Roles of the histone methyltransferase G9a in the development and differentiation of mesenchymal tissues

Hisashi Ideno, Kazuhisa Nakashima* and Akira Nifuji*

Department of Pharmacology, School of Dental Medicine, Tsurumi University, 2-1-3 Tsurumi, Tsurumi-ku, Yokohama City, Kanagawa 230-8501, Japan

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Abstract The expression of cell lineage-specific genes during cell differentiation and development is regulated by lineage-specific transcription factors. Recent studies have revealed that epigenetic mechanisms, including post-translational modifications in histone proteins and DNA methylation, play important roles in cell lineage determination and further differentiation. Many different post-translational modifications of histone proteins have been identified to date. For example, modifications at the N-terminal ninth lysine residue of histone H3 (H3K9) are associated with the level of gene expression and local chromatin structure. H3K9 is known to have un-, mono-, di-, and trimethylation states, and these methylated states are determined by six H3K9 methyltransferases in mammals. Among these H3K9 methyltransferases, G9a is responsible for mono- and dimethylation of H3K9. G9a-null mice showed embryonic lethality, indicating its critical roles in cell differentiation, organogenesis, and development. Indeed, studies of G9a conditional deletion in vivo and G9a-deficient cells in vitro have suggested that G9a is a multifunctional protein in various cell types. This short review summarizes recent findings regarding the effects of G9a function on the development of mesenchymal tissues, such as muscle, adipose, and skeletal tissues.

Keywords: G9a, cell differentiation, development, epigenetics

Introduction Harmonized locomotion is achieved through interactions of several mesenchymal tissues, including bones, cartilage, skeletal muscles, and tendons/ligaments. These tissues comprise a locomotive system that permits fluid movement and structural integrity and strength of the joint. Failures in this system result in pain, limited range of motion, loss of muscle strength, and poor balance in aged people; this condition is called locomotive syndrome1). One of the etiologies of locomotive syndrome is obesity, in which mesenchyme-derived adipose tissue plays a central role. Thus, mesenchymal cells are critical elements for the development and pathogenesis of locomotive system dysfunction.

The molecular mechanisms governing the differentiation, development, and pathological abnormalities of mesenchymal cells and tissues have been under extensive investigation for the last three decades. A transcription factor essential for a certain cell type governs the specific gene expression profile and functions of a certain cell lineage. One well-supported mechanisms of tissue-specific gene transcription are described as recognition and binding of genomic cis-elements of genes by tissue-specific transcription factors2-5). Recent extensive studies, however, have revealed that the nucleosome structure comprising chromatin is a dynamic component of the machinery regulating gene transcription6). Histone modifications change nucleosome structure, thus affecting the functions of tissue-specific transcription factors.

In this short review, we describe recent findings regarding the functions of a histone modification enzyme, G9a, in several mesenchymal tissues, particularly muscle, adipose, and skeletal tissues.

Epigenetic modifications and G9a

The nucleosome structure is composed of a highly conserved histone octamer, which includes two pairs of the histones H2A, H2B, H3, and H4. In this structure, 145-147 base pairs of DNA are wrapped around the octamer of histone proteins7). The N-terminal tails of the histones protrude from the packed nucleosome and are subjected to post-translational modifications, such as methylation, acetylation, phosphorylation, and ubiquitination (Fig. 1)8). More than 50 modifications have been identified in the N-terminal tails of histones. These acquired post-translational modifications are associated with chromatin
structure rearrangement and transcriptional accessibility of transcription factors by altering the higher order of the nucleosome structure\(^9\). For example, acetylation and trimethylation of H3K4 (the fourth lysine residue at the N-terminal tail of histone H3) are associated with active gene expression\(^{10,11}\), whereas trimethylation of H3K9 (the ninth lysine residue of histone H3) and H3K27 are associated with gene repression\(^2\).

Methylation of H3K9 is regulated by site-specific histone methyltransferases and demethylases producing mono-, di-, and trimethylation (H3K9me1, -me2, and -me3, respectively; Fig. 2). H3K9me3 is enriched in transcriptionally inactive heterochromatin\(^13\), whereas H3K9me1 and H3K9me2 are generally associated with gene bodies and regulatory regions, both heterochromatin and euchromatin, and are correlated with both activation and repression of transcription\(^{14,15}\). In mammals, six enzymes have been identified as H3K9-specific histone methyltransferases (HMTs; Fig. 3): G9a, Glp, Eset, Suv39h1, Suv39h2, and Prdm2\(^{15-20}\). All HMTs share a conserved Su (var) 3-9, Enhancer of zeste, Trithorax (SET) domain, which is necessary for their methylation activities\(^21\).

Among them, G9a (EHMT2) catalyzes H3K9me1 and H3K9me2\(^22\).

G9a has long-form and short-form splicing isoforms. The functional differences between these isoforms are not well understood. G9a has a SET domain, transactivation domain (TAD), glutamate-rich domain, cysteine-rich domain, and ankyrin repeat domain (ANK)\(^{23,24}\). G9a recognizes and binds to H3K9me1 and H3K9me2 through ANKs\(^25\). While G9a has been shown to be expressed in different tissues, its expression levels vary\(^{15}\). Moreover, G9a is overexpressed in various human cancers, including lung cancer and prostate carcinoma\(^{26,27}\). Because knockdown of G9a inhibits cancer cell growth, G9a has become an anticancer drug target\(^28\). In addition to catalyzing H3K9me1 and H3K9me2, many studies have suggested that G9a binds to and methylates non-histone proteins, including transcription factors\(^29\).

G9a also plays critical roles in embryonic development; indeed, G9a-deficient mice show embryonic lethality around embryonic day 9.5 (E9.5)\(^30\). Analysis of G9a conditional knockout mice in vivo and G9a-deficient cells in vitro suggests that G9a is a multifunctional protein in a wide range of cell types, including musculoskeletal cells.

The functions of G9a in mesenchymal tissue development

**Myogenesis.** Skeletal muscle development involves commitment of mononuclear progenitor cells and their further differentiation into multinuclear myotubes through cellular fusion\(^31\). The transcription factors involved in the stimulation of myogenic differentiation include MyoD, Myf5, myogenin, MRF4, and members of the MEF2 family. MyoD and Myf5 are expressed in undifferentiated myoblasts and activate the expression of myogenin. Myogenin then activates the expression of MEF2s, in combination with MyoD or myogenin, to promote the expression of myogenin. Myogenin then activates the expression of MEF2s, in combination with MyoD or myogenin, to promote the myogenic differentiation program\(^32\). Muscle-specific G9a-knockout mice have not been reported; thus, the function of G9a in skeletal muscle development in vivo remains unclear.

The roles of G9a in myogenesis in vitro have been extensively examined. During myotube formation, C2C12...
myogenic cells and primary myoblasts show increased expression of MyoD, myogenin, and Mef2D, with reciprocal reduction of G9a expression\textsuperscript{33,34). In these cells, overexpression of G9a inhibits myotube formation, whereas knockdown of G9a promotes myotube formation and the expression of myogenic genes \textit{in vitro}\textsuperscript{33), suggesting that G9a is a negative regulator of myotube formation.

Two distinct molecular mechanisms have been proposed to explain the negative action of G9a. The homeoprotein Msx1 inhibits myotube formation through direct binding with G9a. The G9a-Msx1 heterodimer promotes the enrichment of H3K9me2 on downstream genes, such as MyoD and Myf5\textsuperscript{35). Likewise, the basic helix-loop-helix protein Sharp-1 inhibits myotube formation by forming a heterodimer complex with G9a. The formation of the Sharp-1-G9a complex results in the accumulation of H3K9me2 at the myogenin promoter\textsuperscript{36,37).}

Molecular biological analyses have also revealed that G9a binds directly to MyoD and Mef2D and catalyzes the methylation of these proteins. Methylation of MyoD and Mef2D reduces their transcriptional activity\textsuperscript{34). In addition, methylated MyoD at K104 by G9a is degraded through a ubiquitination-dependent pathway\textsuperscript{33). Therefore, G9a plays a role as a repressor of myogenic differentiation through epigenetic modification of myogenic transcription factor genes and/or through direct modification of the gene products (Fig. 4).

\textbf{Adipogenesis.} Adipogenesis is the process of differentiation of multipotent mesenchymal stem cells into preadipocytes (referred to as the determination phase) and subsequent differentiation into lipid droplet-rich mature

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\textbf{Fig. 3} Schematic representation of the domain structures of H3K9 methyltransferases in mammals. The domains within H3K9-specific HMTs are displayed. SET domain catalyzes methylation. Ankyrin repeats recognize H3K9me1 and H3K9me2. The Tudor domain recognizes H3K9me2 and H3K9me3. The Chromodomain recognizes H3K9me2 and H3K9me3.

\textbf{Fig. 4} A model of G9a-mediated regulation during myogenic differentiation. G9a-Msx1 heterodimer represses MyoD and Myf5 gene expressions through enrichment of H3K9 dimethylation (H3K9me2) at these promoters. G9a-Sharp-1 methylate H3K9 at Myogenin promoter. Transcriptional activity of MyoD and Mef2D are repressed through direct methylation (m) of these proteins by G9a.
adipocytes (referred to as the terminal differentiation phase). Adipocyte differentiation is regulated by members of two distinct families of transcription factors, the CCAAT enhancer-binding protein (C/EBP) family (C/EBP-α, C/EBP-β, and C/EBP-δ) and the peroxisome proliferator-activated receptor family (PPAR-γ). C/EBP-β is expressed in the early phase, and C/EBP-α and PPAR-γ are induced in the late phase during differentiation5,38).

The expression of G9a is high in pre-adipocytes, but low in mature adipocytes. Adipose-specific G9a knockout mice show increased size and weight of white adipose tissue (WAT) and brown adipose tissue (BAT), suggesting that G9a inhibits adipose tissue development in vivo39).

Several mechanisms have been proposed to explain the negative effects of G9a on adipogenesis. G9a represses the expression of C/EBP-α and PPAR-γ through accumulation of H3K9me2 within their enhancer regions, promoters, and entire gene lengths. G9a expression levels are inversely correlated with those of C/EBP-α and PPAR-γ both in vivo and in vitro39,40). G9a directly binds to C/EBP-β, methylates C/EBP-β (K39), and reduces C/EBP-β transcriptional activity41). Thus, G9a is a negative regulatory factor of adipogenesis (Fig. 5).

**Chondrogenesis and Osteogenesis.** In early development in mice, long bone elongation occurs through growth plate formation. After overt differentiation of chondrocytes from mesenchymal condensations, three types of unique cells are generated in growth plates: proliferating, prehypertrophic, and hypertrophic chondrocytes. These chondrocytes show harmonized proliferation and differentiation. After the cartilage matrix is secreted and calcified, chondrocytes are resorbed, and osteoblasts appear and deposit bone matrix.

G9a is expressed at very low levels in mesenchymal cell condensations at E12.5. G9a is predominantly expressed in prehypertrophic and hypertrophic chondrocytes and osteoblasts at E16.542). During osteogenic and chondrogenic differentiation, Runx2, a key transcription factor involved in osteoblastic differentiation, interacts with G9a. In vitro reporter gene assays have shown that G9a promotes the transcriptional activation of Runx243). Deficiency in GLP, which is structurally related and shares an 80% sequence identity with G9a, results in impaired bone formation at the nasal bones and calvarial bone, and growth retardation after birth44). To date, the function of G9a in skeletal development in vivo has not been elucidated.

**Conclusion**

Classically, tissue-specific transcription factors are thought to determine tissue-specific gene expression during cell differentiation. Recently, however, studies have shown that cell differentiation is coordinated in a much more complex manner, with interactions between genetic factors and epigenetic modulators. Moreover, in addition to regulation of H3K9 methylation, G9a has been shown to methylate lysine residues of non-histone proteins and regulate the functions of these proteins. G9a is also a negative regulator of the terminal differentiation of myoblasts and adipocytes. In contrast, G9a is required for proper differentiation, survival, and lineage commitment of adult or somatic stem cells45). Further studies are required to delineate the physiological and pathological roles of G9a in vivo during development and differentiation.

**Conflict of Interests**

The authors declare that there is no conflict of interests regarding the publication of this article.

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