Review

Osteoblast versus Adipocyte: Bone Marrow Microenvironment-Guided Epigenetic Control

Jiao Li^{a, b} Bin Zuo^{a, c} Li Zhang^c Liming Dai^{a, c} Xiaoling Zhang^{a, c}

^aDepartment of Orthopedic Surgery, Xinhua Hospital, Shanghai JiaoTong University School of Medicine (SJTUSM), Shanghai, China; ^bDepartment of Cell Biology and Genetics, Zunyi Medical College, Zunyi, China; ^cThe Key Laboratory of Stem Cell Biology, Institute of Health Sciences, Shanghai Institutes for Biological Sciences (SIBS), Chinese Academy of Sciences (CAS), and Shanghai Jiao Tong University School of Medicine (SJTUSM), Shanghai, China

Keywords

Osteoporosis · Bone marrow microenvironment · Mesenchymal stem cell differentiation · Epigenetic · Osteoblast · Adipocyte

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

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 indispensable 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 transmits 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 PPAR γ 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, ER α and ER β . Once activated by estrogen, ERs positively regulate osteoblastic differentiation by crosstalking with multiple osteogenic signals (BMPs, Wnts, TGF β , 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 PPAR γ . ERs are sharing a similar pool of cofactors with PPAR γ , which provides a platform for mutual interactions between these two nuclear hormone receptors. It was found that activated ER prevents PPAR γ from recruiting its cofactors 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 cofactors are indispensable in activating target gene transcription by building active epigenetic signatures, losing these factors inhibits ligand-activated PPAR γ transcriptional activity. Consistent with its important role in regulating MSC determination, mice with constitutively active ER α in osteoblasts have much higher BMD than normal controls [52]. However, a clinical investigation found that the expression of ER α is much lower in postmenopausal women due to higher DNA methylation at its promoter [53]. The underlying molecular mechanism of ER α 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/EBP β and C/EBP δ [54]. Once expressed, C/EBP β binds to C/EBP α promoter and recruits corepressors HDAC1 and mSin3A to inhibit gene transcription [55]. GC releases C/EBP β from its corepressors and recruits coactivator P300/CBP to C/EBP α 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/EBP β 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/EBP α 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 TGF β 1 [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 (TNF α), 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, TNF α has been reported to act upstream of other cytokines and to play the primary role in inflammation. Several lines of evidence show that TNF α inhibits both the commitment and the maturation of osteoblasts at multiple levels. In vitro studies found that TNF α inhibits Runx2 mRNA transcription and promotes protein degradation in a proteasome-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, TNF α blocks both BMP2- and TGF β -induced Smad signaling pathway through upregulating Smad7 and/or promoting proteolysis of Smads [91–93]. Besides, TNF α also downregulates both the expression and the function of another osteogenic key factor, Osx. The inhibitory effect of TNF α on Osx expression is mediated by MEK1/ERK1 signal and Prx1, a homeobox protein [94, 95]. The NF- κ B pathway is one of the main signaling pathways activated by proinflammatory cytokines. TNF α stimulation promotes NF- κ B separation from its inhibitor, and subsequent translocation into nucleus. The function of NF- κ B 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- κ B on osteoblastic genes such as Runx2 and Osx are still unclear [98]. miRNAs might be involved in the inhibition of NF- κ B on osteoblastic differentiation. Once activated by the inflammatory microenvironment, NF- κ B 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 TGF β) 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-

sistance. $\text{TNF}\alpha$ 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 $\text{TNF}\alpha$ -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 PPAR γ , excessive FFAs and their metabolites released into bone marrow during aging or GC treatment would affect gene expression by activating PPAR γ and drive osteoblasts trans-differentiate into adipocytes [102, 103]. PPAR γ also belongs to the NR superfamily of transcription factors. Normally, PPAR γ interacts with corepressors and inhibits target gene expression [104]. Upon ligand activation, corepressors are degraded while coactivators are recruited to PPAR γ , which then induces target gene expression through binding to PPAR response elements as a heterodimer with retinoid X receptor (RXR) [104]. Activation of PPAR γ 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 PPAR γ 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 PPAR γ 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 micro-environmental 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 dys-differentiation 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, PPAR γ [119], NF- κ B, and factors involved in insulin sensitivity [93].

The epigenetic mechanism plays an 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.

Acknowledgement

This work was supported by grants from the National Natural Science Foundation of China (No. 81772347), Science and Technology Commission of Shanghai Municipality (No. 16430723500), Shanghai Municipal Education Commission-Gaofeng Clinical Medicine Grant Support (No.20161314), Shanghai Shen Kang hospital development center (No. 16CR2036B), and Unit Foundation of Guizhou Science and Technology Department, Zunyi Science and Technology Bureau, Zunyi Medical College ([2015] 7553).

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.

References

- Meunier P, Aaron J, Edouard C, Vignon G. Osteoporosis and the replacement of cell populations of the marrow by adipose tissue. A quantitative study of 84 iliac bone biopsies. *Clin Orthop Relat Res*. 1971 Oct;80(80):147–54.
- Verma S, Rajaratnam JH, Denton J, Hoyland JA, Byers RJ. Adipocytic proportion of bone marrow is inversely related to bone formation in osteoporosis. *J Clin Pathol*. 2002 Sep;55(9):693–8.
- Yeung DK, Griffith JF, Antonio GE, Lee FK, Woo J, Leung PC. Osteoporosis is associated with increased marrow fat content and decreased marrow fat unsaturation: a proton MR spectroscopy study. *J Magn Reson Imaging*. 2005 Aug;22(2):279–85.
- Bennett JH, Joyner CJ, Triffitt JT, Owen ME. Adipocytic cells cultured from marrow have osteogenic potential. *J Cell Sci*. 1991 May;99(Pt 1):131–9.
- Beresford JN, Bennett JH, Devlin C, Leboy PS, Owen ME. Evidence for an inverse relationship between the differentiation of adipocytic and osteogenic cells in rat marrow stromal cell cultures. *J Cell Sci*. 1992 Jun;102(Pt 2):341–51.
- Muruganandan S, Roman AA, Sinal CJ. Adipocyte differentiation of bone marrow-derived mesenchymal stem cells: cross talk with the osteoblastogenic program. *Cell Mol Life Sci*. 2009 Jan;66(2):236–53.
- Post S, Abdallah BM, Bentzon JF, Kassem M. Demonstration of the presence of independent pre-osteoblastic and pre-adipocytic cell populations in bone marrow-derived mesenchymal stem cells. *Bone*. 2008 Jul;43(1):32–9.
- Lunyak VV, Rosenfeld MG. Epigenetic regulation of stem cell fate. *Hum Mol Genet*. 2008 Apr;17 R1:R28–36.
- Scheideler M, Elabd C, Zaragosi LE, Chiellini C, Hackl H, Sanchez-Cabo F et al. Comparative transcriptomics of human multipotent stem cells during adipogenesis and osteoblastogenesis. *BMC Genomics*. 2008 Jul;9(1):340.
- Chen XD, Dusevich V, Feng JQ, Manolagas SC, Jilka RL. Extracellular matrix made by bone marrow cells facilitates expansion of marrow-derived MSCs and prevents their differentiation into osteoblast. *J Bone Miner Res*. 2007;22:1943–56.

- 11 Hassan MQ, Tare R, Lee SH, Mandeville M, Weiner B, Montecino M et al. HOXA10 controls osteoblastogenesis by directly activating bone regulatory and phenotypic genes. *Mol Cell Biol*. 2007 May;27(9):3337–52.
- 12 Linkhart TA, Mohan S, Baylink DJ. Growth factors for bone growth and repair: IGF, TGF beta and BMP. *Bone*. 1996 Jul;19(1 Suppl):1S–12S.
- 13 Bismar H, Klöppinger T, Schuster EM, Balbach S, Diel I, Ziegler R et al. Transforming growth factor beta (TGF-beta) levels in the conditioned media of human bone cells: relationship to donor age, bone volume, and concentration of TGF-beta in human bone matrix in vivo. *Bone*. 1999 Jun;24(6):565–9.
- 14 Kang JS, Alliston T, Delston R, Derynck R. Repression of Runx2 function by TGF-beta through recruitment of class II histone deacetylases by Smad3. *EMBO J*. 2005 Jul;24(14):2543–55.
- 15 Tang Y, Wu X, Lei W, Pang L, Wan C, Shi Z et al. TGF-beta1-induced migration of bone mesenchymal stem cells couples bone resorption with formation. *Nat Med*. 2009 Jul;15(7):757–65.
- 16 Wang YK, Chen CS. Cell adhesion and mechanical stimulation in the regulation of mesenchymal stem cell differentiation. *J Cell Mol Med*. 2013 Jul;17(7):823–32.
- 17 McBeath R, Pirone DM, Nelson CM, Bhadriraju K, Chen CS. Cell shape, cytoskeletal tension, and RhoA regulate stem cell lineage commitment. *Dev Cell*. 2004 Apr;6(4):483–95.
- 18 Gemini-Piperni S, Milani R, Bertazzo S, Peppelenbosch M, Takamori ER, Granjeiro JM et al. Kinome profiling of osteoblasts on hydroxyapatite opens new avenues on biomaterial cell signaling. *Biotechnol Bioeng*. 2014 Sep;111(9):1900–5.
- 19 Boutahar N, Guignandon A, Vico L, Lafage-Proust MH. Mechanical strain on osteoblasts activates autophosphorylation of focal adhesion kinase and proline-rich tyrosine kinase 2 tyrosine sites involved in ERK activation. *J Biol Chem*. 2004 Jul;279(29):30588–99.
- 20 Engler AJ, Sen S, Sweeney HL, Discher DE. Matrix elasticity directs stem cell lineage specification. *Cell*. 2006 Aug;126(4):677–89.
- 21 Tong W, Brown SE, Krebsbach PH. Human embryonic stem cells undergo osteogenic differentiation in human bone marrow stromal cell microenvironments. *J Stem Cells*. 2007;2(3):139–47.
- 22 Li J, Zhang N, Huang X, Xu J, Fernandes JC, Dai K et al. Dexamethasone shifts bone marrow stromal cells from osteoblasts to adipocytes by C/EBPalpha promoter methylation. *Cell Death Dis*. 2013 Oct;4(10):e832.
- 23 Fan Q, Tang T, Zhang X, Dai K. The role of CCAAT/enhancer binding protein (C/EBP)-alpha in osteogenesis of C3H10T1/2 cells induced by BMP-2. *J Cell Mol Med*. 2009 Aug;13(8b 8B):2489–505.
- 24 Takada I, Mihara M, Suzawa M, Ohtake F, Kobayashi S, Igarashi M et al. A histone lysine methyltransferase activated by non-canonical Wnt signalling suppresses PPAR-gamma transactivation. *Nat Cell Biol*. 2007 Nov;9(11):1273–85.
- 25 Song L, Liu M, Ono N, Bringham FR, Kronenberg HM, Guo J. Loss of wnt/ β -catenin signaling causes cell fate shift of preosteoblasts from osteoblasts to adipocytes. *J Bone Miner Res*. 2012 Nov;27(11):2344–58.
- 26 Rodríguez JP, Astudillo P, Ríos S, Pino AM. Involvement of adipogenic potential of human bone marrow mesenchymal stem cells (MSCs) in osteoporosis. *Curr Stem Cell Res Ther*. 2008 Sep;3(3):208–18.
- 27 Rodríguez JP, Montecinos L, Ríos S, Reyes P, Martínez J. Mesenchymal stem cells from osteoporotic patients produce a type I collagen-deficient extracellular matrix favoring adipogenic differentiation. *J Cell Biochem*. 2000 Sep;79(4):557–65.
- 28 Benisch P, Schilling T, Klein-Hitpass L, Frey SP, Seefried L, Raaijmakers N et al. The transcriptional profile of mesenchymal stem cell populations in primary osteoporosis is distinct and shows overexpression of osteogenic inhibitors. *PLoS One*. 2012;7(9):e45142.
- 29 Chen TL. Inhibition of growth and differentiation of osteoprogenitors in mouse bone marrow stromal cell cultures by increased donor age and glucocorticoid treatment. *Bone*. 2004 Jul;35(1):83–95.
- 30 Moerman EJ, Teng K, Lipschitz DA, Lecka-Czernik B. Aging activates adipogenic and suppresses osteogenic programs in mesenchymal marrow stroma/stem cells: the role of PPAR-gamma2 transcription factor and TGF-beta/BMP signaling pathways. *Aging Cell*. 2004 Dec;3(6):379–89.
- 31 Bai XC, Lu D, Bai J, Zheng H, Ke ZY, Li XM et al. Oxidative stress inhibits osteoblastic differentiation of bone cells by ERK and NF-kappaB. *Biochem Biophys Res Commun*. 2004 Jan;314(1):197–207.
- 32 Basu S, Michaëlsson K, Olofsson H, Johansson S, Melhus H. Association between oxidative stress and bone mineral density. *Biochem Biophys Res Commun*. 2001 Oct;288(1):275–9.
- 33 Chen Y, Chen L, Yin Q, Gao H, Dong P, Zhang X et al. Reciprocal interferences of TNF- α and Wnt1/ β -catenin signaling axes shift bone marrow-derived stem cells towards osteoblast lineage after ethanol exposure. *Cell Physiol Biochem*. 2013;32(3):755–65.
- 34 Chen Y, Gao H, Yin Q, Chen L, Dong P, Zhang X et al. ER stress activating ATF4/CHOP-TNF- α signaling pathway contributes to alcohol-induced disruption of osteogenic lineage of multipotential mesenchymal stem cell. *Cell Physiol Biochem*. 2013;32(3):743–54.
- 35 Almeida M, Martin-Millan M, Ambrogini E, Bradsher R 3rd, Han L, Chen XD et al. Estrogens attenuate oxidative stress and the differentiation and apoptosis of osteoblasts by DNA-binding-independent actions of the ERalpha. *J Bone Miner Res*. 2010 Apr;25(4):769–81.
- 36 Feng YL, Tang XL. Effect of glucocorticoid-induced oxidative stress on the expression of Cbfa1. *Chem Biol Interact*. 2014 Jan;207:26–31.

- 37 Chen Q, Liu K, Robinson AR, Clauson CL, Blair HC, Robbins PD et al. DNA damage drives accelerated bone aging via an NF- κ B-dependent mechanism. *J Bone Miner Res*. 2013 May;28(5):1214–28.
- 38 Saeed H, Abdallah BM, Ditzel N, Catala-Lehnen P, Qiu W, Amling M et al. Telomerase-deficient mice exhibit bone loss owing to defects in osteoblasts and increased osteoclastogenesis by inflammatory microenvironment. *J Bone Miner Res*. 2011 Jul;26(7):1494–505.
- 39 Nicolaije C, Diderich KE, Botter SM, Priemel M, Waarsing JH, Day JS et al. Age-related skeletal dynamics and decrease in bone strength in DNA repair deficient male trichothiodystrophy mice. *PLoS One*. 2012;7(4):e35246.
- 40 Bai XC, Lu D, Bai J, Zheng H, Ke ZY, Li XM et al. Oxidative stress inhibits osteoblastic differentiation of bone cells by ERK and NF-kappaB. *Biochem Biophys Res Commun*. 2004 Jan;314(1):197–207.
- 41 Almeida M, Han L, Martin-Millan M, O'Brien CA, Manolagas SC. Oxidative stress antagonizes Wnt signaling in osteoblast precursors by diverting beta-catenin from T cell factor- to forkhead box O-mediated transcription. *J Biol Chem*. 2007 Sep;282(37):27298–305.
- 42 Turker I, Zhang Y, Rehman J. Oxidative stress as a regulator of adipogenesis. *FASEB J*. 2007;21:830–5.
- 43 Kang MI, Kim HS, Jung YC, Kim YH, Hong SJ, Kim MK et al. Transitional CpG methylation between promoters and retroelements of tissue-specific genes during human mesenchymal cell differentiation. *J Cell Biochem*. 2007 Sep;102(1):224–39.
- 44 Krum SA, Chang J, Miranda-Carboni G, Wang CY. Novel functions for NF κ B: inhibition of bone formation. *Nat Rev Rheumatol*. 2010 Oct;6(10):607–11.
- 45 Matsumoto Y, Otsuka F, Takano-Narazaki M, Katsuyama T, Nakamura E, Tsukamoto N et al. Estrogen facilitates osteoblast differentiation by upregulating bone morphogenetic protein-4 signaling. *Steroids*. 2013 May;78(5):513–20.
- 46 Matsumoto Y, Otsuka F, Takano M, Mukai T, Yamanaka R, Takeda M et al. Estrogen and glucocorticoid regulate osteoblast differentiation through the interaction of bone morphogenetic protein-2 and tumor necrosis factor-alpha in C2C12 cells. *Mol Cell Endocrinol*. 2010 Aug;325(1-2):118–27.
- 47 Zhao JW, Gao ZL, Mei H, Li YL, Wang Y. Differentiation of human mesenchymal stem cells: the potential mechanism for estrogen-induced preferential osteoblast versus adipocyte differentiation. *Am J Med Sci*. 2011 Jun;341(6):460–8.
- 48 Kouzmenko AP, Takeyama K, Ito S, Furutani T, Sawatsubashi S, Maki A et al. Wnt/beta-catenin and estrogen signaling converge in vivo. *J Biol Chem*. 2004 Sep;279(39):40255–8.
- 49 Foo C, Frey S, Yang HH, Zellweger R, Filgueira L. Downregulation of beta-catenin and transdifferentiation of human osteoblasts to adipocytes under estrogen deficiency. *Gynecol Endocrinol*. 2007 Sep;23(9):535–40.
- 50 Foryst-Ludwig A, Clemenz M, Hohmann S, Hartge M, Sprang C, Frost N et al. Metabolic actions of estrogen receptor beta (ERbeta) are mediated by a negative cross-talk with PPARgamma. *PLoS Genet*. 2008 Jun;4(6):e1000108.
- 51 Jeong S, Yoon M. 17 β -Estradiol inhibition of PPAR γ -induced adipogenesis and adipocyte-specific gene expression. *Acta Pharmacol Sin*. 2011 Feb;32(2):230–8.
- 52 Ikeda K, Tsukui T, Horie-Inoue K, Inoue S. Conditional expression of constitutively active estrogen receptor α in osteoblasts increases bone mineral density in mice. *FEBS Lett*. 2011 May;585(9):1303–9.
- 53 Lv H, Ma X, Che T, Chen Y. Methylation of the promoter A of estrogen receptor alpha gene in hBMSC and osteoblasts and its correlation with homocysteine. *Mol Cell Biochem*. 2011 Sep;355(1-2):35–45.
- 54 Yeh WC, Cao Z, Classon M, McKnight SL. Cascade regulation of terminal adipocyte differentiation by three members of the C/EBP family of leucine zipper proteins. *Genes Dev*. 1995 Jan;9(2):168–81.
- 55 Wiper-Bergeron N, Wu D, Pope L, Schild-Poulter C, Haché RJ. Stimulation of preadipocyte differentiation by steroid through targeting of an HDAC1 complex. *EMBO J*. 2003 May;22(9):2135–45.
- 56 Wiper-Bergeron N, Salem HA, Tomlinson JJ, Wu D, Haché RJ. Glucocorticoid-stimulated preadipocyte differentiation is mediated through acetylation of C/EBPbeta by GCN5. *Proc Natl Acad Sci USA*. 2007 Feb;104(8):2703–8.
- 57 Steger DJ, Grant GR, Schupp M, Tomaru T, Lefterova MI, Schug J et al. Propagation of adipogenic signals through an epigenomic transition state. *Genes Dev*. 2010 May;24(10):1035–44.
- 58 Koromila T, Baniwal SK, Song YS, Martin A, Xiong J, Frenkel B. Glucocorticoids antagonize RUNX2 during osteoblast differentiation in cultures of ST2 pluripotent mesenchymal cells. *J Cell Biochem*. 2014 Jan;115(1):27–33.
- 59 Zhang YY, Li X, Qian SW, Guo L, Huang HY, He Q et al. Down-regulation of type I Runx2 mediated by dexamethasone is required for 3T3-L1 adipogenesis. *Mol Endocrinol*. 2012 May;26(5):798–808.
- 60 Brunetti G, Faienza MF, Piacente L, Ventura A, Oranger A, Carbone C et al. High dickkopf-1 levels in sera and leukocytes from children with 21-hydroxylase deficiency on chronic glucocorticoid treatment. *Am J Physiol Endocrinol Metab*. 2013 Mar;304(5):E546–54.
- 61 Butler JS, Queally JM, Devitt BM, Murray DW, Doran PP, O'Byrne JM. Silencing Dkk1 expression rescues dexamethasone-induced suppression of primary human osteoblast differentiation. *BMC Musculoskelet Disord*. 2010 Sep;11(1):210.

- 62 Hurson CJ, Butler JS, Keating DT, Murray DW, Sadler DM, O'Byrne JM et al. Gene expression analysis in human osteoblasts exposed to dexamethasone identifies altered developmental pathways as putative drivers of osteoporosis. *BMC Musculoskelet Disord*. 2007 Feb;8(1):12.
- 63 Yao W, Cheng Z, Busse C, Pham A, Nakamura MC, Lane NE. Glucocorticoid excess in mice results in early activation of osteoclastogenesis and adipogenesis and prolonged suppression of osteogenesis: a longitudinal study of gene expression in bone tissue from glucocorticoid-treated mice. *Arthritis Rheum*. 2008 Jun;58(6):1674–86.
- 64 Ko JY, Chuang PC, Chen MW, Ke HC, Wu SL, Chang YH et al. MicroRNA-29a ameliorates glucocorticoid-induced suppression of osteoblast differentiation by regulating β -catenin acetylation. *Bone*. 2013 Dec;57(2):468–75.
- 65 Smith E, Frenkel B. Glucocorticoid inhibit the transcriptional activity of LEF/TCF in differentiating osteoblast in a GSK-3 β -dependent and independent manner. *J Biol Chem*. 2005;280:2388–94.
- 66 Wang FS, Chuang PC, Lin CL, Chen MW, Ke HJ, Chang YH et al. MicroRNA-29a protects against glucocorticoid-induced bone loss and fragility in rats by orchestrating bone acquisition and resorption. *Arthritis Rheum*. 2013 Jun;65(6):1530–40.
- 67 Krahle H, Michaelis U, Pieper HG, Quack G, Montag M. Stimulation of bone growth through sports. A radiologic investigation of the upper extremities in professional tennis players. *Am J Sports Med*. 1994 Nov-Dec;22(6):751–7.
- 68 Leblanc AD, Schneider VS, Evans HJ, Engelbretson DA, Krebs JM. Bone mineral loss and recovery after 17 weeks of bed rest. *J Bone Miner Res*. 1990 Aug;5(8):843–50.
- 69 Keyak JH, Koyama AK, LeBlanc A, Lu Y, Lang TF. Reduction in proximal femoral strength due to long-duration spaceflight. *Bone*. 2009 Mar;44(3):449–53.
- 70 Bonewald LF. Osteocytes as dynamic multifunctional cells. *Ann N Y Acad Sci*. 2007 Nov;1116(1):281–90.
- 71 Hoey DA, Kelly DJ, Jacobs CR. A role for the primary cilium in paracrine signaling between mechanically stimulated osteocytes and mesenchymal stem cells. *Biochem Biophys Res Commun*. 2011 Aug;412(1):182–7.
- 72 Jia YY, Li F, Geng N, Gong P, Huang SJ, Meng LX et al. Fluid flow modulates the expression of genes involved in the Wnt signaling pathway in osteoblasts in 3D culture conditions. *Int J Mol Med*. 2014 May;33(5):1282–8.
- 73 Shirakawa J, Ezura Y, Moriya S, Kawasaki M, Yamada T, Notomi T et al. Migration linked to Fucci-indicated cell cycle is controlled by PTH and mechanical stress. *J Cell Physiol*. 2014 Oct;229(10):1353–8.
- 74 Loisel AE, Lloyd SA, Paul EM, Lewis GS, Donahue HJ. Inhibition of GSK-3 β rescues the impairments in bone formation and mechanical properties associated with fracture healing in osteoblast selective connexin 43 deficient mice. *PLoS One*. 2013 Nov;8(11):e81399.
- 75 Grimston SK, Watkins MP, Stains JP, Civitelli R. Connexin43 modulates post-natal cortical bone modeling and mechano-responsiveness. *Bonekey Rep*. 2013 Nov;2:446.
- 76 Friedl G, Schmidt H, Rehak I, Kostner G, Schauenstein K, Windhager R. Undifferentiated human mesenchymal stem cells (hMSCs) are highly sensitive to mechanical strain: transcriptionally controlled early osteochondrogenic response in vitro. *Osteoarthritis Cartilage*. 2007 Nov;15(11):1293–300.
- 77 Mirza S, Greenwood M, Blunn G. The effect of tensile forces on the differentiation of mesenchymal stem cells. *Bone*. 2007;40:S213–66.
- 78 Kim KM, Choi YJ, Hwang JH, Kim AR, Cho HJ, Hwang ES et al. Shear stress induced by an interstitial level of slow flow increases the osteogenic differentiation of mesenchymal stem cells through TAZ activation. *PLoS One*. 2014 Mar;9(3):e92427.
- 79 Arnsdorf EJ, Tummala P, Castillo AB, Zhang F, Jacobs CR. The epigenetic mechanism of mechanically induced osteogenic differentiation. *J Biomech*. 2010 Nov;43(15):2881–6.
- 80 Li Y, Ge C, Long JP, Begun DL, Rodriguez JA, Goldstein SA et al. Biomechanical stimulation of osteoblast gene expression requires phosphorylation of the RUNX2 transcription factor. *J Bone Miner Res*. 2012 Jun;27(6):1263–74.
- 81 Mai ZH, Peng ZL, Zhang JL, Chen L, Liang HY, Cai B et al. miRNA expression profile during fluid shear stress-induced osteogenic differentiation in MC3T3-E1 cells. *Chin Med J (Engl)*. 2013;126(8):1544–50.
- 82 Romacho T, Elsen M, Röhrborn D, Eckel J. Adipose tissue and its role in organ crosstalk. *Acta Physiol (Oxf)*. 2014 Apr;210(4):733–53.
- 83 Dong X, Bi L, He S, Meng G, Wei B, Jia S et al. FFAs-ROS-ERK/P38 pathway plays a key role in adipocyte lipotoxicity on osteoblasts in co-culture. *Biochimie*. 2014 Jun;101:123–31.
- 84 Maurin AC, Chavassieux PM, Frappart L, Delmas PD, Serre CM, Meunier PJ. Influence of mature adipocytes on osteoblast proliferation in human primary cocultures. *Bone*. 2000 May;26(5):485–9.
- 85 Suganami T, Nishida J, Ogawa Y. A paracrine loop between adipocytes and macrophages aggravates inflammatory changes: role of free fatty acids and tumor necrosis factor alpha. *Arterioscler Thromb Vasc Biol*. 2005 Oct;25(10):2062–8.
- 86 Suganami T, Tanimoto-Koyama K, Nishida J, Itoh M, Yuan X, Mizuarai S et al. Role of the Toll-like receptor 4/NF-kappaB pathway in saturated fatty acid-induced inflammatory changes in the interaction between adipocytes and macrophages. *Arterioscler Thromb Vasc Biol*. 2007 Jan;27(1):84–91.

- 87 Kintscher U, Hartge M, Hess K, Foryst-Ludwig A, Clemenz M, Wabitsch M et al. T-lymphocyte infiltration in visceral adipose tissue: a primary event in adipose tissue inflammation and the development of obesity-mediated insulin resistance. *Arterioscler Thromb Vasc Biol*. 2008 Jul;28(7):1304–10.
- 88 Gilbert L, He X, Farmer P, Rubin J, Drissi H, van Wijnen AJ et al. Expression of the osteoblast differentiation factor RUNX2 (Cbfa1/AML3/Pebp2alpha A) is inhibited by tumor necrosis factor-alpha. *J Biol Chem*. 2002 Jan;277(4):2695–701.
- 89 Kaneki H, Guo R, Chen D, Yao Z, Schwarz EM, Zhang YE et al. Tumor necrosis factor promotes Runx2 degradation through up-regulation of Smurf1 and Smurf2 in osteoblasts. *J Biol Chem*. 2006 Feb;281(7):4326–33.
- 90 Jeon EJ, Lee KY, Choi NS, Lee MH, Kim HN, Jin YH et al. Bone morphogenetic protein-2 stimulates Runx2 acetylation. *J Biol Chem*. 2006 Jun;281(24):16502–11.
- 91 Eliseev RA, Schwarz EM, Zuscik MJ, O'Keefe RJ, Drissi H, Rosier RN. Smad7 mediates inhibition of Saos2 osteosarcoma cell differentiation by NFkappaB. *Exp Cell Res*. 2006 Jan;312(1):40–50.
- 92 Guo R, Yamashita M, Zhang Q, Zhou Q, Chen D, Reynolds DG et al. Ubiquitin ligase Smurf1 mediates tumor necrosis factor-induced systemic bone loss by promoting proteasomal degradation of bone morphogenetic signaling proteins. *J Biol Chem*. 2008 Aug;283(34):23084–92.
- 93 Li Y, Li A, Strait K, Zhang H, Nanes MS, Weitzmann MN. Endogenous TNFalpha lowers maximum peak bone mass and inhibits osteoblastic Smad activation through NF-kappaB. *J Bone Miner Res*. 2007 May;22(5):646–55.
- 94 Lu X, Beck GR Jr, Gilbert LC, Camalier CE, Bateman NW, Hood BL et al. Identification of the homeobox protein Prx1 (MHox, Prrx-1) as a regulator of osterix expression and mediator of tumor necrosis factor α action in osteoblast differentiation. *J Bone Miner Res*. 2011 Jan;26(1):209–19.
- 95 Lu X, Gilbert L, He X, Rubin J, Nanes MS. Transcriptional regulation of the osterix (Ox, Sp7) promoter by tumor necrosis factor identifies disparate effects of mitogen-activated protein kinase and NF kappa B pathways. *J Biol Chem*. 2006 Mar;281(10):6297–306.
- 96 Gerritsen ME, Williams AJ, Neish AS, Moore S, Shi Y, Collins T. CREB-binding protein/p300 are transcriptional coactivators of p65. *Proc Natl Acad Sci USA*. 1997 Apr;94(7):2927–32.
- 97 Mukherjee SP, Behar M, Birnbaum HA, Hoffmann A, Wright PE, Ghosh G. Analysis of the RelA:CBP/p300 interaction reveals its involvement in NF- κ B-driven transcription. *PLoS Biol*. 2013 Sep;11(9):e1001647.
- 98 Dong J, Jimi E, Zhong H, Hayden MS, Ghosh S. Repression of gene expression by unphosphorylated NF-kappaB p65 through epigenetic mechanisms. *Genes Dev*. 2008 May;22(9):1159–73.
- 99 Liao L, Yang X, Su X, Hu C, Zhu X, Yang N et al. Redundant miR-3077-5p and miR-705 mediate the shift of mesenchymal stem cell lineage commitment to adipocyte in osteoporosis bone marrow. *Cell Death Dis*. 2013 Apr;4(4):e600.
- 100 Shen WJ, Liu LF, Patel S, Kraemer FB. Hormone-sensitive lipase-knockout mice maintain high bone density during aging. *FASEB J*. 2011 Aug;25(8):2722–30.
- 101 Guo W, Pirtskhalava T, Tchonia T, Xie W, Thomou T, Han J et al. Aging results in paradoxical susceptibility of fat cell progenitors to lipotoxicity. *Am J Physiol Endocrinol Metab*. 2007 Apr;292(4):E1041–51.
- 102 Wang D, Haile A, Jones LC. Dexamethasone-induced lipolysis increases the adverse effect of adipocytes on osteoblasts using cells derived from human mesenchymal stem cells. *Bone*. 2013 Apr;53(2):520–30.
- 103 Elbaz A, Wu X, Rivas D, Gimble JM, Duque G. Inhibition of fatty acid biosynthesis prevents adipocyte lipotoxicity on human osteoblasts in vitro. *J Cell Mol Med*. 2010 Apr;14(4):982–91.
- 104 Tontonoz P, Spiegelman BM. Fat and beyond: the diverse biology of PPARgamma. *Annu Rev Biochem*. 2008;77(1):289–312.
- 105 Dilworth FJ, Chambon P. Nuclear receptors coordinate the activities of chromatin remodeling complexes and coactivators to facilitate initiation of transcription. *Oncogene*. 2001 May;20(24):3047–54.
- 106 Rosenfeld MG, Lunyak VV, Glass CK. Sensors and signals: a coactivator/corepressor/epigenetic code for integrating signal-dependent programs of transcriptional response. *Genes Dev*. 2006 Jun;20(11):1405–28.
- 107 Yoshiko Y, Oizumi K, Hasegawa T, Minamizaki T, Tanne K, Maeda N et al. A subset of osteoblasts expressing high endogenous levels of PPARgamma switches fate to adipocytes in the rat calvaria cell culture model. *PLoS One*. 2010 Jul;5(7):e11782.
- 108 Lecka-Czernik B, Gubrij I, Moerman EJ, Kajkenova O, Lipschitz DA, Manolagas SC et al. Inhibition of Osf2/Cbfa1 expression and terminal osteoblast differentiation by PPARgamma2. *J Cell Biochem*. 1999 Sep;74(3):357–71.
- 109 Jeon MJ, Kim JA, Kwon SH, Kim SW, Park KS, Park SW et al. Activation of peroxisome proliferator-activated receptor-gamma inhibits the Runx2-mediated transcription of osteocalcin in osteoblasts. *J Biol Chem*. 2003 Jun;278(26):23270–7.
- 110 Pino AM, Ríos S, Astudillo P, Fernández M, Figueroa P, Seitz G et al. Concentration of adipogenic and proinflammatory cytokines in the bone marrow supernatant fluid of osteoporotic women. *J Bone Miner Res*. 2010 Mar;25(3):492–8.
- 111 Hess R, Pino AM, Ríos S, Fernández M, Rodríguez JP. High affinity leptin receptors are present in human mesenchymal stem cells (MSCs) derived from control and osteoporotic donors. *J Cell Biochem*. 2005 Jan;94(1):50–7.

- 112 Astudillo P, Ríos S, Pastenes L, Pino AM, Rodríguez JP. Increased adipogenesis of osteoporotic human-mesenchymal stem cells (MSCs) characterizes by impaired leptin action. *J Cell Biochem*. 2008 Mar;103(4):1054–65.
- 113 Thomas T, Gori F, Khosla S, Jensen MD, Burguera B, Riggs BL. Leptin acts on human marrow stromal cells to enhance differentiation to osteoblasts and to inhibit differentiation to adipocytes. *Endocrinology*. 1999 Apr;140(4):1630–8.
- 114 Luo XH, Guo LJ, Yuan LQ, Xie H, Zhou HD, Wu XP et al. Adiponectin stimulates human osteoblasts proliferation and differentiation via the MAPK signaling pathway. *Exp Cell Res*. 2005 Sep;309(1):99–109.
- 115 Scotece M, Conde J, Abella V, López V, Pino J, Lago F et al. Bone metabolism and adipokines: are there perspectives for bone diseases drug discovery? *Expert Opin Drug Discov*. 2014 Aug;9(8):945–57.
- 116 Sano S, Izumi Y, Yamaguchi T, Yamazaki T, Tanaka M, Shiota M et al. Lipid synthesis is promoted by hypoxic adipocyte-derived exosomes in 3T3-L1 cells. *Biochem Biophys Res Commun*. 2014 Mar;445(2):327–33.
- 117 Müller G, Schneider M, Biemer-Daub G, Wied S. Microvesicles released from rat adipocytes and harboring glycosylphosphatidylinositol-anchored proteins transfer RNA stimulating lipid synthesis. *Cell Signal*. 2011 Jul;23(7):1207–23.
- 118 Gurkan UA, Akkus O. The mechanical environment of bone marrow: a review. *Ann Biomed Eng*. 2008 Dec;36(12):1978–91.
- 119 Kawai M, Devlin MJ, Rosen CJ. Fat targets for skeletal health. *Nat Rev Rheumatol*. 2009 Jul;5(7):365–72.

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.