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MicroRNAs: Important Epigenetic Regulators in Osteoarthritis

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Abstract: Multiple mechanisms are implicated in the development of primary osteoarthritis (OA), in which genetic and epigenetic factors appear to interact with environmental factors and age to initiate the disease and stimulate its progression. Changes in expression of microRNAs (miRs) contribute to development of osteoarthritis. Numerous miRs are involved in cartilage development, homeostasis and degradation through targeting genes expressed in this tissue. An important regulator of gene expression in human cartilage is miR-140, which directly targets a gene coding aggrecanase ADAMTS-5, that cleaves aggrecan in cartilage. This miR is considered a biological marker for cartilage and its level significantly decreases in OA cartilage. On the other hand, increased expression of miR-146a in early OA inhibits two other cartilage-degrading enzymes: MMP13 and ADAMTS4, and may provide a useful tool in developing treatments for OA. The *COL2A1* gene, encoding collagen type II, which is the most abundant structural protein of the cartilage, is silenced by miR-34a and activated by miR-675. Every year, new targets of cartilage miRs are validated experimentally and this opens new possibilities for new therapies that control joint destruction and stimulate cartilage repair. At the same time development of next-generation sequencing technologies allows to identify new miRs involved in cartilage biology.

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MicroRNAs AS IMPORTANT MOLECULES IN A VARIETY OF BIOLOGICAL PROCESSES

The importance of miRs in different biological processes has already been documented. Intensive research has established that miRs are powerful regulators of gene expression. These molecules, which are typically 22 nucleotide long, are produced from larger precursors that contain approximately 70 nucleotides, by enzymes belonging to the Argonaute family and the RNase III, Dicer [1]. After incorporation of miR into the RNA-induced silencing complex (RISC), suppression of the translation or degradation of the target mRNA occurs, resulting in an inhibitory effect on the synthesis of protein product of the gene. The RISC complex is guided to its mRNA target by a single miR strand, which binds imperfectly to its complementary sequence in the 3'UTR of the target mRNA [1]. Thus far, more than 2500 human mature miRs have been discovered (http://www.mirbase.org/). A single gene is usually under control of several miRs [2]. Each miR binds to many targets and based on data from bioinformatics, the assumption has been made that more than 30% of the genes are regulated by miRs [2]. Discovery of the sequences encoding miRs in the human genome has opened new horizons in human genetics. Computational analysis reveals that the human genome may contain information about 50 thousand miRs. MiRs are involved in many regulatory processes. An abnormal expression as well as impaired binding of these molecules to the target sequences in gene transcripts, might be the cause of many diseases. MiR-146 expression results in increased suppression of NFκB activity, which reduces metastatic potential of breast cancer cells [3]. Abnormal expression of miR-1 might result in congenital heart disease [4]. Analysis of families with Parkinson's disease (PD) revealed that miRs contribute to the pathogenesis of this disease [5]. Increased expression of the gene *FGF20* encoding fibroblast growth factor 20 is caused by the risk allele at rs12720208 single nucleotide polymorphism (SNP) that blocks the binding site for miR-433 in the gene transcript. This could potentially provide a novel mechanism for the PD risk [5]. Decrease in the signaling occurring in the midlife, is an important element of human aging process. Some miRs are abnormally expressed in midlife affecting cell cycle, DNA repair, oxidative stress responses and apoptosis, which are under their control. It is likely that further studies on function of miRs in the aging process will allow for better understanding of the aging process and might offer new therapeutic procedures to improve the quality of life. In 2010, Hackl *et al.* analyzed expression of miRs in endothelial cells, replicated $CD8^+$T cells, renal proximal tubular epithelial cells, skin fibroblasts and mesenchymal stem cells from young and old donors [6]. He found that miR-17, miR-19b, miR-20a, and miR-106a are down-regulated during human aging process [6].

The purpose of this report is to review and discuss recent literature on the role of miRs in the development of os-

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teoarthritis, which is one of the major diseases in the aging population.

OSTEOARTHRITIS

Osteoarthritis is a chronic degenerative joint disorder and a major cause of disability in the elderly. Approximately 10% of men and 18% of women over the age of 60 are affected with osteoarthritis. Approximately 80% of those affected with OA have significant movement limitations and 25% are unable to perform activities of daily living [http://www.who.int/chp/topics/rheumatic/en/]. OA is characterized by progressive structural changes in the articular cartilage, accompanied by new bone formation, changes in the subchondral bone and a low-grade synovitis [7]. The disease eventually leads to the loss of joint function, pain and immobility [8]. Despite high frequency of the disease, its cause is still not completely elucidated. Many factors may play a role in its onset and progression including: age, obesity, overuse or genetics. Articular cartilage undergoes several molecular changes during its lifespan, one of these being chondrocyte activity. Over time, chondrocytes synthesize less aggrecans and proteoglycans and become more susceptible to mechanical stress and joint loading [9].

Early alterations include increased water content and a decrease in the size and uniformity of aggrecan molecules due to the loss of function of linking proteins involved in binding chondroitin and keratan sulfate [9, 10]. In OA, the responsiveness of chondrocytes to mechanical stimuli and growth factors decreases. This leads to the loss of cartilage tensile strength, which is accompanied by its stiffness, and contributes to age-related changes in the structure and function of the articular surface. It has been observed that in OA, chondrocytes have a limited capacity to restore the damaged articular surface due to their degenerative phenotype, increased expression of the cellular markers of senescence (e.g. β-galactosidase) and increased DNA damage [9, 11].

Osteoarthritis is classified as primary or idiopathic, when the cause of the disease is unknown. Secondary OA develops as a result of joint injuries, inflammatory conditions, or developmental and metabolic disorders. It occurs in younger adults, while the primary OA is most frequent in the elderly [9]. OA most often develops gradually. It starts with soreness or stiffness of joints and progresses causing moderate to severe pain, which interferes with daily activities such as walking, climbing stairs etc. Other most common features and symptoms of OA are deterioration of posture, pain during walking, and a limited range of motion.

Primary OA most commonly occurs in the weight-bearing joints such as hips, knees and lumbar spine. It also affects the cervical spine, small joints of the hand, thumb or big toe. However, it rarely affects other joints like elbows, wrists or shoulders.

Genetic factors play a significant role in the idiopathic form of OA. Association studies and genome-wide association studies (GWAS) identified single nucleotide polymorphisms in genes important for cartilage development and homeostasis, that predispose to OA [12, 13]. In addition, analysis of the multi-generation families with OA revealed new candidate genes for this disorder [14]. The list of currently known OA candidate genes includes: *ADAM12, AGC1, ASPN, COL2A1, COL11A1, ENPP1, FRZB, GDF5, HFE, IL1, IL4, IGF-1, MATN3* and is still expanding [15-18]. However, predisposing polymorphisms in these genes do not appear to account for most cases of the primary OA and consequently the main cause of this disorder remains unknown. Articular cartilage destruction is considered a crucial feature in OA. Subchondral sclerosis, synovial inflammation and osteophyte formation that also occur during disease progression, result in a severe pain, restricted range of joint motion, extremity deformation and soft tissues contracture. Cartilage damage is characterized by degeneration of the extracellular matrix (ECM). Matrix degrading enzymes that belong to the ADA family (disintegrin and matrix metalloproteinase family) and ADAMTS family (disintegrin and metalloproteinase with thrombospondin motifs family) play a significant role in this process due to their ability to degrade major components of the ECM, type II collagen and/or aggrecans [19].

The clinical treatment of OA still remains unsatisfactory. Non-steroidal anti-inflammatory drugs (NSAIDs), steroids and hyaluronic acid have a limited effect in decreasing the symptoms of OA, and have failed to bring the function of articular cartilage to normal. In addition, some NSAIDs and steroids cause side effects including gastrointestinal ulcer or dyspepsia [20-22].

MicroRNAs AND OSTEOARTHRITIS

Numerous reports prove that miRs are expressed in healthy cartilage and cartilage affected by OA. It has been shown that expression of the aggrecanase gene *ADAMTS-5*, is elevated in the OA cartilage and that this gene is directly regulated by miR-140 (Table **1**). Moreover, in miR-140 knockout mouse, the level of *ADAMTS-5* is significantly increased [23]. In 2009, Tardif *et al.* [24] showed that insulin-like growth factor-binding protein 5 (IGFBP-5), which binds directly IGF-1, is a direct target of miR-140 (Table **1**). Yamasaki *et al.* [25] indicated that miR-146 might play a role in pathogenesis of OA [25]. His study revealed that miR-146 is highly expressed in low grade OA cartilage, and that the stimulation by IL-1β induces miR-146 expression [25]. Increased level of miR-146 caused by inflammatory cytokines could be involved in repression of the *MMP13* gene, encoding collagenase-3, which is the major enzyme involved in progressive erosion of articular cartilage (Table **1**). Taganov *et al.* [26] proposed that miR-146, which is induced by IL-1β at the early-stage of OA, may control cytokine signaling pathways by down-regulating IL-1 receptor-associated kinase 1 (IRAK1) and the TNF receptor-associated factor 6 (TRAF6) levels (Table **1**). Recent analysis of IL-1β induced miR-146a in rat OA chondrocytes revealed that this miR induces *VEGF* expression and inhibits expression of *SMAD4* (Table **1**), which plays a significant role in anabolic TGF-β pathway [27]. Results indicate that chondrocyte apoptosis was triggered in response to changes in expression profiles of genes regulated by miR-146a [27].

Iliopoulos *et al.*, [28] analyzed expression of 365 miRs in the articular cartilage obtained from patients with OA undergoing total knee replacement surgery, and from individuals with no history of joint disease. They detected 16 miRs that

are differentially expressed in cartilage of patients with OA compared to the normal cartilage. Nine of these miRs were up-regulated and seven down-regulated [28]. Jones *et al.* [29] studied 157 human miRs and identified 17 that showed expression differences in the normal and late-stage OA cartilage. Among these are miR-9, miR-98 and miR-146 that reduce IL-1β mediated production of TNF-α (Table **1**). Moreover, miR-9 also decreased basal level of TNF-α. Akhtar *et al.* [30] investigated expression of 352 human miRs in chondrocytes stimulated by interleukin-1β. This group identified 42 miRs that were down-regulated, two miRs were up-regulated and there were no differences in the expression of the remaining 308 miRs. This study also showed that over-expression of miR-27b plays a role in the inhibition of *MMP*-13 expression in chondrocytes stimulated by IL-1β (Table **1**). Further studies of Abouheif *et al.* (2010) demonstrated that IL-1β induced also miR-34a expression while silencing of miR-34a reduced IL-1β-mediated down-regulation of *COL2A1* [31]. In 2010, Dudek *et al.* showed that over-expression of miR-675 increases *COL2A1* expression level and expression of miR-675 is regulated by chondrogenic transcription factor SOX9 [32]. In 2013, Song *et al.* analyzed gene expression patterns in osteoarthritic chondrocytes isolated from patients with knee OA and found that miR-9 directly regulates the *PRTG* gene encoding protogenin (Table **1**), which is involved in the activation of caspase-3 signaling and an increase of chondrocyte apoptosis [33]. Expression of miR-9 is significantly reduced in OA chondrocytes [33]. A decrease of expression in OA chondrocytes was also observed in case of miR-488, which silences gene coding zinc transporter *SLC39A8/ZIP8*, that has a direct effect on MMP-13 activity (Table **1**), [34]. Suppression of *ZIP8* expression in an animal model of OA results in reduction of cartilage degeneration [34].

Table 1. MicroRNAs, that change expression patterns in osteoarthritis models and their targets.

MicroRNAs, in OA	Target gene	Reference
miR-140	*ADAMTS-5*	Miyaki *et al.*, 2010
	IGFBP-5	Tardif *et al.*, 2009
miR-146	*MMP13*	Yamasaki *et al.*, 2009
	IRAK1, TRAF6	Taganov *et al.,* 2006
	SMAD, VEGF	Li *et al.,* 2012
miR-9	TNF-α	Jones *et al.*, 2009
miR-98		
miR-146		
miR-27b	*MMP13*	Akhtar *et al.*, 2010
miR-34a	*COL2A1*	Abouheif *et al.*, 2010
miR-9	*PRTG*	Song *et al.*, 2013
miR-488	*ZIP8*	Song *et al.*, 2013

Kato *et al.* in 2014 analyzed miRs in exosomes isolated from a medium of human synovial fibroblasts [35]. They found that 50 miRs showed different expression profiles in exosomes isolated from IL-1β stimulated synovial fibroblasts, compared to synovial fibroblasts that were not stimulated.

Since the participation of miRs in numerous biological processes has been recognized, further insight into the molecular mechanisms involved in OA initiation and progression may lead to the development of new therapies that will help to control joint destruction and stimulate cartilage repair [36]. One of the most important tasks is identification and functional validation of all target genes that are regulated by miRs differentially expressed in affected versus unaffected tissue. In addition, comparisons of how miRs are expressed in different joints may provide joint-specific findings about expression patterns.

CONCLUDING REMARKS

Osteoarthritis is the most prevalent form of arthritis that particularly affects highly developed populations, causing pain and disability. Age, genetic and epigenetic factors seem to play significant role in development of the primary form of osteoarthritis. The increasing number of individuals affected by this disease directly correlates with an increase of knee- and hip-replacement surgeries, generating significant medical costs for society. It is therefore important to explore new methods of treatment for this debilitating disease. The most challenging problem in the therapeutic use of miRs is the development of safe and efficient systems for the delivery of these small unstable molecules into the joints. Recent report about exosomes suggests that they may play significant role in osteoarthritis initiation as well as in transportation and communication among cells in the joint [35]. These microvesicles stimulated by IL-1β can deliver cytokines and miRs to articular chondrocytes and up-regulate expression of *MMP-13* and *ADAMTS-5* [35]. Future studies utilizing next-generation sequencing of the RNA content of exosomes derived from synovial fluid, may reveal new potential biomarkers for osteoarthritis. RNA sequencing allows for the identification of a low number of transcripts and the potential detection of exosomal miRs that were previously not identified via microarrays. Additionally, comparisons of material from exosomes of affected and unaffected individuals may reveal new information about miRs, which are exosomal biomarkers. Manipulation of exosome secretion is currently considered a promising therapeutic strategy. Abnormally expressed miRs (e.g. miR-146a) in OA are promising prognostic markers. Development of RNA interference technologies may lead to the development of new nucleic acid drugs in the therapy of OA [37]. Therapies with miRs inhibitors like antagomirs could become particularly useful in suppressing function of miRs, which contribute to development of osteoarthritis [37].

CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflict of interest.

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