Exosomes rewire the cartilage microenvironment in osteoarthritis: from intercellular communication to therapeutic strategies

Yuangang Wu¹, Jiao Li², Yi Zeng¹, Wenchen Pu², Xiaoyu Mu², Kaibo Sun¹, Yong Peng²✉ and Bin Shen¹✉

Osteoarthritis (OA) is a prevalent degenerative joint disease characterized by cartilage loss and accounts for a major source of pain and disability worldwide. However, effective strategies for cartilage repair are lacking, and patients with advanced OA usually need joint replacement. Better comprehending OA pathogenesis may lead to transformative therapeutics. Recently studies have reported that exosomes act as a new means of cell-to-cell communication by delivering multiple bioactive molecules to create a particular microenvironment that tunes cartilage behavior. Specifically, exosome cargos, such as noncoding RNAs (ncRNAs) and proteins, play a crucial role in OA progression by regulating the proliferation, apoptosis, autophagy, and inflammatory response of joint cells, rendering them promising candidates for OA monitoring and treatment. This review systematically summarizes the current insight regarding the biogenesis and function of exosomes and their potential as therapeutic tools targeting cell-to-cell communication in OA, suggesting new realms to improve OA management.

INTRODUCTION
Osteoarthritis (OA) is a chronic low-degree inflammatory disease mainly characterized by progressive degeneration of articular cartilage, thickening of the subchondral bone, synovial inflammation, meniscus and ligament degeneration, and osteophyte formation. The well-established risk factors for OA include age, sex, obesity, trauma, metabolism, and joint biomechanics. The chronic pain and dysfunction caused by OA affect over 250 million people worldwide, which severely reduces the life quality of individuals and represents a considerable socioeconomic burden. Currently, drug therapy serves as a fundamental strategy in the overall treatment of OA. Throughout the whole treatment process, most patients need short-term or long-term medication, including nonsteroidal anti-inflammatory drugs (NSAIDs), opioids, and drugs for intra-articular injection (e.g., hyaluronic acid and glucocorticoid). However, the current drug treatment of OA suggested by international guidelines is merely aimed at remission of disease symptoms, without substantial interruption of the destructive process or restoration of lesioned cartilage in OA. As for patients with end-stage OA, joint arthroplasty surgery represents a prevalent treatment modality, although sometimes the functional outcome can be unsatisfactory. Moreover, joint replacement requires more revision surgery in the case of complications such as infection and prosthetic fracture. Therefore, it is essential to clarify the molecular mechanisms underlying OA occurrence and progression to facilitate new therapies for future clinical needs.

Joint cartilage tissue is in a complex microenvironment that contains not only chondrocytes but also a variety of non-chondrocyte types, such as adipocytes, synovial cells, mesenchymal stem cells (MSCs), endothelial cells, and immune cells. These cellular components can crosstalk with each other by secreting a variety of metabolic factors and inflammatory factors through paracrine, autocrine, and endocrine pathways and jointly maintain articular cartilage homeostasis, which is, however, heavily disrupted in OA. Accumulating evidence suggests that altered communication between chondrocytes and the surrounding tissues may directly or indirectly affect the progression of OA (Fig. 1). Upon exposure to risk factors that promote joint vulnerability, dysfunctional chondrocytes release excessive protease matrix-degrading enzymes, such as matrix metalloproteinases (MMPs) and a disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS), leading to the degradation of extracellular matrix (ECM). These degradative products are released into the synovial fluid, where they act as damage-associated molecular patterns (DAMPs) to trigger the inflammatory response of adjacent synovial cells (e.g., synovial fibroblasts, macrophages, and mast cells). Subsequently, OA-related immune components, including proinflammatory cytokines (e.g., interleukin-1β (IL-1β), interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNF-α)), growth factors (e.g., transforming growth factor-beta, (TGF-β)), chemokines and adipokines, are aggregated and further promote the activity of MMPs and ADAMTSs, initiating vicious feedback of local tissue damage and low-grade inflammation.

Therefore, exploring how cells communicate within cartilage microenvironments may help to unveil the pathogenesis of OA and explore new strategies for future treatment of OA. Recently, exosomes have emerged as a new medium involved in cell-to-cell communication. A variety of cellular components, in particular noncoding RNAs (ncRNAs), can be preferentially...
Capsules into exosomes and then delivered to the surrounding or distant environment. Exosomal cargoes can regulate the biological activity of recipient cells, thus shuttling intercellular signals and triggering various physiological and pathological processes.

Multiple cell lineages in the osteoarthritic joint are able to behave as active aggressors or passive responders by secreting or internalizing exosomes during disease onset and progression. Better characterization of exosomal cargoes might facilitate the...
identification of new indicators and inform targeted therapy for OA. In this review, we highlight the important role of exosomes in the progression of OA and discuss the prospects and challenges of their utility as clinical biomarkers or therapeutic agents for OA patients.

**THE BIOGENESIS AND RELEASE OF EXOSOMES**

Exosomes, ranging from 40 to 160 nm in diameter, are lipid-bilayer extracellular vehicles (EVs) secreted by most eukaryotic cells and abundant in various body fluids, including blood, urine, saliva, breast milk, pleural effusion, and synovial fluid (Fig. 2). Typically, exosomes contain a group of common membrane and cytoplasmic proteins, including membrane transport and fusion proteins (Rab GTPases, annexin, integrin, and fibronectin), tetratransins (CD9, CD63, CD81, and CD82), heat shock proteins (Hsp20 and Hsp27), and lipid-related proteins. The biogenesis of exosomes initiates with the double invagination of the plasma membrane to generate early endosomes, which subsequently mature to intracellular multivesicular bodies (MVBs), the intermediates within the endosomal system. During this transition, exosomes, or essentially speaking, intraluminal vesicles (ILVs), are formed within the luminal space of MVBs. This process involves a continuum of mechanisms and particular sorting machinery to sequester cargoes on microdomains of the limiting membranes of MVBs, followed by inward membrane budding and fission of small vesicles harboring separated cytosol. The center of these machineries is the ESCRT complex required for transport (ESCRT), which acts as a driver of membrane shaping and scission of ILVs. The ESCRT machinery is mainly composed of four distinct subcomplexes known as ESCRT-0, -I, -II, and -III. ESCRT-0 and ESCRT-I are involved in cargo aggregation on the microdomains of MVB membranes. ESCRT-II is required for the assembly and recruitment of the ESCRT-III subcomplex, while ESCRT-III performs the budding and fission of ILVs into the MVB lumen. Notably, exosomes are still formed even when the ESCRT complex is exhausted, suggesting an ESCRT-independent mechanism for exosome biogenesis. Wei et al. demonstrated that upon epidermal growth factor (EGF) stimulation, Rab GTPase 31 (RAB31) is phosphorylated by the epidermal growth factor receptor (EGFR) and further interacts with flotillin proteins in lipid raft microdomains to drive EGF entry into ILVs in an ESCRT-independent manner. Therefore, diverse machinery can act on the endosomal system to target and package cargoes scheduled for secretion, and their involvement in activation/inhibition, proliferation/apoptosis signaling pathways. Fig. 2 shows the processes of exosome biogenesis and release.
and contribution also shift due to cell types and the signals or stimuli that cells receive, leading to spatiotemporal dynamics of the exosomal compositional repertoire.

Upon maturation, MVBS are basically targeted to lysosomes for degradation or alternatively transported along with microtubules to the plasma membrane for secretion, but the mechanism to balance the secretion and degradation of MVBS remains largely unknown. Once docked at the plasma membrane, the accumulated nondegradable MVBS fuse with the membrane to release ILVs as exosomes, which primarily rely on the common secretory machinery and can be promoted by several Rab family members, such as Rab27a and Rab27b. After secretion into the extracellular space, the double phospholipid membrane of exosomes protects its content from premature degradation during transportation and enables site-specific anchoring toward recipient cells. Subsequently, exosomes can interact with recipient cells in various ways, depending on cell types and the origin of exosomes. They can be maintained on the surface of recipient cells and directly activate surface receptors to elicit functional responses (e.g., antigen presentation). Alternatively, they can be internalized by recipient cells via direct fusion with the cell plasma membrane or via a variety of endocytic pathways, including clathrin-mediated endocytosis, micropinocytosis, phagocytosis, and caveolin- or lipid raft-mediated uptake. Following the above internalization pathways, exosomes release their contents into the cytoplasm of recipient cells and can also be degraded by lysosomes for recycling.

FUNCTION OF EXOSOMES

The unique mechanisms governing the biogenesis of exosomes confer on them distinct sets of components, varying from proteins to nucleic acids and lipids. Their surface molecules enable physical interactions with cell populations, and their onboard cargoes initiate biological responses and phenotypic changes in recipient cells. Thus, exosomes have emerged as novel vehicles for intercellular communication, allowing the dynamic exchange of material and information between cells, tissues, and organ systems. The current interest in the field has focused on exploring the biological activity of exosomes in maintaining homeostatic balance or as a driver or consequence of pathological processes.

Extensive studies have shown that exosomes serve as a key medium for cell–cell communication in joint cartilage tissue and are involved in the pathogenesis of OA. Cartilage-derived exosomes can impinge on the biological behavior of surrounding stromal cells, ultimately creating an appropriate microenvironment for cartilage growth. At the same time, stromal cells in the cartilage microenvironment can also release exosomes carrying anabolic/catabolic and anti-/proinflammatory factors, which in turn affect chondrocyte proliferation, apoptosis, and inflammatory responses, as well as the synthesis and catabolism of the cartilage ECM. Thus, exosome-mediated complex interactions between chondrocyte and stromal cells ensure cartilage homeostasis, while the perturbation of these processes triggers different forms of imbalances that can cause inflammation and thickening of cartilage tissue, eventually initiating or accelerating the progression of OA.

EXOSOMAL NCRNAS IN OA

Comprehensive profiling of exosomes derived from joint tissues and fluids has demonstrated that they are enriched in lncRNAs, miRNAs, and circRNAs. These molecules can be preferentially loaded into exosomes and shuttled between chondrocytes or surrounding stromal cells. Moreover, the proportion of these cargoes varies depending on the donor cell types and OA staging, suggesting their properties to indicate disease status. Consequently, the focus on characterizing exosomal cargoes in OA is currently growing, and diverse exosomal molecules have been documented to exhibit distinct expression profiles in the gain or maintenance of OA phenotypes. lncRNAs are endogenous small ncRNAs with a length of approximately 22 nucleotides. The biogenesis of miRNAs is precisely processed via a series of RNA polymerases, RNases, and RNA-binding proteins (RBPAs). In many cases, miRNAs bind to the 3’ untranslated regions (UTRs) of messenger RNA (mRNA) to cause translational repression or target mRNA degradation. Abundant evidence supports the selective enrichment of miRNAs in exosomes originating from articular tissues. Importantly, exosomal miRNAs exhibit distinct patterns between OA and non-OA samples. A miRNA microarray performed on exosomes from primary chondrocytes suggested that 22 miRNAs were upregulated and 29 were down-regulated in OA chondrocyte-secreted exosomes compared to the normal group. Another study examined exosomes from the synovial fluid of OA patients and healthy subjects, and the profiling of exosomal contents revealed a remarkable difference in miRNA expression, implying altered intercellular communication within the OA microenvironment.

LncRNAs are designated noncoding transcripts with a length of more than 200 nucleotides. Similar to miRNA processing, most lncRNAs are transcribed via polymerase II but probably undergo inefficient splicing. Based on their subcellular localization, lncRNAs can coordinate with diverse nuclear acids and proteins to regulate gene expression at multiple levels. The important roles of lncRNAs in OA progression have emerged. For example, lncRNA NEAT1 could inhibit chondrocyte proliferation and promote apoptosis by functioning as a sponge for miR-543. Of particular interest is the existence of certain lncRNAs in exosomes isolated from body fluids, with differential expression profiles between OA patients and healthy populations, indicating their preferential packaging into exosomes and potential involvement in OA evolution.

Unlike the typical linear ncRNAs, circRNAs form a closed-loop structure primarily produced from pre-mRNAs via back-splicing, enduring them with inherent resistance to exonuclease-mediated decay and thus greater stability than linear RNAs. The recent advance in RNA sequencing (RNA-seq), together with the specialized computational pipelines, has led to an explosion in circRNA characterization. Most circRNAs are predominantly distributed in the cytoplasm and execute their functions by acting as “miRNA sponges”, protein “decoys” or scaffolds, or by coding regulatory peptides. It is worth noting that circRNAs are also prevalent in various exosomes and can be transferred to recipient cells as influential messengers. The field to characterize exosomal circRNAs in OA is in infancy. A subset of exosomal circRNAs originating from human chondrocytes and MSCs actively participate in chondrogenesis and cartilage degradation. A future comprehensive illustration of exosomal cargo will provide new insight into the role of circRNAs in OA pathology.

OA is a progressive joint disorder typically driven by cartilage loss/breakdown, subchondral bone remodeling, and synovial inflammation. Exosomal ncRNAs from different joint cells, such as chondrocytes, MSCs, fibroblast-like synoviocytes (FLSs), osteoclasts, osteoblasts, alter the proliferation, apoptosis, inflammatory response and differentiation, abilities of target cells, thus possibly disrupting joint tissue homeostasis and affecting the development of OA. In the following section, we summarize the current studies on the specific roles and mechanisms of exosomal miRNAs (Table 1), lncRNAs (Table 2), and circRNAs (Table 3) in the progression of OA.

Exosomal ncRNAs and cartilage loss in OA

Articular cartilage loss represents the major hallmark of OA. The maintenance of cartilage integrity relies largely on the cartilage ECM, which provides the structural basis for the functional properties of
articulated cartilage by formulating a dense and highly organized collagenous network mainly comprised of type II collagen, as well as proteoglycans, particularly aggrecan.85 As the unique cellular component of articular cartilage, chondrocytes are solely responsible for ECM synthesis. The dynamic balance between anabolic and catabolic metabolism in the ECM is disrupted in the pathological condition of OA, where the synthesis of ECM no longer compensates for the loss of matrix integrity.14 MMPs and ADAMTS are two main families of proteinases that lead to the degradation of ECM, in particular MMP13 and ADAMTS5,86,87 which can be produced by chondrocytes or other joint cell lineages and induced by cytokines such as IL-1β and TNF-α in the extracellular space. Deletion of MMP13 and ADAMTS5 in mice can effectively control the degradation of ECM and the progression of OA degeneration.87,88 Therefore, the underlying mechanism to trigger the imbalance of ECM metabolism is multifactorial, involving dynamic crosstalk between joint cells within the cartilage microenvironment.

Chondrocytes are sparsely embedded within the ECM and constitute 2–5% of the cartilage tissue.89 Although residing in a metabolically “resting” state, chondrocytes can respond to mechanical stimuli or active biomolecules in the surrounding microenvironment in a positive or negative manner, leading to variations in the phenotype and metabolism of chondrocytes themselves and eventual changes to the composition of the ECM. Notably, the interaction between chondrocytes and their surroundings can be mediated by exosomes carrying various ncRNAs.

Exosomal ncRNAs regulate chondrocyte proliferation in OA. Cartilage degeneration in the aged joint is tightly related to the decreased number of chondrocytes, which fail to regenerate the ECM properly. In the initial stage of OA, chondrocytes manifest transiently increased proliferation ability, which is interpreted as a repair response.89 Exosomal ncRNAs exhibit significant roles in regulating OA chondrocyte proliferation. Wang and colleagues82 investigated the potential function of exosomal miRNAs in a rat model. They found that miR-135b was upregulated in rat MSC-derived exosomes upon transforming growth factor-β1 (TGF-β1) stimulation and promoted the proliferation of rat chondrocyte C5.18 cells. Mechanistically, miR-135b negatively regulates the expression of Sp1, a transcription factor that inhibits the cell cycle by activating p15INK4b and p21WAF1/Cip1 promoters,90,91 suggesting the pro-proliferative role of MSC-derived exosomal miRNAs.

![Fig. 3 The biogenesis and functions of miRNAs, lncRNAs, and circRNAs.](image-url)
Liu and colleagues observed that exosomal IncRNA KLF3-AS1 derived from human MSCs induced OA chondrocyte proliferation and chondrogenic genes such as type II collagen (COL2A1) and aggregan but suppressed the expression of MMP13 and its direct upstream transcription factor runt-related transcription factor 2 (RUNX2). The results also demonstrated that exosomal IncRNA KLF3-AS1 acted as an endogenous sponge for miR-206, thus relieving miR-206-mediated suppression of G-protein-coupled receptor kinase interacting protein-1 (GIT1), a scaffold protein that can bridge multiple protein interaction networks to promote chondrocyte proliferation. Knockdown of GIT1 significantly reversed exosomal IncRNA KLF3-AS1-mediated chondrocyte proliferation, highlighting the significance of the exosomal IncRNA KLF3-AS1/miR-206/GIT1 axis in tuning chondrocyte function and OA progression.
Beyond miRNAs and IncRNAs, the crucial role of EV-derived circRNAs in cell proliferation has emerged. Circular RNA homologous domain interacting protein kinase 3 (circHIPK3), produced from exon 2 of the HIPK3 gene, is highly abundant in mammalian cells as well as exosomes shed by them and exhibits multifaceted roles in controlling cell growth across tissue types by sponging diverse miRNAs. 74,95 Li and colleagues 74 confirmed that MSC-EV-derived circHIPK3 tended to sponge miR-124-3p, a well-known antiproliferative miRNA, thus remarkably promoting chondrocyte growth and attenuating inflammation responses. 74
OA chondrocyte injury. These studies suggest that exosomal ncRNAs may be involved in OA development by regulating chondrocyte proliferation.

**Exosomal ncRNAs regulate chondrocyte apoptosis in OA.** In the late stage of OA, cartilage is characterized by hypcellularity and lacunar emptying due to excessive chondrocyte apoptosis. Chondrocyte apoptosis damages the synthesis and catabolic pathways of ECM contents, which eventually leads to ECM cartilage loss. In turn, the decrease in ECM contents further exacerbates chondrocyte apoptosis, thus entering a vicious cycle of chronic degeneration. Chondrocyte apoptosis can be triggered by multiple factors, including mechanical stress, cytokines (e.g., IL-1β), and reactive oxygen species (ROS). Several studies have emphasized the involvement of exosomal ncRNAs in these apoptotic pathways.

Articular cartilage absorbs mechanical stress to stabilize the joints, constantly exposing chondrocytes to a combination of different forces. High levels of cyclic loading cause chondrocyte death and the extent of the lesion is increased with impact intensity. In response to cyclic loading, chondrocytes produce aberrant levels of ROS through NADPH oxidase (NOX). With the development of OA, the expression of the mammalian target of rapamycin (mTOR), a major negative regulator of mammalian autophagy, is upregulated. A study by Wu and colleagues demonstrated that infrafatellar fat pad (IPFP) MSC (MSCIPFP)-derived exosomes could significantly enhance autophagy levels by inhibiting mTOR, which ultimately rescued IL-induced OA-like gene expression (MMP13 and ADAMTSS) in chondrocytes. Subsequently, exosomal RNA-seq and luciferase reporter assays revealed that miR-100-5p could target the mTOR 3'UTR among the predicted miRNAs. Intra-articular injection of antagonim-100-5p significantly demolished the remedial effect of MSCIPFP-Exos on damaged cartilage in a mouse model of surgical destabilization of the medial meniscus (DMM). Therefore, these results suggest the potential of exosomal miRNAs originating from MSCIPFP to protect articular cartilage from OA injury. However, evidence regarding the role of exosomal IncRNAs and circRNAs in OA-related autophagy is still lacking.

**Exosomal ncRNAs and inflammatory microenvironment in OA.** OA has been previously considered a "wear disease." However, cumulative evidence suggests the contribution of chronic low-grade inflammation to OA symptoms and progression. Indeed, degraded ECM components or cartilage fragments accumulate in the articular cavity and are viewed as DAMPs that provoke inflammatory responses in peripheral stromal cells, including FLSs and macrophage-like synoviocytes. As a result, multiple proinflammatory factors, such as cytokines and chemokines, are released into the synovial fluid and in turn aggravate the loss of chondrocyte phenotypic stability and the degradation of ECM, causally creating a microenvironment favoring cartilage lesions. Sustained low-grade inflammation in this microenvironment alters the secretory profile of synovial tissue, particularly exosomal content. Currently, the engagement of exosomes in maintaining the OA inflammatory network has received extensive scrutiny.

**Exosome-mediated crosstalk between FLSs and chondrocytes.** FLSs, also termed synovial fibroblasts, populate the intimal lining layer of the synovial membrane. They normally secrete joint fluid that lubricates the articular cartilage. In an osteoarthritic state, however, these cells tend to express excessive MMPs and ADAMTS that tip the balance between cartilage synthesis and catabolism towards tissue destruction and behave as inflammatory aggressors through
the release of exosomal cargoes, including potentially regulatory miRNAs. NanoString analysis demonstrated that the levels of 50 miRNAs differ between exosomes from IL-1β-stimulated and nonstimulated FLSs. FLS-derived exosomes can be readily endocytosed by chondrocytes, suggesting tight contact between these cell lineages via exosomes carrying miRNAs. miR-126-3p, a transcriptional modulator of chronic inflammation and innate immunity, is significantly reduced in synovial exosomes in OA patients, while treatment of exosomes from FLSs overexpressing miR-126-3p effectively suppresses apoptosis and inflammatory cytokine levels (IL-1β, IL-6, and TNF-α) in rat chondrocytes. Consistently, exosomal miRNA-126-3p exerts anti-apoptotic and anti-inflammatory effects on articular cartilage in vivo, indicating that FLSs modulate cartilage inflammatory responses via exosomal miRNAs.

In addition to miRNAs, some exosomal IncRNAs, such as IncRNA-H19 and IncRNA-PCGEM1 were involved in FLS-chondrocyte communication. H19 was significantly decreased in chondrocytes treated with IL-1β, whereas overexpressed exogenous H19 in FLSs can be transferred to chondrocytes through exosomes. Exosomal H19 was able to serve as a sponge for miR-106-5p, a well-characterized driver of inflammatory bone loss by targeting tissue inhibitor of metalloproteinase 2 (TIMP2), therefore weakening MMP13 and ADAMTS5 expression in chondrocytes. Conversely, the upregulation of IncRNA-PCGEM1 was observed in OA patient cartilage and in exosomes isolated from OA FLSs. Exosomal PCGEM1 facilitated IL-1β-induced chondrocyte apoptosis and cartilage matrix degradation.

In a persistent chronic inflammatory environment, FLSs also become passively responding effectors of exosomes shed by chondrocytes, suggesting mutual regulation between FLSs and chondrocytes to promote OA inflammation. When exosomes from IL-1β-treated chondrocytes are applied to OA synovium, a marked elevation in MMP13, IL-1β, TNF-α, and cyclooxygenase-2 (COX-2) was observed compared with nontreated exosomes. This potential positive feedback loop between FLSs and chondrocytes highlights the role of exosomes in contributing to the complexity of OA inflammatory networks.

Role of exosomes in macrophage-involved inflammation. FLSs are interdigitated with macrophage-like synoviocytes to constitute a delicate structure of the synovial intima. Abrupt activation or “polarization” of synovial macrophages by cartilage matrix catabolic molecules and cytokines drive synovial inflammation and OA progression. The polarization phenotype of macrophages varies greatly depending on the stimuli of the local environment (Fig. 5). For example, lipopolysaccharide (LPS), interferon-gamma (IFN-γ), or granulocyte-macrophage colony-stimulating factor (GM-CSF) can induce M0 macrophages into an M1 phenotype, producing proinflammatory cytokines (TNF-α, IL-1β, and IL-6) to elicit proinflammatory responses. In contrast, interleukin-4 (IL-4) or interleukin-13 (IL-13) stimulates M0 macrophages into an M2 phenotype to secrete anti-inflammatory cytokines (IL-10) and growth factors (TGF-β), ultimately suppressing inflammation and promoting tissue repair. In addition, M1 and M2 macrophages display distinct markers. The M1 phenotype highly expresses membrane receptor cluster differentiation 86 (CD86), toll-like receptor 4 (TLR4), and inducible nitric oxide synthase (iNOS), while the M2 phenotype is characterized by cluster differentiation 163 (CD163). The accumulation of M1-polarized macrophages in the synovial intima is the morphological characteristic of synovial inflammation, and the polarization status highly correlates with OA severity. In this regard, the progression of OA can be alleviated by facilitating the reprogramming of macrophages from the proinflammatory M1 to the anti-inflammatory M2 subset. The involvement of exosomes in determining macrophage phenotype and behavior in the inflamed OA joint has been highlighted. Exosomes isolated from TGF-β-treated bone MSCs increased the percentage of CD163-positive macrophages (M2) in OA rat tissues. Meanwhile, these exosomes guide the phenotypic switch of LPS-primed macrophages from M1 to M2. Among the exosomal contents, miR-135b was highly expressed and probably actively involved in M2 transition by targeting mitogen-activated protein kinase 6 (MAPK6), thereby alleviating cartilage damage. Exosomes are also linked to the inflammatory behavior of polarized macrophages. Upon the stimulation of exosomes isolated from the synovial fluid of OA patients, GM-CSF-primed M1 macrophages release abundant IL-1β and chemokines, indicating that exosomes in synovial fluid serve as a key mediator to create the inflammatory microenvironment in OA. Another study found that exosome-like vesicles originating from OA chondrocytes can be internalized by LPS-primed macrophages and further enhance inflammation activation and subsequent IL-1β processing. Mechanistically, these vesicles likely inhibit autophagy-related 4B (ATG4B) expression by delivering its upstream modulator miR-449a-5p to target macrophages. Subsequently, the decreased autophagy promoted the production of mitochondrial ROS, a potential signal to promote IL-1β production, suggesting the proinflammatory role of exosomes released from OA chondrocytes. Instead, exosomes from normal chondrocytes restore mitochondrial dysfunction and trigger the macrophage response towards the M2 phenotype. Alternatively, macrophages can modulate chondrocyte behavior via exosomes. Upon IL-4 or IL-13 treatment, RAW264.7 murine macrophages and mouse bone marrow-derived macrophages undergo M2 polarization, with a dramatic increase in IncRNA MM2P. MM2P upregulates the expression of SRY-box transcription factor 9 (SOX9) in M2 macrophages either by interacting with the RNA-binding protein FUS to stabilize SOX9 mRNA or by blocking SHP2-mediated dephosphorylation of signal transducer and activator of transcription 3 (STAT3), which promotes SOX9 transcription. The subsequent elevated SOX9 mRNA and protein can be packaged into exosomes and transferred to mouse chondrocytes, where they can promote the secretion of ECM components. Collectively, mutual crosstalk between macrophages and other cell populations is largely attributed to exosomes released from them.

Exosomal ncRNAs and subchondral bone remodeling in OA

The articular cartilage, calcified cartilage, and subchondral bone constitute osteochondral units that work in concert to support the functional loading and motion of the joint, while progressive heterogeneous changes in the osteochondral tissues occur during OA evolution. ECM loss in the OA cartilage layer reduces its ability to absorb mechanical pressure and leads to excessive loading on the subchondral bone. Gradually, however, the fine balance between osteoclast-mediated bone resorption and osteoblast-mediated bone formation is perturbed, bringing about heterogeneity in the subchondral density or stiffness that creates local shear forces to intensify cartilage deformation and further damage. Hence, OA is no longer considered a simple subject bothering articular cartilage but rather an entire joint disorder is concurrently driven by subchondral bone remodeling and subsequent neovascular invasion into the synovium and articular cartilage.

During abnormal subchondral bone remodeling, the formation of microcracks, combined with abnormal vascularization, facilitates molecular transport between cartilage and subchondral bone. In view of this, the crosstalk between the subchondral and cartilage layers may be elevated in OA. Subchondral osteocytes can induce phenotypic changes in OA chondrocytes. This interaction might be conducted in an exosome-dependent fashion. For example, Wu et al isolated and purified exosomes from osteoblasts in the non-sclerotic or sclerotic regions of human OA subchondral bone. After co-culture with chondrocytes, exosomes from sclerotic subchondral bone increased the percentage of CD163-positive macrophages (M2) in OA rat tissues.
genes and reduced the expression of chondrocyte-specific markers. RNA-seq confirmed the high enrichment of miR-210-5p in the exosomes of OA sclerotic osteoblasts. Exosomal miR-210-5p increased hypertrophic and ECM-degradative gene expression and inhibited cellular aerobic respiration in chondrocytes, a feature frequently observed in OA conditions. Another study demonstrated a compelling case of pathological exosomal miRNA communication between osteoclasts and neighboring chondrocytes in OA. Following anterior cruciate ligament transection (ACLT) surgery to establish a mouse OA model, a set of miRNAs
Exosomes rewire the cartilage microenvironment in osteoarthritis: from... Wu et al.

Exosomal cargoes as potential biomarkers for OA

CLINICAL SIGNIFICANCE OF EXOSOMES IN OA

Potential therapeutic strategies based on exosomes

As a promising therapeutic delivery system in OA therapy. MSCs exhibit excellent performance in tissue repair and regeneration and serve as promising seed cells in cartilage engineering approaches. These advantages are at least partially based on the paracrine effects of cellular exosomes that carry various ncRNAs to promote proliferation and inhibit inflammation in recipient cells. Therefore, MSC-derived exosomes have been considered a promising delivery system for OA therapy.

Recently, several studies have attempted to use naïve MSC-derived exosomes to treat OA in vivo and in vitro models.
Two individual studies demonstrated that bone marrow MSC-derived exosomes display a beneficial therapeutic effect on OA by transferring regulatory miRNAs to cartilage and balancing the synthesis and degradation of ECM. However, they focused on different miRNAs in bone marrow MSC-derived exosomes, and which cargo dominates the delay of cartilage degeneration remains unclear. To address this, some studies manipulate donor MSCs by virtue of transfection with genes of interest. miR-140, one of the most important miRNAs in chondrocytes, plays a central role in both cartilage development and homeostasis. Tao et al. overexpressed miR-140-5p in synovial MSCs using a lentiviral system and showed consistently elevated levels of miR-140-5p in the exosomes shed by these cells. Compared with non-modified exosomes, miR-140-5p-abundant exosomes showed an enhanced ability to trigger chondrocyte proliferation and migration without hindering ECM secretion in vitro and successfully prevented cartilage degeneration in OA rat models. Similarly, transfection of dental pulp stem cells (DPSCs) with miR-140-5p mimics enriches miR-140-5p in their exosomes, and administration of these exosomes remarkably improves joint conditions in a rat OA model.

In addition to the genetic manipulation of exosomal cargoes, targeted modification of exosomes also serves as a promising OA treatment approach to deliver cargoes into chondrocytes across the dense and nonvascular ECM. To this end, dendritic cells are transfected with plasmids encoding fusing protein of chondrocyte-affinity peptide (CAP) and lysosome-associated membrane glycoprotein 2b (Lamp2b), which allows the release of exosomes displaying CAP-Lamp2b on the surface. The engineered exosomes are subsequently loaded with miR-140 via electroporation and are capable of transferring miR-140 into deep cartilage regions, thus alleviating OA progression in a rat model.
These findings may allow targeted regulation of intercellular communication and point toward a potential cell-free therapy of OA alternatives to cell-based MSC therapy.

Certainly enhanced cargo delivery conducted by exosomes hinders cartilage homeostasis, which may be blocked in OA therapy. Nevertheless, the biogenesis of exosomes relies on normal cellular processes. Disrupting their activity in specific cell types or tissues remains challenging. Indeed, preferential suppression of exosomal ncRNA crosstalk to manipulate physiological or pathological processes has not yet been evidenced in vivo.

**CONCLUSIONS AND PROSPECTS**

Exosomes represent a novel mode of cell-to-cell communication in joint tissues. Exosomal contents, especially ncRNAs, are selectively packaged and transferred to recipient cells to regulate their biological behaviors. Certain exosomal ncRNAs showed different expression patterns between the synovial fluid of OA patients and healthy controls, making them signs of disease onset and promising biomarkers that may inform inflammation types and disease status. Accumulating evidence also highlights novel therapeutic strategies based on exosomal ncRNAs, some of which have shown promising outcomes in vivo.

Despite the recent progress made in characterizing exosomes in OA, some challenges remain unresolved in this field, particularly in terms of the identification of the cellular origin of exosomes. Indeed, exosomes in body fluids are often heterogeneous vesicles of various origins, making it difficult to identify and isolate joint-specific exosomes in OA samples. Next, the involvement of exosomes in cell-to-cell communication is achieved by the delivery of specific bioactive molecules. However, how these molecules are selectively packaged into exosomes is unclear, and further studies are needed to reveal detailed mechanisms underlying exosome biogenesis. To date, only a small fraction of exosomal cargo has been identified in OA, most of which are ncRNAs. How other exosomal contents function in OA requires further investigation.

OA pathology is marked by abnormal regulation of gene expression in multiple ways. Nevertheless, a considerable portion of exosomal studies have focused merely on cartilage, synovium, or subchondral bone in isolation, ignoring the fact that OA is a whole joint disorder. In the case of viewing these in vitro studies, however, caution must be taken in regard to the pathophysiological correlation, such as whether the stimuli can pathologically mimic the inducers of arthritis. Additionally, the field to examine the therapeutic potential of exosomal ncRNAs is still in its infancy. Current studies confirm that exosomes can be engineered to deliver therapeutic factors to recipient cells for therapeutic applications, but one premise is that exosomes must be able to accurately find their targets and release their cargo to target the specificity of tissues such as chondrocytes and synovial cells. Hence, more studies are needed to identify specific markers on the membrane of joint cell populations. Accordingly, exosomes with surface modifications are designed to identify these markers, enabling targeted intervention of intercellular crosstalk. Moreover, OA is a chronic degenerative disease and the progression of OA can be controlled using “stepped therapy” at different stages. Therefore, how to accurately subtype OA by exosomes, especially in the early stage of OA, is crucial for the clinical treatment of OA. In addition, previous studies have evaluated the feasibility of sampling patient blood or synovial fluid to isolate exosomes for OA diagnosis. Non-invasive methods for exosome acquisition from other body fluids (e.g., urine, saliva) are expected to relieve the pain of patients. With an increasing exploration of these unknowns, we believe that exosomes will become feasible in the future diagnosis and treatment of OA.
13. Zhou, Q., Cai, Y., Jiang, Y. & Lin, X. Exosomes in osteoarthritis and cartilage injury: advanced development and potential therapeutic strategies. *Int J. Biol. Sci.* **16**, 1811–1820 (2020).

14. Liu-Bryan, R. & Terkeltaub, R. Emerging regulators of the inflammatory process in osteoarthritis. *Nat. Rev. Rheumatol.* **11**, 35–44 (2015).

15. Loeser, R. F., Goldring, S. R., Scanzello, C. R. & Goldring, M. B. Osteoarthritis: a disease of the joint. *Arthritis Rheum.* **64**, 1697–1709 (2012).

16. Kalluri, R. & Lebleu, V. S. The biology, function, and biomedical applications of exosomes. *Science* **367**, 640 (2020).

17. Tkach, M. & Thery, C. Communication by extracellular vesicles: where we are and where we need to go. *Cell* **164**, 1226–1232 (2016).

18. Medeloski, J. Exosomes and ectosomes in intercellular communication. *Curr. Biol.* **28**, R435–R444 (2018).

19. van Niel, G., D’Angelo, G. & Raposo, G. Shedding light on the cell biology of extracellular vesicles. *Nat. Rev. Mol. Cell Biol.* **19**, 213–228 (2018).

20. Parada, N., Romero-Trujillo, A., Georges, N. & Alcayaga-Miranda, F. Camou...

21. Fan, S. J. et al. Glutamine deprivation alters the origin and function of cancer cell exosomes. *EMBO J.* **39**, e103009 (2020).

22. Liu, S. et al. M1-like macrophage-derived exosomes suppress angiogenesis and exacerbate cardiac dysfunction in a myocardial infarction microenvironment. *Basic Res. Cardiol.* **115**, 22 (2020).

23. Larios, J., Mercier, V., Roux, A. & Grunenberg, J. ALIX- and ESCRT-III-dependent sorting of tetraspanins to exosomes. *J. Cell Biol.* **219**, e201904113 (2020).

24. Tobon-Aroyave, S. I., Celis-Mejia, N., Cordoba-Hidalgo, M. P. & Isaia-Guzman, D. M. Decreased salivary concentration of CD9 and CD81 exosome-related tetraspanins may be associated with the periodontal clinical status. *J. Clin. Periodontol.* **46**, 470–480 (2019).

25. Reddy, V. S., Madala, S. K., Trinath, J. & Reddy, G. B. Extracellular small heat shock proteins: exosomal biogenesis and function. *Cell Stress Chaperones* **23**, 441–454 (2018).

26. Paolicelli, R. C., Bergamini, G. & Rajendran, L. Cell-to-cell communication by extracellular vesicles: focus on microglia. *Neuroscience* **405**, 148–157 (2019).

27. Safdar, A., Saleem, A. & Tarnopolsky, M. A. The potential of endurance exercise-derived exosomes to treat metabolic diseases. *Nat. Rev. Endocrinol.* **12**, 504–517 (2016).

28. Vietri, M., Radulovic, M. & Stenmark, H. The many functions of ESCRTs. *Nat. Rev. Mol. Cell Biol.* **18**, 5–17 (2017).

29. Vietri, M., Radulovic, M. & Stenmark, H. The many functions of ESCRTs. *Nat. Rev. Mol. Cell Biol.* **21**, 25–42 (2020).

30. Betti, M. F. et al. Syndecan-syntenin-ALIX regulates the biogenesis of exosomes. *Nat. Cell Biol.* **14**, 677–685 (2012).

31. Stufler, S., Wegner, C. S., Stenmark, H. & Brech, A. Multivesicular endosomal biogenesis in the absence of ESCRTs. *Traffic* **10**, 925–937 (2009).

32. Wei, D. H. et al. Rab31 marks and controls an ESCRT-independent exosome pathway. *Cell Res.* **31**, 157–177 (2021).

33. Ostrowski, M. et al. Rab27a and Rab27b control different steps of the exosome secretion pathway. *Nat. Cell Biol.* **12**, 19–U61 (2010).

34. Song, L. et al. KIBRA controls exosome secretion via inhibiting the proinflammatory degradation of Rab27a. *Nat. Commun.* **10**, 1639 (2019).

35. O’Brien, K., Breyne, K., Ughetto, S., Laurent, L. C. & Breakefield, X. O. DNA delivery by extracellular vesicles in mammalian cells and its applications. *Nat. Rev. Mol. Cell Biol.* **21**, 585–606 (2020).

36. Raimondo, F., Morosi, L., Chinello, C., Magni, F. & Pitto, M. Advances in membranous vesicle and exosome proteomics improving biological understanding and biomarker discovery. *Front. Immunol.* **11**, 709–720 (2020).

37. Mulcahy, L. A., Pink, R. C. & Carter, D. R. Routes and mechanisms of extracellular vesicle uptake. *J. Extracell Vesicles* **3** (2014).

38. Parada, N., Romero-Trujillo, A., Georges, N. & Alcayaga-Miranda, F. Camouflage strategies for therapeutic exosomes evasion from phagocytosis. *J. Adv. Res.* **31**, 61–74 (2021).

39. Huang, Y. et al. Influence of species and processing parameters on recovery and content of brain tissue-derived extracellular vesicles. *J. Extracellular Vesicles* **9**, 1785746 (2020).

40. Toh, W. S., Lai, R. C., Hui, J. H. P. & Lim, S. K. MSC exosome as a cell-free MSC therapy for cartilage regeneration: Implications for osteoarthritis treatment. *Semin. Cell Dev. Biol.* **67**, 56–64 (2017).

41. Ashgar, S., Litherland, G. J., Lockhart, J. C., Goodyear, C. S. & Crilly, A. Exosomes in intercellular communication and implications for osteoarthritis. *Rheumatology* **59**, 57–68 (2020).

42. Di Nicola, V. Degenerative osteoarthritis a reversible chronic disease. *Regen. Ther.* **15**, 149–160 (2020).

43. Kölhe, R. et al. Gender-specific differential expression of exosomal miRNA in serum fluid of patients with osteoarthritis. *Sci. Rep.* **7**, 2029 (2017).
Exosomes rewire the cartilage microenvironment in osteoarthritis: from… Wu et al.

77. Wu, J. Y. 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. *Biomaterials* **206**, 87–100 (2019).
78. Tan, F., Wang, D. & Yuan, Z. The fibroblast-like synoviocyte-derived exosomal non-coding RNA H19 alleviates osteoarthritis progression through the miR-106b-5p/TIMP2 axis. *Inflammation* **43**, 1498–1509 (2020).
79. Zenobi, E., Xing, W., Dey, G., Xiao, S. L. & Li, F. Fibroblast-like synoviocyte-derived exosomal PCGEM1 accelerates IL-1 beta-induced apoptosis and cartilage matrix degradation by miR-142-5p/RUNX2 in chondrocytes. *Immunol. Invest.* **1–18** (2021).
80. Dai, J. et al. Osteouesta-derived exosomal let-7a-5p targets Smad2 to promote the hypertrophic differentiation of chondrocytes. *Am. J. Physiol. Cell Physiol.* **2020**.
81. Cui, Y., Luan, J., Li, H., Zhou, X. & Han, J. Exosomes derived from mineralizing osteoblasts promote cell osteogenic differentiation by alteration of microRNA expression. *FEBS Lett.* **590**, 189–192 (2016).
82. Wang, R., Xu, B. & Xu, H. G. TGF-beta 1 promotes chondrocyte proliferation by regulating Sp1 through MSC-exosomes derived miR-133B. *Cell Cycle* **17**, 2411–2422 (2018).
83. Liu, Y. et al. MSC-derived exosomes promote proliferation and inhibit apoptosis of chondrocytes via IncRNA-KLF5-AS1/miR-206/GIT1 axis in osteoarthritis. *Cell Cycle* **17**, 2756–2765 (2018).
84. Mao, G. P. et al. Exosomes derived from miR-92a-3p-overexpressing human mesenchymal stem cells enhance chondrogenesis and suppress cartilage degradation via targeting WNT5A. *Stem Cell Res. Ther.* **9**, 247 (2018).
85. Mow, J. K., Ou, G. Q. & Weaver, V. M. Extracellular matrix assembly: a multiscale deconstruction. *Nat. Rev. Mol. Cell Biol.* **15**, 771–785 (2014).
86. Latouche, A. et al. Systemic inhibition of IL-6/STAT3 signaling protects against experimental osteoarthritis. *Ann. Rheum. Dis.* **76**, 748–755 (2017).
87. Glasson, S. S. et al. Deletion of active ADAMTS5 prevents cartilage degradation in a murine model of osteoarthritis. *Nature* **434**, 644–648 (2005).
88. Little, C. B. et al. Matrix metalloproteinase 13-depletion of articular chondrocytes. *Ann. Rheum. Dis.* **65**, 1595–1598 (2006).
89. Goldring, M. B. Update on the biology of the chondrocyte and new approaches to treating cartilage diseases. *Best. Pr. Res. Clin. Rheumatol.* **20**, 1003–1025 (2006).
90. Feng, X. H., Lin, X. & Derynck, R. Smad2, Smad3 and Smad4 cooperate with Sp1 to induce p130(SNK) transcription in response to TGF-beta. *EMBO J.* **19**, 5178–5193 (2000).
91. Kavurma, M. M. & Khachigian, L. M. Sp1 inhibits proliferation and induces apoptosis in vascular smooth muscle cells by repressing p21WAF1/Cip1 transcription and cyclin D1-Cdk4/p21WAF1/Cip1 complex formation. *J. Biol. Chem.* **278**, 32537–32543 (2003).
92. Zhang, L. Q. et al. Integrin-beta 1 regulates chondrocyte proliferation and apoptosis through the upregulation of G1T1 expression. *Int. J. Mol. Med.* **35**, 1074–1080 (2015).
93. Chen, P., Gu, W. L., Gong, M. Z., Wang, J. & Li, D. G. G1T1 gene deletion delays inflammation and cartilage degradation in a murine model of osteoarthritis. *Osteoarthr. Cartil.* **23**, 901–945 (2005).
94. Xiao, X., Leng, X. & Zhang, Q. The current state of nanoparticle-induced macrophage factor gene expressions in synovium. In ORS 2012 Annual Meeting, *San Francisco*, Poster No. 0708 (2012).
95. Xie, J. W. et al. Alpha defensin-1 attenuates surgically induced osteoarthritis in association with promoting M1 to M2 macrophage polarization. *Osteoarthr. Cartil.* **29**, 1048–1059 (2021).
96. Sun, Y., Zuo, Z. & Kuang, Y. An emerging target in the battle against osteoarthritis: macrophage polarization. *Int. J. Mol. Sci.* **21**, 8513 (2020).
97. Taylor, P. R. et al. Macrophage receptors and immune recognition. *Annu. Rev. Immunol.* **23**, 391–433 (2005).
98. Xiao, L. et al. Production of recombinant human umbilical cord mesenchymal stem cells inhibit osteoblast activity. *Cell Disco.* **15**, 1088–1104 (2015).
99. Chen, P., Gu, W. L., Gong, M. Z., Wang, J. & Li, D. G. G1T1 gene deletion delays chondrocyte differentiation and healing of tibial plateau fracture through suppressing proliferation and apoptosis of chondrocyte. *Bmc Musculoskel. Dis.* **18**, 320 (2017).
100. Zheng, Q. et al. Circular RNA profiling reveals an abundant circHIPK3 that regulates cell growth by sponging multiple miRNAs. *Nat. Commun.* **7**, 11215 (2016).
101. Wang, Y. et al. Exosomal cHippK3 released from hypoxia-pretreated cardiomyocytes regulates oxidative damage in cardiac microvascular endothelial cells via the miR-29a/IGF-1 pathway. *Oxid. Med. Cell Longev.* **2019**, 7954657 (2019).
102. Hwang, H. S. & Kim, H. A. Chondrocyte apoptosis in the pathogenesis of osteoarthritis. *Int. J. Mol. Sci.* **16**, 26035–26054 (2015).
103. Li, X. 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. *Cell Biol. Int.* **45**, 2096–2106 (2021).
104. Wang, X. et al. Exosomes isolated from bone marrow mesenchymal stem cells exhibit a protective effect on osteoarthritis via IncRNA LRYMA-AS1-GPRR-mir-6515-Sp. *Front. Cell Dev. Biol.* **9**, 644380 (2021).
105. Zhu, C., Shen, K., Zhou, W., Hu, L. & Li, F. Fibroblast-like synoviocyte-derived exosomal miRNA-135b inhibits cartilage cell death induced by GF in a murine model of osteoarthritis. *Immunol. Immunopathol.* **52**, 2373–2373 (2009).
106. Li, X. et al. Horizontal cross-talk between monocyte-derived cells modulates primary chondrocytes. *Cell Death Disc.* **11**, 763 (2020).
107. Goldring, S. R. & Goldring, M. B. Changes in the osteochondral unit during osteoarthritis: structure, function and cartilage-bone crosstalk. *Arthritis Rheum.* **62**, 632–644 (2016).
108. Chen, L. Z. et al. Horizontal fissuring at the osteochondral interface: a novel and unique pathological feature in patients with obesity-related osteoarthritis. *Ann. Rheum. Dis.* **79**, 811–818 (2020).
109. Yuan, X. D. et al. Bone-cartilage interface crosstalk in osteoarthritis: potential pathways and future therapeutic strategies. *Osteoarthropathy* **22**, 1077–1089 (2014).
110. Burr, D. B. & Gallant, M. A. Bone remodeling in osteoarthritis. *Nat. Rev. Rheumatol.* **8**, 665–673 (2012).
111. Sun, W. et al. Osteouesta-derived microRNA-containing exosomes selectively inhibit osteoblast activity. *Cell Discov.* **2**, 16015 (2016).
112. Sanchez, C. et al. Subchondral bone osteoblasts induce phenotypic changes in articular chondrocytes. *Osteoarthropathy* **13**, 988–997 (2000).
113. Li, X. et al. Exosomes: roles and therapeutic potential in osteoarthritis. *Bone Res.* **8**, 25 (2020).
114. Li, Z., Huang, Z. & Bai, L. Cell interplay in osteoarthritis. *Front. Cell Dev. Biol.* **9**, 720477 (2021).
115. Wu, X., Crawford, R., Xiao, Y., Mao, X. & Prasadam, I. Osteoarthritis subchondral bone release exosomes that promote cartilage degeneration. *Cells* **10**, 251 (2021).
