Functional Mechanism of Mesenchymal Stem Cell-Derived Exosomes in Articular Cartilage Regeneration

Chao Song *

Department of Life Science, Sichuan University, Chengdu, China

* Corresponding author: chaosong@stu.scu.edu.cn

Abstract. As the aging of the population, a chronic degenerative joint disease - Osteoarthritis (OA), its incidence and severity are increasing. Compared with cells, the immunogenicity of the exon is low and the immunomodulatory performance is excellent. Various types of mesenchymal stem cell-derived exosomes (MSC-EXOs) are now being used to treat cartilage injury and disease as target drugs or delivery vehicles. A thorough examination of the existing evidence is required in light of the emerging evidence on the effective mechanism and method of MSC-EXOs in OA. This article reviews the specific mechanism and latest research progress of MSCs derived exosomes in OA treatment. It mainly summarizes three mechanisms of action of exosomes: a. Enhancing extracellular matrix (ECM) and preventing cartilage degradation; b. Promoting chondrocyte migration, proliferation, migration, and inhibiting chondrocyte apoptosis, c. Influencing immune protection or immune destruction signals. In addition, the difficulties of exosomes in the clinical treatment of OA are briefly reviewed, with the hope of providing ideas for developing biomaterials scaffolds and clinical treatment of MSC-EXOs.

Keywords: Osteoarthritis, Mesenchymal Stem Cell, Exosomes, Chondrocytes.

1. Introduction

OA is a chronic and degenerative disease, which is characterized by pathological imbalances in the degradation and repair process of the entire joint and its components. Synovium and articular cartilage are the two typical secondary inflammatory changes in OA, especially in the complex disease that includes bio-mechanical changes in joint composition, metabolic changes, and joint inflammation, resulting in an abnormal balance of synovial joints. Research shows that 9.6% of males and 18.0% of females over sixty have symptoms of OA [1]. For a long time, the definition and terminology of osteoarthritis has been a controversial topic around the loss of integrity of articular cartilage. Osteoarthritis is a dynamic active change caused by an imbalance of breakdown as well as rehabilitation with double joint tissues such as subchondral bone, cruciate ligament, hyaline articular cartilage, capsule, and synovium, and lateral collateral ligament. The interleukin-1 (IL-1) family contains numerous pro-inflammatory and anti-inflammatory proteins. Innate immunity and inflammation are both mediated by IL-1. IL-1, IL-6, and IL-8 as pro-inflammatory cytokines, together with a variety of pro-catabolic mediators via their signalling pathways, are the main pathophysiological mechanisms in OA. NF-kβ and mitogen-activated protein kinase signalling also play a role in responding and reprogramming switching pathways in transcriptional networks.

There are many clinical treatments for OA. Although surgery and drug therapy can alleviate pain, they cannot solve articular cartilage regeneration and repair. MSCs have a strong ability to regenerate and repair cartilage, and they can differentiate into chondrocytes and regulate immunity. MSCs have become the most widely studied treatment method for OA cells. Many researches reveal that MSCs differentiate into chondrocytes and participate in tissue repair mainly through their paracrine pathway, in which the secreted exosomes may play a key role.

Exosomes (EXOs) released by various cell types are double-layer micro membrane bound vesicles whose diameter is 30–150 nm. Exosomes are derived from endosomes formed by plasma membrane endocytosis. Bioactive substances such as mRNA and protein congregate in the inner bodies of cells, giving rise to multicystic endosomes or intracavitary vesicles. Exosomes are finally released via membrane fusion after further processing. These microbubbles are crucial in cell communication by

participating in the process of information transmission between cells. In addition, exosomes can also be used as stable carriers to deliver lipids, proteins, miRNA, circRNA, lncRNA, and other bioactive substances to target cells. Exosomes are vital for intercellular communication and cellular immune response. Because of their higher stability, immunogenicity, and no cytotoxicity, exosomes can reduce the immune response brought on by stem cell transplantation. Therefore, exosomes are easier to be used for clinical treatment. In OA treatment, EXOs from MSCs, as a "cell-free" treatment method, have attracted more and more attention. However, the mechanisms of MSC-EXOs in treating OA disease have not been determined yet. This article reviews the latest research on the mechanism and progress of MSCs derived exosomes in OA treatment, hoping to provide ideas for developing biomaterials scaffolds and clinical treatment.

2. Mechanisms of MSC-Exosomes in Articular Cartilage Regeneration

Exosomes can derive from various cells. Adipose and bone marrow cells are still the MSCs' most common sources, which are used to derive EXOs for OA treatment. In addition to the traditional MSC-based sources, different origins of EXOs have now been evaluated. These include infrapatellar fat pad-derived exosomes (IPFP-MSC), synovium-derived exosomes (SMSC), adipose-derived exosomes (ADMSC), amniotic fluid-derived exosomes, and umbilical cord-derived exosomes (UC-MSC) (Figure 1). Although EXOs come from many sources, its functionality varies by its production environment. For instance, the synovial EXOs content of diseased individuals is different from that of normal individuals, and it is also different in various joint diseases. Although the specific regulatory mechanisms of EXOs from different sources on OA treatment are different, their functions are roughly the same and can be classified.

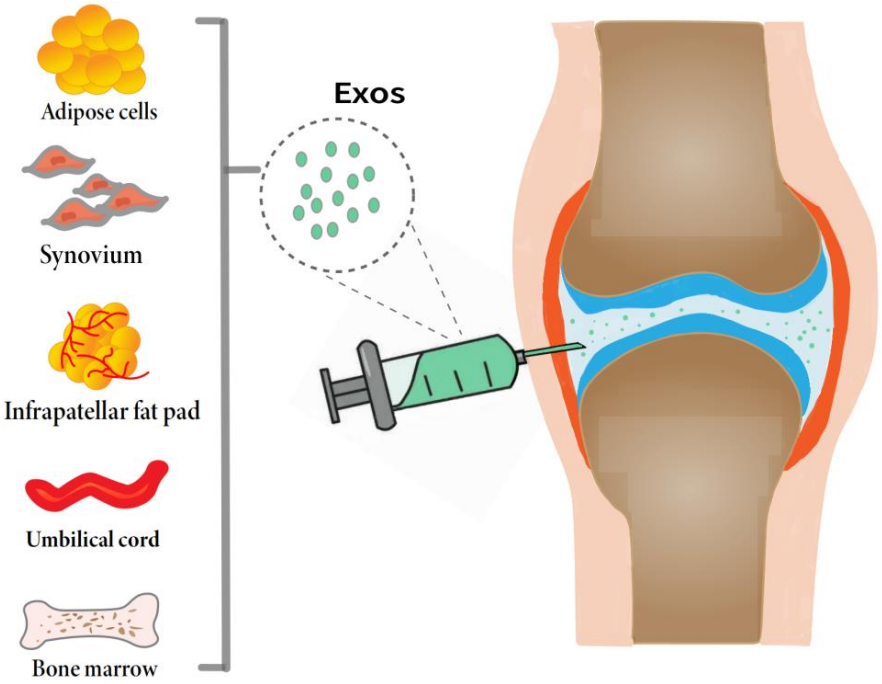

Figure 1. EXOs derived from various of cells. (Photo credit: Original)

There are many cargos in exosomes, such as mRNA, protein, long non-coding RNA (lncRNA, cirRNA), and miRNA. Exosomes have the ability to alter gene expression through targeting gene directly, can change gene downstream functions, and processes associated with the target cells' physiology or pathology. The RNA load in exons has drawn wide attention from researchers, especially miRNAs. Extensive physiological activities, such as cartilage metabolism as well as degenerative processes in OA and other illnesses, are crucially regulated post-transcriptionally by miRNAs (table 1) [2]. Short non-coding RNAs called miRNAs regulate several biological processes. They control the amount of degradation by directly interacting with the complementary regions of

the target mRNAs' 30 UTR. In addition to operating within cells, exosomes create miRNAs, which are then transported to nearby or distant cells in order to regulate genes expression and cell function.

Table 1. Function of various miRNA for OA treatment

Function	miRNA
Prevent cartilage degradation	miR-145, miRNA-140, miR-582-3p
Prevent articular cartilage destruction	miR-204
Increase cartilage repair	mi-107
Increase chondrocyte proliferation	miR-138, miR-101, miR-132, miR-210, miR-29a, miR-4784, miR-210-3p
Prevent cartilage degradation	miR-221
Encourage cartilage regeneration	miR-149-5p
Reduce metabolic enzyme activity	miR-1
Chondrocyte autophagy inhibition	miR-130a
Inhibit apoptosis of chondrocytes	miR-132s, miR-766-3p, miR-582-3p, miR-138, miR-455-3p, miR-93-5p
Reduce chondrocyte inflammation	miR-335-5p, miR-582-39, miR-106a-5p

2.1. Enhancing ECM and preventing cartilage degradation

OA is characterized by changes in extracellular matrix composition and structure (Figure 2a). Two main components of articular cartilage's extracellular matrix, aggrecan and collagen type II (col-II), both play roles in forming a functioning cartilage matrix. The breakdown of cartilage extracellular matrix protein can cause cartilage degradation. ADAMTS4, with thrombospondin motifs 4, is a metalloproteinase and disintegrin, also a zinc-dependent protease that belongs to the large ADAMTS family. Matrix metalloproteinase 13 (MMP-13) belongs to the matrix metalloproteinase family and is a collagenase. During the progression of OA, enhanced expression of collagenases (e.g MMP-13), as well as two aggrecanases such as metalloproteinase and disintegrin with ADAMTS-5 and ADAMTS-4, both lead to notable reductions in col-II and aggrecan. EXOs may mediate ECM through various mechanisms. miR-100-5p from IPFP-MSCs enhances chondrocytes autophagy through mTOR pathway, and inhibits chondrocyte apoptosis, reduces catabolic factors, promotes ECM synthesis, and promotes anabolic factors [3]. Insulin-like growth factors (IGFs) are a group of secreted peptide hormones that play critical roles in cellular growth and development as well as organism function. In chondrocytes, MSC-derived CD73, TGF-β, and IGF have the ability to stimulate AKT, ERK, as well as AMPK signalling pathways. MSC exosomes restored matrix and overall joint homeostasis through ptomoting s-GAG synthesis and inhibiting IL-1-induced NO and MMP13 development [4]. miR-92a-3p derived from MSC-EXOs can inhibit cartilage degradation as well as WNT5A expression in MSCs and PHCs, however, cartilage proliferation and the upregulation of matrix gene are increased in MSCs and OA primary human chondrocytes (PHCs) [5]. Exosome-transported circRNA_0001236 overexpression can inhibit MMP13 in chondrogenesis, and inhibit cartilage degradation and promote chondrogenesis through the miR-3677-3p/Sox9 axis [6]. miR-125a-5p can accelerate chondrocytes migration and alleviate matrix degradation through up-regulated the expression of aggrecan, col-II and SOX9, while down-regulate MMP-13 expression [7]. The collagen type II alpha 1 chain (COL2A1) gene encodes the alpha-1 chain of type II procollagen. Type II collagen is the major component of cartilage, which is comprised of three identical alpha-1 chains. miR-106-5p mimics significantly reduced chondrocyte migration and proliferation, increased matrix degradation through promoting the expression of ADAMTS5 and MMP13, while decreased aggrecan and COL2A1 expression. By targeting the miR-106b-5p/TIMP2 axis, lncRNA H19 promotes chondrocyte migration, proliferation, as well as inhibits matrix degradation in OA [8].

2.2. Promoting chondrocyte migration, proliferation, hypertrophy, and inhibiting chondrocyte apoptosis

To maintain cartilage health, chondrocyte migration and proliferation play key roles, which are both downregulated in OA (Figure 2b). EXOs regulate cellular proliferation by altering gene expression or by reversing the inhibiting effect of TNF as well as IL1 on chondrocytes proliferation and migration [9]. Exosomes derived from UC-MSCs activated TGF1 and Smad2/3 signalling pathways, while EXOs extracted from synovial MSCs activated YAP with Wnt5a and Wnt5b. Through the miR-124-3p/MYH9 axis, EVs-circHIPK3 induced MSC-EXOs-mediated chondrocyte migration and proliferation, while suppressing chondrocyte apoptosis. In OA, MSCs-EVs can enhance the expression of aggrecan, SOX9, and COL2A1, as well as suppress the expression of the RUNX family transcription factor 2 (Runx2) and MMP-13 [10]. TGFβ-1 can stimulate chondrocyte proliferation through the way of suppressing Sp1 via miR-135b derived from MSC-EXOs [11]. MiR-216a-5p stimulates chondrocyte migration and proliferation while suppressing JAK2. as well as inhibiting apoptosis. HIF-1α is likely to be a vital therapeutic target for OA through regulating chondrocyte metabolism and inflammation, which causes hypoxic BMSCs to release EXOs. Through the miR-216a-5p/JAK2/STAT3 pathway, BMSC-EXOs play a role in promoting chondrocyte migration, proliferation, and apoptosis inhibition [12].

Exosomes can prevent apoptosis through enhancing anti-apoptosis gene expression. hMSCs-EXOs increase the expression of Survivin and Bcl-2 which are both anti-apoptosis genes, while decreasing CCP3+ apoptotic cells. BMMSCs-EXOs and hMSCs-EXOs can also inhibit MAPK, p38, ERK, and turn on the AKT pathway. hESC-EXOs reduced the number of CCP3+ apoptotic cells, as well as suppressed Bax gene expression in a occlusal joint OA model. Exosomes containing lncRNA H19 reduced the Bax/Bcl-2 ratio in protein as well as mRNA levels in Osteoarthritis chondrocytes [13].

Exosomal RNAs may have anti-apoptotic properties as well. (Figure 2b). Exosomes derived from UCMSCs that contain the lncRNA H19 can play a vital role in blocking miR-29b-3p through being competitive intracellular RNA, thereby increasing the expression of FoxO3. FoxO3 then stimulates cartilage development in vitro by inhibiting chondrocyte apoptosis [14]. MiR-100-5p originated from IPFP-MSCs has the ability to prevent the production of mTOR protein, which can result in autophagy [3]. MiR-31-5p protects oxidatively stressed EPCs from ER-stress-induced apoptosis and calcification [15]. Through miR-100-5p, derived from hucMSCs-EXOs, targeting NADPH oxidase 4 (NOX4), it can inhibit apoptosis and reactive oxygen species (ROS) production in primary articular chondrocytes [16].

2.3. Influencing immune protection or immune destruction signals

Exosomes derived from MSC can reduce inflammation by inhibiting pro-inflammatory factor expression while increasing anti-inflammatory factor expression. MSCs may secrete interleukin IL-6, IL-8, (IL)-1β, MMP-13 as well as MMP-1 down regulate inflammatory signals in osteoarthritis cartilage, but can also increase anti-inflammatory IL-10. miR-129-5p inhibits IL-1β-mediated HMGB1 upregulation by targeting 3'UTR end of the gene, remarkably declines the apoptosis and inflammatory response of chondrocytes. The expression of proinflammatory markers like the interleukin NF-kB, (IL)-6, which are reduced through being injected with exosomes, while the expression of the anti-inflammatory cytokine IL10 is increased [17]. Mir-145 and mir-221 levels are higher in periosteal cells treated with exosomes, which can promote periosteal cell proliferation and chondrogenic potential, respectively [18]. Circulation 0001846 used the miR-149-5p/WNT5B axis to modulate IL-1β-induced chondrocyte cell damage. The knockdown of circ 0001846 inhibited IL-1β-mediated chondrocyte migration, proliferation, apoptosis, inflammation, invasion, and extracellular matrix degradation [19].

Furthermore, in osteoarthritic models, exosomes increase M2 macrophage polarization while decreasing M1 macrophage production. Proinflammatory M1-polarized macrophages can increase cartilage inflammation and degradation, whereas M2 macrophage polarization can significantly

reduce cartilage damage and facilitate repair. TGF-β1 stimulated BMSCs promote M2 SM polarisation by inhibiting MAPK6 expression via exosomal miR-135b, thereby improving cartilage damage [20].

In sum, MSC EXOs mainly promote chondrogenesis in OA models by a) enhancing extracellular matrix and preventing cartilage degradation, b) promoting chondrocyte migration, proliferation, and hypertrophy, as well as suppressing chondrocyte apoptosis, c) influencing immune protection or immune destruction signals (Table 2, Figure 2).

Table 2. Functional mechanism of MSC-EXOs in articular cartilage regeneration

Origin	Key Cargo	Target RNA, Protein or Pathway	Mechanical	Function	Ref
IPFP-MSCs	miR-100-5p	mTOR, PIK3	COL2A1 and aggrecan ↑ MMP13 and ADAMTS5 ↓	a, c	[3]
ESC-MSCs	CD73, IGF	AKT, ERK, AMPK	s-GAG matrix synthesis↑ NO and MMP13 ↓	a	[4]
BM-MSCs	miR-92a-3p	WNT 5A	aggrecan, COMP, and COL2A1↑	a	[5]
BM-MSCs	circRNA_0001236	miR-3677-3p/Sox9 axis	CoL2a1 and Sox9↑ MMP13↓	a	[6]
HS-MSCs	miR-129-5p	HMGB1, NF-κB	Inhibits IL-1β-mediated upregulation of HMGB1	c	[17]
BM-MSCs	miR-31-5p	ATF6	ER-stress-related apoptosis and calcification↓	b	[15]
BM-MSCs	miR-125a-5p	E2F2	COL2A1, aggrecan, and Sox9↑ MMP13↓	a	[7]
ADSCs	miR-145 miR-221	TnF-α	Collagen type II and β-catenin↑ TnF-α↓	b, c	[18]
HucMSCs	NM	miR-100-5p/NOX4 axis	Cyclic strain-induced ROS production↓ apoptosis ↓	b	[16]
BM-MSCs	miR-216a-5p	JAK2/STAT3	JAK2↓	b	[12]
MSC-EXOTGF-β1	miR-135b	Sp1	Sp1↓	b	[11]
SM-MSCs	Lnc RNA H19	MiR-106-5p/TIMP2 axis	CoL2a1 and ACAN↑ MMP13 and ADAMTS5↓	a, b	[8]
BMSC-EXOTGF-β1	miR-135b	MAPK6	M2 polarization of SMs↑	c	[20]
MSCs	Circ_0001846	miR-149-5p/WNT5B axis	Wnt5B↓	a, b, c	[19]
MSCs	circHIPK3	miR-124-3p/MYH9	COL2A1, SOX9 and Aggrecan↑ Runx2 and MMP-13 ↓	a, b	[10]

NM, not mention;

a. Enhancing ECM and preventing cartilage degradation;

b. Promoting chondrocyte migration, proliferation, hypertrophy, as well as suppressing chondrocyte apoptosis;

c. Influencing immune protection or immune destruction signals.

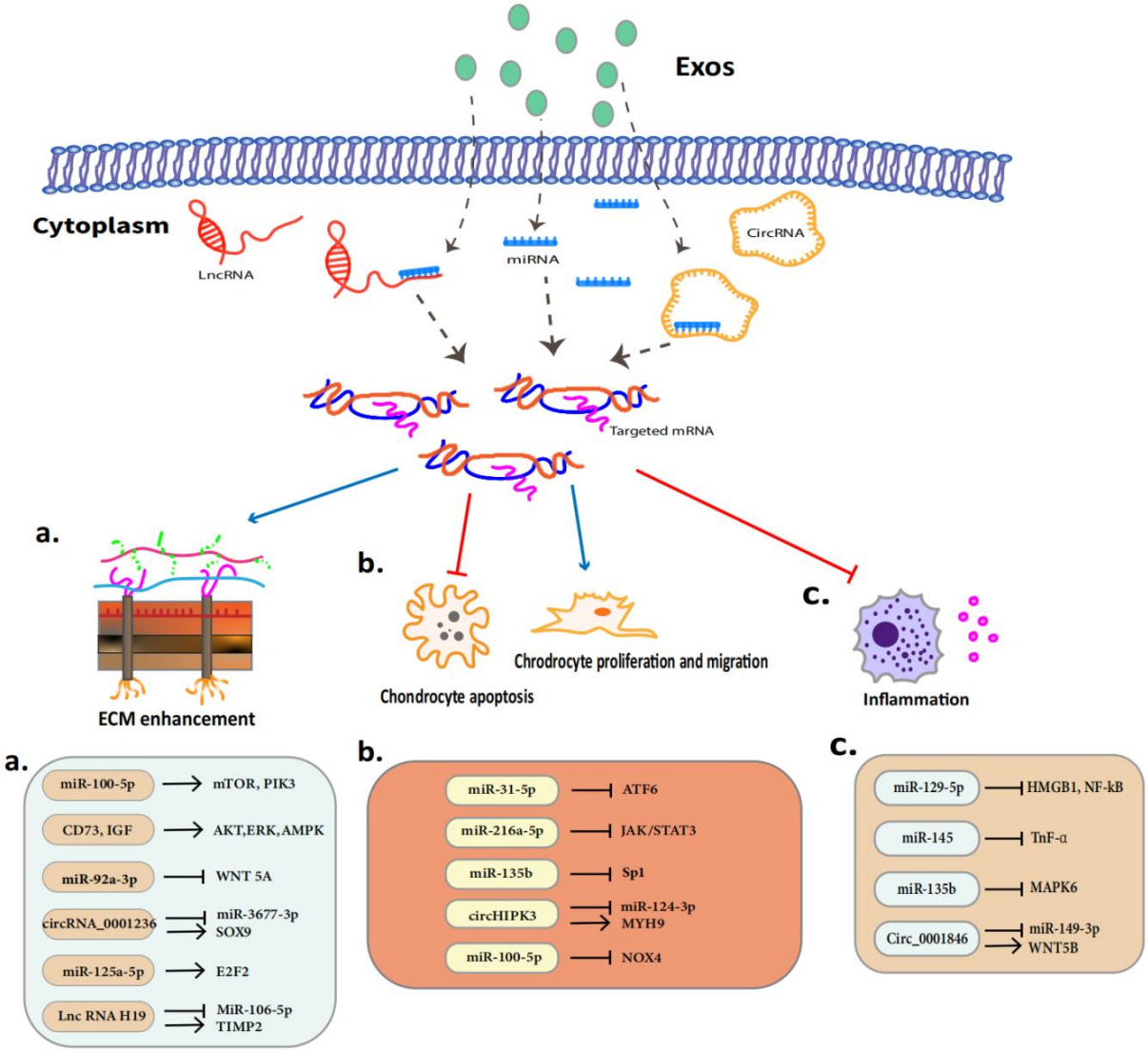

Figure 2. Functional Mechanism of MSC-EXOs in Articular Cartilage Regeneration.

(Photo credit: Original)

a. Enhancing ECM and preventing cartilage degradation;

b. Promoting chondrocyte migration, proliferation, hypertrophy, as well as suppressing chondrocyte apoptosis;

c. Influencing immune protection or immune destruction signals.

3. Discussion

Numerous researchers are now recommending cell-free therapy instead of traditional cell therapy for many reasons. The heterogeneity of living cells, in general, results in inconsistent efficacy. Long term expansion in vitro may result in cell aging as well as dedifferentiation, reducing therapeutic potential. In addition, stem cells are susceptible to tumorigenesis and mutation. Tumor formation is an impediment to the clinical application of iPSC and ESC. Extracorporeal therapy is a type of acellular therapy that can be used to compensate for the shortcomings of cell therapy. EXOs have the advantages of easier to process and store than cells, with lower production costs and less time required. Moreover, exosomes can be developed into nanoscale carriers for delivering therapeutic drugs to target cells. However, the disability of replication and reproduction in vivo are the two most significant disadvantages of exons. Furthermore, due to its short half-life, multiple doses are necessary in order to achieve the desired therapeutic effect. More importantly, no adverse events were

reported in the studies, indicating that the exon's immunogenicity is low and its immunomodulatory performance is excellent.

However, despite the fact that numerous preclinical researches have shown that EOXs can promote cartilage regeneration, the efficacy is still at the early stages. In addition, the precise mechanism and signal cascade of exosome-mediated cartilage repair and regeneration remain unclear. Therefore, more research should be done on the mechanism of action. Also, the study on the efficacy of EXOs in cartilage regeneration is still limited to small animal models. In animal models, the optimal source as well as dose of exosomes for osteoarthritis treatment have yet to be determined. Therefore, more clinical relevance should be used for future research in large animal models to investigate the dosage, safety, as well as effectiveness for exosomes treatment.

4. Conclusion

With the progress of treatment technology based on MSC-EXOs, the pathogenesis of early OA disease is gradually being effectively controlled. MSC treatment technology considerably modulate the pathological milieu and lead to increased repair of damaged articular tissue. MSC-EXOs, along with their multiple genetic cargos, effectively facilitate cell-cell interactions in the direction of extracellular matrix synthesis and degradation, as well as chondrocyte migration, proliferation, and apoptosis, reducing inflammation, thus facilitating recovery from knee OA, allowing for the restoration of damaged cartilage tissue. To obtain a consistent and optimal treatment result, exosome treatment must be standardized. As a result, more researches on OA treatment are required to be responsible for determining the best cell origin. culture conditions, exon dose, administration frequency, and administration method to achieve the best therapeutic effect while avoiding side effects. The current technology is expected to produce therapeutic EXO for OA to resolve the limitations of currently available OA drugs, thereby reducing symptoms and possibly regenerating lesioned joints.

References

[1] Zavatti M, Beretti F, Casciaro F, et al. Comparison of the therapeutic effect of amniotic fluid stem cells and their exosomes on monoiodoacetate-induced animal model of osteoarthritis[J]. Biofactors. 2020, 46 (1): 106 - 117.

[2] Jeyaraman M, Muthu S, Shehabaz S, et al. Current understanding of MSC-derived exosomes in the management of knee osteoarthritis[J]. Exp Cell Res. 2022: 113274.

[3] Wu J, Kuang L, Chen C, et al. miR-100-5p-abundant exosomes derived from infrapatellar fat pad MSCs protect articular cartilage and ameliorate gait abnormalities via inhibition of mTOR in osteoarthritis[J]. Biomaterials. 2019, 206: 87 - 100.

[4] Zhang S, Teo K Y W, Chuah S J, et al. MSC exosomes alleviate temporomandibular joint osteoarthritis by attenuating inflammation and restoring matrix homeostasis[J]. Biomaterials. 2019, 200: 35 - 47.

[5] Mao G, Zhang Z, Hu S, et al. Exosomes derived from miR-92a-3p-overexpressing human mesenchymal stem cells enhance chondrogenesis and suppress cartilage degradation via targeting WNT5A [J]. Stem Cell Res Ther. 2018, 9 (1): 1 - 13.

[6] Mao G, Xu Y, Long D, et al. Exosome-transported circRNA_0001236 enhances chondrogenesis and suppress cartilage degradation via the miR-3677-3p/Sox9 axis[J]. Stem Cell Res Ther. 2021, 12 (1): 1 - 14.

[7] Xia Q, Wang Q, Lin F, et al. miR-125a-5p-abundant exosomes derived from mesenchymal stem cells suppress chondrocyte degeneration via targeting E2F2 in traumatic osteoarthritis[J]. Bioengineered. 2021, 12 (2): 11225 - 11238.

[8] Tan F, Wang D, Yuan Z J I. The fibroblast-like synoviocyte derived exosomal long non-coding RNA H19 alleviates osteoarthritis progression through the miR-106b-5p/TIMP2 axis[J]. Inflammation. 2020, 43 (4): 1498 - 1509.

[9] Liu C, Li Y, Yang Z, et al. Kartogenin enhances the therapeutic effect of bone marrow mesenchymal stem cells derived exosomes in cartilage repair[J]. Nanomedicine (Lond). 2020, 15 (3): 273 - 288.

[10] Guo Z, Wang H, Zhao F, et al. Exosomal circ-BRWD1 contributes to osteoarthritis development through the modulation of miR-1277/TRAF6 axis [J]. Arthritis Res Ther. 2021, 23 (1): 1 - 14.

[11] Wang R, Xu B, Xu H J C C. TGF-β1 promoted chondrocyte proliferation by regulating Sp1 through MSC-exosomes derived miR-135b[J]. Cell Cycle. 2018, 17 (24): 2756 - 2765.

[12] Rong Y, Zhang J, Jiang D, et al. Hypoxic pretreatment of small extracellular vesicles mediates cartilage repair in osteoarthritis by delivering miR-216a-5p[J]. Acta Biomater. 2021, 122: 325 - 342.

[13] Yan L, Liu G, Wu X J J O O T. Exosomes derived from umbilical cord mesenchymal stem cells in mechanical environment show improved osteochondral activity via upregulation of LncRNA H19 [J]. Orthop Translat. 2021, 26: 111 - 120.

[14] Yan L, Liu G, Wu X J C, et al. The umbilical cord mesenchymal stem cell-derived exosomal lncRNA H19 improves osteochondral activity through miR-29b-3p/FoxO3 axis[J]. Clin Transl Med. 2021, 11 (1): e255.

[15] Xie L, Chen Z, Liu M, et al. MSC-derived exosomes protect vertebral endplate chondrocytes against apoptosis and calcification via the miR-31-5p/ATF6 axis[J]. Mol Ther Nucleic Acids. 2020, 22: 601 - 614.

[16] Li X, Wang Y, Cai Z, et al. Exosomes from human umbilical cord mesenchymal stem cells inhibit ROS production and cell apoptosis in human articular chondrocytes via the miR-100-5p/NOX4 axis [J]. Cell Biol Int. 2021, 45 (10): 2096 - 2106.

[17] Qiu M, Liu D, Fu Q J L S. MiR-129-5p shuttled by human synovial mesenchymal stem cell-derived exosomes relieves IL-1β induced osteoarthritis via targeting HMGB1[J]. Life Sci. 2021, 269: 118987.

[18] Zhao C, Chen J Y, Peng W M, et al. Exosomes from adipose-derived stem cells promote chondrogenesis and suppress inflammation by upregulating miR-145 and miR-221[J]. Mol Med Rep. 2020, 21 (4): 1881 - 1889.

[19] Zhu C, Shen K, Zhou W, et al. Exosome-mediated circ_0001846 participates in IL-1β-induced chondrocyte cell damage by miR-149-5p-dependent regulation of WNT5B [J]. Clin Immunol. 2021, 232: 108856.

[20] Wang R, Xu B J C, Research T. TGF-β1-modified MSC-derived exosomal miR-135b attenuates cartilage injury via promoting M2 synovial macrophage polarization by targeting MAPK6 [J]. Cell Tissue Res. 2021, 384 (1): 113 - 127.