Molecular Therapy
Nucleic Acids
Review

Biological roles and therapeutic potential of circular RNAs in osteoarthritis

Xingjia Mao,[1,5] Yanyan Cao,[2,3,5] Zijian Guo,[1] Linlin Wang,[4] and Chuan Xiang[1]

[1]Department of Orthopedics, The Second Hospital of Shanxi Medical University, Taiyuan 030000, China; [2]MicroNano System Research Center, Taiyuan University of Technology, Taiyuan, China; [3]College of Information Science and Engineering, Hebei North University, Zhangjiakou 075000, China; [4]Department of Basic Medicine Sciences, Zhejiang University School of Medicine, Hangzhou 310000, China

Osteoarthritis (OA) is a common and disabling joint disorder that is mainly characterized by cartilage degeneration and narrow joint spaces. The regulatory functions of non-coding RNAs (long non-coding RNAs, microRNAs [miRNAs], and circular RNAs [circRNAs]) in OA progression have attracted considerable attention, and the function of circular RNAs in the context of OA has been an increasingly popular research topic in the last 6 years. Recent studies have reported that various circRNAs can delay or aggravate diverse aspects of the OA process, including extracellular matrix formation, apoptosis, proliferation, inflammation, and autophagy, via circRNA/miRNA/mRNA pathways. Thus, circRNAs and related pathways are potential therapeutic targets for OA. Our review provides comprehensive information about circRNAs, including their biogenesis, functions, and characteristics, and it reveals their critical roles in the pathogenesis of OA via a large regulatory network of sponges. Considering their regulatory functions and characteristics, we hypothesize that circRNAs not only can be transferred through bodily fluids to serve as diagnostic biomarkers, but they can also be released from mesenchymal stem cell-derived exosomes and delivered to OA chondrocytes acting as therapeutic circRNAs. Further investigations of the in-depth molecular mechanisms of action of circRNAs in OA are expected to provide effective and safe OA treatment strategies.

INTRODUCTION

Osteoarthritis (OA) is a chronic and progressive cartilage degeneration disease with a high morbidity and disability rate and is characterized by cartilage degeneration, osteophyte formation, thickening of subchondral bone, synovial inflammation, meniscal injuries, and ligament deterioration.[1,2] At present, more than 500 million people worldwide are being affected by OA,[3] and the peak of incidence is around the age of 75 years.[1] Multiple factors leading to OA include aging, sex, obesity, genetics, metabolic environment, and joint alignment;[1] however, the exact molecular mechanisms regulating OA pathogenesis remain elusive, and no effective interventions or therapies,[4] with the exception of surgery, can slow or reverse OA progression.[4,5] Chondrocytes are the only cell type present in the mature cartilage and undergo pathological changes with considerable involvement of non-coding RNAs when OA occurs.[4] Therefore, the exploration of the pathophysiological and molecular mechanisms regulating chondrocytes in OA is of critical significance.

Non-coding RNAs, including long non-coding RNAs (lncRNAs), microRNAs (miRNAs), and circular RNAs (circRNAs), influence biological processes via the modifications of DNA structure, RNA transcription, and protein translation.[4,6] As techniques such as RNA sequencing (RNA-seq) and bioinformatics analysis have been advanced, an increasing number of circRNAs have been discovered, and the principles behind their formation and biological functions have been progressively revealed.[7] The functions of circRNAs are coming into focus, and increasing evidence has shown that circRNAs play key regulatory roles in diverse cellular processes, such as cell proliferation, apoptosis, differentiation, and invasion.[8] In addition, several remarkable characteristics of circRNAs, including stability,[9] specificity,[10] conservatism,[11] and universality,[12] have been identified as potential biomarkers for diagnostics and as therapeutic targets in diseases,[13] such as ciRS-7 in Alzheimer's disease,[14] circ-PRMT5 in breast cancer,[15] hsa_circ_0000658 in osteosarcoma,[16] circRIMS1 in bladder cancer,[17] and circVMA21 in intervertebral disc degeneration.[18]

Since 2015, the number of studies on circRNAs in OA has been increasing, and related publications have predominantly originated from China (Figure 1). Increasing evidence has shown that circRNAs are closely associated with chondrocyte proliferation, apoptosis, inflammation, autophagy, and ECM metabolism in OA[19–21] and delay or aggravate OA progression.[22,23] Moreover, the circRNAs/miRNAs/mRNAs axis was pointed out to have a significant role in OA progression,[24] and circRNAs can act as sponges of miRNAs to inhibit the translation of mRNAs, thus participating in the occurrence and development of OA.[22] Therefore, circRNAs with significant regulatory roles may become new biological markers and therapeutic

https://doi.org/10.1016/j.omtn.2021.04.006.

[5]These authors contributed equally

Correspondence: Linlin Wang, Department of Basic Medicine Sciences, Zhejiang University School of Medicine, Hangzhou 310000, China.
E-mail: wanglinlin@zju.edu.cn
Correspondence: Chuan Xiang, Department of Orthopedics, The Second Hospital of Shanxi Medical University, Taiyuan 030000, China.
E-mail: chuanxiang@sxmu.edu.cn

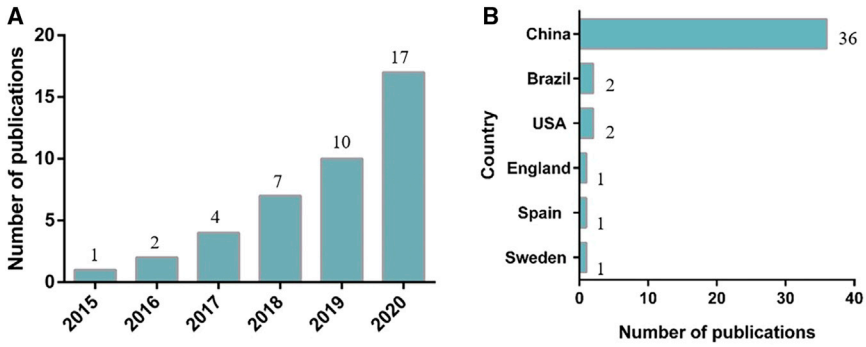

Figure 1. Status of circRNA research in OA
(A) The annual number of publications related to circRNA research in OA in the past 6 years. (B) The sum of publications related to circRNA research in OA from the top six countries.

targets in OA; however, the mechanisms of action of circRNAs in OA pathogenesis need to be examined in detail.[25] The present review provides an overview of biological roles and therapeutic potential of circular RNAs in OA, which may contribute to the understanding of the molecular mechanism of OA pathogenesis and provide novel potential targets for OA diagnosis and therapy.

BIOGENESIS, FUNCTIONS, AND CHARACTERISTICS OF circRNAs

Biogenesis of circRNAs

Precursor mRNAs (pre-mRNAs) undergo spliceosome-mediated splicing to generate linear mRNAs[26] and backsplicing to generate circRNAs with the assistance of RNA polymerase II (RNA Pol II);[27] in this reaction, a downstream 5′ splice site (ss) is bound to an upstream 3′ ss, and the final RNA circle is ligated by a 3′-5′ phosphodiester bond at the junction site.[28,29] The patterns of overlap between the dominant circular and linear transcripts can be used to classify circRNAs into the following three categories according to shared exons: (1) overlapped, both use the same subset of exons; (2) partially overlapped, some but not all exons are shared; and (3) not overlapped, no exons are shared (Figure 2). Furthermore, circRNAs can be divided into three classes according to the genomic origin of exons and introns: circular intronic RNAs (ciRNAs), exonic circRNAs (ecircRNAs), and exon-intron circRNAs (EIciRNAs)[26,30] (Figure 3).

Functions of circRNAs

The biological functions of circRNAs have been studied in a minor fraction of the molecules and have been described in detail;[28,31–33] however, most circRNAs have been proposed to act as miRNA sponges.[32,34] Intronic circRNAs contribute to RNA-mediated inheritance and epigenetics in the cytoplasm, whereas ecircRNAs act as miRNA sponges to regulate miRNAs in the cytoplasm.[35,36] Moreover, circRNAs can interact with proteins, including those acting as RNA-binding protein (RBP)-related protein sponges/decoys,[27,37,38] enhancing particular protein functions by forming RNA-protein complexes,[30,32] acting as scaffolds for specific enzymes and substrates,[39–41] and recruiting proteins to specific locations or subcellular compartments.[42] Another intriguing function involves cap-independent translation of circRNA-encoded peptides;[43–46] however, most circRNAs are universally acknowledged to be non-coding[47] (Figure 4).

Regulatory characteristics of circRNAs

circRNAs are involved in the intracellular RNA regulatory network and are closely related to the occurrence and development of diseases,[48,49] including OA[50]. The information on the circRNA/miRNA/mRNA pathways in diseases has been recently expanded, with these pathways providing novel directions for the pathogenesis, diagnosis, and therapy of the diseases.[26,51] The competitive endogenous RNA (ceRNA) network has revealed new mechanisms by which RNAs regulate each other at the posttranscriptional level. Various ceRNA molecules, including mRNA, lncRNA, circRNA, and pseudogene species, regulate the expression levels of target genes via miRNA response elements (MREs). It has been reported that circRNAs contain a large number of miRNA binding sites[26,52,53] and can function as miRNA sponges or ceRNAs that competitively inhibit the activity of miRNAs via MREs[22,54] and silence genes by binding to mRNA. However, the mechanisms involved in the pathological process in OA are poorly understood.

BIOLOGICAL ROLES OF circRNAs IN CHONDROCYTES IN OA

The development of OA involves numerous types of cells, including chondrocytes, osteoblasts, osteoclasts, and synoviocytes. Chondrocytes play the main role in OA pathogenesis and have gained the most attention. Thus far, the studies on the role of circRNAs in OA mainly focused on chondrocytes, and the main investigated features include the degradation of extracellular matrix (ECM), apoptosis of chondrocytes, production of inflammatory cytokines, and reductions in proliferation and autophagy. Corresponding interventions to influence these pathological processes can delay the progression of OA. Detailed investigations into the biogenesis, biological functions, and characteristics of circRNAs have demonstrated that circRNAs are involved in numerous aging-related diseases, including cancer,[55] cardiovascular diseases,[56] and neurodegenerative disorders.[57] In this study, we classify OA-related circRNAs based on their involvement in ECM formation (Figure 5), apoptosis (Figure 6), proliferation, inflammation, and autophagy (Figure 7) of chondrocytes and focus on the regulatory functions of circRNAs to demonstrate that certain circRNAs or related pathways can be used as diagnostic and prognostic biomarkers for OA treatment (Table S1).

ROLES OF circRNAs IN ECM FORMATION

The ECM is mainly composed of glycosaminoglycan (GAG), aggrecan, and collagen, and the balance of catabolic and anabolic processes in the ECM is important for ECM homeostasis. ECM degradation is one of the central and most critical features of OA pathogenesis and is

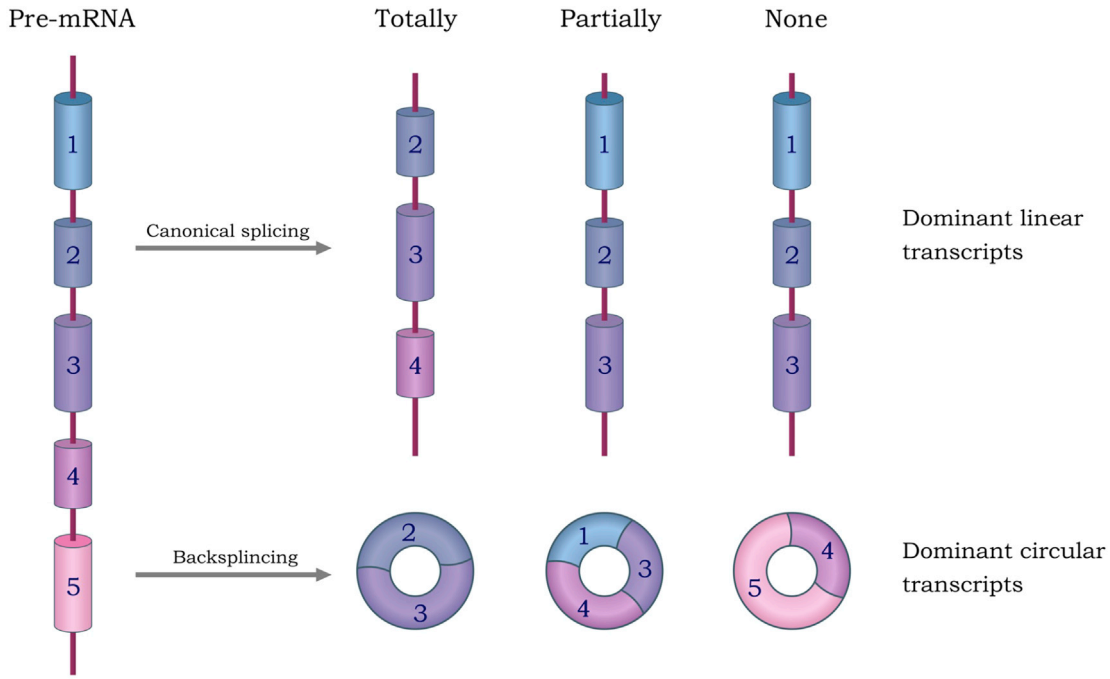

Figure 2. Classification of dominant linear transcripts and dominant circular transcripts

Linear transcripts and circular transcripts can be produced by pre-mRNA. In terms of the patterns of overlap between dominant circular and linear transcripts, circRNAs could be classified into overlapped, partially overlapped, and not overlapped.

mainly due to the upregulation of matrix-degrading enzymes, including those in the matrix metalloproteinase (MMP) family and ADAMTS (a disintegrin and metalloproteinase with thrombospondin motifs) family.[58,59] Current studies on the roles of circRNAs in the ECM mainly focus on the promotion or inhibition of ECM formation. Next, we discuss two opposite effects of circRNAs on ECM formation.

circRNAs that promote ECM formation

circPDE4D derived from the phosphodiesterase 4D (PDE4D) gene was significantly downregulated in OA cartilage. Knocking down circPDE4D downregulated GAG and aggrecan, whereas the matrix catabolic enzymes MMP3, MMP13, ADAMTS4, and ADAMTS5 were significantly upregulated,[59] leading to ECM degradation. circRNAs are known to act as miRNA sponges to eliminate miRNA functions. circPDE4D acts as a sponge to abrogate the function of miR-103a-3p via direct binding, and silencing miR-103a-3p reversed circPDE4D-short hairpin RNA (shRNA)-induced ECM degradation. The targets of circPDE4D and miR-103a-3p include FGF18, a member of the fibroblast growth factor (FGF) family, which is the only gene with cartilage regeneration properties that simultaneously enhances anabolism and suppresses catabolism in healthy controls (HCs).[60] FGF18 was identified as a direct target of miR-103a-3p and was able to reverse an increase in the expression of MMP3, MMP13, ADAMTS4, and ADAMTS5 in circPDE4D-deficient HCs. Thus, the circPDE4D/miR-103a-3p/FGF18 axis was shown to be a potential and critical target for the prevention of ECM degradation in OA.

In addition, circCDK14 (hsa_circ_0001722), which is derived from exons 3 and 4 of the CDK14 gene, was identified as a key factor protecting ECM formation during OA.[61] The overexpression of circCDK14 increased the levels of SRY-related high mobility group-box 9 (SOX9) and collagen II, downregulated MMP3 and MMP13, and inhibited the interleukin (IL)-1β-induced inflammatory response. Similar to other circRNAs, circCDK14 acts as a miRNA sponge. Zhao and colleagues[61] confirmed that miR-125a-5p binds to circCDK14 and is an important downstream target that counteracts the effects of circCDK14 in OA chondrocytes. Furthermore, Smad2 is an important signal transduction protein of the transforming growth factor β (TGF-β) signaling pathway; the 3′ UTR of Smad2 mRNA contains sequences complementary to miR-125a-5p and is thus thought to be downstream of circCDK14/miR-125a-5p. Smad2 overexpression counteracted the effects of circCDK14 knockdown and the miR-125a-5p mimic on SOX9 and collagen II to protect OA chondrocytes. Notably, the circCDK14/miR-125a-5p/Smad2 axis was able to maintain the ECM of chondrocytes but did not downregulate MMP3 or MMP13. Thus, the circCDK14/miR-125a-5p/Smad2 axis provides a potential molecular therapeutic target for OA treatment.

Additionally, circRNA serpin family E member 2 (circSERPINE2, hsa_circ_0008365) plays a role in the modification of ECM homeostasis and has been systematically identified as a protective circRNA in OA. Knocking down circSERPINE2 expression promoted the expression of MMP3, MMP13, and ADAMTS4 and decreased the levels of

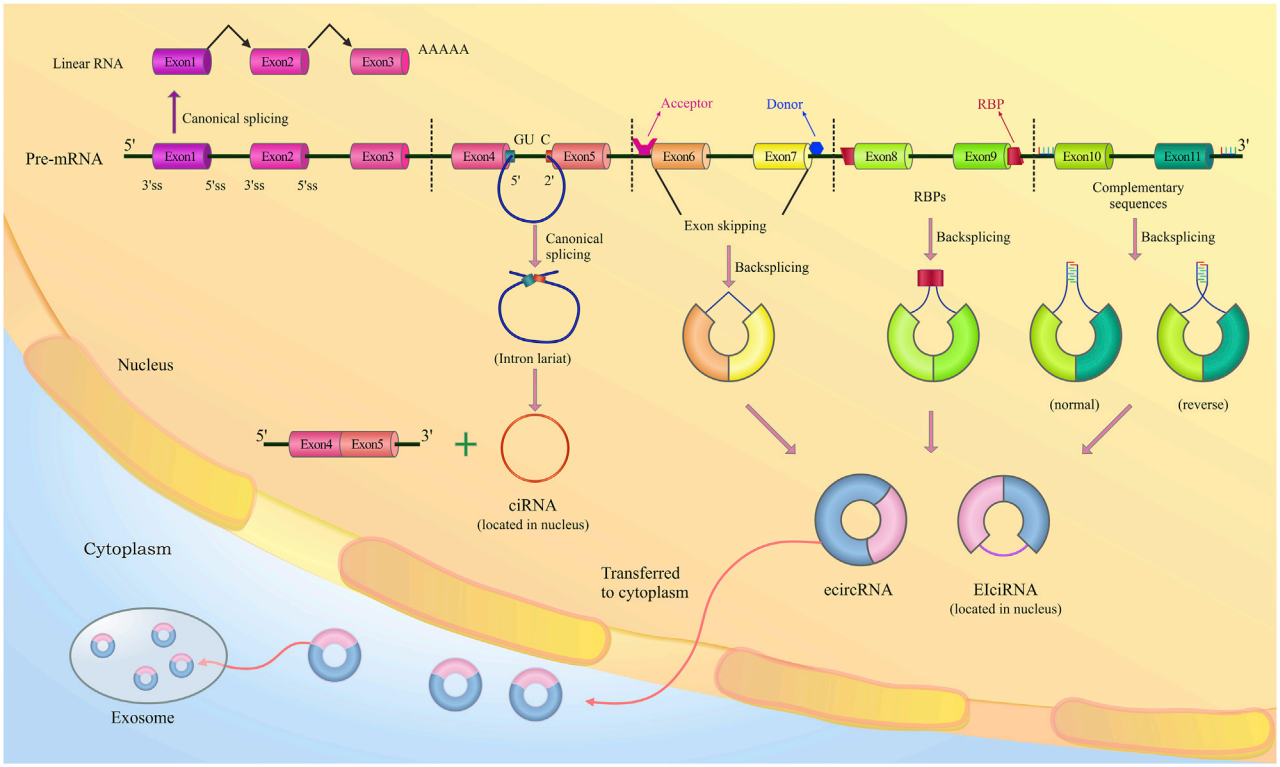

Figure 3. Models of circRNAs biogenesis

ciRNAs are generated by canonical splicing from 2′-5′ intronic lariat and mainly located in the nucleus. ecircRNAs are only derived from exons and mainly located in the cytoplasm, representing more than 80% of total circRNAs. EIciRNAs, containing exon and intron regions, are mainly located in the nucleus. The processes of ecircRNAs and EIciRNAs are characterized by exon skipping, RNA-binding proteins (RBPs), or complementary sequences including normal and reverse pairing. The circRNAs in cytoplasm can be transported by exosomes.

SOX9, collagen type II alpha 1 (COL2A1), and aggrecan, clearly indicating the anticatabolic effects of circSERPINE2 in HCs.[22] Furthermore, circSERPINE2 was able to bind to miR-1271, and E26 transformation-specific (ETS)-related gene (ERG) is a putative target of miR-1271. The effects of miR-1271 on chondrogenic phenotypes were mediated by ERG. Therefore, the circSERPINE2/miR-1271/ERG axis is a novel target for the promotion of ECM formation.

circRNAs that inhibit ECM formation

circRNF121 (hsa_circ_0023404) was increased in chondrocytes induced by IL-1β *in vitro* and is produced by backsplicing exon 2 and exon 3 regions of RNF121 pre-mRNA.[62] The overexpression of circRNF121 increased the levels of MMP13 and ADAMTS5 and decreased the levels of aggrecan and collagen II, indicating that circRNF121 overexpression induces ECM degradation. Thus, the aberrant expression of circRNF121 can mediate OA progression by promoting ECM degradation. miR-665 was selected as the most vintage-correlated miRNA and was negatively correlated with circRNF121. Furthermore, circRNF121 is a direct target of miR-665, and MYD88 is a direct target of miR-665. The expression of MYD88 was increased in cells transfected with circRNF121 and decreased after transfection of cells with a miR-665 mimic, demonstrating that circRNF121 functions as a sponge of miR-665 and indirectly regulates MYD88. Therefore, the circRNF121/miR-665/MYD88 axis provides a promising therapeutic strategy to prevent ECM degradation for OA treatment.

It has been reported that circRNA-CDR1as (cerebellar degeneration-related protein 1 antisense transcript) was upregulated in OA chondrocytes, and silencing of circRNA-CDR1as increased collagen II and decreased MMP13, effects that were reversed by the overexpression of circRNA-CDR1as.[63] circRNA-CDR1as contains the binding sites for other miRNAs and functions as a sponge in various diseases.[64,65] Yu and colleagues[63] investigated whether circRNA-CDR1as functions as a sponge of a certain miRNA to regulate OA. The results showed that circRNA-CDR1as directly targeted miR-641 as a sponge and modulated its downstream functions similar to other circRNAs. In addition, FGF-2 expression was upregulated in OA chondrocytes. Silencing FGF-2 downregulated MMP13, IL-6, and RUNX2 and upregulated collagen II, and knocking down circRNA-CDR1as or miR-641 mimics decreased the levels of FGF-2, phosphorylated (p-)MEK, and p-ERK. Therefore, the circRNA-CDR1as/miR-641/FGF-2 axis may be a potential target for the prevention of the degradation of ECM in the cartilage in OA.

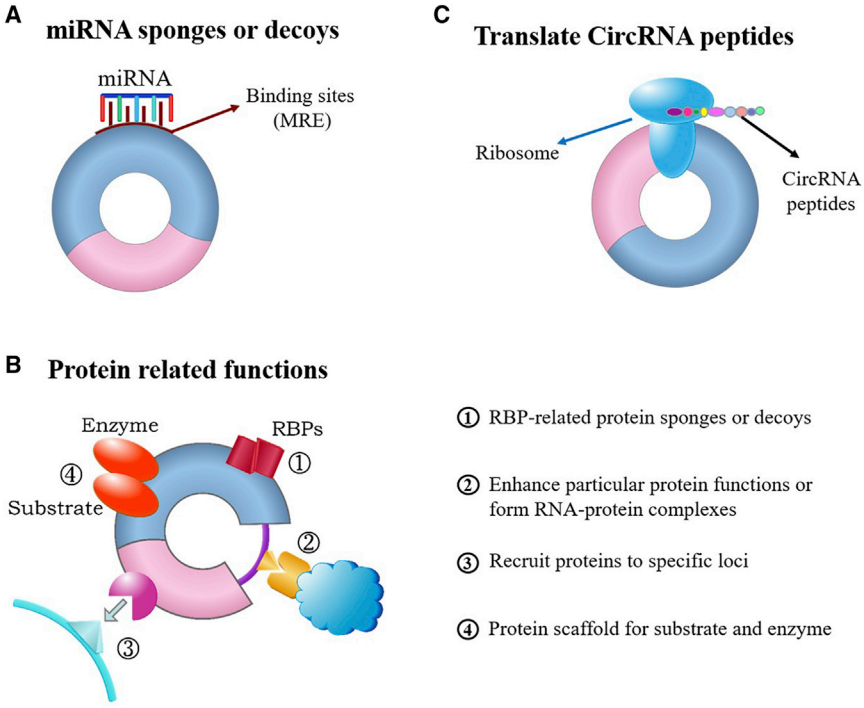

Figure 4. Functions of circRNAs
(A) circRNAs can act as sponges of miRNAs and bind to miRNAs directly via binding sites. (B) circRNAs can act as RBP-related protein sponges or decoys to regulate their functions indirectly and enhance particular protein functions or form RNA-protein complexes. In addition, circRNAs can recruit proteins to specific loci and provide protein scaffold for substrate and enzyme. (C) circRNAs can translate circRNA peptides under certain circumstances.

circRNA_Atp9b (circ_15898) is derived from the chr18:80734143|80934058(−) region of the Atp9b gene and was significantly upregulated in IL-1β-induced mouse chondrocytes, suggesting that circRNA_Atp9b may play a key role in IL-1β-induced chondrocytes. Knocking down circRNA_Atp9b dramatically increased the level of collagen II and decreased the levels of MMP13, IL-6, and COX-2. These data indicate that knocking down circRNA_Atp9b protects against IL-1β-induced ECM degradation and the production of inflammatory factors. In addition, circRNA_Atp9b directly targets miR-138-5p by functioning as a sponge but does not regulate miR-138-5p expression. The effects of circRNA_Atp9b on IL-1β-induced chondrocytes were confirmed to be mediated by targeting miR-138-5p.[66] Thus, the circRNA_Atp9b/miR-138-5p axis may be a part of a potential therapeutic strategy for OA.

The level of MMP13 is increased in chondrocytes induced by tumor necrosis factor (TNF)-α and IL-1β, and the overexpression of MMP13 promotes ECM degradation. circTMBIM6, circRNA-CER, and circRNA.33186 were also upregulated in OA. Wang and colleagues[67] confirmed that circTMBIM6 binds to miR-27a and that the overexpression of circTMBIM6 downregulates miR-27a expression. Ao and colleagues[68] confirmed that circRNA-CER functions as a sponge of miR-136, and Zhu and colleagues[23] confirmed that miR-127-5p is the only binding target of circRNA.33186. In addition, direct binding of MMP13 to miR-27a, miR-136, and miR-127-5p indicated that MMP13 is downstream of circTMBIM6/miR-27a, circRNA-CER/miR-136, and circRNA.33186/miR-127-5p. The TGF-β, JNK, and ERK pathways target MMP13[69,70] and may regulate MMP13 via the circRNA-related pathways.[68] Thus, the circTMBIM6/miR-27a/MMP13 axis, circRNA-CER/miR-136/MMP13 axis, and circRNA.33186/miR-127-5p/MMP13 axis may be potential therapeutic targets for OA.

ROLES OF circRNAs IN APOPTOSIS

Chondrocyte apoptosis plays a key role in the pathogenesis of OA. Several confirmed pathways are involved in chondrocyte apoptosis, such as the FAS/apoptosis antigen 1 (APO-1) pathway,[71] the cysteine-aspartic protease (caspase) family of proteins,[72] and the nitric oxide (NO) pathway.[73] Therefore, investigation of the mechanisms of chondrocyte apoptosis is essential for the identification of potential approaches to OA treatment. Chondrocyte apoptosis is associated with ECM degradation, cell proliferation, and inflammation, and certain circRNAs play multiple roles, such as circRNF121 and circSERPINE2. Recently, the roles of circRNAs with proapoptotic and antiapoptotic effects in chondrocyte apoptosis in OA were investigated, and the information is presented in the following section.

circRNAs that promote apoptosis

circCDH13 (hsa_circ_0040646) plays proapoptotic and procatabolic roles and is produced by backsplicing of the exon 9 and exon 10 regions of the CDH13 gene on chromosome (chr)16. Upregulated circCDH13 has been detected in OA chondrocytes and in HCs treated with IL-1β and TNF-α.[74] Knocking down circCDH13 downregulated MMP13 and ADAMTS5 and increased COL2A1 and aggrecan; all of these effects were reversed by the overexpression of circCDH13. circCDH13 can bind to miR-296-3p, acting as a sponge, and the downstream targets of circCDH13/miR-296-3p were investigated. The results showed that the 3′ UTR of phosphatase and tensin homolog (PTEN) mRNA is directly targeted by miR-296-3p; the effects of PTEN on MMP13, ADAMTS5, aggrecan, and COL2A1 were consistent with the effects of circCDH13 and were reversed by miR-296-3p mimics. The overexpression of circCDH13 contributed to apoptosis via the miR-296-3p/PTEN pathway.

A pronounced reduction in miR-127-5p and a robust increase in circ_0136474 and MMP13 in OA tissues were revealed by Guan and colleagues.[75] The authors demonstrated that miR-127-5p is

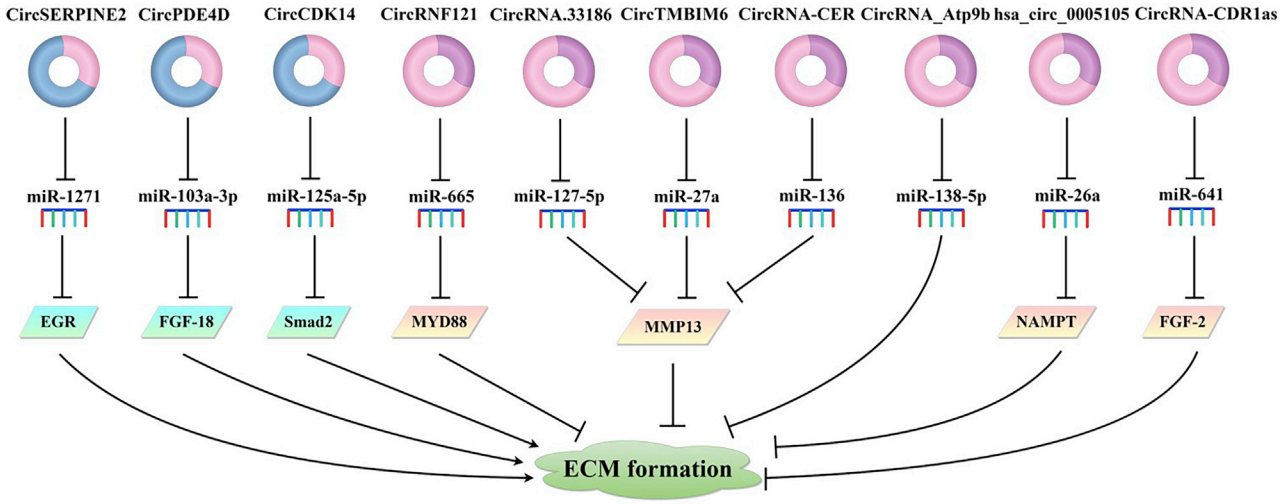

Figure 5. circRNAs that promote or inhibit ECM formation

circSERPINE2, circPDE4D, and circCDK14 can promote ECM formation by acting as miRNA sponges, whereas circRNF121, circRNA.33186, circTMBIM6, circRNA-CER, circRNA_Atp9b, hsa_circ_0005105, and circRNA-CDR1as can inhibit ECM formation. Solid arrow indicates induction; bar-headed line indicates inhibition.

targeted by circ_0136474 and that overexpression of circ_0136474 can decrease miR-127-5p expression. In addition, miR-127-5p can target MMP13, and the negative correlation of miR-127-5p and MMP13 was confirmed. The overexpression of circ_0136474 or a miR-127-5p inhibitor increased the levels of caspase-3 and BAX but downregulated the expression of Bcl-2, indicating an increase in the apoptosis rate, which can be reversed by si-circ_0136474 and miR-127-5p mimics. In contrast, si-circ_0136474 or miR-127-5p repressed IL-1β, TNF-α, and IL-17, which downregulated MMP13. In addition, miR-127-5p can be targeted by circRNA.33186 and MMP13.[23] In summary, circ_0136474 and circRNA.33186 sponge miR-127-5p to upregulate MMP13 in OA, and the circ_0136474/miR-127-5p/MMP13 axis and the circRNA.33186/miR-127-5p/MMP13 axis may provide new therapeutic strategies for OA.

circRNAs that inhibit apoptosis

circRNA-9119 was significantly decreased in IL-1β-treated chondrocytes, suggesting that it is closely involved in OA progression.[76] Sun and colleagues[76] confirmed that circRNA-9119 inhibits the apoptosis of chondrocytes after IL-1β induction, as reflected by the upregulation of Bcl-2 and downregulation of BAX. Bioinformatics analysis showed that miR-26a is a target of circRNA-9119 and that PTEN is targeted by miR-26a. circRNA-9119 functions as a ceRNA by sequestering miR-26a.[77,78] A decrease in PTEN in IL-1β-induced chondrocytes was restored via the upregulation of circRNA-9119 expression. Thus, miR-26a expression is downregulated via circRNA-9119 to increase PTEN expression in IL-1β-induced chondrocytes. The PTEN-related pathway is associated with apoptosis of various cells.[79,80] The levels of the factors downstream of PTEN, which represent PTEN activity, including COX-2, phosphatidylinositol 3-kinase (PI3K), Akt, and p-Akt, were reduced in IL-1β-induced chondrocytes, and this effect was reversed by the activation of PTEN. In summary, the circRNA-9119/miR-26a/PTEN axis is an ideal target for OA treatment.

circRNA HIPK3 (circHIPK3) was reported to be associated with the occurrence and development of various diseases;[81,82] low expression of circHIPK3 significantly promotes apoptosis in OA, and miR-124 is significantly downregulated. A dual-luciferase assay confirmed that circHIPK3 binds to miR-124, and their levels are negatively correlated. A decrease in circHIPK3 and the overexpression of miR-124 can enhance chondrocyte apoptosis, increasing the mRNA and protein levels of caspase-3. Considering that miR-124 serves as a sponge of SOX8 in non-small cell lung cancer, Tang and colleagues[82] investigated whether miR-124 also targets SOX8 in OA chondrocytes. The results indicated that miR-124 can directly regulate SOX8 and that the miR-124 level is negatively correlated with SOX8 expression. Overall, circHIPK3 inhibits apoptosis of OA chondrocytes by acting as a sponge of miR-124 via SOX8, and the circHIPK3/miR-124/SOX8 axis provides a novel mechanism for the inhibition of chondrocyte apoptosis in OA therapy.

As noted earlier in this review, circSERPINE2 protects ECM metabolism and regulates the apoptosis of OA chondrocytes. Xia and colleagues[83] found that the overexpression of circSERPINE2 mitigated IL-1β-induced chondrocyte apoptosis by decreasing caspase-3. OA-associated miR-495 has been confirmed as a target of circSERPINE2, and TGF-β receptor 2 (TGFBR2) is targeted by miR-495. These correlations confirm that circSERPINE2 can reduce miR-495 abundance and promote TGFBR2 expression in chondrocytes by competitively binding miR-495. Thus, the circSERPINE2/miR-495/TGFBR2 axis may be a potential target for the prevention of chondrocyte apoptosis in OA.

ROLES OF circRNAs IN PROLIFERATION

In addition to the roles of circRNAs in ECM formation and apoptosis, their effects on the balance of chondrocyte proliferation are involved in the development of OA. Several research groups have recently

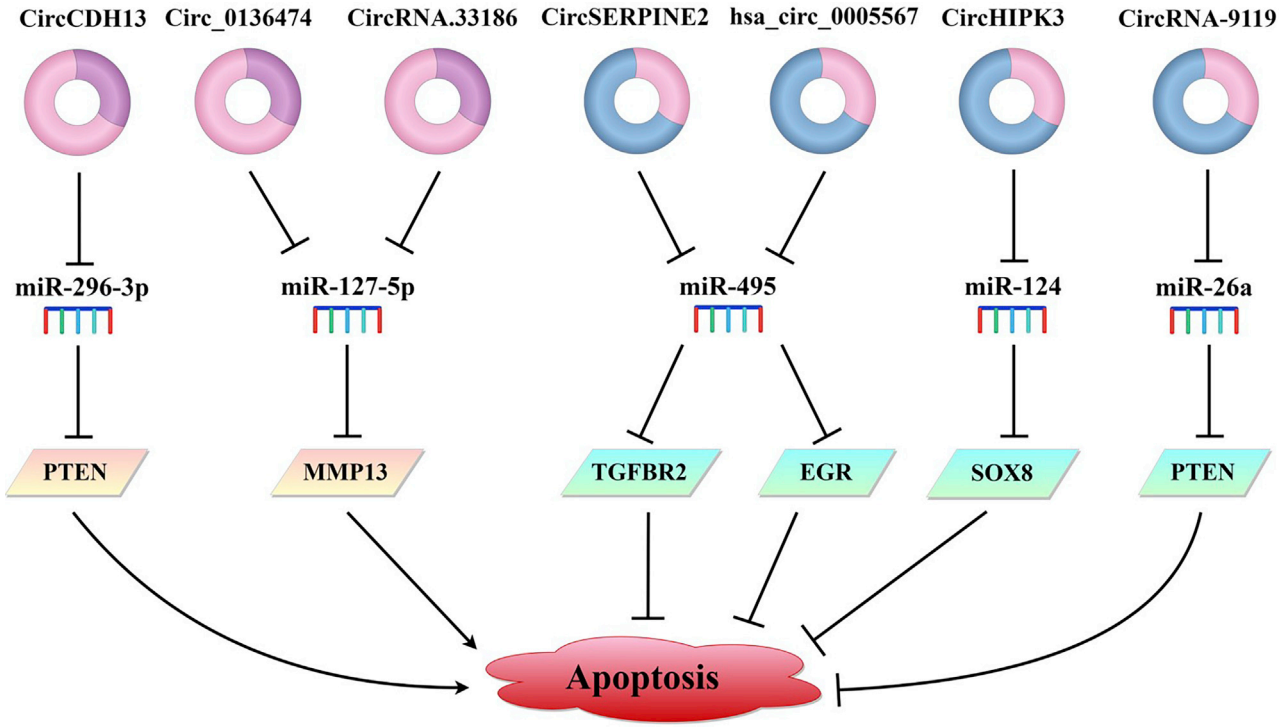

Figure 6. circRNAs that promote or inhibit chondrocyte apoptosis

circCDH13 can promote chondrocyte apoptosis by acting as the sponge of miR-296-3p, and circ_0136474 and circRNA.33186 can promote chondrocyte apoptosis by acting as the sponge of miR-127-5p. However, circSERPINE2, hsa_circ_0005567, circHIPK3, and circRNA-9119 can inhibit chondrocyte apoptosis with protective roles.

studied the roles of circRNAs in chondrocyte proliferation in OA. In the following section, we summarize recent findings on the effects of circRNAs on chondrocyte proliferation.

circRNAs that promote proliferation

The downregulated expression of hsa_circ_0045714 and the upregulated expression of miR-193b are negatively correlated in chondrocytes treated with TNF-α.[48] Insulin-like growth factor 1 receptor (IGF1R) is an important target gene of miR-193b that inhibits IGF1R expression; however, hsa_circ_0045714 notably upregulates IGF1R expression and antagonizes the inhibition of IGF1R expression by miR-193b. IGF1R is a member of the insulin-like growth factor receptor family and has been considered an important target gene affected by miR-193b. Upon IGF1 binding, IGF1R activates the PI3K and mitogen-activated protein kinase (MAPK) signaling pathway and regulates cell proliferation, differentiation, and apoptosis through autophosphorylation. Both hsa_circ_0045714 and IGF1R can promote cell proliferation, whereas miR-193b does not have this effect. In fact, miR-193b can promote apoptosis by silencing hsa_circ_0045714 and thus IGF1R. Moreover, IGF1R siRNA can restrain the function of hsa_circ_0045714, and this effect can be reversed by IGF1R overexpression.[48] Thus, hsa_circ_0045714 promotes the proliferation of chondrocytes through the miR-193b target gene IGF1R, and the hsa_circ_0045714/miR-193b/IGF1R axis provides a new possibility for OA treatment based on circRNAs.

circRNAs that inhibit proliferation

circRNA.33186 is a 536-nt circRNA derived from the Umad1 gene on chr6:8373906|8427185(+) and is significantly upregulated in OA chondrocytes, and silencing circRNA.33186 considerably alleviates OA *in vivo*.[23] Zhu and colleagues[23] demonstrated that knocking down circRNA.33186 can reverse a decrease in the proliferation of chondrocytes. Furthermore, the authors confirmed that miR-127-5p was the only binding target of circRNA.33186 and that the level of miR-127-5p was negatively correlated with the expression of circRNA.33186. Then, Zhu and colleagues investigated the interaction between circRNA.33186 and miR-127-5p, and the results showed that a reduction in MMP13 expression caused by silencing circRNA.33186 was significantly restored by a miR-127-5p inhibitor. Thus, circRNA.33186 promotes OA pathogenesis by functioning as a sponge for miR-127-5p. In addition, miR-127-5p functions by regulating MMP13. Overall, the circRNA.33186/miR-127-5p/MMP13 axis can be considered a potential target for OA therapy.

circPSM3 can inhibit the proliferation and metabolism of gastric cancer by directly targeting miR-296-5p.[55] Shi and colleagues[19] investigated the role of circPSM3 in OA and found that the expression of circPSM3 was notably increased in OA chondrocytes and that cell proliferation and the levels of the proliferation-related PCNA molecule were increased due to circPSM3 silencing. The levels of cell differentiation-related molecules, including BMP2, BMP4, BMP6, and

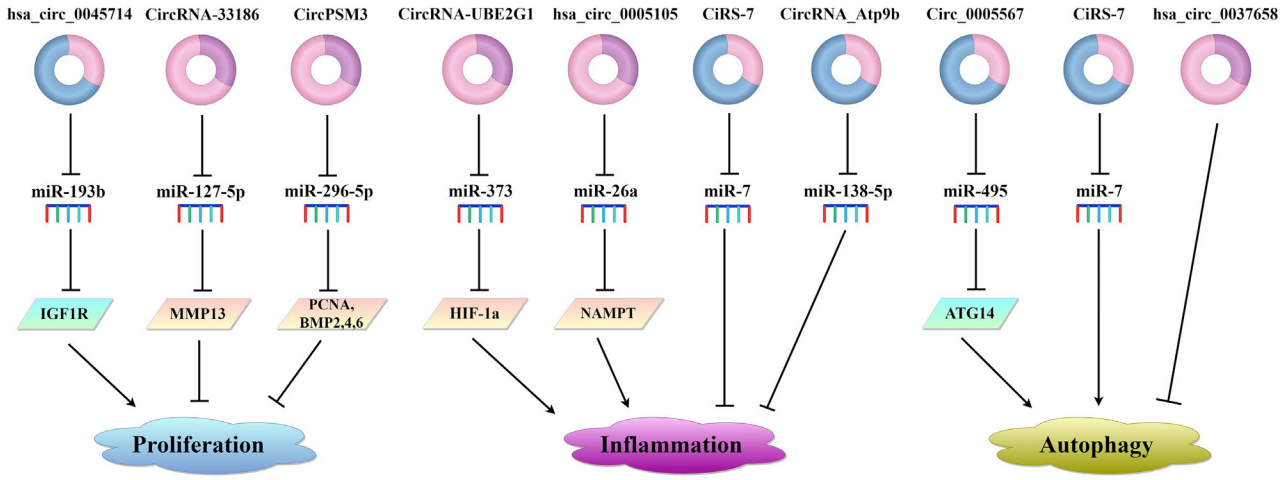

Figure 7. circRNAs that promote or inhibit proliferation, inflammation, and autophagy

hsa_circ_00045714 can promote proliferation, ciRS-7 and circRNA_Atp9b can inhibit inflammation, circ_000557 and ciRS-7 can promote autophagy, and all are protective. In contrast, circRNA.33186 and circPSM3 can inhibit proliferation, circRNA-UBE2G1 and hsa_circ_0005105 can promote inflammation, and hsa_circ_0037658 can inhibit autophagy.

RUN2, were increased. In addition, silencing circPSM3 in OA chondrocytes can significantly promote miR-296-5p expression, which is negatively correlated with the level of circPSM3; subsequent investigation confirmed that circPSM3 may influence the proliferation and differentiation of chondrocytes by acting as a sponge of miRNA-296-5p in OA. overall, the CircPSM3/miRNA-296-5p axis provides a theoretical basis for OA treatment.

ROLES OF circRNAs IN INFLAMMATION

OA is an inflammation-related disease, and the related symptoms include joint swelling and pain;[72,84] thus, the activation of inflammation is a critical feature of OA pathogenesis. Abnormal increases in inflammatory cytokines can lead to the destruction and degradation of ECM and inhibit the synthesis and repair of chondrocytes, resulting in a vicious cycle of ECM damage,[85] including the role of hsa_circ_0005105. In the next section, we provide a review of the pro-inflammatory and anti-inflammatory roles of circRNAs in OA chondrocytes.

circRNAs that promote inflammation

circRNA-UBE2G1 (hsa_circ_0008956), which is derived from the UBE2G1 gene, and HIF-1a were upregulated in the OA tissues, and the levels of both molecules were positively correlated with modified Mankin scores; in contrast, miR-373 expression was downregulated and negatively correlated with modified Mankin scores.[8] Chen et al.[8] determined the mechanism of action of circRNA-UBE2G1 in OA and found that miR-373 harbors a binding site for circRNA-UBE2G1. In addition, HIF-1a was identified as an important factor; the results showed that miR-373 mimics significantly reduced HIF-1a expression, and this effect was reversed by miR-373 inhibitors, suggesting that miR-373 is targeted by HIF-1a. Moreover, HIF-1a was positively correlated with circRNA-UBE2G1 and negatively correlated with miR-373, and the overexpression of circRNA-UBE2G1 and HIF-1a or a miR-373 inhibitor can increase the levels of IL-1β, IL-6, and TNF-α.[8] Thus, circRNA-UBE2G1 regulates HIF-1a expression through miR-373 sponging, and the circRNA-UBE2G1/miR-373/HIF-1a axis may be a potential target for the prevention of inflammation for OA treatment.

IL-1β-induced chondrocytes are suitable models of inflammatory chondrocytes. A significant increase in the expression of hsa_circ_0005105 was detected in IL-1β-induced chondrocytes by Zhang and colleagues,[86] and miR-26a was inhibited in IL-1β-induced chondrocytes and was negatively correlated with hsa_circ_0005105. In addition, miR-26a can counteract the effects of hsa_circ_0005105. Nicotinamide phosphoribosyltransferase (NAMPT), also known as visfatin or pre-B cell colony-enhancing factor (PBEF), was identified as a target of miR-26a. hsa_circ_0005105 was shown to contribute to the expression of prostaglandin E2 (PGE2), IL-6, and IL-8; however, miR-26a inhibited the expression of these factors, and downregulated NAMPT can reverse this function of hsa_circ_0005105. Thus, hsa_circ_0005105 enhances the expression of the inflammatory factors by binding to the miR-26a target NAMPT, thereby aggravating OA progression. Overall, the hsa_circ_0005105/miR-26a/NAMPT axis provides a novel strategy for the prevention of inflammation in OA.

circRNAs that inhibit inflammation

ciRS-7, also known as Cdr1as, is considered an endogenous competitive RNA inhibitor of miR-7 and acts as a "super sponge" of miR-7.[54] In OA chondrocytes, the expression of ciRS-7 is significantly downregulated; in contrast, miR-7 is upregulated. The results of a study by Huang and colleagues[4] indicated that transfection of ciRS-7 siRNA and miR-7 mimic enhanced inflammatory cytokine release, which was reversed by ciRS-7 cDNA and a miR-7 inhibitor. Thus, the ciRS-7/miR-7 axis contributes to inflammation in OA chondrocytes and provides an anti-inflammatory approach for OA therapy.

ROLES OF circRNAs IN AUTOPHAGY

Autophagy represents the ability of the cells to prevent their own death and to protect cells against apoptosis.[87] Autophagy is an important trigger of apoptosis and has been reported to be closely associated with the progression of OA. Protective autophagy occurs in the initial degenerative phase of OA and is reduced as cartilage gradually degrades. Activation of the PI3K/AKT/mTOR pathway, which is the fundamental intracellular signaling pathway, can inhibit autophagy.[88] Therefore, autophagy-related circRNAs also are worthy of further detailed investigation.

circRNAs that promote autophagy

The expression of circ_0005567 was significantly lower in chondrocytes induced by IL-1β, and circ_0005567 overexpression suppressed the apoptosis induced by IL-1β, an effect that can be abrogated by knocking down circ_0005567.[89] Gui and colleagues[89] determined the relationship between circ_0 005567 expression and chondrocyte autophagy, which is based on the chondroprotective role of autophagy. The results of the study showed that circ_0005567 expression upregulated autophagy-related markers, namely, LC3 and beclin-1, and the ratio of LC3-II/LC3-I. However, an inhibitor of autophagy, 3-methyladenine (3-MA), reversed the promotion of autophagy mediated by circ_0005567 overexpression. In brief, the overexpression of circ_0005567, which is a therapeutic target, attenuated IL-1β-induced chondrocyte apoptosis by restoring autophagy deficiency in OA chondrocytes. circ_0005567 can depress ATG14 expression by functioning as a miR-495 sponge. Thus, the role of circ_0005567 in the promotion of autophagy is mediated by sponging miR-495 to decrease ATG14 expression. Overall, the circ_0005567/miR-495/ATG14 axis can be a promising therapeutic target in the regulation of autophagy in OA therapy.

In addition, Huang and colleagues[88] speculated that the regulatory effects of the ciRS-7/miR-7 axis on cartilage degradation and autophagy defects mediated by IL-17A was closely associated with the activation of the PI3K/AKT/mTOR pathway and demonstrated that the ciRS-7/miR-7 axis downregulates IL-17A-mediated PI3K/AKT/mTOR activation, autophagy damage, and ECM degradation.

circRNAs that inhibit autophagy

It was also reported that hsa_circ_0037658 was notably upregulated in OA.[90] The correlation between hsa_circ_0037658 and autophagy in CHON-001 cells treated with IL-1β was investigated, and the results showed that knocking down hsa_circ_0037658 can notably attenuate this effect by inducing autophagy.[20] A decrease in collagen II and aggrecan and an increase in MMP13 induced by IL-1β were reversed by hsa_circ_0037658 shRNAs. LC3 plays regulatory roles in autophagy; the level of LC3 was considerably decreased and the apoptosis rate was obviously increased in CHON-001 cells induced by IL-1β, and both effects were reversed by hsa_circ_0037658 shRNAs. In addition, the expression of ATG5 and beclin-1 was downregulated by IL-1β, and the effect was reversed by hsa_circ_0037658 shRNAs; the expression of p62 and AIF was upregulated by IL-1β and inhibited by hsa_circ_0037658 shRNAs. Thus, knocking down hsa_circ_0037658 inhibited OA progression by inducing autophagy, and hsa_circ_0037658 may serve as a potential target for OA treatment. However, the correlation between hsa_circ_0037658 and miRNAs in OA remains unclear, and the interaction between miRNAs and autophagy-related proteins is poorly understood.

CONCLUSIONS AND PERSPECTIVES

OA is a common arthritis with multifactorial pathogenesis, and it is a serious public health issue with tremendous economic burden worldwide. Cartilage degeneration is the main pathological characteristic, and previous studies have shown that aberrant biological functions of chondrocytes, including ECM formation, apoptosis, inflammation, proliferation, and autophagy, are causally involved in OA pathogenesis. Increasing evidence, as provided in our review, has indicated that circRNAs with regulatory effects are critical for OA pathogenesis. However, it is difficult to select a therapeutic for the prioritization of various classes of non-coding RNAs (miRNAs, lncRNAs, and circRNAs) due to the uncertainty of their importance in OA development.

Studies on circRNAs in OA are currently popular, although investigations of the roles of circRNAs in chondrocytes in OA are still at a relatively early stage. Recent studies have confirmed that circRNAs participate in the pathogenesis of OA in chondrocytes, including ECM formation, apoptosis, proliferation, inflammation, and autophagy, providing novel insight into the pathological and mechanistic aspects of OA. Due to the biological characteristics of circRNAs, their roles as biomarkers in OA have been promulgated,[13] such as hsa_circ_003213 in peripheral blood and hsa_circ_0104595 in synovial fluid.[91]

Regarding the therapeutic effects of circRNAs, our review has described the known mechanisms and signaling pathways that may be involved in OA; however, certain challenges remain to be addressed. First, the roles of only a small number of circRNAs have been studied in chondrocytes in OA; thus, additional circRNAs need to be identified and further investigated. Second, in addition to circRNAs themselves, circRNA-related pathways contain other downstream molecules that are directly or indirectly regulated by circRNAs; therefore, the studies need to determine whether circRNA-related pathways should be considered as a whole to achieve clinical therapeutic effects or whether only circRNAs should be targeted. OA is known to involve the entire joint, including subchondral bone and synovium; hence, the roles of circRNAs in osteoblasts, osteoclasts, and synoviocytes deserve additional attention, and more efforts are required a attain a comprehensive understanding of the pathogenesis of OA. Finally, the clarification of the delivery of circRNAs is essential. Previous studies have reported that circRNAs can be highly enriched in exosomes in bodily fluids.[12,13] The emerging recognition that circRNAs can be transferred by exosomes suggests a new perspective on the possible transfer of circRNAs through synovial fluid by exosomes; thus, circRNAs in the synovial fluid may be used as biomarkers for the diagnosis of OA. In addition, biotherapy and gene therapy are current research trends in disease treatment, and extracellular vesicles have shown great potential as the vehicle

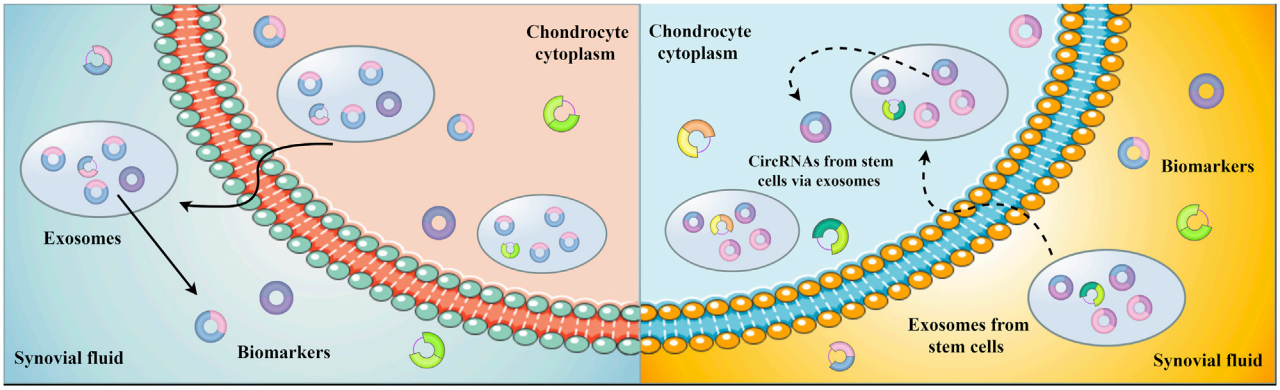

Figure 8. Potential diagnostic and therapeutic roles of circRNAs

As attributed to their characteristics, circRNAs may be transferred from chondrocyte cytoplasm to synovial fluid by exosomes and be detected for the diagnosis of OA. In addition, MSC-derived exosomes may contain circRNAs and transfer them into chondrocyte cytoplasm to protect chondrocytes in OA.

to selectively and accurately deliver drugs into a specific site of tissue.[92] Mesenchymal stem cell (MSC)-derived exosomes have biochemical potential for cartilage regeneration in OA therapy.[93,94] Therefore, we speculate that transferring protective circRNAs into chondrocytes in OA may be one of the therapeutic mechanisms of MSC-derived exosomes (Figure 8). However, the best way to achieve exosome-specific delivery of circRNA-based therapeutics remains undefined. Moreover, in addition to the diagnostic biomarker and possible therapeutic target of circRNAs, other novel directions need to be explored as well. In summary, further studies are essential to determine whether circRNAs and related pathways can be used to develop clinical therapies.

Current strategies for OA treatment are tiered and palliative; therefore, modifying OA progression, including slowing, halting, and reversing the progression via molecular mechanisms, is essential.[1] Although exploiting circRNAs for the treatment of OA is premature, circRNAs have been shown to be potential biomarkers and therapeutic targets for novel clinical strategies in OA. Considering the in-depth investigation of circRNAs and the development of biotechnology, treatments for OA may be achieved by restoring the expression of downregulated circRNAs or silencing aberrantly upregulated circRNAs. To the best of our knowledge, the role of circRNAs as miRNA sponges is among the critical functions of circRNAs, suggesting that artificial therapeutic agents mimicking the structures and functions of endogenic circRNA sponges can regulate the downstream miRNA/mRNA pathways in OA. In addition, siRNA and genome-editing tools could be used for suppressing the synthesis of circRNAs, which should be at a low expression state.[91,95] Moreover, circRNAs are closely associated with gene medicine and translational medicine, both of which are undergoing an upsurge worldwide; hereafter, safer and more effective strategies based on circRNAs and related pathways will be developed.

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.omtn.2021.04.006.

ACKNOWLEDGMENTS

This work was supported by a grant from the National Natural Science Foundation of China (no. 81972075) and by the Shanxi Province Science and Technology Research Project (no. 201803D421050).

AUTHOR CONTRIBUTIONS

X.M. and Y.C. wrote the manuscript, Z.G. retrieved literature, and L.W. and C.X. critically revised the manuscript. All authors have read and approved the final manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.

REFERENCES

1. Hunter, D.J., and Bierma-Zeinstra, S. (2019). Osteoarthritis. Lancet *393*, 1745–1759.
2. Mao, X., Fu, P., Wang, L., and Xiang, C. (2020). Mitochondria: Potential targets for osteoarthritis. Front. Med. (Lausanne) *7*, 581402.
3. Hunter, D.J., March, L., and Chew, M. (2020). Osteoarthritis in 2020 and beyond: A *Lancet* Commission. Lancet *396*, 1711–1712.
4. Zhou, X., Jiang, L., Fan, G., Yang, H., Wu, L., Huang, Y., Xu, N., and Li, J. (2019). Role of the ciRS-7/miR-7 axis in the regulation of proliferation, apoptosis and inflammation of chondrocytes induced by IL-1β. Int. Immunopharmacol. *71*, 233–240.
5. Carr, A.J., Robertsson, O., Graves, S., Price, A.J., Arden, N.K., Judge, A., and Beard, D.J. (2012). Knee replacement. Lancet *379*, 1331–1340.
6. Turner, A.M., and Morris, K.V. (2010). Controlling transcription with noncoding RNAs in mammalian cells. Biotechniques *48*, ix–xvi.
7. Lei, W., Feng, T., Fang, X., Yu, Y., Yang, J., Zhao, Z.A., Liu, J., Shen, Z., Deng, W., and Hu, S. (2018). Signature of circular RNAs in human induced pluripotent stem cells and derived cardiomyocytes. Stem Cell Res. Ther. *9*, 56.
8. Chen, G., Liu, T., Yu, B., Wang, B., and Peng, Q. (2020). circRNA-UBE2G1 regulates LPS-induced osteoarthritis through miR-373/HIF-1a axis. Cell Cycle *19*, 1696–1705.
9. Memczak, S., Jens, M., Elefsinioti, A., Torti, F., Krueger, J., Rybak, A., Maier, L., Mackowiak, S.D., Gregersen, L.H., Munschauer, M., et al. (2013). Circular RNAs are a large class of animal RNAs with regulatory potency. Nature *495*, 333–338.
10. Zhou, Z., Du, D., Chen, A., and Zhu, L. (2018). Circular RNA expression profile of articular chondrocytes in an IL-1β-induced mouse model of osteoarthritis. Gene *644*, 20–26.
11. Han, B., Chao, J., and Yao, H. (2018). Circular RNA and its mechanisms in disease: From the bench to the clinic. Pharmacol. Ther. *187*, 31–44.

12. Li, Y., Zheng, Q., Bao, C., Li, S., Guo, W., Zhao, J., Chen, D., Gu, J., He, X., and Huang, S. (2015). Circular RNA is enriched and stable in exosomes: A promising biomarker for cancer diagnosis. Cell Res. *25*, 981–984.

13. Zhang, Z., Yang, T., and Xiao, J. (2018). Circular RNAs: Promising biomarkers for human diseases. EBioMedicine *34*, 267–274.

14. Lukiw, W.J. (2013). Circular RNA (circRNA) in Alzheimer's disease (AD). Front. Genet. *4*, 307.

15. Wu, D., Jia, H., Zhang, Z., and Li, S. (2021). circ-PRMT5 promotes breast cancer by the miR-509-3p/TCF7L2 axis activating PI3K/AKT pathway. J. Gene Med. *23*, e3300.

16. Jiang, X., and Chen, D. (2021). Circular RNA hsa_circ_0000658 inhibits osteosarcoma cell proliferation and migration via the miR-1227/IRF2 axis. J. Cell. Mol. Med. *25*, 510–520.

17. Wang, F., Fan, M., Cai, Y., Zhou, X., Tai, S., Yu, Y., Wu, H., Zhang, Y., Liu, J., Huang, S., et al. (2020). Circular RNA circRIMS1 acts as a sponge of miR-433-3p to promote bladder cancer progression by regulating CCAR1 expression. Mol. Ther. Nucleic Acids *22*, 815–831.

18. Cheng, X., Zhang, L., Zhang, K., Zhang, G., Hu, Y., Sun, X., Zhao, C., Li, H., Li, Y.M., and Zhao, J. (2018). Circular RNA VMA21 protects against intervertebral disc degeneration through targeting miR-200c and X linked inhibitor-of-apoptosis protein. Ann. Rheum. Dis. *77*, 770–779.

19. Ni, J.L., Dang, X.Q., and Shi, Z.B. (2020). circPSM3 inhibits the proliferation and differentiation of OA chondrocytes by targeting miRNA-296-5p. Eur. Rev. Med. Pharmacol. Sci. *24*, 3467–3475.

20. Sui, C., Liu, D., Que, Y., Xu, S., and Hu, Y. (2021). Knockdown of hsa_circ_0037658 inhibits the progression of osteoarthritis via inducing autophagy. Hum. Cell *34*, 76–85.

21. Zhu, H., Hu, Y., Wang, C., Zhang, X., and He, D. (2020). circGCN1L1 promotes synoviocyte proliferation and chondrocyte apoptosis by targeting miR-330-3p and TNF-α in TMJ osteoarthritis. Cell Death Dis. *11*, 284.

22. Shen, S., Wu, Y., Chen, J., Xie, Z., Huang, K., Wang, G., Yang, Y., Ni, W., Chen, Z., Shi, P., et al. (2019). circSERPINE2 protects against osteoarthritis by targeting miR-1271 and ETS-related gene. Ann. Rheum. Dis. *78*, 826–836.

23. Zhou, Z.B., Huang, G.X., Fu, Q., Han, B., Lu, J.J., Chen, A.M., and Zhu, L. (2019). circRNA.33186 contributes to the pathogenesis of osteoarthritis by sponging miR-127-5p. Mol. Ther. *27*, 531–541.

24. Li, H.Z., Lin, Z., Xu, X.H., Lin, N., and Lu, H.D. (2018). The potential roles of circRNAs in osteoarthritis: A coming journey to find a treasure. Biosci. Rep. *38*, BSR20180542.

25. Liu, Q., Zhang, X., Hu, X., Yuan, L., Cheng, J., Jiang, Y., and Ao, Y. (2017). Emerging roles of circRNA related to the mechanical stress in human cartilage degradation of osteoarthritis. Mol. Ther. Nucleic Acids *7*, 223–230.

26. Ren, S., Lin, P., Wang, J., Yu, H., Lv, T., Sun, L., and Du, G. (2020). Circular RNAs: Promising molecular biomarkers of human aging-related diseases via functioning as an miRNA sponge. Mol. Ther. Methods Clin. Dev. *18*, 215–229.

27. Ashwal-Fluss, R., Meyer, M., Pamudurti, N.R., Ivanov, A., Bartok, O., Hanan, M., Evantal, N., Memczak, S., Rajewsky, N., and Kadener, S. (2014). circRNA biogenesis competes with pre-mRNA splicing. Mol. Cell *56*, 55–66.

28. Li, X., Yang, L., and Chen, L.L. (2018). The biogenesis, functions, and challenges of circular RNAs. Mol. Cell *71*, 428–442.

29. Wilusz, J.E. (2018). A 360° view of circular RNAs: From biogenesis to functions. Wiley Interdiscip. Rev. RNA *9*, e1478.

30. Li, Z., Huang, C., Bao, C., Chen, L., Lin, M., Wang, X., Zhong, G., Yu, B., Hu, W., Dai, L., et al. (2015). Exon-intron circular RNAs regulate transcription in the nucleus. Nat. Struct. Mol. Biol. *22*, 256–264.

31. Liu, J., Liu, T., Wang, X., and He, A. (2017). Circles reshaping the RNA world: From waste to treasure. Mol. Cancer *16*, 58.

32. Kristensen, L.S., Andersen, M.S., Stagsted, L.V.W., Ebbesen, K.K., Hansen, T.B., and Kjems, J. (2019). The biogenesis, biology and characterization of circular RNAs. Nat. Rev. Genet. *20*, 675–691.

33. Xiao, M.S., Ai, Y., and Wilusz, J.E. (2020). Biogenesis and functions of circular RNAs come into focus. Trends Cell Biol. *30*, 226–240.

34. Piwecka, M., Glažar, P., Hernandez-Miranda, L.R., Memczak, S., Wolf, S.A., Rybak-Wolf, A., Filipchyk, A., Klironomos, F., Cerda Jara, C.A., Fenske, P., et al. (2017). Loss of a mammalian circular RNA locus causes miRNA deregulation and affects brain function. Science *357*, eaam8526.

35. Zhao, Z.J., and Shen, J. (2017). Circular RNA participates in the carcinogenesis and the malignant behavior of cancer. RNA Biol. *14*, 514–521.

36. Zhang, H.D., Jiang, L.H., Sun, D.W., Hou, J.C., and Ji, Z.L. (2018). circRNA: A novel type of biomarker for cancer. Breast Cancer *25*, 1–7.

37. Dudekula, D.B., Panda, A.C., Grammatikakis, I., De, S., Abdelmohsen, K., and Gorospe, M. (2016). CircInteractome: A web tool for exploring circular RNAs and their interacting proteins and microRNAs. RNA Biol. *13*, 34–42.

38. Abdelmohsen, K., Panda, A.C., Munk, R., Grammatikakis, I., Dudekula, D.B., De, S., Kim, J., Noh, J.H., Kim, K.M., Martindale, J.L., and Gorospe, M. (2017). Identification of HuR target circular RNAs uncovers suppression of PABPN1 translation by *CircPABPN1*. RNA Biol. *14*, 361–369.

39. Du, W.W., Fang, L., Yang, W., Wu, N., Awan, F.M., Yang, Z., and Yang, B.B. (2017). Induction of tumor apoptosis through a circular RNA enhancing Foxo3 activity. Cell Death Differ. *24*, 357–370.

40. Du, W.W., Yang, W., Liu, E., Yang, Z., Dhaliwal, P., and Yang, B.B. (2016). Foxo3 circular RNA retards cell cycle progression via forming ternary complexes with p21 and CDK2. Nucleic Acids Res. *44*, 2846–2858.

41. Zeng, Y., Du, W.W., Wu, Y., Yang, Z., Awan, F.M., Li, X., Yang, W., Zhang, C., Yang, Q., Yee, A., et al. (2017). A circular RNA binds to and activates AKT phosphorylation and nuclear localization reducing apoptosis and enhancing cardiac repair. Theranostics *7*, 3842–3855.

42. Chen, N., Zhao, G., Yan, X., Lv, Z., Yin, H., Zhang, S., Song, W., Li, X., Li, L., Du, Z., et al. (2018). A novel *FLI1* exonic circular RNA promotes metastasis in breast cancer by coordinately regulating TET1 and DNMT1. Genome Biol. *19*, 218.

43. Pamudurti, N.R., Bartok, O., Jens, M., Ashwal-Fluss, R., Stottmeister, C., Ruhe, L., Hanan, M., Wyler, E., Perez-Hernandez, D., Ramberger, E., et al. (2017). Translation of circRNAs. Mol. Cell *66*, 9–21.e7.

44. Begum, S., Yiu, A., Stebbing, J., and Castellano, L. (2018). Novel tumour suppressive protein encoded by circular RNA, circ-SHPRH, in glioblastomas. Oncogene *37*, 4055–4057.

45. Zhang, M., Huang, N., Yang, X., Luo, J., Yan, S., Xiao, F., Chen, W., Gao, X., Zhao, K., Zhou, H., et al. (2018). A novel protein encoded by the circular form of the *SHPRH* gene suppresses glioma tumorigenesis. Oncogene *37*, 1805–1814.

46. Zhang, M., Zhao, K., Xu, X., Yang, Y., Yan, S., Wei, P., Liu, H., Xu, J., Xiao, F., Zhou, H., et al. (2018). A peptide encoded by circular form of *LINC-PINT* suppresses oncogenic transcriptional elongation in glioblastoma. Nat. Commun. *9*, 4475.

47. You, X., Vlatkovic, I., Babic, A., Will, T., Epstein, I., Tushev, G., Akbalik, G., Wang, M., Glock, C., Quedenau, C., et al. (2015). Neural circular RNAs are derived from synaptic genes and regulated by development and plasticity. Nat. Neurosci. *18*, 603–610.

48. Li, B.F., Zhang, Y., Xiao, J., Wang, F., Li, M., Guo, X.Z., Xie, H.B., Xia, H., and Chen, B. (2017). hsa_circ_0045714 regulates chondrocyte proliferation, apoptosis and extracellular matrix synthesis by promoting the expression of miR-193b target gene IGF1R. Hum. Cell *30*, 311–318.

49. Li, Z., Chen, X., Xu, D., Li, S., Chan, M.T.V., and Wu, W.K.K. (2019). Circular RNAs in nucleus pulposus cell function and intervertebral disc degeneration. Cell Prolif. *52*, e12704.

50. Xiao, K., Yang, Y., Bian, Y., Feng, B., Li, Z., Wu, Z., Qiu, G., and Weng, X. (2019). Identification of differentially expressed long noncoding RNAs in human knee osteoarthritis. J. Cell. Biochem. *120*, 4620–4633.

51. Su, Q., and Lv, X. (2020). Revealing new landscape of cardiovascular disease through circular RNA-miRNA-mRNA axis. Genomics *112*, 1680–1685.

52. Qu, S., Yang, X., Li, X., Wang, J., Gao, Y., Shang, R., Sun, W., Dou, K., and Li, H. (2015). Circular RNA: A new star of noncoding RNAs. Cancer Lett. *365*, 141–148.

53. Panda, A.C. (2018). Circular RNAs act as miRNA sponges. Adv. Exp. Med. Biol. *1087*, 67–79.

54. Hansen, T.B., Jensen, T.I., Clausen, B.H., Bramsen, J.B., Finsen, B., Damgaard, C.K., and Kjems, J. (2013). Natural RNA circles function as efficient microRNA sponges. Nature *495*, 384–388.

55. Rong, D., Lu, C., Zhang, B., Fu, K., Zhao, S., Tang, W., and Cao, H. (2019). circPSMC3 suppresses the proliferation and metastasis of gastric cancer by acting as a competitive endogenous RNA through sponging miR-296-5p. Mol. Cancer *18*, 25.

56. Li, M., Ding, W., Tariq, M.A., Chang, W., Zhang, X., Xu, W., Hou, L., Wang, Y., and Wang, J. (2018). A circular transcript of *ncx1* gene mediates ischemic myocardial injury by targeting miR-133a-3p. Theranostics *8*, 5855–5869.

57. Kondo, M.A., Mohan, A., and Mather, K.A. (2020). Going around in circles: Deciphering the role of circular RNAs in neurodegenerative disease. Curr. Opin. Psychiatry *33*, 141–147.

58. Mouw, J.K., Ou, G., and Weaver, V.M. (2014). Extracellular matrix assembly: A multiscale deconstruction. Nat. Rev. Mol. Cell Biol. *15*, 771–785.

59. Wu, Y., Hong, Z., Xu, W., Chen, J., Wang, Q., Chen, J., Ni, W., Mei, Z., Xie, Z., Ma, Y., et al. (2021). Circular RNA circPDE4D Protects against Osteoarthritis by Binding to miR-103a-3p and Regulating FGF18. Mol. Ther. *29*, 308–323.

60. Tachmazidou, I., Hatzikotoulas, K., Southam, L., Esparza-Gordillo, J., Haberland, V., Zheng, J., Johnson, T., Koprulu, M., Zengini, E., Steinberg, J., et al.; arcOGEN Consortium (2019). Identification of new therapeutic targets for osteoarthritis through genome-wide analyses of UK Biobank data. Nat. Genet. *51*, 230–236.

61. Shen, P., Yang, Y., Liu, G., Chen, W., Chen, J., Wang, Q., Gao, H., Fan, S., Shen, S., and Zhao, X. (2020). circCDK14 protects against osteoarthritis by sponging miR-125a-5p and promoting the expression of Smad2. Theranostics *10*, 9113–9131.

62. Wang, T., Hao, Z., Liu, C., Yuan, L., Li, L., Yin, M., Li, Q., Qi, Z., and Wang, Z. (2020). LEF1 mediates osteoarthritis progression through circRNF121/miR-665/MYD88 axis via NF-κB signaling pathway. Cell Death Dis. *30*, 598.

63. Zhang, W., Zhang, C., Hu, C., Luo, C., Zhong, B., and Yu, X. (2020). Circular RNA-CDR1as acts as the sponge of microRNA-641 to promote osteoarthritis progression. J. Inflamm. (Lond.) *17*, 8.

64. Chen, H., Mao, M., Jiang, J., Zhu, D., and Li, P. (2019). Circular RNA CDR1as acts as a sponge of miR-135b-5p to suppress ovarian cancer progression. OncoTargets Ther. *12*, 3869–3879.

65. Yuan, W., Zhou, R., Wang, J., Han, J., Yang, X., Yu, H., Lu, H., Zhang, X., Li, P., Tao, J., et al. (2019). Circular RNA Cdr1as sensitizes bladder cancer to cisplatin by upregulating APAF1 expression through miR-1270 inhibition. Mol. Oncol. *13*, 1559–1576.

66. Zhou, Z.B., Du, D., Huang, G.X., Chen, A., and Zhu, L. (2018). Circular RNA Atp9b, a competing endogenous RNA, regulates the progression of osteoarthritis by targeting miR-138-5p. Gene *646*, 203–209.

67. Bai, Z.M., Kang, M.M., Zhou, X.F., and Wang, D. (2020). circTMBIM6 promotes osteoarthritis-induced chondrocyte extracellular matrix degradation via miR-27a/MMP13 axis. Eur. Rev. Med. Pharmacol. Sci. *24*, 7927–7936.

68. Liu, Q., Zhang, X., Hu, X., Dai, L., Fu, X., Zhang, J., and Ao, Y. (2016). Circular RNA related to the chondrocyte ECM regulates MMP13 expression by functioning as a miR-136 "sponge" in human cartilage degradation. Sci. Rep. *6*, 22572.

69. Zhang, X., Ziran, N., Goater, J.J., Schwarz, E.M., Puzas, J.E., Rosier, R.N., Zuscik, M., Drissi, H., and O'Keefe, R.J. (2004). Primary murine limb bud mesenchymal cells in long-term culture complete chondrocyte differentiation: TGF-β delays hypertrophy and PGE2 inhibits terminal differentiation. Bone *34*, 809–817.

70. Fan, Z., Söder, S., Oehler, S., Fundel, K., and Aigner, T. (2007). Activation of interleukin-1 signaling cascades in normal and osteoarthritic articular cartilage. Am. J. Pathol. *171*, 938–946.

71. Kühn, K., and Lotz, M. (2001). Regulation of CD95 (Fas/APO-1)-induced apoptosis in human chondrocytes. Arthritis Rheum. *44*, 1644–1653.

72. Tu, J., Huang, W., Zhang, W., Mei, J., and Zhu, C. (2020). The emerging role of lncRNAs in chondrocytes from osteoarthritis patients. Biomed. Pharmacother. *131*, 110642.

73. Ren, H., Yang, H., Xie, M., Wen, Y., Liu, Q., Li, X., Liu, J., Xu, H., Tang, W., and Wang, M. (2019). Chondrocyte apoptosis in rat mandibular condyles induced by dental occlusion due to mitochondrial damage caused by nitric oxide. Arch. Oral Biol. *101*, 108–121.

74. Zhou, Z., Ma, J., Lu, J., Chen, A., and Zhu, L. (2021). Circular RNA circCDH13 contributes to the pathogenesis of osteoarthritis via circCDH13/miR-296-3p/PTEN axis. J. Cell. Physiol. *236*, 3521–3535.

75. Li, Z., Yuan, B., Pei, Z., Zhang, K., Ding, Z., Zhu, S., Wang, Y., Guan, Z., and Cao, Y. (2019). circ_0136474 and MMP-13 suppressed cell proliferation by competitive binding to miR-127-5p in osteoarthritis. J. Cell. Mol. Med. *23*, 6554–6564.

76. Chen, C., Yin, P., Hu, S., Sun, X., and Li, B. (2020). Circular RNA-9119 protects IL-1β-treated chondrocytes from apoptosis in an osteoarthritis cell model by intercepting the microRNA-26a/PTEN axis. Life Sci. *256*, 117924.

77. Zhang, L., Liu, X., Che, S., Cui, J., Liu, Y., An, X., Cao, B., and Song, Y. (2018). circRNA-9119 regulates the expression of prostaglandin-endoperoxide synthase 2 (PTGS2) by sponging miR-26a in the endometrial epithelial cells of dairy goat. Reprod. Fertil. Dev. *30*, 1759–1769.

78. Zhang, Z., Zhang, X., Zhang, Y., Li, J., Xing, Z., and Zhang, Y. (2020). Retraction. J. Mol. Neurosci. *70*, 1926.

79. Weng, L., Brown, J., and Eng, C. (2001). PTEN induces apoptosis and cell cycle arrest through phosphoinositol-3-kinase/Akt-dependent and -independent pathways. Hum. Mol. Genet. *10*, 237–242.

80. Honjo, S., Osaki, M., Ardyanto, T.D., Hiramatsu, T., Maeta, N., and Ito, H. (2005). COX-2 inhibitor, NS398, enhances Fas-mediated apoptosis via modulation of the PTEN-Akt pathway in human gastric carcinoma cell lines. DNA Cell Biol. *24*, 141–147.

81. Chen, G., Shi, Y., Liu, M., and Sun, J. (2018). circHIPK3 regulates cell proliferation and migration by sponging miR-124 and regulating AQP3 expression in hepatocellular carcinoma. Cell Death Dis. *9*, 175.

82. Wu, Q., Yuan, Z.H., Ma, X.B., and Tang, X.H. (2020). Low expression of circRNA HIPK3 promotes osteoarthritis chondrocyte apoptosis by serving as a sponge of miR-124 to regulate SOX8. Eur. Rev. Med. Pharmacol. Sci. *24*, 7937–7945.

83. Zhang, Q., Qiao, X., and Xia, W. (2020). circSERPINE2 weakens IL-1β-caused apoptosis and extracellular matrix degradation of chondrocytes by regulating miR-495/TGFBR2 axis. Biosci. Rep. *40*, BSR20201601.

84. Castrogiovanni, P., Di Rosa, M., Ravalli, S., Castorina, A., Guglielmino, C., Imbesi, R., Vecchio, M., Drago, F., Szychlinska, M.A., and Musumeci, G. (2019). Moderate physical activity as a prevention method for knee osteoarthritis and the role of synoviocytes as biological key. Int. J. Mol. Sci. *20*, 511.

85. Wojdasiewicz, P., Poniatowski, Ł.A., and Szukiewicz, D. (2014). The role of inflammatory and anti-inflammatory cytokines in the pathogenesis of osteoarthritis. Mediators Inflamm. *2014*, 561459.

86. Wu, Y., Zhang, Y., Zhang, Y., and Wang, J.J. (2017). circRNA hsa_circ_0005105 upregulates NAMPT expression and promotes chondrocyte extracellular matrix degradation by sponging miR-26a. Cell Biol. Int. *41*, 1283–1289.

87. Noda, N.N., Wang, Z., and Zhang, H. (2020). Liquid-liquid phase separation in autophagy. J. Cell Biol. *219*, e202004062.

88. Zhou, X., Li, J., Zhou, Y., Yang, Z., Yang, H., Li, D., Zhang, J., Zhang, Y., Xu, N., Huang, Y., and Jiang, L. (2020). Down-regulated ciRS-7/up-regulated miR-7 axis aggravated cartilage degradation and autophagy defection by PI3K/AKT/mTOR activation mediated by IL-17A in osteoarthritis. Aging (Albany NY) *12*, 20163–20183.

89. Zhang, J., Cheng, F., Rong, G., Tang, Z., and Gui, B. (2020). hsa_circ_0005567 activates autophagy and suppresses IL-1β-induced chondrocyte apoptosis by regulating miR-495. Front. Mol. Biosci. *7*, 216.

90. Xiang, S., Li, Z., Bian, Y., and Weng, X. (2019). RNA sequencing reveals the circular RNA expression profiles of osteoarthritic synovium. J. Cell. Biochem. *120*, 18031–18040.

91. Zhang, W., Qi, L., Chen, R., He, J., Liu, Z., Wang, W., Tu, C., and Li, Z. (2021). Circular RNAs in osteoarthritis: Indispensable regulators and novel strategies in clinical implications. Arthritis Res. Ther. *23*, 23.

92. Vader, P., Mol, E.A., Pasterkamp, G., and Schiffelers, R.M. (2016). Extracellular vesicles for drug delivery. Adv. Drug Deliv. Rev. *106* (Pt A), 148–156.

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

94. Miyaki, S., and Lotz, M.K. (2018). Extracellular vesicles in cartilage homeostasis and osteoarthritis. Curr. Opin. Rheumatol. *30*, 129–135.

95. Yu, C.X., and Sun, S. (2018). An emerging role for circular RNAs in osteoarthritis. Yonsei Med. J. *59*, 349–355.