0%	−4.40 [−6.84, −1.96]
Heterogeneity: $\delta^2 = 10.57$, df = 7 ($P = 0.16$); $I^2 = 34\%$								
Test for overall effect: $Z = 3.53$ ($P = 0.0004$)								
1.3.3 12 months								
Kuah at al. 2018a	−5	5	8	−6	8	2	5.2%	1.00 [−10.62, 12.62]
Kuah et al. 2018b	−8	6	8	−6	8	2	5.0%	−2.00 [−13.84, 9.84]
Matas et al. 2018a	9.5	7.4	9	9.2	9.4	4	6.5%	0.30 [−10.10, 10.70]
Matas et al. 2018b	2.6	2.3	9	9.2	9.4	4	8.1%	−6.60 [−15.93, 2.73]
Lu et al. 2019	−7.04	8.06	26	−4.64	6.41	24	43.4%	−2.40 [−6.42, 1.62]
Lamo-Espinosa et al. 2020	16.7	11.6	24	15.5	11.9	26	16.5%	1.20 [−5.32, 7.72]
Zhang et al. 2018a	8.92	10.31	36	23.67	18.66	18	8.2%	−14.75 [−24.00, −5.50]
Zhang et al. 2018b	14.61	15.17	36	23.67	18.66	18	7.1%	−9.06 [−19.00, 0.88]
Subtotal (95% CI)			156			98	100.0%	−3.26 [−5.91, −0.61]
Heterogeneity: $\delta^2 = 10.71$, df = 7 ($P = 0.15$); $I^2 = 35\%$								
Test for overall effect: $Z = 2.41$ ($P = 0.02$)								
Test for subgroup differences: $\delta^2 = 1.02$, df = 2 ($P = 0.60$); $I^2 = 0\%$								

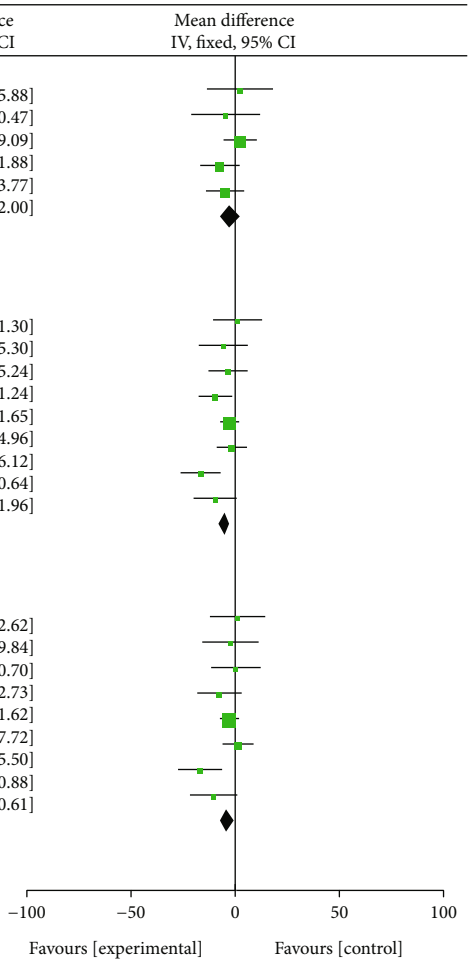

Figure 7: WOMAC physical function.

Study or subgroup	Experimental Mean	Experimental SD	Experimental Total	Control Mean	Control SD	Control Total	Weight	Std. mean difference IV, random, 95% CI
Vega et al. 2015	30	11.62	15	42	19.36	15	39.5%	−0.73 [−1.47, 0.01]
Gan et al. 2014	4.13	1.01	6	17.93	1.27	6	21.2%	−11.10 [−16.64, −5.57]
Tan et al. 2013	5.19	1.12	36	10.42	1.08	36	39.2%	−4.70 [−5.62, −3.79]
Total (95% CI)			**57**			**57**	**100.0%**	**−4.49 [−8.21, −0.77]**
Heterogeneity: $\tau^2 = 8.95$; $\delta^2 = 53.20$, df = 2 ($P < 0.00001$); $I^2 = 96\%$								
Test for overall effect: $Z = 2.37$ ($P = 0.02$)								

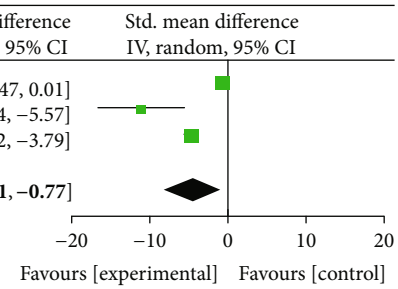

Figure 8: Lequesne.

that there is no significant difference in the safety of MSC injection and the control group [1.20 (0.97, 1.48), $P = 0.09$] (Figure 13). The other RCTs such as Hernigou et al. [29], Lamo-Espinosa et al. [31], Matas et al. [38], Garza et al. [46], Lamo-Espinosa et al. [47] all reported no serious adverse events.

3.7. Publication Bias of Primary Outcomes. The primary outcomes were tested for publication bias, and the results showed that these primary outcomes (12 months) are less likely to have publication bias (WOMAC pain: $P = 0.138$; WOMAC stiffness: $P = 0.142$; WOMAC physical function: $P = 0.536$; adverse events: $P = 0.188$), while VAS may have publication bias ($P = 0.083$) (Figure 14).

3.8. Quality of Evidence. The evidence at 12-month follow-up was judged to be moderate to very low (Table 2). The quality of WOMAC physical function and adverse events were moderate; the quality of WOMAC pain and WOMAC stiffness were moderate; the quality of VAS was very low (Table 2).

Study or subgroup	Experimental Mean	Experimental SD	Experimental Total	Control Mean	Control SD	Control Total	Weight	Mean difference IV, random, 95% CI
2.2.1 Bone marrow-3 months								
Lamo-Espinosa et al. 2020	4.6	3.3	24	4.3	3.3	26	100.0%	0.30 [−1.53, 2.13]
Subtotal (95% Cl)			24			26	100.0%	0.30 [−1.53, 2.13]
Heterogeneity: Not applicable								
Test for overall effect: $Z = 0.32$ ($P = 75$)								
2.2.3 Adipose-3 months								
Kuah et al. 2018a	−1.5	2.5	8	−0.4	3.2	2	16.0%	−1.10 [−5.86, 3.66]
Kuah et al. 2018b	−3.5	2.5	8	−0.4	3.2	2	16.0%	−3.10 [−7.86, 1.66]
Hong et al. 2019	−7.31	3.52	16	2.06	6.84	16	20.5%	−9.37 [−13.14, −5.60]
Zhang et al. 2018a	6.81	4.76	36	11.5	5.94	18	23.9%	−4.69 [−7.84, −1.54]
Zhang et al. 2018b	6.25	5.28	36	11.5	5.94	18	23.4%	−5.25 [−8.49, −2.01]
Subtotal (95% CI)			104			56	100.0%	−4.95 [−7.44, −2.46]
Heterogeneity: $\tau^2 = 4.15$; $\delta^2 = 8.39$, df = 4 ($P = 0.08$); $I^2 = 52\%$								
Test for overall effect: $Z = 3.90$ ($P < 0.0001$)								
2.2.4 Bone marrow-6 months								
Lamo-Espinosa et al. 2020	4.3	3.6	24	4.4	3	26	100.0%	−0.10 [−1.95, 1.75]
Subtotal (95% Cl)			24			26	100.0%	−0.10 [−1.95, 1.75]
Heterogeneity: Not applicable								
Test for overall effect: $Z = 0.11$ ($P = 0.92$)								
2.2.5 Umbilical cord-6 months								
Matas et al. 2018a	2.1	1.4	9	4.2	3.8	4	50.7%	−2.10 [−5.93, 1. 73]
Matas et al. 2018b	2.4	1.7	9	4.2	3.8	4	49.3%	−1.80 [−5.69, 2.09]
Subtotal (95% Cl)			18			8	100.0%	−1.95 [−4.68, 0.78]
Heterogeneity: $\tau^2 = 0.00$; $\delta^2 = 0.01$, df = 1 ($P = 0.91$); $I^2 = 0\%$								
Test for overall effect: $Z = 1.40$ ($P = 0.16$)								
2.2.6 Adipose-6 months								
Kuah et al. 2018a	−1.8	1.8	8	−1	2.5	2	12.2%	−0.80 [−4.48, 2.88]
Kuah et al. 2018b	−3.4	1.7	8	−1	2.5	2	12.2%	−2.40 [−6.06, 1.26]
Lu at al. 2019	−2.62	2.21	26	−1.48	1.53	24	14.4%	−1.14 [−2.19, −0.09]
Hong et al. 2019	−7.94	3.84	16	3.38	5.73	16	12.5%	−11.32 [−14.70, −7.94]
Zhang et al. 2018a	4.17	5.55	36	12.36	6.92	18	12.2%	−8.19 [−11.87, −4.51]
Zhang et al. 2018a	4.17	5.55	36	12.36	6.92	18	12.2%	−8.19 [−11.87, −4.51]
Zhang et al. 2018b	4.78	5.91	36	12.36	6.92	18	12.1%	−7.58 [−11.31, −3.85]
Zhang et al. 2018b	4.78	5.91	36	12.36	6.92	18	12.1%	−7.58 [−11.31, −3.85]
Subtotal (95% CI)			202			116	100.0%	−5.81 [−8.96, −2.66]
Heterogeneity: $\tau^2 = 17.66$; $\delta^2 = 63.59$, df = 7 ($P < 0.00001$); $I^2 = 89\%$								
Test for overall effect: $Z = 3.61$ ($P = 0.0003$)								
2.2.7 Bone marrow-12 months								
Lamo-Espinosa et al. 2020	4.1	3.6	24	4.5	3.2	26	100.0%	−0.40 [−2.29, 1.49]
Subtotal (95% Cl)			24			26	100.0%	−0.40 [−2.29, 1.49]
Heterogeneity: Not applicable								
Test for overall effect: $Z = 0.41$ ($P = 0.68$)								
2.2.8 Umbilical cord-12 months								
Matas et al. 2018a	3.7	2.6	9	4.3	3.5	4	46.0%	−0.60 [−4.43, 3.23]
Matas et al. 2018b	1.1	1.3	9	4.3	3.5	4	54.0%	−3.20 [−6.73, 0.33]
Subtotal (95% Cl)			18			8	100.0%	−2.00 [−4.60, 0.59]
Heterogeneity: $\tau^2 = 0.00$; $\delta^2 = 0.96$, df = 1 ($P = 0.33$); $I^2 = 0\%$								
Test for overall effect: $Z = 1.51$ ($P = 0.13$)								
2.2.9 Adipose-12 months								
Kuah et al. 2018a	−3.5	2	8	−0.4	2.4	2	16.4%	−3.10 [−6.70, 0.50]
Kuah et al. 2018b	−1.2	1.7	8	−0.4	2.4	2	16.5%	−0.80 [−4.33, 2.73]
Lu et al. 2019	−2.63	2.36	26	−1.44	1.85	24	18.2%	−1.19 [−2.36, −0.02]
Hong et al. 2019	−8	4.77	16	5.69	4.29	16	16.8%	−13.69 [−16.83, −10.55]
Zhang et al. 2018a	5.08	6.02	36	13.61	7.3	18	16.1%	−8.53 [−12.43, −4.63]
Zhang et al. 2018b	5.53	6.34	36	13.61	7.3	18	16.0%	−8.08 [−12.04, −4.12]
Subtotal (95% CI)			130			80	100.0%	−5.83 [−10.21, −1.45]
Heterogeneity: $\tau^2 = 27.10$; $\delta^2 = 68.87$, df = 5 ($P < 0.00001$); $I^2 = 93\%$								
Test for overall effect: $Z = 2.61$ ($P = 0.009$)								
Test for subgroup differences: $\delta^2 = 25.77$. df = 7 ($P = 0.0006$); $I^2 = 72.8\%$								

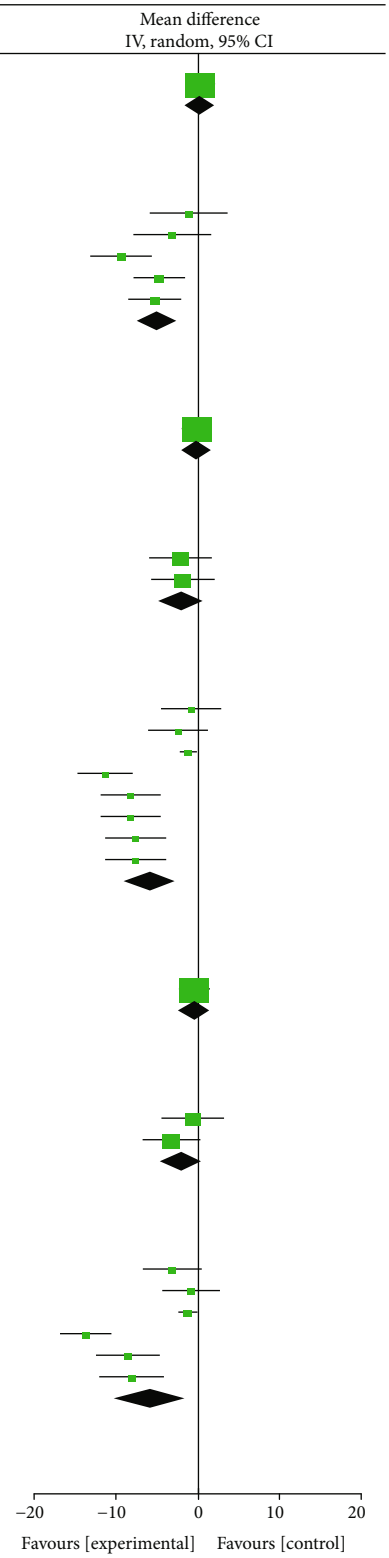

Figure 9: WOMAC pain-different cell sources.

4. Discussion

This systematic review and meta-analysis included 28 RCTs involving 1494 participants. In general, intra-articular injection of MSCs may relieve pain (reduce WOMAC pain and VAS) and joint stiffness (reduce WOMAC stiffness) and improve joint function (reduce WOMAC physical function). MSCs may also improve knee arthritis (decrease Lequesne). From the time point of view, the relief of pain by MSCs begins at most the third month after its injection, and the

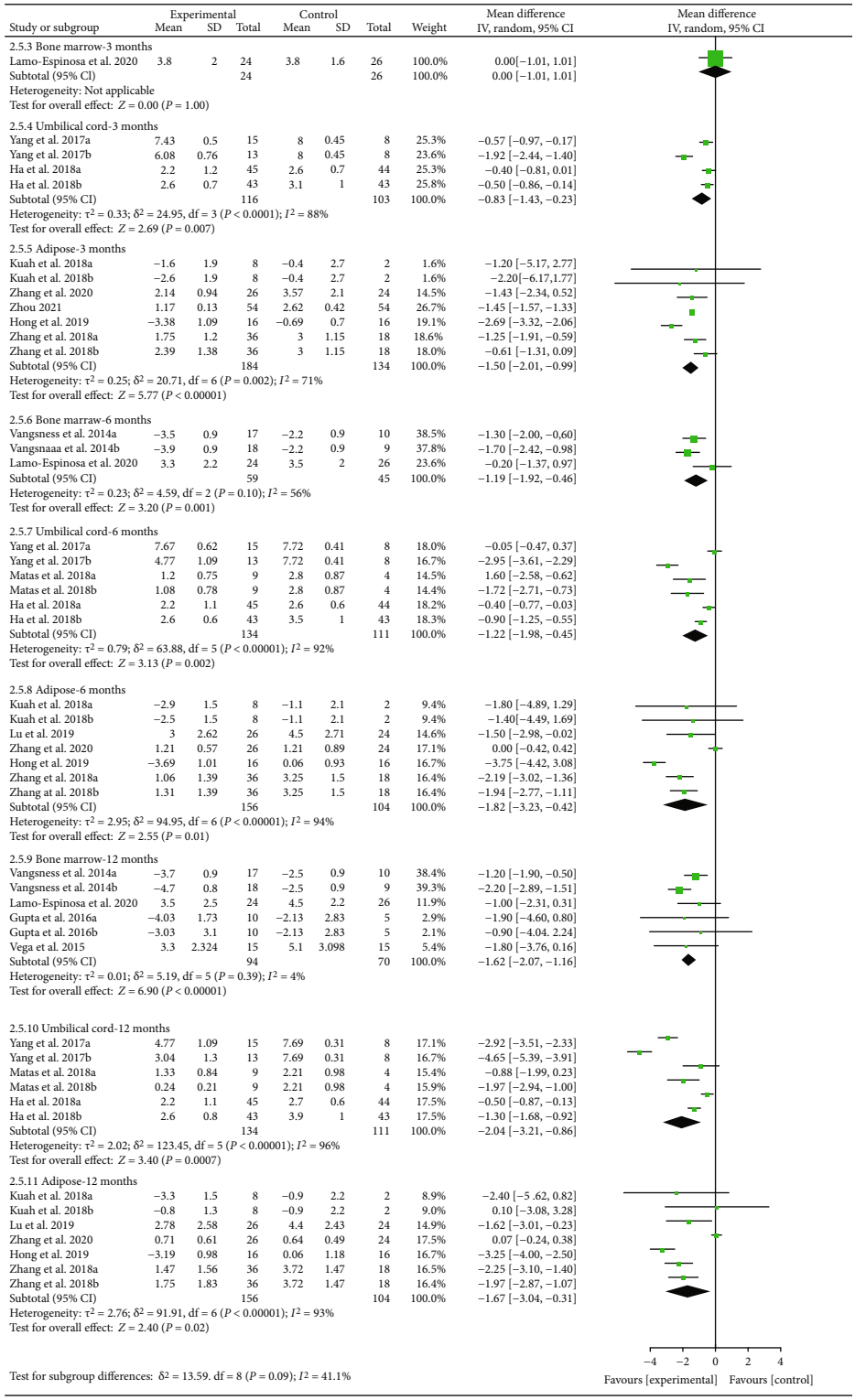

Figure 10: VAS-different cell sources.

effective time lasts for at least 12 months. The improvement of MSCs on stiffness and physical function starts at most 6 months after injection, and the effective time lasts for at least 12 months. Based on MCID, the changes of WOMAC pain, WOMAC stiffness, WOMAC physical function, and VAS have clinical significance. The WOMAC score scale can effectively reflect the condition of patients before and after treatment, such as the degree of satisfaction of patients, and has high reliability for the assessment of OA. VAS is more sensitive and comparable and can reflect the pain level of patients. The improvement of these results is clinically meaningful, suggesting that MSCs transplantation may be

Study or subgroup	Experimental Mean	Experimental SD	Experimental Total	Control Mean	Control SD	Control Total	Weight	Mean difference IV, random, 95% CI
2.3.4 Bone marrow-3 months								
Lamo-Espinosa et al. 2020	2.3	2.2	24	1.9	1.4	26	100.0%	0.40 [−0.63, 1.43]
Subtotal (95% CI)			24			26	100.0%	0.40 [−0.63, 1.43]
Heterogeneity: Not applicable								
Test for overall effect: $Z = 0.76$ ($P = 0.45$)								
2.3.5 Adipose-3 months								
Kuah et al. 2018a	−0.7	2.5	8	−0.2	1.2	2	16.3%	−0.50 [−2.90, 1.90]
Kuah et al. 2018b	−1.1	0.9	8	−0.2	1.2	2	20.0%	−0.90 [−2.68, 0.88]
Hong et al. 2019	−2.19	1.8	16	1.94	2.49	16	21.7%	−4.13 [−5.64, −2.62]
Zhang et al. 2018a	1.08	1.7	36	2.22	3.18	18	21.3%	−1.14 [−2. 71, 0.43]
Zhang et al. 2018b	1.97	2.38	36	2.22	3.18	18	20.7%	−0.25 [−1.91, 1.41]
Subtotal (95% CI)			104			56	100.0%	−1.45 [−2.97, 0.06]
Heterogeneity: $\tau^2 = 2.17$; $\delta^2 = 15.11$, df = 4 ($P = 0.004$); $I^2 = 74\%$								
Test for overall effect: $Z = 1.88$ ($P = 0.06$)								
2.3.6 Bone marrow-6 months								
Lamo-Espinosa et al. 2020	2	1.9	24	2.2	1.6	26	100.0%	−0.20 [−1.18, 0.78]
Subtotal (95% CI)			24			26	100.0%	−0.20 [−1.18, 0.78]
Heterogeneity: Not applicable								
Test for overall effect: $Z = 0.40$ ($P = 0.69$)								
2.3.7 Umbilical cord-6 months								
Matas et al. 2018a	1.4	1.2	9	1.1	1.8	4	48.1%	0.30 [−1.63, 2.23]
Matas et al. 2018b	0.9	0.9	9	1.1	1.8	4	51.9%	−0.20 [−2.06, 1.66]
Subtotal (95% CI)			18			8	100.0%	0.04 [−1.30, 1.38]
Heterogeneity: $\tau^2 = 0.00$; $\delta^2 = 13$, df = 1 ($P = 0.71$); $I^2 = 0\%$								
Test for overall effect: $Z = 0.06$ ($P = 0.95$)								
2.3.8 Adipose-6 months								
Kuah et al. 2018a	−1	0.8	8	−0.2	0.9	2	16.9%	−0.80 [−2.16, 0.56]
Kuah et al. 2018b	−1.2	0.7	8	−0.2	0.9	2	17.0%	−1.00 [−2.34, 0.34]
Lu et al. 2019	−0.69	1.49	26	−0.52	1.26	24	19.1%	−0.17 [−0.93, 0.59]
Hong et al. 2019	−2.5	1.59	16	2.44	2.56	16	16.4%	−4.94 [−6.42, −3.46]
Zhang et al. 2018a	0.78	1.68	36	2.56	3.56	18	15.4%	−1.78 [−3.51, −0.05]
Zhang et al. 2018b	1.17	2.02	36	2.56	3.56	18	15.2%	−1.39 [−3.16, 0.38]
Subtotal (95% CI)			130			80	100.0%	−1.64 [−3.02, −0.25]
Heterogeneity: $\tau^2 = 2.46$; $\delta^2 = 32.52$, df = 5 ($P < 0.00001$); $I^2 = 85\%$								
Test for overall effect: $Z = 2.32$ ($P = 0.02$)								
2.3.9 Bone marrow-12 months								
Lamo-Espinosa et al. 2020	2.1	1.9	24	2.1	1.6	26	100.0%	−0.00 [−0.98, 0.98]
Subtotal (95% CI)			24			26	100.0%	−0.00 [−0.98, 0.98]
Heterogeneity: Not applicable								
Test for overall effect: $Z = 0.00$ ($P = 1.00$)								
2.3.10 Umbilical cord-12 months								
Matas et al. 2018a	1.7	2.1	9	1.7	1.4	4	36.4%	0.00 [−1.94, 1.94]
Matas et al. 2018b	0.6	0.8	9	1.7	1.4	4	63.6%	−1.10 [−2.57, 0.37]
Subtotal (95% CI)			18			8	100.0%	−0.70 [−1.87, 0.47]
Heterogeneity: $\tau^2 = 0.00$; $\delta^2 = 0.79$, df = 1 ($P = 0.38$); $I^2 = 0\%$								
Test for overall effect: $Z = 1.17$ ($P = 0.24$)								
2.3.11 Adipose-12 months								
Kuah et al. 2018a	−0.3	0.5	8	−0.7	0.7	2	18.5%	0.40 [−0.63, 1.43]
Kuah et al. 2018b	−1.2	1.8	8	−0.7	0.7	2	16.4%	−0.50 [−2.08, 1.08]
Lu et al. 2019	−0.67	1.61	26	−0.44	1.26	24	19.2%	−0.23 [−1.03, 0.57]
Hong et al. 2019	−2.25	2.11	16	2.69	2.57	16	16.2%	−4.94 [−6.57, −3.31]
Zhang et al. 2018a	0.92	2.14	36	2.89	3.77	18	15.1%	−1.97 [−3.85, −0.09]
Zhang et al. 2018b	1.56	2.95	36	2.89	3.77	18	14.7%	−1.33 [−3.32, 0.66]
Subtotal (95% CI)			130			80	100.0%	−1.34 [−2.79, 0.10]
Heterogeneity: $\tau^2 = 2.65$; $\delta^2 = 33.86$, df = 5 ($P < 0.00001$); $I^2 = 85\%$								
Test for overall effect: $Z = 1.83$ ($P = 0.07$)								
Test for subgroup differences: $\delta^2 = 10.33$. df = 7 ($P = 0.17$). $I^2 = 32.2\%$								

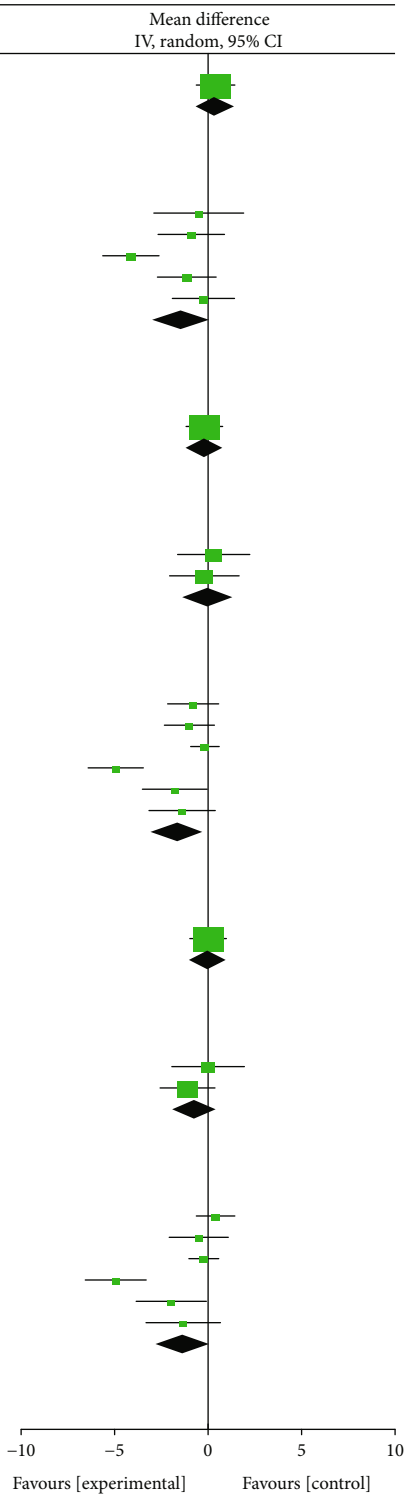

Figure 11: WOMAC stiffness-different cell sources.

an effective regimen for the treatment of OA. From a cellular point of view, (1) regarding pain, existing studies have shown that bone marrow-derived stem cells begin to take effect at least 12 months after injection, while umbilical cord and adipose derived cells begin to take effect up to 3 months after injection, and the effect lasts at least 12 months. (2) Regarding stiffness, adipose-derived cells begin to take effect up to 6 months after injection, and the effect lasts at least 12 months, while the bone marrow-derived and umbilical cord-derived cells did not show obvious effect. (3) Regarding physical function, umbilical cord and adipose-derived cells begin to take effect up to 6 months after injection, and the

Study or subgroup	Experimental Mean	Experimental SD	Experimental Total	Control Mean	Control SD	Control Total	Weight	Mean difference IV, fixed, 95% CI
2.4.4 Bone marrow-3 months								
Lamo-Espinosa et al. 2020	17.6	12.6	24	15.5	12.6	26	100.0%	2.10 [−4.89, 9.09]
Subtotal (95% CI)			24			26	100.0%	2.10 [−4.89, 9.09]
Heterogeneity: Not applicable								
Test for overall effect: $Z = 0.59$ ($P = 0.56$)								
2.4.5 Adipose-3 months								
Kuah et al. 2018a	−4	1	8	−6	10	2	13.0%	2.00 [−11.88, 15.88]
Kuah et al. 2018b	−10	6	8	−6	10	2	11.9%	−4.00 [−18.47, 10.47]
Zhang et al. 2018a	12.67	12.27	36	19.08	15.7	18	36.3%	−6.41 [−14.70, 1.88]
Zhang et al. 2018b	14.83	10.51	36	19.08	15.7	18	38.8%	−4.25 [−12.27, 3.77]
Subtotal (95% CI)			88			40	100.0%	−4.20 [−9.19, 0.80]
Heterogeneity: $\delta^2 = 1.04$, df = 3 ($P = 0.79$); $I^2 = 0\%$								
Test for overall effect: $Z = 1.65$ ($P = 0.10$)								
2.4.6 Bone marrow-6 months								
Lamo-Espinosa et al. 2020	14.9	11.8	24	16.3	11.1	26	100.0%	−1.40 [−7.76, 4.96]
Subtotal (95% CI)			24			26	100.0%	−1.40 [−7.76, 4.96]
Heterogeneity: Not applicable								
Test for overall effect: $Z = 0.43$ ($P = 0.67$)								
2.4.7 Umbilical cord-6 months								
Matas et al. 2018a	10.3	7.2	9	13.3	6.9	4	42.3%	−3.00 [−11.24, 5.24]
Matas et al. 2018b	5	3.1	9	13.3	6.9	4	57.7%	−8.30 [−15.36, −1.24]
Subtotal (95% CI)			18			8	100.0%	−6.06 [−11.42, 0.70]
Heterogeneity: $\delta^2 = 0.92$, df = 1 ($P = 0.34$); $I^2 = 0\%$								
Test for overall effect: $Z = 2.21$ ($P = 0.03$)								
2.3.8 Adipose-6 months								
Kuah et al. 2018a	−5	5	8	−6	7	2	8.7%	1.00 [−9.30, 11.30]
Kuah et al. 2018b	−11	5	8	−6	7	2	8.7%	−5.00 [−15.30, 5.30]
Lu et al. 2019	−6.65	7.11	26	−4.32	7.24	24	58.3%	−2.33 [−6.31, 1.65]
Zhang et al. 2018a	7.14	8.57	36	21.67	17.17	18	13.1%	−14.53 [−22.94, −6.12]
Zhang et al. 2018b	13.25	13.42	36	21.67	17.17	18	11.3%	−8.42 [−17.48, 0.64]
Subtotal (95% CI)			114			64	100.0%	−4.55 [−7.59, −1.51]
Heterogeneity: $\delta^2 = 8.42$, df = 4 ($P = 0.08$); $I^2 = 53\%$								
Test for overall effect: $Z = 2.93$ ($P = 0.003$)								
2.4.9 Bone marrow-12 months								
Lamo-Espinosa et al. 2020	16.7	11.6	24	15.5	11.9	26	100.0%	1.20 [−5.32, 7.72]
Subtotal (95% CI)			24			26	100.0%	1.20 [−5.32, 7.72]
Heterogeneity: Not applicable								
Test for overall effect: $Z = 0.36$ ($P = 0.72$)								
2.4.10 Umbilical cord-12 months								
Matas et al. 2018a	9.5	7.4	9	9.2	9.4	4	44.6%	0.30 [−10.10, 10.70]
Matas et al. 2018a	2.6	2.3	9	9.2	9.4	4	55.4%	−6.60 [−15.93, 2.73]
Subtotal (95% CI)			18			8	100.0%	−3.52 [−10.47, 3.42]
Heterogeneity: $\delta^2 = 0.94$, df = 1 ($P = 0.33$); $I^2 = 0\%$								
Test for overall effect: $Z = 0.99$ ($P = 0.32$)								
2.4.11 Adipose-12 months								
Kuah et al. 2018a	−5	5	8	−6	8	2	7.6%	1.00 [−1 0.62, 12.62]
Kuah et al. 2018b	−8	6	8	−6	8	2	7.3%	−2.00 [−13.84, 9.84]
Lu et al. 2019	−7.04	8.06	26	−4.64	6.41	24	63.0%	−2.40 [−6.42, 1.62]
Zhang et al. 2018a	8.92	10.31	36	23.67	18.66	18	11.9%	−14.75 [−24.00, −5.50]
Zhang et al. 2018b	14.61	15.17	36	23.67	18.66	18	10.3%	−9.06 [−19.00, 0.88]
Subtotal (95% CI)			114			64	100.0%	−4.27 [−7.46, −1.08]
Heterogeneity: $\delta^2 = 7.58$, df = 4 ($P = 0.11$); $I^2 = 47\%$								
Test for overall effect: $Z = 2.62$ ($P = 0.009$)								
Test for subgroup differences: $\delta^2 = 6.50$. df = 7 ($P = 0.48$); $I^2 = 0\%$								

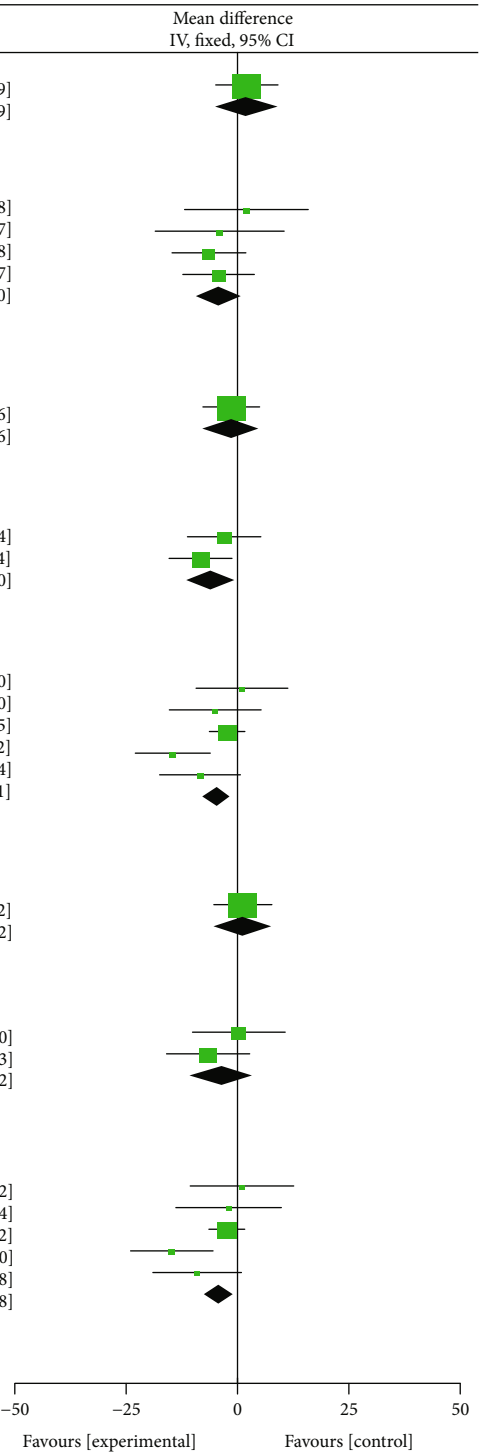

Figure 12: WOMAC physical function-different cell sources.

effect lasts at least 12 months; while bone marrow-derived cells did not show obvious effect. Safety studies have shown that the adverse events of intra-articular injection of MSCs are similar to those of the control group. It could be considered that the addition of MSCs would not increase the incidence of adverse events.

The quality of evidence assessments shows that the qualities of WOMAC physical function and adverse events were moderate; the qualities of WOMAC pain and WOMAC stiffness were moderate; the quality of VAS was very low. However, because there are few studies related to umbilical cord-derived cells (only 1 RCT reported extractable

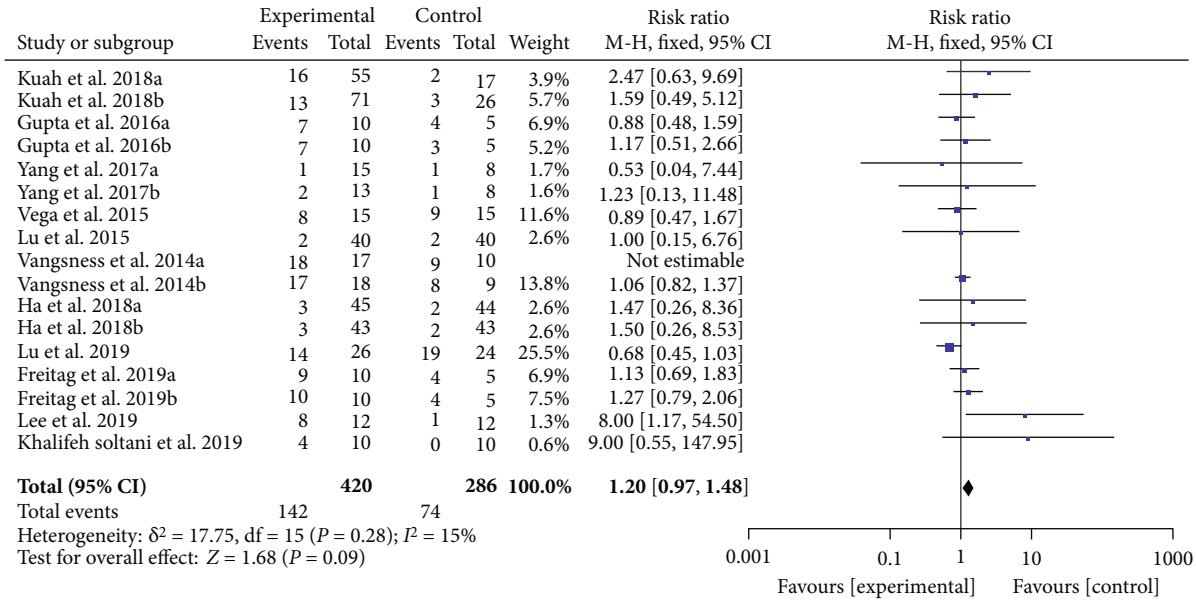

Figure 13: Adverse events.

WOMAC data), more studies on umbilical cord-derived MSCs are needed in the future. In addition, since most of the data reported by RCTs are between 3 months and 12 months, it is impossible to compare the efficacy of 3 months before and after 12 months. Therefore, based on the current evidence, we can only speculate that the onset time of MSCs therapy is no later than 3 months, and the duration of the effect is no earlier than 12 months. More follow-up data are needed in the future to further revise the conclusions.

The dose of RCTs included in this study is basically between 1 ∗ 10^7 and 1 ∗ 10^8. The clinical data of "Stem Cell Translational Medicine" showed that the Chilean research team used double-dose UC-MSCs to treat knee arthritis more effectively than the single-dose group (cell volume: 2∗10^7). Regardless of single-dose or double-dose treatment, the therapeutic effect of the MSC group was better than that of the hyaluronic acid control group. Only patients treated with MSC had significant improvement in pain and knee joint function (WOMAC-A score). And during the 12th-month follow-up period, no serious adverse events occurred [38]. Another study reported that 12 patients with moderate/severe KOA aged 45-65 received different doses of MSC treatment. The injection doses of the 3 groups were 1 ∗ 10^6, 1 ∗ 10^7, and 5 ∗ 10^7. After 12 months, the pain level and quality of life of all patients have been significantly improved, and at all tested doses, MSC injection is safe, and the test results show that the higher the dose of MSC, the better the effect [59]. In addition, the researchers believe that the number of stem cells used is also important for cartilage regeneration. Jo and other Korean researchers injected adipose MSCs into 18 patients with knee osteoarthritis and divided the patients into a low-dose group (1.0 ∗ 10^7), a medium-dose group (5.0 ∗ 10^7) and a high-dose group (1.0 ∗ 10^8). Studies have shown that the three groups can improve knee joint function and relieve knee pain within 2 years, but only the high-dose group has a statistically significant clinical improvement within 2 years, and the clinical improvement of the middle and low-dose group tends to degenerate after 1 year [60].

Regarding cell sources, the included RCTs mainly involve bone marrow, umbilical cord, adipose, and placenta-derived MSCs. Current research shows that MSCs are easy to accept gene modification, have anti-immune ability, and have strong self-renewal ability [61]. Migliorini et al. found that patients who received bone marrow MSCs (BMSCs) treatment in the early degenerative stage had a good prognosis, significantly improved joint pain and functional scores, and greatly improved the quality of life and recreational activities [62]. A 4-month follow-up study showed that MSCs were effective and safe in the treatment of knee arthritis [63]. In addition, MSCs still have good clinical efficacy and safety in the treatment of patients with mild or moderate knee OA [64]. In the research on the mechanism of BMSCs promoting OA repair, firstly, under certain in vitro induction conditions, BMSCs can differentiate into a variety of cells. Common methods include dexamethasone, sodium α-glycerophosphate, and other small molecules to induce MSCs to differentiate into osteoblasts/chondrocytes. Secondly, exosomes (various noncoding RNAs and cytokines, etc.) secreted by BMSCs can promote the repair of osteoarthritis (such as promoting cartilage repair and inhibiting inflammation) [65]. Meanwhile, BMSCs transplantation has many advantages, mainly in the simplicity of acquisition and the value of isolation and culture, easy expansion, and high differentiation potential [66]. However, studies have shown that the differentiation and proliferation ability of BMSCs is unstable during the culture process [67], and the process of extracting bone marrow is traumatic. Some studies compared human cord blood MSCs and BMSCs in vitro induced culture expansion and differentiation potential. They found that both worked well in osteoblast differentiation capacity and could be transplanted as

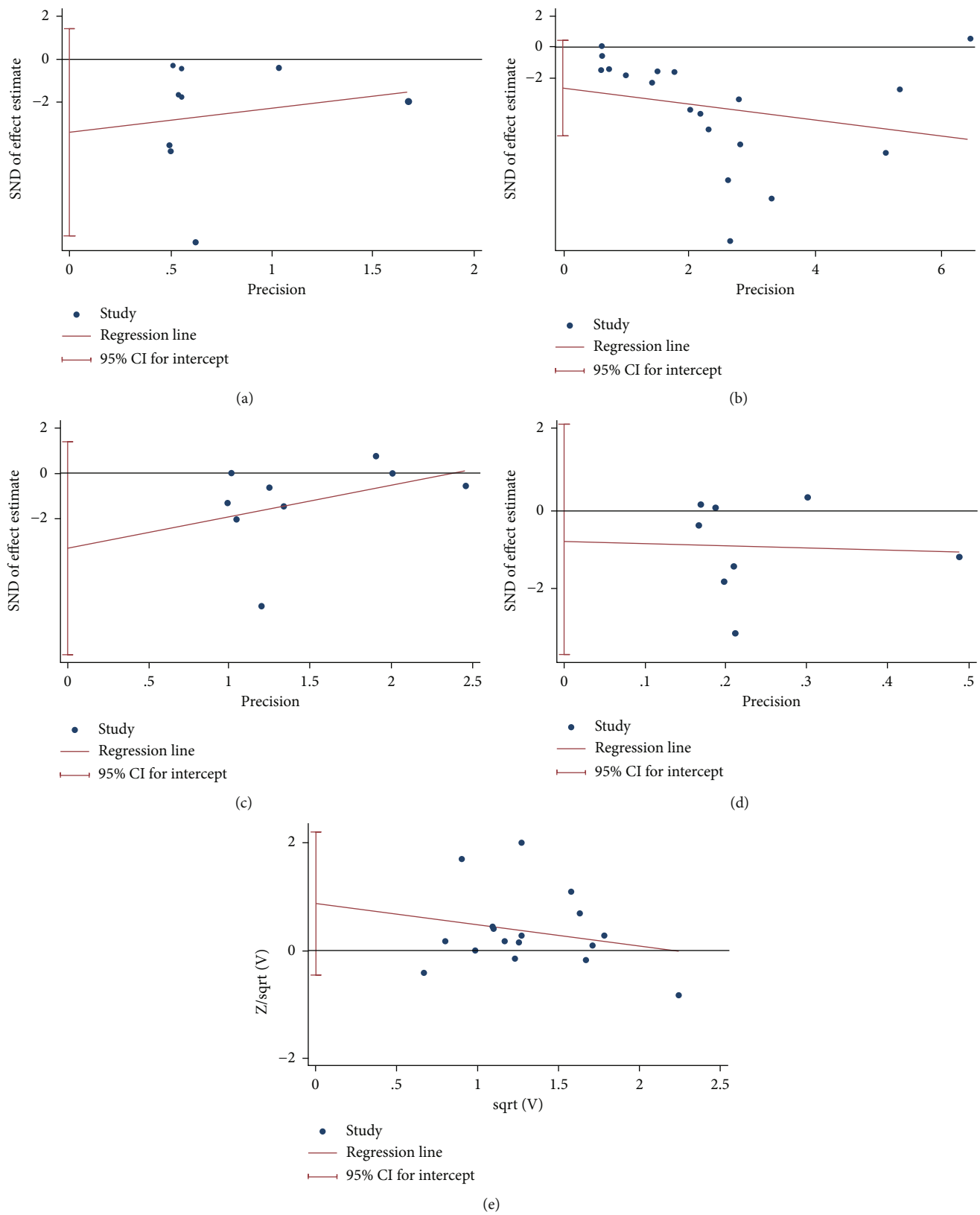

Figure 14: Publication bias: (a) WOMAC pain; (b) VAS; (c) WOMAC stiffness; (d) WOMAC physical function; (e) adverse events.

Table 2: Quality of evidence.

Outcomes	Illustrative comparative risks∗(95% CI) Assumed risk **Control**	Corresponding risk **Adverse event**	**Relative effect (95% CI)**	**No of participants (studies)**	**Quality of the evidence (GRADE)**	**Comments**
WOMAC pain-12 months		The mean WOMAC pain-12 months in the intervention groups was 4.29 lower (7.12 to 1.47 lower)		286 (9 studies)	⊕⊕⊝⊝ low[1,2]	
VAS-12 months		The mean VAS-12 months in the intervention groups was 1.77 lower (2.43 to 1.12 lower)		669 (19 studies)	⊕⊝⊝⊝ very low[1,2,3]	
WOMAC stiffness-12 months		The mean WOMAC stiffness-12 months in the intervention groups was 0.99 lower (1.95 to 0.03 lower)		286 (9 studies)	⊕⊕⊝⊝ low[1,2]	
WOMAC physical function-12 months		The mean WOMAC physical function-12 months in the intervention groups was 3.26 lower (5.91 to 0.61 lower)		254 (8 studies)	⊕⊕⊕⊝ moderate[1]	
Adverse events	259 per 1000	Study population 310 per 1000 (251 to 383)	RR 1.2 (0.97 to 1.48)	706 (17 studies)	⊕⊕⊕⊝ moderate[1]	
	125 per 1000	Moderate 150 per 1000 (121 to 185)				

∗The basis for the assumed risk (e.g. the median control group risk across studies) is provided in footnotes. The corresponding risk (and its 95% confidence interval) is based on the assumed risk in the comparison group and the relative effect of the intervention (and its 95% CI). CI: confidence interval; RR: risk ratio; GRADE Working Group grades of evidence. High quality: Further research is very unlikely to change our confidence in the estimate of effect. Moderate quality: Further research is likely to have an important impact on our confidence in the estimate of effect and may change the estimate. Low quality: Further research is very likely to have an important impact on our confidence in the estimate of effect and is likely to change the estimate. Very low quality: We are very uncertain about the estimate. [1]Downgraded one level due to serious risk of bias (random sequence generation, allocation concealment, blinding, and incomplete outcomes), and most of the data comes from the RCTs with moderate risk of bias. [2]Downgraded one level due to the probably substantial heterogeneity. [3]Downgraded one level due to the potential publication bias.

seed cells in the treatment of OA [68, 69]. BMSC has a strong osteogenic potential and has a certain effect on the treatment of OA, but the amount of MSCs in adult bone marrow is small; and due to the limitation of age, the sources of BMSCs are limited, and the clinical effects of BMSCs from different donors are also different [70]. A research found that there is a kind of bone marrow concentrate (BMAC), which shows a good application prospect in the treatment of OA [71]. MSCs in BMAC are rich in a variety of exosomes and paracrine cell growth factors, and in addition to their good repairing effects, they also have immunomodulatory effects, which have potential value for improving the clinical application of OA and regenerative medicine.

Adipose-derived stem cell (ADSC) adipose tissue is also an important source of MSCs. Current studies have shown that ADSCs have the potential to differentiate into mesoderm-derived cells, such as bone/chondrocytes, adipocytes, and muscle cells [72, 73]. A larger RCT involving 110 patients with OA found that microadipose tissue-derived cells and bone marrow concentrate-derived cell injections in patients with knee OA can significantly improve pain and function and thus improve the clinical symptoms of patients. This suggests that cells from these two tissues have a good effect in ameliorating OA, with no significant difference between the two [74]. In addition, ADSCs have many clinical advantages over BMSCs in view of the current preparation and collection of MSCs. For example, ADSC is more convenient and convenient in material acquisition, without ethical restrictions, and has the advantages of strong in vitro expansion ability, low culture difficulty, and strong ability to differentiate into chondrocytes. Therefore, given these advantages, ADSCs are expected to be used in the treatment of OA in the future. Despite the above advantages, the potential clinical problems in the treatment of OA still need to be solved in the future, such as the ability of ADSCs to induce osteogenic differentiation, and its mechanism still need to be further elucidated; in addition, the transplantation of ADSCs into the patient's body requires a material scaffold adapted to human biology as a transplant carrier [75]. Nasb et al. first combined low-intensity pulsed ultrasound and ADSC in the treatment of knee joint OA [76]. The results of this study show that the combination of ADSCs with low-intensity pulsed ultrasound can significantly improve the clinical effect of treatment compared with the comparison of transplantation of ADSCs alone, and the safety is also better than that of transplantation alone. This also provides a reference value for subsequent clinical trials. With the in-depth research on the regulatory mechanism and safety of ADSCs in the future, the treatment of OA with ADSCs will have broader clinical application prospects [76].

A study also reported the therapeutic progress of human cord blood-derived mesenchymal stem cells (hUCB-MSCs) in OA. It is mainly isolated and cultured from umbilical cord blood, and at the same time, it expresses mesenchymal characteristic markers and can be differentiated into bone/cartilage/adipocytes, indicating that Huck-MSCs have multidirectional differentiation potential and high plasticity [77]. Park et al. also used a mixture of cord blood mesenchymal stem cells and hyaluronic acid to repair a woman with knee cartilage injury. One year after the operation, it was found that knee joint function and pain were significantly relieved, and imaging confirmed that there was a normal shape of new cartilage [78]. Song et al. investigated OA patients receiving allogeneic umbilical cord blood mesenchymal stem cell therapy. They were followed up for two years, and the clinical symptoms and quality of life of the patients at the initial 1 and 2 years were significantly improved, and there were no adverse reactions or complications. After at least 2 years of follow-up, hUCB-MSCs implantation is effective in treating knee osteoarthritis [79]. Park et al. followed up to 7 years in patients with OA who were treated with the allogeneic hUCB-MSCs and hyaluronic acid hydrogel complex. In this process, 7 patients underwent treatment were evaluated by arthroscopy and NMR. As a result, none of the 7 patients had adverse reactions, and the symptoms of OA were effectively improved, which proved the effectiveness and safety of hUCB-MSCs [80].

In recent years, clinical research results of using placental MSCs to treat OA have been reported. There is a recent clinical trial for the treatment of knee OA through intra-articular injection of placental MSCs (Trial registration number: IRCT2015101823298N). Twenty patients with symptomatic knee OA were randomly divided into two groups and injected with placental mesenchymal stem cells or saline, respectively. The results showed that the quality of life, daily activities, and exercise of the placental mesenchymal stem cell injection group were significantly improved, and the symptoms of OA were significantly reduced [50].

The advantages of this research are as follows: this systematic review and meta-analysis explores the potential of MSCs as a safe treatment for OA. Compared with previous systematic reviews and meta-analyses [11–18], this meta-analysis conducted a subgroup analysis of RCTs according to source and follow-up time and preliminarily summarized the onset time, duration of efficacy, and tissue origin of MSCs treatment. This meta-analysis also adopted a more stringent risk of bias assessment tool (RoB2) and introduced MCID and found that the efficacy of MSCs may be clinically meaningful.

The limitations of this research were as follows: (1) there are few RCTs involved in some outcomes (such as the WOMAC pain-bone marrow subgroup) so that reliable conclusions cannot be drawn, and more RCTs reporting these outcomes are needed in the future. (2) Due to the source and specific culture conditions of MSCs, the injection site, the injection dose, and the differences between the countries and regions where the research was conducted and the patients included in the research, the RCTs have high heterogeneity and potential risk of bias. This in turn affects the generalization of clinical evidence for the findings. In future clinical trials, more accurate clinical conclusions can be drawn through higher quality study designs. (3) The included RCTs are mainly in Chinese and English, and RCTs in other languages may not be included. Therefore, RCTs reported in other languages may be considered in the future to provide better reference information for clinical

treatment. (4) The follow-up time of RCTs is more than 3 months and less than 12 months, resulting in the failure to evaluate the results within 3 months and beyond 12 months. Therefore, RCTs with longer follow-up time and follow-up at more time points are needed in the future.

5. Conclusion

At present, OA still seriously threatens human health and quality of life. The development of regenerative medicine and innovative stem cell technology provides a unique opportunity to treat this disease. This systematic review collects RCTs of all types of stem cells to treat OA, in order to make a comprehensive summary, description, and characteristic analysis of these studies. This study shows that MSCs may have a good curative effect in the treatment of OA, the onset time is no later than 3 months, and the time to maintain the curative effect is no less than 12 months. In addition, based on current evidence, it can be considered that ADSC may have a better efficacy because of its early onset of action and a longer duration of efficacy. Regarding safety, MSCs may be considered a safe therapy. However, these results should be generalized with caution due to the generally low quality of evidence and RCTs.

Glossary

RCT: Randomized controlled trial
TCM: Traditional Chinese medicine
RR: Risk ratio
CI: Confidence interval.

Data Availability

All data generated or analyzed during this study are included in this published article.

Conflicts of Interest

We declare no competing interests.

Authors' Contributions

Zhiyong Long and Tianqing Zhang contributed equally to this work.

Supplementary Materials

PRISMA 2020 checklist: Checklist; Table S1: search Strategies for PubMed and Embase. *(Supplementary Materials)*

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Research Article

Wharton's Jelly-Derived Mesenchymal Stem Cells with High Aurora Kinase A Expression Show Improved Proliferation, Migration, and Therapeutic Potential

Sun Jeong Kim,[1,2] **Sang Eon Park**,[1,2] **Jang Bin Jeong**,[1,2] **Shin Ji Oh**,[1,2,3] **Alee Choi**,[1] **Yun Hee Kim**,[1] **Suk-joo Choi**,[4] **Soo-young Oh**,[4] **Gyu Ha Ryu**,[5,6] **Hong Bae Jeon**,[1] **and Jong Wook Chang**[1,2,5]

[1]*Stem Cell Institute, ENCell Co. Ltd, Seoul 06072, Republic of Korea*
[2]*Cell and Gene Therapy Institute, Samsung Medical Center, Seoul 06351, Republic of Korea*
[3]*Department of Health Sciences and Technology, SAIHST, Sungkyunkwan University, Seoul 06351, Republic of Korea*
[4]*Department of Obstetrics and Gynecology, Samsung Medical Center, Seoul 06351, Republic of Korea*
[5]*Department of Medical Device Management and Research, SAIHST, Sungkyunkwan University School of Medicine, Seoul 06351, Republic of Korea*
[6]*The Office of R&D Strategy & Planning, Samsung Medical Center, Seoul 06351, Republic of Korea*

Correspondence should be addressed to Hong Bae Jeon; jhb@encellinc.com
and Jong Wook Chang; jongwook.chang@samsung.com

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Mesenchymal stem cells (MSCs) are effective therapeutic agents that contribute to tissue repair and regeneration by secreting various factors. However, donor-dependent variations in MSC proliferation and therapeutic potentials result in variable production yields and clinical outcomes, thereby impeding MSC-based therapies. Hence, selection of MSCs with high proliferation and therapeutic potentials would be important for effective clinical application of MSCs. This study is aimed at identifying the upregulated genes in human Wharton's jelly-derived MSCs (WJ-MSCs) with high proliferation potential using mRNA sequencing. Aurora kinase A (AURKA) and dedicator of cytokinesis 2 (DOCK2) were selected as the upregulated genes, and their effects on proliferation, migration, and colony formation of the WJ-MSCs were verified using small interfering RNA (siRNA) techniques. mRNA expression levels of both the genes were positively correlated with the proliferation capacity of WJ-MSCs. Moreover, AURKA from human WJ-MSCs regulated the antiapoptotic effect of skeletal muscle cells by upregulating the chemokine (C motif) ligand (XCL1); this was further confirmed in the mdx mouse model. Taken together, the results indicated that AURKA and DOCK2 can be used as potential biomarkers for proliferation and migration of human WJ-MSCs. In particular, human WJ-MSCs with high expression of AURKA might have therapeutic efficacy against muscle diseases, such as Duchenne muscular dystrophy (DMD).

1. Introduction

Mesenchymal stem cells (MSCs) are multipotent stromal cells isolated from various tissues, including bone marrow, fat tissue, placenta, and Wharton's jelly (WJ). Since MSCs can maintain stemness, regulate self-renewal, differentiate into various cell types, and restore damaged tissues and cells, they have been used to treat various diseases [1–3].

Various proteins secreted from MSCs are known to have therapeutic potentials and play an important role in tissue repair. Proteins secreted by MSCs have shown therapeutic effects on diverse diseases, such as Alzheimer's disease and

Duchenne muscular dystrophy (DMD) [4, 5]. Among the MSC-secreted proteins, the chemokine (C motif) ligand (XCL1) was reported to suppress apoptosis in skeletal muscle [6]. Based on such paracrine activities, WJ-MSCs have been suggested as effective therapeutic agents for various diseases, since they can control the effects of antiapoptosis, anti-inflammation, and immunomodulation via secretion of proteins [7, 8].

Although a sufficient number of MSCs would be required for their application in clinical trials, it is often difficult to achieve due to limitations, such as differences in proliferation capacity and production yield of MSCs across donors [9, 10]. MSCs with poor proliferation ability need prolonged culture to reach sufficient number; however, this inevitably induces cellular senescence, which potentially causes poor clinical outcomes [11]. Therefore, identification of MSC markers associated with high proliferative activity and therapeutic potential would be important for successful clinical application of MSCs. Early selection of WJ-MSCs with high proliferation ability might have positive clinical effects, since they can shorten the time to attain sufficient number of MSCs, avoiding long-term cultures that cause aging. Moreover, if the therapeutic effect can be predicted in advance, it can help clinical applications by reducing unnecessary effort or time. Therefore, early selection of cells with good treatment potential, using appropriate markers, can reduce the cost and shorten the time of attainment of MSCs, which would be beneficial for expanding MSCs under good manufacturing practice (GMP) conditions.

In this study, we identify proliferation-related factors in human WJ-MSCs; mRNA sequencing was performed to select the upregulated genes in WJ-MSCs with high proliferation potential, and Aurora kinase A (AURKA) and dedicator of cytokinesis 2 (DOCK2) were selected as the potential candidates.

2. Materials and Methods

2.1. Ethics Statement. This study was approved by the Institutional Animal Care and Use Committee of the Samsung Biomedical Research Institute (SBRI) at Samsung Medical Center. The SBRI complies with the Institute of Laboratory Animal Resources guidelines. The umbilical cords were collected from pregnant mothers with their prior consent, in accordance with the guidelines approved by the institutional review board of Samsung Medical Center (IRB#2016-07-102).

2.2. Cell Culture. WJ-MSCs were isolated and cultured according to the procedure described in a previous report [6]. For coculture experiments, mouse myoblast C2C12 cells (ATCC CRL-1772; American Type Culture Collection, Rockville, MD, USA) were seeded at 1×10^5 cells/well in a 6-well plate, and apoptosis was induced by culturing them in serum-free medium for 24 h. WJ-MSCs were seeded at 1×10^5 cells/insert in trans-well inserts (pore size 1 μm, BD Biosciences, Franklin Lakes, NJ, USA) and cocultured with apoptosis-induced C2C12 cells. C2C12 cells were cultured with or without WJ-MSCs for 24 h under serum-starvation conditions.

2.3. mRNA Sequencing and Data Analysis. Total RNA was extracted using TRIzol reagent (Invitrogen, Waltham, MA, USA), and libraries were prepared therefrom using the SMARTer Stranded RNA-Seq Kit (Clontech Laboratories, Inc., CA, USA). High-throughput sequencing was performed as paired-end 100 sequencing using a HiSeq 2500 (Illumina, Inc., San Diego, CA, USA). Gene expression levels were evaluated according to Read Count (RC) using BEDTools [12], and normalization of the expression values was based on the quantile normalization method by edgeR within R [13]. Data mining and graphic visualization were conducted using the ExDEGA (E-biogen, Inc., Seoul, Korea) and MeV software. Distance was analyzed using the Euclidean distance metric, and linkage method was selected for average linkage clustering.

2.4. siRNA Transfection. All siRNAs were purchased from Bioneer Corporation (Daejeon, Korea); the sequences of AURKA-specific siRNA were (sense) 5′-GUGCAAUAACC UUCCUAGU-3′ and (antisense) 5′-ACUAGGAAGGU UAUUGCAC-3′ while those of DOCK2-specific siRNA were (sense) 5′-CUGAGAAUGACUUCCUAC-3′ and (antisense) 5′-UGUAGGAAGUCAUUCUCAG-3′. WJ-MSCs were grown in growth medium till 50% confluence and then transfected with siRNA using Lipofectamine RNAiMAX (Invitrogen) according to the manufacturer's protocol. All siRNAs were transfected at a final concentration of 25 nM in serum-free medium. The control used a nonsilencing scramble RNA (siNC) with at least four mismatches with any human, mouse, or rat gene.

2.5. Real-Time Quantitative Reverse Transcription Polymerase Chain Reaction (Real-Time qRT-PCR). Total RNA was isolated from WJ-MSCs using an AccuPrep Universal RNA Extraction kit (Bioneer), according to the manufacturer's protocol. Real-time qRT-PCR was performed with a QuantStudio 6 Flex Real-Time PCR System (Thermo Fisher Scientific Inc., Waltham, MA, USA) and 2x Power SYBR Green Master Mix (Applied Biosystems, Waltham, MA, USA) using the following program: 95°C for 10 min, followed by 40 cycles of 95°C for 15 s, 56°C for 30 s, and 72°C for 30 s. Primers were purchased from Bioneer Corporation, and the primer sequences were as follows: AURKA, forward 5′-GGCCAC TGAATAACACCCAAA-3′ and reverse 5′-AGAGGGCGA CCAATTTCAAAG-3′; DOCK2, forward 5′-TTTCAACAC CGTTCTGGAGG-3′ and reverse 5′-TCAGCGTTCTTAGG ATTGGC-3′; and GAPDH, forward 5′-GAAGGTGAAGG TCGGAGT-3′ and reverse 5′-TGGCAACAATATCCAC TTTACCA-3′. All PCR reactions were performed in triplicate, and comparative quantification of each target gene was normalized to GAPDH expression using the $2^{-\Delta\Delta Ct}$ method.

2.6. Western Blot Analysis. Cells were lysed with RIPA buffer (BIOSESANG, Sungnam, Korea) containing a protease inhibitor cocktail (AMRESCO, Solon, OH, USA) and

incubated on ice for 20 min. Proteins were obtained via centrifugation at 15,000 × g for 30 min and separated using SDS-PAGE and transferred to membranes. The membranes were blocked with 5% skim milk at room temperature (RT) for 1 h and incubated overnight at 4°C with the following primary antibodies: anti-AURKA (Invitrogen), anti-p-AKT, anti-p-ERK, anti-XCL1 (R&D, Minneapolis, MN, USA), antiprotein kinase B (AKT), antiextracellular signal-regulated kinase (ERK), antipoly ADP ribose polymerase (PARP), anticleaved caspase-3, antifocal adhesion kinase (FAK), anti-p-FAK, anti-c-Jun N-terminal kinases (JNK), anti-p-JNK (Cell Signaling Technology, Danvers, MA, USA), anti-annexin V, antifibronectin (Abcam, Cambridge, MA, USA), anti-DOCK2, and anti-β-actin (Santa Cruz Biotechnology, Dallas, TX, USA). After washing with TBST, the membranes were incubated with HRP-conjugated secondary antibodies at RT for 1 h. The membranes were then washed with TBST, and protein bands detected using a gel imaging system (Amersham Imager 600, GE Healthcare, Buckinghamshire, UK). Band intensities were measured using ImageJ (National Institutes of Health (NIH), Bethesda, MD, USA) and then normalized to that of β-actin.

2.7. Flow Cytometric Analysis. Harvested MSCs were washed in PBS and blocked with 2% FBS for 30 min at RT. WJ-MSCs were then incubated with the following antibodies for 20 min at RT in the dark: CD11b, CD14, CD19, CD34, CD44, CD45, CD73, CD90, CD105, CD166, and HLA-DR (BD Biosciences, Franklin Lakes, NJ, USA). At least 10,000 events were counted using the BD FACSVerse flow cytometer (BD Biosciences). Flow cytometry was performed for appropriate isotype controls as well.

2.8. Migration Assay. The migration of WJ-MSCs was estimated using a wound healing assay. WJ-MSCs were seeded at 1×10^5 cells/well in 12-well plates and transfected with siRNA. They were incubated for 48 h and then treated with mitomycin C (10 μg/ml, Sigma, St. Louis, MO, USA) in MEM-α without serum for 2 h. The center of cell monolayer was scratched using a 200 μl pipette tip, and the wells were rinsed with the serum-free MEM-α; thereafter, cells were incubated with serum-free MEM-α for 24 h. Images were captured using a microscope (Olympus CKX41, Olympus, Tokyo, Japan). The area of wound was quantified by ImageJ. Migration capacity of WJ-MSCs was measured by calculating the percentage of wound closure [14]. Wound healing assays were analyzed using 15 fields for each group.

2.9. Clonogenic Assay. WJ-MSCs were seeded in 6-well plates at 1,000 cells/well. After incubation for 14 days, the WJ-MSCs were fixed with ice-cold 100% methanol and stained with 1% crystal violet solution (Sigma) for 30 min. Cells were then rinsed with distilled water, colonies with more than 50 cells were counted, and pictures of the plate were captured with a camera. Clonogenic assays were performed in triplicate, and each well had 1,000 cells to start with.

2.10. Cell Proliferation. Proliferation assays were performed at 0, 24, 72, and 144 h after seeding the transfected cells. After cells were seeded in 96-well plates at 2×10^3 cells/well; cell proliferation was confirmed using the Cell Counting kit-8 (CCK-8, Dojindo, Tokyo, Japan), according to the manufacturer's instructions. OD values were measured at 450 nm using a microplate reader (xMark™ Microplate Absorbance Spectrophotometer, Bio-Rad Laboratories, Inc., Hercules, CA, USA). To determine the number of cells, cells were seeded in 12-well plates at 3×10^3 cells/cm^2 in triplicate. WJ-MSC proliferation was assessed by the doubling time and fold change.

2.11. Live/Dead Staining. Apoptosis was detected using the Apoptosis/Necrosis Detection Kit (Abcam) according to the manufacturer's instructions. Apoptotic cells were stained with Apopxin Green Indicator, and viable cells were stained with CytoCalcein Violet 450. Images were acquired using a microscope (Olympus IX51, Olympus) and analyzed using ImageJ.

2.12. Animals. Mdx (C57BL/10ScSn-Dmdmdx/J(mdx) (JAX#001801)) and normal control (C57BL/10ScSnJ (JAX#000476)) mice were purchased from Jackson Laboratory (Bar Harbor, ME, USA). Two- to five-month-old mdx mice were injected with 5×10^4 WJ-MSCs in 100 μl of PBS via the tail vein. Seven days after injection, the mice were sacrificed using isoprene.

2.13. Immunohistochemistry and Sirius Red Staining. The gastrocnemius muscles of mdx mice were harvested, fixed with 4% paraformaldehyde, and sectioned into paraffin blocks of 4 μm thickness. IHC using annexin V was performed to confirm apoptosis. Tissue samples were incubated with annexin V antibody (Abcam), followed by incubation with goat anti-rabbit IgG secondary antibody (Alexa Fluor® 594 AffiniPure, Thermo Fisher Scientific) and counterstaining with Hoechst 33342 (Thermo Fisher Scientific). Fluorescence images were acquired using an LSM 700 confocal microscope (Carl Zeiss Meditec, Jena, Germany). Relative intensity of annexin V was calculated by dividing the intensity of annexin V fluorescence by the number of nuclei. Sirius Red staining was performed to observe muscle fibrosis, in accordance with standard procedures. Stained images were obtained under a ScanScope AT (Leica Microsystems, Buffalo Grove, IL, USA) and analyzed using ImageJ.

2.14. An Alu-Based Real-Time PCR. Genomic DNA was isolated from the gastrocnemius and thigh muscles of mdx mice using a Gentra Puregene Tissue kit (Qiagen Inc., Valencia, CA, USA), according to the manufacturer's protocol. Real-time PCR was performed under the following conditions: 95°C for 10 min, followed by 40 cycles of 95°C for 15 s and 68°C for 1 min. The forward and reverse primers for Alu were 5′-GTCAGGAGATCGAGACC ATCCC-3′ and 5′-TCCTGCCTCAGCCTCCCAAG-3′, respectively [15]. All experiments were conducted in triplicate using standards mixed with human DNA extracted from human MSCs and mouse DNA extracted from the liver of mdx mice.

2.15. Statistical Analysis. All data are shown as means ± standard error of the mean (SEM) of three or more

replicates. Statistical comparisons between two groups were performed by two-tailed Student's t-test, and multiple comparisons across more than two groups were analyzed by one-way analysis of variance (ANOVA) with Tukey's or Dunnett's multiple-comparison post hoc test or Duncan's multiple range test. Differences were considered statistically significant at $p < 0.05$. Correlation coefficient (r) was measured using Pearson's correlation analysis. The SPSS Statistics 23 software (IBM Corp., Armonk, NY, USA) was used for all analyses.

3. Results

3.1. AURKA and DOCK2 Were Significantly Upregulated in WJ-MSCs with High Proliferation Capability. To divide WJ-MSCs into two groups according to their proliferative capacity, we estimated the doubling time of WJ-MSCs and classified them into a proliferation-high (P-high) group and a proliferation-low (P-low) group [16]. The doubling time was significantly shorter in the P-high group (28.4 ± 1.2 h) than in the P-low group (46.5 ± 1.6 h) (Figure 1(a)).

To confirm the difference in mRNA levels between the groups, mRNA sequencing was performed using the RNA isolated from each group. Heat map indicated the mean of 33 differentially expressed genes related to cell differentiation, cell proliferation, cell growth, cell adhesion, and chemotaxis in the P-high group compared to that in the P-low group (Figure 1(b)). Upregulated genes in the P-high group (compared to those in the P-low group) included coronin-1A (CORO1A), syntaxin-1B (STX1B), high mobility group box 1 (HMGB1), DOCK2, AURKA, solute carrier family 9, subfamily A (SLC9A4), cholinergic/acetylcholine receptor M3 (CHRM3), natriuretic peptide receptor C/guanylate cyclase C (NPR3), retinoic acid receptor responder protein 2 (RARRES2), and annexin A3 (ANXA3). AURKA and DOCK2 were selected for further experiments, since their mRNA levels were significantly higher in the P-high group than in the P-low group ($p < 0.05$) (Figure 1(c)).

To confirm the role of AURKA and DOCK2 in WJ-MSCs, we suppressed the expression of these genes via transfection with siAURKA and siDOCK2. Real-time qRT-PCR and western blotting were performed thereafter to confirm whether the expression of AURKA and DOCK2 had indeed decreased (Figures 1(d) and 1(e)). Results indicated that siRNA transfection effectively knocked down AURKA and DOCK2 in WJ-MSCs. Flow cytometric analysis was performed to determine whether siRNA transfection affected the stemness of WJ-MSCs. As shown in Supplementary Figure 1, knockdown of AURKA and DOCK2 had no effect on the stemness of WJ-MSCs.

3.2. Knockdown of AURKA and DOCK2 Inhibited Proliferation and Migration of WJ-MSCs. Since AURKA and DOCK2 are known to regulate cell proliferation and migration [17–19], we confirmed their functions through gene suppression using siRNA transfection. To confirm whether knockdown of AURKA and DOCK2 decreased the proliferation of WJ-MSCs, cell counting and CCK-8 assays were performed at 0, 24, 72, and 144 h after seeding the transfected cells. Doubling time was 31.1 ± 0.4 h in the siNC-transfected group (control), 55.3 ± 0.8 h in the siAURKA-transfected group, and 59.2 ± 0.8 h in the siDOCK2-transfected group, hence suggesting that suppression of AURKA and DOCK2 increased the doubling time of WJ-MSCs. Fold changes were calculated by dividing each OD value by that at 0 h. Compared to the siNC-transfected group, the siAURKA- and siDOCK2-transfected groups showed significantly decreased cell proliferation over time, the effect being notable in the siDOCK2-transfected group (Figure 2(a)). Clonogenic assays were performed for two weeks to confirm whether AURKA and DOCK2 affected cell survival over a prolonged period of time. The plating efficiency of WJ-MSCs used in this experiment was 3.9%, and clonogenicity of the siAURKA- and siDOCK2-transfected groups was significantly reduced compared to that in the siNC-transfected group ($p < 0.001$) (Figure 2(b)). Degree of wound closure was significantly decreased in the siAURKA-transfected group and siDOCK2-transfected group compared to that in the siNC-transfected group ($p < 0.001$). Downregulation of AURKA and DOCK2 inhibited the migration of WJ-MSCs (Figure 2(c)). We performed western blotting to identify the effect of AURKA and DOCK2 on the phosphorylation of AKT, ERK, FAK, and JNK, which are involved in migration and cell proliferation [20–24]. AURKA knockdown decreased the phosphorylation of AKT and FAK while DOCK2 knockdown decreased the phosphorylation of AKT, ERK, and JNK ($p < 0.05$) (Figure 2(d)). Collectively, the results demonstrated that AURKA affects colony formation, migration, and cell proliferation through the AURKA/AKT/FAK signaling pathway while DOCK2 affects the same through AKT, ERK, and JNK.

3.3. mRNA Expression Levels of AURKA and DOCK2 Were Significantly Negatively Correlated with Doubling Time of WJ-MSCs. We checked the doubling time (Figure 3(a)) and mRNA expression levels of AURKA and DOCK2 genes in WJ-MSCs isolated from ten donors (Figure 3(b)) and verified the correlation between mRNA expression levels of these genes and doubling time through Pearson's correlation analysis (Figure 3(c)). The doubling time showed a strong negative correlation with mRNA expression levels of AURKA ($r = -0.757$, $p < 0.01$) and a moderate negative correlation with those of DOCK2 ($r = -0.536$, $p < 0.01$) [25] (Figure 3(c)). Together, the results suggest that AURKA and DOCK2 are associated with proliferation of WJ-MSCs.

Based on the above results, we selected three WJ-MSCs to confirm the protein levels of AURKA and DOCK2 and phosphorylation of AKT, ERK, FAK, and JNK; MSC_A had the highest expression of AURKA, MSC_C had the highest expression of DOCK2, and MSC_J had the lowest expression of both the genes. MSC_A and MSC_C showed no difference in the protein expression of AURKA and DOCK2. On the other hand, the protein expression of AURKA and DOCK2 decreased prominently in MSC_J compared to that in MSC_A and MSC_C ($p < 0.001$) (Figure 3(d)). Similarly, although the phosphorylation of AKT, ERK, FAK, and JNK did not differ between MSC_A and MSC_C, it decreased significantly in MSC_J than in

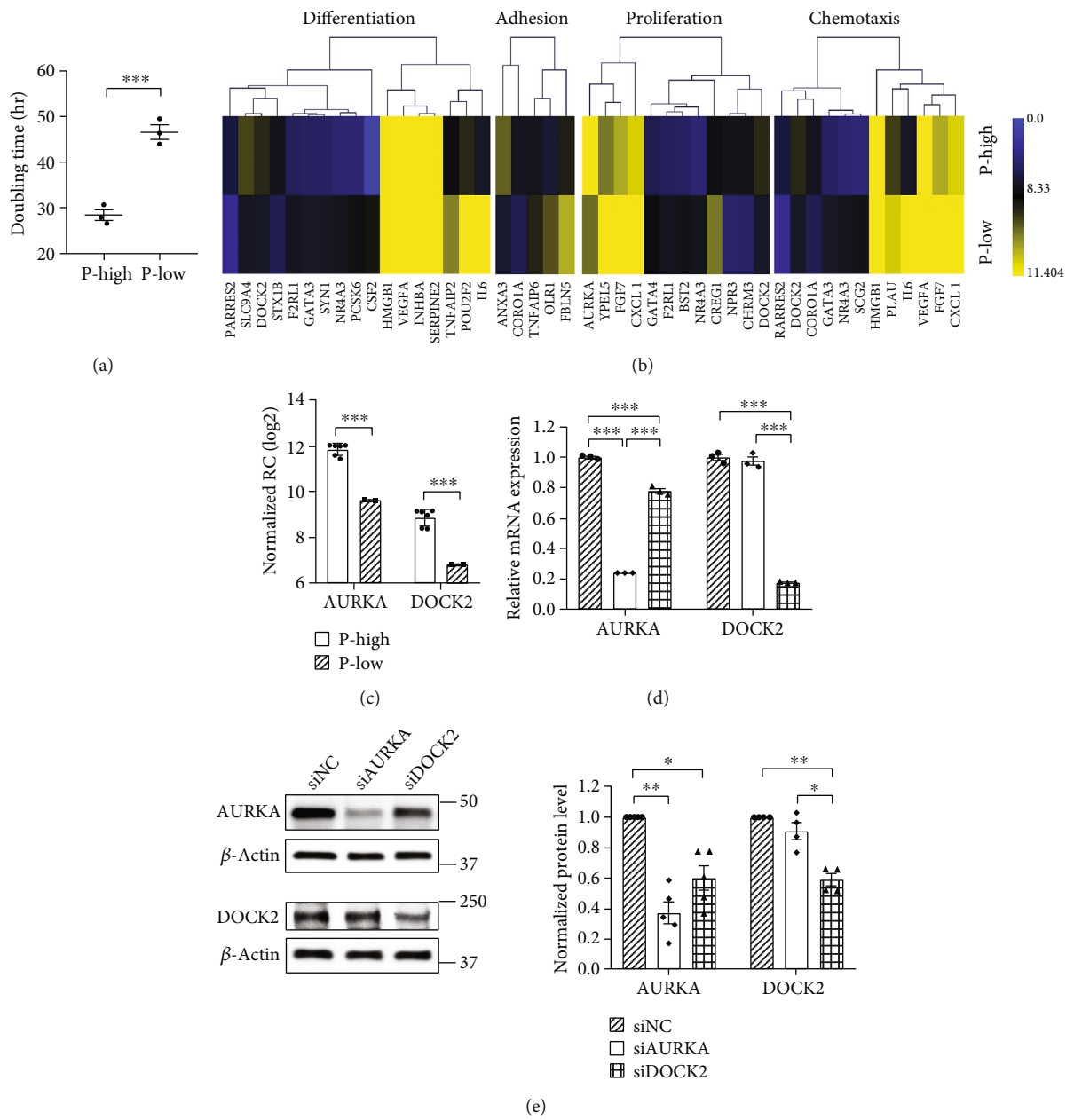


Figure 1: Comparative analysis of gene expression profiles of WJ-MSCs according to their proliferative capacity. (a) Doubling time of WJ-MSCs was measured, and the cells were classified into the proliferation-high (P-high) and proliferation-low (P-low) groups. (b) Heat map shows 33 differentially expressed genes related to cell differentiation, secretion, cell proliferation, cell adhesion, chemotaxis, and cell growth in both groups. (c) AURKA and DOCK2 genes expressed more significantly in the P-high group than in the P-low group. DEG selection criteria: significant fold changes ($p < 0.05$), fold change > 3, normalized read count (RC) > 6. (d) mRNA expression levels of AURKA and DOCK2 decreased at 48 h after siRNA transfection. (e) Suppression of AURKA and DOCK2 expression was examined by western blotting at 72 h after siRNA transfection. Normalized protein level indicates the value converted based on the siNC group. Data are shown as means ± SEM. Two-tailed Student's t-test (a, c). One-way ANOVA with Tukey's multiple comparisons test (d, e). $^{***}p < 0.001$, $^{**}p < 0.01$, and $^{*}p < 0.05$.

both ($p < 0.05$) (Figure 3(e)). The results suggested that AURKA and DOCK2 affect the phosphorylation of kinases associated with proliferation and migration of WJ-MSCs.

3.4. WJ-MSCs with High Expression of AURKA and DOCK2 Were More Effective in Inhibiting Apoptosis of C2C12 Cells than Those with Low Expression of Both. An apoptosis induction model was established in vitro to verify whether AURKA and DOCK2 are involved in therapeutic effects. Degree of apoptosis was determined by the expression of apoptosis marker proteins and fluorescence staining of apoptotic cells; apoptosis was induced in C2C12 cells cultured in serum-starved medium compared to that in serum-containing medium (Supplementary Figure 2). Next, the apoptosis-induced C2C12 cells and WJ-MSCs were cocultured to determine whether the antiapoptotic effect

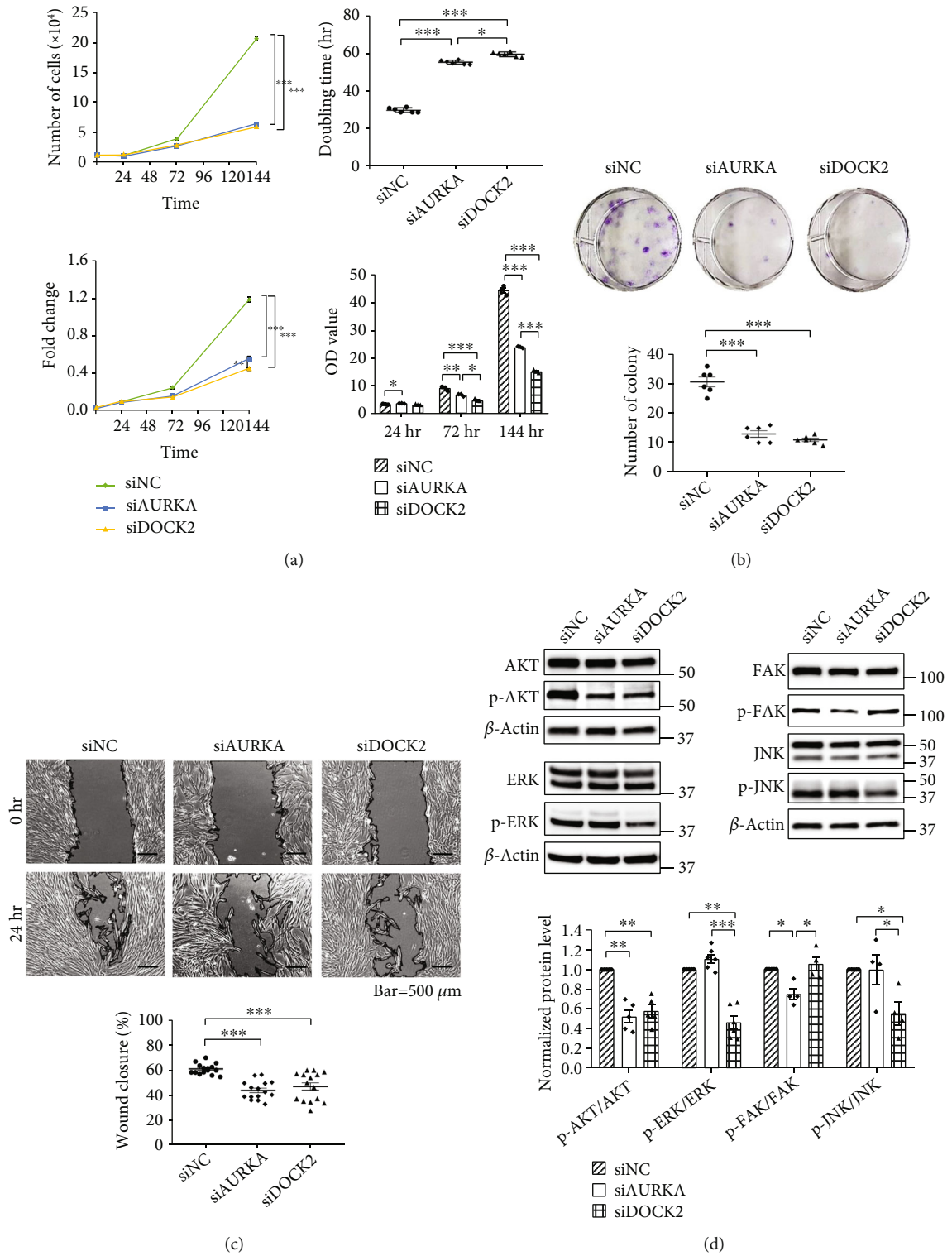


Figure 2: Knockdown of AURKA and DOCK2 inhibited cell proliferation, colony formation, and migration of WJ-MSCs. (a) Proliferation was evaluated by cell counting and CCK-8 assay at 0, 24, 72, and 144 h after seeding the siRNA-transfected cells. (b) Colony formation assays were performed in triplicate, with 1,000 cells per well to start with. Colonies were stained and counted two weeks after plating. (c) Migration was examined by wound healing assay. Knockdown of AURKA and DOCK2 inhibited the migration of WJ-MSCs. (d) Downregulation of AURKA inhibited phosphorylation of AKT and FAK while that of DOCK2 inhibited phosphorylation of AKT, ERK, and JNK. Normalized protein level indicates the value converted based on the siNC group. Data are shown as means ± SEM. One-way ANOVA with Dunnett's multiple comparison test (b–d), and Tukey's multiple comparisons test (a). $^{***}p < 0.001$, $^{**}p < 0.01$, and $^{*}p < 0.05$.

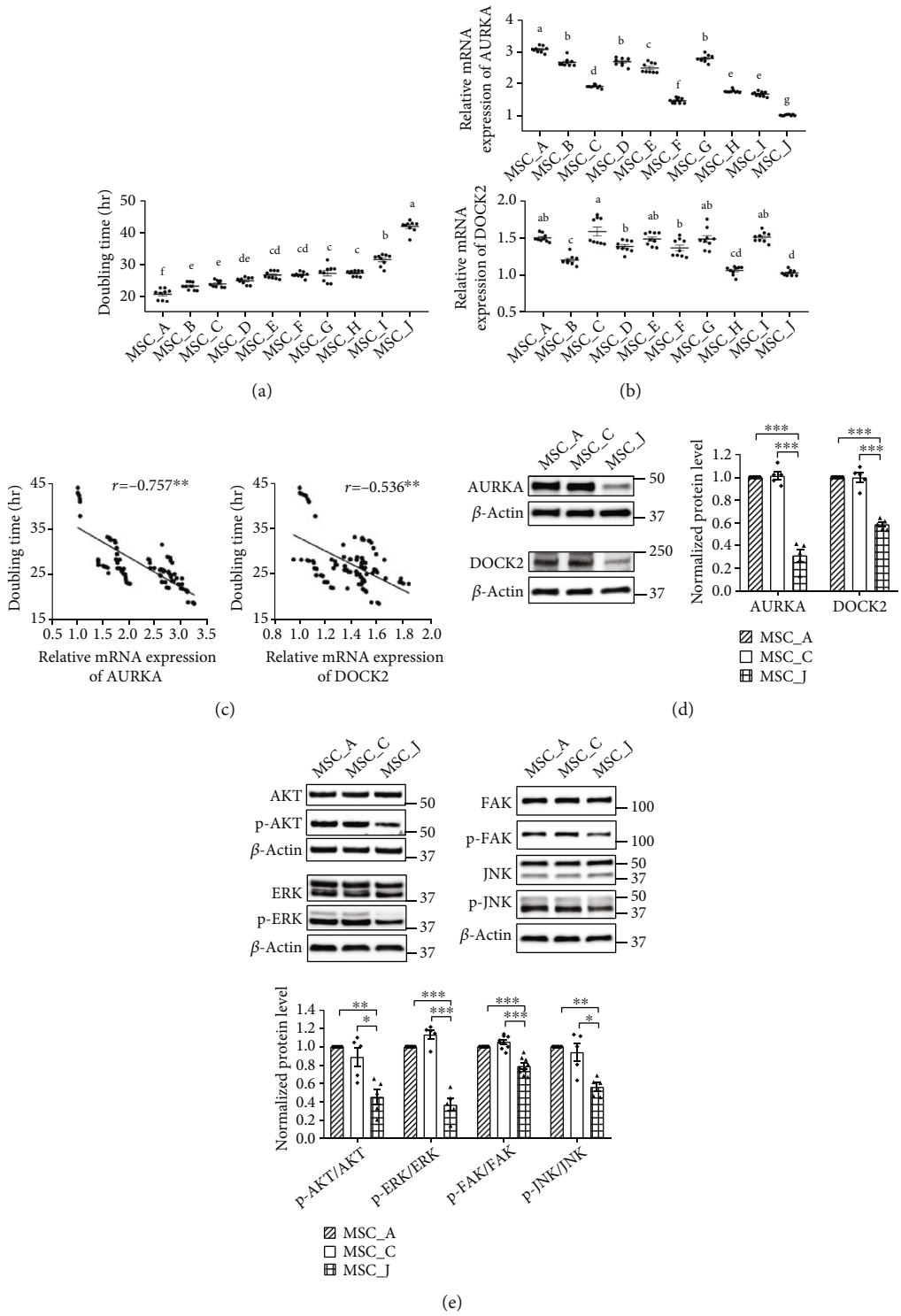


Figure 3: Correlation between doubling time and mRNA expression of AURKA and DOCK2 in WJ-MSCs. (a) The graph shows doubling time in ten lots of WJ-MSCs. (b) Real-time qRT-PCR analysis showed mRNA expression levels of AURKA and DOCK2 in WJ-MSCs. Relative mRNA expression indicates the value converted based on that of MSC_J. Different letters above bars mean significant difference from others (one-way ANOVA followed by Tukey's multiple range test, $p < 0.05$). (c) Pearson's correlation analysis confirmed a strong negative relationship between mRNA expression levels of AURKA and doubling time across the lots of WJ-MSCs. mRNA expression levels of DOCK2 and doubling time of WJ-MSCs showed a moderate negative relationship. (d) Western blotting of the protein levels of AURKA and DOCK2 in three WJ-MSCs. (e) Western blotting of the phosphorylation level of AKT, ERK, FAK, and JNK in three WJ-MSCs. Normalized protein level indicates the value converted based on that of MSC_A. Data are shown as means ± SEM. One-way ANOVA with Tukey's multiple comparisons test (a, b) and Dunnett's multiple comparisons test (d, e). $^{***}p < 0.001$, $^{**}p < 0.01$, and $^{*}p < 0.05$.

varied depending on the mRNA levels of AURKA and DOCK2. Antiapoptotic effects were compared using MSC_A with the highest expression of AURKA, MSC_C with the highest expression of DOCK2, and MSC_J with the lowest expression of both the genes. In apoptosis-induced C2C12 cells cocultured with MSC_A or MSC_C, the expression of cleaved caspase-3 and cleaved PARP decreased remarkably ($p < 0.001$) compared to that in apoptosis-induced C2C12 cells (control). However, in apoptosis-induced C2C12 cells cocultured with MSC_J, expression of cleaved caspase-3 and cleaved PARP decreased gently compared to that in the control group ($p < 0.05$) and increased significantly compared to that in the MSC_A and MSC_C groups (cleaved caspase-3, $p < 0.05$; cleaved PARP, $p < 0.001$) (Figure 4(a)). Apoptosis kits were used to identify the apoptotic rate among the total cells, where viable cells were stained blue and apoptotic cells were stained green. Fluorescence images were representative of the apoptosis-induced C2C12 cells 24 h after coculture with WJ-MSCs. Apoptotic rate in the control group was $53.42 \pm 1.52\%$ while that significantly decreased in all groups of C2C12 cocultured with MSCs ($p < 0.001$). However, among MSCs, apoptotic rate in the MSC_J groups increased significantly compared to that in the MSC_A and MSC_C groups ($p < 0.05$) (Figure 4(b)). These results showed MSC_J to be less effective in suppressing apoptosis of C2C12 cells compared to MSCs _A and MSC_C.

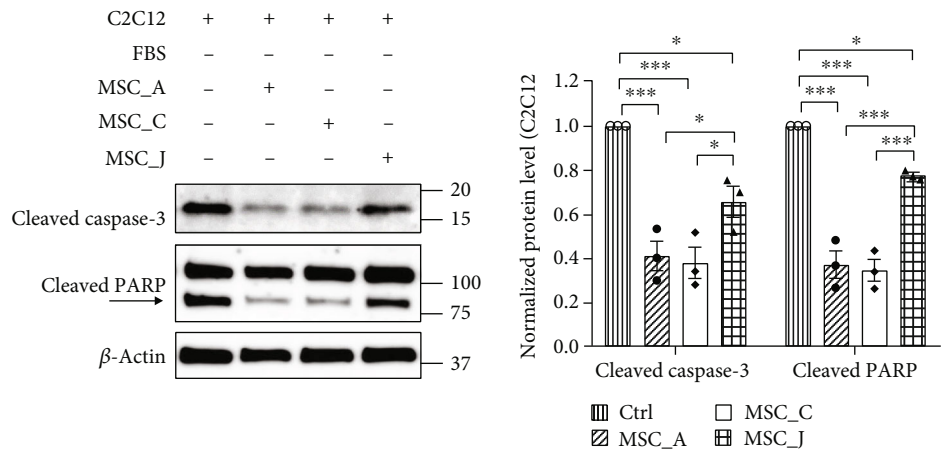
3.5. Knockdown of AURKA Suppressed Antiapoptotic Effect of WJ-MSCs through Reduction of XCL1 Protein Expression. To directly determine whether the antiapoptotic effect of WJ-MSCs on skeletal muscle cells could be related to the expression of AURKA and DOCK2, we suppressed AURKA and DOCK2 through siRNA transfection. After apoptosis-induced C2C12 cells (control) were cultured with WJ-MSCs, siNC-transfected WJ-MSCs, siAURKA-transfected WJ-MSCs, and siDOCK2-transfected WJ-MSCs, western blotting was performed to confirm the protein expression of cleaved caspase-3 and cleaved PARP. The apoptosis-induced C2C12 cells cocultured with siDOCK2-transfected WJ-MSCs showed no significant difference in the expression of apoptotic markers compared to the siNC-co-cultured group. Meanwhile, the expression of cleaved caspase-3 and cleaved PARP significantly increased in the apoptosis-induced C2C12 groups cocultured with siAURKA-transfected WJ-MSCs compared to that in both the siNC-co-cultured group and the siDOCK2-co-cultured group ($p < 0.05$) (Figure 5(a)). Fluorescence images were representative of the apoptosis-induced C2C12 cells after coculture with WJ-MSCs. Similar to western blotting results, apoptotic rate in apoptosis-induced C2C12 cocultured with WJ-MSCs decreased compared to that in the control group ($p < 0.001$). However, the apoptotic rate increased significantly in the siAURKA group compared to the siNC group and siDOCK2 group ($p < 0.01$) (Figure 5(b)). When AURKA was knocked down in WJ-MSCs, both protein expression of apoptosis markers and apoptotic rate were significantly increased in apoptosis-induced C2C12 cells than in both the siNC group and siDOCK2 group. Results indicated that the antiapoptotic effect of WJ-MSCs on skeletal muscle cells is affected by AURKA.

Our previous study had shown that XCL1 secreted by WJ-MSCs prevented the apoptosis of C2C12 [6]. Therefore, we investigated whether AURKA in WJ-MSCs could be related to an antiapoptotic effect, when cocultured with C2C12 cells, via XCL1. Expression of XCL1 protein in the siAURKA-transfected group was reduced ($p < 0.01$) compared to that in the siNC-transfected group (Figure 5(c)), indicating that AURKA inhibits apoptosis by upregulating XCL1 protein in skeletal muscle cells.

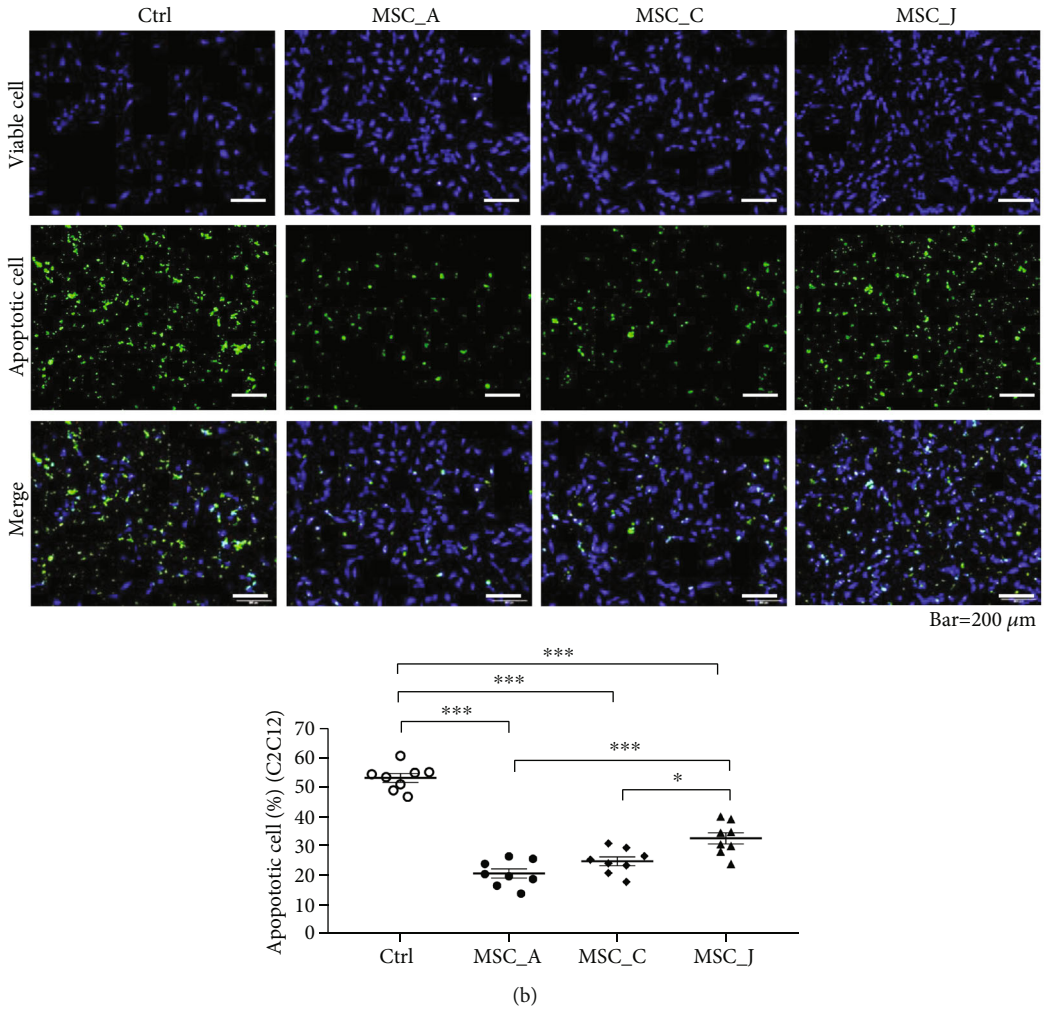
3.6. Expression Levels of AURKA Were Related to Antiapoptotic Effect of WJ-MSCs on Skeletal Muscle of the mdx Mouse. Since the antiapoptotic effect of WJ-MSCs is influenced by AURKA and the difference in mRNA levels of DOCK2 between donors is only slight, we selected WJ-MSCs based on the mRNA level of AURKA and conducted subsequent animal experiments. To evaluate whether AURKA expression and proliferative capacity of WJ-MSCs were effective in suppressing apoptosis in DMD pathology, we confirmed the protein expression of annexin V using western blotting and immunohistochemistry in the gastrocnemius muscles of mdx mouse (a DMD model). Experiments were performed 7 days after intravenous (IV) injection of WJ-MSCs with different AURKA mRNA levels and proliferative capacity into the mice. MSC_A had the highest expression of AURKA and the shortest doubling time, whereas MSC_E had the median value of both AURKA expression and doubling time, and MSC_J had the lowest expression of AURKA and the longest doubling time. Compared to that in the mdx control mice, protein expression of annexin V was significantly decreased in MCS_ A- and MCS_E-injected mdx mice; however, there was no difference in MSC_J-injected mdx mice. Annexin V levels in MSC_J-injected mdx mice were higher than in MSC_A-injected mdx mice and MSC_E-injected mdx mice ($p < 0.05$). In MCS_ A-, MCS_E-, and MSC_J-injected mdx mice, the cleaved caspase-3 protein levels were significantly reduced compared to that in the mdx control mice. The level of cleaved caspase-3 in MSC_J-injected mdx mice was significantly higher than that in MSC_A-injected mdx mice ($p < 0.01$) (Figure 6(a)). IHC results were similar as above. In MSC-injected mdx mice, the relative intensity of annexin V fluorescence significantly decreased compared to that in mdx control mice ($p < 0.001$). The MSC_J-injected mdx mice showed a relatively stronger intensity of annexin V fluorescence than the MSC_A-injected mdx mice ($p < 0.05$) and MSC_E-injected mdx mice ($p = 0.07$) (Figure 6(b)), thereby indicating that WJ-MSCs, with low levels of AURKA expression, are ineffective in suppressing muscle cell death.

4. Discussion

The deviation among MSCs in terms of cell growth and yield, depending on donors, has been one of the major limitations in their application to cell therapy [9, 10]. Selection of MSCs with high proliferation capacity and therapeutic potency would be crucial in the initial stage of



(a)



(b)

Figure 4: WJ-MSCs with high expression of AURKA and DOCK2 were more effective in reducing apoptosis of mouse skeletal myoblast cells (C2C12). (a) Inhibition of apoptosis was evaluated by protein expression levels of cleaved caspase-3 and cleaved PARP in apoptosis-induced C2C12 cells after coculture with WJ-MSCs. Normalized protein level indicates the value converted based on that of the control group. (b) Representative fluorescence images showed antiapoptotic effect of WJ-MSCs in apoptosis-induced C2C12 (green, apoptotic cells; blue, viable cells). The chart quantified the apoptotic cell rate of each group. Data are shown as means ± SEM. One-way ANOVA with Tukey's multiple comparisons test. $^{***}p < 0.001$, $^{**}p < 0.01$, and $^{*}p < 0.05$.

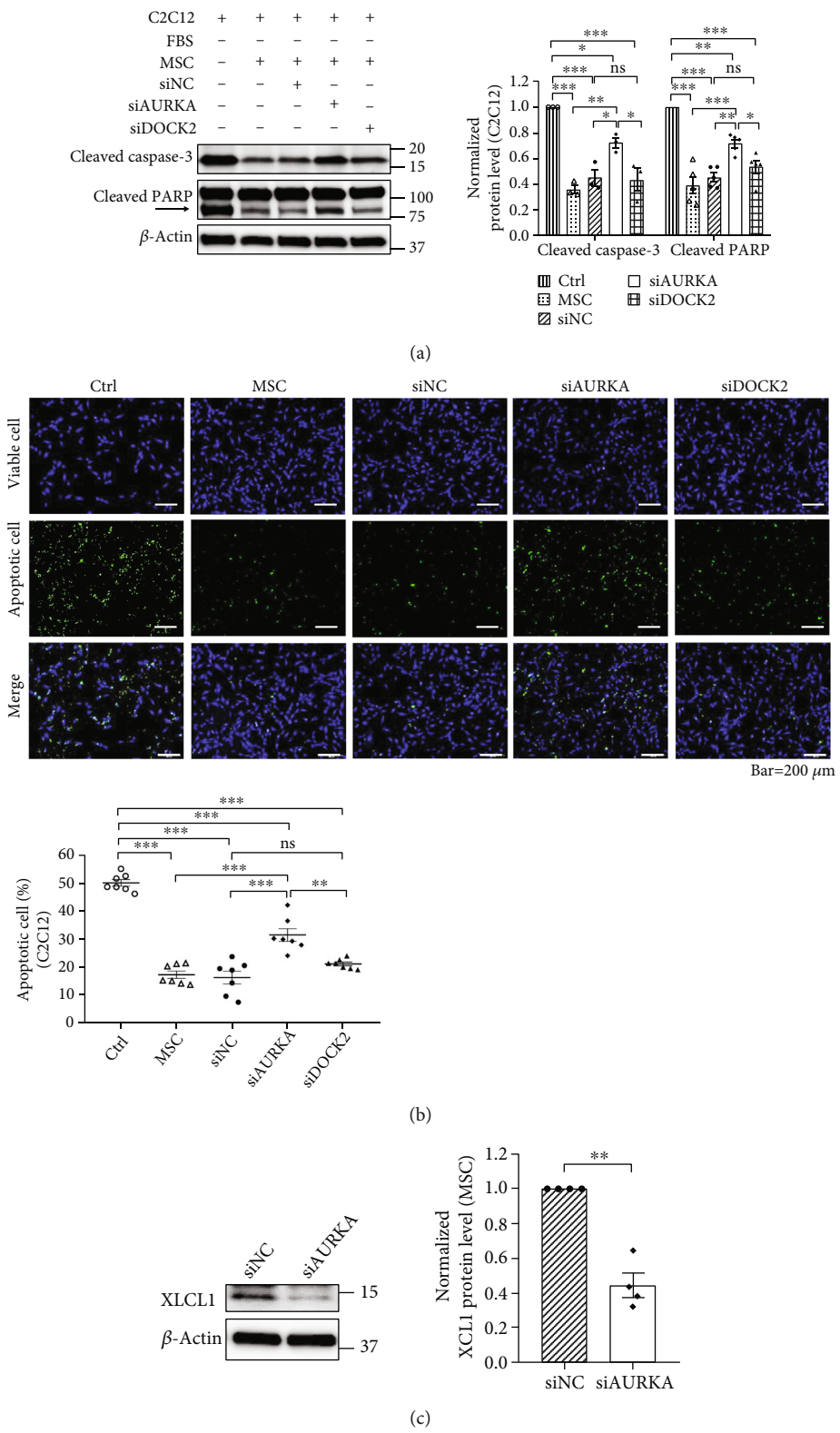


Figure 5: Knockdown of AURKA inhibited antiapoptotic effect of WJ-MSCs and was accompanied by the reduction of XCL1 protein. (a) Western blotting of the protein expression levels of cleaved caspase-3 and cleaved PARP in apoptosis-induced C2C12 cells. Normalized protein level indicates the value converted based on that of the control group. (b) Representative fluorescence images showed whether the suppression of AURKA and DOCK2 inhibited antiapoptotic effects of WJ-MSC in C2C12 (green, apoptotic cells; blue, viable cells). (c) Western blotting of the expression level of XCL1 protein in siAURKA-transfected WJ-MSCs. Normalized protein level indicates the value converted based on that of the siNC group. Data are shown as means ± SEM. One-way ANOVA with Tukey's multiple comparisons test (a, b). Two-tailed Student's t-test (c). $^{***}p < 0.001$, $^{**}p < 0.01$, and $^{*}p < 0.05$.

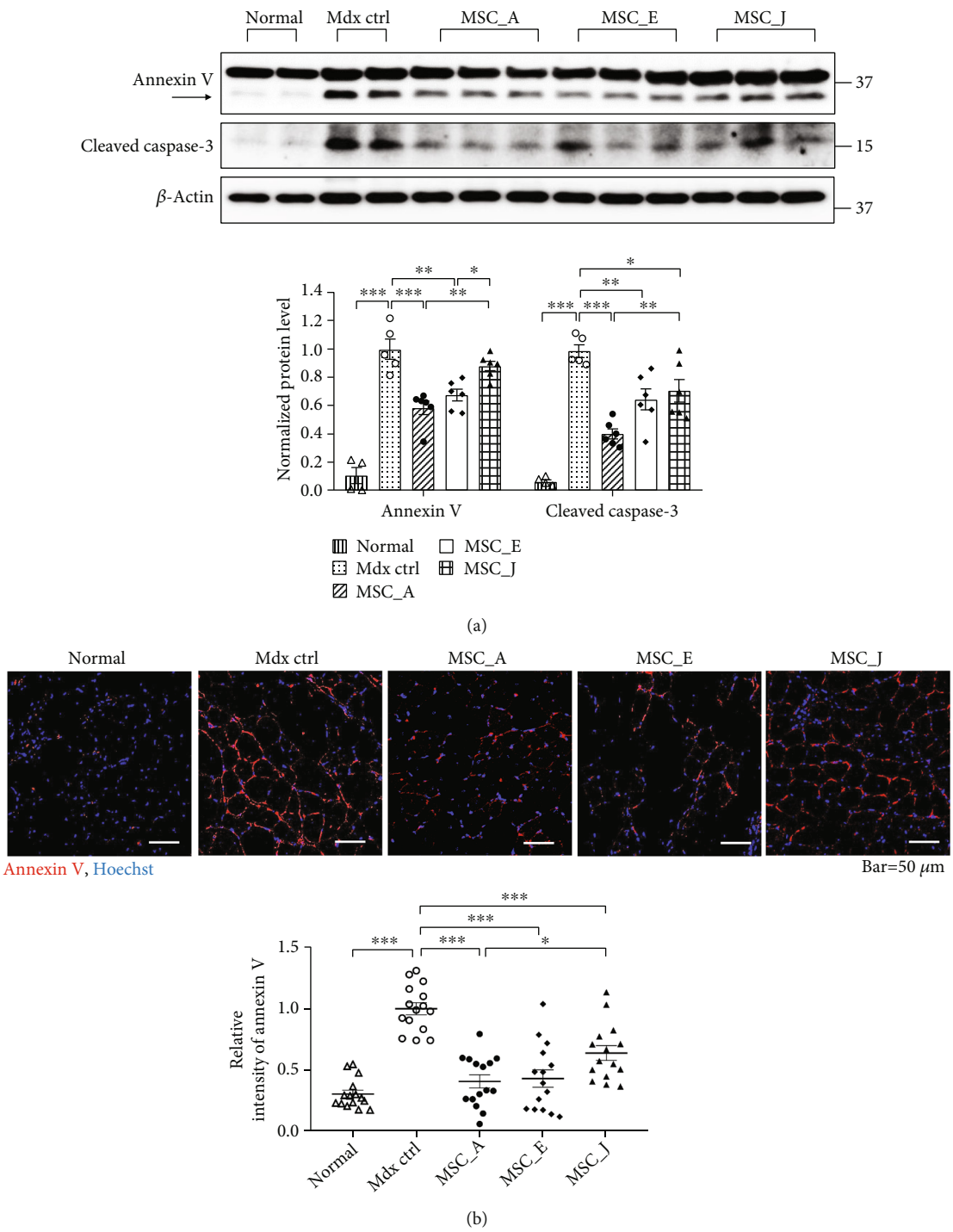


Figure 6: Antiapoptotic effect based on the differences in AURKA mRNA expression of WJ-MSCs in the skeletal muscle of mdx mice. (a) Protein expression levels of annexin V and cleaved caspase-3 were confirmed using western blotting. (b) Immunohistochemistry of annexin V was conducted in the gastrocnemius muscles of mdx mice (red, annexin V; blue, Hoechst). Relative intensity of annexin V indicates the value converted based on that of the mdx control group. Data are shown as means ± SEM. One-way ANOVA with Tukey's multiple comparison test (a) and Dunnett's multiple comparison test (b). $^{***}p < 0.001$, $^{**}p < 0.01$, and $^{*}p < 0.05$.

manufacturing MSCs as a medicinal product to resolve the donor variation. In this study, we classified WJ-MSCs isolated from various donors into two groups, namely, proliferation-high (P-high) and proliferation-low (P-low), based on their proliferation capacity, and performed gene expression profiling to examine the difference in gene expression between the two groups. We identified AURKA and DOCK2 as cell proliferation markers among the upregulated genes in the P-high group compared to that in the P-low group (Figure 1). A previous study comparing WJ-MSCs

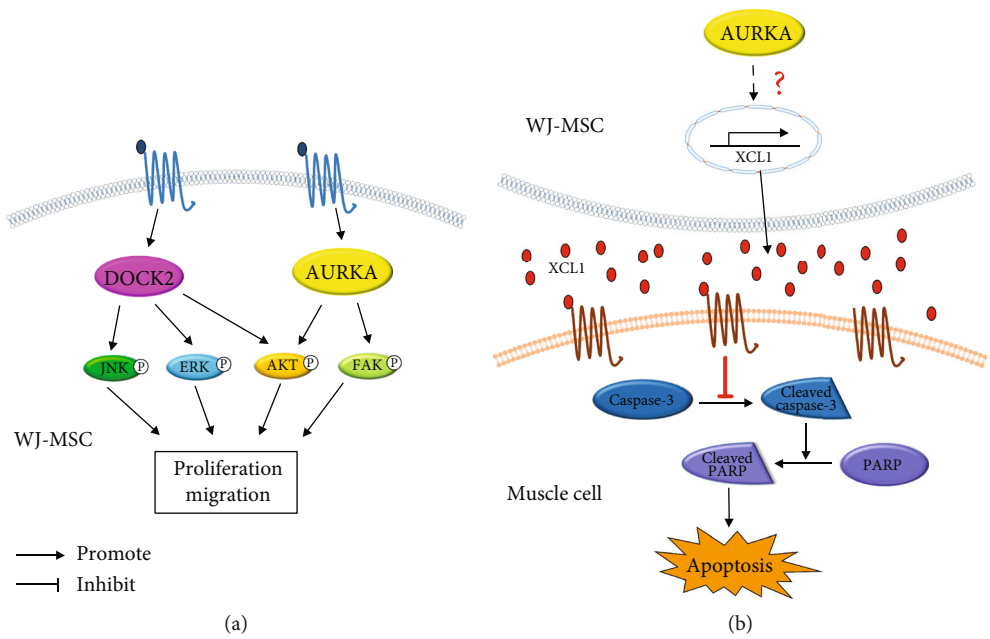


Figure 7: Both AURKA and DOCK2 contributed to cell proliferation and migration of WJ-MSCs, and AURKA of WJ-MSCs inhibited apoptosis in mdx mouse through XCL1. (a) In WJ-MSCS, both AURKA and DOCK2 promoted the proliferation and migration of WJ-MSCs through phosphorylation of kinases. (b) In WJ-MSCs, AURKA stimulated XCL1 transcription, causing XCL1 secretion from WJ-MSCs. The secreted XCL1 bound to the chemokine receptors of muscle cells, thereby inhibiting the cleavage of caspase-3 and PARP and suppressing the apoptosis of muscle cells.

and BM-MSCs has reported that cell cycle-related genes, such as Aurora kinase B (AURKB), cyclin D2 (CCND2), and cell division cycle 25C (CDC25C), are involved in the proliferation capacity of WJ-MSCs [26]. Another study comparing the proliferation capacity of WJ-MSCs in hypoxia and normoxia showed growth factors, such as fibroblast growth factor-17 (FGF-17), vascular endothelial growth factor A (VEGFA), and insulin-like growth factor binding protein 3 (IGFBP3), to be involved in the proliferation of WJ-MSCs [27]. We identified novel factors related to the donor-dependent variation in proliferation capacity of WJ-MSCs by examining differential gene expression under the same conditions in WJ-MSCs originating from different donors.

Results demonstrated that the knockdown of AURKA and DOCK2 diminished both cell proliferation and migration rate; knockdown of AURKA reduced phosphorylation of AKT and FAK while that of DOCK2 reduced the phosphorylation of AKT, ERK, and JNK (Figure 2). These results indicated the involvement of AURKA and DOCK2 in cell proliferation and migration by activating AKT and FAK via AURKA and by activating AKT, ERK, and JNK via DOCK2 (Figure 7(a)). AURKA, a centrosome-associated serine/threonine kinase, has been shown to modulate essential mitotic events, including centrosome maturation, bipolar spindle assembly, and G2-M transition [28–30] and promote cell survival, migration, invasion, and proliferation in various cancer cells by activating AKT and FAK [17, 31, 32]. DOCK2, an atypical guanine nucleotide exchange factor (GEF), is involved in lymphocyte migration by regulating the actin cytoskeleton [19], as well as the motility and polarity during neutrophil chemotaxis [33] in immune cells. It also promotes cell proliferation, invasion, and migration by phosphorylation of AKT, ERK, and JNK in cancer cells [18, 34, 35]. Although AURKA and DOCK2 have been extensively reported to be closely related to cell proliferation and migration of cancer cells, the function of these two molecules in WJ-MSCs has not yet been studied extensively. In our current study, we demonstrated, to the best of our knowledge, for the first time that AURKA and DOCK2 regulate the proliferation and migration of human WJ-MSCs. In WJ-MSCs derived from ten different donors, the mRNA expression levels of AURKA and DOCK2 were significantly negatively correlated with doubling time (Figure 3(c)), which implied that AURKA and DOCK2 are potential biomarkers to identify and select the cells with high proliferation capacity in the initial stage of development of cell therapy to control the donor-dependent variation in medicinal products.

Among the factors determining the therapeutic potential of WJ-MSCs, enhanced migration and proliferation capacities definitely contribute more to the therapeutic effect of MSCs in various diseases [36–39]. Since AURKA and DOCK2 are involved in cell proliferation and migration, we applied in vitro apoptosis models to determine whether WJ-MSCs with high expression of both genes could be more effective in treating muscle disease. As expected, WJ-MSCs with high expression of AURKA and DOCK2 were more effective in suppressing apoptosis (Figure 4). However, knockdown results of both genes

showed that the factor inhibiting apoptosis was AUKRA, not DOCK2, and AURKA regulated protein expression of XCL1, known to inhibit apoptosis [6] (Figure 5). We also demonstrated the anti-poptotic effect of AURKA in in vivo model. An enhanced antiapoptotic effect was observed in the limbs of mdx mice, a DMD mouse model, injected with WJ-MSCs highly expressing AURKA compared to that in mice injected with WJ-MSCs expressing AURKA at a low level (Figure 6). These findings indicated that AURKA in WJ-MSCs is not only involved in proliferation and migration but also in suppressing the apoptosis of skeletal muscle cells by regulating XCL1 expression. AKT and FAK have been reported to be involved in the transcription of chemokine genes [40, 41]; chemokines promote myoblast proliferation, which affects muscle repair [42, 43]. Therefore, we hypothesized that in WJ-MSCs, some kinases activated by AURKA might induce the transcription of XCL1, a family of chemokines; XCL1 protein is, hence, increased in cells, eventually promoting the secretion of XCL1 protein. It may then reach and bind to the chemokine receptors of muscle cells, thereby causing inhibition of apoptosis in the muscle tissue by reducing the cleavage of caspase-3 and PARP (Figure 7(b)).

Lee et al. [44] have demonstrated that MSCs with enhanced proliferation and migration due to ethionamide were retained longer when injected into mouse brain and were expected to show better therapeutic effect than naïve MSCs in the treatment of brain diseases. Therefore, WJ-MSCs with enhanced proliferation and migration, due to high expression of AURKA, probably showed improved apoptotic effect in the mdx mouse due to the higher number of WJ-MSCs retained in the muscle tissue than the WJ-MSCs with low expression of AURKA. In fact, the number of WJ-MSCs with low expression of AURKA, which remained in the limb muscle tissue of mdx mouse, was found to be less than that of WJ-MSCs highly expressing AURKA (Supplementary Figure 3(a)). In addition, the WJ-MSCs with low-level expression of AURKA showed less therapeutic effect on muscle fibrosis, a pathological hallmark of DMD [45], than those with high-level expression of AURKA. Not only expression of fibronectin, a fibrosis marker, but also accumulation of collagen was significantly reduced in the muscle tissue of mdx mice injected with MSCs with high-level expression of AURKA compared to that in mice injected with MSCs expressing low-level AURKA (Supplementary Figure 3(b), (c)). The results collectively suggested that MSCs highly expressing AURKA are more effective in ameliorating muscle fibrosis since the cells could migrate to limb muscle faster and stay in the muscle tissue longer.

In this study, expression of AURKA was found to regulate the capacity of cell proliferation and migration, demonstrating a treatment effect in DMD. Therefore, we proposed the mRNA expression level of AURKA to possibly be a useful standard for identifying and selecting WJ-MSCs for the development and manufacture of cell therapy products for DMD treatment. However, the downstream signaling pathways of AURKA, which are involved in the transcription of XCL1 in WJ-MSCs, are yet to be identified. Further studies on these would be required, and the factors regulating fibrosis in skeletal muscle would need to be identified.

5. Conclusions

This study was the first to confirm that AURKA and DOCK2 contribute to the proliferation and migration of WJ-MSCs. Especially, AURKA can be utilized as a strong potential selection marker for the proliferation of WJ-MSCs, and early selection of WJ-MSCs with it could not only reduce production costs but also enable large-scale production of WJ-MSCs for clinical applications. In addition, since AURKA mediated antiapoptosis processes of skeletal muscle cell by regulating XCL1, the selection of WJ-MSCs based on the level of AURKA could be an alternative option to improve their therapeutic efficacy in treating muscle diseases, such as DMD.

Data Availability

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

Conflicts of Interest

The authors declare no conflict of interest.

Acknowledgments

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Supplementary Materials

Supplementary Figure 1: siRNA transfection of AURKA and DOCK2 in WJ-MSCs did not affect cell stemness. Stemness was evaluated using flow cytometry, by identifying the expression of MSC-positive markers (CD44, CD73, CD90, CD105, and CD166). Expression of MSC-negative markers was confirmed using the hematopoietic markers (CD11b, CD14, CD19, CD34, CD45, and HLA-DR). Supplementary Figure 2: C2C12 cells were incubated in serum-free medium for 24 h to cause apoptosis. (a) The C2C12 cells were cultured with or without FBS for 24 h. (b) The apoptosis markers were upregulated in C2C12 cultured without FBS. Normalized protein levels indicate the value converted based on the FBS (-) group. (c) Representative fluorescence images showed apoptosis induction due to serum deficiency in C2C12 (green, apoptotic cells; blue, viable cells). The chart

quantified the apoptotic rate of each group. Data are shown as means ± SEM. Two-tailed Student's t-test. $^{***}p < 0.001$ and $^{**}p < 0.01$. Supplementary Figure 3: residual cell number and antifibrotic effect according to the differences in AURKA mRNA expression of WJ-MSCs in the skeletal muscle of mdx mice. (a) The number of residual WJ-MSCs in mouse leg muscle (gastrocnemius muscle and thigh muscle) was identified using human Alu using real-time PCR. (b) Protein expression levels of fibronectin were confirmed using western blot. Normalized protein level indicates the value converted based on the mdx control group. One-way ANOVA with Tukey's multiple comparison test. (c) Fibrotic area was confirmed based on collagen deposition through Sirius Red staining of the gastrocnemius muscles of mdx mice. Data are shown as means ± SEM. One-way ANOVA with Duncan's multiple range test (a) and Tukey's multiple comparisons test (b, c). $^{***}p < 0.001$, $^{**}p < 0.01$, and $^{*}p < 0.05$. *(Supplementary Materials)*

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