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Epigenetic Regulation in Chondrogenesis

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Abstract

Epigenetics is an essential mechanism to control gene expression and fundamental cellular processes. DNA methylation in CpG-rich promoters correlates with gene silencing. Histone modification including histone acetylation and deacetylation determines the stability of the chromatin structure. Condensed chromatin (heterochromatin), which has a higher-order histone-DNA structure, prevents the access of transcriptional activators to their target genes. The fundamental unit of eukaryotic chromatin consists of 146 bp of DNA wrapped around a histone octamer. Posttranslational modifications of the histone tail and the chromatin remodeling complex disrupt histone-DNA contacts and induce nucleosome mobilization. Histone acetylation of specific lysine residues in the histone tail plays a crucial role in epigenetic regulation. Histone acetylation is a dynamic process regulated by the antagonistic actions of 2 families of enzymes - the histone acetyltransferases (HATs) and the histone deacetylases (HDACs). The balance between histone acetylation and deacetylation serves as a key epigenetic mechanism for transcription factor-dependent gene expression and the developmental process. We review emerging evidence that DNA methylation, histone acetylation modified by HAT and/or HDAC, and transcription factor-associated molecules contribute to a mechanism that can alter chromatin structure, gene expression, and cellular differentiation during chondrogenesis.

KEYWORDS: epigenetics, DNA methylation, histone acetylation and HAT, histone deacetylation and HDAC, chondrogenesis

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Review

Epigenetic Regulation in Chondrogenesis

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Epigenetics is an essential mechanism to control gene expression and fundamental cellular processes. DNA methylation in CpG-rich promoters correlates with gene silencing. Histone modification including histone acetylation and deacetylation determines the stability of the chromatin structure. Condensed chromatin (heterochromatin), which has a higher-order histone-DNA structure, prevents the access of transcriptional activators to their target genes. The fundamental unit of eukaryotic chromatin consists of 146 bp of DNA wrapped around a histone octamer. Posttranslational modifications of the histone tail and the chromatin remodeling complex disrupt histone-DNA contacts and induce nucleosome mobilization. Histone acetylation of specific lysine residues in the histone tail plays a crucial role in epigenetic regulation. Histone acetylation is a dynamic process regulated by the antagonistic actions of 2 families of enzymes - the histone acetyltransferases (HATs) and the histone deacetylases (HDACs). The balance between histone acetylation and deacetylation serves as a key epigenetic mechanism for transcription factor-dependent gene expression and the developmental process. We review emerging evidence that DNA methylation, histone acetylation modified by HAT and/or HDAC, and transcription factor-associated molecules contribute to a mechanism that can alter chromatin structure, gene expression, and cellular differentiation during chondrogenesis.

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Functional musculoskeletal systems depend on the coordinated development of cartilage, bone, tendon, ligament, and muscle [1]. Genetic (DNA) codes play a key role in normal development and appropriate gene expression, called "genetic regulation", this sequential expression is interrupted or altered by genetic disorders. Although tissue-derived mesenchymal stem cells (MSCs) contain the same genetic information, each cell has a different gene expression and specific cellular function. The sequential differentiation of mesenchymal progenitors is tightly controlled by many transcriptional parameters. Tissue-specific transcription factors regulate the spatiotemporal gene expressions by associating with cis-acting elements such as promoters, enhancers, and silencers in the DNA sequences [2]. Transcriptional coactivators and corepressors also have important roles in transcriptional regulation. Since the decade of the genome project, "epigenetic regulation" (such as DNA methylation, histone modification, and chromatin remodeling) has been highlighted. Epigenetics ("epi" means "above") is defined as gene-regulating activity that does not involve changes in the underlying DNA code. The fundamental unit of eukaryotic chromatin,

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the nucleosome, consists of 146 bp of genomic DNA wrapped around a histone octamer (2 sets each of H2A, H2B, H3, and H4 core histones) [3]. DNA methylation is not only essential for mammalian development but also is a major epigenetic mechanism [2, 4, 5]. Histone modification can alter the condensed chromatin (heterochromatin) structure by influencing histone-DNA and histone-histone contacts [6, 7]. In addition, the chromatin remodeling complex disrupts histone-DNA contacts and induces nucleosome mobilization [8]. The epigenetic marking system "histone code", which modifies the synergistic or antagonistic effects of chromatin-associated proteins on histone-packaged DNA, is a critical mechanism to regulate the transcriptional status of gene expression for coordinated development [9].

Chondrogenesis derived from mesenchymal condensation is a dynamic process in endochondral bone formation. The sequential differentiation and maturation steps of chondrocytes are regulated by transcription factors and growth factors such as the Sry-type high mobility group box (Sox) genes, the runt-related Runx genes, and the transforming growth factor (TGF)-β superfamily [10–12]. Sox9, which encodes a high mobility group DNA-binding domain, has been identified as a master transcription factor in chondrogenesis [13]. Mutations in the *Sox9* gene underlie the congenital dwarfism syndrome, campomelic dysplasia [14]. Mouse chimeras using *Sox9* (-/-) embryonic stem cells shows that *Sox9* (-/-) cells are excluded from cartilage tissues and are unable to express chondrocyte-specific genes such as α1(II) collagen (*Col2a1*) [15]. In the genital ridge, however, *Col2a1* is not expressed despite abundant *Sox9* expression [16]. In addition, *Sox9* overexpression in chondrocytes produces a phenotype of dwarfism [17]. These findings suggest that additional mechanisms cooperatively regulate the Sox9-dependent chondrogenesis. The TGF-β superfamily is a multifunctional growth factor for many cellular processes such as proliferation, differentiation, and apoptosis [18]. In chondrocyte differentiation, TGF-β stimulation is necessary for MSC-derived primary chondrogenesis [19]. On the other hand, chondrocyte maturation is inhibited by TGF-β [20]. These conflicting effects of TGF-β during chondrogenesis might depend on chromatin structure and/or the epigenetics of each differentiated stage. Several pathways following the activation of TGF-β receptors such as Smad2, Smad3, and mitogen-activated protein kinase have been identified as key intracellular signals in response to TGF-β treatments [18, 21]. We have previously demonstrated that TGF-β-regulated Smad3 activates the Sox9-dependent transcription on the chromatin structure [22]. Smad3 also associates with other transcription factors, such as the osteogenic inducer Runx2 and the myogenic factor MyoD, and the coactivator p300, which has an intrinsic histone acetyltransferase (HAT) activity [23–25]. From these findings, chondrogenesis is considerably regulated by the cross-talk among transcription factors, growth factor signals, histone modification, and chromatin structure.

Epigenetics is an essential mechanism to control gene expression and fundamental cellular processes such as proliferation and differentiation [26–28]. In this review, we focus on epigenetic regulation during chondrogenic differentiation.

DNA Methylation

DNA methylation is an important epigenetic mechanism for the stable silencing and appropriate maintenance of gene expression [2–4]. DNA methylation in mammals occurs in the cytosine of the CpG nucleotide via DNA methyltransferases. CpG islands, regions with more than 500 bp and a G/C content larger than 55%, are localized in the promoter of about 40% of all mammalian genes and are normally maintained in nonmethylated forms [3, 29]. CpG-rich promoters of chondrogenic-related genes, such as *Sox9, Runx2, chondromodulin-I*, and *fibroblast growth factor receptor 3*, are hypomethylated during synovium-derived chondrogenesis [30]. In addition, the demethylation status of the *chondromodulin-I* promoter is correlated with *chondromodulin-I* gene expression through the binding of Sp3 as a transcriptional activator [31]. The *Col2a1* gene is also less methylated in chondrocytes than in fibroblasts [32]. CpG methylation-based gene silencing of α1(X) collagen (*Col10a1*), a hypertrophic marker for differentiated chondrocytes, is consistently established in cartilage tissues and articular chondrocytes. However, the demethylation of the *Col10a1* promoter correlates with *Col10a1* induction during MSC-derived chondrogenesis [33]. These reports suggest that the state of DNA methylation regulates chondrogenic gene expression and cellular

differentiation along with chondrogenesis (Fig. 1). The patterns of genomic methylation are also associated with several human diseases such as ATR-X, Fragile X, and ICF syndromes [34]. DNA methylation is important in determining the gene expression pattern observed in osteoarthritic chondrocytes [35]. On the other hand, CpG methylation does not seem to have a central role in the switch of *aggrecan* promoter activity in articular chondrocytes [36]. Further investigations will be required to understand the relationship between DNA methylation and chondrocyte differentiation.

Histone Modification

Posttranslational histone modification including acetylation, methylation, phosphorylation, ubiquitination, and ADP-ribosylation determines the stability and/or instability of the chromatin structure [7, 9]. External to the histone fold domain, approximately 25% of the core histone mass is contained within the histone tail domain [6]. Histone tails, located at the N-termini of all histones and the C-terminus of H2A, are modified by many enzymes such as HAT, histone deacetylase (HDAC), and histone methyltransferase.

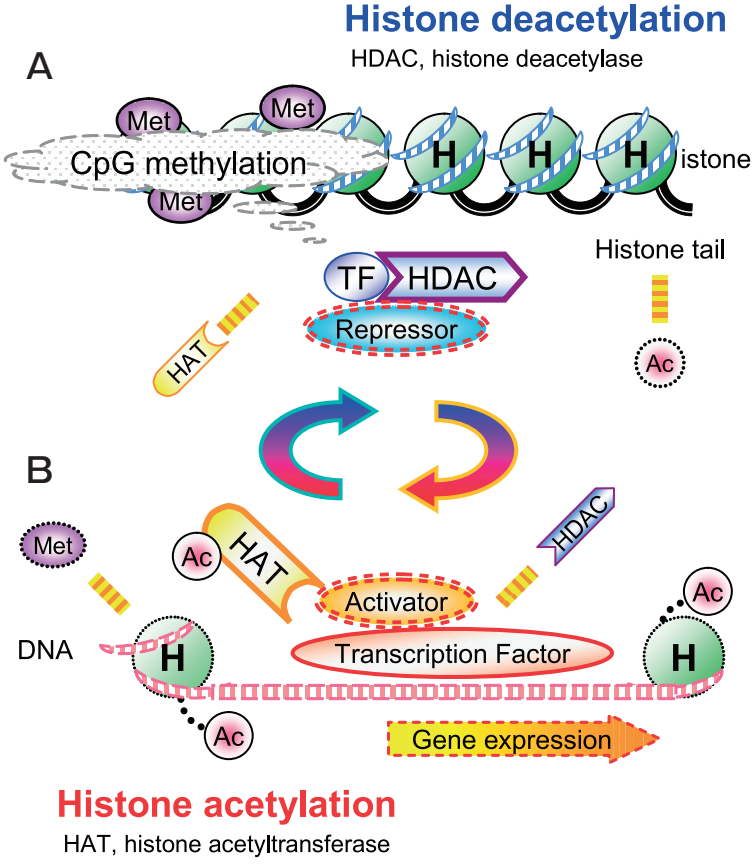

Fig. 1 Epigenetic regulation in the balance between histone acetylation and deacetylation. **A**, Schematic representation of a condensed heterochromatic structure. CpG islands are methylated in the promoter of the inactive (repressed) genes on chromatin. DNA and deacetylated histone form a higher-order chromatin structure via stable histone tails. In heterochromatic regions, the transcription factor (TF) cannot either recognize or associate with its DNA-binding sequence. The repressing molecule and signal (Repressor) favorably associate with TF via the recruitment of corepressor HDAC; **B**, Schematic model of accessible euchromatic environment. TF, the coactivator HAT, and activating molecule (Activator) (*e.g.* Sox9, p300, and Smad3/4 transcriptional complex) cooperatively induce histone acetylation. The chromatin structure changes from an inactive to accessible form by histone acetylation. In the relaxed euchromatin, other transcriptional complexes cooperatively enhance the transcription of the activated gene. H, histone; Ac, acetylation; Met, methylation.

The N-termini of histone tails interact with DNA and protein in chromatin. Furthermore, histone tails are critical for the self-assembly of condensed chromatin fibers into higher-order structures [6]. The degree of chromatin folding directly influences the activity of DNA in transcription, replication, and recombination [37]. Therefore, histone acetylation of specific lysine residues in the histone tail plays a fundamental role in epigenetic transcriptional regulation (Fig. 1).

Histone Acetylation and HAT Coactivator

Recent biochemical and genetic studies have identified several multisubunit HAT complexes such as coactivator p300 and its paralog CREB-binding protein, the MYST family, and GNAT superfamily members [37, 38]. The multifunctional coactivator p300 has an important role for gene expression and cellular differentiation. p300 acts as a protein scaffold and a bridging factor for the assembly of the transcriptional apparatus. In addition, the HAT activity of p300 has the potential to facilitate transcriptional activity by modulating the chromatin structure [39]. In chondrogenesis, p300 stimulates transcription factor-mediated chromatin disruption. Coactivator p300 directly associates with the master chondrogenic factor Sox9, and activates Sox9-dependent transcription [40]. We have previously reported that Sox9-dependent transactivation is induced by p300-mediated histone acetylation of chromatin [41]. *In vitro* transcription and S1 nuclease assays have revealed that p300 potentiates Sox9-dependent transcription on a chromatinized DNA template and is associated with hyperacetylated histones [41]. In addition, histone hyperacetylation using the HDAC inhibitor trichostatin A (TSA) enhances Sox9-regulated cartilage matrix gene expressions (*COL2A1* and *aggrecan*) in human chondrocytes [41]. HDAC inhibitors, including TSA and FK228, have the synergistic potential to induce *Sox9* expression via enhanced recruitment of nuclear factor Y (NF-Y) to the proximal promoter of *Sox9* [42]. p300 also acts as a transcriptional coactivator of cartilage homeoprotein-1 (Cart1), which is involved in skeletal development, through direct interaction with Cart1. Deletion and mutagenesis analyses have identified that the 131st lysine of Cart1 is acetylated by p300-HAT, and that Cart1 acetylation is critical in the association with p300 as well as p300-dependent transactivation [43]. Gene activation of the *cartilage oligomeric matrix protein* (*COMP*), encoding a noncollagenous matrix protein expressed predominantly in cartilage, is cooperatively regulated by Sox9, Sox5, Sox6, and p300 [44]. The *COMP* promoter contains a positive and negative regulatory element. Sox9 directly binds to the positive regulatory element and activates *COMP* expression by associating with p300 [44]. On the other hand, the leukemia/lymphoma-related factor transcriptional repressor inhibits *COMP* gene expression by recruiting HDAC1 as a negative regulatory element of the *COMP* promoter [45]. Tat-interactive protein-60 (Tip60) is a member of the MYST family involved in a wide range of regulatory functions in various organisms [38]. Coactivator Tip60, which mainly acetylates H4, increases Sox9/Sox5-dependent *Col2a1* transcription by associating with Sox9 on chromatin [46]. The basic helix-loop-helix transcription factor, Scleraxis, and its partner, E47, cooperatively stimulate Sox9-dependent transcription through the formation of a transcriptional complex with p300 [47]. These findings suggest that coactivator-induced histone acetylation triggers the transcriptional activation of chondrogenic genes and has a fundamental role in epigenetic regulation during chondrogenesis.

Histone Deacetylation and HDAC Corepressor

Acetylation of lysine residues in histone tails neutralizes their positive charge, thereby relaxing the chromatin (euchromatin) structure. This interferes with the generation of higher-order chromatin structures, and increases the accessibility of transcription factors to their target genes [48]. Histone deacetylation induced by HDACs has a crucial role in chromatin compaction and transcriptional repression. Therefore, the balance between histone acetylation and deacetylation serves as a key regulatory mechanism for gene expression, developmental processes, and disease states [49]. HDACs lack intrinsic DNA-binding activity and are recruited to target genes via their direct association with transcriptional activators and repressors, as well as their incorporation into multiprotein transcriptional complexes [48]. Mammalian genomes encode 11 HDAC proteins with a highly conserved deacetylase domain. Recent analyses of *HDAC* knockout mice have revealed specific functions

of individual HDACs in development and disease [49]. In chondrogenesis, HDAC4 has a central role in skeletal development [50]. HDAC4, which is expressed in prehypertrophic chondrocytes, regulates chondrocyte hypertrophy and endochondral bone formation by interacting with and inhibiting the activity of Runx2 [50]. *HDAC4*-null mice display premature ossification of developing bone due to early induction of chondrocyte hypertrophy, and die during the first week of life owing to ectopic ossification, which prevents the expansion of the rib cage and leads to an inability to breathe [50]. Adenoviral *HDAC4* overexpression promotes synovial stem cell-derived chondrogenesis but inhibits its hypertrophic differentiation [51]. In cultured chondrocytes, total HDAC activity and *Col2a1* expression decrease along with the dedifferentiation in passaged chondrocytes, but recover during the redifferentiation in pellet-cultured chondrocytes [52]. This phenomenon seems to be caused by HDAC-mediated transcriptional suppression of *Wnt-5a*, which inhibits *Col2a1* expression [52]. On the other hand, transcription factor Snail inhibits the expression of the *Col2a1* gene by associating with HDAC1 and HDAC2 [53]. These reports suggest that histone deacetylation is complicatedly modified by transcription factor-related HDACs. The balance between histone acetylation and deacetylation is tightly regulated by many transcriptional complexes during chondrogenic differentiation and maturation (Fig. 1).

Growth Factors and Epigenetics

Growth factors, cytokines, and nonproteinaceous chemical compounds including dexamethasone, vitamin D_3, prostaglandin E2, and ascorbic acid influence gene expression and cellular differentiation during chondrogenesis [54]. Intracellular signaling activated by the ligand-receptor complex also plays an important role in epigenetic regulation by modulating the association between transcription factors and coactivators/corepressors. The TGF-β superfamily and its signal mediators are well-documented in the field of chondrocyte differentiation, maturation, and degeneration [55]. TGF-β-regulated Smad3, but not Smad2, promotes MSC-derived primary chondrogenesis through the activation of Sox9 via p300 recruitment [56]. Smad3 stabilizes the association between Sox9 and coactivator p300 by forming a transcriptional apparatus with Sox9/p300 [56]. Phosphorylated Smad3/4, Sox9, and p300 cooperatively activate the Sox9-dependent transcription on a chromatinized DNA template [22]. Bone morphogenetic protein (BMP)-2, a member of the TGF-β superfamily, stimulates *Sox9* expression by increasing the association between NF-Y-p300 complex and *Sox9* promoter. BMP-2 also induces histone hyperacetylation and methylation at the *Sox9* gene on chromatin [57]. Chondrocyte terminal differentiation is stimulated by the activation of BMP-regulated Smad1/5/8 and inhibited by the TGF-β-regulated Smad2/3 pathway [55]. Although the interaction between Smad1 and Runx2 is essential for Runx2-dependent transcription, the Runx2-Smad3 complex inhibits the function of Runx2 [58]. The BMP-activated Smad1/4 complex associates with Nkx3.2, expressed in the sclerotome and developing cartilage, by facilitating the recruitment of corepressor HDAC1 [59]. The studies cited demonstrate that the interaction between the transcription factor and TGF-β/BMP-dependent Smad can result in either transcriptional activation or repression (Fig. 1). The growth factor-regulated signal may have the potential to activate (or repress) target gene expression by stabilizing the association between the transcription factor and the coactivator (or corepressor).

Epigenetics in Chondrogenic Differentiation

Epigenetics plays an essential role in determining the course of cellular differentiation and gene expression modulation without disrupting genetic information. DNA methylation correlates with gene silencing during chondrogenesis. Histone acetylation and deacetylation, which mainly modulate a relaxed and/or condensed chromatin structure, are precisely regulated by transcription factor-related complex formation with coactivator HATs and with corepressor HDACs, respectively. The "epigenetic code" maintains subconscious developmental memories in chondrogenic differentiation [60, 61].

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