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Review

Epigenetics of cartilage diseases☆

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ABSTRACT

Osteoarticular diseases, such as arthritis or osteoarthritis, are multifactorial diseases with an underlying genetic etiology that are challenging to study. Genome-Wide Association studies (GWAS) have identified several genetic loci associated with these diseases. Epigenetics is a complex mechanism of chromatin and gene modulation through DNA methylation, histone deacetylation or microRNA, which might contribute to the inheritability of disease. Some of these mechanisms have been studied for decades in other diseases or as part of the aging process, where epigenetic changes seem to play an important role. With the implementation of better technological tools, such as the Illumina next generation sequencing, altered methylation of DNA has been linked to articular diseases and these mechanisms have been shown to regulate metalloprotease (MMP) expression and cartilage matrix integrity. Some miRNA have also been identified and more extensively characterized, such as delineation of the role played by miR-140 in chondrogenesis, followed by the discovery of numerous miRNA potentially involved in the epigenetic regulation of osteoarthritic disease. Histone deacetylases have long been linked to aging, particularly with respect to the Sirtuin family with Sirt1 as the major player. Because aging is the major risk factor for osteoarthritis, the involvement of Sirtuins in the etiology of osteoarthritis has been suggested and investigated. All of these fine regulations together shed new light on cartilage disease pathophysiology. We present in this short review an update of the role of these pathways in articular diseases.

1. Introduction

Epigenetics, known as early as the 1940's, has been extensively studied for more than a decade in chronic diseases such as arthritis, osteoarthritis (OA) and cartilage diseases. Epigenetic changes also arise as a consequence of the normal aging process and have been shown to be altered during aging. Osteoarthritis is a multifactorial disease that can impact all joint tissues, including cartilage, synovial membrane and fluid, subchondral bone, meniscus, and intrapatellar fat pad. The inflammation process is characterized by e flares, cartilage degradation and subchondral bone modifications. Aging is one of the OA risk factors. Rheumatoid arthritis leads to cartilage degeneration and joint deformation, due to a chronic autoimmune inflammation with a major role played by the synovium and its cells. We now know much more about mechanisms and regulation of chromatin in these diseases and the specific role played by chromatin modifiers. Epigenetic analysis, particularly the assessment of DNA methylation, clearly shows how genetic effects are modulated by the genome and the environment. Three major mechanisms of genetic regulation have emerged so far. Among these, a role for histone deacetylases, non-coding RNAs such as microRNA (miRNA), along with DNA methylation of CpG dinucleotides have been identified [1–5].

2. Histone deacetylases

Histones, which are the proteins enveloped by DNA to form the nucleosomes, can undergo modifications that alter chromatin conformation, leading to the modulation of gene promoter exposure to transcription factors and influencing the binding of chromatin-associated factors. Acetylation/deacetylation of histones and methylation are the primary mechanisms studied in osteoarthritis and arthritis. Acetylation is carried out by histone acetyl transferases (HAT) and is critical to transcription factor binding and initiation of gene expression. In contrast, deacetylation is carried out by histone deacetylases (HDAC) and leads to repression of gene expression. The balance between HAT and HDAC, the HAT/HDAC ratio, seems to be influenced by biological treatments used in arthritis, confirming an important role of histone modifications in the disease [6]. Moreover, the balance between Ac/de-Ac

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has a strong impact on the chondrocyte phenotype. Histone modifications, on the other hand, are recognized as important regulators of the immune system and, therefore, are of interest in the deciphering of arthritis pathogenesis. HDAC1, HDAC2 and HDAC7 are upregulated in osteoarthritic chondrocytes. Inhibition of HDAC 7 by siRNA has been shown to suppress IL-1β-mediated induction of MMP-13, thereby contributing to cartilage degradation in human knee OA chondrocytes [7]. For this reason, HDAC inhibitors (HDACi) have been developed (TSA, MS-275, BA) and have shown to prevent cartilage degradation in vitro and, more specifically, degradation following stress-induced damage [8]. HDAC activity decreases during human chondrocyte dedifferentiation, and use of an HDAC inhibitor has been shown to prevent the re-differentiation of dedifferentiated chondrocytes, suggesting a crucial role for HDACs in the chondrocyte phenotype maintenance [9]. Among the histone deacetylases, the Sirtuins family, which is composed of seven members so far, seems to be playing an active and pivotal role in chromatin regulation in cartilage. Sirt1 to Sirt7 have been involved in arthritic disease modulation and their localization in the cell varies. Sirt1, Sirt6 and Sirt7 are found in the nucleus, Sirt1 and Sirt2 in the cytosol and Sirt3, Sirt4 and Sirt5 in the mitochondria, giving them a unique role depending on their location.

Sirt1 is by far the most studied Sirtuin in the arthritic field. Sirt1 is a nicotinamide adenine dinucleotide (NAD^+) dependent deacteylase, similar to Sirt2, -3, -5 and -6, meaning that its activation is coupled to the conversion of intracellular NAD^+ into nicotinamide (NAM). Sirt1 deacetylates lysine residues (removes CH3) on the DNA or on other substrates. Sirt1 has been shown to be involved in the pathogenesis of OA in many in vitro and in vivo studies, playing a role in apoptosis, survival, promotion of cartilage matrix, and skeletal homeostasis. More recently, Sirt1 activity has been shown to correlate with TNF-α and IL-6 levels in arthritic PBMC from patients with rheumatoid arthritis, suggesting Sirt1 mediated epigenetic regulation in arthritis [10]. The use of Sirt1 inhibitors (such as Resveratrol) has facilitated the deciphering of some mechanisms, such as closely linking its enzymatic function to hypoxia [11]. Disruption of Sirt1 in chondrocytes has recently been observed to cause accelerated progression of OA under mechanical stress and aging [12]. Set7/9, a histone methyltransferase, prevents the histone deacetylase activity of Sirt1, impacting *Col2A1* gene transcription [13]. Several single-nucleotide polymorphisms (SNPs) in the *Sirt1* gene have been shown to be associated with a variety of diseases, including the metabolic syndrome recently revealed to be involved in knee OA, as well as longevity [14]. Finally, Shakibaei et al. have shown that Sirt1 is required for and plays a major role in promoting chondrogenic differentiation of mesenchymal stem cells [15]. Sirt2 is a cytosolic Sirtuin and is implicated in cell cycle regulation. Some of the Sirt2 targets include the acetyl transferase p300, transcriptional regulator, and nuclear factor-κB, NF-κB subunit p65, regulating the wide repertoire of NF-κB dependent genes, and modulation of inflammation. Sirt3, Sirt4 and Sirt5, known as mitochondrial Sirtuins, help the cells to reduce energy consumption. The involvement of theses Sirtuins in ATP production may protect against reactive oxygen species (ROS) production and aging. Sirt3-dependent mitochondrial adaptations, by stimulating pathways associated with calorie restriction, might play a central role in longevity and delay of aging. Sirt3 protects cells from oxidative stress by reducing ROS and de-acetylating SOD2, an antioxidant enzyme [16]. Sirt4 plays a role in cell metabolism. The balance between Sirt3 and Sirt4 may regulate glycerol phosphate dehydrogenase (GDH) and depletion of Sirt4 increases mitochondrial and fatty acid enzymes, as well as Sirt1. Sirt5 plays a role in the urea cycle. Deficiency of Sirt6, another nuclear Sirtuin and the mammalian homologue of Sir2 (NAD^+-dependent histone deactylase from yeast) leads to low insulin growth factor 1 (IGF-1) genome instability and early death [17]. Its deficiency leads also to the alteration of hundreds of genes, since it is a transcriptional regulator. Piao et al. have recently shown that Sirt6 regulates postnatal growth plate differentiation and proliferation, implying that Sirt6 can directly control proliferation and differentiation of chondrocytes [18]. Sirt7, the only nucleolar sirtuin, associates with active ribosomal RNA and binds to histones and RNA polymerase I to stimulate transcription (Table 1).

Table 1
Sirtuins (Sirt) localizations, targets and functions in articular diseases.

Histone deacetylation Sirtuins localization/targets	Function in articular diseases
Sirt-1 cytosolic and nuclear H4K16, H3K4, p53, H1K26 PGC1α, NF-κB, FOXO, Notch, HIFα, PI3K, TOR	Mitochondria regulation, apoptosis, cell survival, tissue regeneration, chondrogenic differentiation, stress response. Skeletal homeostasis, promotion of cartilage matrix
Sirt-2 cytosolic H4K16, tubulin, PAR-3, FOXO, CD20 PGC1α	Genome integrity, oxidative catabolism Modulation of inflammation
Sirt-3 mitochondrial GDH	Oxidative stress
Sirt-4 mitochondrial ADP-ribosylation	Fatty acid oxidation
Sirt-5 mitochondrial	ROS modulation
Sirt-6 nuclear H3K9, H3K56	Genome stability, telomere silencing
Sirt-7 nucleolar	Binds to histones and RNApol -> transcription Modulation of HIFα and β (linked to hypoxia), p 53. Pro-survival molecule

3. Histone methylation

The most studied general epigenetic mechanism is DNA methylation. DNA methylation is mediated by two different sets of enzymes, DNA methyl transferases (DNMT3) and maintenance methyltransferases (DNMT1). Methylation, addition of a methyl (CH3) group to the 5th carbon of a cytosine nucleotide, occurs at cytosine-guanine dinucleotides (CpG) found in CpG islands on DNA, often clustered with genes promotors. Methylation is a very stable marker that is detectable in urine, plasma and serum. DNA methylation has been studied in arthritis and osteoarticular diseases. The first analysis of total DNA methylation by chromatography years ago in OA did not show any difference relative to controls with respect to the levels of DNMT1 and DNMT3 [19]. However, the use of new technologies such as Illumina sequencing has brought new insights to this area of research. The profile of methylation and candidate genes has been determined in arthritis. Altered methylation has been shown in the promoters of IL1β, MMP-3, -9, -13 and ADAM-TS4. The profiles of nitric oxide synthase 2 (NOS2) and Leptine (LEP) correlate with differences in gene expression in OA cartilage. Altered methylation on gene promotors is associated with pathogenic mechanisms, which are currently being elucidated. A very recent study assessing DNA methylation for preserved and lesional articular cartilage in end-stage disease has shown 2324 correlations of the methylation status on the 9838 genes transcribed. Hypo- and hypermethylation were observed for 62 and 25 active CpG covering 70 unique genes involved in developmental and extracellular matrix maintenance pathways, indicating a possible reactivation of endochondral ossification [20]. Hypomethylation of the MMP-13 promotor correlates with the binding of transcription factor HIFα. Hypomethylation of the NOS2 enhancer correlates with NF-κB binding. These mechanisms might underlie the altered extracellular matrix present in diseased cartilage [21,22]. A recent study compared chondrocytes dispensed from the tibial plateau of OA or healthy control DNA methylation patterns. Ninety-one sites were identified and a significant