Review

Osteoblast versus Adipocyte: Bone Marrow Microenvironment-Guided Epigenetic Control

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Abstract
The commitment and differentiation of bone marrow mesenchymal stem cells (MSCs) is tightly controlled by the local environment ensuring lineage differentiation balance and bone homeostasis. However, pathological conditions linked with osteoporosis have changed the bone marrow microenvironment, shifting MSCs’ fate to favor adipocytes over osteoblasts, and consequently leading to decreased bone mass with marrow fat accumulation. Multiple questions related to the underlying mechanisms remain to be answered. As recent findings have confirmed the fundamental role of the epigenetic mechanism in connecting environmental signals with gene expression and stem cell differentiation, a regulatory network in the bone marrow microenvironment, epigenetic modulation, gene expression, and MSC differentiation begins to emerge. This review discusses how pathological environmental factors affect MSCs’ fate by

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epigenetic modulating lineage-specific genes. We conclude that manipulating local environments and/or the epigenetic regulatory machinery that target the adipocyte differentiation pathway might be a therapeutic implication of bone loss diseases such as osteoporosis.

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Introduction

The formation of bone tissue in the embryo and the maintenance of bone homeostasis in the adult are attributed to the activity of bone marrow stem cells called mesenchymal stem cells (MSCs), whose dysfunction may give rise to bone diseases like osteoporosis. It has been observed that decreased bone mineral density (BMD) is accompanied by marrow fat accumulation under pathological conditions characterized by bone loss since 1971 [1–3]. As both osteoblasts and adipocytes are originated from MSCs, it is likely that predisposition of bone marrow MSCs to adipocyte versus osteoblast lineage is a contributing factor to this phenomena. The fact that osteoblasts and adipocytes can be converted to each other under certain conditions indicates a high degree of plasticity between these two lineages [4, 5]. Even though their destinies are intertwined and share a variety of genetic, hormonal and environmental factors [6], the mere presence of fat in bone marrow does not mean that osteoblast precursors are being exclusively forced down to the adipocyte pathway [7], which raises unsolved questions like what ultimately determines bone marrow MSC fate. Like many other types of stem cells, the fate of MSCs is tightly controlled by the local microenvironment. The bone marrow microenvironment, an extraordinarily heterogeneous and dynamic system, is generated by the functional relationship among different cells found in bone marrow via locally produced soluble factors, allowing for autocrine, paracrine, and endocrine activities. Physiological bone marrow provides an environment for osteoblast differentiation and the maintenance of bone homeostasis, whereas pathological conditions have changed the bone marrow microenvironment, leading to shifted MSC differentiation pathway.

The epigenetic mechanism refers to modifications on chromatin that define, at least in part, chromatin structure and gene expression level without affecting the DNA sequence. Well-studied epigenetic modifications include DNA methylation of CpG dinucleotides, histone post-translational modifications, and incorporation of histone variants, noncoding RNA regulation of target genes, and chromatin remodeling enzymes that modify the interactions between DNA and histone complex. Slight variations of these epigenetic modifications might result in the changes of local chromatin configuration or chromatin accessibility, hence affecting gene expression level. Stem cell research in the past decades has demonstrated that the epigenetic mechanism acts as a bridge that links the extracellular microenvironment with gene expression regulation [8]. Since the epigenetic mechanism also plays an important role in both osteoblastic and adipocytic differentiation regulation [9], this review will discuss how the bone marrow microenvironment modulates MSCs’ fate choice between these two lineages under both physiological and pathological conditions, by epigenetic mechanisms.
Osteoblastic/Adipocytic Differentiation Control under Physiological Conditions

MSCs pass through a sequence of events controlled by hormones and transcriptional factors ensuring proper osteoblast development of phenotype and functional properties until they enter osteocyte phenotype and/or undergo apoptosis. The bone marrow microenvironment plays a fundamental role for MSC maintenance and osteogenic process through providing signals from local systemic factors and extracellular matrix [10]. MSCs undergo osteogenic commitment under signals such as bone morphogenetic protein 2 (BMP2) in the bone marrow. During this commitment stage, the gene expression profile does not change greatly, but genome-wide changes of epigenetic modifications take place to establish a specific signature for osteoblastic differentiation [9]. HoxA10, one of the early markers activated at this stage, helps to establish an active epigenetic signature including H3K4 methylation and histone acetylation by recruiting epigenetic enzyme complexes on osteoblastic gene promoters [11]. Yet, the expression of these genes is inhibited by bone marrow factors until pre-osteoblasts migrate to the bone-forming surface [12, 13]. Transforming growth factor-beta 1 (TGFβ1), which is one of the most abundant cytokines in the bone marrow, mediates the silencing of osteogenic genes through promoting interaction between Runx2 and its corepressors histone deacetylase 4/5 (HDAC4/5) while inducing pre-osteoblast migration to the bone-forming surface [14, 15]. Recruitment of corepressors by Runx2 to its target promoters silences osteoblastic genes in precursors while keeping them poised for activation. The bone-forming surface provides a stiff, elastic microenvironment that immediately triggers the focal adhesion kinase (FAK) pathway in attached pre-osteoblasts, leading to cytoskeleton rearrangement and a more spread cell shape [16, 17]. FAK and/or local soluble factors (BMPs, Wnts, and their agonists) secreted by bone cells further activate osteogenic signals such as Wnt, ERK, MAPK, and PI3-K/Akt [18, 19], under whose actions the inhibitory epigenetic marks are erased and osteoblastic specific genes are highly expressed [20, 21]. Therefore, pre-osteoblasts undergo a maturation process that eventually leads to the formation of mineralized tissue.

Adipocytic differentiation of MSCs in the physiological bone microenvironment is tightly regulated. BMP2, TGFβ1, and Wnt signals have all been reported to inhibit adipocytic differentiation. Among these signals, the inhibitory mechanisms of both canonical and noncanonical Wnt pathways on adipogenesis are most well studied. Our previous work found that canonical Wnt signaling that is activated during BMP2-induced osteoblastic differentiation silences the expression of adipogenic master factor CCAAT/enhancer binding protein alpha (C/EBPα) by mediating the recruitment of DNA methyltransferases 3a and 3b (Dnmt3a/3b) to its promoter [22]. Expression silencing of C/EBPα as a result of DNA hypermethylation is indispensible for osteoblastic differentiation, as both impaired DNA methylation and overexpression of C/EBPα convert well-differentiated osteoblasts into adipocytes [23]. Noncanonical Wnt signal suppresses another adipogenic master factor peroxisome proliferator-activated receptor gamma (PPARγ) activity through the CamKII-TAK1-TAB2-NLK pathway. Once activated by Wnt5a, NLK promotes PPARγ to interact with H3K9 methyltransferase SETDB1, which is recruited by the former to its target promoters to repress gene expression by H3K9 trimethylation [24]. The inhibition of both the expression and the activity of adipogenic transcriptional factors by bone-forming environmental factors makes an assurance for irreversible differentiation of bone marrow MSCs into osteoblastic lineage.
Changed Bone Marrow Microenvironment under Pathological Conditions Impairs MSCs’ Fate Determination

With advanced age, estrogen deficiency, chronic glucocorticoid (GC) treatment, and decreased mechanical loading, bone loss is accompanied by fat infiltration. It has been hypothesized for years that shifted MSC differentiation fate to favor adipocytes over osteoblasts is a contributing factor for this phenomenon. Even though there is no direct evidence to prove that the accumulated fat in bone marrow is originated from adipogenesis of marrow MSCs, the changed differentiation potential of MSCs under the pathological conditions mentioned above has been observed both in vitro and in vivo. MSCs from aging, ovariectomized, long-term GC-treated, or mechanical unloading mice are more likely to form adipocytes, whereas osteogenic induction is more difficult than in normal controls [25–29]. Pathological bone marrow is characterized by higher levels of reactive oxygen species (ROS), inflammatory cytokines, excessive free fatty acids (FFA), reduced levels of TGFβ1, BMP2, and Wnts [30], and elevated levels of BMP2 and Wnt inhibitors [28]. In such circumstances, both MSC migration to the bone-forming surface and subsequent osteoblastogenesis are severely impaired. The molecular mechanism of how these altered environmental factors shift MSCs’ fate is far from understood. Genome-wide changes of the epigenetic signature caused by pathological environmental factors during MSC determination might be an explanation [9]. From the limited data available to date, evidence indicating that these bone marrow environmental factors under pathological conditions shift MSC fate through altering epigenetic modulation on lineage-specific genes is beginning to emerge.

The Impact of Pathological Bone Marrow on MSCs’ Fate Determination

Aging

Oxidative stress and inflammation are the main reasons that cause age-related bone loss. The effect of an inflammatory microenvironment on MSC differentiation will be discussed in the next part of this review. Evidence from pharmacological and genetic studies has provided support for a deleterious effect of oxidative stress in bone and has strengthened the idea that an increase in ROS represents a pathophysiological mechanism underlying bone loss caused by advanced age [31, 32], alcohol exposure [33, 34], estrogen deficiency [35], and GC treatment [36]. A high level of ROS is believed to be one of the main reasons for MSC apoptosis as well as inhibited osteoblastogenesis by inducing DNA damage and impairing telomerase activity [37, 38]. In vivo studies with a DNA repair deficient mouse model have confirmed that accumulation of DNA damage interferes with normal skeletal maintenance, leading to reduced osteoblast precursor numbers and decreased bone strength [39]. Besides, ROS suppresses the osteoblastic differentiation process of MSCs, manifested by a reduction of differentiation markers including alkaline phosphatase (ALP), collagen type I alpha I (Col1a1), and phosphorylated Runx2 [40]. One of the reasons for ROS-impaired osteoblastogenesis lies in the regulatory activities of Forkhead box O (FoxO), the transcriptional factor that activates the expression of free radical scavenging enzymes under oxidative stress. FoxO expression is upregulated in aged bone marrow and is transported into the nucleus after ROS-induced activation, where it transcripts target gene expression through forming heterodimer with β-catenin [41]. As β-catenin is an indispensable factor for transcriptional activity of T-cell factor (TCF), the downstream transcriptional factor of the canonical Wnt pathway, competitively bound by FoxO leading to suppressed Wnt target genes. Considering its important role in maintaining
osteoblastic/adipocytic differentiation balance, downregulation of canonical Wnt signal results in promoted adipogenesis over osteoblastogenesis. Besides, oxidative stress itself has been shown to directly modulate adipogenic differentiation. Adipogenesis is accompanied with the generation of ROS, while high doses of ROS markedly induce adipocytic differentiation [42]. An elevated expression level of PPARγ in MSCs cultured in an oxidative environment due to a lower CpG methylation level at its promoter might be an explanation for the positive effect of ROS on adipogenesis [43]. Since the bone marrow microenvironment is exposed to oxidative stress under pathological conditions, oxidative stress itself may provide a favorable adipogenic environment resulting in fat accumulation.

Estrogen Deficiency

Estrogen deficiency is another main cause of osteoporosis in postmenopausal women. Estrogen plays a critical role in maintaining the physiological bone marrow microenvironment by diminishing excessive ROS and inhibiting inflammation at multiple levels [35, 44]. Therefore, deficiency in estrogen results in elevated ROS and proinflammatory cytokines in bone marrow, leading to a pathological environment with oxidative stress and inflammation that is similar to aged bone marrow. Besides, estrogen also directly maintains the differentiation balance of osteoblasts versus adipocytes through binding to its receptors in MSCs. Estrogen receptor (ER) belongs to the nuclear receptor (NR) superfamily. Two types of ERs have been identified, ERα and ERβ. Once activated by estrogen, ERs positively regulate osteoblastic differentiation by crosstalking with multiple osteogenic signals (BMPs, Wnts, TGFβ, and PI3K/Akt) [45–47]. As a specific example, researchers found that estrogen enhances canonical Wnt signaling pathway by preventing β-catenin from degradation and promoting its nuclear translocation. A physical interaction between ER and β-catenin has been found fundamental for the effect of estrogen on the Wnt/β-catenin pathway [48, 49]. The crosstalk between ERs and osteogenic signals not only promotes osteoblastogenesis, but also helps to prevent adipogenesis of bone marrow MSCs. In addition, as a member of the NR superfamily of transcriptional factors, ERs also regulate target gene expression through direct binding to DNA elements. The transcriptional activity of ERs needs the interaction with their cofactors. Most of their identified cofactors are epigenetic modification enzymes, such as histone methyltransferase SETD6 and CARM1 (coactivator associated arginine methyltransferase 1). These cofactors either activate or repress gene expression by building epigenetic marks once they are recruited to ER target promoters. However, no studies have been done to investigate the epigenetic mechanism of ER-regulated gene expression during osteoblastic differentiation. Yet, several reports indicated that the epigenetic mechanism plays a critical role in ER-inhibited adipogenesis by regulating the transcriptional activity of PPARγ. ERs are sharing a similar pool of cofactors with PPARγ, which provides a platform for mutual interactions between these two nuclear hormone receptors. It was found that activated ER prevents PPARγ from recruiting its coactivators such as steroid receptor coactivator 1 (SRC1), transcriptional intermediary factor 2 (TIF2), and CREB-binding protein (CBP) to its target promoters [50, 51]. Since these coactivators are indispensable in activating target gene transcription by building active epigenetic signatures, losing these factors inhibits ligand-activated PPARγ transcriptional activity. Consistent with its important role in regulating MSC determination, mice with constitutively active ERα in osteoblasts have much higher BMD than normal controls [52]. However, a clinical investigation found that the expression of ERα is much lower in postmenopausal women due to higher DNA methylation at its promoter [53]. The underlying molecular mechanism of ERα promoter hypermethylation is still unclear. Higher levels of homocysteine in osteoblasts of
these women might be correlated with this phenomenon [53]. Considering its effect on environmental factors, a lack of estrogen leads to inflammatory bone marrow with oxidative stress. In such a microenvironment, combined with a lack of ER signaling, impaired osteoblastic differentiation, but excessive adipocyte formation of MSCs, is expected.

**GC Treatment**

GC is widely used as immune suppressor in the clinical treatment of autoimmune diseases and chronic inflammation. However, long-term and/or high-dose GC therapy leads to severe side effects of bone loss and low BMD. In the bone marrow of both GC-induced osteoporotic patients and animal models, a vast body of adipose tissue is found. Isolated MSCs from the bone marrow of these patients and animal models are favored to form adipocytes even under osteogenic inductions, indicating that the MSC differentiation potential has been changed under chronic GC treatment [22]. GC is one of the key inducers of adipogenesis by activating the transcription of C/EBPβ and C/EBPδ [54]. Once expressed, C/EBPβ binds to C/EBPα promoter and recruits corepressors HDAC1 and mSin3A to inhibit gene transcription [55]. GC releases C/EBPβ from its corepressors and recruits coactivator P300/CBP to C/EBPα promoter [55, 56]. Albeit its important role in adipogenesis, GC is not needed in mature adipocytes [57]. It was found that GC receptor functions transiently with other proteins (C/EBPβ and P300) to propagate a gene expression program by establishing an active epigenetic signature on target promoters [57], providing a memory of an earlier adipogenic signal. Therefore, under such an environment with a high dose of GC, MSCs are adipocytic determined as a result of genome-wide established epigenetic signature by GC-mediated mechanism. Besides, GC also mediates the inhibition of osteogenic transcriptional factors and signaling pathways through, at least in part, the epigenetic mechanism. In in vitro osteoblast cultures, treatment with GC leads to downregulation of Runx2 and Osterix (Osx) expression levels and inhibition of transcriptional activity of Runx2 by recruiting HDAC1 to its target promoters [58, 59]. However, the main mechanism of GC in osteoblastic/adipocytic differentiation regulation is modulating the Wnt signaling pathway. Clinical investigations and mouse model experiments have confirmed that chronic GC treatment elevates Wnt inhibitor levels (Dickkopf-1 [DKK1] and secreted Frizzled-related protein [sFRP]) in sera as well as bone marrow [60–63]. Besides, GC also blocks this pathway through, first, downregulating β-catenin level [64] and, second, impairing TCF transcriptional activities through recruiting HDAC1 to their target promoters [65]. As discussed before, inhibition of the Wnt pathway leads to impaired osteogenesis, but promotes adipocytic differentiation. Our previous research proved that during osteoblastogenesis, the inhibited Wnt/β-catenin pathway by dexamethasone treatment promotes C/EBPα expression due to downregulated DNA methylation at its promoter [22]. In addition, changed microRNA expression profiles might also help to mediate the inhibitory effect of GC on osteoblastic differentiation [64, 66]. Thereby, a conclusion could be drawn through these in vivo and in vitro studies that excessive GC in bone marrow helps to establish both active and repressive epigenetic signatures on adipogenic and osteogenic promoters, respectively, by networking with lineage-related cell signals and transcriptional factors.

**Mechanical Environment**

Biomechanical force is one of the major factors that determine the form, differentiation, and remodeling of skeleton tissue. It has long been found that increased skeleton mass is associated with weight-bearing exercises [67], and conversely, dramatic losses in bone density are associated with bed rest and space flight [68, 69]. External biomechanical loading is trans-
duced into bone marrow and generates a unique mechanical environment composed of intramedullary pressure and the fluid flow shear stress (FFSS) generated by pressure gradients. These mechanical forces are responded by sensitive cells through re-distribution of intracellular stresses called cytoskeleton, and are transmitted directly into the nucleus to modify gene expression. Osteocytes, which are exquisitely sensitive to mechanical strain [70], are regarded as chief mechano-sensors in adaptive bone. They detect and transduce mechanical signals initiated by whole bone mechanical loading and mediate these signals via releasing soluble factors that regulate osteoblastogenesis of MSCs [71]. Candidates for these soluble factors include Wnts, Sclerostin, and TGFβ1 [72]. In response to these factors, MSCs migrate to the bone-forming surface and differentiate into osteoblasts [73]. In addition to secreted soluble factors, mechanical signals can also be transmitted from osteocytes to osteoblastic lineage through a gap junction. MSCs, pre-osteoblasts, osteoblasts, osteocytes, and bone-lining cells all form gap junctions, creating a continuous network and enabling intercellular communication. Of note, FFSS induces conformational changes of Connexin 43 (Cx43) and opens its hemichannels [74, 75]. Deficiency of Cx43 results in lower response of bone to mechanical signals, indicating that Cx43-mediated communication plays a pivotal role in mechanical signal transduction among bone cells. Besides, MSCs have been proven to be responsive to mechanical signals [76, 77]. Cell shape regulates commitment of human MSCs to adipocyte or osteoblast fate. hMSCs allowed to adhere, flatten, and spread underwent osteogenesis, while unspread, round cells became adipocytes [16, 17]. RhoA/ROCK signal is activated by cytoskeleton tension, which further activates osteogenic signals, such as ERK1/2 [19], and promotes nuclear translocation of osteogenic transcriptional factors, such as TAZ (transcriptional coactivator with PDZ-binding motif) [78]. RhoA/ROCK signal may serve as the main switch that regulates the osteoblastic/adipocytic differentiation balance under mechanical conditions, expressing dominant-negative RhoA committed hMSCs to become adipocytes, while constitutively active RhoA caused osteogenesis [17].

How the activated signals and kinases by mechanical stimuli regulate the lineage-specific gene expression profile is still not fully understood. However, recent studies begin to shed light on the epigenetic role in mechanical force-regulated osteoblastic gene expression. The first identified epigenetic mechanism during mechanical-stimulated osteoblastic differentiation is DNA methylation on osteopontin (OPN) promoter. FFSS promotes DNA demethylation on OPN promoter, which is consistent with its increased expression level under such a condition [79]. These epigenetic modulations are regulated by mechanical stimuli-activated cell signals. Yet, limited data are available to picture the regulatory networks among cell signals, epigenetic modulations, and gene expression regulation in a mechanical microenvironment. As a specific example, Li et al. [80] found that FFSS activates pERK and promotes the latter to bind to Runx2-targeted promoters, which leads to histone acetylation and elevated gene expression. Besides, the miRNA expression profile is also greatly changed in pre-osteoblasts under mechanical stimulation [81]. Several mechano-sensitively expressed miRNAs have been identified. Targets of these miRNAs include osteoblastic markers and signaling pathway components. Therefore, these mechano-sensitive miRNAs help to establish the osteoblastic phenotype by responding to mechanical signals.

The Impact of Increased Adipose Tissue on Osteoblastic Differentiation

Changed environmental factors under the pathological conditions discussed above all attribute to the differentiation of additional adipocytes from a stem cell pool. Fat tissue is now considered as an endocrine organ capable of expressing and secreting many different auto-
crine, paracrine, and endocrine factors [82]. In vitro culture assays have found that osteoblastic differentiation is inhibited in adipocyte-conditioned media or when cocultured with adipocytes [83, 84], indicating the negative effect of adipocyte-secreted factors on osteoblastogenesis. Therefore, the increased adipose tissue has a severe impact on the bone marrow microenvironment by its secreted factors, accelerating bone loss and fat accumulation. These factors include proinflammatory cytokines, FFAs, adipokines, and exosomes.

Proinflammatory Cytokines
An inflammatory microenvironment is the main characteristic of pathological bone marrow. Chronic inflammation is known to mediate bone loss by accelerating osteoclastic bone resorption. Accumulated marrow fat plays a major role as initiator of bone marrow inflammation by expression and secretion of proinflammatory cytokines such as tumor necrosis factor alpha (TNFα), IL-6, and monocyte chemoattractant protein-1 (MCP-1) [85, 86]. These cytokines promote a cascade of events that result in the recruitment of inflammatory T-lymphocyte subsets, mast cells, monocytes, and macrophages from the blood [87]. These infiltrated immune cells in adipose tissue secrete more proinflammatory factors and together contribute to an inflammatory microenvironment. Under these conditions, osteoblast-mediated bone formation cannot compensate for bone resorption, suggesting a direct inhibitory effect of the inflammatory environment on osteoblastogenesis. Among these cytokines, TNFα has been reported to act upstream of other cytokines and to play the primary role in inflammation. Several lines of evidence show that TNFα inhibits both the commitment and the maturation of osteoblasts at multiple levels. In vitro studies found that TNFα inhibits Runx2 mRNA transcription and promotes protein degradation in a proteosome-dependent manner by upregulating smurf1/2 protein levels [88, 89]. BMP2 would protect Runx2 from Smurf-catalyzed proteolysis by stimulating Runx2 acetylation in a smad-dependent manner [90]. However, under pathological conditions, TNFα blocks both BMP2- and TGFβ-induced Smad signaling pathway through upregulating Smad7 and/or promoting proteolysis of Smads [91–93]. Besides, TNFα also downregulates both the expression and the function of another osteogenic key factor, Osx. The inhibitory effect of TNFα on Osx expression is mediated by MEK1/ERK1 signal and Prx1, a homeobox protein [94, 95]. The NF-κB pathway is one of the main signaling pathways activated by proinflammatory cytokines. TNFα stimulation promotes NF-κB separation from its inhibitor, and subsequent translocation into nucleus. The function of NF-κB as a transcriptional factor needs coactivator P300/CBP [96, 97], which improves gene expression through histone acetylation at target promoters. However, the molecular mechanisms and epigenetic roles in the inhibitory effect of NF-κB on osteoblastic genes such as Runx2 and Osx are still unclear [98]. miRNAs might be involved in the inhibition of NF-κB on osteoblastic differentiation. Once activated by the inflammatory microenvironment, NF-κB upregulates the expression level of miR-3077-5P and miR-705 during osteoblastogenesis, which target Runx2 and HoxA10, respectively [99]. Both Runx2 and HoxA10 deficiency results in automatic adipogenesis, indicating their determinate roles in osteoblastic determination of MSCs. As a combined result of blocked cell signals (BMP2, Wnt, and TGFβ) and downregulated transcriptional factors (Runx2, Osx, and HoxA10), MSCs from inflammatory bone marrow tend to form adipocytes over osteoblasts in in vitro cultures.

Free Fatty Acids
In an inflammatory microenvironment, both differentiation and function of adipocytes are severely affected. Proinflammatory cytokines are important mediators of insulin re-
TNFα suppresses the expression of many proteins that are required for insulin sensitivity, such as insulin receptor, glucose transporter type 4 (Glut4), and adiponectin. Consequently, FFAs uptake and lipogenesis is inhibited, while FFA release is accelerated as a result of TNFα-stimulated lipolysis. The cytotoxic effect of FFAs is termed lipotoxicity. These FFAs generate more ROS during oxidation and contribute to systemic dysfunction [100]. Under such conditions, even pre-adipocytes become increasingly susceptible to lipotoxicity [101]. Especially under saturated FFAs, adipocytes express more proinflammatory cytokines, setting up a vicious cycle that accelerates osteoblast dysfunction and bone loss. As natural ligands for PPARγ, excessive FFAs and their metabolites released into bone marrow during aging or GC treatment would affect gene expression by activating PPARγ and drive osteoblasts trans-differentiate into adipocytes [102, 103]. PPARγ also belongs to the NR superfamily of transcription factors. Normally, PPARγ interacts with corepressors and inhibits target gene expression [104]. Upon ligand activation, corepressors are degraded while coactivators are recruited to PPARγ, which then induces target gene expression through binding to PPAR response elements as a heterodimer with retinoid X receptor (RXR) [104]. Activation of PPARγ during osteoblastogenesis inhibits the transcriptional activity of Runx2 and turns osteoblasts into adipocytes [107–109] by mechanisms that might include epigenetic modulation. Like ERs, most PPARγ cofactors belong to epigenetic modification enzymes. In fact, it is common for NRs to integrate their ligand signals into epigenetic code through interaction with epigenetic modification enzymes [105, 106]. Yet, the specific molecular mechanism of PPARγ in osteoblastogenesis that cooperates with epigenetic modulation is far from understood.

Adipokines

Cytokines secreted from adipose tissue are termed adipokines, such as leptin, adiponectin, chemerin, omentin, and resistin, which have profound effects on surrounding cells. In osteoporotic bone marrow of postmenopausal women, the levels of leptin and adiponectin were found significantly decreased [110]. Besides, the affinity of leptin receptors on osteoporotic MSCs is much lower [111], indicating decreased leptin signal activity. As bone marrow microenvironmental factors, the changed adipokine signals may affect the osteoblastic/adipocytic differentiation balance. In vitro tests confirmed that both leptin and adiponectin have positive effects on osteoblast proliferation and differentiation, but negatively regulate adipogenesis through their receptors expressed on both lineages [112–114]. The role of other adipokines in MSC differentiation is less clear; however, recent findings suggest that most of these adipokines may play a role in regulating bone metabolism and remodeling [115].

Exosomes

Cultured adipocytes reportedly release exosomes that may play a role in cell-to-cell communication [116]. Exosomes are membrane vesicles and carry a cargo of proteins, lipids, and nuclear acids. Adipocyte-secreted exosomes are demonstrated to contain specific transcripts and miRNAs, which are transported into recipient cells and are involved in the upregulation of lipogenesis and cell size [117]. The cargo components may differ with adipogenic stages and/or under pathological conditions [116]. Until now, no research has been conducted to investigate the possible differences of exosomes secreted by bone marrow adipose tissue under osteoporotic conditions and the effects these exosomes may have on MSCs determination and osteoblastogenesis.
Conclusion and Therapeutic Implications

The stem cell differentiation paradigm is based on the progression of cells through generations of daughter cells that eventually become restricted and committed to 1 lineage, resulting in fully differentiated cells. As the commitment and differentiation process is tightly controlled by their supporting microenvironment, changed environmental signals result in an abnormal differentiation pathway of stem cells. Progressive changes in the bone marrow microenvironment occur under pathological conditions linked with bone loss, leading to shifted MSC differentiation to favor adipocytes over osteoblasts. In osteoporotic bone marrow that is caused by aging, estrogen deficiency, chronic GC treatment, and decreased mechanical loading, the microenvironment has some similarities, which are characterized by increased oxidative stress, suppressed osteogenic signals, and elevated osteoblastic inhibitors. The effects of pathological environmental factors combined together that predetermine adipogenic fate of bone marrow MSCs inhibit the osteoblastogenic pathway and induce dysdifferentiation of pre-osteoblasts into adipocytes. As a consequence of marrow obesity, pathological bone marrow is aggravated by chronic inflammation with progressive immune cell infiltration and greatly increased proinflammatory cytokines. An inflammatory marrow microenvironment accelerates bone loss by osteoclastic bone resorption and promotes FFA releasing by impairing adipocyte functions. Excessive FFAs in the bone marrow set up a vicious cycle by aggravating oxidative stress and inflammation. Besides, accumulated adipose tissue in osteoporotic bone marrow can also change the mechanical environment. Clinical investigation has found that intramedullary hydrostatic pressure is greatly increased in osteoporotic bone, whereas overall FFSS is significantly decreased [118]. Together, the changed biochemical and mechanical environment contributes to osteoporosis by way of altering the microenvironment of the bone marrow progenitors, precursors, and stem cells (Fig. 1).

These common features of the bone marrow microenvironment under pathological conditions make it possible to investigate common therapeutic targets to treat bone loss. Traditional therapeutic strategies to combat bone loss and osteoporosis have centered almost exclusively on anti-bone resorption agents designed to prevent further bone breakdown in patients already at high risk for fracture. As preventing further bone resorption cannot ameliorate low BMD in these patients, attention has been focused on the development of anabolic agents to actively rebuild lost bone mass. Considering the reciprocal relationship between adipocytes and osteoblasts, as well as the contributing role of adipose tissue in pathological changes of bone tissue, targeting adipocyte to treat bone disease would be a promising therapeutic strategy. Potential targets were listed as leptin, PPARγ [119], NF-κB, and factors involved in insulin sensitivity [93].

The epigenetic mechanism plays and important role as a link between the MSC microenvironment and cell fate control. Bone loss is a continuous process not only caused by gene polymorphisms but very likely also by epigenetic modulations of gene expression changes. Since the unbalanced osteoblastic/adipocytic differentiation pathways are likely to be modulated by abnormal epigenetic modulations of lineage-related genes, manipulating epigenetic machinery in MSCs may have a great prospective in alleviating osteoblastic differentiation and bone formation under pathological conditions. There are, however, some aspects of detailed molecular mechanisms that need to be comprehended before a more complete picture on the epigenetic regulatory network is attained. For instance, pivotal information about how epigenetic machinery is built on specific target promoters is still missing, and regulatory mechanisms of environmental signals on the network among epigenetic modification enzymes, tran-
scriptional factors, and target genes are far from understood. These unsolved questions restrict drug target selection and new drug design. Thorough understanding of epigenetic roles and related regulatory mechanisms may provide promising therapeutic targets for bone diseases.

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**Statement of Ethics**

Ethical approval for this investigation was obtained from the Research Ethics Committee of Xinhua Hospital, Shanghai JiaoTong University School of Medicine.

**Disclosure Statement**

The authors declare no conflict of interest.

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Fig. 1. Under pathological conditions, altered bone marrow environmental factors shift MSCs to favor adipocytes over osteoblasts. With advanced age, estrogen deficiency, chronic GC treatment, and decreased mechanical loading, the bone marrow environment has been altered with increased oxidative stress, decreased osteogenic signaling factors (BMPs, Wnts, and TGFβ), elevated osteoblast inhibitors (DKK1, sFRP, and sclerostin), marrow inflammation (immune cell infiltration and high level of proinflammatory cytokines), excessive FFAs, and changed adipokines and exosomes. These factors shift bone marrow MSC differentiation to favor adipocytes over osteoblasts. Furthermore, excessive adipose tissue secretes factors (proinflammatory cytokines, FFAs, adipokines, and exosomes) and sets up a vicious cycle that aggravates the bone marrow microenvironment and accelerates bone loss.