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The Role of MicroRNAs and their Targets in Osteoarthritis

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Abstract

MicroRNA regulation and expression has become an emerging field in determining the mechanisms regulating a variety of inflammation-mediated diseases. Recent studies have focused on specific microRNAs that are differentially expressed in case of osteoarthritis. Furthermore, several targets of these miRNAs important in disease progression have also been identified. In this review, we focus on microRNA biogenesis, regulation, detection, and quantification with an emphasis on cellular localization and how these concepts may be linked to disease processes such as osteoarthritis. Next, we review the relationship of specific microRNAs to certain features and risk factors associated with osteoarthritis such as inflammation, obesity, autophagy, and cartilage homeostasis. We also identify selected microRNAs that are differentially expressed in osteoarthritic tissue, but have unidentified targets and functions in the disease pathogenesis. Lastly, we highlight the potential use of microRNAs for therapeutic purposes, and also point to certain remedies that regulate microRNA expression.

Keywords

osteoarthritis; microRNA; gene regulation; inflammation; cartilage

Introduction

Osteoarthritis (OA) is a debilitating pathological condition that causes significant pain and stiffness in the joints and affects millions of Americans [1]. Characteristics of OA typically include breakdown of the articular cartilage, synovitis, chondrocyte apoptosis, inflammation, and remodeling of the subchondral bone. Risk factors for the development of OA include obesity, mechanical stress, genetic predisposition, gender, and aging. Currently, treatment options are pain management and symptom control. The underlying molecular mechanisms of disease pathogenesis are still under investigation. An area that is currently subject of intense investigations is the role of microRNAs in the development and progression of OA.

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Compliance with Ethics Guidelines

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Micro ribonucleic acids (microRNAs or miRNAs) are non-coding single-stranded RNAs that were first identified in *C. elegans* in the 1990s [2]. They function as post-transcriptional regulators of gene expression through base-pairing with “Seed Sequence” normally located in the 3’ untranslated region (UTR) of target mRNAs. Changes in miRNA expression have been associated with several disease processes including obesity [3], cardiovascular diseases [4], and cancer [5]. Much interest has been gained in the area of miRNAs as biomarkers for disease activity due to their stability and ease of detection in the body fluids.

Many investigators have used miRNA expression profiling arrays to identify specific miRNAs that are differentially expressed in OA and other diseases. These studies have been reviewed elsewhere [6-8]. This review focuses on microRNAs formation, processing, localization, regulation, detection, and quantification with a view to generate interest in studies exploring these aspects in OA disease pathogenesis. Furthermore, we also review the specific miRNAs that have been linked to OA and identify their specific targets. Lastly, we describe several approaches currently under development involving use of miRNAs in targeted therapeutics.

miRNA Formation and Processing

MicroRNAs are ubiquitously expressed in a variety of different organisms, yet many of them are phylogenetically conserved indicating an important role in evolution [9]. Generally, clusters of miRNAs are transcribed from a single polycistronic transcription unit; however, certain individual miRNAs originate from separate promoters [10]. Transcription of miRNA genes is mediated primarily by RNA polymerase II and to some extent by RNA polymerase III in case of viral miRNAs [11-13]. The primary miRNA transcript (pri-miRNA) contain a hairpin structure at the 3’ end that is cleaved and processed in the nucleus by the enzyme Drosha releasing one or more precursor miRNAs (pre-miRNAs) [14]. Drosha, along with the critical co-factor DiGeorge syndrome critical region gene 8 (DGCR8), forms a large complex known as the microprocessor complex [15]. Once the processing of the pre-miRNA is complete, the pre-miRNA is exported from the nucleus to the cytoplasm by *Exportin 5*, a member of the nuclear transport receptor family [16]. Once exported to the cytoplasm, the pre-miRNA is recognized by Dicer, an RNase III type endonuclease responsible for cleavage of pre-miRNAs in the cytoplasm, resulting in the generation of ~22 nucleotide long mature miRNA duplex [17-19]. The mature miRNA duplex is then loaded onto one of the Argonaute (AGO) family members forming an effector complex known as the miRNA-induced silencing complex (miRISC) [20, 21]. Following loading of the miRNA duplex, unwinding of the immature miRNA occurs, resulting in one mature miRNA strand of approximately 22 nucleotides, while the other strand (passenger strand, usually denoted by an asterisk) is degraded.

miRNA Localization

MicroRNAs localize to different subcellular compartments such as the mitochondria, endoplasmic reticulum, P-bodies, nucleus, and nucleolus [22]. Furthermore, mature miRNAs are secreted from cells via exosomes and can be detected in plasma and other bodily fluids [23, 24]. Localization and distribution of miRNAs in subcellular compartments and

exosomes can be influenced by several factors. These include protein modifications based on cellular conditions, and the availability of AGO family members and other non-AGO miRNA-binding proteins [22]. The presence of AGOs is critical for miRNA stability, as the overexpression of Ago genes enhances mature miRNA accumulation [25]. Interestingly, certain DNA/RNA binding proteins such as Translin, TAR DNA binding protein 43 (TDP-43), and heterogeneous nuclear ribonucleoprotein E2 (hnRNP E2) can regulate the stability of certain miRNAs in a manner independent of AGO proteins [26-28]. These components are referred to as non-AGO components or non-AGO proteins and only a few such proteins have been identified so far. In order to determine the location-specific function of miRNAs, it is important to consider the components of the miRISC complex, including the AGO family member and other non-AGO miRISC components in the overall experimental design.

Processing bodies (P-bodies; PBs) are sites of mRNA surveillance that are enriched with AGO family members and other RNA decay factors such as deadenylases and GW-182 [29]. Previous studies have shown that miRNAs and their targets localize at PBs [30], suggesting that the location-specific function of miRNAs in PBs involves the decay and storage of miRNA targets and other RNA decay factors. What role this may have in disease pathogenesis is not clear at present, since it is not known if this function is altered in OA chondrocytes or other tissues of the affected joints.

AGO family members, as well as Dicer, localize in endomembranes, or specifically the endoplasmic reticulum (ER) and multivesicular bodies [31]. In the case of the role of the ER in miRNA-mediated post-transcriptional regulation of gene expression, the RNA binding proteins such as TRBP localize within the ER and are required for loading specific miRNAs to AGO family members within the ER membrane [32]. It is unknown whether there are alterations in the function of this miRISC complex and the fate of the target mRNAs in ER in OA. Multivesicular bodies (MVBs) are membrane-bound compartments responsible for sorting molecules to be secreted from the cell via exosomes, recycled back to the Golgi, or degraded in the lysosome [22]. MVB formation and turnover play regulatory roles in miRNA silencing. For example during MVB formation, the depletion of certain components for endosomal sorting required for transport (ESCRT) prevent miRNA-induced silencing, while blocking the MVB turnover pathway results in enhanced miRNA-mediated silencing and increased generation of miRISC complexes [33, 34]. These data indicate that the MVB specific-miRNA function may be related to miRNA loading and recycling in the cell. The fusion of MVBs with the plasma membrane results in the release of exosomes to the extracellular environment. These secreted exosomes contain mature miRNAs and miRISC components which regulate the local microenvironment. Much attention is now being drawn to secreted miRNAs as they have the ability to modulate gene expression of neighboring cells and in a variety of cell types, including those in the immune system, neuronal synapses, and cancer [35-37].

Interestingly, it has been documented that mature miRNAs and miRISC components exist within the nucleus and nucleolus [38-41]. Previous reports indicated that nuclear miRNAs are involved in epigenetic regulation through silencing or activating gene promoters [42]. MicroRNA-21 and -29b are preferentially enriched in the nucleus [38, 43] and genome wide

data indicate that localization of mature miRNAs within the nucleus is more frequent than thought previously [40]. The nucleolus is the major sub-compartment within the nucleus and is involved in ribosome biogenesis, cell cycle control, and signaling [44]. Several miRNAs, including miRNA-191, miRNA-484, miRNA-193b, and miRNA-93, are found in the nucleolus [45, 46], and actively traffic to the cytoplasm in a manner dependent on the transport protein *Exportin1* [47].

miRNAs and associated miRISC components also exist within the mitochondria [48-50]. Several studies have identified a variety of pre- and mature miRNAs in mitochondria (also referred to as mitomiRs) in liver and muscle [49-51] including miR-1 [52], miR-223 [50], miR-155 [50], and miR-130a [49]. Previous studies suggest that miRNAs localize to the mitochondria to regulate mitochondria-specific mRNA targets [53] based on the discovery that only AGO2, and not Dicer, was found in isolated mitochondria [52, 54]. Interestingly, the mitomiR expression profile changes under certain pathological conditions. For example, in a model of type I diabetes the miRNA expression profile in mitochondria isolated from mouse liver revealed high level expression of miR-494, miR-202-5p, miR-134, and miR-155 [50]. Another study showed that miR-181c shuttles from the nucleus to the cytoplasm in cases of myocardial infarction and regulates mitochondrial complex IV and reactive oxygen species (ROS) levels [54]. Several functions of these mitomiRs have been proposed, including regulation of ubiquitination, apoptosis, and important biological pathways such as transforming growth factor (TGF), Wnt, p53, and cell cycle [55]. These studies indicate that miRNAs located in the mitochondria play important roles in the disease processes. Alterations in mitochondrial activity are associated with OA. Dysregulation of mitochondrial function can result in increased chondrocyte apoptosis, enhanced ROS production, and inflammation [56]. It would be interesting to determine if certain mitomiRs known to be involved in mitochondrial dysfunction are also associated with or play a role in OA disease progression?.

miRNA Regulation

MicroRNA expression is a tightly controlled process that can be regulated at different levels associated with miRNA formation and processing. These include proteins and molecules related to miRNA transcriptional regulation (RNA PolII), nuclear processing (Drosha and DGCR8), nuclear export (exportin-5), and pre-miRNA processing (RISC complex). Modifications of these molecules linked to miRNA processing can occur through several mechanisms, including DNA methylation, histone deacetylation, gene mutation, and DNA copy alteration [57-59].

MicroRNA expression can also undergo intrinsic regulation which involves alteration in the RNA sequence and/or structure that affect the maturation and turnover of mature miRNAs. These changes include single nucleotide polymorphisms, miRNA tailing, RNA editing, and RNA methylation [21]. Single nucleotide polymorphisms (SNPs) within miRNA genes can affect their biogenesis or their specificity to target mRNAs [60]. Tailing involves the addition of nucleotides at the 3' end of the miRNA molecule modifying its expression. This process occurs in both the pre-miRNA and mature miRNA [61]. The most extensively studied proteins involved in miRNA tailing are Lin28 and the terminal uridylyl transferase (TUT)

family of enzymes [62-64]. RNA editing occurs by the conversion of adenosine to inosine through the adenosine deaminase acting on RNA (ADAR) family of enzymes [65, 66]. Previous work showed that this process occurs in pri-miRNA transcripts and that miRNA editing causes insufficient Dicer processing [67]. Three ADAR genes, ADAR1, ADAR2, and ADAR3, exist in vertebrates [68-70]. ADAR1 knockout mice are embryonically lethal; however, ADAR2 mice are viable but have dysfunctional brain development resulting in abnormal behavior and hearing [71, 72]. Mutations in these enzymes have been linked to human diseases such as amyotrophic lateral sclerosis (ALS), measles, and several different types of cancers [73]. These RNA modifying enzymes regulate several critical miRNAs including the let-7 family, miR-376, and miR-411 [74]. miRNA methylation occurs through the methyltransferase enzyme BCDIN3D [75]. The BCDIN3D enzyme O-methylates the 5' monophosphate end of pre-miRNA, thereby disrupting Dicer processing.

Detection and Quantification of miRNAs

The detection and quantification of miRNAs constitute a useful strategy for identifying novel mechanisms of gene regulation and disease state. MicroRNAs can be extracted from cells, live tissue, fixed tissue, plasma, serum, and other bodily fluids and subjected to miRNA profiling by several approaches, including quantitative RT-PCR, hybridization microarrays, RNA sequencing, and pri- and pre-miRNA quantification [76]. Quantitative RT-PCR assays such as TaqMan, SmartChip, and Biomark are well established and can be used for absolute quantification, but the disadvantage of this approach is that it cannot identify novel miRNAs. Hybridization-based methods or arrays enable the analyses of a large number of samples for known miRNAs. Several variations of the hybridization approach have been developed, including fluorescent labeling of the miRNA in a sample for subsequent hybridization to DNA-based probes or beads on the array [76, 77]. Disadvantages of this technique include lower specificity and difficulty in quantification. Next-generation RNA-sequencing in combination with bioinformatics allows for the identification of known and novel miRNAs. The general technique is to prepare a cDNA library from the small RNAs in the sample of interest followed by sequencing of millions of cDNA molecules in the library in a single run [78]. Several different RNA-sequencing platforms have been established which include high-throughput next-generation sequencing [78], smaller-scale next-generation sequencing [79], and single-molecule sequencing [80]. These methods are highly accurate and have the potential to distinguish miRNAs of similar sequence. A disadvantage of these methods is that absolute quantification cannot be determined and substantial computational support is needed for data analyses.

miRNA Expression in Osteoarthritis

The roles of miRNAs in musculoskeletal development and the pathology of OA were first discovered using genetically modified animal models with knockout or overexpression of genes critical for miRNA biogenesis and processing. Previous studies showed that global knockouts of molecules critical for miRNA processing result in embryonic lethality [81-83]. Through the use of tissue-specific knockout mice, studies have revealed the importance of miRNA processing enzymes in chondrocyte homeostasis. For example, Dicer deficiency in Col2 α 1-expressing cells leads to early postnatal lethality and reveals abnormal skeletal

growth and development due to a reduction of proliferating chondrocytes [84]. Furthermore, Droscha and DGCR8 deficiency in Col2 α 1-expressing cells also showed abnormal skeletal development similar to that seen in the Dicer-deficient mice [85]. Interestingly, mice in which Droscha deletion was induced postnatally in articular cartilage Prg4-expressing cells develop mild-OA naturally due to increased chondrocyte death and decreased extracellular matrix production [85]. Taken together this suggests that dysregulation of miRNAs and miRNA processing enzymes may be contributory in the development of OA pathology.

Studies with genetically modified mice have linked specific miRNAs to OA pathology. One of the first studies showed that miR-140 knockout mice have abnormal skeletal growth and develop early signs of OA characterized by proteoglycan loss and fibrillation in the articular cartilage [86]. This same study also showed that mice overexpressing miR-140 were resistant to antigen-induced arthritis primarily through its direct regulation of a disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS)-5 [86]. Therefore, miR-140 is a major contributor to cartilage homeostasis and may serve as a protective factor in cases of OA.

Several studies have taken advantage of bioinformatic techniques in order to determine the specific miRNAs that are differentially expressed in OA. Iliopoulos et al. (2008) identified 16 differentially expressed miRNAs (9 upregulated and 7 downregulated) in normal and OA cartilage using TaqMan miRNA microarray assays [87]. Jones et al. (2009) identified 17 differentially expressed miRNAs in chondrocytes and 30 differentially expressed miRNAs in osteoarthritic bone [88]. Interestingly, two of these miRNAs, miR-9 and miR-98, were upregulated in both OA cartilage and bone. Akhtar et al. (2010) discovered 44 differentially expressed miRNAs (2 upregulated and 42 significantly downregulated) in OA chondrocytes after treatment with IL-1 β [89]. Diaz-Prado et al. (2012) characterized 7 differentially expressed miRNAs in normal and OA chondrocytes, including miR-149, miR-582-3p, miR-1227, miR-634, miR-576-5p, miR-641, and miR-483-5p [90]. Borgnio-Cuadra et al. (2014) examined the expression of circulating miRNAs in patients with OA and identified 12 differentially expressed miRNAs [91]. Although these studies have discovered several miRNAs that are differentially expressed in OA, the specific molecular targets of several of these miRNAs and their function in OA have yet to be confirmed.

miRNAs in inflammation and matrix degradation in osteoarthritis

Inflammation within the joints is one of the main contributors to OA [92]. Pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF- α) interleukin (IL)-1 and IL-6) and cytokine-inducible cyclooxygenase-2 (COX-2), matrix metalloproteinases (MMPs), nitric oxide (NO), and reactive oxygen species (ROS) are highly upregulated in OA [93]. These pro-inflammatory mediators act on a variety of different cell types in the affected joints, including chondrocytes, synoviocytes, osteoblasts, osteoclasts, and macrophages [6]. In OA, several miRNAs that regulate pro-inflammatory factors, at least *in vitro*, are differentially expressed.

miRNA regulation of TNF- α , IL-1, and IL-6 in osteoarthritis

Several miRNAs that are downregulated in OA may have protective functions. These include miR-130 which regulates TNF- α levels [88, 94], and miR-149 which regulates the levels of several inflammatory cytokines, including TNF- α , IL-1, and IL-6 [95].

Several miRNAs regulate IL-1 and IL-6 either directly or indirectly through targeting other proteins. Monocyte chemoattractant protein-induced protein 1 (MCP-1) is downregulated in cases of OA and is a negative regulator IL-6 expression [96]. In this study, miR-9 was shown to directly target the “seed-sequence” in the 3'UTR of MCP-1 mRNA resulting in its downregulation and in enhanced IL-6 expression in human OA chondrocytes.

Interestingly, miRNA-139 also targets MCP-1 and is highly expressed in OA chondrocytes [97]. IL-1 β mRNA is a potential target of miR-483, which is upregulated in murine cartilage samples [98].

miRNA regulation of COX-2 in osteoarthritis

Several miRNAs are involved in the post-transcriptional regulation of COX-2. For example, miR-199* directly targets COX2 mRNA and may have an anti-inflammatory effects on OA cartilage [99]. Furthermore, miR-558 which directly targets COX-2 mRNA is downregulated in OA chondrocytes, which might contribute to the high levels of COX-2 expression in OA [100]. Withaferin A (WFA) is a natural compound isolated from the medicinal plant *Withania somnifera* known for its anti-inflammatory properties [101, 102]. Interestingly, WFA upregulates miR-25 which reduces COX-2 protein expression in chondrocytes [103].

miRNA regulation of matrix metalloproteinases and aggrecanases

A key characteristic of OA is the breakdown of the cartilage matrix. Of the MMPs that are important in OA pathogenesis MMP-13 is an important proteinase involved in degrading the collagen network and its mRNA is a target of several microRNAs that are downregulated in OA including miR-27b [89], miR-27a [104], miR-148a [105], miR-320 [106], miR-127-5p [107] and miR-411 [108]. Interestingly, other miRNAs that are upregulated in cases of OA also target MMP-13 including miR-9 [88], miR-140 [109], and miR-181b [110]. Certain miRNAs regulate the level of MMPs indirectly through targeting other molecules. For example, miR-23b is important for chondrogenesis by targeting PRKACB and downregulating MMP-9 [111]. Furthermore, p16INK4a accumulates in OA and enhances the mRNA expression of MMP1 and MMP13 [112]. Interestingly, p16INK4a is targeted by miR-24 which is downregulated in OA.

A given microRNA may regulate not just one, but several target genes related to OA progression. For example, miR-105 and miR-148 are downregulated in OA and probably have a protective function as these miRNAs are known to target Runx2, ADAMTS4, ADAMTS5, ADAMTS7 and ADAMTS12, MMP-13, and COL10 [113]. Studies have also shown that miR-15a targets ADAMTS5 and may have a protective function in OA [114].

Several studies have linked certain miRNAs to aging and OA progression. A previous report from Ukai et al (2012) revealed that miR-320c is downregulated in aging OA samples [115]. This group also showed that miR-320c regulates ADAMTS5 and may have a role as a

protective factor by enhancing chondrogenesis. Furthermore, miR-377 is upregulated in OA samples and is induced by PKC signaling [87, 116]. Interestingly, bioinformatics analysis indicated that this miRNA may also target the mRNA of ADAMTS5.

miRNA regulation of obesity and lipid metabolism genes in OA

Obesity is a risk factor for the development of OA and studies have shown that progression of OA is directly correlated with obesity and BMI [117]. Several genes related to lipid metabolism are altered in cases of OA. Furthermore, certain microRNAs that are altered in OA are target genes of lipid metabolism that contribute to the onset of the disease. For example, miR-22 is highly expressed in OA and functions by directly targeting PPARA and BMP-7 [87]. Leptin, a small polypeptide produced by adipose tissue, is positively correlated with BMI, fat mass, and body weight in patients with OA [118, 119]. Interestingly, a previous report indicated that miR-29a regulates leptin expression by using matching miRNA and protein data, which may explain the correlation that exists between obesity and OA [87].

The dysregulation of cholesterol synthesis and efflux may also contribute to OA [120, 121]. MicroRNA-33a regulates genes related to cholesterol synthesis and this miRNA could be a novel target for treatment of OA [122]. Obesity has also been linked with chronic inflammation in chondrocytes. Xie et al (2015) showed that miR-26a inhibits fatty-acid induced activation of NF- κ B and that this miRNA is downregulated in patients with OA, possibly providing an explanation regarding the high levels of expressed genes known to be regulated by NF- κ B [123].

miRNA regulation of apoptosis and autophagy in OA

The degradation of the cartilage matrix can be attributed, in part, to the reduced number of surviving chondrocytes. Previous studies have shown that chondrocyte apoptosis is stimulated in aging and OA [124, 125]. Several miRNAs target apoptotic genes in chondrocytes. For example, miR-34a, which may target Col2a1, is upregulated in IL-1 β -treated chondrocytes, and the inhibition of miR-34a effectively reduces chondrocyte apoptosis [126]. Li et al (2012) found that miR-146a is highly upregulated in IL-1 β treated chondrocytes, in surgically-induced arthritis in animals, and in response to mechanical stress [127, 128]. Furthermore, this miRNA directly targets the 3'-UTR of Smad4 mRNA resulting in an increase of VEGF and chondrocyte apoptosis. Furthermore, microRNA-9 is downregulated in OA chondrocytes and protects against chondrocyte apoptosis by targeting protogenin, a chondrocyte inhibitory factor [129]. Mitochondrial and peroxisomal dysfunction can contribute to cell death by apoptosis. MicroRNA-223 is upregulated in OA and is associated with chondrocyte apoptosis through peroxisomal regulation [130, 131]. Recent evidence indicates that histone deacetylases (HDACs) may be therapeutic targets for inflammatory diseases as these molecules are highly expressed in OA chondrocytes [132, 133]. Interestingly miR-222 reduces chondrocyte apoptosis and cartilage destruction by targeting HDAC4 [134]. Members of the TNF receptor superfamily (TNFRSF) upon activation are important inducers of apoptosis [135]. Previous studies have shown that death receptor 6 (TNFRSF21) is upregulated in OA and is targeted by miR-210, which is

downregulated in OA [136, 137]. Hypoxia inducible factor-1- α (HIF-1 α) regulates chondrogenesis as well as apoptosis and autophagy in chondrocytes [138]. Interestingly, miRNA-195 is highly expressed in OA samples and stimulates chondrocyte apoptosis by targeting HIF-1 α [91, 139].

Autophagy is a homeostatic self-renewal process that involves the degradation of cytoplasmic components and recycling of macromolecules within the cell. Recent evidence has indicated that autophagy may have a protective function in the pathogenesis of OA [140-142]. Furthermore, enhanced autophagy correlates with decreased apoptosis in arthritis. Several miRNAs target molecules that are involved in autophagy. Zhang *et al* (2015) has shown that miR-146a, along with hypoxia and HIF-1 α , stimulates autophagy by targeting Bcl-2 and inhibiting its expression [143]. Another study has shown that miR-155 suppresses autophagy by downregulating the expression of several autophagy genes including Ulk1, FoxO3, Atg14, Atg5, Gabarapl1, and Map1lc3 [144].

miRNA regulation of chondrogenesis and cartilage homeostasis

MicroRNAs target key signaling mediators in cases of OA. For example, the miRNA-29 family targets NF- κ B, Smad, and WNT [145]. A number of miRNAs that are upregulated in OA chondrocytes are also important regulators of chondrogenesis. For example, several miRNAs in OA target members of the TGF β signaling pathway. These miRNAs include miR-16-5p and miR-337, both of which target Smad3 [146] and TGF β R2 [147], respectively. A number of other miRNAs may target SOX9 and COL2A1 in chondrocytes including miR-101 [148], miR-675 [149], and miR-200a [150]. Interestingly, inhibition of these miRNAs may enhance chondrogenesis and have a protective function in OA conditions. MicroRNA-138 represses COL2A1 in chondrocytes through directly targeting the transcription factors, Sp-1 and HIF-2 α [151]. Since miRNA-138 is highly expressed in dedifferentiated chondrocytes, it may be a potential target for future therapeutic applications.

Other reports have shown that miRNAs regulate chondrocyte homeostasis through by targeting relatively uncommon pathways. For example, Li *et al* (2015) showed that miR-30b is highly expressed in OA cartilage [152]. This miRNA targets ERG, a member of the ETS family of transcription factors that is important for chondrocyte differentiation. Two other examples of miRNAs that regulate chondrocyte metabolism are miR-137 and miR-483-5p which target Runx2 [153] and members of the MAPK family [154], respectively. Interestingly, Runx2 inhibits chondrocyte proliferation and hypertrophy [155]. Therefore, miRNA regulation of Runx2 may be a critical pathway in the progression of OA pathology.

It is now well established that epigenetic regulation by methyltransferase enzymes is important for chondrocyte differentiation and may play a role in OA pathology [156-158]. MicroRNA-370 and miR-373 target hydroxymethyltransferase-2 (SHMT-2) and methyl-CpG-binding protein-2 (MECP-2) [159]. Chromatin modifications mediated by SHMT-2 and MECP-2 influence MMP13 gene expression and viability in chondrocytes. Overexpression of miR-370 or miR-373 reduces MMP13 expression and inhibits apoptosis in chondrocytes by downregulating SHMT-2 and MECP-2 [159].

miRNAs altered in OA cartilage samples, but with unknown target and function in chondrocytes

Several miRNAs that are highly upregulated or downregulated in OA still do not have identified and confirmed target genes in chondrocytes. Interestingly, a few of these miRNAs do have targets that have been identified in other cell types. For example miR-34b is highly upregulated in OA chondrocytes [88]; however, its direct role in chondrocytes has not yet been determined. Interestingly, this miRNA represses Smad3 resulting in the inhibition in cancer metastasis [160]. Furthermore, miR-98 is highly expressed in OA chondrocytes but has no confirmed target; however, it is an important regulator in intervertebral disc degeneration, where it targets IL-6 [161]. Several miRNAs that are downregulated in OA samples also have unidentified roles in chondrocyte homeostasis. For example, miR-107 is downregulated in OA cartilage [88]; however, its role in chondrocyte metabolism is yet to be determined. MicroRNA-107 is highly expressed under hypoxic conditions and targets HIF-1 β in endothelial progenitor cells [162]. It would be interesting to determine if this miRNA would have similar effects in chondrocytes, as hypoxia and members of the HIF-1 family are important for chondrogenesis [163, 164].

Current and Future Therapies

The use of miRNAs as diagnostic tools for a variety of diseases has become a provocative idea. An even more intriguing idea that is currently being developed is therapeutic targeting of certain dysfunctional miRNAs in disease processes such as OA. Currently, there exist three main approaches to target miRNAs, which include expression vectors (miRNA sponges), small-molecule inhibitors, and antisense oligonucleotides (ASOs) [165]. Most attention has been paid to the use of ASO, particularly those that target miRNAs directly (anti-miRs) to inhibit their function; however, chemically unmodified DNA or RNA oligonucleotides have poor outcomes *in vivo* applications. Several different chemical modifications have been considered when determining the best delivery approach. These include liposome-based methods, nanoparticle-based methods, and antibody-based methods [165]. Although the delivery of these anti-miRs has potential for future applications there are still a few obstacles that exist. These obstacles include delivery issues and hybridization-independent and associated off-target effects.

Several studies have shown the beneficial use of alternative remedies for alleviation of OA symptoms; however, very few studies have directly linked these therapies with miRNAs and the disease process. Resveratrol, examined in previous studies, may prevent the progression of OA through several different mechanisms [166-168]. Interestingly, resveratrol modulates the expression of a number of miRNAs involved in inflammation and disease progression that including miR-663, miR-155, and miR-2 [169-171]. Future focus should emphasize mechanisms underlying the activities of alternative remedies and their roles in regulating miRNAs linked to OA progression.

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Figure 1.

Cellular Location of Specific miRISC Components and miRNAs

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Table 1
Differentially Expressed miRNAs and their Targets in Cartilage

The table presented in this manuscript are original and has not been borrowed from any other previously published resource.

<u>Upregulated</u>	<u>Target Gene(s)</u>	<u>Mechanism in Cartilage</u>	<u>Reference</u>
miRNA-22	PPAR α , BMP7, Aggrecan	Lipid Metabolism	Iliopoulos et al 2008
miRNA-675	Col2a1	Chondrocyte metabolism	Steck et al 2012
miRNA-155	SHIP1, FoxO3, Ulk1, Atg14, Atg5, Gabarapl1, and Map1lc3	Inflammation and autophagy	Kurowska-Stolarska et al 2011
miRNA-193b	TGF β 2, TGF β R3, SOX9, Col2	Chondrocyte Differentiation and Homeostasis	Hou et al 2015 and Ukai et al 2012
miRNA-483-5p	MAPK	Chondrocyte Differentiation and Homeostasis	Diaz-Prado 2012 and Yang et al 2015
miRNA-483	BMP7, TGF β , IL-1 β , MMP13	Inflammation	Qi et al 2013 and Iliopoulos et al 2008
miRNA-377	ADAMTS5	Matrix Degradation in Human Nucleus Pulposus cells	Tsirimoniaki et al 2013 and Iliopoulos et al 2008
miRNA-103	KLF4	ND	Iliopoulos et al 2008 and Hartmann et al 2016
miRNA-16	Bcl2	ND	Iliopoulos et al 2008 and An et al 2012
miRNA-30b	ERG	Chondrocyte Differentiation and Homeostasis	Iliopoulos et al 2008 and Li et al 2015
miRNA-23b	PRKACB	Chondrocyte Differentiation and Homeostasis	Ham et al 2014 and Iliopoulos et al 2008
miRNA-509	ND	ND	Iliopoulos et al 2008
miRNA-9	MMP13, MCP1	Matrix Degradation	Jones et al 2009 and Makki et al 2015
miRNA-25	COX2	Inflammation	Jones et al 2009 and Kim et al 2014
miRNA-34b	Smad3	ND	Jones et al 2009 and Liu et al 2013
miRNA-98	IL-6	ND	Jones et al 2009 and Ji et al 2015
miRNA-137	Runx2	Chondrocyte Differentiation and Homeostasis	Jones et al 2009 and Zhang et al 2011
miRNA-182	TIAM1	ND	Jones et al 2009 and Hu et al 2015
miRNA-185	ND	ND	Jones et al 2009
miRNA-200a	Col2a1 and SOX9	Chondrocyte Differentiation and Homeostasis	Jones et al 2009 and Umeda et al 2015
miRNA-211	TGF β R2	ND	Jones et al 2009 and Chu et al 2013
miRNA-299	ND	ND	Jones et al 2009
miRNA-342	ID4 and Estrogen receptor	ND	Jones et al 2009 and Crippa et al 2014
miRNA-29	Smad, NF-kB, WNT	Chondrocyte Differentiation and Homeostasis	Le et al, 2015
miRNA-34a	Col2a1, iNOS	Chondrocyte Apoptosis	Abouheif et al, 2010, Jones et al 2009, Dunn et al 2009
miRNA-146a	VEGF, Smad4, Bcl2	Chondrocyte Apoptosis and Autophagy	Li et al, 2012, Borgonio-Cuadra et al 2014 and Zhang et al 2015
miRNA-223	NF1A, MCSFR	Chondrocyte Apoptosis	Li et al, 2012, Kim et al, 2014, Iliopoulos et al 2008
miRNA-181b	MMP13	Matrix Degradation	Song et al 2013

Upregulated	Target Gene(s)	Mechanism in Cartilage	Reference
miRNA-602	SHH	Chondrocyte Differentiation and Homeostasis	Akhtar et al 2015
miRNA-608	SHH	Chondrocyte Differentiation and Homeostasis	Akhtar et al 2015
miRNA-33a	ABCA1 and ApoA1	Chondrocyte metabolism	Kostopoulou et al 2015
miRNA-16-5p	Smad 3	Chondrocyte Differentiation and Homeostasis	Li et al 2015
miRNA-145	Smad3, Sox9	Chondrocyte Differentiation and Homeostasis	Yang et al 2014 and Martinez-Sanchez et al 2012
miRNA-101	Sox9	Chondrocyte Differentiation and Homeostasis	Dai et al 2012
miRNA-21	GDF5	Chondrocyte Differentiation and Homeostasis	Zhang et al 2014
miRNA-20b	HIF1 α and VEGF	ND	Borgnio-Cuadra et al 2014 and Xue et al 2015
miRNA-29C	Integrin β 1 and MMP2	ND	Borgnio-Cuadra et al 2014 and Wang et al 2013
miRNA-93	MMP3	Matrix Degradation in Human Nucleus Pulposus cells	Borgnio-Cuadra et al 2014 and Jing and Jiang 2015
miRNA-126	VCAM1	ND	Borgnio-Cuadra et al 2014 and Harris et al 2008
miRNA-184	GAS1	ND	Borgnio-Cuadra et al 2014 and Li et al 2016
miRNA-186	ND	ND	Borgnio-Cuadra et al 2014
miRNA-195	HIF1 α	Chondrocyte Apoptosis	Borgnio-Cuadra et al 2014 and Bai et al 2015
miRNA-345	Smad1	ND	Borgnio-Cuadra et al 2014 and Chen et al 2016
miRNA-885-5p	CDK2, MCM5	ND	Borgnio-Cuadra et al 2014 and Afanasyeva et al 2011
miRNA-139	MCPIP1	Inflammation	Makki et al 2015
miRNA-199a-3p	SOX9, Col2	Chondrocyte Differentiation and Homeostasis	Ukai et al 2012

Downregulated	Target Gene(s)	Mechanism in Cartilage	Reference
miRNA-130a	TNF α	Inflammation	LI et al 2015
miRNA-149	TNF α , IL-1, IL-6	Inflammation	Santini et al 2014 and Jones et al 2009
miRNA-210	DR6 and NF-kB	Inflammation, Chondrocyte Apoptosis	Zhang et al 2015 and Iliopoulus et al 2008
miRNA-337	TGF β R2	Chondrocyte Differentiation and Homeostasis	Zhong et al, 2012 and Iliopoulus et al 2008
miRNA-558	COX2	Inflammation	Park et al 2013
miRNA-27b	MMP13	Matrix Degradation	Akhtar et al 2010
miRNA-27a	MMP13, IGFBP5	Matrix Degradation	Tardif et al 2009
miRNA-148a	MMP13, ADAMTS5, ColX	Matrix Degradation	Vonk et al 2014 and Jones et al 2009
miRNA-21	GAS5, GDF5	Autophagy	Zhan et al, 2014, Song et al 2014 and Diaz-Prado 2012
miRNA-582-3p	PGGT1B, LRRK2, DIXDC1	ND	Diaz-Prado et al 2012 and Uchino et al 2013

Downregulated	Target Gene(s)	Mechanism in Cartilage	Reference
miRNA-1227	ND	ND	Diaz-Prado et al 2012
miRNA-634	mTOR	ND	Diaz-Prado et al 2012 and Cong et al 2015
miRNA-576-5p	ND	ND	Diaz-Prado et al 2012
miRNA-641	ND	ND	Diaz-Prado et al 2012
miRNA-29a	Leptin, MMP13, TIMP1	Chondrocyte Metabolism	Li et al 2015 and Iliopoulos et al 2008
miRNA-140	ADAMTS5, MMP13, TIMP1, SP1	Matrix Degradation	Li et al 2015, Yang et al 2011, and Iliopoulos et al 2008
miRNA-25	COX2	Inflammation	Iliopoulos et al 2008 and Kim et al 2014
miRNA-26a	NFkB	Obesity and Inflammation	Xie et al 2015 and Iliopoulos et al 2008
miRNA-373	SHMT-2 and MECP-2	Matrix Degradation and Chondrocyte Apoptosis	Song et al 2015 and Iliopoulos et al 2008
miRNA-107	HIF1 β	ND	Jones et al 2009 and Meng et al 2012
miRNA-130b	CYP2C9	ND	Jones et al 2009 and Rieger et al 2015
miRNA-9	Protogenin	Chondrocyte Apoptosis	Song et al 2013
miRNA-127-5p	MMP13	Matrix Degradation	Park et al 2013
miRNA-222	HDAC4	Chondrocyte Apoptosis	Song et al 2015
miRNA-320	MMP13	Matrix Degradation	Meng et al 2016
miRNA-411	MMP13	Matrix Degradation	Wang et al 2015
miRNA-105	Runx2, ADAMTS4, ADAMTS5, ADAMTS7, and ADAMTS12	Chondrocyte Differentiation and Matrix Degradation	Ji et al 2016
miRNA-24	P16INK4a	Chondrocyte Differentiation and Apoptosis	Philipot et al 2014
miRNA-199a	COX2	Inflammation	Akhtar et al 2012
miRNA-488	ZIP8	Matrix Degradation	Song et al 2013
miRNA-15a	ADAMTS5	Matrix Degradation	Lu et al 2015
miRNA-335-5p	WNT, DKK1	Mesenchymal Stem Cell Differentiation	Tornero-Esteban et al 2015
miRNA-370	SHMT2 and MECP2	Matrix Degradation and Chondrocyte Apoptosis	Song et al 2015
miRNA-320c	ADAMTS5	Matrix Degradation	Ukai et al 2015
miRNA-138	Sp1 and HIF2 α	Chondrocyte Differentiation	Seidl et al. 2016 and Akhtar et al. 2010