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REVIEW ARTICLE

Epigenetic regulation of gene expression in osteoarthritis

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Abstract Osteoarthritis (OA) is the most common form of joint disease and the leading cause of chronic disability in middle-aged and older populations. The development of disease-modifying therapy for OA currently faces major obstacles largely because the regulatory mechanisms for the function of joint tissue cells remain unclear. Previous studies have found that the alterations in gene expression of specific transcription factors (TFs), pro- or anti-inflammatory cytokines, matrix proteinases and extracellular matrix (ECM) proteins in articular cartilage may be involved in the development of OA. However, the regulatory mechanisms for the expression of those genes in OA chondrocytes are largely unknown. The recent advances in epigenetic studies have shed light on the importance of epigenetic regulation of gene expression in the development of OA. In this review, we summarize and discuss the recent studies on the regulatory roles of various epigenetic mechanisms in the expression of genes for specific TFs, cytokines, ECM proteins and matrix proteinases, as well the significance of these epigenetic mechanisms in the pathogenesis of OA.

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Introduction

Osteoarthritis (OA) is the most common form of arthritis in the U.S. and affects approximately 27 million Americans.¹ As OA mainly occurs in weight-bearing joints, such as the knee and hip, OA has long been thought of as a mechanical issue.² However, there is a growing body of evidence supporting the notion that OA is a result of the interaction between mechanical and molecular events in the affected joint.³ There is no single specific cause that has been

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identified for OA to date. Some risk factors, including age, gender, obesity, joint injury, genetic and mechanical abnormalities, have been shown to be associated with the development of OA.⁴ However, how these risk factors trigger the onset of OA still need to be elucidated. While OA is a disease of the whole joint and may affect all of the joint tissues, articular cartilage degradation is a major hallmark of OA.⁵ Aberrant gene expression of specific transcription factors (TFs), cytokines, matrix proteinases and extracellular matrix (ECM) structural proteins (e.g., collagens and proteoglycans) in articular chondrocytes (ACs) of human OA and animal models of OA samples has been documented. Nevertheless, the underlying regulatory mechanism for the expression of those genes in OA cartilage is not fully understood.

"Epigenetics" is referred to as changes in gene expression caused by mechanisms other than changes in the underlying DNA sequences. DNA methylation and histone modification are the two best-studied classic epigenetic regulatory mechanisms, which regulate the transcriptional activity of a cell in the nucleus. DNA methylation is a biochemical process where a methyl group is added to the cytosine or adenine, mainly at the C5 position of CpG dinucleotides, by DNA methyltransferase (DNMT). DNA hypermethylation suppresses gene transcription, while DNA hypomethylation enhances gene transcription. Histone modifications are enzymatic post-translational modifications which include methylation, acetylation, phosphorylation, sumoylation and ubiquitination.^{6,7} These modifications primarily occur within the amino-terminal tails of histone proteins that regulate gene expression by changing the chromatin structure.⁸

A broader definition of "epigenetics" has been proposed by Egger et al as heritable changes in gene expression that are not coded in the DNA sequences.⁹ In this regard, non-coding RNAs (ncRNAs) which possess epigenetic-like properties have also been taken into account as one of the epigenetic mechanisms.^{10,11} ncRNAs are functional RNA molecules that regulate gene expression but do not translate into proteins. ncRNAs can be mainly divided into short ncRNAs (<30 nucleotides) and long ncRNAs (lncRNAs, >200 nucleotides). Short ncRNAs include microRNAs (miRNAs), short interfering RNAs (siRNAs) and piwi-interacting RNAs

(piRNAs).¹² In general, miRNAs function to modify the protein expression mainly at the post-transcriptional level in cytoplasm by binding to a specific target messenger RNA (mRNA) with a complementary sequence to induce cleavage, degradation or block translation.¹³ Recent progress in the study of ncRNAs has revealed the importance of ncRNAs in development and diseases.^{14,15}

Given the importance of epigenetics in normal development as well as cancer and age-related diseases,¹¹ recent studies on epigenetics in OA have provided new insights into the pathogenesis of OA and new targets to develop potential therapeutic strategies for OA. In this review, we will focus on the epigenetic mechanisms for the expression of TFs, cytokines, matrix proteinases and ECM proteins in ACs, as well as their significance in the pathogenesis of OA (Table 1).

TFs

TFs are the proteins that bind to specific DNA sequences and control the transcriptional rate of the target genes from genomic DNA to mRNA, which then translate into protein in the cytoplasm. Therefore, abnormal expression of TFs has been found to be involved in the development of many diseases, including OA.

Nfat1 (NFAT1/NFATc2) is a member of the Nuclear Factor of Activated T-cells (NFAT) transcription factor family originally identified as a regulator of the expression of cytokine genes during the immune response.^{34,35} NFAT1 has recently been shown to play an important role in maintaining the permanent cartilage phenotype in adult mice. *Nfat1* knockout (*Nfat1*^{-/-}) mice exhibit normal skeletal development, but display over-expression of numerous matrix-degrading proteinases and proinflammatory cytokines and loss of collagen-2 and aggrecan during the initiation stage of OA. These initial changes are followed by articular chondrocyte clustering, formation of chondrocytes, progressive articular surface destruction, formation of subchondral bone cysts, and exposure of thickened subchondral bone.¹⁶

Our recent studies have demonstrated that NFAT1 regulates the chondrocyte function through its age-dependent

Table 1 Gene expression changes mediated by epigenetic mechanisms in osteoarthritic chondrocytes.

Category	Gene	Expression ^a	Epigenetic regulation ^b			References
			DNA methylation	Histone modification	microRNA	
TFs	<i>Nfat1</i>	↓ (m)	↔	✓	↔	16,17
	<i>SOX9</i>	↓ (h)	✓	✓	✓	18–20
Cytokines	<i>IL-1B</i>	↑ (h)	✓	↔	✓	21–23
	<i>TNF-alpha</i>	↑ (h)	↔	↔	✓	23
Proteinases	<i>ADAMTS4</i>	↑ (h)	✓	✓	✓	24–27
	<i>ADAMTS5</i>	↑ (h)	✓	✓	✓	20,26,27
	<i>MMP-13</i>	↑ (h)	✓	✓	✓	24,27,28
ECM proteins	<i>COL2A1</i>	↓ (h)	↔	✓	✓	20,29–31
	<i>COL9A1</i>	↓ (h)	✓	↔	↔	32
	<i>ACAN</i>	↓ (h)	X	✓	✓	20,33

^a Gene expression information is cited from the references of this manuscript. ↓: decrease; ↑: increase; m: mouse; h: human.

^b Gene expression changes are associated with specific epigenetic alterations (✓), or not (X), or unknown (↔).

expression in mouse articular cartilage. NFAT1 expression in wild-type articular chondrocytes was low in the embryonic, but high in the adult stage (2–6 months old). Our epigenetic studies¹⁷ revealed that an increase in NFAT1 expression in ACs is associated with increased H3K4me2 (a histone modification linked to transcriptional activation); while a decrease in NFAT1 expression in ACs is correlated with increased H3K9me2 (a histone modification linked to transcriptional repression). Knockdown of lysine-specific demethylase-1 (Lsd1) in embryonic ACs up-regulates NFAT1 expression concomitant with increased H3K4me2 at the *Nfat1* promoter. Knockdown of Jmjc-containing histone demethylase-2a (Jhdm2a) in 6-month ACs down-regulates NFAT1 expression concomitant with increased H3K9me2 at the *Nfat1* promoter. These results suggest that the age-dependent NFAT1 expression in ACs is regulated by dynamic histone methylation.¹⁷ Further study should be directed to investigate the expression of NFAT1 in aged articular cartilage and its underlying epigenetic mechanisms as well as the role of NFAT1 in the development of OA in humans.

SOX9 is a master transcription factor for chondrogenesis during the development of the skeletal system, in cooperation with SOX5 and SOX6.^{36,37} Although mice with conditional postnatal deletion of *Sox9* in articular cartilage did not develop OA even by the age of 18 months,³⁸ later OA usually is associated with decreased SOX9 expression in humans.³⁹ Kim et al recently reported that down-regulated SOX9 expression in advanced hip OA chondrocytes is mediated by DNA methylation and histone modification, including histone methylation and acetylation.¹⁸ Moreover, miRNA-145 has been identified as an inhibitor of SOX9 expression in human chondrocyte; increased miRNA-145 directly represses SOX9 expression, causing reduced expression of COL2A1 and aggrecan and an increased level of matrix metalloproteinases 13 (MMP13).¹⁹ In addition, miRNA-199a-3p and miRNA-193b has been found to down-regulate SOX9 expression.²⁰ While SOX9 is considered a typical anabolic factor in articular cartilage, the response of cultured chondrocytes to forced expression of SOX9 has been controversial. Kyriotes et al found that overexpression of SOX9 itself was unable to restore the chondrocyte phenotype in dedifferentiated osteoarthritic chondrocytes,⁴⁰ whereas Cucchiari et al reported that r-AAV mediated SOX9 gene transfer up-regulated the expression levels of proteoglycans and type II collagen in normal and OA ACs.⁴¹ Therefore, more studies are needed to determine whether the epigenetically regulated change in SOX9 expression in articular cartilage is the cause or the result of OA.

Cytokines

Cytokines are small proteins mostly secreted by immune cells that function as signaling messengers in the immune system. It has been well-documented that cytokines play important roles in the development of rheumatoid arthritis (RA) which is a typical autoimmune disease in human joints.⁴² A new concept that OA is a joint disease of inflammation involving immune reaction has been proposed based on findings of the aberrant expression of cytokines in human OA and animal models of OA.⁴³ Although many

cytokines have been implicated in OA, interleukin 1- β (IL-1 β) and tumor necrosis factor- α (TNF- α) are the two main proinflammatory cytokines contributing to the degradation of articular cartilage.^{44,45}

The epigenetic regulation of IL-1 β expression in OA cartilage has been well documented. Hashimoto et al found that specific CpG sites at -299 of the *IL-1 β* promoter has a significant impact on its promoter activity and methylation of these sites results in marked suppression of its transcriptional activity in human ACs.²¹ Demethylation of these sites increases the transcriptional response of IL-1 β to inflammatory cytokines in human ACs.²² In addition, Santini et al found miR-149 is down-regulated in OA chondrocytes, and a functional study showed that this miRNA regulates the production of TNF- α , IL-1 β and IL-6.²³ Other than being regulated by epigenetic mechanisms, IL-1 β also modulates epigenetic events in OA cartilage, because stimulation of OA chondrocytes with IL-1 β can affect miRNA production.⁴⁶ Moreover, the overall methylation status in different histological zones of human cartilage was found to be different upon IL-1 β stimulation.⁴⁷ Both DNA methylation and histone modification are involved in the control of TNF- α expression⁴⁸; however, the epigenetic status of TNF- α in OA chondrocytes remains to be elucidated.

Matrix proteinases

The expression of matrix proteinases, including collagenases, aggrecanases and matrix metalloproteinases (MMPs), is relatively low in normal articular cartilage. Matrix proteinases are required for cartilage turnover but elevated in OA, which are deleterious factors for ECM degradation.^{49,50} ADAMTS (a disintegrin and metalloproteinase with thrombospondin motifs) -4 and -5 are two major aggrecanases which have been shown to play important role in development of OA.^{51–54} MMP13, a major type II collagen-degrading collagenase, not only contributes to the onset of OA, but also contributes to irreversible joint damage during the progression of OA.^{55,56} In addition to being regulated by transcription factors and cytokines, proteinase expression is also regulated by DNA methylation.²⁴ For example, Cheung et al found that increased ADAMTS-4 expression was mediated by the loss of DNA methylation at specific CpG sites in the *ADAMTS-4* promoter in OA chondrocytes.²⁵ In OA cartilage specifically, differentially and highly expressed lncRNA-CIR not only controls the expression of collagen and aggrecan but also regulates the expression of MMP13 and ADAMTS-5.²⁶ Moreover, several miRNAs have also been found to regulate ADAMTS-5 expression in human OA cartilage.²⁰ In a study of cultured SW1353 chondrosarcoma cells and primary human chondrocytes, Young et al demonstrated that histone deacetylase (HDAC) inhibitors decreased the level of collagenolytic enzymes in conditioned culture medium by down-regulating the expression of MMPs and ADAMTSs, and the elevated HDAC7 expression in cartilage from OA patients was associated with up-regulated MMP13 gene expression,²⁸ implicating that histone modification, especially acetylation, may play a role in the control of those proteinases in the pathogenesis of OA.²⁷

Extracellular matrix proteins

Collagen and proteoglycan are the major ECM protein components of articular cartilage. The maintenance of the normal amount and architecture of these components are required for articular cartilage to fulfill its mechanical properties.^{57,58} In humans, collagen gene mutations account for a family of spondyloepiphyseal dysplasias, which are associated with early-onset of OA.⁵⁹ Mice that lack *Col9a1* or bear a small deletion mutation in type II collagen gene display OA-like cartilage degradation.^{60,61} Adult articular cartilage is an avascular tissue, in which chondrocytes, the unique cellular component, do not normally divide, but maintain low-turnover replacement of the ECM. Therefore, degeneration of articular cartilage ECM is the major feature of OA.^{49,62} As discussed above, the up-regulated matrix proteinases contribute to ECM disruption. Decreased ECM synthesis activity of chondrocytes regulated by epigenetic mechanisms accounts for the loss of ECM in OA cartilage.

Histone acetyl-transferase CBP/P300 and the Class III NAD-dependent histone deacetylase Sirtuin 1 (SirT1) have been shown to co-regulate *COL2A1* mRNA expression in cooperation with SOX9.^{29,30} Moreover, a recent study using human chondrocytes found that histone methyltransferase Set7/9 elevated trimethylated lysine 4 on histone 3 on *COL2A1* promoter, resulting in increased *COL2A1* expression.³¹ In a study on the correlation between gene methylation and expression of aggrecan in chondrocytes, Pochl et al failed to find a significant correlation of *ACAN* mRNA expression levels and DNA methylation status between normal aged and osteoarthritic chondrocytes. This result suggests that DNA methylation does not play a central role in switching off *ACAN* promoter activity in human adult ACs.³³ Although the methylation status of *COL2A1* has been studied in differentiated and dedifferentiated chondrocytes,⁶³ little is known about the role of DNA methylation in *COL2A1* expression in OA chondrocytes. DNA methylation has also been found to control the decreased expression of *COL9A1* mRNA in OA chondrocytes. Imagawa

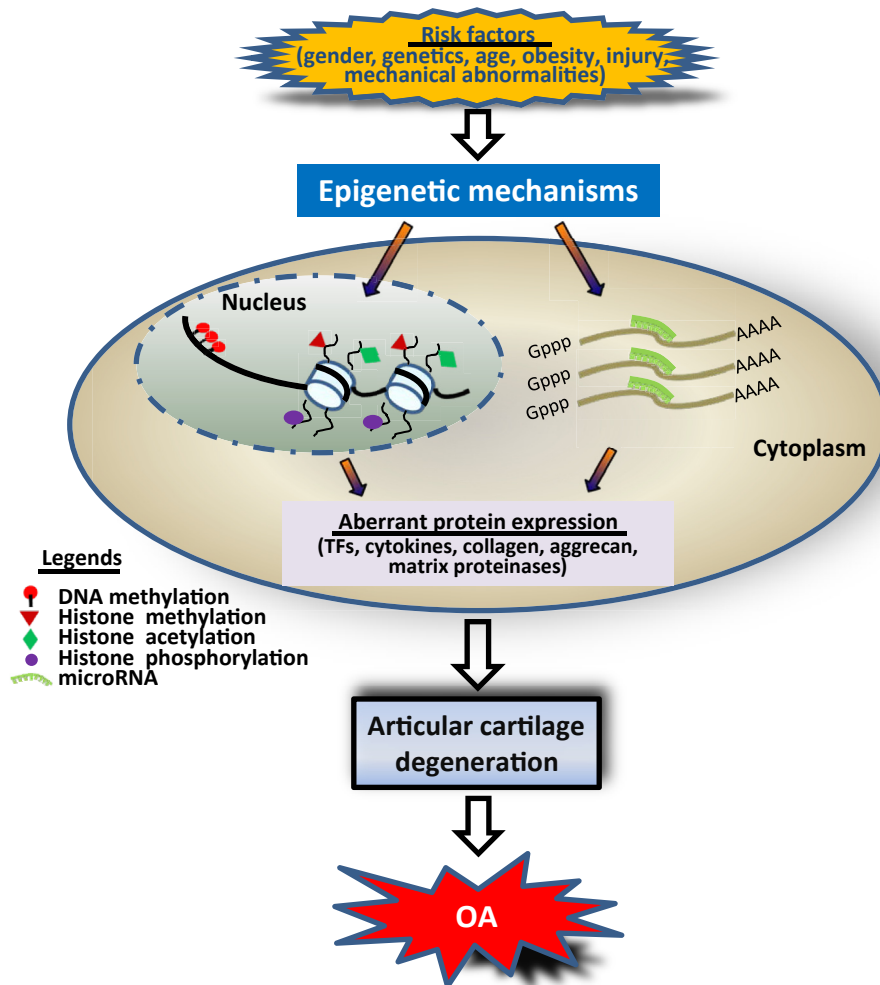


Figure 1 Possible roles of epigenetic changes in the pathogenesis of OA. Under the accumulative effect of risk factors, chondrocytes undergo epigenetic events including DNA methylation and histone modifications that occur in the nucleus, and miRNAs which function in the cytoplasm. This results in aberrant expression of TFs, cytokines, collagen, aggrecan, and matrix proteinases. Abnormal expression of these factors may disrupt the balance of anabolic and catabolic activity and compromise cartilage homeostasis, leading to articular cartilage degradation and the development of OA.

et al recently reported that CpG sites in *COL9A1* promoter were hypermethylated and that this hypermethylated CpG attenuated SOX9 binding to the *COL9A1* promoter, resulting in down-regulation of *COL9A1* expression in OA cartilage.³² In addition to the regulation of SOX9 expression, miRNA-199a-3p and miRNA-193b also control the expression of *COL2A1* and aggrecan in human OA chondrocytes.²⁰

Conclusions and future directions

As epigenetics is a molecular link of environmental factors to the development of diseases,^{64,65} the advance of epigenetics has greatly enhanced our understanding of the pathogenesis of multifactorial diseases, such as cancer and OA. Accumulative influence of risk factors which trigger epigenetic events such as DNA methylation, histone modifications and miRNAs in chondrocytes, may result in aberrant gene expression of TFs, cytokines, collagen and aggrecan, and matrix proteinases. Abnormal expression of these genes may compromise the balance of anabolic and catabolic activity and disrupt cartilage homeostasis, leading to cartilage degradation, which is the key step of the development of OA (Fig. 1). Moreover, the formation of positive feedback loops among TFs, cytokines and matrix proteinases during the development of OA may partially explain why OA is irreversible once occurs.⁶⁶

With the use of new techniques, especially the application of second generation sequencing in the study of epigenetics, the era of epigenetic study in the pathogenesis of OA is coming.⁶⁷ Global analysis of epigenetic modifications in OA is being undertaken and more detailed epigenetic alterations in OA will be identified.^{47,68–70} The upcoming epigenetic findings may not only broaden our knowledge to appreciate the molecular mechanisms underlying the development of OA, but also promote the development of new drugs for the treatment of OA.⁷¹

Conflicts of interest

The authors declare no conflict of interest.

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