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Review Article

Adipocyte differentiation of bone marrow-derived mesenchymal stem cells: Cross talk with the osteoblastogenic program

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Abstract. Bone marrow mesenchymal stem cells (MSCs) are multipotent cells, which among other cell lineages, give rise to adipocytes and osteoblasts. Within the bone marrow, the differentiation of MSCs into adipocytes or osteoblasts is competitively balanced; mechanisms that promote one cell fate actively suppress mechanisms that induce the alternative lineage. This occurs through the cross talk between complex signaling pathways including those derived from bone morphogenic proteins (BMPs), wingless-type MMTV integration site (Wnt) proteins, hedgehogs, delta/jagged proteins, fibroblastic growth factors (FGF), insulin, insulin-like growth factors (IGF), and transcriptional regulators of adipocyte and osteoblast differentiation including peroxisome proliferator-activated receptor-γ (PPARγ) and runt-related transcription factor 2 (Runx2). Here, we discuss the molecular regulation of bone marrow adipogenesis with emphasis on signals that interact with osteoblastogenic pathways and highlight the possible therapeutic implications of these interactions.

Keywords. Adipocyte, bone, bone morphogenic proteins, differentiation, mesenchymal stem cell, osteoblast, peroxisome proliferator-activated receptor-γ.

Introduction

Bone is a specialized connective tissue that provides structural support to skeletal muscle, physical protection to vital organs such as the brain and heart, a reserve for minerals such as calcium and phosphate, and a continuous source of hematopoietic stem cells for regeneration of cells of the blood and immune system. Given these vital roles, loss of normal bone structure and function is associated with various diseases, most notably osteoporosis. Throughout life, bone is constantly remodeled through the processes of bone formation by osteoblasts and bone resorption by osteoclasts. Developmentally, osteoclasts are derived from hematopoietic stem cell precursors of the monocyte/macrophage lineage located in the blood and the bone marrow [1], while osteoblasts originate from bone marrow MSCs [2]. In normal, healthy bone, a balance of bone formation/resorption is achieved in large measure through the coordinated differentiation of these cell types from their stem cell precursors. Bone marrow MSCs are a multipotent cell type that can give rise not only to osteoblasts, but also to a range of other cell types including adipocytes, chondrocytes, and myoblasts (Fig. 1) [3]. Among these potential

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fates, differentiation to the osteoblast and adipocyte lineages has particular relevance to the maintenance of normal bone homeostasis. For example, considerable evidence exists to support that a shift in MSC differentiation to favor the adipocyte lineage over the osteoblast lineage can directly contribute to imbalances in bone formation/resorption and ultimately lead to bone loss. This shift of MSC differentiation to the adipocyte lineage may contribute to the progressive increase in adipocyte formation and decrease in osteoblast number that coincides with age-related bone loss [4]. In support of this reciprocal relationship, numerous *in vitro* experiments performed with bone marrow-derived MSCs have demonstrated that factors that induce adipogenesis inhibit osteoblast formation [5, 6] and, likewise, factors that promote osteoblastogenesis inhibit adipocyte formation [7]. Furthermore, the majority of conditions associated with bone loss, including aging, glucocorticoid treatment, increased cortisol production, and osteoporosis, also coincide with increased marrow adiposity (as reviewed in [8, 9]). Adipocytes may further influence bone remodeling through the secretion of fatty acids and adipokines with paracrine actions that may influence the development and function of stem cell precursors as well as mature cell types such as osteoblasts and osteoclasts [10]. Thus, given the close association between adipocyte and osteoblast formation, the potential exists to prevent or treat bone loss by inhibiting bone marrow adipogenesis.

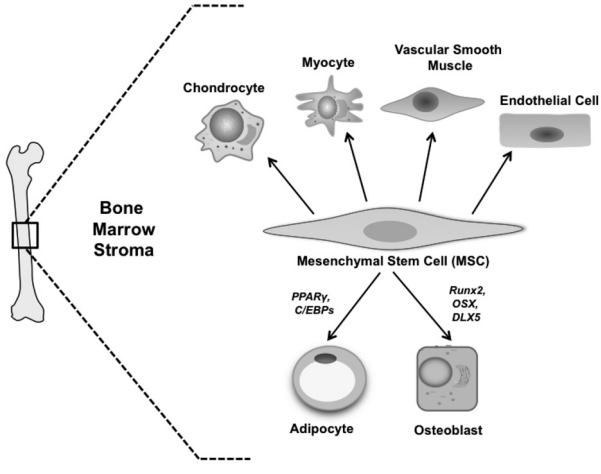

Figure 1. Developmental fates of bone marrow mesenchymal stem cells. Mesenchymal stem cells (MSCs) derived from the bone marrow have the ability to differentiate into multiple cell types (*e.g.,* chondrocyte, myocyte). Adipocyte differentiation from MSCs requires activation of several key transcription factors such as PPARγ and C/EBPs. Other transcription factors such as Runx2, OSX, and DLX5 promote osteoblast differentiation.

In bone marrow, the developmental fate of MSCs is largely determined by the expression of specific groups of transcription factors that act as *molecular switches* to drive the differentiation of uncommitted precursors down a specific lineage. For example, expression of the transcription factors Runx2 and osterix (OSX) are the main determinants of MSC osteoblastogenesis [11, 12]. In contrast, the transcription factor PPARγ (peroxisome proliferator-activated receptor-γ) is the key factor that drives the adipogenic differentiation of MSCs [13]. This review highlights the signaling mechanisms involved in bone marrow adipogenesis and discusses the therapeutic potential to reduce or prevent bone loss through modulation of these signals.

Adipocyte differentiation of MSCs

Although adipocyte formation is likely a multistep process involving many cellular intermediates, adipogenesis is typically described in the context of two major phases: the *determination* phase and the *terminal differentiation* phase. In the determination phase, multipotent MSCs become committed to the adipocyte lineage and lose their ability to differentiate into other mesenchymal lineages. In this phase, committed preadipocytes are morphologically indistinguishable from their precursors. During the terminal differentiation phase, fibroblastic preadipocytes are converted to spherical, mature adipocytes that can synthesize and transport lipids, secrete adipocyte-specific proteins, and that contain the machinery necessary for insulin sensitivity [14]. In addition to these two phases, several cellular models of adipogenesis (*e.g.,* murine 3T3-L1) also require a period of *mitotic clonal expansion*, involving one or two rounds of cell division prior to adipogenesis. In human bone marrow MSCs, clonal expansion is not required for the adipogenic differentiation [15]. However, despite that mitotic clonal expansion has not been unequivocally established as a requisite for bone marrow adipogenesis, it is clear that some cell cycle proteins that act to regulate mitosis also act to regulate some aspects of adipocyte differentiation in this tissue [16].

Functionally, adipogenesis reflects a fundamental shift in gene expression patterns within uncommitted MSCs that promotes and culminates in the phenotypic properties that define mature adipocytes [13]. Adipogenesis is driven by a complex and well-orchestrated signaling cascade (Fig. 2) involving regulated changes in the expression and/or activity of several key transcription factors, most notably PPARγ and several members of the CCAAT/enhancer-binding family of proteins (C/EBPs) (reviewed in [13]).

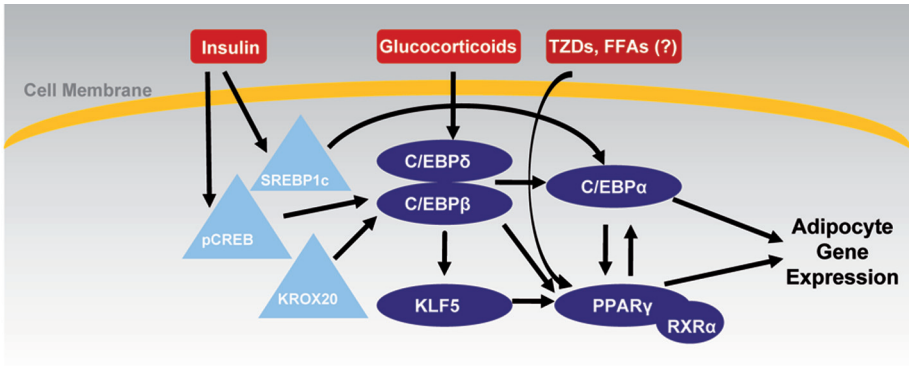

Figure 2. Adipogenic induction involves a complex cascade of transcription factors. Extracellular signals promote adipogenesis by modifying the expression and/or activity of various adipogenic transcription factors. While CEBPα and PPARγ (as a heterodimer with RXRα) are generally regarded as the ultimate critical regulators of this process, experimental evidence supports important intermediary roles for other transcription factors including sterol regulatory element binding protein-1c (SREBP1c), phosphorylated cAMP response element-binding protein (pCREB), the zinc finger transcription factor KROX-20, Kruppel-like factor 5 (KLF5) as well as CEBPβ and CEBPδ.

PPARγ: The master regulator of adipogenesis

PPARγ is a member of the nuclear hormone receptor gene superfamily of ligand-activated transcription factors. PPARγ is expressed as two isoforms (PPARγ1 and PPARγ2) that are generated from alternative promoter usage and splicing; with PPARγ2 being the predominant isoform found in adipose tissue. PPARγ1 is expressed at lower levels in adipose tissue as well as in many other cell types and tissues including macrophages, breast, and prostate [17–19]. Although some debate exists regarding the precise role of each isoform in adipogenesis, it is established that both PPARγ1 and PPARγ2 expressions are highly induced in adipogenesis, supporting a role for both in the induction of the adipogenic process [20, 21].

PPARγ is commonly referred to as the master regulator of adipogenesis, because no factor has yet been identified that can induce normal adipogenesis in its absence. Furthermore, ectopic expression and activation of PPARγ are sufficient to induce adipocyte differentiation of many cell types that are not normally destined for this lineage [18]. In further support of a dominant role for PPARγ in adipogenesis, all critical cell signaling pathways involved in adipogenesis converge on PPARγ and most factors that stimulate adipogenesis ultimately exert their effect through regulation of this transcription factor (as reviewed in [13, 14]). Although targeted disruption of the PPARγ gene by conventional means is embryonic lethal, inducible and selective deletion of the PPARγ gene in adipose tissue of adult mice resulted in a loss of both brown and white adipocytes followed by their subsequent replacement with adipocytes containing PPARγ [22]. In a similar study, adipose selective knockout of PPARγ was found to cause a significant reduction in adipocyte function, which then contributed to progressive lipodystrophy, insulin resistance, and many other metabolic defects [23].

Similar to other models of adipocyte differentiation, bone marrow MSC adipogenesis is characterized by a dramatic increase in PPARγ expression [24]. This increased expression of PPARγ in adipogenesis directly activates or induces the expression of the majority of genes that characterizes the adipocyte phenotype including fatty acid synthase, Glut 4, acetyl CoA carboxylase, adipocyte-selective fatty acid binding protein, and the insulin receptor. In addition, treatment of primary bone marrow MSCs as well as MSC lines with PPARγ agonists promotes adipogenesis [25]. Thus, it is most generally believed that promotion of adipogenesis by PPARγ involves not only an increase in the expression but also a ligand-dependent activation of this transcription factor. However, the presence of a ligand does not appear to be a requisite to maintain the differentiation of mature adipocytes, but rather for commitment of cells to the adipocyte lineage. This was made evident with the observation that differentiation of nonadipogenic fibroblasts with forced expression of PPARγ, required PPARγ activation via exposure to exogenous ligand, whereas the adipogenic differentiation of preadipocytes occurred in the absence of a ligand [26]. Exogenous ligands for PPARγ include derivatives of long-chain polyunsaturated acids as well as thiazolidinediones (TZDs), synthetic agonists that are clinically exploited for their insulin sensitizing actions [27].Therefore, although PPARγ expression is critical for both the determination and differentiation phases

of adipogenesis, it appears that the ligand is only required in the determination phase. Several endogenous fatty acid-derived molecules have been shown to bind and activate PPARγ [26, 28], however none have been directly implicated in adipogenesis. Furthermore, the recent observation that ectopic expression of a mutant form of PPARγ lacking a functional ligand-binding domain was able to support adipocyte differentiation of PPARγ-null fibroblasts (see [29]), has called into question the requirement for ligand activation in this process. Whether this finding is a consequence of overexpression of the mutant form (10–20 greater than normal) or is a more generalized phenomenon that also relates to cells with a greater multipotent character such as MSCs requires further investigation.

The adipogenic C/EBPs

The C/EBPs belong to the basic-leucine zipper class of transcription factors and of the six isoforms that have been identified, those expressed in adipocytes include: C/EBPα, C/EBPβ, C/EBPγ, C/EBPδ, and transcription factor homologous to CCAAT/enhancer-binding protein (CHOP). Within adipose, C/EBPα, C/EBPβ, and C/EBPδ promote adipogenesis, while C/EBPγ and CHOP appear to inhibit adipogenesis by forming dimers with and subsequently inactivating C/EBPβ (as reviewed in [30]). Adipogenesis in immortalized cell lines (*e.g.*, 3T3-L1, 3T3-F442A) and bone marrow-derived MSCs support a rapid induction of C/EBPβ and C/EBPδ that precedes and contributes to the subsequent induction of the major adipogenic transcription factors, C/EBPα and PPARγ [31,32]. Interestingly, in human MSCs, but not in most preadipocyte cell lines, adipogenesis is characterized by both early and late rounds of C/EBPβ and C/EBPα induction, prior to terminal differentiation, suggesting involvement not only in the differentiation phase, but also in the determination phase of MSC adipogenesis [24]. Lending partial support to this observation is the finding that ectopic expression of C/EBPβ is able to drive adipocyte formation in nonadipogenic cell lines in the presence of hormonal inducers [33]. The adipogenic role of C/EBPβ and C/EBPδ has been further elucidated through various gain- and loss-of-function experiments. Ectopic expression of C/EBPβ in 3T3-L1 preadipocytes was sufficient to stimulate adipogenesis, whereas ectopic expression of C/EBPδ in the preadipocytes accelerated the formation of mature adipocytes only in the presence of hormonal inducers [31]. Further support of their proadipogenic role derives from results in which embryonic fibroblasts with a knockdown in expression of both C/EBP forms (β and δ) exhibited a significant reduction in their ability to form adipocytes. Interestingly, it seems that either C/EBPδ or C/EBPβ may be able to compensate to some degree for the loss of the other, because embryonic stem cells with a knockdown of either the β or δ form led to a reduction in adiposity less than the magnitude of the double knockout [34]. While *in vivo* studies using mice lacking these two C/EBP forms have produced some equivocal results, they, nonetheless, support a role for both C/EBPβ and C/EBPδ in adipogenesis. For example, mice lacking C/EBPβ expression have slightly reduced adipose tissue; however, this may be attributable to abnormalities in metabolism and not in adipogenesis. In contrast, the double knockout of C/EBPβ and C/EBPδ, demonstrate significant reduction in adiposity [34].

Although the expression of C/EBPβ and C/EBPδ are not required for the induction of C/EBPα and PPARγ expression [34], they appear to promote adipogenesis in large part through their synergistic induction of these two dominant adipogenic transcription factors. Following its induction, C/EBPα directly activates several adipogenic genes necessary for the development of mature adipocytes, including PPARγ. Moreover, once activated, PPARγ and C/EBPα stimulate each other's expression, which then remains elevated for the lifetime of mature adipocytes [18, 35]. Considerable evidence supports a critical role of C/EBPα in adipogenesis. For instance, ectopic expression of C/EBPα is able to drive the adipogenic differentiation of cell lines that otherwise do not undergo adipogenesis [33, 36]. Moreover, overexpression of C/EBPα in preadipocyte cell lines is sufficient to induce their differentiation into adipocytes [35, 36], while C/EBPα antisense expression in these cells abrogates their adipogenic potential [37]. In further support of the adipogenic role of C/EBPα, fibroblasts lacking C/EBPα expression have a significantly reduced adipogenic potential and PPARγ level, which are reversed upon the addition of PPARγ. In the complementary experiment, the adipogenic potential of fibroblasts lacking PPARγ was not rescued by the viral expression of C/EBPα, suggesting that C/EBPα promotes adipogenesis in a PPARγ-dependent manner [38]. *In vivo*, interpretation of the role of C/EBPα in adipogenesis using traditional mouse knockout models is confounded by severe defects of hepatic metabolic function and lethality. Nonetheless, rescue of these mice with liver specific expression of C/EBPα [39], or targeted replacement of the C/EBPα locus with C/EBPβ [40], results in dramatically reduced amounts of white, but not brown, adipose tissue. And, as levels of PPARγ in white adipose tissue may be more dependent on induction by C/EBPα than in brown adipose tissue, this would explain the contrasting findings in

white versus brown adipose tissue in knockout mice. Thus, C/EBPs are key contributors of adipogenesis that act in large measure through modulation of PPARγ expression and function.

Interaction of adipogenic and osteoblastogenic signaling pathways

The differentiation fate of MSCs within the bone marrow is determined in large measure by a complex interplay of extracellular mediators such as growth factors, hormones, and nutrients that affect the expression and activation of lineage-specific transcription factors. This also includes pharmacologic agents such as TZDs, which are potent PPARγ agonists and thereby promote adipocyte differentiation of MSCs. Thus, optimal conditions entailing appropriate levels and spectra of growth factors, nutrients, and hormones are likely required for the normal osteoblastogenesis and the suppression of adipogenesis. Changes in this physiological milieu likely underlie the preponderance of bone marrow adipocytes associated with aging, disease, and other conditions characterized by bone loss.

1. PPARγ. Considerable data supports an antiosteoblastogenic role of the master inducer of adipogenesis, PPARγ. For example, naturally occurring PPARγ ligands such as 15-deoxy-delta (12,14)-PGJ_2 as well as the potent TZD rosiglitazone have been shown to promote adipogenesis and inhibit osteoblastogenesis of primary bone marrow MSCs [41, 42]. However, it should be noted that the nature of the agonist for PPARγ is particularly significant as the partial agonist GW0072 has been shown to block MSC osteoblastogenesis without promoting adipogenesis [41]. Different responses to individual PPARγ ligands have also been noted *in vivo*. For example, chronic treatment of mice with troglitazone, a low-affinity TZD, resulted in an increase in bone marrow adipocytes with no effect on bone mass or volume [43], whereas treatment with rosiglitazone, a high-affinity TZD, resulted in an increase in bone marrow adiposity with an associated decrease in bone mineral density (BMD or Bone mass), rate of bone formation, and trabecular bone volume [44, 45]. These works also demonstrated that the inhibition of bone formation by rosiglitazone occurred in part through the suppression of the dominant osteoblastogenic transcription factors, Runx2, OSX, and Dlx5 [44, 45], whereas the low-affinity PPARγ agonist, netoglitazone, had no effect on the levels of Runx2 or Dlx5 [42]. In further support of the inverse osteoblast/adipocyte relationship, researchers demonstrated that an insufficiency in PPARγ led not only to decreased marrow adipogenesis but also to increased osteoblastogenesis *in vitro* and increased trabecular bone volume *in vivo* [46]. Moreover, *in vivo* studies involving mutations in the PPARγ gene have also gone some distance in demonstrating inverse changes in marrow adipose and bone formation. For example, mice with a congenital mutation in the PPARγ2 locus, which have reduced expression of both PPARγ in white adipose tissue, exhibit such a large increase in bone formation that insufficient space is left in the marrow cavity for hematopoiesis, which relocates to extramedullary sites [47]. Correspondingly, heterozygous PPARγ-deficient mice have increased bone mass at both young and old ages, due to either the direct or indirect influence of PPARγ signaling on major osteoblast pathways. This was established by the finding that culture of bone marrow MSCs derived from these heterozygous mice had increased expression of major osteoblast transcription factors including Runx2 and OSX. In line with these observations, aged-mice and mouse models of premature aging (SAMP6) exhibit increased expression of PPARγ, fatty bone marrow and decreased expression of Runx2 [48]. In humans, recent clinical studies have found that diabetic patients treated with TZDs reported an increased rate of fractures [49], highlighting the necessity to find a TZD that is 'bone sparing'. Collectively, these studies support an inverse relationship between osteoblast and adipocyte formation, especially in the context of PPARγ as a stimulator of bone marrow adipogenesis and an inhibitor of osteoblastogenesis. More importantly, these studies demonstrate the importance of this relationship with respect to bone physiology, and bone loss in particular.

2. Bone morphogenic proteins (BMPs). The BMPs are the largest family of proteins from the transforming growth factor-β (TGF-β) superfamily of growth factors. These proteins are important regulators of cell growth, proliferation, differentiation, migration, apoptosis as well as development and function of a variety of tissues including the bone, cartilage, heart, brain, gut, kidneys, skin, endothelium, and vascular smooth muscle [50]. More than 20 different BMPs have been identified and of these BMP-2, BMP-4, BMP-6, and BMP-7 are the well-established mediators of osteoblast and/or adipocyte differentiation from MSCs [51–57]. The intracellular effects of BMPs are mediated by interaction with cell surface serine-threonine kinases BMP receptors (BMPRs). Two distinct subtypes of BMPRs (type I and type II) are involved in BMP signaling that is initiated by the binding of BMP ligand with a type II receptor such as the BMP type II receptor (BMPR-II) or activin type II

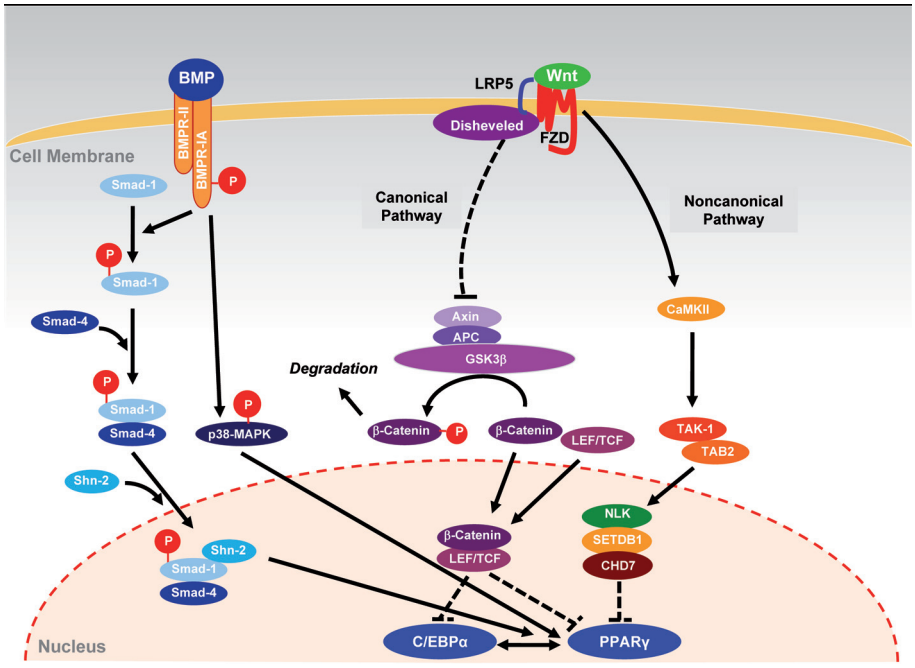

Figure 3. Regulation of adipogenic transcriptional factors (C/EBPα and PPARγ) by extracellular signaling molecules (BMPs and Wnts). BMP binding with BMPR-II results in the recruitment, phosphorylation and consequent activation of BMPR-IA. Activation of BMPR-IA results in further activation of the Smad and p38-MAPK signaling pathways. The activation of Smad1 is initiated by phosphorylation events required for complex formation with Smad4. This Smad1/4 complex can directly interact with Shn-2 and C/EBPα on the PPARγ promoter thus, resulting in the transcriptional activation of PPARγ. The p38-MAPK signaling pathway also ultimately converges at the transcriptional activation of PPARγ. The Wnt proteins bind to FZDs that direct intracellular canonical and non-canonical signals. The canonical downstream signals that enhance the stability of β-catenin repress PPARγ transactivation via the LEF/TCF pathway while the non-canonical Wnt signals that activate CaMKII and MAPK kinase kinases such as TAK-1 and TAB2 repress PPAR-γ transactivation via the activation of NLK that forms chromatin associated complexes with chromodomain helicase DNA binding protein 7 (CHD7), SETDB1 and PPARγ.

receptor (ActR-II). BMP binding results in the recruitment, phosphorylation, and consequent activation of type I BMPRs [50, 58, 59]. Seven distinct type I BMPRs have been identified. Among these, BMPR-IA (also called ALK-3), BMPR-IB (also called ALK-6), and activin receptor I (also called ALK-2) have roles in adipose and skeletal formation [60]. The phosphorylation of type I BMPR initiates subsequent phosphorylation and activation of downstream intracellular signals in the cytosol such as the canonical mothers against decapentaplegic (Smad), p38 mitogen-activated protein kinase (MAPK), and c-Jun N-terminal kinase (JNK) signaling pathways [50, 61, 62]. Smads are the most important and well-characterized immediate downstream intracellular signaling molecules activated by the BMPs. Ultimately, the activated Smads form oligomeric protein complexes that are translocated to the nucleus where they regulate transcription of target genes (Fig. 3) [50, 58, 59].

Several members of the BMP family are secreted by osteoblasts and regulate bone mass in an autocrine and paracrine fashion by directly inducing osteoblast differentiation of MSCs and by enhancing the function of differentiated osteoblasts in matrix production [63, 64]. Genetically modified mouse models for BMP receptors, BMP ligands, or their endogenous inhibitors have confirmed the essential role of BMP signaling in bone formation [65–71]. BMP signaling converges on OSX through Runx2-dependent and Runx2-independent pathways to stimulate osteoblastogenesis, with Smads and p38-MAPK being the most important intermediate mediators of these effects (Fig. 4) [72–75]. While BMP signaling is critical for skeletal development and homeostasis through actions on osteoblasts, evidence also exists for an adipogenic role of this signaling pathway. This is explained in part by recognizing that the downstream events mediated by BMPs are highly dependent on the type of receptor involved and that this ultimately determines the specificity of intracellular signals for an adipogenic or osteoblastogenic differentiation. In general, activation of BMPR-IA is considered adipogenic and BMPR-IB osteoblastogenic; however, this is by no means exclusive [60]. Thus, while interference with BMPR-IA function blocks adipogenesis in tissue culture, conditional (osteoblast-selective) disruption of BMPR-IA interferes with bone remodeling in mice [70]. The lineage determinant effects of BMPs on

MSC differentiation also appear to be highly sensitive to dose. For example, differentiation of C3H10T1/2 mouse embryonic stem cells into adipocytes occurs preferentially at lower concentrations of BMP-2, while osteoblast and chondrocyte differentiation is favored at higher concentrations [76]. Consistent with other adipogenic stimuli, the effect of BMPs (such as BMP-2) on promoting adipocyte differentiation ultimately converges on PPARγ [77, 78].

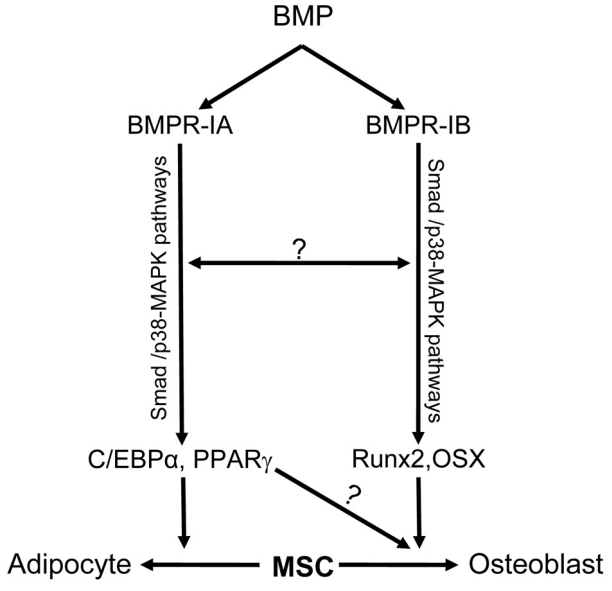

Figure 4. Dual role of BMP signaling pathways on adipocyte and osteoblast differentiation of MSCs. The divergence of the adipocyte or osteoblast lineage from a common MSC precursor is regulated by the interaction of BMPs with distinct types of receptors, *viz*. BMPR-IA and BMPR-IB. Interaction with BMPR-IA is generally adipogenic, while that with BMPR-IB is osteoblastogenic. However, some of the downstream signaling events such as the Smad and p38-MAPK phosphorylation appear to be common between the two lineages and there is an essential requirement of both receptors for normal bone formation. The downstream signals converge at C/EBPα and PPARγ for adipocyte differentiation and at Runx2 and OSX for osteoblast differentiation. While these transcriptional factors are generally considered charactaristic for each of the lineages, recent evidence indicates that PPARγ also positively regulates osteoblast differentiation of MSCs.

Similar to the BMP-induced osteoblast differentiation of MSCs, concomitant activation of both the Smads and p38-MAPK has been implicated in the downstream adipogenic effects of BMPs (Fig. 4) [77]. Induction of PPARγ transactivation through Smad signaling involves a cooperative interaction with the zinc finger transcription factor Schnurri (Shn)-2 and C/EBPα. These interdependent signaling components play a synergistic role during adipogenesis (Fig. 3) [79]. Mechanistically, Smad1/4 signaling promotes the nuclear entry of Shn2 and is essential for the binding of Shn2 to the PPARγ promoter [79]. Consistent with this model, overexpression of the Smad1 antagonist Smad6 in the C3H10T1/2 MSC line blocked PPARγ expression and adipocyte differentiation induced by BMP-2 [77]. In the same cell model, inhibition of p38-MAPK abrogated BMP-2-induced adipocyte differentiation and transcriptional activation of PPARγ, while activation of p38-MAPK increased adipogenesis and transcriptional activation of PPARγ [77]. Recent evidence indicates that BMP signaling is involved in the earliest stages of adipocyte differentiation and is an important determinant of the commitment of MSCs to the adipocyte lineage. For example, forced commitment of the C3H10T1/2 MSC line to a preadipocyte cell lineage (A33) using the DNA methyltransferase inhibitor 5-azacytidine results in elevated expression of BMP-4 [55, 80]. Functionally, this endogenous BMP-4 expression appears to be critical for elaboration of the preadipocyte phenotype as treatment with the BMP-4 antagonist noggin inhibited adipocyte differentiation of A33 cells in response to hormonal inducers [80]. Although the hormonal inducers alone fail to stimulate adipogenesis of C3H10T1/2 stem cells, overexpression or application of exogenous BMP-4 promotes commitment of these cells to the adipocyte lineage and subsequent adipocyte differentiation in response to hormonal inducers [55, 81, 82]. Consistent with BMP receptors as determinant of the lineage outcome (*e.g.*, osteoblast versus adipocyte) of BMP signaling, A33 cells lack BMPR-1B expression and exhibit elevated levels of BMPR-1A expression compared with the parental C3H10T1/2 line. In a recent study, BMP-2, BMP-4, BMP-6, BMP-7, or BMP-9 were reported to promote both osteoblastogenic and adipogenic lineage commitment and terminal differentiation of MSCs in a mutually exclusive fashion [83]. As expected, overexpression of Runx2 enhanced BMP-induced osteoblastogenic differentiation, while knockdown of Runx2 expression diminished osteoblastogenesis. However, knockdown or overexpression of PPARγ was also shown to impair or stimulate, respectively, both BMP-induced osteoblastogenesis and adipogenesis. These findings identify an unexpected role of the master adipogenic transcription factor PPARγ in BMP-induced osteoblastogenesis and highlight the complexity and overlapping influence of BMP signaling on lineage determination in MSCs.

In summary, BMPs have stimulatory effects on both osteoblast and adipocyte differentiation of MSCs. While much has been learned regarding the molecular mechanisms by which BMPs achieve adipogenic or osteoblastogenic effects, the basis of selectivity for either lineage is only just beginning to be elucidated.

3. The Wnt signaling pathway. The Wnts are a group of secreted cysteine-rich glycoproteins that regulate developmental processes through their involvement in various aspects of cell biology including cell migration, proliferation, and differentiation. Intracellularly, Wnt signaling involves complex interactions among various proteins that can be broadly subclassified as canonical or noncanonical [84]. Canonical Wnt signaling is initiated by the binding of extracellular Wnts such as Wnt3a to cell surface expressed frizzled receptors (FZDs) that form complexes with other proteins including the low-density lipoprotein receptor (LRP5/6) coreceptor, and proteins of the disheveled (DSH) family. Wnt-induced complex formation results in activation of DSH, which in turn, inhibits a second complex of proteins that includes axin, glycogen synthase kinase 3 (GSK3), and the adenomatosis polyposis coli (APC) protein. GSK3 is a constitutively active kinase that phosphorylates cytoplasmic β-catenin, a protein, which regulates the activity and subcellular localization of many transcription factors. Phosphorylation by GSK3 is a destabilizing event that promotes proteolytic degradation of β-catenin. Inhibition of the axin/GSK3/APC complex by Wnt signaling stabilizes the cytoplasmic pool of β-catenin and results in an increase in the amount of β-catenin that can enter the nucleus (Fig. 3). In the nucleus, the β-catenin undergoes heterodimerization with other family of proteins such as the lymphoid enhancer-binding factor/T cell factor (LEF/TCF) transcription factor family by displacing the corepressors of such proteins [84]. In this manner, the canonical signaling pathway for Wnts ultimately results in a selective change in the expression of genes relevant to cell growth and differentiation. Similar to the canonical pathway, noncanonical Wnt signaling pathways are initiated by the binding of Wnts such as Wnt1 and Wnt5a to cell surface FZDs. However, beyond this point, the canonical and noncanonical pathways diverge such that the latter is β-catenin independent. In contrast to canonical Wnt signaling, the noncanonical mechanisms are somewhat heterogeneous and involve various intracellular mediators [85, 86]. One example is the calcium-dependent Wnt/Ca^{2+} pathway, which stimulates intracellular calcium release to regulate the activity of calcium-sensitive enzymes such as Ca^{2+}-calmodulin-dependent protein kinase-II (CaMKII), protein kinase C, and calcineurin. Downstream targets of these enzymes include TAK-1 (TGF-β activated kinase-1)/NLK (nemo-like kinase) and Elk-1 and nuclear factor of activated T-cells transcription factors. Another example is the planar cell polarity (PCP) pathway that is DSH-dependent and involves activation of Rho and Rac GTPases as well as c-jun NH2-terminal kinase (JNK). Ultimately, both noncanonical pathways result in selective changes in gene expression in target cells (Fig. 3). Almost every component of the Wnt signaling pathway has been identified to be both present and functional in human MSCs [87]. Functionally, a large body of experimental evidence supports a role for canonical Wnt signaling in self-renewal as well as lineage-selective determination of MSCs.

A clear relationship has been established between Wnt signaling and bone mass in humans and mice. Canonical Wnt signaling is believed to promote bone formation by several mechanisms including stem cell renewal [88], stimulation of osteoblast proliferation [89], and induction of osteoblastogenesis [89]. In humans, perhaps the most striking evidence for the critical role of Wnt signaling in bone formation is the development of pseudo-glioma syndrome characterized by extremely low bone mass or, alternatively, extremely high bone mass phenotypes associated with loss- or gain-of-function LRP5 mutations, respectively [90–92]. Conditional targeted disruption of the β-catenin gene in the mesenchymal precursors of both chondrocytes and osteoblasts in mice resulted in embryos devoid of skeletal bone [93]. This defect was ascribed to a lack of osteoblast differentiation owing to an arrest at the early progenitor stage. Consistent with this, stabilization of β-catenin by treatment with the GSK3 inhibitor lithium chloride induces the expression of early osteoblast marker genes such as alkaline phosphatase (AKP) in the C3H10T1/2 stem cell line [94]. The related observation that disruption of β-catenin signaling causes spontaneous adipocyte conversion of various cell types both *in vivo* and *in vitro* [95–97], also underscores the potential importance of canonical Wnt signaling as a determinant of osteoblast versus adipocyte differentiation of bone marrow-derived MSCs. Importantly, this determinant function appears to involve both promotion of osteoblastogenesis and active repression of adipogenesis. Pharmacological treatments that activate Wnt signaling pathways and/or β-catenin in MSCs stimulate osteoblastogenesis and suppresses adipogenesis [98]. For example, while Wnt activation in tissue culture models does not affect C/EBPβ or C/EBPδ, induction of PPARγ and C/EBPα is blocked [97]. Furthermore, constitutive Wnt signaling favors the expression of osteoblast genes at the expense of adipocyte genes [99]. Consistent with this, suppression of Wnt signaling by dominant-negative or neutralizing antibody approaches stimulates adipogenesis in tissue culture models [95, 97]. Functionally, activation of β-catenin signaling through canonical Wnt signaling inhibits adipogenesis primarily by blocking the induction of the critical adipogenic transcription factors C/EBPα and PPARγ [95, 97, 100,

101]. Conversely, PPARγ induction is known to suppress the β-catenin signaling during adipogenesis [95, 97, 100, 101]. Furthermore, the canonical Wnt ligand Wnt3a, suppressed both PPARγ-induced C/EBPα expression and C/EBPα-induced PPARγ expression, suggesting that the mutual upregulation of PPARγ and C/EBPα expression is a potential target of β-catenin signaling [102]. Surprisingly, the overexpression of both PPARγ and C/EBPα could not fully rescue Wnt suppressed adipogenesis, indicating that β-catenin signaling not only blocks the induction of these transcriptional factors but also interferes with their transcriptional activities [102]. Suppression of adipogenesis is believed to involve cyclin D1 and c-myc as these two critical cell cycle control proteins that can inhibit PPARγ and C/EBPα, respectively, are activated by β-catenin [103–109]. Several Wnt ligands, including Wnt1, Wnt3a, and Wnt10b have been shown to inhibit adipogenesis through the inhibition of PPARγ and C/EBPα [95, 101, 102].

The noncanonical signaling pathway has also been implicated as a determinant of osteoblast versus adipocyte differentiation of MSCs. Recently, Takada et al. [110] described a mechanism by which Wnt5a suppresses adipogenic PPARγ transactivation in bone marrow-derived MSCs with a concomitant induction of the key osteoblastogenic transcription factor Runx2. Mechanistically, Wnt-dependent CaMKII activation in MSCs stimulates an intracellular signaling cascade that involves activation of NLK through a MAPK kinase kinase TAK-1/TAB2 dependent mechanism (Fig. 3). NLK-dependent phosphorylation of SET domain bifurcated 1 (SETDB1) leads to the formation of chromatin-associated complexes that include ligand-bound PPARγ. The end result of this complex formation is suppression of PPARγ-regulated gene expression, suppression of adipogenesis, and promotion of osteoblastogenesis. To date, there have been no reports linking the Wnt/PCP pathway as a determinant of osteoblast versus adipocyte differentiation of MSCs.

In summary, substantial evidence indicates that promotion of bone formation involves activation of the osteoblastogenic differentiation program in bone marrow-derived MSCs, concomitant with an active suppression of the adipogenic program through both canonical and noncanonical mechanisms.

4. TAZ (transcriptional co-activator with PDZ-binding motif). TAZ – also known as WW domain–containing transcription regulator 1 (WWTR1) – was initially identified by its ability to interact with 14-3-3 proteins, a highly conserved family of signal transduction proteins involved in cell differentiation, apoptosis, and cell cycle regulation [111]. Recent evidence has implicated TAZ as modulator of MSC cell differentiation through direct interactions with the regulatory regions of the master osteoblastogenic and adipogenic transcription factors, Runx2 and PPARγ, respectively [112, 113]. While both Runx2 and PPARγ have similar binding motifs within their activation domains, the outcome of interaction with TAZ differs substantially in that Runx2 gene transactivation is stimulated, while the PPARγ transactivation is repressed [112, 114]. Functionally, this translates to a net osteoblastogenic influence of TAZ during MSC differentiation. Consistent with this, shRNA mediated depletion of endogenous TAZ in 3T3-L1 preadipocytes and MSCs resulted in increased adipogenesis versus osteoblastogenesis [112]. Furthermore, an increase in TAZ expression during osteoblast differentiation and a converse decrease of TAZ levels during adipocyte differentiation have been reported [113]. BMP-2 has been reported to induce TAZ expression and this is believed to be an important upstream event required for Runx2 induction, Runx2-dependent osteocalcin gene expression, and the osteoblastogenic influence of BMP signaling [112]. In humans, it was recently reported that the osteoblastogenic potential of the MSCs isolated from multiple myeloma patients was decreased significantly, and that TAZ expression of these cells was lower than that of healthy donors [115]. In culture, both MSC osteoblastogenesis and TAZ expression were partially restored by neutralizing antibodies to TNFα; suggesting that this cytokine is a negative regulator of TAZ expression. This finding is consistent with the reported inhibitory effects of TNFα on osteoblastogenesis and bone formation [116]. Additional studies, particularly with *in vivo* models, will help to further elucidate the role of TAZ in bone marrow osteoblastogenesis/adipogenesis and the implications for disorders of bone metabolism.

5. Other extracellular signal modifiers. Members of the hedgehog, delta/jagged, FGF, and IGF families of proteins have been identified as extracellular ligands that modulate adipocyte differentiation of bone marrow-derived MSCs along with potential interactions on osteoblastogenic differentiation pathways. Among these, the role of hedgehog signaling in osteoblastogenic/adipogenic differentiation has been the best described [117–119]. Members of the Ci/Gli family of zinc-finger proteins are the ultimate transcriptional effectors of the hedgehog-signaling pathway. In a mechanism analogous to canonical Wnt signaling, the interaction of hedgehogs with cell surface receptors of the patched family ultimately rescues Ci/Gli transcription factors from proteolytic degradation and thereby promotes their nuclear

accumulation and transactivation activity (Fig. 5). Hedgehogs such as sonic hedgehog (SHH), Indian hedgehog, and desert hedgehog as well as Gli are highly expressed in MSCs [117]. Adipocyte differentiation of human MSCs is characterized by a decrease in hedgehog signaling primarily as a consequence of decreased Gli expression [117]. Conversely, hedgehog activation was shown to interfere with adipocyte differentiation by reducing the adipogenic induction of C/EBPα and PPARγ as well as lipid accumulation [117]. While previous data identify these adipogenic transcription factors as putative downstream targets of hedgehog signaling, other studies showing that PPARγ expression could overcome the hedgehog induced adipogenic blockade (see [120]) are suggestive of a further upstream targets of hedgehog signaling in the adipogenic program. The decrease of Gli expression observed during adipogenesis in human MSCs is consistent with the down regulation of Gli1, Gli2, and Gli3 with enhanced fat formation reported during diet-induced obesity or genetically obese *ob/ob* mouse models [120]. Expression of a dominant negative form of Gli2 has been shown to enhance adipocyte differentiation of 3T3-L1 cells and treatment of these cells with hedgehogs such as SHH was found to not only decrease the expression of adipocyte marker genes such as PPARγ and aP2, but also simultaneously increase the expression of osteoblast marker genes such as Runx2 and AKP [117, 120]. A synergistic interaction between SHH and BMP-2 via Smad signaling has been reported to potentiate BMP-induced osteoblast differentiation of MSCs, while the SHH alone was able to block the adipocyte lineage commitment both in the presence and in the absence of BMP-2 [119]. Other studies have provided evidence in support of an interaction between the SHH and BMP-2 signaling in promoting osteoblastogenesis through the Gli3 and Smads, respectively, at the level of downstream effectors [121].

The delta and jagged family of proteins are the extracellular ligands for the notch receptors. The role of notch signaling in the adipogenic differentiation is highly complex, and in many aspects remains only poorly elucidated. Existing evidence comes largely from studies of the 3T3-L1 model and provides support for both an inhibitory role and, alternatively, an absolute requirement for notch signaling in adipogenic differentiation (Fig. 5) [122, 123]. Exposure to jagged1 or overexpression of the notch target gene Hes-1 in 3T3-L1 cells blocks PPARγ and C/EBPα induction in response to differentiation inducers [123]. However, virus-mediated expression of either PPARγ or C/EBPα in these cells rescued normal adipocyte differentiation in the context of high notch signaling suggesting that the Hes-1 may act upstream of PPARγ and C/EBPα to inhibit adipogenesis [123]. Paradoxically, in the same study reduction of endogenous Hes-1 expression by RNAi was also associated with inhibition of adipogenesis, suggesting an additional obligatory role for this gene and perhaps a role for notch signaling in adipogenesis. Other studies have provided additional evidence for an essential requirement of notch-1 for adipogenesis of 3T3-L1 cells and have proposed a mechanism that involves modulation of the expression of a known inhibitor of adipocyte differentiation, DLK1/Pref1 [122, 123]. Thus, it is likely that multiple notch-regulated intracellular signaling pathways exist that result in inhibition or, alternatively, stimulation of adipogenesis (Fig. 5). Constitutive notch signaling achieved by overexpression of the intracellular domain of notch (notchIC) in murine fibroblast MC3T3 or the murine bone marrow-derived mesenchymal ST-2 cell line has been reported to result in preferential differentiation of these cells to adipocytes versus osteoblasts [124]. This suppression of osteoblastogenic differentiation was mediated by suppression of Wnt/β-catenin signaling, thereby establishing an interaction between Wnt and notch signaling during MSC fate decisions [124, 125].

FGFs are recognized as strong stimulators of both adipogenesis and osteoblastogenesis (Fig. 5). *In vitro* studies have demonstrated the strong adipogenic effects of FGFs such as FGF1, FGF2, and FGF10 in the presence of adipocyte differentiation stimuli [126–129]. Similarly, other studies have shown that the FGF2 (basic FGF) stimulates the proliferation and osteoblastogenic differentiation of bone marrow MSCs in the presence of stimuli for osteoblastogenic differentiation [130–133]. Consistent with these *in vitro* studies, several *in vivo* reports support a dual role of FGFs in enhancing both osteoblastogenesis and adipogenesis. For example, FGF2 has been shown to stimulate adipose generation from subcutaneously implanted preadipocytes on collagen scaffolds or reconstituted basement membranes [134–136]. Other studies using osteopenic ovariectomized animal models [137–142] or healthy growing rats [143] have shown a strong stimulatory effect of FGF2 on bone formation. However, other studies have identified suppressive effects of FGF1 (acidic FGF) on osteoblast differentiation [144, 145]. For example, FGF2 treatment of osteoblastic MC3T3-E1 cells was reported to decrease TAZ levels and osteoblast marker gene expression concomitant with increased adipocyte marker gene expression [146]. Thus, similar to the experimental evidence for notch signaling, it is likely that the impact of FGFs on adipogenesis and osteo-

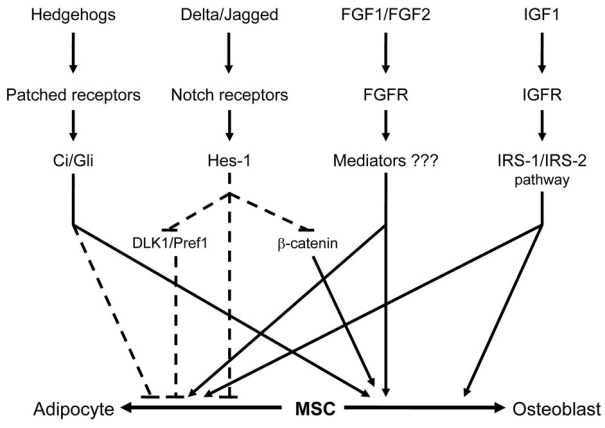

Figure 5. The complex interplay of extracellular signaling molecules in adipocyte and osteoblast differentiation of MSCs. Hedgehogs bind to the Patched family of receptors and the downstream signals generated through this interaction lead to the stabilization of Ci/Gli transcriptional factors that ultimately stimulate osteoblastogenesis and inhibit adipogenesis of MSCs. The delta/jagged family of proteins bind to the notch receptors that can activate the notch target gene, Hes-1. The inhibition of DLK1/Pref1 and β-catenin by Hes-1 can result in the stimulation of adipocyte differentiation and inhibition of osteoblast differentiation processes, respectively. Conversely, Hes-1 can also interfere with adipocyte differentiation if present in excess. The FGFs and IGFs stimulate both adipocyte and osteoblast differentiation of MSCs with the net result influenced strongly by the effect of other extracellular modifiers of differentiation. While the downstream signaling cascade for FGF signaling is not yet precisely established, the activation of IRS1 and IRS2 adapter proteins are the crucial events in the immediate downstream of IGF signaling for both adipocyte and osteoblast differentiation of MSCs.

blastogenesis is highly dependent upon the mode of signaling, the nature of the ligand, and the existence of other extracellular stimuli.

Insulin and IGF1 utilize many common molecular events in their signaling pathways and have highly homologous tyrosine kinase receptors (insulin receptor, IR and insulin-like growth factor receptor, IGFR, respectively) that can bind either ligand, albeit with lower affinity than the cognate ligand [147, 148]. Ligand binding activates IR/IGFR to phosphorylate a variety of intracellular proteins, most notably insulin receptor substrates (IRS-1 and IRS-2; Fig. 5). This initiates an intracellular signaling cascade that ultimately affects the activity of key intracellular targets including phosphatidylinositol 3-kinase (PI 3-kinase) and MAPK pathways that induce the nuclear activation of transcriptional factors involved in proliferation and differentiation processes [149]. IGFR signaling has been shown to stimulate MSC proliferation as well as both adipogenic and osteoblastogenic differentiation (Fig. 5) [150–154]. Although initial studies indicated a role for IGF only in terminal differentiation [150], subsequent studies employing IGF1 overexpression or blocking antibodies targeting the IGFR, revealed a role for this signaling pathway during both early osteoblast and adipocyte differentiation of human MSCs [151, 152]. IGF1 alone or synergistically with BMP-2, stimulates OSX expression in MSCs through protein kinase (MAPK) and protein kinase D-dependent pathways [61] and the adapter proteins, IRS-1 and IRS-2 are believed to be crucial to the osteoblastogenic influence of this pathway [155, 156]. Consistent with this, mice lacking IRS-1 or IRS-2 exhibit impaired osteoblast differentiation with severe osteopenia [155, 156]. However, while osteoblast-targeted disruption of the IGFR or overexpression of IGF1 in mice resulted in decreased and increased bone formation, respectively, osteoblast number was largely unchanged [154, 157]. Thus, while IGFR signaling is clearly implicated in bone metabolism, the relative importance for osteoblast differentiation versus the function of mature osteoblasts *in vivo* remains in question. Experimental evidence supports an adipogenic role for both insulin and IGF1, although at present, little direct information is available regarding adipogenesis of bone marrow MSCs [158, 159]. In mouse models of impaired IGFR signaling, reduced adipose tissue formation as well as impaired proliferation and differentiation of preadipocyte precursors has been reported [153, 160]. Forced expression of the adipocyte differentiation inhibitory factor Pref1 in 3T3-L1 cells reduces IGFR expression and IGFR-dependent MAPK activation [159]. Conversely, exogenous insulin or IGF1 rescued MAPK activation and adipocyte differentiation in the context of Pref1 overexpression, thereby establishing a mechanistic link between these two signaling pathways with opposing effects on adipocyte differentiation [159]. Consistent with reports of the osteoblastogenic effects of IGFR signaling, the downstream events that promote adipocyte differentiation are mediated by IRS-1 and IRS-2. Activation of these adapter proteins and associated PI 3-kinase pathways ultimately signals to the nucleus for activation of adipogenic transcription factors such as PPARγ and C/EBPα resulting in the induction of adipocyte differentiation [161, 162]. Thus, IGFR signaling has been implicated in both osteoblast and adipocyte differentiation and function. Additional studies will be required to further elucidate how selectivity for each lineage is determined and the interplay, if any, between the osteoblastogenic and adipogenic signaling cascades.

Relevance of bone marrow adipogenesis to bone loss and therapeutic prospects

An early pathological association between bone marrow adipogenesis and osteoporosis came from

the work of Meunier [163], who reported a marked accumulation of adipocytes in iliac crest biopsies prepared from elderly osteoporotic women. Subsequent studies reporting increased bone marrow adiposity in osteoporotic postmenopausal women and a negative correlation between bone formation and marrow adiposity confirmed these findings [164–166]. MRI studies have revealed that in elderly women with low bone mineral density (BMD), bone marrow adiposity increases the risk for compression fractures [167]. Other MRI studies have noted an increase in bone marrow adiposity in osteoporosis [168] and that postmenopausal women have double the bone marrow fat of premenopausal women [169]. Furthermore, women with low BMD have significantly greater marrow adiposity compared with age-matched women with normal BMD [169]. Diseases such as diabetes are also associated with reduced BMD, increased marrow adiposity, and higher risk for fracture [170]. Skeletal unloading caused by inactivity or immobility has been associated with decreased bone formation as well as increased conversion of bone marrow MSCs to adipocytes compared with osteoblasts [171]. Both chronic glucocorticoid therapy and endogenous overproduction of cortisol are clearly associated with marked bone marrow adiposity, low BMD, and an increased risk for fracture [172–174]. Bone marrow MSCs isolated from postmenopausal osteoporotic patients exhibit increased adipocyte marker gene expression and a propensity for adipocyte differentiation compared with those from healthy subjects with normal bone mass [175, 176]. Collectively, these studies indicate that changes in the physiological milieu with aging, menopause, and various disease states, favor the differentiation of bone marrow MSCs into adipocytes versus osteoblasts. Ultimately, this may alter the balance of bone formation/resorption and thus, contribute to a loss of bone mass and increase the risk for bone fracture.

Given the increasing body of evidence that bone marrow adipogenesis is detrimental to bone formation; does this provide opportunities for novel therapeutic approaches to treat disorders of bone metabolism? Implantation of recombinant human BMP (complexed with carrier matrices) at the site of injury has shown great potential to promote healing of some bone fractures and to promote spinal fusion in patients with spinal defects [177–179]. It is likely that the osteoblastogenic effect of BMPs on bone marrow MSCs contributes substantially to the therapeutic benefit. However, as discussed previously, BMP signaling can also be proadipogenic for MSCs. This knowledge, combined with the more recent findings that higher doses of BMPs promote the development of bone resorbing osteoclasts, [180] renders predictions of the long-term utility of this approach difficult. Furthermore, given the short half-life and potential for undesirable effects at sites other than bone, the prospect of systemic BMP administration for the treatment of chronic bone disorders, such as osteoporosis, remains uncertain [181]. Compared with BMP signaling, there is more evidence for opposing osteoblastogenic/anti-adipogenic effects of Wnt signaling in bone marrow MSCs. Administration of small molecule inhibitors of GSK3, such as lithium chloride, is beneficial in models of progressive bone loss in mice [182]. Intriguingly, in both $LRP^{-/-}$ and SAMP6 (a model of senescence-induced bone loss) mice, lithium chloride treatment increased bone formation rates, osteoblast numbers and reduced bone marrow adiposity *in vivo* [182]. Utilizing another approach, treatment of mice with neutralizing antibodies to Dickkopf-1, a soluble inhibitor of Wnt signaling, promoted osteoblast formation and improved bone density in a model of cancer-induced bone loss [183]. The limited retrospective analyses of patients receiving chronic lithium chloride treatment for various psychiatric disorders have provided conflicting evidence regarding the benefits of this therapy to bone fracture risk [184, 185]. Although targeting Wnt signaling is promising, the regulatory role of this pathway in diverse cellular functions raises concerns of undesirable effects associated with chronic, systemic modulation. Treatment of rodents with the PPARγ agonists such as TZDs decreases BMD, bone formation, and trabecular bone volume concomitant with increasing bone marrow adipogenesis. [44, 45, 186]. In humans, recent clinical studies have reported an increased bone loss and rate of fractures in diabetic individuals treated with TZDs [49, 187]. Intriguingly, PPARγ was recently reported to also be a positive regulator of osteoclast differentiation in mice [188]. Heterozygous PPARγ knockout mice have increased bone mass, as well as reduced adipogenesis and enhanced osteoblastogenesis of bone marrow MSCs [46, 189]. Similarly, reservatol has been reported to inhibit bone marrow MSC adipogenesis and promote osteoblast marker gene expression, an effect attributed to antagonism PPARγ [190]. However, while the PPARγ antagonist bisphenol-A-diglycidyl ether (BADGE) was shown to prevent bone marrow adiposity in type 1 diabetic mice, this was not associated with an improvement in bone density [191]. Thus, at this early stage, the benefit of PPARγ modulation as a means of treating bone loss remains uncertain.

Concluding remarks

A growing body of experimental evidence supports a causative role for bone marrow adipogenesis in the bone loss associated with aging, chronic drug treatment, and pathogenic disorders such as diabetes and osteoporosis. While information regarding the underlying mechanisms is only beginning to emerge, it is likely that predisposition of bone marrow MSCs to the adipocyte versus osteoblast lineage is a contributing factor. Thus, identification and thorough characterization of signaling pathways that influence or determine the phenotypic fate of MSCs has the potential to provide promising, novel pharmacologic targets. Given the limitations of current therapies for disorders of bone loss such as osteoporosis [192], this is an attractive prospect. However, further research and fundamental problems of target tissue selectivity need to be addressed before the potential of these approaches can be fully realized.

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