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Review

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The complex landscape of microRNAs in articular cartilage: biology, pathology, and therapeutic targets

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The disabling degenerative disease osteoarthritis (OA) is prevalent among the global population. Articular cartilage degeneration is a central feature of OA; therefore, a better understanding of the mechanisms that maintain cartilage homeostasis is vital for developing effective therapeutic interventions. MicroRNAs (miRs) modulate cell signaling pathways and various processes in articular cartilage via posttranscriptional repression of target genes. As dysregulated miRs frequently alter the homeostasis of articular cartilage, modulating select miRs presents a potential therapeutic opportunity for OA. Here, we review key miRs that have been shown to modulate cartilage-protective or -destructive mechanisms and signaling pathways. Additionally, we use an integrative computational biology approach to provide insight into predicted miR gene targets that may contribute to OA pathogenesis, and highlight the complexity of miR signaling in OA by generating both unique and overlapping gene targets of miRs that mediate protective or destructive effects. Early OA detection would enable effective prevention; thus, miRs are being explored as diagnostic biomarkers. We discuss these ongoing efforts and the applicability of miR mimics and antisense inhibitors as potential OA therapeutics.

Introduction

MicroRNAs (miRs) are a class of small, noncoding RNAs that regulate cellular processes through RNA silencing and posttranscriptional regulation of gene expression. Primary miR (pri-miR) transcripts may originate from intergenic, intronic, or exonic regions of host genes (1). In the nucleus, pri-miRs are transcribed and then cleaved by the ribonuclease Drosha to produce a precursor-miR (pre-miR) (2). Exportin 5 transports pre-miRs to the cytoplasm where they undergo a final cleavage by the endonuclease Dicer, giving rise to mature miRs (3). Mature miRs bind to complementary messenger RNA (mRNA) sequences of target genes via the RNA-induced silencing complex (RISC). Interactions between miRs and targets with a high degree of complementarity result in mRNA degradation, while imperfect interactions between miR and target transcripts usually result in translational repression (4). Until recently, the main focus of miR research has been to identify downstream target genes and biological functions. Researchers are now exploring upstream signaling pathways that regulate miR expression. miRs are mostly regulated by the promoters of their host genes, which are transcriptionally induced in *cis* or *trans* by transcription factors (5, 6). For example, let-7e and miR-98 are estradiol-regulated miRs that reduce c-Myc and E2F2 expression in breast cancer cells (7). Estrogen can also act indirectly by inducing c-Myc to bind to the miR-17–92 promoter (8). miR-146a, which exhibits cartilage-destructive effects, is induced by LPS in an NF-κB–dependent manner in human monocytes (9). Despite substantial progress in understanding miR expression patterns in osteoarthritis (OA), their regulatory mechanisms are still widely unknown. Bioinformatic algorithms predict that each miR can regulate hundreds of mRNA targets and individual genes are regulated by multiple miRs, thereby mediating a diverse array of biological functions across most signaling cascades. Some miRs target multiple genes in the same pathway (intrapathway), while others target genes across diverse pathways (universal), emphasizing the importance of miRs in regulating whole signaling cascades (10).

Not surprisingly, dysregulation of miR expression contributes to pathologies of various diseases, including cancers, neurodegenerative diseases, and metabolic disorders (11–13).

In the past few years, the OA research community has been considerably interested in miRs, especially as potential OA biomarkers and therapeutic targets. In this review, we provide a comprehensive summary of miRs known to be involved in cartilage-protective or -destructive mechanisms. Using computational approaches, we further highlight the complexity of miR regulation in articular cartilage by identifying unique and overlapping gene targets and signaling pathways of miRs that mediate protective or destructive effects to expand on their biological relevance and therapeutic potential. Finally, we discuss the current understanding of miRs as OA biomarkers and future strategies that may facilitate translation of miR-targeted therapies from bench to bedside.

Cartilage homeostasis and OA

Healthy articular cartilage is paramount to normal and pain-free joint function. Articular cartilage is a 2- to 4-mm-thick tissue of hyaline cartilage comprising chondrocytes surrounded by an extracellular matrix (ECM). The ECM primarily contains type II collagen, proteoglycans, and non-collagenous proteins, which are structurally and spatially organized for optimum tensile strength and resistance to compressive forces (14). Risk factors such as age, sex, genetics, and obesity adversely affect matrix quality, resulting in cartilage function decline (15–18). Articular cartilage degeneration, particularly in appendicular joints, is central to the clinical syndrome of OA. OA pathologies also include increased chondrocyte proliferation and fibro-cartilage formation resulting from the synthesis of abnormal matrix components, predominantly type I collagen (19). Fibrocartilage lacks the appropriate biomechanical properties of articular cartilage and, con-sequently, undergoes degeneration (20). Intact articular cartilage that borders fibrocartilage subsequently degenerates, resulting in OA progression.

Articular cartilage cannot self-heal and, as this tissue lacks vessels and innervation, is unable to take advantage of vascular system–invoked reparative processes. As Hunter observed in 1743, cartilage "once destroyed, is not repaired" (21). Over time, catabolic activity outcompetes anabolic attempts, disrupting cartilage homeostasis. Other joint components, including the subchondral bone and synovium, contribute to cartilage destruction and OA progression through various mechanisms, such as catabolic enzyme expres-sion, paracrine modulation of neighboring cells via inflammatory cytokines, and release of other molecular regulators, including miRs (22–24).

miRs and articular cartilage

miRs that are differentially expressed in cartilage, synovial fluid, and blood of patients with OA compared with those from healthy individuals likely contribute to OA pathophysiology (25–31). For instance, a miR signature comprising 9 increased and 7 decreased miRs was identified in human OA cartilage compared with normal cartilage (26). Proteomic analysis of predicted gene targets of OA-associated miRs identified SRY-box 11 (SOX11), CCR3, and WW domain–containing oxidoreductase (WWOX), all of which are dif-ferentially expressed in OA chondrocytes and may alter cartilage homeostasis. Thus, microarray screening can identify candidate miRs that can be used to predict mRNA targets, proteins, and pathways that may contribute to OA pathogenesis. This approach identifies potential therapeutic targets for further research.

Cellular and tissue maintenance mechanisms are critical for preservation of cartilage integrity and function. Autophagy is fundamental in maintaining chondrocyte homeostasis and adjusts cell metabolism in response to various stresses by removing damaged and unnecessary intracellular organelles and proteins (32). During OA, autophagy-related proteins are markedly reduced in articular cartilage and contribute to cartilage degeneration (33). miRs regulate autophagy machinery in cartilage by directly targeting 3′-UTRs of autophagy genes, including beclin1 (*BECN1*) and autophagy-associated gene 5 (*ATG5*) (34, 35). miRs can also be packaged in 30- to 100-nm-sized extracellular vesicles called exosomes (36). Exosomes present another level of communication between joint tissues and the systemic circulation, as they are released into the synovial fluid and influx into joints from circulating blood via the vascularized synovial membrane (37, 38). Thus, miRs not only function as cell-autonomous regulatory molecules but also mediate cell-to-cell and tissue-to-tissue communication in joints. Overall, some miRs are involved in mediating protective mechanisms within the joint, while others have destructive consequences contributing to OA-associated molecular changes. We summarize miRs that have been shown to modulate cartilage-protective or -destruc-tive mechanisms in OA in Figure 1, and describe their functional roles in Supplemental Tables 1 and 2 (supplemental material available online with this article; https://doi.org/10.1172/jci.insight.121630DS1).
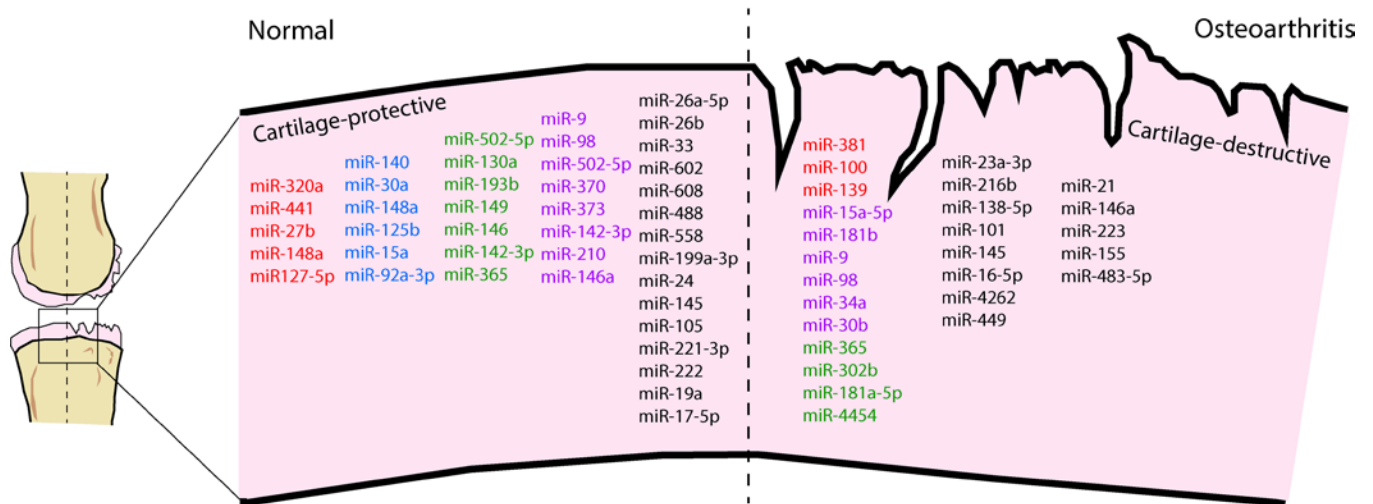
Figure 1. miRNAs involved in cartilage-protective and cartilage-destructive mechanisms. miRNAs highlighted in red directly target MMPs. miRNAs highlighted in blue directly target ADAMTSs. miRNAs highlighted in green regulate inflammatory pathways. miRNAs highlighted in purple regulate cellular apoptosis mechanisms. The remaining miRNAs either maintain or disrupt the articular cartilage via alternate mechanisms. See Supplemental Tables 1 and 2.

miRs involved in cartilage-protective mechanisms

Inflammation and catabolic enzyme–regulating miRs. In OA, articular cartilage degradation triggers inflammatory responses and cytokine production in surrounding tissues of the joint. These inflammatory molecules stimulate further ECM catabolism via increased protease production in chondrocytes (22). miR expression is affected by proinflammatory cytokines, which contribute to differential expression of target genes that promote OA progression. Furthermore, miRs can directly modulate proinflammatory cytokine expression. For instance, inhibition of miR-203 in LPS-treated mouse chondrocytes reduces cell viability, increases apoptosis, and further stimulates proinflammatory cytokine production (39). Maternally expressed gene 3 (MEG3) is a long noncoding RNA (lncRNA) that acts as a competing endogenous RNA, or sponge, for miR-203. MEG3 knockdown modulates LPS-induced inflammation by increasing miR-203 and decreasing proinflammatory cytokines (39). Overall, these studies suggest that miR-203 may have an antiinflammatory role in OA. miR-92a-3p, which is involved in late chondrogenesis of human mesenchymal stem cells (hMSCs), is downregulated in human OA cartilage and in response to IL-1β in vitro (40, 41). A disintegrin-like metalloproteinase with thrombospondin type 1 motifs (ADAMTS-4 and -5, also known as aggrecanase-1 and -2) and histone deacetylase 2 (HDAC2) are validated miR-92a-3p targets; thus, IL-1β–mediated decreases of miR-92a-3p likely promote aggrecan degradation in OA (40, 41).

Several other miRs also protect cartilage from proteolytic ECM destruction by modulating ADAMTS-5 expression. miR-140 is one of the first miRs shown to contribute to articular cartilage development and homeostasis, and normal endochondral bone development (42, 43). miR-140 is markedly downregulated in human OA cartilage compared with normal cartilage (26, 44). Moreover, miR-140 knockout in mice accelerates proteoglycan loss and fibrillation of articular cartilage by dysregulating ADAMTS-5 expression (43). In rats, intra-articular injection of miR-140 restores ECM homeostasis and prevents OA progression (45). Similarly to miR-140, miR-30a, which directly targets *Adamts5*, is decreased by IL-1β–induced activator protein (AP1) expression in human chondrocytes in vitro and in OA tissues in vivo (46). Thus, decreased expression of miR-140 and miR-30a in chondrocytes, in part through inflammatory signaling, may contribute to increased aggrecanase activity and the catabolic shift in OA.

Some miRs protect against OA by modulating the expression of transcription factors induced by inflammatory signaling pathways. For instance, miR-210, which is downregulated in OA tissue (26), promotes survival of LPS-treated rat chondrocytes by inhibiting NF-κB signaling and apoptosis (47). In OA chondrocytes miR-210 overexpression inhibits HIF-3α expression, further promoting chondrocyte proliferation and ECM deposition (48). Similarly, injection of miR-210–expressing lentivirus into rats with surgically induced OA reduces select cytokine levels in synovial fluid (47).

Other miRs target components of inflammatory signaling pathway receptor complexes to modulate inflammatory signaling–induced joint degeneration. For example, miR-502-5p, which is downregulated in OA articular tissues and IL-1β–induced chondrocytes (49), targets the 3′-UTR of the gene encoding TNF receptor–associated factor 2 (TRAF2), inhibiting NF-κB signaling and protecting chondrocytes from IL-1β–induced apoptosis (49). miR-145, a Sox9-mediated chondrogenic inhibitor, is downregulated in both TNF-α–induced chondrocytes and OA cartilage, resulting in induction of downstream matrix-degrading enzymes (50, 51). miR-145, regulated by p65, inhibits cartilage degradation by suppressing mitogen-activated protein kinase 4 (MKK4), which decreases matrix-degrading enzyme production and inactivates c-JUN N-terminal kinase (JNK) and p38 (50).

As nitric oxide (NO) production promotes cartilage degeneration, miRs that inhibit inducible NO synthase (iNOS) expression may impart protective effects. For instance, while miR-26a-5p directly targets IL-1β–induced iNOS in human OA chondrocytes, it is also downregulated by IL-1β signaling in chondrocytes (52), limiting its protective function. In addition to MMP-19, miR-193b-3p inhibits iNOS in human chondrocytes. miR-193b-3p promotes chondrogenic differentiation of hMSCs but is reduced in OA and in IL-1β–treated chondrocytes (53, 54). Increasing miR-193b-3p expression may inhibit degradation of ECM components and moderate inflammation through regulation of iNOS (54).

Thus, selective miRs are capable of imparting protective effects in cartilage and maintaining tissue homeostasis by targeting inflammatory signaling and cartilage catabolic components. However, reductions in expression of cartilage-protective miRs as part of OA pathogenesis may contribute towards altered cartilage homeostasis and eventual degradation.

Senescence-regulating miRs. Both intrinsic and extrinsic mechanisms contribute to chondrocyte senescence as articular cartilage ages. Premature cell cycle arrest can occur due to stress-induced premature senescence (SIPS), such as in response to extrinsic stresses including oxidative stress and inflammation (55). Senescent chondrocytes exhibit a senescent secretory phenotype (SSP) characterized by increased IL-6, IL-1, MMPs, and growth factors. Accumulation of SSP chondrocytes alters articular cartilage homeostasis and drives aging-related cartilage destruction (56). $p16^{INK4a}$, a marker of cellular senescence, is highly expressed in human OA cartilage (57), and chondrocytes with $p16^{INK4a}$ overexpression produce significantly higher MMP1 and MMP13 levels compared with control chondrocytes (58). Bioinformatic analysis identified miR-24, which is significantly downregulated in OA cartilage, as a negative regulator of $p16^{INK4a}$. Transfection of a miR-24 antagonist in vitro markedly increases $p16^{INK4a}$ and MMP1 secretion, indicating that miR-24 is protective against $p16^{INK4a}$-induced senescence and cartilage catabolism (58). In addition to preventing senescence, miR-24 promotes cell proliferation and inhibits chondrocyte apoptosis in rats, possibly via regulating the proto-oncogene *c-Myc* and downregulating MAPK signaling (59), activities that likely help in reducing OA pathogenic processes.

miRs involved in cartilage-destructive mechanisms

Chondrocyte maturation transcription factor–regulating miRs. miRs can promote cell differentiation and increase catabolic effector gene expression by targeting various transcription-regulating factors. For example, miR-139 is increased in OA articular cartilage and inhibits cell proliferation and viability by suppressing expression of eukaryotic translation initiation factor 4 G2 (EIF4G2) and insulin-like growth factor 1 receptor (IGF1R), which is involved in cell proliferation (60, 61). miR-381, involved in late chondrogenesis and endochondral ossification, is increased in various models of cartilage degeneration and directly inhibits the expression of histone deacetylase 4 (HDAC4), a key regulator of runt-related transcription factor 2 (RUNX2) and MMP13, promoting a catabolic chondrocyte phenotype (62, 63). Mechanical stress, which contributes to articular cartilage catabolic processes and disrupts cartilage homeostasis (64), regulates expression of miR-365 via cyclical loading in vitro and in vivo (65). miR-365 is highly expressed in cartilage from primary OA patients and those with trauma-induced OA (65) and, like miR-381, directly inhibits HDAC4, promoting chondrocyte hypertrophy and catabolic enzyme expression (65, 66). A separate study showed that miR-365 is downregulated in OA cartilage, thus sparing cartilage from increased catabolic gene expression, in part, by sustaining expression of HIF-2α, another target gene of miR-365 (67). These discrepancies in miR-365 detection in OA could result from OA cartilage donor age, disease stage, or control-tissue criteria, all of which must be considered when interpreting results and selecting miR targets for therapeutic development.

Similarly to OA cartilage, miRs present in synovial fluid can help distinguish between early- and late-stage radiographic knee OA. miR-23a-3p is significantly increased in synovial fluid late-stage OA compared with early-stage OA, consistent with expression patterns in arthritic cartilage compared with normal tissue, and in

synovium explants treated with IL-1β compared with untreated explants (30, 68). miR-23a-3p overexpression in OA cartilage suppresses ECM synthesis by targeting SMAD3 (68). In contrast, IL-1β signaling inhibits miR-23a-3p expression in cartilage explants (30). Collectively, these studies indicate that a negative-feedback regulatory mechanism involving inflammatory signals could manage expression of miR-23a-3p in OA.

miR-101 is increased in IL-1β–stimulated chondrocytes and mono-iodoacetate–induced (MIA-induced) OA models, and promotes OA progression, in part, by regulating expression of type II collagen (*Col2a1*) and aggrecan by decreasing *Sox9*, the essential transcription factor for chondrocyte differentiation (69, 70). In vivo, miR-101 also induces cartilage degradation–related genes including *Il6*, *Adamts1*, *Adamts5*, and periostin (*Postn*) (70). Antisense-mediated inhibition of miR-101 protects cartilage from MIA-induced degradation in rats, highlighting the feasibility of miR inhibitors as therapeutic options for OA (discussed further below). miR-145, which also directly targets SOX9 (71), is increased in human OA chondrocytes and is further enhanced by IL-1β stimulation (72). Like miR-23a-3p, *Smad3* is another direct target of miR-145 (72, 73). Thus, miRs target a number of key transcription factors expressed as part of OA molecular pathology that modulate the chondrocyte phenotype and cartilage homeostasis towards a catabolic phenotype.

Apoptosis-regulating miRs. An accumulating body of evidence suggests that chondrocyte apoptosis reduces cellularity and compromises ECM maintenance, leading to articular cartilage degradation and OA progression (74). Selected miRs are vital regulators of this cell death process. miR-98 promotes chondrocyte apoptosis, as miR-98 silencing reduces chondrocyte apoptosis and inhibits cartilage degradation in rat models of OA (75). Apoptosis-promoting effects of miR-98 are, in part, mediated through regulation of the antiapoptotic target gene B cell lymphoma 2 (*Bcl-2*) (76). Moreover, in vitro expression of miR-139 and miR-9 in chondrocytes reduces BCL2 and B cell lymphoma–extra large (BCLXL), promoting caspase 3/7 activity–induced apoptosis (61). miR-9 expression is significantly downregulated in both human and rat knee OA cartilage compared with normal tissues, inversely correlates with its direct target NF-κB1, and indirectly targets IL-6 and MMP13 (77, 78). Thus, modulation of miR-9 expression in OA may enhance chondrocyte proliferation and suppress apoptosis. miR-181a regulates OA pathogenesis by inducing chondrocyte apoptosis via downregulating glycerol-3-phosphate dehydrogenase 1–like protein (GPD1L) (79) and tumor suppressor PTEN (80). GDP1L regulates the hydroxylation of HIF-1α, which is vital for chondrocyte homeostasis (81). In LPS-treated chondrocytes, miR-146a expression is increased and targets CXCR4, deactivating the PI3K/AKT and Wnt/β-catenin pathways, subsequently reducing chondrocyte viability, promoting apoptosis, and increasing expression of inflammatory cytokines (82). Additional roles of miR-146a in OA pathophysiology are discussed below.

miR-34a is a p53-targeted miR with apoptotic and antiproliferative effects in some human cancers (83, 84). This miR is induced by IL-1β in rat chondrocytes and promotes apoptosis, induces iNOS expression, and decreases *Col2a1* expression (85). miR-4262, which targets SIRT1, is increased in TNF-α–treated rat chondrocytes and promotes chondrocyte apoptosis and inhibits autophagy, contributing to OA pathogenesis (86). Loss of matrix synthesis proteins and elevation of matrix-degrading enzymes, such as MMP-13 and ADAMTS-5, was also observed downstream of miR-4262 (86).

In facet joint (FJ) cartilage, both miR-181a-5p and miR-4454 are upregulated. Moreover, expression of these miRs positively correlated with FJ OA severity (87). This study showed that both miRs promote chondrocyte apoptosis, inflammation, and catabolic activity in FJ cartilage.

Autophagy-regulating miRs. In cartilage, age-related loss of autophagy has been associated with cell death and cartilage degeneration, while adequate autophagy signaling is essential for maintaining cartilage homeostasis (88–90). A number of miRs increased in OA pathology target autophagy-related genes, shifting cartilage homeostasis towards catabolism. For instance, miR-155 is upregulated in human OA knee cartilage compared with normal cartilage, based on next-generation sequencing. Analysis revealed multiple putative autophagy-related miR-155 gene targets, including *ATG3*, GABA type A receptor–associated protein-like 1 (*GABARAPL1*), *ATG5*, *ATG2B*, lysosome-associated membrane protein 2 (*LAMP2*), and forkhead box O3 (*FOXO3*) (35). In human chondrocytes, miR-155 mimic reduced mRNA and protein levels of autophagy-related target genes, as well as other nonpredicted targets, including unc-51–like autophagy-activating kinase 1 (*ULK1*), *ATG14*, and microtubule-associated protein 1 light chain 3 (*MAP1LC3*) (35), possibly through indirect mechanisms. miR-146a is also upregulated in OA cartilage and contributes to cartilage degeneration; however, in later disease stages, miR-146a is substantially downregulated compared with normal tissue (91–93). Aberrant miR-146a expression also contributes to pathogenesis of systemic lupus erythematosus (SLE), psoriasis, and Sjögren's syndrome, demonstrating its role in various rheumatic

diseases (94–96). Besides regulating chondrocyte apoptosis in response to mechanical injury, miR-146a promotes autophagy under hypoxic conditions (92, 97). HIF-1α induces miR-146a, leading to decreased expression of the autophagy inhibitor BCL-2. Therefore, miR-146a likely promotes both cartilage destruction and autophagy-mediated chondrocyte homeostasis, depending on disease stage. mir-17-5p belongs to the oncogenic miR-17–92 cluster, which is essential for normal skeletal growth and embryonic development (98). miR-17-5p decreases autophagy in OA chondrocytes by targeting the crucial autophagy regulator p62 (also known as SQSTM-1) (99). Compared with normal tissue, miR-20 expression is elevated in human OA chondrocytes and suppresses autophagy partly through inhibition of target gene *ATG10* (100). miR-30b targets autophagy pathway regulators BECN1 and ATG5 (34), and overexpression of this miR in TNF-α–treated chondrocytes suppresses autophagy and ECM gene expression while upregulating pro-apoptotic genes. Inhibition of one or combinations of these miRs could protect against OA pathogenesis by promoting autophagy and associated homeostatic mechanisms in chondrocytes.

Synovial membrane contribution to joint pathology via miRs

Fibroblast-like synoviocytes (FLSs) respond to inflammatory mediators, including IL-1β, TNF-α, and ligands of TLR2, TLR3, and TLR4, by modulating miR expression profiles (101). For example, miR-155 increases in response to proinflammatory stimuli and inhibits MMP3 and MMP1 expression, thereby suppressing FLS proliferation and invasion (102, 103). TNF-α–mediated NF-κB activation induces the miR-17–92 cluster, which includes miR-18a and miR-19b, in FLSs. miR-18a increases secretion of MMP1 and inflammatory cytokines (104), whereas miR-19b increases basal cytokine production to exacerbate inflammatory activation of FLSs (105). Elevated miR-203 levels also facilitate FLS activation by increasing NF-κB–mediated expression and secretion of MMP1 and IL-6 (106). Thus, miR-18a, miR-19b, and miR-203 may contribute to FLS-mediated cartilage destruction and immune cell infiltration by aggravating the activated FLS phenotype.

The miR-29 family is highly expressed in human cartilage during end-stage OA and immediately after destabilization of the medial meniscus (DMM) surgery in mice, highlighting its involvement in early disease and maintaining articular cartilage homeostasis (107). However, miR-29 expression is diminished in end-stage knee OA synovium and associates with synovial lining hypertrophy, inflammation, and fibrosis (108). Intra-articular miR-29a injection maintains synovial homeostasis by targeting VEGF and inhibiting OA-related angiogenesis. Interestingly, reduced miR-29 is also implicated in fibrogenesis of systemic sclerosis, an autoimmune rheumatic disease (109). miR-125b-5p expression increases with OA severity (110), and miR-125b-5p overexpression inhibits OA synovial cell proliferation and promotes apoptosis by targeting synoviolin (SYVN1), suggesting miR-125b-5p upregulation is an attempt to attenuate synovial hyperplasia and fibrosis (110). Thus, miR-29 family miRs and miR-125b-5p expression may be part of an effort to maintain normal synovial function rather than contribute to pathologic OA disease progression. miRs in FLSs also target other noninflammatory, noncatabolic pathways that contribute to homeostasis or promote pathological states (see Supplemental Tables 1 and 2).

Unique and overlapping targets of cartilage-protective and -destructive miRs

As detailed above, miRs can regulate both cartilage-protective and -destructive mechanisms, resulting in a complex signaling network. To allow investigation of a broader biological relevance and therapeutic potential of miRs in the maintenance of articular cartilage homeostasis, we used an integrative computational biology approach to identify unique and overlapping gene targets, and signaling pathways of miRs that have been reported to mediate cartilage-protective and -destructive mechanisms (Supplemental Tables 1 and 2).

First, we used mirDIP (http://ophid.utoronto.ca/mirDIP) ver. 4.1.6.6 to identify gene targets for selected miRs (111). To increase the quality of predicted targets, we focused only on predictions with very high support (top 1%), resulting in some miRs not having any targets. Second, NAViGaTOR (http://ophid.utoronto.ca/navigator) ver. 3.0.3 was used to further annotate, visualize, and prioritize identified gene targets (Figure 2) (112). This analysis identified both unique and overlapping targets, as well as genes regulated by many miRs exhibiting cartilage-protective or -destructive mechanisms. Interestingly, most of these prioritized targets (39 out of 47, see Figure 2) exhibit binding activity or transcription factor functions. Link protein N-terminal peptide (LPP) is predicted to be regulated by a high number of both protective and destructive miRs. This glycoprotein strengthens the binding between aggrecan and hyaluronan (HA) and can function as a growth factor to stimulate synthesis of type II collagen and proteoglycans in human artic-
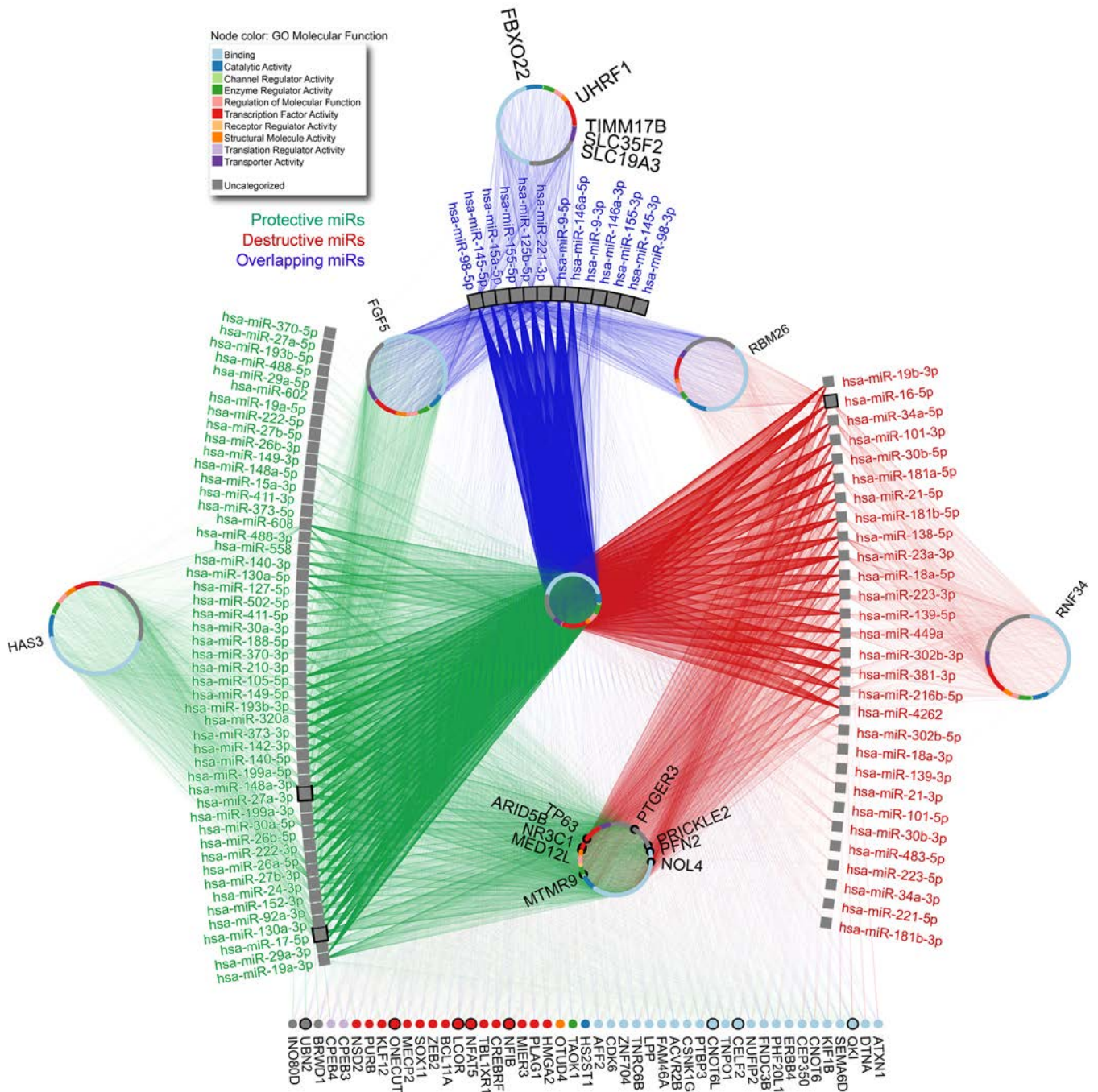
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Figure 2. Unique and overlapping gene targets regulated by miRNAs involved in cartilage-protective and -destructive mechanisms. Considering protective and destructive miRNAs, we used mirDIP ver. 4.1.6.6 portal to identify high-confidence mRNA targets. The resulting network was further annotated with gene ontology (GO) molecular function in NAViGaTOR version 3.0.3. Edge color corresponds to specific or overlapping miRNA-gene relationships.

ular cartilage and intervertebral disc (113–115). Given the number of LPP-targeting miRs, miR-mediated LPP regulation may substantially contribute to maintaining ECM integrity in OA. HA is synthesized by chondrocytes and is integral for articular cartilage structure and function; thus, modifications in size and concentration of HA can affect cartilage homeostasis (116). Our prediction indicates that HA synthase 3 (HAS3) is exclusively regulated by miRs exhibiting cartilage-protective mechanisms, suggesting that these miRs could play a role in modulating HA polymers in cartilage (117). Ring finger protein 34 (RNF34), also known as human ring finger homologous to inhibitor of apoptosis protein type (hRFI), is among the predicted genes targeted by only those miRs that exhibit cartilage-destructive mechanisms. hRFI has not

been investigated in OA; however, it has antiapoptotic functions in various cancers (118, 119). Thus, select cartilage-destructive miRs may promote apoptosis by targeting RNF34. Most gene targets predicted by mirDIP have not been implicated or investigated in OA, apart from a select few. Owing to the vast number of connected miRs, the putative targets highlighted in Figure 2 are potentially significant contributors to the pathogenesis of OA. Third, we performed a comprehensive pathway enrichment analysis of identified miR targets using pathDIP (http://ophid.utoronto.ca/pathDIP) ver. 3.0.27.2 (120). We used extended pathway associations that integrate core pathways with gene-pathway associations predicted using physical protein interactions from IID (http://ophid.utoronto.ca/iid) ver. 04-2018 (121).

A total of 2,224 pathways were significantly ($P < 0.05$; Bonferroni corrected for multiple testing) enriched for protective miR gene targets (including WNT pathways [$n = 35$; $P < 2.36 \times 10^{-6}$], TGF pathways [$n = 28$; $P < 5.05 \times 10^{-8}$], epidermal growth factor receptor [ERBB] pathways [$n = 24$; $P < 3.17 \times 10^{-4}$], and TNF pathways [$n = 18$; $P < 1.78 \times 10^{-3}$]). Active EGFR signaling maintains structural, functional, and mechanical properties of articular cartilage (122). Reduced EGFR signaling dramatically accelerates cartilage degeneration and OA progression in mouse models (122, 123). Term enrichment analysis of significantly enriched OA-associated pathways identified cancer, MAPK, and WNT as the most frequent terms. Extracellular stimuli, including proinflammatory cytokines, transduce signals into the nucleus, in part through MAPK signaling, activating genes that promote cellular development, proliferation, and apoptosis (124). MAPK signaling is required for chondrogenic regulation and cartilage development (125, 126) but can also negatively regulate articular chondrocyte stability by mediating catabolic responses to inflammatory cytokines. In fact, p38 MAPK signaling promotes OA chondrocyte apoptosis in response to proinflammatory stimuli (127).

A total of 2,169 pathways were significantly enriched for destructive miR targets (including WNT pathways [$n = 36$; $P < 1.39 \times 10^{-2}$], TGF pathways [$n = 28$; $P < 5.55 \times 10^{-10}$], and ERBB pathways [$n = 24$; $P < 1.96 \times 10^{-2}$]). Term enrichment analysis of OA-destructive miR targets revealed a high frequency of the terms MAPK, cancer, WNT, and regulation. The TGF-β pathway is a key signaling pathway in OA, is necessary for normal cartilage development (128), and serves both protective and destructive roles in the synovial joint (129–132). Similarly, pathway enrichment analysis predicts TGF-β signaling to be influenced by both protective and destructive miRs. WNT signaling is also among the pathways enriched in both protective and destructive miR targets, supporting previous findings that propose cartilage homeostasis depends on fine-tuning of WNT signaling and not binary activation or suppression (133).

miRs as biomarkers

As they are stable, highly sensitive, and easy to detect, miRs can be valuable OA biomarkers. Extracellular miRs in plasma, serum, or urine could be used to noninvasively diagnose or prognosticate OA severity. miRs in OA synovial fluid are similar to those secreted by synovial tissues, suggesting that these miRs potentially originate from synovial membrane tissue; however, there is no correlation between plasma and synovial fluid miRs (134). An initial screen of 752 miRs in synovial fluid from patients with early- and late-stage radiographic knee OA identified a panel of miRs capable of differentiating knee OA stage (30). miR-378-5p was detectable in late-stage OA synovial fluid but largely undetectable in early-stage OA synovial fluid, thereby providing a distinct synovial fluid miR signature with potential to predict early- from late-stage radiographic knee OA. Interestingly, increased serum miR-378 is being investigated as a biomarker for renal cell carcinoma and gastric cancer, demonstrating multiple diagnostic potentials of miR-378 in different biofluids (135, 136).

While miR-132 has diagnostic potential to differentiate healthy controls from patients with OA or rheumatoid arthritis (RA), it does not differentiate RA and OA patients. In contrast, miR-16, miR-146a, miR-155, and miR-223 are enriched in RA synovial fluid compared with OA synovial fluid, and can differentiate between patient cohorts, supporting miRs in synovial fluid as biomarkers of specific arthropathies compared with those in plasma (134). However, plasma miR expression profiles in patients with early-to-intermediate radiologic knee OA compared with healthy controls identified 12 of 380 analyzed miRs as highly expressed in OA that could clearly differentiate OA individuals from healthy controls (31). As discussed above, miR-181a-5p and miR-4454 positively correlate with the degree of facet cartilage destruction (87); however, detection of these miRs in blood and a correlation with cartilage tissue levels is required for future use as noninvasive biomarkers.

In a large prospective population-based study, 12 (miR-122, -25, - 28-3p, -93, -140, -191, -342-3p, -146b, -454, -885-5p, let-7b, and let-7e) of 377 analyzed miRs were differentially detected in serum of OA patients (29). Further validation revealed that circulating let-7e levels inversely associate with the severity of knee or hip OA. Serum levels of miR-454 and miR-885-5p also differentiate between individuals receiving joint

arthroplasty and healthy controls; however, these associations are not consistently significant. Thus, specific miRs, such as let-7e, and to a lesser extent miR-454 and miR-885-5p, have potential to predict severe knee and hip OA (29). These miRs have not been investigated for early OA detection.

A recent screen of 2,549 miRs revealed that miR-140-3p, miR-671-3p, and miR-33b-3p are downregulated in the serum of OA patients compared with healthy individuals, with miR-140-3p and miR-671-3p also downregulated in OA articular cartilage compared with healthy cartilage (137). These results complement previous studies showing reduced miR-140 in OA cartilage and knee synovial fluid (26, 43, 44, 138). Target gene analysis revealed that these miRs are involved in regulating metabolic processes, such as lipid and cholesterol metabolism, that could affect OA progression (139). Larger, independent cohorts with diverse demographic and anthropometric characteristics are needed to validate these miRs as reliable biomarkers.

There is a great need for reliable biomarkers to detect early OA, as symptoms only begin to surface after cartilage is degraded past the point of intrinsic repair. While differences in miR expression profiles are evident between mouse models of posttraumatic OA and inflammatory arthritis, dysregulation of serum miRs between mouse models of arthritis and controls at early stages of disease are not detectable, suggesting circulating miRs may not be useful predictors of early cartilage degeneration. However, miRs in cartilage correlate with early disease. For example, miR-146 is highly expressed in low-grade OA cartilage and decreases with increasing cartilage degeneration, suggesting miR-146 could be an early OA indicator (140). miR-146 is also markedly downregulated in cartilage obtained from patients undergoing total knee replacements (end-stage OA) compared with normal cartilage (27). Furthermore, miR-29–family expression increases immediately upon surgical induction in a murine cartilage injury model (107). Thus, changes in miR-29–family expression appear to correlate with OA onset, while miR-146 may indicate severity. Overall, serum miRs in OA are not yet a reliable tool for detecting early stages of cartilage damage but can be predictive of OA progression.

Kung et al. recently profiled early OA mouse cartilage and subchondral bone miR expression to gain further insight into potential regulators of OA initiation (141) and discovered 139 mouse cartilage–specific miRs dysregulated early (1 and 6 weeks) after DMM surgery. Bioinformatic analysis revealed that OA pathology–associated miR-mRNA target interactions overlap with previously identified dysregulated human miRs, suggesting that these miRs (miR-15/16-5p, miR-26b-5p, miR-30c-5p, miR-98-5p, miR-149-5p, miR-210-3p, and miR-342-3p) associate with both OA initiation and progression. A similar study was conducted in an attempt to identify dysregulated synovial tissue miRs in early OA; however, no differential miR expression was observed between DMM and control mice (142).

miR mimics and antisense inhibitors as therapeutic agents

Early therapeutic intervention is crucial to improve OA patient outcomes. Reliable and accurate miR biomarkers for OA will be valuable for early diagnosis; however, specific miRs for disease progression monitoring, determination of treatment responses, or therapeutic targeting are also of interest. Several miR antagonists and replacement therapies are being studied preclinically and in clinical trials (143). The miR-122–specific inhibitor miravirsen showed promising results in a completed phase IIa clinical trial of patients with chronic hepatitis C (ClinicalTrials.gov Identifier NCT01200420). The miR-34 mimic MRX34 reduces cell proliferation in multiple cancers and inhibits the formation of cancer stem cells in preclinical studies (144–146). In 2013, MRX34 was the first miR mimic to enter clinical trials; however, the phase 1 study was halted in September of 2016 due to multiple immune-related severe adverse events (ClinicalTrials.gov Identifier NCT01829971). Miragen Therapeutics has miR antagonist antimiR-155 and miR mimic promiR-29b in clinical trials to treat cutaneous T cell lymphoma and fibrosis, respectively (ClinicalTrials.gov Identifier NCT02580552 and NCT02603224). Interestingly, miR-155 is upregulated in OA chondrocytes and contributes to autophagy dysfunction and OA pathogenesis; however, in vitro inhibition of miR-155 is chondroprotective by enhancing autophagy (35). One advantage of miR therapeutics for treating OA is the ability to locally deliver treatment via intra-articular injection. The encapsulated and isolated structure of a synovial joint allows for fewer off-target effects and adverse events that can result from systemic exposure (as seen with MRX34), where miR expression and function may differ from pathological or homeostatic roles in the joint. miR-targeted therapy appears to be a promising therapeutic avenue; however, off-target effects are possible due to multiple gene targets of miRs (direct or indirect).

In preclinical and clinical studies, mesenchymal stem/stromal cells (MSCs) can protect and repair articular cartilage (147–161), and the MSC secretome, by way of exosomes, has been shown to possess paracrine factors required to mediate tissue repair (162–165). In accordance with these findings, injec-

tion of MSC-derived exosomes on a weekly basis repairs osteochondral defects in a rat model (166). The cartilage regenerative effects of embryonic stem cell–derived (ESC-derived) MSC exosomes were also investigated in a DMM-induced mouse OA model (167). Intra-articular injection of ESC-derived MSC exosomes twice a week for 4 weeks prevented cartilage destruction, increased type II collagen, and reduced ADAMTS5 expression. This approach represents a cell-free, lipid-based, safer therapeutic approach for administration of disease-modifying factors and eliminates challenges of cell-based MSC therapies. Of particular relevance, Chen et al. showed that besides proteins and lipids, biologically functional pre-miRs are enriched in secreted exosomes and can exert their functions after being readily taken up by cells (168). Experiments in which argonaut-2 (Ago2), a regulator of the biological function of miRs, is knocked down showed that miR composition mediates the neuroprotective effect of exosomes for treatment of degenerative ocular disease (169). Tao et al. showed that overexpressed miR-140-5p in synovial MSC exosomes promotes chondrocyte proliferation and migration, thereby delaying progression in an OA rat model (170). Thus, it is possible to manipulate the miR content in exosomes to modulate miR expression in the joint and restore joint homeostasis. While further characterization of MSC-exosome miRs is required, these studies show a potential application of MSC-exosome miRs — with or without miR modification — for OA treatment. Additionally, synthetic lipid–based vesicles resembling exosomes could be generated with a subset of miR mimics and antisense inhibitors to mediate OA disease modification (171). Thus, exosomes could serve as a delivery tool for OA-modulating miRs.

Conclusions

Research of OA-associated miRs is still in its infancy and further work is required before translation to clinical application. Research advances in various fields highlight the potential of miRs as indicators of disease activity and therapeutic targets, with preclinical animal models of OA producing encouraging results. miRs are not purely tissue specific and many remain to be identified. This further emphasizes the need for unbiased, comprehensive, sequencing-based assays combined with systematic computational analysis to identify OA miRs for diagnosis, prognosis, and treatment. Of note, some miRs that actively maintain articular cartilage homeostasis (protection or destruction) may operate through common gene targets and signaling pathways, as identified by our integrated computational analysis. Such comprehensive analysis may open up new therapeutic avenues for targeting multiple miRs by modulating common downstream gene targets or signaling pathways. These data also suggest that targeting miRs for therapeutic benefit may pose significant challenges, as miRs operate through multiple gene targets and signaling pathways.

Author contributions

HE and MK were responsible for the conception and design of the review. HE was responsible for data analysis and interpretation and was the major contributor in drafting the review. JR contributed to the design, critical revision, and editing of the review. IJ analyzed data, performed computational analyses for miR gene target and pathway predictions, and interpreted the data. All authors read and approved the final manuscript to be published.

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