

The Epigenomic Landscape in Osteoarthritis

Tommie C. Simon¹ · Matlock A. Jeffries^{1,2}

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Abstract

Purpose of Review Epigenomics has emerged as a key player in our rapidly evolving understanding of osteoarthritis. Historical studies implicated epigenetic alterations, particularly DNA methylation, in OA pathogenesis; however, recent technological advances have resulted in numerous epigenome-wide studies examining in detail epigenetic modifications in OA. The purpose of this article is to introduce basic concepts in epigenetics and their recent applications to the study of osteoarthritis development and progression.

Recent Findings Epigenetics describes three major phenomena: DNA modification via methylation, histone sidechain modifications, and short noncoding RNA sequences which work in concert to regulate gene transcription in a heritable fashion. Cartilage has been the most widely studied tissue in OA, and differential methylation of genes involved in inflammation, cell cycle, TGF β , and HOX genes have been confirmed several times. Bone studies suggest similar findings, and the intriguing possibility of epigenetic changes in subchondral bone during many OA processes. Multiple studies have demonstrated the involvement of certain noncoding RNAs, particularly miR-140, in OA development via modulation of key catabolic factors.

Summary Although much work has been done, much is still unknown. Future epigenomic studies will no doubt continue to widen our understanding of extraarticular tissues and OA pathogenesis, and studies in animal models may offer glimpses into epigenome alterations in the earliest stages of OA.

Keywords Osteoarthritis · Epigenetics · DNA methylation · Review

Introduction

Osteoarthritis (OA) is a chronic, debilitating musculoskeletal disease characterized by progressive loss of joint function leading to significant pain, mobility loss, and functional limitation. OA is the leading cause of chronic disability in the USA, affecting 40% of adults over the age of 70 [1], and is the most rapidly growing major health condition worldwide [2]. A variety of factors including age, gender, genetics, mechanical trauma, and local inflammatory processes all contribute to the development and progression of osteoarthritis [3]. The osteoarthritic joint is characterized by cartilage degradation without an appropriate healing response, sclerosis of underlying subchondral bone, and synovial inflammation [4]. Although many genetic studies have been performed, a strong genetic component has yet to be identified, particularly for knee and hip OA, with only a handful of susceptibility genes confirmed, all with relatively mild disease contribution (hazard ratios of <2) [5]. Several studies have suggested that age-related changes to epigenetic processes may be a potential cause of late-onset human diseases such as osteoarthritis [2], and recent reports have demonstrated an association between epigenetic changes and the development and progression of knee and hip OA. Understanding these epigenetic

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✉ Matlock A. Jeffries
matlock-jeffries@ouhsc.edu

¹ Division of Rheumatology, Immunology, and Allergy, University of Oklahoma Health Sciences Center, Oklahoma City, OK, USA

² Oklahoma Medical Research Foundation, Arthritis and Clinical Immunology Program, 825 NE 13th St., Laboratory MC400, Oklahoma City, OK, USA

contributions may not only provide insights into disease pathogenesis, but also potentially facilitate novel biomarkers and disease therapeutics. The goal of this review is to present highlights from published literature related to the epigenetics of osteoarthritis. We will introduce each of the three major epigenetic control mechanisms and explore the most recent reports of how changes in each of these have been implicated in OA.

Epigenetics

To achieve appropriate homeostasis in changing environments, cells must be able to adapt while maintaining their ability to express a certain phenotype with a shared codex of underlying genomic sequence [1]. Epigenetics, defined as heritable changes in gene expression that occur in the absence of mutations of underlying genomic DNA, is a common mechanism whereby organisms alter transcriptomic patterns in response to both external and internal environmental cues. Epigenetic regulation occurs by a few common mechanisms, including cytosine genomic DNA methylation, histone modifications or variation, and noncoding RNA. We will consider each of these separately (Table 1).

DNA Methylation

The most widely studied epigenetic control mechanism is DNA methylation. The addition of a methyl group to the 5' position of cytosine most commonly occurs in CpG dinucleotides, mediated by DNA methyltransferases. Active demethylation of cytosine residues is a multistep process involving oxidation to a hydroxymethyl intermediate, mediated in part by ten-eleven-ten (TET) DNA glycosylases [6]. Methylation

of certain regulatory regions, most commonly within the 5' untranslated (5'UTR) regions upstream of a target gene, is of particular relevance to transcription. Additional regions of epigenetic control include CpG-enriched regions known as CpG islands and shores. Finally, recent studies have also implicated epigenetic control of gene transcription from distant enhancer elements [7].

DNA methylation studies can be broadly classified into two categories: candidate gene studies, which examine the methylation state of CpGs within or around a particular gene or set of genes in detail, and genome-wide DNA methylation surveys, which have traditionally utilized microarray techniques to query DNA methylation status of many CpG sites (a few per gene) throughout the genome. As DNA methylation patterns, along with other epigenetic marks, vary from tissue to tissue, it would follow that separate analyses of each tissue of interest in a given disease would be crucial to assembling a complete picture of the epigenetic landscape. Although most studies in OA have focused on articular cartilage, there have been a few descriptions of other tissues, as will be discussed below.

DNA Methylation in OA, Candidate Gene Methylation Studies

The role of DNA methylation in the pathogenesis of osteoarthritis was suggested as far back in the literature as 1985, when Fernández et al. proposed a role for DNA methylation in the regulation of types I and II collagen in chick embryos [8]. The first specific examination of DNA methylation in OA came from Roach and colleagues in 2005 [9], who used a candidate gene approach to examine the DNA methylation state of a few key catabolic matrix degradation enzyme genes, including *ADAMTS4*, *MMP3*, *MMP9*, and *MMP13* [9]. They described demethylation of the proximal promoter regions of these

Table 1 Overview of epigenetic changes in osteoarthritis

	DNA methylation changes	Histone sidechain modifications	microRNAs
Articular cartilage	Globally: hypomethylated Matrix degradation genes: <i>ADAMTS4</i> , <i>MMP3</i> , <i>MMP9</i> , <i>MMP13</i> Inflammatory genes: <i>IL1B</i> , <i>IL6</i> , <i>IL8</i> Signaling/transcription factors: <i>DIO2</i> , <i>HOX</i> family, <i>NFAT1</i> , <i>ROR2</i> , <i>RUNX1</i> , <i>RUNX2</i> , <i>SOX9</i> , <i>TGF-beta</i> , <i>VIT</i> , <i>Wnt</i> pathway	Globally: increased HDAC1, HDAC2 Collagen genes: <i>COL2A1</i> suppressed by HDAC1, HDAC2 signaling/transcription factors: <i>SOX9</i> , Sirtuin 1, <i>Nfat1</i>	miR-140: decreased expression, multiple known OA-related gene targets miR-145: increased expression, targets <i>SOX9</i> , <i>MMP13</i> miR-27a, miR-145: reduced expression, target <i>MMP13</i> miR-483-5p: increased expression, intraarticular antagonist delays murine OA large surveys' pathway analysis: TGF-beta, Wnt, Erb, mTOR targeted
Subchondral bone	Globally: hypomethylated signaling: <i>Wnt</i> pathway, <i>HOX</i> family, <i>TGF-beta</i>	No studies yet published	miR-9, miR-98, miR-146 30 differentially expressed in one study, pathway analysis: inflammatory processes targeted

genes associated with increased expression in hip cartilage chondrocytes obtained from late-stage hip OA patients compared to control chondrocytes from neck of femur fracture (#NOF) patients. In 2007, Iliopoulos et al. demonstrated differential methylation of leptin in eroded hip and knee articular cartilage from end-stage OA patients compared to histologically normal cartilage from within the same joint [10]. The expression level of leptin, a key adipokine implicated in obesity, was inversely correlated with the methylation status of its proximal promoter. Interestingly, knockdown of leptin by transfection of short interference RNA in vitro resulted in the concomitant reduction of MMP13 gene expression in cultured primary chondrocytes.

More recent candidate gene studies have revealed the variety of mechanisms by which epigenetic changes can contribute to potential pathogenesis. One recurring theme is the modification of binding of transcription factors by epigenetic variation. In 2014, Reynard and colleagues examined in detail the methylation state of a particular location within *GDF5*, a member of the transforming growth factor-beta family [11••]. *GDF5* is regulated by DNA methylation in cartilage, and is demethylated in OA, with concomitant upregulation of gene expression. Interestingly, the 5' untranslated region (UTR) of this gene also contains a single nucleotide polymorphism (SNP), rs143383, which is a genetic susceptibility loci for knee OA. When the 5'UTR containing each allele of this SNP (C/T) was cloned into a luciferase reporter, the C allele (which created an unmethylated CpG dinucleotide) exhibited significantly greater reporter activity than did the T allele. Furthermore, forced DNA methylation reduced luciferase expression more effectively in the C allele compared to the T allele. This was due to differences in binding of the transcription factors SP1, SP3, and DEAF1. In 2013, de Andres and colleagues demonstrated that an alterations in DNA methylation within NF- κ B enhancer elements of the inducible nitric oxide synthase (iNOS) gene augment the ability of this inflammatory transcription factor to activate iNOS, which is overexpressed in OA chondrocytes [12]. Similarly, Papathanasiou et al. in 2015 described epigenetic regulation of sclerostin (*SOST*), a key target of both Wnt and BMP-Smad signaling [13]. Beyond demethylation and increased expression in knee and hip articular chondrocytes compared to #NOF controls, they also demonstrated that pharmacological demethylation of *SOST* by treatment of in vitro chondrocyte cultures with 5-Azacytidine rendered them sensitive to BMP-2 signaling activation by facilitating the binding of the Smad 1/5/8 transcription factors. Several other studies have confirmed the association of epigenetic gene alteration and transcription factor binding changes of key genes in OA, including *SOX9*, *MMP13* [14], *COL9A1*, and others [15–17].

Differential DNA methylation of inflammatory genes has also been associated with OA in human chondrocytes. For example, the 2015 study by Takahashi and colleagues

demonstrated a 38-fold higher expression of interleukin 8 (*IL8*) in hip OA patients compared to #NOF controls [18••]. This increase in transcription was accompanied by demethylation of the proximal promoter region of *IL8*, and in vitro methylation of an *IL8* promoter-reporter construct blunted the ability of the transcription factors NF- κ B, AP-1, and C/EBP to activate this gene. IL-1 β is also epigenetically regulated. In 2013, Hashimoto [14, 19] and colleagues demonstrated that methylation of CpG sites located within the proximal promoter of the *IL-1 β* gene inversely correlated with gene transcription in OA chondrocytes. Interestingly, IL-1 β can in and of itself modulate the epigenetic status of other genes. In an abstract presented at the 2012 American College of Rheumatology meeting, Akhtar and Haqqi showed that both maintenance and de novo expression levels of DNA methyltransferases DNMT1, DNMT3a, and DNMT3b were increased in cultures of OA chondrocytes following IL-1 β stimulation in vitro [20].

DNA Methylation in OA, Genome-Wide DNA Methylation Studies

Although instructive, candidate gene DNA methylation analyses fail to give a broad picture of epigenetic changes in OA tissues. Furthermore, since the particular regions studied are not necessarily standardized, comparisons among multiple studies can be difficult. Following their introduction several years ago, genome-wide DNA methylation techniques have largely supplanted candidate assays, and they possess several advantages. First, they allow for a more complete analysis of global epigenetic modifications associated with a particular cell type. Furthermore, they generally allow multiple OA data sets to be compared directly, as the specific CpG sites tested are standardized in each array. Finally, their widespread use in multiple diseases and tissue types allows for direct comparisons of analyses of tissues in other disease states, which are particularly useful as OA-free controls. The clear majority of genome-wide DNA methylation assays performed in OA to date have leveraged Illumina beadchip technology, and fall into two fundamental groups of study design: (1) comparison of eroded/end-stage OA tissue with non-eroded/early-stage tissue within the same joint and (2) comparison of end-stage OA tissue with “disease-free” cadaveric or #NOF control tissue. Comparisons have also been made between knee and hip cartilage tissue and between cultured chondrocytes derived from OA and control patients, although there is some controversy regarding the potential of ex vivo culture to alter epigenetic patterns.

The first such study was published in 2014 by Fernández-Tajes et al. [21••]. This study utilized the Illumina HumanMethylation 27 k array, which provides site-specific CpG DNA methylation information on about 27,000 CpG sites throughout the genome, covering over

half of coding genes (14,500). Their sample set consisted of knee OA patient cartilage collected at the time of total joint replacement and cadaveric knee samples. They identified 91 differentially methylated CpG sites, with the runt-related transcription factor *RUNXI* being most differentially (hypo) methylated among OA cases. Differentially methylated genes were overrepresented among inflammatory gene ontology and the mitogen-activated protein kinase (MAPK) pathways. Perhaps more importantly, though, supervised clustering of differentially methylated genes identified a subset of OA patients that was distinct from both other cases and control specimens. The methylation sites that defined this subgroup clustered strongly among extracellular matrix genes and a variety of genes related to cytokine production, inflammatory regulation, and inflammatory activation motifs. Later in 2014, our group published a genome-wide DNA methylation analysis of hip OA patients, comparing eroded with intact cartilage from within the same joint of 24 hip OA patients using the broader Illumina HumanMethylation 450 k platform [22••]. This newer technology allows for quantitation of around 470,000 CpG sites throughout the genome, and covers nearly all coding genes that contain an average of 17 CpG sites per gene. We identified 550 differentially methylated CpG sites among hip OA patients, again with *RUNXI* being the most differentially methylated gene, and again identifying several inflammatory pathways. Interestingly, we did not encounter a distinct inflammatory cluster of patients upon supervised clustering, although this may have been related to differences in our definition of “differential methylation.” There have been several additional studies published using this same 450 k pathway and examining both hip and knee OA [22••, 23–27]. Of note, Reynard et al. and den Hollander et al. have examined in detail the similarities between varying studies, stratified by study design, and have highlighted many consistent findings [28••, 29]. First, the cartilage DNA methylome is distinct when comparing knee to hip samples regardless of the presence or absence of OA. Secondly, differential methylation of genes related to development and differentiation is a feature of both knee and hip OA cartilage compared to either intact cartilage from the same joint or #NOF control cartilage. Finally, one can identify a cluster of “inflammatory” samples in both hip and knee OA. These are characterized by, among others, differential methylation of tumor necrosis factor (TNF) genes, IL1 and IL6, and a variety of matrix-degrading enzymes including MMPs and ADAMTS5. Consistent among nearly all studies have been a strong transforming growth factor beta (TGF β) and fibroblast growth factor (FGF) signatures. Also important to note is a strong concordance among both the CpG sites identified as differentially methylated in similar studies performed by different groups in different

countries and the magnitude of changes of DNA methylation patterns in these groups (unpublished, comparison by L. Reynard of Jeffries et al., den Hollander et al., and Reynard et al. hip and knee OA cartilage methylome datasets).

Two groups have gone on to examine the impact of epigenetic variation on genetic susceptibility to OA and gene transcription patterns from a genome-wide perspective. The first study, published by den Hollander et al. in 2015 [30], identified 31 genes for which changes in DNA methylation were affected by local genetic variation, and 26 genes for which changes in gene expression were affected by both local epigenetic patterns and genetic variation. Three were of note. *VIT*, which encodes the protein vitrin, is involved in extracellular integrin signaling and contains a von Willebrand factor domain [31]. *ROR2*, receptor tyrosine kinase-like orphan receptor 2, is involved in osteoprotegerin/RANKL signaling [32], and *WLS*, Wnt ligand secretion mediator, is involved in endochondral ossification [33]. A second study, published by Rushton et al. in 2015, performed a methylation quantitative trait loci (meQTL) analysis in knee and hip OA cartilage samples, focusing on 16 previously identified European OA genetic susceptibility loci [34]. They identified four meQTLs containing nine CpG sites, wherein genetic variation was associated with alteration in local DNA methylation patterns. Interestingly, the effects of these variations on gene transcription patterns were seen both in diseased and disease-free tissue, and several of these had been reported previously in other non-cartilage joint tissues. Similar effects have also been noted in the iodothyronine deiodinase 2 gene (*DIO2*), where DNA methylation levels mediate the OA susceptibility of SNP locus *rs225014* [35]. Interestingly, *Dio2* knockout mice are relatively protected from damage in a forced-exercise OA model [36].

To date, there have been two published analyses of genome-wide DNA methylation patterns in non-cartilage OA tissues. The first, published by our group in 2015, characterized the methylome of subchondral bone underlying eroded and intact cartilage sections of end-stage OA hip patient joints [37••]. We identified roughly an order of magnitude more differentially methylated CpG sites in subchondral bone than the matched overlying cartilage. Interestingly, 44% of the genes differentially methylated in cartilage were also differentially methylated in subchondral bone, the vast majority having similar methylation patterns. Thirty-two proposed OA susceptibility genes were differentially methylated, suggesting epigenetic inactivation as an alternative to genetic mutation in gene disruption. Gene ontology analysis revealed a strong TGF β signature and overrepresentation of genes involved in various cytokine pathways. A second study conducted by Zhang et al. later in 2016 studied knee OA specimens, dividing subchondral bone from three distinct regions of the tibial plateau, corresponding to overlying cartilage

scored as displaying early, intermediate, and late disease [38]. Interestingly, they were able to determine that DNA methylation changes occurring both in subchondral bone and cartilage appear first in the subchondral bone compartment. Gene ontology analysis demonstrated overrepresentation of genes involved in both stem cell development and differentiation (Oct4 in pluripotency) and a cluster of factors from the homeobox family (HOX). The HOX family of genes, in particular, is shared among many DNA methylation analyses of OA cartilage [29]. Taken together, the authors suggest that epigenetic aberrations in OA may begin in subchondral bone and later spread to overlying cartilage. This hypothesis is indeed intriguing, but will need confirmation in future studies.

Histone Modification

Histones are highly conserved proteins that function to stabilize, organize, and condense DNA within the limited confines of the cell nucleus. Composed of repeating octamers, they contain dimers of each of the four core histone proteins (H2A, H2B, H3, and H4) and wrap genomic DNA around their outer surfaces. Typically, histone tails are positively charged due to amine groups present on lysine and arginine amino acids; this positive charge facilitates binding of negatively charged phosphate groups on the nucleotide backbone of DNA. They coordinate their packaging role in concert with several other DNA binding proteins and alter the local chromatin geography resulting in facilitation of transcription or repression. The ability of histones to interact with requisite additional DNA packaging proteins is controlled by post-transcriptional modifications of the amino acid sidechains attached to various histone amino acid residues themselves, including acetylation, methylation, ubiquitination, sumoylation, deimination, and poly (ADP)-ribosylation, among others [39]. The most well-known (and widely studied) histone modifications are acetylation and methylation. Acetylation neutralizes the positive charges on histones by oxidizing amine residues to amides and decreases the ability of histones to bind to DNA, thus preventing chromatin condensation and allowing access of the gene transcription machinery to the underlying code. Deacetylation presents a positively charged histone tail, encouraging high affinity binding between the DNA backbone and histones, resulting in chromatin condensation, and thus preventing transcription. Like DNA, histones may also undergo methylation via histone methyltransferases and demethylation via histone demethylases, which both have the ability to alter gene transcription. Methylation of histones results in different transcriptional outcomes depending upon the particular residue involved; for example, the addition of three methyl groups to lysine 27 of histone 3 (H3K27) is generally repressive, whereas methylation of lysine 4 in histone 3 (H3K4) is generally activating [40].

Histone Modification in OA

Few studies have sought to determine specific histone sidechain patterns in particular genomic regions associated with OA. There have been, however, many studies examining the effects of more general alterations of global histone sidechain patterns. Histone deacetylase-1 (HDAC1) and HDAC2 levels are elevated in both chondrocytes and the synovium from OA patients compared to controls [41, 42]. These studies also demonstrated that a novel carboxy-terminal domain of HDAC1 and HDAC2 work in concert with the transcriptional repressor Snail 1 to repress the expression of the collagen gene *COL2A1*. Several studies have examined the effects of pharmacological inhibition of HDAC activity (HDACi) in OA model systems.

The broad-spectrum HDACi trichostatin A, for example, prevents IL1 β -induced matrix metalloproteinase upregulation in human chondrocyte cultures in vitro [43]. In 2013, Culley and colleagues confirmed this and further found that inhibition of Class I HDACs via two other agents (valproic acid and MS275) was sufficient to prevent IL1 β -induced upregulation of the matrix metalloproteinase, MMP-13 [44]. Trichostatin A (TSA) also has a potential role in the treatment and/or prevention of OA. A study by Nasu et al. demonstrated the efficacy of systemic TSA in reducing the expression of various matrix metalloproteinases, as well as measures of overall cartilage destruction in a collagen antibody-injection OA mouse model [45]. Culley et al. confirmed this in the post-traumatic disruption of the medial meniscus (DMM) mouse model, where systemic HDACi treatment (with TSA) reduced OA histopathologic scores [44]. The broad-spectrum HDACi vorinostat inhibits IL1 β -induced upregulation of various matrix metalloproteinases as well as nitric oxide production in chondrocytes in vitro, selectively acting through p38 and ERK1/2 inhibition [46]. Similar results have been obtained through selective inhibition of HDAC7 in vitro [47]. Interestingly, the latter study also demonstrated significant upregulation of HDAC7 in cartilage from knee OA patients compared to normal controls.

SOX9, a master transcription factor regulating chondrogenesis, is itself controlled both by DNA methylation and by histone modification. Kim et al. in 2013 demonstrated in advanced hip OA cartilage a variety of changes in the *SOX9* promoter, including both increases in histone H3K9 and H3K27 methylation and decreases in H3K9, 15, 18, 23, and 27 acetylation [15]. Sirtuins are a class of NAD-dependent histone deacetylases, which have been implicated in OA pathology. Along with SOX9, Sirtuin 1 (*Sirt1*) regulates the expression of the collagen gene *COL2A1* in human chondrocytes [48, 49]. Similarly, another histone-modifying enzyme, the methyltransferase Set7/9, controls *COL2A1* expression by increasing trimethylated lysine 4 (H3K4) [50]. Interestingly, increases in the nuclear factor of activated T-cells gene (*Nfat1*), itself also epigenetically controlled both

by DNA methylation and histone acetylation, cause further epigenetic modifications in articular chondrocytes by globally increasing histone 3 lysine 9 dimethylation (H3K9me2) [51]. Furthermore, the expression of *Nfat1* in wild-type mice increases with age, and in *Nfat1* knockout mice, OA-like changes do not develop until later in adulthood. This group went on to show that expression of *Nfat1* in embryonic articular chondrocytes is correlated with histone 3 lysine 4 dimethylation (H3K4me2) at the *Nfat1* promoter. H3K9me2 has also been evaluated in the context of the promoter of the gene encoding microsomal prostaglandin E synthase 1 (*mPGES-1*). Elevation of lysine-specific histone demethylase (LSD1) contributes to both histone demethylation and gene induction at this promoter following IL1 β stimulation in human OA cartilage samples [52]. As mentioned earlier, the field is lacking large studies of genome-wide histone modification patterns in OA, particularly integrated studies of DNA methylation patterns and gene expression data to identify new targets of epigenetic dysregulation; this should be a focus of future research efforts.

Noncoding RNAs

The field of noncoding RNA research is still relatively young, with new categories of RNAs being described with regularity. The first and most widely studied of these RNA subtypes are microRNAs (miRNA). Composed of small, non-protein-coding nuclear-DNA-encoded RNAs of around 22 nucleotides in length, miRNAs modulate gene expression by three principal mechanisms. First, certain types bind to complementary sites on the 3' tails of messenger RNA transcripts and target them for degradation by the RNA-induced silencing complex (RISC), composed of microRNAs bound to target mRNA and various cleavage proteins, notably including members of the Argonaut family [53]. A second mechanism involves the binding and destabilization of mRNAs without cleavage, and a third is the reduction in the efficiency of ribosomal translation [53]. Several novel functions of miRNAs have been proposed, and several new classes of larger noncoding RNA with important transcriptional significance have been described recently, including long noncoding RNAs (lncRNA), Piwi-interacting RNAs (piRNAs), small nucleolar RNAs (snoRNAs), and others [54, 55].

Noncoding RNAs and OA

Like DNA methylation, there has been significant interest in miRNAs and OA, not only in disease pathogenesis but also for potential therapeutic and diagnostic applications. Like DNA methylation studies, miRNAs have been evaluated both by targeted (candidate) studies and by expression analyses of hundreds of miRNAs simultaneously in a target tissue on large arrays. The first such assay was

performed by Iliopoulos et al. [56]. They examined the expression pattern of 365 microRNAs using a TaqMan array in a group of 33 cartilage samples from patients with knee and hip OA compared to normal human cartilage from #NOF patients. They identified an OA signature consisting of 16 miRNAs that were differentially expressed; these were validated by real-time PCR and Northern blot analysis. Interestingly, beyond the presence or absence of OA, they found that miRNA expression correlated with patient body mass index (BMI), itself the second most impactful OA risk factor after age. In addition, they performed a protein quantitation analysis and identified miRNA-gene target pairs using these data, identifying several important relationships (including miR-140:*ADAMTS5* and miR-509:*SOX9*). Finally, they identified miR-22 as a regulator of *PPARA* and *BMP7*, which, in turn, in chondrocytes, regulate gene expression of *IL1 β* and *MMP13*, both key promoters of inflammation and cartilage destruction, as described earlier.

Subsequently, Jones et al. approached this topic a bit more widely by examining both OA cartilage and subchondral bone using real-time RT-PCR, rather than an array technique [57••]. They identified 17 miRNAs, which were differentially expressed by more than fourfold comparing OA and normal cartilage; interestingly, they found more (30) miRNAs differentially expressed in subchondral bone. miR-9, miR-98, and miR-146 were shared between the two tissue types, and pathway analysis suggested that these were involved in inflammatory processes; particularly in IL1 β -induced tumor necrosis factor alpha (TNF α) production. Recently, Diaz-Prado and colleagues performed a broad miRNA analysis of cartilage samples from hip OA patients and healthy donors using an Agilent microarray, which tests 723 microRNAs [58]. It is important to note that, in this case, samples were cultured *ex vivo* as micropellets before miRNA quantitation. Following an extensive statistical analysis, they identified 7 mRNAs which were significantly differentially expressed with a fold-change cutoff of >1.5, including miR-483-5p, miR-149-3p, miR-582-3p, miR-1227, miR-634, miR-576-5p, and miR-641. Although there was no overlap with the previous studies' findings, ontological analysis revealed overrepresentation of the TGF β , Wnt, Erb, and mammalian target of rapamycin (mTOR) signaling, previously described in OA. In a quite interesting study, in 2016, Li et al. examined the extracellular miRNA signature in synovial fluid of early-stage vs. late-stage knee OA patients (defined radiographically) using a panel of 752 miRNAs [59••]. They identified and validated a set of 14 differentially expressed miRNAs, including three expressed only in late-stage OA, raising the possibility of future miRNA-based diagnostics.

Although these large-scale projects have come to some discordant results, there are a few miRNAs that are consistently associated with OA pathology. miR-140, a

conserved miRNA that is important for both chondrogenesis and osteogenesis, is generally found as highly expressed in normal cartilage [60–63]. Furthermore, decreased expression of miR-140 has been reported in OA chondrocytes [64]. A miR-140 knockout mouse developed by Miyaki and colleagues manifested a mildly hypoplastic skeletal phenotype and grossly normal cartilage when young; however, mice developed OA-like changes as they aged [65]. This age-related OA pathology was associated with upregulation of *Adams5*, which, the authors showed, was directly regulated by miR-140. Further confirming its importance, miR-140 knockout mice exhibited accelerated post-traumatic OA and transgenic mice overexpressing miR-140 were protected from IL-1 β -induced arthritis. In addition, another gene overexpressed in OA chondrocytes, insulin-like growth factor binding protein 5 (*IGFBP-5*), is a direct target of miR-140 [64]. Finally, a recent study confirmed that forced overexpression of miR-140 in vitro in human chondrocytes inhibits the upregulation of inflammatory genes and stimulates chondrogenesis [66].

Several other miRNAs specifically linked to OA are worth consideration. miR-145 has been identified as a direct inhibitor of the key chondrogenic regulator SOX9 in human chondrocytes, resulting subsequently in decreased expression of *COL2A1*, and it is associated with increased expression of *MMP13* [67]. Additionally, miR-193b and miR-199a also downregulates SOX9 [68]. This same group also demonstrated that these two miRNAs are downregulated with aging. miR-27a is decreased in OA chondrocytes compared to #NOF controls; furthermore, adiposity contributes to downregulation of this miRNA [64, 69]. Like miR-22 and miR-145, *MMP13* is a direct target of miR-27a, and *MMP13* gene expression is inversely correlated with miR-145 levels [70]. Finally, miR-146, previously detected in a large-scale miRNA survey, is associated with OA-related pain in human glial cells [71].

The targeting of particular miRNAs may also play a role in OA diagnosis and therapy. For example, a recent study demonstrated that intraarticular delivery of an antagonist of miR-483-5p (previously identified as overexpressed in OA chondrocytes) delayed the progression of OA in mice using the DMM post-traumatic model, specifically by modulating matrilin 3 and tissue inhibitor of metalloproteinase 2 (TIMP-2) [72]. A second study published in 2014 examined intraarticular injection of miRNA-210 in partially transected anterior cruciate ligaments of rats and found increased healing by the enhancement of angiogenesis through upregulation of VEGF and FGF2 expression, along with increases in transcription of *Col2a1* [73]. Despite tissue evidence of miRNA dysregulation in OA, a very recent study in mice failed to identify a circulating serum miRNA profile associated with early OA damage [74].

Conclusion

Epigenetic research in OA is burgeoning. Even though it is still a relatively young field of inquiry, it has contributed many exciting developments to both our understanding of OA pathogenesis and offered insight into novel treatments and diagnostic strategies for this poorly understood but quite common disease. Specifically, our increasing knowledge of DNA methylation, histone sidechain modification, and the miRNA landscape of OA cartilage and subchondral bone has contributed to our understanding of the complexity and breadth of mechanisms in different tissues involved in OA, and has reiterated previous notions of the inflammatory components of OA. Furthermore, modification of the epigenome, either globally with HDAC inhibitors or methyltransferase inhibitors, or more directly via specific miRNAs, offers hope for specific disease-modifying agents that are presently lacking in our quite limited OA pharmacological armamentarium. Further research in OA epigenetics will no doubt reveal additional insights into the basic mechanisms underlying the delicate balance of anabolism and catabolism, as well as integrate our fragmented understanding of the various tissue responses involved in the early stages of OA.

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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