Turkish Journal of Biology

Volume 40 | Number 5 Article 9

1-1-2016

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HEYDARABAD, MILAD ZADI; VATANMAKANIAN, MOUSA; NIKASA, MINA; and HAGH, MAJID FARSHDOUSTI (2016) "Epigenetic regulation of specific transcription factors in osteogenicdifferentiation of mesenchymal stem cells," *Turkish Journal of Biology*: Vol. 40: No. 5, Article 9. https://doi.org/10.3906/biy-1507-6

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Turkish Journal of Biology

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Review Article

Turk J Biol
(2016) 40: 1040-1049
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doi:10.3906/biy-1507-6

Epigenetic regulation of specific transcription factors in osteogenic differentiation of mesenchymal stem cells

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Received: 02.07.2015 • **Accepted/Published Online:** 23.02.2016 • **Final Version:** 08.11.2016

Abstract: An abundance of experiments have been performed to clarify the differentiation process of mesenchymal stem cells (MSCs). Osteogenic differentiation and in vitro conditions favoring these cell lineages have attracted the attention of many researchers. Moreover, the gene expression profile during MSC differentiation toward its main specialized cells (bone, cartilage, and adipose cells) has been mostly understood. In the last decades another layer of investigation has attempted to clarify the epigenetic mechanisms underlying MSC differentiation into its specialized cells. It has been shown that as MSCs progress through the differentiation process, more nonspecific genes undergo DNA methylation to prevent differentiation into improper cell fates. Moreover, promoters of lineage-specific genes are strongly hypomethylated in MSCs during differentiation. The main objective of this review is to address the role of major epigenetic mechanisms such as DNA methylation, histone modifications, and noncoding RNAs, especially miRNAs, in osteoblastic differentiation of MSCs and to summarize all the previous studies that have determined the epigenetic alterations of the nuclear genome during osteoblastic differentiation of MSCs.

Key words: Epigenetic, MSCs, osteogenic differentiation, specific transcription factors

1. Introduction

Mesenchymal stem cells (MSCs) have acquired remarkable attention for their capacity as a tool for tissue regeneration and other therapeutic purposes. Therefore, along with this purpose, many investigators have attempted to identify MSCs specific markers. Although there is no consensus among authors regarding these markers, the International Society of Cryotherapy (ISCT) has suggested that human MSCs can be described using the following criteria: first, MSCs adhere to plastic surfaces under standard cell culture conditions; second, MSCs are positive for CD105, CD73, and CD90; and are negative for CD45, CD44, CD14, or CD11b, CD79α or CD19, and HLA-DR surface markers; third, MSCs have a differentiation potential to osteoblasts, adipocytes, and chondrocytes in vitro (Dominici et al., 2006). Samsonraj et al. (2015) presented additional phenotypic criteria to ISCT minimum criteria through which the evaluation of MSC therapeutic potency is improved and thus the MSCs can be applied for medical applications with greater quality. Their study proposed that various populations of MSCs have different capacities for clinical application such as ectopic bone formation. The author discussed that the surface markers proposed by ISCT do not sufficiently predict the in vivo ability of MSCs regarding their therapeutic potentials. They introduced surface markers including STRO-1 and PDGFR-α as markers for differentiating the MSCs with high and low capacity for ectopic bone formation. They also concluded that MSCs with greater colony-forming efficacy, predominant small-size cells, and greater growth capacity are more potent to form ectopic bone (Samsonraj et al., 2015).

It should be mentioned that multipotent cell populations derived from adipose tissue, bone marrow, and skeletal muscle have all been confirmed to meet the above-mentioned criteria (Morizonoc et al., 2003; Delorme et al., 2006; Kern et al., 2006; Péault et al., 2007; da Silva Meirelles et al., 2008). These different MSC populations have several common characteristics. For instance, adipocyte-derived stem cells (ASCs) and bone marrow MSCs (BMMSCs) have similar gene expression profiles (Boquest et al., 2005; Shahdadfar et al., 2005; Pedemonte et al., 2007) and surface

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molecules (Kern et al., 2006; da Silva Meirelles et al., 2008), and also share a similar potential for differentiation (De Ugarte et al., 2003; Kern et al., 2006). Increasing evidence regarding the function, phenotype, transcriptome, and recently epigenetics has suggested that MSCs isolated from different tissues are closely related; it is considered that all the MSCs originated from a common ancestor (Sørensen et al., 2010). Despite this evidence, there are still several debates concerning discrepancies among different origin-derived MSCs. A number of studies have demonstrated that the MSCs from distinct origins have unequal potential for transdifferentiation into neuronal lineage. It has been shown that these MSC-derived neuronal cells have various requirements to be achieved from MSCs and also harbor nonidentical characterization markers. Furthermore, different source specific MSC-derived neuronal cells do not present similar potential to be used for in vivo regeneration purposes (Taran et al., 2014). A study by Karaöz et al. has also proposed a more efficient differentiation of dental pulp-derived stem cells into neuronal and epithelial lineage compared with bone marrow-derived stem cells. It has been also shown that these stem cells derived from the two origins (dental pulp and bone marrow) show dissimilar gene expression profile, proliferation ability, phenotypic features, ultrastructural, and differentiation properties (Karaöz et al., 2011). Another controversial study, conducted by De Miguel et al., supposed a different capacity of MSCs originated from various tissues regarding their immunomodulatory effects upon the transplantation. Taken together, further attempts may be needed to shed light on whole in vitro and in vivo discrepancies between these multiple site-derived MSCs regarding their specific characteristics and potentials as well as the opposing results taken from the challenging features (De Miguel et al., 2012).

Recently, many investigators have attempted to perform a wide variety of experiments in order to clarify the various mechanisms contributing to MSC multilineage differentiation. Gene expression profiling, structural analysis, and proteomic profiles of different specialized cells originated from MSCs have all been investigated so far. Interestingly, none of them have yet clearly characterized the differentiation process of MSC toward its different cell fates. In the last decades, epigenetics has attracted significant attention as a powerful machine in the regulation of gene expression. This regulatory mechanism has been proposed to be involved in different evolutionary stages of vertebrates, gene regulations during stem cell self-renewal and lineage fate specification, and many other cell intrinsic molecular pathways. In this review, we address various epigenetic mechanisms through which MSCs are committed to be differentiated into osteoblasts.

2. Epigenetic mechanisms in development and stem cell differentiation

Epigenetic is used to describe heritable alterations in gene expression patterns that do not involve the nucleotide sequences (Holliday and Pugh, 1996). Epigenetic changes are responsible for diversity of structure and function of cells in multicellular organisms. This diversity is a result of differential expression of genes that originated through development and are maintained during mitosis (Jaenisch and Bird, 2003). The same mechanism occurs during stem cell differentiation into specialized cells. During stem cell differentiation, a highly coordinated process occurs in which the genes responsible for the differentiation become upregulated and those that are responsible for stem cell self-renewal and pluripotency become suppressed (Kouzarides, 2007). Among the most studied epigenetic mechanisms are stable but reversible alterations in DNA methylation at the promoter region of genes and histone modifications by which the accessibility of genes to the transcription factors becomes modified (Qiu, 2006; Vincent and Van Seuningen, 2009). DNA methylation is most likely restricted to CpG dinucleotides, while histone modifications encompass a wide range of modifications such as acetylation, phosphorylation, methylation, ubiquitination, biotinylation, and SUMOylation. Upregulation and downregulation of genes occur as a result of a highly complicated crosstalk between the epigenetic code including DNA methyltransferases, histone acetyltransferases, histone deacetylases, histone methyltransferases, histone demethylases, etc. (Santos-Rosa and Caldas, 2005). Recently, micro-RNAs (miRNA) are added as a new layer of epigenetic machinery and have made these mechanisms more complex. These small noncoding RNAs target the 3′-UTR of mRNA and subsequently exert their effect in mRNA degradation and translation repression (Bartel, 2004; Croce and Calin, 2005). It has also recently been demonstrated that miRNAs can silence the transcriptional activity of genes through chromatin remodeling (Gonzalez et al., 2008).

2.1. DNA methylation

Generally, DNA methylation is defined as covalent binding of a methyl group (CH_3) to the carbon 5 of cytosine at CpG dinucleotide sites of a DNA sequence. This enzymatic reaction occurs to produce 5-methylcytosine (5-mc) by which the genes become suppressed. A cluster of CpG sequences, known as CpG islands, are located near the promoter region of almost 30% of genes (Miranda and Jones, 2007). Methylation at these sites is strongly associated with suppression of genes. Furthermore, demethylation of the sites is correlated with the gene activation and allows the gene to be available to transcription factors (Bestor, 1990; Bird, 1993). In addition, DNA methylation has been confirmed to silence the transposons (Miranda

and Jones, 2007). DNA methylation plays an important role in many critical processes including long-term gene silencing (Hoffman and Hu, 2006; Klose and Bird, 2006), X chromosome inactivation, appropriate development (Razin and Shemer, 1995; Mann, 2001; Young and Beaujean, 2004; Morgan et al., 2005), and genome imprinting (Reik et al., 1990). Methylation of DNA is accomplished through a family of enzymes known as DNA methyl transferases (DNMTs) that use S-adenosylmethionine as methyl donor (Bird, 1985). Several types of DNMTs are responsible for DNA methylation during development. DNMT3a and DNMT3b are de novo transferases that are strongly expressed in developing mouse embryo and enhance the overall de novo DNA methylation after implantation (Okano et al., 1999). DNMT1 is not capable of de novo DNA methylation, but maintains the methylation status after mitosis (Li, 2002). During the mitosis, DNMT1 identifies hemimethylated DNA and catalyzes the methylation of daughter cells and thereby ensures the fidelity of methylation profile (Jaenisch and Bird, 2003). DNMT3a and DNMT3b are also proposed to act as demethylase; however, weak DNA MTase activity has been reported for DNMT2. It should be mentioned that the detailed mechanisms of demethylation are yet to be fully determined (Li et al., 2013).

2.2. Histone modifications

Within the nuclei of eukaryotic cells, DNA and related proteins are packaged and form a specialized structure known as chromatin. The chromatin can be loosely packaged as euchromatin, which allows the genes to be transcripted, or can be tightly organized as heterochromatin, a state in which the genes become repressed. The fundamental unit of the chromatin is called nucleosome, which consists of 147 bp of DNA wrapping 8 core histone proteins (two subunits of each H2A, H2B, H3, and H4). Histone proteins have a predominantly globular nature and their interaction with DNA strands can alter gene expression. These small basic proteins comprise unstructured N-terminal tails that can undergo several posttranslational modifications to regulate the expression of genes (Kouzarides, 2007). The most clearly investigated histone modifications are acetylation and methylation, which are accomplished by histone acetyl transferases and histone methyl transferases, respectively. Acetylation on the N-terminal tail of histones H3 and H4 is mostly correlated with activation of gene expression, while di- and trimethylation of lysine 9 of H3 (H3K9) and trimethylation of lysine 27 of H3 (H3K27) are associated with chromatin compaction and inactivation of gene expression. Histone methylation-mediated gene silencing is predominantly exerted through the recruitment of heterochromatin protein 1 (HP1) and polycomb group (PcG) proteins (Bartel, 2004; Olive et al., 2009). Similar to methylation changes, histone modifications have significant variability during stem cell differentiation. These characteristics are crucial for chromatin rearrangement and programming the global transcription after differentiation. In particular, the genes regulated through development comprise either active or repressive histone marks. This bivalency in chromatin structure allows the genes encoding specific transcription factors to be activated after immediate need for differentiation (Bernstein et al., 2006). Moreover, PcG complexes facilitate the stabilization of chromatin in a repressive state and have been indicated to act as direct silencer of differentiation regulators including Otx2, Satb2, and Tbx3 factors (Pasini et al., 2007). B cell Moloney murine leukemia virus insertion region protein 1 (BMI-1) is a key regulatory component of PcG complexes that serves as a self-renewal regulator of adult stem cells (Vincent and Van Seuningen, 2012).

3. Specific transcription factors in osteoblastic differentiation of MSCs

In a monolayer culture, supplements specific for each cell lineage lead to upregulation of the lineage specific transcription factors and thereby differentiate the MSCs toward the related cell fates. For example, osteogenic supplements ultimately lead to upregulation of osteoblast specific transcription factors. A well-studied transcription factor that is upregulated upon MSC commitment toward osteogenic differentiation is core binding factor alpha 1 (CBFα1), also known as RUNX2 (Ducy, 2000; Yamaguchi et al., 2000). In addition to Runx2, other transcription factors such as TAZ may cooperate to promote the differentiation toward osteoblasts. TAZ is a transcription coactivator that is able to coactivate Runx2-dependent gene transcription in murine MSCs (Hong et al., 2005). Several bone-specific genes including osteocalcin (OC), collagen I, osteopontin, bone sialo protein (BSP), and the parathormon receptor (PTHR) can be activated by Runx-2- dependent gene activation (Ducy, 2000). Furthermore, it has been discovered that MSCs isolated from a murine source, which was transduced with the osterix gene, induced MSC bone differentiation. This discovery introduced the osterix gene as another inducer for MSC bone differentiation (Tu et al., 2006). It has previously been shown that two homeotic genes, MSX2 and DLX5, contribute to regulate the development of mineralized tissues, such as bone, cartilage, and teeth (Ryoo et al., 1997). Furthermore, Bialek et al. indicated that in the early stages of skeletal development, Twist-1 and -2 are upregulated in Runx2-expressing cells (Bialek et al., 2004). Using whole transcriptome analysis, it was shown that zinc finger and BTB domain containing 16 (ZBTB16) are overexpressed throughout osteoblastic differentiation (Morsczeck et al., 2009). It was also confirmed that Atf4, a leucine zipper-

containing transcription factor, controls early osteogenesis and skeletal growth by activation of osteocalcin (Ocn) in osteoblasts (Wang et al., 2012).

4. DNA methylation in osteogenic differentiation

Differentiation of MSCs toward osteoblasts is a complicated process that is tightly regulated by a network of signaling pathways and transcription factors (Deng et al., 2008). A well-known master regulator for osteogenic differentiation of MSCs is Runt-related transcription factor 2 (Runx-2), which is highly expressed during bone development and maturation (Ducy et al., 1999; Karsenty, 2000; Komori, 2006; Farshdousti Hagh et al., 2012; Vincent and Van Seuningen, 2012). Interestingly, numerous coactivators and corepressors may overshadow the Runx-2 activity and generate a new layer of Runx-2 transcriptional regulation (Komori, 2002; Lian et al., 2003; Komori, 2006). In addition, accumulating evidence has supported the involvement of epigenetic machinery by histone modifications and chromatin remodeling than DNA methylation in the regulation of Runx-2 activity and thus osteogenic differentiation of MSCs (Li et al., 2004; Farshdousti Hagh et al., 2015). Generally, epigenetic regulation alters the binding ability of Runx-2 and other transcription factors by changing the chromatin structure at the promoter region. The most clearly investigated promoter for osteogenic lineage is the osteocalcin (OC) promoter, which possesses binding sites for various factors essential for the activation of genes specific for osteogenic differentiation such as Runx-2 (Stein et al., 1997, 2004; Bird and Wolffe, 1999; Villagra et al., 2002; Shen et al., 2003). For example, noticeable hypermethylation at the osteocalcin (OC) gene promoter has been observed. This hypermethylation was confirmed to be associated with a condensed chromatin structure (Villar-Garea and Esteller, 2004). Subsequent experiments have revealed that CpG methylation of the osteocalcin promoter region diminishes during in vitro osteoblastic differentiation of MSCs (Villagra et al., 2002). After differentiation by mechanical stimulus, upregulation of bone-specific genes has been found to be due to reduced CpG methylation of the genes (Arnsdorf et al., 2010; Karakaş et al., 2014). Furthermore, we observed that promoter methylation status did not change in RUNX2, distal-less homeobox5 (DLX5), or bone sialoprotein (BSP) genes during MSC osteoblastic differentiation, whereas osterix (OSX) and receptor tyrosine kinase-like orphan receptor 2 (ROR2) gene promoters showed a dynamic change in methylation pattern and hypomethylated during osteoblastic differentiation of MSCs (Tarfiei et al., 2011; Farshdousti Hagh et al., 2012; Saki et al., 2013).

Involvement of epigenetics in osteogenic differentiation of MSCs has also been confirmed by Dansranjavin et al. (2009). They reported that downregulation of stemness genes including Brachyury and LIN28 during osteogenic and adipogenic differentiation of MSCs was basically due to DNA hypermethylation, which predominantly occurs at their promoter region (Seo et al., 2004). Hsiao et al. also verified that the thyroid hormone receptor interactor 10 (Trip 10) is controlled by epigenetic regulation as bone marrow-derived MSCs are induced to differentiate toward osteogenic lineage. To determine if the gene suppression was mediated by DNA methylation they transfected a methylated Trip 10 promoter DNA into MSCs. Their results demonstrated an accumulative methyl-cytosin at the endogenous Trip 10 promoter, Trip 10 downregulation, and progressive MSC to neuron and osteocyte differentiation (Hsiao et al., 2010). In contrast to that study, lack of a remarkable alteration in DNA methylation of the promoter during in vitro differentiation of MSCs into osteocytes was reported by Kang et al. (2007).

Other key regulators by which MSC specialized into osteocytes is regulated are skeletal loading and loading-induced dynamic fluid flow (Dehority et al., 1999; Li et al., 2004; Kreke et al., 2005; Triplett et al., 2007). To address whether these regulators exert their function via epigenetic mechanisms, Arnsdorf et al. (2010) designed an experiment in which MSCs were differentiated into osteogenic lineage by mechanical forces, and then evaluated the methylation status of osteopontin (OPN), an essential factor for bone remodeling. They confirmed a strong association between mechanically induced differentiation and significantly reduced DNA methylation at the promoter of the OPN gene. They have also demonstrated that this reduction in the level of DNA methylation was correlated with decreased expression of OPN and enhanced osteogenic differentiation. It has also been shown that using biological components including media supplemented with growth factor β-glycerolphosphate, ascorbic acid, and dexamethasone for differentiation yields similar results. It is not surprising that epigenetic alteration in osteoblast-specific genes occurs during osteogenic differentiation of MSCs. Nevertheless, a recent report proposed that these alterations are beyond the specific genes and may occur on a global scale during osteogenic differentiation of MSCs. For instance, promoter hypermethylation of brachyury, which leads to suppression of brachyury, is correlated with MSC osteo-induction (Dansranjavin et al., 2009). Studies reviewing the epigenetic regulation of osteogenic differentiation of mesenchymal stem cells are mentioned in the Table.

Table. Epigenetic regulation of osteogenic differentiation of mesenchymal stem cells.

Epigenetic mechanism	Target gene	Finding	Reference
DNA methylation	OC	Promoter hypomethylation leads to osteogenic differentiation.	Villagra et al. (2002)
DNA methylation	OPN	Osteogenic differentiation is promoted by mechanically induced DNA demethylation.	Arnsdorf et al. (2010)
DNA methylation	Brachyury	DNA methylation in promoter region is associated with osteogenic differentiation.	Dansranjavin et al. (2009)
DNA methylation	Trip 10	Promoter DNA methylation correlated with enhanced osteogenic differentiation	Hsiao et al. (2010)
DNA methylation	RUNX2 DLX5 BSP OSX	No change in methylation status of RUNX2, DLX5, and BSP genes, but hypomethylation status of OSX gene during osteoblastic differentiation of MSCs	Farshdousti Hagh et al. (2015)
DNA methylation	LIN28	Promoter hypermethylation leads to LIN28 downregulation during osteogenic differentiation.	Dansranjavin et al. (2009)
Histone modification	OC	H3 and H4 acetylation is associated with expression of OC and osteogenic differentiation	Shen et al. (2003)
Histone modification	HOXA10	Chromatin acetylation mediated by HOXA10 and methylation of H3K4 promotes transcription of osteogenic genes. Methylation of H3K4 and H3k36 is correlated with expression of AP-2α and subsequent osteogenic differentiation.	Hassan et al. (2007)
Histone modification	AP-2α	Mutation in demethylation- related proteins (e.g., BCOR) have strong correlation with the OFCD syndrome.	Fan et al. (2009)
Histone modification	BCOR	Promotes H3K4 and H3K36 methylation of BCOR gene in MSCs results in osteo-dentinogenic gene transcription in MSCs and thus empowers the potential of MSCs to be differentiated toward osteogenic and dentinogenic lineage.	Fan et al. (2009)
Histone modification	CDK1	CDK1 activation can lead to trimethylation of H3K27 at Thr 487, a mark for chromatin repressive state, and eventually enhance the osteogenic differentiation of MSCs.	Wei et al. (2011)

OC: osteocalcin; OPN: osteopontin; Trip 10: thyroid hormone receptor interactor-10; BCOR: BCL_6 corepressor; OFCD: oculo-facial-cardio-dental; CDK1: Cyclin-dependent kinase 1; AP-2α: activating protein 2; Dlx-5: distal-less homeobox 5 gene; LIN28: Lin-28 homolog A.

5. Histone modifications during osteogenic differentiation

Histone acetylation is another epigenetic mechanism that is reported to contribute during osteogenesis. Shen et al. (2003) examined the chromatin-related mechanism through which transcription activity of bone-specific osteocalcin gene is promoted during both normal osteoblast differentiation and cell growth secluded ROS 17/2.8 osteosarcoma cells. Using the chromatin immunoprecipitation (ChIP) technique, they evaluated the acetylation of histones H3 and H4 at the promoter of the osteocalcin gene during and after cell proliferation. According to their findings, a high level of acetylation at H3 and H4 histones either at the promoter or coding region of the osteocalcin gene was observed during the proliferative phase of osteoblast differentiation. Moreover, they reported a strong association between acetylation of core histones and active expression of the osteocalcin gene in confluent ROS 17/2.8 cells as well as mature osteoblasts (Shen et al., 2003). Conversely, another study, in which the microarray technique was used to determine the role of histone modifications (H3K9Ac and H3K9Me2) during osteogenesis, showed a decreased level of global H3K9Ac at the gene promoter, while many promoters showed a high level of H3K9Me2 after the induction of human osteocyte differentiation (Tan et al., 2009). This discrepancy can be attributed to either the cell types (cell line or MSCs) or the technique (ChIP or microarray)

applied in each experimental procedure. Lee et al. (2006) preferred to determine the level of HDAC and the acetylation degree in order to investigate the reverse role of histones deacetylation in the osteogenesis process. They have confirmed that an important process for osteogenic differentiation is the downregulation of HDAC1 (Lee et al., 2006). MSCs have also been reported to undergo histone methylation, as an epigenetic mechanism, during differentiation toward osteogenic lineage. A previous study reported that HOXA10 leads to activation of Runx2, alkaline phosphatase, osteocalcin, and bone sialoprotein, and thereby specify the MSCs toward osteogenic lineage. They have also supported that this contribution is mediated through overall hyperacetylation of chromatin and H3K4 hypermethylation of the genes.

Hsiao et al. (2010) reported epigenetic modification of Trip 10, a protein that adapts various cellular functions, during differentiation of BM-MSCs. Because of its correlation with H3K27me3 mark, Trip 10 was a candidate to be investigated to determine whether its epigenetic changes could alter the pattern of MSC differentiation. They observed that MSCs transfected with in vitro methylated Trip10 promoter DNA was enriched with methylated cytosine at the endogenous Trip 10 promoter. They also verified that this methylation was correlated with reduced expression of Trip 10 and progressive differentiation toward osteoblasts and neural cells (Hsiao et al., 2010). Furthermore, their study discussed whether the manipulation of MSC epigenetic state can be applied as a therapeutic measure distinct from classic nuclear reprogramming. However, a deep understanding of long-term effects of such a manipulation is needed before the widespread use of this therapeutic modality in humans.

Fan et al. (2009) investigated whether mutation in the BCL-6 co-repressor (BCOR) gene (encoding BCOR protein that acts as a corepressor when it binds to a promoter DNA element) promotes histone H3K4 and H3K36 methylation in MSCs. This can in turn reactivate the osteo-/dentinogenic gene transcription in MSCs and thus empower the potential of MSCs to be differentiated toward osteogenic and dentinogenic lineage.

In addition, it was deciphered that AP-2α is most likely to be responsible for the osteogenic/dentinogenic capacity of MSCs. Methylation of lysine 4 and 36 (H3K4 and H3K36) of H3 at the promoter of AP-2α is associated with gene upregulation (Klose et al., 2006; Shi and Whetstine, 2007). The normal function of BCOR is the demethylation of H3K4me3 and H3K36me2 (Tsukada et al., 2006; Frescas et al., 2007), but a malfunction occurs when it mutates (Fan et al., 2009). The methylation prevents the binding of BCL-6 to the promoter of the AP-2α gene, and thus allows the AP-2α to undergo uncontrolled transcription. In conclusion, a mutant BCOR that has lost its demethylating activity leads to uncontrolled differentiation of MSCs toward osteo-/dentinogenic lineage, an event that occurs in oculo-facial-cardio-dental (OFCD) syndrome (Fan et al., 2009).

The contribution of histone methylation has also supported in the study by Wei et al. (2011). The study proposed that the activation of cyclin-dependent kinase 1 (CDK1) enhances osteogenic differentiation of MSCs through phosphorylation of zeste homologue 2 (EZH2) enhancer that eventually with the involvement of polycomb repressive complex 2 (PRC2) results in trimethylation of H3K27 at Thr 487, a mark for chromatin repressive state (Wei et al., 2011).

Acetylation of histones H3 and H4, and DNA hypomethylation promote the availability of osteo-inductive transcription factors to OC promoter (Bird and Wolffe, 1999; Villagra et al., 2002; Shen et al., 2003). Moreover, HOXA10 can induce chromatin hyperacetylation and trimethylation of H3K4, which induce structural changes in chromatin. These changes can facilitate the Runx2-mediated activation of genes encoding the markers of osteoblastic phenotype such as OC (Hassan et al., 2007). Interestingly, a newly identified member of the CHD chromatin remodeler family known as CreMM/CHD9 (Shur and Benayahu, 2005; Marom et al., 2006; Shur et al., 2006) has been detected to be associated with MSCs near newly generated adult bones (Benayahu et al., 2009). During osteogenic gene expression, CreMM/CHD9 binds to either Runx2 or OC promoter. The detailed epigenetic mechanism by which CreMM/CHD9 promotes osteogenic differentiation has yet to be fully determined. However, it has been supposed to change the chromatin architecture through DNA-dependent ATPase activity (Benayahu et al., 2009).

Several studies have reported a pivotal role for HDAC during osteogenesis. Cho et al. (2005) reported that the treatment of adipose tissue- and bone marrow-derived pluripotent stromal cells with VPA can activate calcium deposition as well as upregulation of genes specific for osteogenic differentiation of MSCs. They showed that this process is mediated by HDAC inhibition (Cho et al., 2005). Interestingly, despite the fact that p21CIP1/WAF1 was overexpressed in cells pretreated with HDAC inhibitors, the cell proliferation in the osteogenic medium was not affected. These results propose a bypass mechanism after osteogenic induction by which MSCs maintain their proliferation even though p21CIP1/WAF1 is upregulated and block the cell cycle (Lee et al., 2009).

Altogether, according to the above-mentioned studies, osteogenic differentiation of MSCs may be regulated by a network of epigenetic mechanisms including DNA methylation, histone methylation, and acetylation. Further investigation is required to determine whether MSC

osteogenic differentiation involves all the mechanisms simultaneously or only one mechanism induces the differentiation dependent on the culture condition. Studies that reviewed epigenetic regulation of osteogenic differentiation of MSCs are mentioned in the Table.

6. The role of miRNAs in osteogenic differentiation of MSCs

Increasing evidence supports the significance of miRNAs in gene regulation during stem cell differentiation. Among these several miRNA families including miR-29, miR-let-7, and miR-26 are proposed to be involved in osteogenic differentiation of MSCs. Li et al. (2009) have reported several mechanisms through which miR-29b promotes the differentiation of MSCs toward osteogenic lineage. They have shown in reporter assays that activity of COL1A1, COL5A3, and COL4A2 3'-UTR sequences as well as endogenous gene expression was reduced by miR-29b and was promoted by anti-miR-29b. These results suggest a mechanism for the accumulation of regulating collagen protein during the mineralization phase as miR-29b reaches its peak level. The author assumes that this mechanism inhibits fibrosis and induces mineral deposition. It is concluded that miR-29b directly downregulates the inhibitors of osteoblast differentiation including HDAC4, TGF-β, ACVR2A, CTNNBIP1, and DUSP2 proteins by binding to target 3´-UTR sequences in their mRNAs and thereby promotes osteogenic differentiation of MSCs. As a result, miR-29b is a critical regulator for osteoblast differentiation that targets anti-osteogenic factors and remodels bone extracellular matrix proteins (Li et al., 2009).

Recently, it has been shown that a group of miRNAs are downregulated by BMP-2 function. By suppression of these miRNAs, BMP-2 removes the negative control on MSC bone differentiation in nonosseous cells. This is considered a possible mechanism for BMP2-mediated osteogenic induction (Li et al., 2008). Despite all the previous efforts, the specific miRNAs involved in the regulation of bone formation from the committed cells to the mature osteocyte remain to be fully clarified. Li et al. (2008) conducted a large-scale study during which they showed that 22 miRNAs are downregulated in response to BMP2 expression, each of which targets a particular component of the osteogenic pathway. Among these, two representative miRNAs have been well characterized to be involved in direct downregulation of key osteoblastic transcription factors. MiR-133 directly targets Runx2. MiR-135 is another key factor that has been demonstrated to target Smad5, an essential transducer of osteogenic signal mediated by BMP2 (Li et al., 2008).

7. Conclusion

Using MSCs for the treatment of critical size bone defects, bone metabolic disorders, degeneration of cartilage tissue, and cerebral and heart ischemia has attracted the main focus of many investigators. According to previous reports, differentiation toward bone, cartilage, and adipose tissues is the main characteristic of MSCs. This capacity has made MSCs a regenerative agent for multiple tissue damage, especially bone and cartilage injuries that are almost incurable or are minimally cured by current therapies. Using MSCs in regenerative medicine is accomplished in two main strategies. One strategy applies undifferentiated cells and allows them to differentiate toward a proper lineage at the site of injury. However, differentiation into unwanted lineage at the defective site is a disadvantage of this strategy. An alternative strategy is the full differentiation of MSCs toward a desired cell fate before transplantation. This strategy requires an inevitable step before the transplantation for in vitro differentiation of MSCs for cell-based therapy of tissue defects. Conclusively, the differentiation process of MSC must be clearly understood, particularly the regulatory mechanisms through which MSCs rearrange the lineage specific genes. Many epigenetic changes occur during osteoblastic differentiation of MSCs. Extensive knowledge was obtained in mechanisms that regulate osteoblastic differentiation of MSCs, but further research is needed for safe therapeutic use of MSCs in regenerative medicine. Efficient epigenetic manipulation such as controlling the expression of HDACs, miRNA targeting, and DNA methylation alterations may bring a promising gateway through controlling the fate of MSCs toward bone tissue. These in conjugation with the induction of differentiation factors perhaps secreted by differentiated chondrocytes in the repair sites could enable us to more efficiently control the fate of MSCs into chondrocyte lineage.

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