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†The authors, as well as the first authors, contributed equally to this work

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Authors for correspondence: Yuting Wang, Juan Li and Shishu Huang, E-mails: 3183428801@qq.com; lijuan@scu.edu.cn; h0794062@scu.edu.cn

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Epigenetics and bone diseases

Tu Huang[1,2,†], Xiu Peng[2,†], Zhenxia Li[1], Quan Zhou[2], Shishu Huang[2], Yuting Wang[3], Juan Li[1] and Youqiang Song[4]

[1]State Key Laboratory of Oral Diseases, Department of Orthodontics, West China School of Stomatology, Sichuan University, Chengdu 610041, PR China; [2]Department of Orthopedic Surgery, West China Hospital, Sichuan University, Chengdu, 610041, China; [3]Department of Human Resource Management, West China Hospital, Sichuan University, Chengdu, 610041, China and [4]Department of Biochemistry, Faculty of Medicine, University of Hong Kong, Hong Kong, China

Abstract

Owing to the development of new technologies, the epigenome, a second dimensional method for genome analysis has emerged. Epigenetic mechanisms, including DNA methylation, histone modifications and noncoding RNAs, regulate gene expression without changing the genetic sequence. These epigenetic mechanisms normally modulate gene expression, trans-generational effects and inherited expression states in various biological processes. Abnormal epigenetic patterns typically cause pathological conditions, including cancers, age-related diseases, and specific cartilage and bone diseases. Facing the rapidly developing epigenetic field, we reviewed epigenetic mechanisms and their involvement with the skeletal system and their role in skeletal development, homeostasis and degeneration. Finally, we discuss the prospects for the future of epigenetics.

1. Introduction

The epigenome is a multitude of chemical compounds that instruct the genome how to function. These functions include DNA methylation, chromatin modifications, nucleosome positioning and alterations in the noncoding RNA (ncRNA) profile (Verma, 2015), which may affect gene expression and its regulation without altering the DNA sequence. With the rapid development of bio-technologies, many unknown epigenetic phenomena have been revealed, which has led to an increasing interest in epigenetic mechanisms of individual development and of diseases.

Epigenetic modulation exerts either a positive or negative feedback pathway, leading to silencing of one of the two X chromosomes in female cells in early development (Gendrel & Heard, 2014), genomic imprinting (Gupta *et al.*, 2014*a*) and paramutation (Brink, 1973). The epigenetic pattern and changed phenotype appear after mitosis or meiosis (Huang *et al.*, 2014). Although various epigenetic programs have been identified, only three main categories are widely accepted, which are as follows: DNA methylation, histone modification and ncRNAs. DNA methylation is the most studied; this includes the methylation of the fifth carbon of cytosine. The DNA methylation pattern is dynamically regulated by DNA methyltransferases (DNMTs) during development. DNA methylation pattern includes endogenous transposable elements repression, chromosome alignment and segregation, second X chromosome control via inactivation in females and modulation of imprinted gene expression. Histone modifications are characterized by histones that can be covalently modified at their flexible N- or C-terminal tails, as well as globular domains. This phenomenon is associated with DNA methylation. It is also regarded as one of the key components of chromatin packaging (Henikoff & Shilatifard, 2011). Histones function both positively and negatively in gene expression regulation; histones are also mainly governed by post-translational histone modifications (PTMs) and specific histone variants (Kimura, 2013). PTMs regulate transcription and other DNA-templated functions, which are dynamically mediated by specific modifying enzymes (Fan *et al.*, 2015). PTMs can be classified into several categories, including lysine acetylation, lysine and arginine methylation, arginine citrullination, lysine ubiquitination, phosphorylation, fatty acylation and ADP-ribosylation, which affect DNA function individually or collectively. NcRNAs are comprised of short and long ncRNAs. These molecules have been highlighted in biological processes with the development of deep sequencing and transcriptome analyses; however, these molecules have been previously regarded as junk RNAs.

Therefore, epigenetic programs are essential to basic biologic events that are associated with physiological and pathological processes, including skeletal genesis, bone remodelling and bone metabolic disorders (Fan *et al.*, 2015). This review will discuss the current knowledge of epigenetics in the skeletal system and will strive to shed new light on the understanding of epigenetic roles in bone and cartilage tissues.

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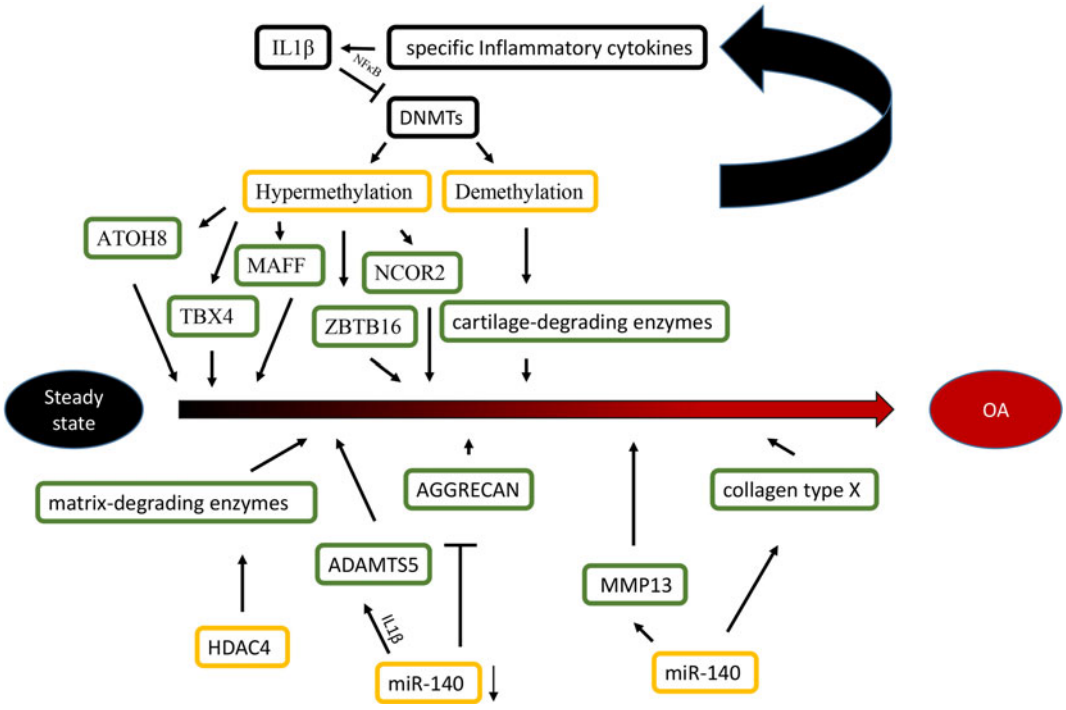


Fig. 1. Epigenetic mechanisms are associated with OA. Specific inflammatory cytokines were shown to accelerate the development of OA by directly targeting DNA methyltransferases, and the level of DNA methylation modulated the expression of specific inflammatory cytokines in chondrocytes reversely. For histone modifications, the overexpression of HDAC4 significantly led to a release of matrix-degrading enzymes, thus, contributing to bone loss associated with OA. For miRNAs, the reduction of miR-140 played an important role in OA progression by targeting ADAMTS5 and AGGRECAN. Conversely, The overexpression of miR-365 may accelerate the development of OA by targeting MMP13 and collagen type X (Col X).

2. DNA methylation

DNA methylation is the methylation of the fifth carbon of cytosine, which is known as one of the most important epigenetic modifications. It plays a considerable role in genome stability, gene expression and individual development in both prokaryotes and eukaryotes.

DNA methylation, which occurs on gene promoters, is linked to transcriptional repression (Chae *et al.*, 2013). Although it happens mostly in CpG sites, non-CpG sites have also been observed to be methylated. However, the role of non-CpG methylation remains unclear. Conversely, gene enhancers that have undergone DNA methylation are correlated with active gene expression (Moore *et al.*, 2013). Except for DNA methylation of promoters and enhancers, DNA methylation emerges on different genomic regions with different functions. DNA methylation, which occurs on intergenic regions, represses the expression of potentially harmful genetic elements when the methylation of the CpG islands impairs the binding of transcription factors, the recruiting of repressive methyl-binding proteins and gene silencing. However, more studies are needed to determine how DNA methylation of the gene body contributes to gene regulation.

DNA methylation is accomplished by DNMTs, including these five enzymes: DNMT1, DNMT3A, DNMT3B, DNMT3L and DNMT2. DNMTs have been reported to be involved in the maintenance and *de novo* of DNA methylation (Uysal *et al.*, 2015). For example, DNMT1 mainly maintains DNA methylation patterns (Elliott *et al.*, 2015). On the other hand, DNMT3A and DNMT3B are essential for *de novo* methylation in early development (Okano *et al.*, 1999). DNMT3L cooperates with both DNMT3A and DNMT3B and enhances the initiation of DNA methylation (Cheng & Blumenthal, 2008). The fifth DNA methyltransferase, DNMT2, targets RNA methylation in mammals, rather than participating in genome methylation. This phenomenon modifies the 38th cytosine residue in the anticodon loop of certain tRNAs and enhances the stability of tRNAs (Ashapkin *et al.*, 2016). For example, DNMT2 affects polypeptide synthesis during haematopoiesis by modulating the stability and fragmentation of tRNAs (Tuorto *et al.*, 2015).

DNA methylation changes the nucleic acid structure and the gene phenotype when it occurs at a certain gene region, which makes DNA methylation a potential new biomarker in biological research (Lee *et al.*, 2016). The earliest approach that detected DNA methylation was through the quantification of total methylated cytosines in a chunk of DNA (Umer & Herceg, 2013). With development in technology, genome-wide analyses, such as next-generation sequencing (NGS) technologies, have been widely used in genome-wide and locus-specific DNA methylation analyses. Particularly, novel approaches emerge with pertinence, sensitivity and speed. For example, the global estimation of 5-methylcytosine content can be detected by high-performance capillary electrophoresis with UV-V detection, liquid chromatography with electrospray ionization mass spectrometric detection and LUminometric Methylation Assay (Berdasco *et al.*, 2009). These methods provide information about the disease process and progress and can be useful in various clinical settings and in drug screening (Umer & Herceg, 2013). Locus-specific DNA methylation analysis provides insights into early epigenetic reprogramming events and identifies rare cells with unique epigenetic signatures (Cheow *et al.*, 2015). Many methods are available, including methylation-specific PCR, MethyLight, combined bisulfite conversion restriction analysis, bisulfite (Sanger) sequencing,

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bisulfite pyrosequencing and methylation-sensitive high-resolution melting (Umer & Herceg, 2013). Although genome-wide and locus-specific analyses provide a comprehensive understanding of DNA methylation, higher coverage and accuracy are required to detect DNA methylation. Firstly, restriction landmark genomic scanning, methylation-specific arbitrarily primed PCR, methylation-sensitive representational differential analysis and amplification of intermethylated sites are widely used, with some inevitable limitations for samples with large quantities of DNA. These approaches are labour-intensive and include complex procedures. Furthermore, microarray-based (DMH, CHARM, HELP assays, MeDIP-Chip and Illumina Infinium) and sequencing-based approaches (bisulfite treatments [BS-Seq, MethylC-Seq, reduced representational bisulfite sequencing (RRBS) and methyl CpG-binding domain (MBD)-isolated genome sequencing (MIGS)]) enhance the sensitivity, simplify the experimental setup and reduce the sequence redundancy in genome-scale DNA methylation analysis (Umer & Herceg, 2013). In addition to these widely used DNA methylation detection methods, some novel approaches have subsequently emerged, which include an electrochemical detection system and a methyl-sensitive fluorescence polarization assay. The former technique targets MBD and a glucose dehydrogenase-fused zinc finger protein (Lee *et al.*, 2016). The latter technique recognizes the palindromic target sequence CCGG through restriction endonucleases, namely, MspI and HpaII (Shiratori *et al.*, 2016). The developments in DNA sequencing technologies, as well as methods to identify and map 5-hydroxymethylcytosine are expected to augment our current understanding of epigenomics.

3. Histone modifications

Eukaryotic DNA is tightly packaged within the 2–10 μm nucleus, which requires several metres of DNA to be compact (Rothbart & Strahl, 2014). These packages are mainly comprised of core nucleosome particles, which are formed of 147 DNA base pairs wrapped around an octamer of histones (two copies each of H2A, H2B, H3 and H4). The central histones modulate the function and dynamics of the chromatin by the presence of specific histone variants and PTMs (Biterge & Schneider, 2014).

On the other hand, histone variants are the key players in the shape of chromatin structure and the regulation of fundamental cellular processes, such as chromosome segregation and gene expression (Vardabasso *et al.*, 2014). Histone variants replace the canonical histones to change DNA expression timings (DNA replication independent) and mRNA characteristics (Biterge & Schneider, 2014). This phenomenon alters the stability, dynamics and accessibility of DNA (Weber & Henikoff, 2014), More importantly, histone variants play important roles in disease progression in certain cases. For example, MacroH2A is a histone variant that is overexpressed in patients with Huntington's disease and steatosis-associated hepatocellular carcinoma (Biterge & Schneider, 2014). H3.3 is the variant of H3 that replaces H3K27me3 in paediatric glioma; it is associated with reduced survival in patients with paediatric glioma (Chan *et al.*, 2013). Furthermore, subsequent studies have reported that other core histone variants, namely H2A.X, H2A.Z, CENP-A and linker histone H1 variants, are linked to biological development of diseases (González-Romero *et al.*, 2012).

For PTMs, covalent modification occurs in histones at their flexible N- or C-terminal tails, as well as globular domains. PTMs also play important roles in many biological processes, including in DNA stability and expression. With the advancement of approaches to biochemical systems, PTMs can be classified into the following categories: lysine acetylation, lysine and arginine methylation, arginine citrullination, lysine ubiquitination, phosphorylation, fatty acylation and ADP ribosylation. These processes are precisely modulated by enzymes that transfer specific chemical groups to implement different modification. Histone acetylation is catalysed by diverse enzymes, in which two converse enzymes, namely, histone acetyltransferases and deacetylases, modulate the acetylation status of histones (Bannister & Kouzarides, 2011). Additionally, a large number of histone methyltransferases catalyse the methylation on the ε-amino group of lysine residues and form the most prevalent histone methylation (Fan *et al.*, 2015). PTMs have diverse functions with enzyme involvement. For example, histone acetylation, which is the so-called opening up of chromatin, makes DNA more accessible to other protein factors (Fan *et al.*, 2015). Histone methylation level is associated with activating transcription, resulting in different chromatin states, influencing aging and aging phenotypes (McCauley & Dang, 2014; Berr *et al.*, 2016). Furthermore, cross-talk between different histone modifications, which may constitute a histone code, have been observed, which may help fine tune overall control (Bannister & Kouzarides, 2011).

Thousands of experiments have provided enormous data repositories in terms of the genome-wide binding pattern of modified histones by ChIP-seq (Rivera & Ren, 2013). Although ChIP-seq is the gold standard for mapping PTMs, limited resolution, dependence on antibodies and the need for large amounts of starting material have limited its application. To break the limitations, researchers have proposed a ChIP-exo technique to provide a single bp accuracy, in which an exonuclease precisely trims the ChIP DNA of the cross-linking site into a small fragment (Rhee & Pugh, 2011). Furthermore, a nano-ChIP-seq protocol, which is combined with a high-sensitivity small-scale ChIP assay and a tailored procedure, generates high-throughput sequencing libraries from scarce amounts of ChIP DNA (Adli & Bernstein, 2011). This nano-ChIP-seq does not only decrease the need for starting materials, but it also makes the entire procedure faster. Additionally, many novel approaches have emerged with comprehensiveness, high-resolution and short setup features, including ChIP-bisulfite-sequencing (ChIP-BS-seq) (Brinkman *et al.*, 2012), bisulfite sequencing of chromatin immunoprecipitated DNA (BisChIP-seq) (Challen *et al.*, 2014) and other high-resolution mass spectrometry assays (Lin & Garcia, 2012).

4. NcRNAs

Previously, mRNA function had been mainly considered to provide protein-coding information only (Kumari & Sampath, 2015). With the development of deep sequencing and transcriptome analysis, only a tiny portion of the biological genome corresponds to protein-coding sequences; in addition, most genomic loci produce large transcripts rather than proteins, which are defined as ncRNAs (Perez *et al.*, 2013). Although these ncRNAs had been regarded as junk RNAs for a long time, they have now been identified to have a role in many physiological processes, including the maintenance of self-renewal, direction of cell lineage (Guan *et al.*, 2013) and expression of hundreds of genes (Varela *et al.*, 2013).

NcRNAs are classified into the following two categories based on their length: small and long ncRNAs (lncRNAs) (Hirose *et al.*, 2014). Small ncRNAs include microRNAs (miRNAs), small interfering RNAs (siRNAs) and Piwi-interacting RNAs (piRNAs). MiRNAs are one of the most widely studied small ncRNAs. A total of 52% of miRNAs are located in human intergenic regions,

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40% lie within the intrinsic regions of genes and the final 8% are exonic (Hsu *et al.*, 2006). Mature miRNAs have similar physiological roles to other RNAs, such as transcriptional activation and inhibition, epigenetic repression and controlled degradation rates (Mott & Mohr, 2015). Furthermore, by targeting the complementary sequence of miRNAs, they modulate over 60% of the translation of protein-coding genes (Mott & Mohr, 2015). Not only in the modulation of gene translation, miRNA also present intricate functions in different tissues (Guo *et al.*, 2010). For example, miR-125b up-regulates and displays oncogenic potential in colon cancer and haematopoietic tumours. Conversely, miR-125b down-regulation contributes to malignant transformation in mammary tumours and hepatocellular carcinoma (Banzhaf-Strathmann & Edbauer, 2014). siRNAs are usually paired with mRNAs with perfect complementarity and are directed in their endonucleolytic cleavage and destruction (Hirose *et al.*, 2014). On the other hand, piRNAs play a pivotal role in germline genome protection from transposons (Hirose *et al.*, 2014). lncRNAs are the second class of ncRNAs, which regulate the RNA and protein content of a cell on the transcriptional and post-transcriptional level (Melissari & Grote, 2016). These molecular mechanisms include dosage compensation, chromatin regulation, genomic imprinting and nuclear organization (Yan *et al.*, 2016). In addition, a small number of lncRNAs modulate the recruitment of RNA polymerase II and induce chromatin remodelling in global or local gene expression in *trans* or *cis* (Shi *et al.*, 2013). Therefore, lncRNAs are present in pathological conditions such as cancer and cardiovascular disease, which makes lncRNAs a novel potential biomarker for clinical usage (Schmitz *et al.*, 2016).

Moreover, a small part of ncRNAs codes for proteins including early nodulin 40 and MtHAP2-1 in plants. At the same time, some mRNAs may modulate post-transcriptional level gene expression, which is independent of their encoded proteins. These two kinds of RNAs are called cncRNAs (Kumari & Sampath, 2015), which have shed new light on the understanding of ncRNAs. A total of 300 alternatively spliced bifunctional RNAs may be observed in the human genome (Ulveling *et al.*, 2011). The exploration of protein-coding and noncoding functions for cncRNA loci may be highlighted in future studies (Kumari & Sampath, 2015).

NcRNA realization has been expanded with the advent of NGS (Ilott & Ponting, 2013). In particular, lncRNAs have been identified by RNA-seq technology (Li *et al.*, 2014). Moreover, RNA-seq data integration detects active transcription to further understand the reported lncRNAs (Chen *et al.*, 2016 *a*). These findings may help to discover novel lncRNAs and may improve the characterization of identified lncRNAs.

5. Bone and cartilage diseases associated with epigenetics

Mesenchymal stromal cells (MSCs) are typical adult progenitor cells, which possess multidifferentiation capacity *in vitro* and *in vivo* (Takahashi *et al.*, 2015). MSCs are one of the promising candidate cells for skeletal regeneration due to their convenient isolation and immune-modulatory capability. The epigenetic changes of MSCs are essential in the differentiation of MSCs into bone and cartilage. These processes include DNA methylation, histone modifications and miRNAs.

(i) Osteogenic and chondrocyte differentiation of MSCs by epigenetics

A coordinated cascade of transcription factors and epigenetic modifications drive gene transcription, and cause specific cell fate, and this is indispensable for terminal differentiation of multipotent stem cells (Meyer *et al.*, 2016). For example, the osteogenic and adipogenic differentiation of MSCs are partly regulated by transcription factors, including peroxisome proliferator-activated receptor-γ (PPAR-γ) and Runt-related transcription factor 2 (Runx2) (James, 2013), and various epigenetic alterations (Glemžaitė & Navakauskienė, 2016). These processes are accompanied by a loss of the Brachyury gene. Brachyury inactivation is associated with the methylation of its promoter, which represses stem cell-associated genes (Dansranjavin *et al.*, 2009). In addition to the Brachyury gene, the cytosine methylation accumulation at the endogenous thyroid hormone receptor interactor 10 (Trip10) promoter reduces Trip10 expression, which accelerates osteogenic differentiation (Hsiao *et al.*, 2010). Osteocalcin (OC) is a non-collagenous bone matrix protein, which is partly regulated by DNA methylation and histone modifications. In MSC osteogenic differentiation *in vitro*, OC expression is usually increased with the decrease in DNA methylation. Additionally, OC is activated by the accumulation of H3 and H4 acetylation. Conversely, OC is inhibited by the decrease in H3 and H4 acetylation in the OC promoter and coding regions. This event occurs in the proliferative period of osteogenic differentiation (Takahashi *et al.*, 2015). For the H3 sub-family, the acetylation levels of H3K9Ac and H3K9me2 are associated with the activation and silencing genes, respectively, which are modulated by vitamin D receptors at specific gene promoters (Tan *et al.*, 2009). For the epigenetic modulation of miRNAs, the function of miRNA has been reported in the inhibition of mRNA translation and degradation (Wei *et al.*, 2012; Takahashi *et al.*, 2015). For example, miR-206 overexpression inhibits the differentiation of osteoblasts by the target of connexin 43 (Inose *et al.*, 2009). MiR-34 inhibits mouse osteoblast proliferation and differentiation by targeting SATB2, which is a nuclear matrix protein involved in osteoblast differentiation (Wei *et al.*, 2012). MiR-27a and miR-489 down-regulate the osteoblast differentiation by targeting PEX7; on the other hand, miR-148b up-regulates the differentiation (Schoolmeesters *et al.*, 2009).

The epigenetic mechanisms of chondrocyte differentiation of MSCs are scarce. In cartilage formation, DNA methylation levels of CpG-rich promoters of chondrocyte-specific genes are mostly at a low level (Ezura *et al.*, 2009). SOX9 is one of the master chondrogenic transcription factors, which is involved in chondrocyte differentiation and cartilage formation (Shi *et al.*, 2015). Age-dependent SOX9 expression is regulated by epigenetic mechanisms (Mak *et al.*, 2015). Epigenetic studies revealed that DNA methylation levels increased at specific CpG islands of the Sox9 gene in mice articular chondrocytes (ACs) at 6 and 12 months old (Zhang *et al.*, 2016). Using the supplementation of 5-azacytidine, which is an inhibitor of DNA methylation, the DNA methylation level is reduced in the Sox9 promoter region, which elevates the level of Sox9 expression in ACs. This finding suggests that expression is associated with DNA methylation (Zhang *et al.*, 2016). Moreover, in DNA methylation, many kinds of histone modifications are involved in chondrocyte differentiation. For example, several transcription factors and coactivators, such as Scleraxis/E47 and p300, cooperatively modulate Sox9-dependent transcription through p300-mediated histone acetylation (Furumatsu & Asahara, 2010). And a novel Runx2 enhancer is localized in the primary osteoblasts and is characterized by the presence of the histone variant H2A.Z, which contains sufficient elements to direct Runx2 expression to osteoblasts (Kawane *et al.*, 2014). For the epigenetic modulation of ncRNAs, miR-101 and HOTTIP are up-regulated by targeting

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DNMT3B, which alters integrin-α1 methylation, which is a key protein of chondrocyte ossification (Kim *et al.*, 2013*a*).

(ii) Skeletal diseases associated with epigenetics

Epigenetic alterations are associated with the aetiology and pathology of bone and cartilage diseases. Osteoarthritis (OA) is a chronic multifactorial disease associated with specific genes. Specific transcription factors (TFs), cartilage-degrading enzymes, pro- and anti-inflammatory cytokines and extracellular matrix proteins contribute to OA development (Zhang & Wang, 2015). More importantly, advanced studies highlight the role of epigenetics in OA, including DNA methylation, histone modifications and miRNAs (Im & Choi, 2013) (Figure 1).

Differences in the methylome between normal and OA knee articular cartilage have been identified, including 929 differentially methylated sites. Most of the methylated sites in OA (69%) are hypomethylated and enriched among gene enhancers in OA cartilage tissue (Jeffries *et al.*, 2014). For epigenetic changes of TFs, some OA related TFs (ATOH8, MAFF, NCOR2, TBX4, ZBTB16 and ZHX2) are significantly hypermethylated and down-regulated in OA cartilage. These results indicate that the DNA methylation level negatively affects the chondrocyte transcriptome and function in OA pathogenesis (Alvarez-Garcia *et al.*, 2016). For cartilage-degrading enzymes, the demethylation of MMP3, MMP9, MMP13 and ADAMTS4 promoters increase their gene expression in OA cartilage (Reynard, 2016). Additionally, inflammatory mediators modulate DNMTs, DNMT3B and DNMT3A, which target DNA methylation directly (Shen *et al.*, 2017). IL-1β is a proinflammatory cytokine guiding the function of immune and proinflammatory cells and decreases the expression of DNMT3B and DNMT3A through NF-κB signalling (Shen *et al.*, 2017). Another inflammatory chemokine, IL-8, shows increased demethylation in the promoter region in OA chondrocytes, which is correlated with enhanced IL-8 expression (Takahashi *et al.*, 2015). Furthermore, the DNA methylation level modulates the expression of specific inflammatory cytokines in chondrocytes reversely. Significant demethylation of CpG sites in the inflammatory chemokine IL-8 promoter increases the expression of IL-8 in the OA chondrocytes (Takahashi *et al.*, 2015).

With increasing data of genome-wide profiling of DNA methylation in OA, the alterations of DNA methylation affect more gene expression in OA. The demethylation of specific promoter and enhancer sites in GDF5, iNOS and SOST increase their gene expression in OA cartilage and isolated chondrocytes. Conversely, the increased methylation of SOX9, DIO2 and COL9 promoters caused reduced expression in OA cartilage (Iliopoulos *et al.*, 2007; Verma and Dalal, 2011; Kim *et al.*, 2013*b*; Gupta *et al.*, 2014*b*; Papathanasiou *et al.*, 2015).

For histone modifications, HDAC4 is one of the key regulators in OA development (Lu *et al.*, 2014). The HDAC4 expression level has a statistically negative correlation with OA severity (Lu *et al.*, 2014). HDAC4 expression reduction significantly leads to a repression of the following matrix-degrading enzymes: MMP1, MMP3, MMP13, ADAMTS4 and ADAMTS5 (Lu *et al.*, 2014). HDAC4 overexpression does not only decrease the expression of IL-1β, Cox2 and iNOS, but it also partially blocks IL-1β mediated effects in catabolic events in human OA chondrocytes (Thompson *et al.*, 2015). However, HDAC4 inhibition has suppressed the expression of inhibited genes (Young *et al.*, 2005). For example, trichostatin A (TSA) and sodium butyrate HDAC inhibitors inhibit cartilage degradation by blocking key MMPs (MMP-1 and MMP-13) and aggrecan-degrading enzymes (ADAMTS4 and ADAMTS5). In addition, HDAC4 inhibitors, including vorinostat, TSA and sodium butyrate, are promising in the potential treatment of OA (Chen *et al.*, 2010; Makki & Haqqi, 2016).

For miRNAs, while normal human articular cartilage expresses miR-140 in chondrocytes, its expression is significantly reduced in OA progression (Zhang *et al.*, 2012). The transfection of miR-140 in chondrocytes down-regulates IL-1β-induced ADAMTS5 expression and alleviates IL-1β-dependent repression of AGGRECAN gene expression (Miyaki *et al.*, 2009). These findings indicate that decreased expression of miR-140 caused the abnormal gene expression profile seen in OA. MiR-365 overexpression in chondrocytes increases the expression of the matrix-degrading enzyme MMP13 and collagen type X, which may accelerate OA development (Yang *et al.*, 2016). Moreover, the interplay of histone modifications and miRNAs have been identified in the aetiology of OA. Subsequently, evidence shows that miR-365 directly targeted HDAC4, which led to HDAC4 expression down-regulation (Yang *et al.*, 2016), which accelerates the development of OA. Furthermore, a study of miR-381 has demonstrated that miR-381 overexpression promotes MMP13 and Runx2 expression by the inhibition of HDAC4, and miR-381 inhibitors increased HDAC4 expression and decreased Runx2 expression (Chen *et al.*, 2016*b*). This finding provides us with a novel therapeutic method for the treatment of OA. This result may inspire researchers to study inhibitors that block the interaction between histone modifications and miRNAs in order to alleviate the severity of OA.

6. Future direction

This review summarizes the current advances in the study of epigenetics and discusses the epigenetic findings pertaining to the skeletal system. In addition to significant technological developments, emerging epigenetics research may provide new understanding of single genes, specific chromosome regions and the whole genome (Adli & Bernstein, 2011). Moreover, modifications can be induced or inhibited by drugs to alleviate or cure disease; this finding needs further investigation. However, many challenges remain unsolved to fully use this epigenetic information. Thus; these challenges will require further research.

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