


Compositional Variation and Functional Mechanism of Exosomes in the Articular Microenvironment in Knee Osteoarthritis

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Abstract

Osteoarthritis (OA) is a major cause of disability worldwide with increasing age. Knee OA (KOA) is the most prevalent type of OA. Recently, it is considered that KOA is a whole joint disease, including articular cartilage, subchondral bone, synovium, ligaments, joint capsules, and muscles around the joint. Exosomes in knee joint are mainly secreted by articular chondrocytes and synoviocytes. They participate in cell and tissue cross-talk by carrying a complex cargo of proteins, lipids, nucleic acids, etc. Under normal conditions, exosomes maintain the microenvironmental homeostasis of the joint cavity. Under pathological conditions, the composition and function of exosomes changes, which in turn, disrupts the balance of anabolism and catabolism of articular chondrocyte and facilitates inflammatory responses, thus accelerating KOA progression. As a regenerative medicine, mesenchymal stem cells (MSCs) are promised to facilitate repair of degenerated cartilage and decelerate OA process. The therapeutic function of MSC mainly depends on MSC-derived exosomes, which can restore the homeostasis of the articular microenvironment. In the future, the specific mechanism of exosomes for OA treatment needs further elucidation, and the treatment effect of exosomes for long-term and/or severe OA needs further exploration.

Keywords

osteoarthritis, exosomes, articular, microenvironment

Introduction

Osteoarthritis (OA) is a chronic musculoskeletal degenerative disease, which accompanies the symptoms of joint swelling, pain, and stiffness. The major risk factor includes ageing, prior joint trauma, gender, mechanical stress, and genetics. The complicated pathogenesis of OA is mainly characterized by articular cartilage degeneration, synovial inflammation, subchondral bone deterioration, osteophyte formation, ligament degeneration, and hypertrophy of the joint capsule^{1,2}. OA has become more common with ageing and the estimated number of patients has reached 250 million worldwide³. This disease seriously affects the quality of patient life, and the prevention and treatment of OA have led to an extreme financial burden to individuals and society^{3,4}. Nowadays, Knee OA (KOA) is the most prevalent type of OA disease in society.

As the vital component of extracellular vesicles (EVs), exosomes play important and complicated roles in the process and treatment of KOA. Increasing evidence has demonstrated that exosomes are involved in a variety of pathological mechanism, including extracellular matrix (ECM) degradation, inflammation, regulation of angiogenesis, and antigen presenting^{5,6}.

Recently, exosomes in the research of pathogenesis and treatment on KOA has got more and more attention. Exosomes have dual roles in maintaining the microenvironmental homeostasis of the joint cavity. In this review, we discuss the current

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research of exosomes in the biological, pathological, and therapeutic actions of knee joint.

Articular Microenvironment and KOA

With the progress of research, KOA is not only a single disease of articular cartilage degeneration but an organ-related disease affecting whole joint tissues, including articular cartilage, subchondral bone, synovium, meniscus, ligaments, joint capsules, and periarticular muscles⁷⁻⁹. Although meniscus dysfunction may be present in asymptomatic knees or in knees with no evidence of OA, meniscal degeneration is one of the most vital risk factors for KOA, and partial meniscectomy is the most common type of orthopedic surgery for inducing KOA animals and promotes KOA progression including cartilage damage and synovitis⁹⁻¹³. In the process of KOA, pathological changes mainly include cartilage degeneration, synovial inflammation, subchondral sclerosis, and osteophyte formation¹⁴. During the early stages of KOA, the modified superficial layer of articular cartilage gradually develops to affect the deep cartilage¹⁵. At the same time, the disequilibrium of anabolism and catabolism of the chondrocyte ECM disrupts the articular cartilage¹⁶. Further, these pathological changes stimulate synovitis and synovial hyperplasia, resulting in increased release of inflammatory factors from synovial cells, which further damages the cartilage¹⁷. Without any intervention, such changes will gradually deteriorate the joint functionality and promote disease progression^{18,19}. The pathology of subchondral bone deterioration involves in the functional imbalance of osteoblasts and osteoclasts²⁰. Osteophyte is caused by intrachondral osteogenesis according to the induction of inflammatory factors in the joints and their abnormal activity at the site²¹.

The homeostasis of the articular microenvironment in KOA is destroyed and accompanied by increased TNF- α , IL-1 β , IL-6, MMP-13, etc^{22,23}. The tissues in the joint are in concordance with articular microenvironment. Therefore, KOA is a disease in which the articular microenvironment is dysregulated or even disordered. Cells *in vivo* secrete EVs into the local environment to affect the microenvironment²⁴. Such EVs are classified into three groups: exosomes, microvesicles, and apoptotic bodies; among them, exosomes and cellular microvesicles are secreted by living cells^{24,25}. The microvesicles are derived from outward budding of the plasma membrane, while exosomes are produced through the inward invagination of the endosomal membrane pathway. Consequently, a specific subset of endosomal proteins has been identified in exosomes²⁵. Exosomes are found in bodily fluids including blood, urine, cerebrospinal liquid, breast milk, and saliva^{26,27}. In the case of KOA, variation in the composition of exosomes helps to understand the disease mechanism, and the effect of specific exosomes on the articular microenvironment provides new insights for the treatment of disease.

Exosomes

The diameter of exosomes ranges from 40 to 160 nm (average 100 nm) enclosed by a phospholipid bilayer. Exosomes contain large amounts of protein, lipid, DNA, RNA, and amino acid metabolite²⁸. They are first produced by endocytosis of the cell membrane and undergo further processing before being released from parent cells via membrane fusion²⁹. After their release, they communicate with neighboring cells or distant cells through endocrine and paracrine pathways. Exosomes can selectively target recipient cells either through direct fusion with the plasma membrane, or by interacting with receptors, or through endocytosis and fusion with endosomes³⁰. Therefore, exosomes can be employed as important signal messengers and carriers to transmit signals to the corresponding target cells, then change their physiological functions and status. Also, exosomes can be used as vehicles for cell excretion to eliminate unused or harmful RNA and proteins.

It was reported that a variety of exosomes from heart cells were involved in angiogenesis, cell migration, proliferation, apoptosis, hypertrophy, and regeneration. Nevertheless, the number and content of exosomes in circulation are different among cells from various sources and in alternate internal environment, such as hypoxia³¹. Similarly, in articular tissues, exosomes derived from different cells are involved in various pathological mechanisms, including ECM metabolism and regulation of inflammation^{7,32,33}. At present, the function of exosomes is limited to preclinical studies and there is no report of clinical case for OA treatment.

The separation of exosomes with high purity is critical to understanding their action mechanisms and for their further application in preclinical and clinical treatment. Currently, the methods of isolation exosomes include ultracentrifugation divided into differential ultracentrifugation and density gradient ultracentrifugation, size-based ultrafiltration, size-exclusion chromatography, polymer precipitation, immunoaffinity purification, and microfluidics-based isolation techniques^{24,34,35}. Each method has its advantage and disadvantage, adapts to different situations. For example, ultracentrifugation is a conventional method suitable for large sample volumes, but not for clinical samples^{35,36}. These methods need to be further improved to promote the application of exosomes. Different methods of exosome separation have no significant differences in exosome characteristics and therapeutic effects for OA treatment in preclinical research^{37,38}.

Exosomes Derived From Articular Chondrocytes and KOA

The lack of blood and lymphatic system in the cartilage tissue makes it difficult to self-repair after injury. Instead, exosomes derived from normal chondrocyte help in maintaining the stability of chondrocytes and their surrounding microenvironment. Chen et al. found that exosomes derived

from normal chondrocytes promote cartilage formation by regulating chondrocyte precursor cells. While the chondrocyte exosomes were added to tissue engineering scaffold, they promoted the formation of regenerated cartilage through the TGF- β /SMAD signaling pathway, where SOX-9 and COL-II were stably expressed³⁹. The study of Liu et al. showed that exosomes derived from articular chondrocytes protected chondrocytes from destruction by down-regulating inflammatory factors and promoted the synthesis of aggrecan and COL-II of ECM, and the exosomes could promote the chondrogenic differentiation of C3H10T1/2 cells⁴⁰. Mao et al. studied the miRNA expression profiling assays in exosomes and found that the expression of miR-95-5p in normal human chondrocyte exosomes was higher than that of KOA patients. Further study showed that miR-95-5p directly targeted HDAC2/8, regulated cartilage development and homeostasis, leading to the elevated expression of Ach3, aggrecan, and Col2A1. Exosomes derived from primary chondrocytes overexpressing miR-95-5p promoted chondrogenic differentiation of mesenchymal stem cells (MSCs), cartilage development, and increased ECM expression in chondrocytes⁴¹. The exosomes produced by chondrocytes act on not only near chondrocytes and chondrocyte precursor cells through the paracrine pathway but also other type cells from other tissues in the articular microenvironment. Zheng et al. found that proteins belonged to mitochondrion were enriched in immune system processes in the exosomes derived from primary articular chondrocytes of C57BL/6 mice compared with those induced by IL-1 β . They validated that exosomes derived from normal chondrocytes eliminated the mitochondrial dysfunction of chondrocytes induced by IL-1 β . Through intra-articular injection, they found that exosomes from normal chondrocytes could repair damaged mitochondria in the chondrocytes from KOA mice induced by anterior cruciate ligament transection surgery in both knee joints. The exosomes promoted the differentiation of synovial macrophages from M1 into M2 type, thereby effectively treating KOA and delaying KOA progression⁴².

Exosomes derived from articular chondrocytes of patients with KOA inhibited ECM formation of chondrocyte by enhancing the release of MMP-1 and MMP-13⁴³. Liu et al. found that exosomes derived from chondrocytes precursor cells induced by IL-1 β promoted the chondrocyte catabolism and inhibited the cartilage formation⁴⁰. Mao reported that exosomes derived from chondrocytes transfected with an antisense inhibitor of miR-95-5p prevented chondrogenic differentiation and reduced cartilage matrix synthesis by enhancing the expression of HDAC2/8⁴¹. Exosomes derived from KOA articular chondrocytes also affect other surrounding tissues and cells in articular cavity. Ni et al. showed that the expression of miR-449a-5p was increased in exosomes derived from KOA chondrocytes. They demonstrated that miR-449a-5p inhibited autophagy of synovial macrophages and increased the release of IL-1 β by suppressing the expression of ATG4B, which in turn, increased inflammation in

KOA and promoted KOA⁴⁴. Besides, it was reported the osteoarthritic chondrocytes showed increasing levels of Cx43 within their exosomes compared to those isolated from healthy donors. Cx43-positive exosomes released by osteoarthritic chondrocytes were involved in the spread of cellular senescence, inflammatory factors, and responsible for the progression of OA among cartilage, synovium, and subchondral bone⁴⁵. Therefore, with the changes in the articular microenvironment of KOA patients, the composition of exosomes derived from articular chondrocyte was changed, thereby destroying the cartilage homeostasis and aggravating the OA progression. However, the changes in the specific composition and corresponding mechanisms of exosome need further exploration (summarized in Table 1).

Exosomes Derived From Synoviocytes and KOA

Synoviocytes mainly referred to as synovial fibroblasts (SFB) and synovial macrophages, and SFB is the main cell type in synovium tissues. Under normal conditions, exosomes derived from synoviocytes were released into articular microenvironment and maintain the homeostasis; nevertheless, under osteoarthritic conditions, exosomes derived from synoviocytes cause the imbalance of anabolism and catabolism of chondrocyte in articular cartilage, and promote the progression of KOA⁴⁶. Treatment with exosomes from IL-1 β stimulated SFB compared to non-stimulated SFB, significantly increased the expression of MMP13 and ADAMTS5, and decreased the expression of COL2A1 and ACAN in the articular chondrocytes. Besides, the proteoglycan release was increased from cartilage explants, and migration and tube formation activity were significantly higher in human umbilical vein endothelial cells. The result suggested that exosomes promoted KOA progression in the inflammatory microenvironment. The NanoString analysis showed that levels of 50 miRNAs were differentially expressed in exosomes from IL-1 β stimulated SFB compared to non-stimulated SFB⁴⁷ (Table 1). Tan et al. investigated the cellular processes of human chondrocytes regulated by FBS-derived exosomes and found the exosomal lncRNA H19-mediated regulation of the miR-106b-5p/TIMP2 axis⁴⁸. In the cases related to KOA, M1 type macrophages can present antigens, activate Th1 responses, and promote inflammation. On the contrary, M2 type macrophages have anti-inflammatory effects and promote cartilage repair by inhibiting inflammation⁴⁹. To date, the effect of macrophage-derived exosomes on OA has not been reported.

The Role and Diagnostic Value of Exosomes in Synovial Fluid in KOA

With the progress of exosome research, the role of exosomes in synovial fluid is being gradually recognized, especially in cartilage homeostasis of KOA⁵⁰. Exosomes in the synovial fluid of healthy joints derive from cells in

Table 1. The Variation and Function of Exosomes Derived From the Articular Microenvironment of Normal and Osteoarthritic Conditions.

Origin	Comparison	Compositional variation	Function	Mechanism	Reference
AC	NC versus OA patients	miR-95-5p	Promote chondrogenic differentiation and cartilage matrix synthesis <i>in vitro</i>	Target HDAC2/8	29
AC	Normal CC versus CC treated with IL-1 β	Proteins belonged to mitochondrion involved in immune system processes	Restore mitochondrial dysfunction in CC <i>in vitro</i> and <i>in vivo</i> ; prevent OA process and polarize macrophage to M2 <i>in vivo</i>	NM	30
SFB	IL-1 β stimulated SFB compared with normal SFB	miR-518-3d, 623, 1537, 532-3p, 1306-3p, 548b-5p, etc.	Inhibits ECM production in AC <i>in vitro</i>	NM	35
SFB	NC versus OA patients	LncRNA H19	Promote chondrocyte proliferation and migration and inhibit matrix degradation <i>in vitro</i>	Target miR-106b-5p/TIMP2 axis	
SF	Late-stage OA patients versus early-stage OA patients	IL1 β , IL2, IL4, IL5, IL6, IL-13, IL17, TNF- α , IFN- γ (cytokines) CCL2, CCL3, CCL15, CXCL8, CXCL9, CXCL12 (chemokines)	Activate PBMC chemotaxis and inhibit chondrocyte proliferation <i>in vitro</i> ; predicted to recruit inflammatory cells and promote joint degeneration <i>in vivo</i>	NM	39
SF	NC versus OA patients (female and male, respectively)	miR-24-3p, 23a-3p, 26a-5p, 4487, 6821-5p, 4508, 4532, 7107-5p, etc. (female specific); miR-6878-3p, 5090, 4749-5p, etc. (male specific)	Predicted that female OA-specific miRNAs are estrogen responsive and target TLR signaling pathways <i>in vivo</i>	NM	40
SF	OA patients versus NC	miR-200c	NM	NM	41
SF	Different stages of OA patients	lncRNA PCGEM1	A biomarker from the early-stage OA to the late-stage OA in patients	Targeted miR-770 as a sponge	42,43

AC: articular chondrocytes; CC: chondrocytes; ECM: extracellular matrix; HUVEC: human umbilical vein endothelial cells; NC: normal controls; NM: not mentioned; OA: osteoarthritis; PBMC: peripheral blood mononuclear cell; SF: synovial fluid; SFB: synovial fibroblasts.

surrounding tissues, mainly including articular cartilage and synovium, and act as valuable cargoes to exchange information between cells. However, with the progression of KOA, the amount and content of exosomes in synovial fluids change drastically and result in adverse effects. Domenis et al. found that exosomes derived from KOA synovial fluid can significantly stimulate M1 macrophages to release IL-1 β , chemokines (CCL8, CCL15, CCL20, and CXCL1), and metalloproteinases (MMP12 and MMP7), thus exacerbating inflammation reaction and cartilage degeneration⁵¹. Gao et al. reported that exosomes from end-stage KOA patients had a higher level of cytokines and chemokines in comparison to profiles of free cytokines and chemokines in soluble synovial fluids. Some specific cytokines and chemokines were elevated in the exosomes of the end-stage patients with KOA compared to the early-stage group (Table 1). Exosomes derived from synovial fluids of end-stage patients recruited inflammatory cells and inhibited cartilage proliferation, thus promoting joint degeneration. This study provided a new perspective for understanding the changes in the internal environment of KOA⁵². Microarray analysis of miRNA from exosomes derived from synovial fluids of KOA patients and healthy

controls showed that exosomal miRNA content was altered in KOA patients and the changes were gender specific. Bioinformatics analysis revealed that female-specific miRNAs in the synovial fluid of exosomes were sensitive to estrogen and participated in Toll-like receptor signaling pathways. Further functional experiments showed that the expression of anabolic genes was decreased, while the expression of catabolic and inflammatory genes was elevated in articular chondrocytes treated with KOA synovial fluid exosomes. Therefore, miRNAs in the synovial fluid exosomes of female patients might be associated with a high incidence of KOA⁵³. The differently expressed cytokines or miRNAs may serve as a potential diagnostic marker for tracking the development and progression of OA⁵²⁻⁵⁴ (Table 1). Zhao et al. firstly analyzed the diagnostic value of the differential components of exosomes in joint fluids. They found that lncRNA PCGEM1 was differentially expressed between the early and late stages of the OA group and the area value under the receiver operating characteristic curve was 0.879, which implied that lncRNA PCGEM1 was a powerful indicator in distinguishing early OA from late-stage OA⁵⁵. Before this study, Kang et al. had reported that lncRNA PCGEM1 could act as a sponge

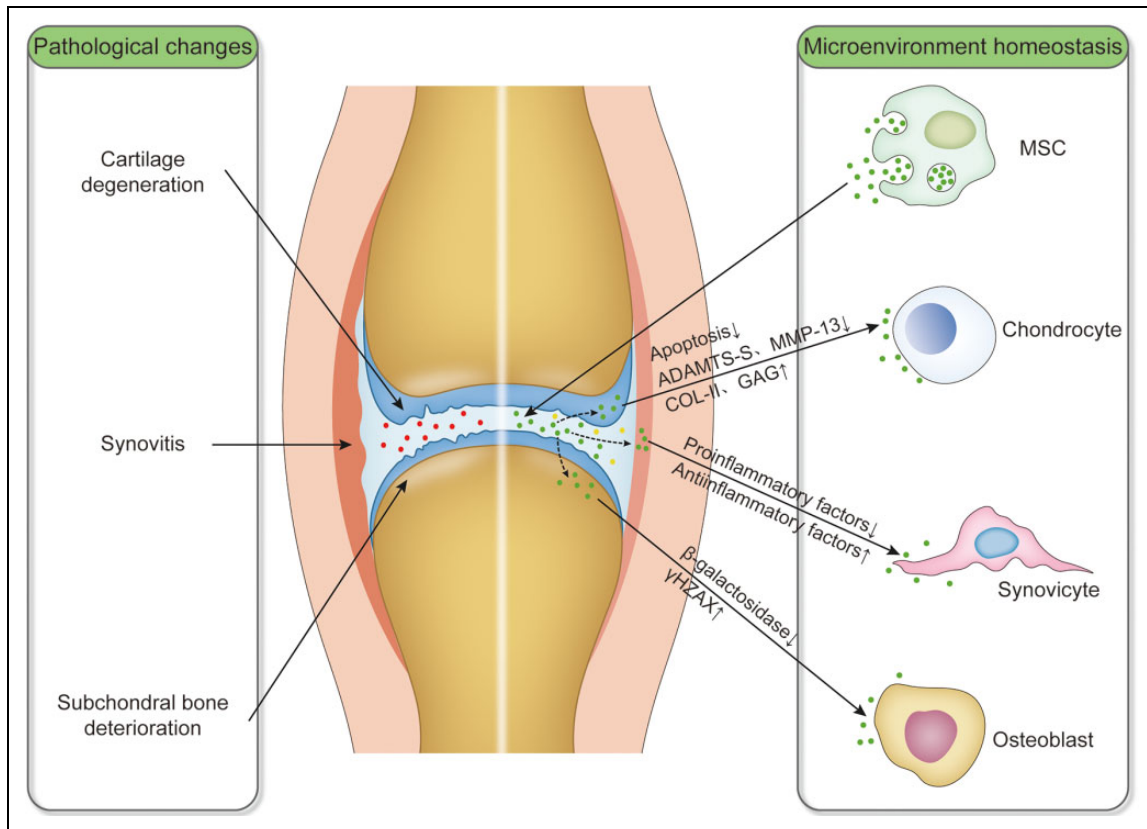


Figure 1. Schematic diagram of intra-articular injection of exosomes derived from MSC that act on articular chondrocytes, synoviocytes, osteoblasts, and maintain articular microenvironment homeostasis. The pathological changes of OA involved in cartilage degeneration, synovitis, and subchondral bone deterioration. Exosomes derived from MSC alter the component contents of synovial fluid. Red dots: exosomes in synovial fluid of OA; green dots: exosomes derived from MSC; yellow dots: exosomes of altered composition in OA after intra-articular injection of exosomes derived from MSC; down arrows: downregulation; and up arrows: upregulation. MSC: mesenchymal stem cell; OA: osteoarthritis.

lncRNA that targeted miR-770 and stimulated the proliferation of osteoarthritic synoviocytes⁵⁶ (Table 1).

Exosomes Derived From MSCs and OA Treatment

Both preclinical and clinical trials have shown that the application of MSC had great potential in the treatment of OA. MSC not only plays a role in free radical scavenging, immune regulation, and cell proliferation but also in tissue homeostasis^{2,57}. Many pieces of evidence showed that the therapeutic function of MSC mainly depended on their paracrine mechanism and the MSC-derived exosomes played a crucial role in promoting its therapeutic effects^{58–62}. Therefore, MSC-derived exosomes from various sources had been reported to maintain the homeostasis of articular microenvironment during the treatment of OA (Fig. 1).

The preclinical therapy of exosomes derived from MSCs on OA and osteochondral defects is mainly applied to mouse and rat OA animals. Exosomes derived from MSCs can inhibit inflammation and apoptosis of chondrocytes, downregulated oxidative stress and senescence of osteoblasts, protect

cartilage and bone from degradation, ameliorate gait abnormality, promote cartilage regeneration, and impede OA progression^{37,38,58–61,63–69}. The therapeutic effects and molecular mechanisms of exosomes derived from MSCs for the treatment of OA and osteochondral defects *in vivo* and *in vitro* are summarized in Table 2. Exosomes targeted articular chondrocytes by regulating p38/ERK/Akt, miR-92a-3p/WNT5A, TGF- β 1/miR-135b/Sp1, lncRNA-KLF3-AS1/miR-206/GIT1 axis, and autophagy-related mTOR signaling pathway, which leads to increased synthesis of COL-II and proteoglycans and reduced production of ADAMTS-5 and MMP-13, thereby inhibiting the apoptosis of target chondrocytes and remodeling the stability of cartilage for KOA therapies^{37,63–66,69} (Table 2). In cardiovascular disease, exosomes derived from MSCs target macrophages by regulating miR-182 and activating S1P/SK1/S1PR1 signaling to initiate their switch from the M1 to M2 phenotype, thereby reducing the release of inflammatory factors and exerting anti-inflammatory effects^{70–72}. Although no studies report about MSC-derived exosomes acting on synovial macrophages in the articular cavity, it is accepted that the

Table 2. Therapeutic Effects and Molecular Mechanisms of Exosomes Derived From MSCs for the treatment of OA and Osteochondral Defects Treatment *In Vivo* and *In Vitro*.

Origin	Isolation methods	Subjects	Therapeutic effects and molecular mechanisms	References
Human MSCs (HuES9)	HPLC fractionation	<i>In vivo</i> : rat osteochondral defects created on the trochlear grooves of the distal femurs. <i>In vitro</i> : NM	<i>In vivo</i> : Exosome-treated defects showed enhanced gross appearance and improved histological scores than the contralateral PBS-treated defects. <i>In vitro</i> : NM	59
Human MSCs (EI-MYC 16.3)	Ultrafiltration	<i>In vivo</i> : rat osteochondral defects created on the trochlear grooves of the distal femurs. <i>In vitro</i> : PRAC	<i>In vivo</i> : exosomes increased cellular proliferation and infiltration, enhanced matrix synthesis and a regenerative immune phenotype <i>In vitro</i> : CD73 in exosomes activated AKT and ERK signaling pathway	58
Human SMSCs	Ultrafiltration	<i>In vivo</i> : KOA rats induced by ACLT and MMT. <i>In vitro</i> : PHC	<i>In vivo</i> : SMSC-140-Exos prevented OA better than SMSC-Exos. <i>In vitro</i> : SMSC-140-Exos enhanced the proliferation and migration of ACs without damaging ECM secretion via RalA	61
Human MSCs	Density gradient ultracentrifugation	<i>In vivo</i> : KOA rats induced by type II collagenase. <i>In vitro</i> : PRAC treated with IL-1 β	<i>In vivo</i> : MSCs exosomes promoted cartilage repair better than those knocking down lncRNA-KLF3-AS1. <i>In vivo</i> : lncRNA KLF3-AS1 enriched in exosomes promoted cell proliferation and suppressed apoptosis	63
Rat MSC	Extraction kit	<i>In vivo</i> : KOA rats induced by medial collateral ligament and MMT. <i>In vitro</i> : rat chondrocyte line (C5.18 cell)	<i>In vivo</i> : exosomes treated with TGF- β 1 promoted cartilage repair better and miR-135b inhibitor inhibited the therapeutic effects. <i>In vitro</i> : TGF- β 1 promoted chondrocyte proliferation by regulating Sp1 through miR-135b enriched in MSC exosomes	64
Human ESC-MSCs	Differential ultracentrifugation	<i>In vivo</i> : KOA mice induced by DMM. <i>In vitro</i> : PMACs treated with IL-1 β	<i>In vivo</i> : protected cartilage and bone from degradation. <i>In vitro</i> : exerted similar chondroprotective and anti-inflammatory	60
Murine BMSCs	Differential ultracentrifugation	<i>In vivo</i> : KOA mice induced by type II collagenase. <i>In vitro</i> : PMAC treated with IL-1 β	<i>In vivo</i> : impeded cartilage destruction and OA process. <i>In vitro</i> : maintained chondrocyte phenotype by increasing Col2a1 synthesis and decreasing ADAMTS5 expression	38
Human BMSCs	Differential ultracentrifugation	<i>In vivo</i> : KOA mice induced by type II collagenase. <i>In vitro</i> : human MSCs, normal and OA PHC	<i>In vivo</i> : MSC-92a-Exos inhibited the progression of early OA and prevented articular cartilage damage better than MSC-Exos. <i>In vitro</i> : MSC-92a-Exos promoted chondrocyte proliferation and matrix genes expression better, and targeted WNT5A expression	65
Human IPFP MSCs	ExoQuick™ (EQ) reagent kit (SBI) and ultrafiltration	<i>In vivo</i> : KOA mice induced by DMM in right knees. <i>In vitro</i> : PHC treated with IL-1 β	<i>In vivo</i> : protected AC from damage and ameliorated gait abnormality; miR-100-5p in exosomes targeted mTOR pathway. <i>In vitro</i> : inhibited cell apoptosis, enhanced matrix synthesis, and enhanced autophagy level partially via mTOR inhibition	37
Human MSCs	Density gradient ultracentrifugation	<i>In vivo</i> : KOA mice induced by type II collagenase. <i>In vitro</i> : PMAC treated with IL-1 β , KOA articular chondrocytes	<i>In vivo</i> : NM. <i>In vitro</i> : increased Col2a1 and aggrecan, and decreased MMP13 and RUX2 expression in KOA chondrocytes; attenuated apoptosis in KOA articular chondrocyte; lncRNA-KLF3-AS1 in exosomes targeted miR-206/GIT1 axis	66

(continued)

Table 2. (continued)

Origin	Isolation methods	Subjects	Therapeutic effects and molecular mechanisms	References
Human ADSCs	Differential ultracentrifugation	<i>In vivo</i> : NM. <i>In vitro</i> : osteoblasts of osteoarthritic patients	<i>In vivo</i> : NM. <i>In vitro</i> : anti-inflammation, and downregulation oxidative stress and senescence in osteoblasts	67
Human BMSCs	Differential ultracentrifugation	<i>In vivo</i> : NM. <i>In vitro</i> : PMAC treated with TNF- α	<i>In vivo</i> : NM. <i>In vitro</i> : inhibited adverse effects of inflammation on cartilage homeostasis and promoted cartilage regeneration	68
Rabbit BMSCs	Ultrafiltration	<i>In vivo</i> : NM. <i>In vitro</i> : primary rabbit chondrocytes	<i>In vivo</i> : NM. <i>In vitro</i> : inhibited MS-induced apoptosis of chondrocytes via p38, ERK, and Akt pathways	69

AC: articular cartilage; ACLT: anterior cruciate ligament transection; ADSCs: adipose-derived MSCs; DMM: destabilization of the medial meniscus; ECM: extracellular matrix; HPLC: high-performance liquid chromatography; IFFP: infrapatellar fat pad MSCs; KOA: knee OA; MMT: medial meniscus transection; MS: mitochondrial dysfunction; MSC: mesenchymal stem cells; MSC-92a-Exos: exosomes derived from BMSCs overexpressing miR-92a-3p; NM: not mentioned; OA: osteoarthritis; PHC: primary human chondrocytes; PMAC: primary mouse articular chondrocytes; PRAC: primary rat articular chondrocytes; SMSCs: synovial MSCs; SMSC-140-Exos: exosomes derived from SMSCs overexpressing miR-140-5p.

exosomes play an important role in regulating macrophage polarization. Exosomes derived from MSCs also target osteoblasts by regulating β -galactosidase involved in aging, to increase the accumulation of γ H2AX, thereby reducing the release of inflammatory mediators for the treatment of KOA⁶⁷.

Therefore, exosomes derived from MSCs showed efficient therapeutic effects on OA and osteochondral defects as similar to MSCs. There are several advantages of exosomes derived from MSCs compared with MSCs for the treatment. Firstly, exosomes can be stored for 2 years at -80°C without loss of biological activity, facilitating the use as an off-the-shelf regenerative medicine⁷³. On the contrary, the storage and transportation of viable MSCs are relatively tricky for clinical application. Secondly, the use of exosomes is safer than cell-based therapy. The viable transplanted MSCs cannot be removed in the event of adverse activity, while exosomes are not permanent and can be easily stopped during the therapeutic process. In addition, the secreted exosomes derived from genetically modified MSCs with viral vectors are small and relatively safer. Thirdly, the quality and quantity of exosomes are accessibly controlled. Exosomes can be produced at a specific growth or differentiated time point of MSCs under controlled conditions, avoiding poor viability or senescence stage following multiple passages of cells. Further, exosomes can be characterized and quantified by nanoparticle tracking analysis, dynamic light scattering, morphological analysis, and protein analysis, facilitating developing therapeutic standards^{24,25}.

However, there are limitations in translating exosomes as regenerative medicine for clinical practice. Firstly, none isolated method can ultimately retain the nature of exosomes. Secondly, it is difficult to yield sufficient quantities of exosomes for animal studies and human clinical trials⁷⁴. It was reported that 1 l of MSC-conditioned media from a total of

about 60 million MSCs yields about 1–2 mg (protein content) EVs, sufficient for experimentation in only a few mice⁷⁵. Nonetheless, compared with direct cell transplantation, exosomes derived from MSCs provide a much simpler, safer, more practical, and more easily controlled solution for KOA treatment.

In short, MSC-derived exosomes can act on intra-articular cells by regulating the anabolism and catabolism of cartilage matrix, thereby improving the inflammatory environment in the joint, and then remodeling the joint homeostasis for the treatment of KOA. In the future, exosomes can also be used as a drug-delivery vehicle or as compound exosomes in tissue engineering scaffolds for OA therapies.

Conclusion and Perspective

KOA is a common joint disease, and at present, the treatment of KOA mainly depends on the drug and surgery treatment. Nevertheless, these treatments still face many problems, such as drug side effects, surgical complications, and financial burden. The steady state of the microenvironment in the joint cavity is crucial to the occurrence and progression of KOA. Exosomes derived from joint tissues are the key factors for microenvironmental homeostasis. Exosomes in normal joints maintain the balance of articular microenvironment; nevertheless, exosomes in OA joints disrupt the balance and further aggravate the disease. Injecting exosomes derived from MSCs into the joint cavity will change the molecular composition and improve the microenvironment of the joint cavity. The specific trophic factors released from exosomes could administrate anti-inflammation and immunoregulation, restore homeostasis of ECM, and promote cartilage regeneration, so that the exosomes repair articular cartilage, ameliorate the symptoms of KOA, and delay the progress of the disease. Although great breakthroughs have been achieved in the research based on exosomal treatment for KOA, the specific mechanism for the

treatment still needs to be further elucidated. For example, the study of exosomes involved in the pathological processes of subchondral bone sclerosis and osteophyte formation is not clear. Also, exosomal treatment has good results on early-stage KOA treatment in the short term, and the effect on long-term and severe KOA needs further observation and exploration.

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