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Review:

Roles of microRNA and signaling pathway in osteoarthritis pathogenesis*

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Abstract: Osteoarthritis (OA) is a common chronic degenerative joint disease, with complicated pathogenic factors and undefined pathogenesis. Various signaling pathways play important roles in OA pathogenesis, including genetic expression, matrix synthesis and degradation, cell proliferation, differentiation, apoptosis, and so on. MicroRNA (miRNA) is a class of non-coding RNA in Eukaryon, regulating genetic expression on the post-transcriptional level. A great number of miRNAs are involved in the development of OA, and are closely associated with different signaling pathways. This article reviews the roles of miRNAs and signaling pathways in OA, looking toward having a better understanding of its pathogenesis mechanisms and providing new therapeutic targets for its treatment.

Key words: MicroRNA, Signaling pathway, Osteoarthritis, Pathogenesis
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1 Introduction

Osteoarthritis (OA) is a common chronic degenerative joint disease, which is characterized by degeneration of the articular cartilage, synovial inflammation, and changes in the periarticular subchondral bone (Pelletier *et al*., 1983; 1992; Gu *et al*., 2014). Although there are a lot of predispositions contributing to OA, including joint injury, heredity, obesity, aging, mechanics, and inflammation (Goldring and Goldring, 2007; Miyaki and Asahara, 2012), its pathogenesis is complicated and not fully understood. The current clinical treatment for OA is unsatisfactory. Drugs such as non-steroidal anti-inflammatory drugs (NSAIDs), selective cyclooxygenase 2 (COX-2) inhibitors, steroids, hyaluronic acid have limited effectiveness in alleviating its symptoms, and fail to reverse the loss of articular cartilage (Shamoon and Hochberg, 2000). Total joint arthroplasty (TJA) is an effective treatment for end stage OA. However, we have to accept its associated risks such as infection, peri-prosthetic fracture, deep vein thrombosis (DVT), and joint dislocation. So it is important to continue in-depth studies on OA pathogenesis, which may help to find new therapeutic targets and methods for treating this disease.

In the developing stages of OA, various signaling pathways play important roles, including nuclear factor-κB (NF-κB) pathways, bone morphogenetic protein (BMP) pathways, transforming growth factor β (TGF-β) pathways, SRY-related protein 9 (SOX9) pathways, insulin-like growth factor (IGF) pathways, and so on. These signaling pathways are involved in chondrocyte metabolisms, cell proliferation, differentiation, apoptosis, synthesis and degradation of the extracellular matrix (ECM), pro-inflammation, and anti-inflammation. If these signaling pathways were able to be interrupted, then the advancement of OA may be either hindered or even inverted.

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MicroRNAs (miRNAs) are a class of naturally occurring, small non-coding RNA molecules about 20–22 nucleotides long, in Eukaryon, regulating the genetic expression on the level of post-transcription by interacting with the 3' untranslated regions (UTRs) (Cheng and Jin, 2012; Lu *et al*., 2014). As more and more miRNAs were discovered, their functions in biological processes are being given greater attention. A large number of current studies have reported that various miRNAs play different roles in OA pathogenesis. It is therefore necessary to have a systematic understanding of miRNA in OA, which will help to provide new therapeutic targets.

With the purpose of developing a foundation for providing a better understanding of OA pathogenesis and new therapeutic targets, we reviewed the miRNAs and signaling pathways which are involved in OA pathogenesis, clarifying their functional mechanisms and showing how they interact with each other.

2 miRNA

Currently, the biochemical progress and mechanisms of miRNA have been identified (Wang and Luo, 2015; Yuan *et al*., 2014): miRNA genes are transcribed to form primary (pri)-miRNAs, which are subjected to cleavage by a miRNA processor (a protein complex composed of Drosh associated with DGCR8) to form a shorter precursor miRNA called a pre-miRNA (Lee *et al*., 2003). A pre-miRNA is transported from the nucleus to the cytoplasm by exportin-5 (Lund *et al*., 2004), and then is sliced by another RNase III, called a Dicer, to form a mature miRNA (Bernstein *et al*., 2001). miRNA is then combined together with Agonature proteins (Agos), the core unit of the RNA-induced silencing complex (RISC) (Bartel, 2004; Farh *et al*., 2005; Calin and Croce, 2006). The miRNA-RISC complex binds the targeted mRNA and mediates the translational repression or degradation of the mRNA (Bartel, 2004).

Although the information about miRNA expression and function in the musculoskeletal system is not fully understood, its importance in cartilage and chondrocyte studies has been established. Loss of a Dicer in the chondrocytes results in a reduction in the number of proliferation chondrocytes by decreased proliferation or accelerated differentiation into post-mitotic hypertrophic chondrocytes (Cobb *et al*., 2005; Kanellopoulou *et al*., 2005). Limb or cartilage specific Dicer deficiency may lead to a severe phenotype with reduced limb size but normal patterning (Harfe *et al*., 2005; Kobayashi *et al*., 2008). As a Dicer plays a key role in miRNA synthesis, the importance of miRNA in biological processes of the musculoskeletal system is self-evident.

Iliopoulos *et al*. (2008) tested the expressions of 365 miRNAs in articular cartilage obtained from patients with OA and total knee arthroplasty (TKA), and from normal individuals without a history of joint disease, finding that 16 miRNAs were differentially expressed in osteoarthritic versus normal cartilage. Hundreds of miRNAs take part in gene expression, cell cycle regulation, ECM metabolism, inflammation process, and so on (Table 1). In the meantime, various inflammation cytokines play important roles in OA by regulating the different miRNAs. For example, interleukin (IL)-1β can increase the expression of miR-491-3p and decrease the expressions of 42 miRNAs, including miR-23-3p, miR-610, and miR-27b (Yasuda, 2011). The degradation of ECM is a feature of articular cartilage degeneration, while collagen II and proteoglycan are important compositions of ECM (Goldring and Goldring, 2010). Matrix metalloproteinases (MMPs) and a disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS) are vital ECM-degrading enzymes, participating in the degradation of collagen II and proteoglycan (Tortorella *et al*., 2009; Li and Wu, 2010). MMP13 can degrade a large number of ECM components, including collagen. Because of its powerful degradation ability, MMP13 is a key factor in the biology studies of articular cartilage and OA pathogenesis (Fosang *et al*., 1996; Knauper *et al*., 1996; Billinghurst *et al*., 1997). ADAMTS4 and ADAMTS5 are major enzymes in the degradation of proteoglycan, and are important targets for treatment of OA (Wittwer *et al*., 2007; Gilbert *et al*., 2008). A great number of miRNAs are involved in the regulation of MMPs, ADAMTSs, and other related factors by using different pathways to control the progress of OA.

3 miRNAs and signaling pathways

3.1 NF-κB signaling pathway

NF-κB proteins constitute a family of ubiquitously expressed transcription factors involved in

Table 1 miRNAs and their targets

miRNA	Target	Species	Effect	Function	Reference
140	MMP13	*Homo sapiens*	↓	Matrix-degrading enzyme	Liang *et al*., 2012
	ADAMTs	*H. sapiens*	↓	Matrix-degrading enzyme	Miyaki *et al*., 2009
	ADAMTs	*Mus musculus*	↓	Matrix-degrading enzyme	Miyaki *et al*., 2010
	ACAN	*H. sapines*	↑	ECM component	Miyaki *et al*., 2009
	CXCL12	*M. musculus*	↓	Signaling	Jones *et al*., 2009
	SMAD3	*M. musculus*	↓	Signaling	Bazzoni *et al*., 2009
	DNPEP	*M. musculus*	↓	Signaling	Ohgawara *et al*., 2009
	HDAC4	*M. musculus*	↓	Transcription	Tuddenham *et al*., 2006
	PDGFRA	*Danio rerio*	↓	Skeletogenesis	Eberhart *et al*., 2008
	IGFBP5	*H. sapiens*	↓	Signaling	Tardif *et al*., 2009
	SP1	*H. sapiens*	↓	Cell cycle regulation	Martinez-Sanchez *et al*., 2012
	RALA	*H. sapiens*	↓	Regulate SOX9	Karlsen *et al*., 2014
146	IRAK1/TRFA6	*H. sapiens*	↓	Signaling	Taganov *et al*., 2006
	TNFα (IL-1-induced)	*H. sapiens*	↓	Inflammation mediators	Jones *et al*., 2009
9	TNFα	*H. sapiens*	↓	Inflammation mediators	Bazzoni *et al*., 2009
	TIR	*H. sapiens*	↓	Signaling	Bazzoni *et al*., 2009
	MMP13 (secretion)	*H. sapiens*	↓	Matrix-degrading enzyme	Jones *et al*., 2009
18A	CCN2	*H. sapiens*	↓	Signaling	Ohgawara *et al*., 2009
21	MMP-1/2/3/9	*H. sapiens*	↓	Matrix-degrading enzyme	Zhang *et al*., 2014
	GDF5	*H. sapiens*	↓	Signaling	Zhang *et al*., 2014
22	BMP-7	*H. sapiens*	↓	Signaling	Iliopoulos *et al*., 2008
	PPRA	*H. sapiens*	↓	Signaling	Iliopoulos *et al*., 2008
27	IGFBP5	*H. sapiens*	↓	Signaling	Tardif *et al*., 2009
	MMP13	*H. sapiens*	↓	Matrix-degrading enzyme	Akhtar *et al*., 2010
34	COL2A1	*H. sapiens*	↓	ECM component	Abouheif *et al*., 2010
	iNOS2	*H. sapiens*	↓	Signaling	Abouheif *et al*., 2010
98	TNFα	*H. sapiens*	↓	Inflammation mediators	Tardif *et al*., 2009
125B	ADAMTS4	*H. sapiens*	↓	Matrix-degrading enzyme	Matsukawa *et al*., 2013
127-5P	MMP13	*H. sapiens*	↓	Matrix-degrading enzyme	Park *et al*., 2013a
145	SOX9	*H. sapiens*	↓	Transcription	Martinez-Sanchez *et al*., 2012
	SOX9	*M. musculus*	↓	Transcription	Yang *et al*., 2011
365	HDAC4	*Gallus gallus*	↓	Transcription	Guan *et al*., 2011
455-3P	ACVR2B	*H. sapines*	↓	Signaling	Swingler *et al*., 2012
	SMAD2				
	CHRD1				
558	COX2 (IL-1β-induced)	*H. sapines*	↓	Inflammation mediators	Park *et al*., 2013b
675	COLA1	*H. sapines*	↑	ECM component	Dudek *et al*., 2010

immunity, stress responses, inflammatory diseases, cell proliferation, and cell death (Oeckinghaus and Ghosh, 2009). NF-κB can be stimulated by pro-inflammatory cytokines, chemokines, stress-related factors, and ECM degradation products (Yasuda, 2011; Rigoglou and Papavassiliou, 2013). The activation of an NF-κB signaling pathway can trigger the expressions for various amounts of immunomodulatory proteins, cytokines, chemokines, proteases, angiogenic factors, and proliferation- or apoptosis-related molecules (Niederberger and Geisslinger, 2008). There are two distinct pathways for activating the NF-κB signaling cascades. The first one, called canonical or classical pathway, is mediated by a tumor

necrosis factor, Toll-like or T-cell receptor (TNF-R, TL-R, and TC-R, respectively), and induces the activation of the inhibitor of nuclear factor κB kinase α (IKKα)/IKKβ/IKKγ-NEMO (NF-κB essential modulator) complex, which results in degradation of the Iκ proteins. The other one, called non-canonical or alternative pathway, involves stimulation of the B-cell activating factor, CD40 or lymphotoxin β receptors (BAFF-R, CD40-R, LTβ-R), and relies on NF-κB-inducing kinase (NIK) that activates the IKKα kinase (Rigoglou and Papavassiliou, 2013).

A great number of miRNAs were found to be involved in the NF-κB signaling pathway. Akhtar *et al*. (2010) reported that the NF-κB signaling pathway could suppress miR-27b, which regulated the expression of MMP13. miR-146a/b has the function of decreasing the expressions of TNF receptor-associated factor 6 (TRAF6) and IL-1 receptor-associated kinase 1 (IRK1) at a post-transcriptional level (Taganov *et al*., 2006). TRAF6 and IRK1 play important roles in triggering the activation of Iκ kinase and JNK, and then the downstream NF-κB and AP-1 transcription factors which result in the up-regulation of the immune-responsive gene (Taganov *et al*., 2006). In IL-1β-stimulated C28/I2 cells, expressions of miRNA-140 and MMP13 were elevated. However, their expressions decreased when the IL-1β-stimulated C28/I2 cells were treated with DHMEQ, an NF-κB inhibitor. So the expressions of miRNA-140 and MMP13 were shown to be NF-κB-dependent. miRNA-140 down-regulates the expression of MMP13, which will be up-regulated when transfecting C28/I2 with anti-miR-140 (Liang *et al*., 2012).

In rheumatoid arthritis synovial fibroblasts (RASFs), TNFα induced the expression of miR-17-92 in an NF-κB-dependent manner. miR-17-92-derived miR-18a contributes to cartilage destruction and chronic inflammation in the joints through a positive feedback loop in the NF-κB signaling (Trenkmann *et al*., 2013). Gantier *et al*. (2012) found that miR-19b controlled NF-κB signaling by suppressing its regulon of negative regulators (including A20/Tnfainp3, Rnf11, Fbx11/Kdm2a, and Zbtb16). What's more, miR-203 up-regulated the expressions of MMP13 and IL-6 through the NF-κB signaling pathway (Stanczyk *et al*., 2011). As miRNA is relatively conservative, the roles that these miRNAs play in rheumatoid arthritis (RA) may also be played in OA (Fig. 1).

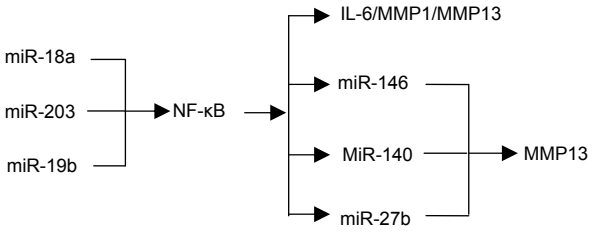

Fig. 1 miRNAs and NF-κB signaling pathway

3.2 BMP signaling pathway and TGF-β signaling pathway

BMP/TGF-β-mediated signaling pathways involve the development of OA and are potent regulatory systems in chondrocytic cell types (Ballock *et al*., 1993; Serra *et al*., 1999; Grimsrud *et al*., 2001). In the growth plate, BMP signaling promotes chondrocyte terminal differentiation through SMAD1/5/8; conversely, TGF-β signaling blocks this process through SMAD2/3 (van der Kraan *et al*., 2012).

By comparing the profiles of RNA associated with Argonature 2 (Ago2) between the wild-type and miR-140$^{-/-}$ chondrocytes, it was found that aspartyl aminopeptidase (*DNPEP*) was identified as a miR-140 target gene. In miR-140$^{-/-}$ chondrocytes, the increased expression of DNPEP showed a mild antagonistic effect on BMP signaling at a position downstream of the SMAD activation and the lower-than-normal basal BMP signaling in miR-140$^{-/-}$ chondrocytes was reversed by applying a *DNPEP* knockdown. miR-140 was essential for normal endochondral bone development and the reduced BMP signaling caused by *DNPEP* up-regulation played a causal role in skeletal defects of miR-140$^{-/-}$ mice (Nakamura *et al*., 2011). The knockdown of miR-140 in limb bud micromass cultures resulted in the arrest of chondrogenic proliferation by regulating SP1, acting downstream from the BMP signaling (Yang *et al*., 2011). miR-140 has plenty of targets conserved between human and chicken and validated *BMP2* as a direct target gene (Nicolas *et al*., 2011). miR-140 targets the CXC group of chemokine ligand 12 (CXCL12) and SMAD3 (Nicolas *et al*., 2008; Pais *et al*., 2010), both of which are involved in chondrocyte differentiation. Through repressing SMAD3, miR-140 suppresses the TGF-β pathway (Tuddenham *et al*., 2006, Araldi and Schipani, 2010). In conclusion, miR-140 promotes chondrocyte terminal differentiation by enhancing the BMP pathway and suppressing the TGF-β pathway.

During BMP2-induced chondrogenesis, miR-199a expression is decreased, indicating that it may function as a suppressor during the early stages in the chondrogenic program (Lin *et al*., 2009). Enforced miR-199a expression in Murine C3H10T1/2 stem cells or in the prechondrogenic cell line ATDC5 suppresses multiple markers of early chondrogenesis, including the type II collagen and cartilage oligomeric matrix protein (COMP), while anti-miR-199a has an opposite, stimulatory effect (Lin *et al*., 2009). SMAD1, a positive downstream mediator of BMP2 signaling, was shown to be a direct target of miR-199a (Lin *et al*., 2009). So the post-transcriptional repression of SMAD1 mediated by miR-199a will be prevented by BMP2-mediated repression of miR-199a.

Functional experiments on selected miR-gene pairs verified the presence of miR-22-regulated BMP7 and peroxisome proliferator-activated receptor α (PPARA) at the RNA and protein levels, respectively (Iliopoulos *et al*., 2008). The up-regulation of miR-22 or the down-regulation of BMP7 and PPARA can result in increases in the IL-1β and MMP13 protein levels (Iliopoulos *et al*., 2008). miR-455-3p appears to regulate TGF-β signaling by suppressing the SMAD2/3 pathway (Swingler *et al*., 2012). In other words, various miRNAs play important roles in chondrocyte differentiation, the regulation of inflammatory factors and ECM-degrading enzymes through the BMP/TGF-β signaling pathway (Figs. 2 and 3).

3.3 SOX9-related signaling pathway

SOX9 is an essential transcription factor regulating the expression of many ECM genes, such as *ACAN* (Bi *et al*., 1999) and *COL2A1* (Bell *et al*., 1997), and is essential for converting medenchymal stem cells (MSCs) into chondrocytes (Kronenberg, 2003). The CAMP-PKA-CREB pathway synergized with SOX9 at the nuclear and cytoplasmic levels to promote BMP2-induced osteochondrogenic differentiation (Zhao *et al*., 2009). TGF-β is shown to stimulate the expression of SOX9 mRNA (Roman-Blas *et al*., 2007; Kim *et al*., 2014). In addition, SMAD3 acts in cooperation with p300 and SOX9 to control gene expression during chondrogenesis (Furumatsu *et al*., 2009).

It was confirmed that miR-140 was directly induced by SOX9 and that the suppression of miR-140 is partially due to the inhibition of SOX9 by Wnt/catenin signaling in the micro mass cultures and the ATDC5 cell line (Yang *et al*., 2011). It was reported that RALA, a small GTPase not previously known to be involved in chondrogenesis, acted as a new direct target of miR-140-5p and showed that a knockdown of RALA during early chondrogenesis led to a significant up-regulation of SOX9 protein expression (Kartsen *et al*., 2014). SOX9 itself is directly targeted by miR-145 during the early stages of chondrogenic differentiation (Yang *et al*., 2011; Martinez-Sanchez *et al*., 2012). Through regulating SOX9, increasing miR-145 leads to down-regulation of the critical cartilage ECM genes (*COL2A1* and

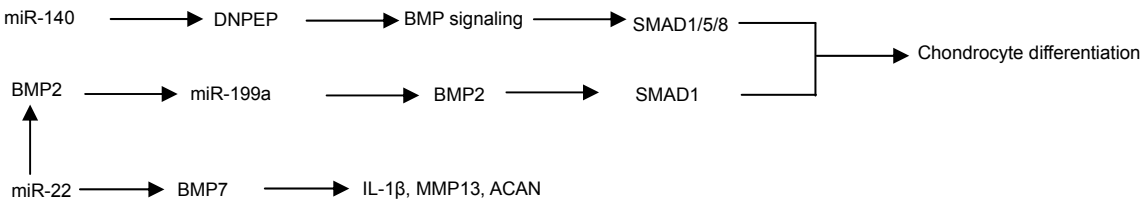

Fig. 2 miRNAs and BMP signaling pathway

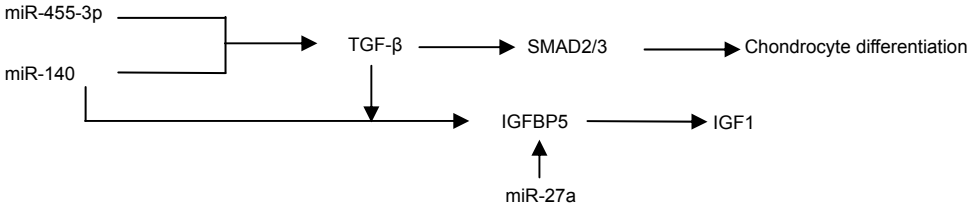

Fig. 3 miRNAs and TGF-β signaling pathway

ACAN) and tissue-specific miRNAs (miR-675 and miR-140), and up-regulation of RUNX2 and MMP13 (Martinez-Sanchez *et al*., 2012). OA cartilage revealed several miRNA-gene target pairs potentially involved in cartilage homeostasis and structure, including miR-509-SOX9 (Iliopoulos *et al.*, 2008).

Multiple signaling pathways and miRNAs result in various bio-effects in articular cartilage through SOX9, which is a key factor in the progress of OA. So controlling the expression of SOX9 may help us to intervene these signaling pathways and miRNAs, providing new treatments for OA (Fig. 4).

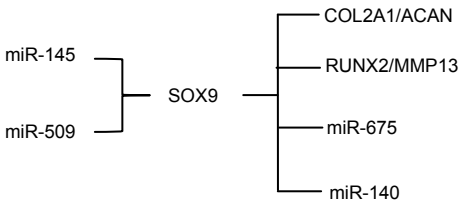

Fig. 4 miRNAs and SOX9 signaling pathway

3.4 IGF signaling pathway

IGF-1, a main anabolic mediator in articular cartilage, enhances cell proliferation and the synthesis of ECM proteins, and inhibits apoptosis through PI3K and ERK (Ashraf *et al*., 2015). Insulin-like growth factor binding proteins (IGFBPs), whose expression is very low in human OA chondrocytes (Tardif *et al*., 2009), are known to play an important role for IGF1 in joint treatment (Jones *et al*., 1993). Increasing the IGFBP5 concentration results in the increase of IGF-1, which is associated with a reduction of cartilage destruction in a dog OA model (Clemmons *et al*., 2002).

Transfection with pre-miR-140 significantly decreased IGFBP5 expression, while transfection with anti-miR-140 had the opposite effect, suggesting that IGFBP5 is a direct target of miR-140 (Ashraf *et al*., 2015). When human OA chondrocytes were treated with TGF-β, the expression of IGFBP5 was increased and the expression of miR-140 was decreased, indicating that both of them are regulated by TGF-β (Ashraf *et al*., 2015). It was found that miR-27a down-regulated the levels of MMP13 and IGFBP5 indirectly, and both of them were up-regulated when transfected with anti-miR-27a (Tardif *et al*., 2009) (Fig. 3).

4 Conclusions

In summary, through different signaling pathways, various miRNAs and their targets play important roles in the OA process, including genetic expression, matrix synthesis and degradation, cell proliferation, differentiation, apoptosis, and so on. Although a larger amount of work has already been done, OA pathogenesis requires future studies to have a better understanding of how it works and the implications for ongoing treatment. The knowledge about the functions of signaling pathways and miRNAs in OA will provide the potential means for diagnosis and treatment of OA.

Compliance with ethics guidelines

Bin XU, Yao-yao LI, Jun MA, and Fu-xing PEI declare that they have no conflicts of interest.

The article does not contain any studies with human or animal subjects performed by any of the authors.

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中文概要

题　目：MicroRNA 与信号通路在骨关节炎发病机制中的作用

概　要：通过综述 microRNA 及信号通路在骨关节炎发病机制中参与基因表达、基质代谢及细胞周期等生理过程的作用，以及整理两者之间的关系，为更好地理解其发病机制，提供了新的治疗靶点及途径。

关键词：MicroRNA；信号通路；骨关节炎；发病机制