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Review

# Fate decision of mesenchymal stem cells: adipocytes or osteoblasts?

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**Mesenchymal stem cells (MSCs), a non-hematopoietic stem cell population first discovered in bone marrow, are multipotent cells capable of differentiating into mature cells of several mesenchymal tissues, such as fat and bone. As common progenitor cells of adipocytes and osteoblasts, MSCs are delicately balanced for their differentiation commitment. Numerous *in vitro* investigations have demonstrated that fat-induction factors inhibit osteogenesis, and, conversely, bone-induction factors hinder adipogenesis. In fact, a variety of external cues contribute to the delicate balance of adipo-osteogenic differentiation of MSCs, including chemical, physical, and biological factors. These factors trigger different signaling pathways and activate various transcription factors that guide MSCs to commit to either lineage. The dysregulation of the adipo-osteogenic balance has been linked to several pathophysiologic processes, such as aging, obesity, osteopenia, osteopetrosis, and osteoporosis. Thus, the regulation of MSC differentiation has increasingly attracted great attention in recent years. Here, we review external factors and their signaling processes dictating the reciprocal regulation between adipocytes and osteoblasts during MSC differentiation and the ultimate control of the adipo-osteogenic balance.**
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Bone is a rigid organ that provides support and physical protection to various vital organs of the body. Throughout the life, bone is in the dynamic balance involving a complex coordination of multiple bone marrow cell types. It is estimated that in adult human body, the entire skeleton is renewed every 7 years. Bone formation by osteoblasts and resorption by osteoclasts are tightly regulated processes responsible for continuous bone remodeling. Osteoclasts originate from hematopoietic stem cell precursors (HSCs) along the myeloid differentiation lineage;[1] whereas osteoblasts are derived from a common progenitor cell with adipocytes, bone marrow mesenchymal stem cells (MSCs).[2,3] The imbalance between bone formation and resorption results in various diseases, such as osteopetrosis, osteopenia, and osteoporosis.[1] These bone malformations also participate in other diseases such as cancer and autoimmunity. As a common progenitor, the tightly controlled lineage commitment of MSCs has a critical role in the maintenance of bone homeostasis. Although a variety of cell types can be derived from MSCs, the commitment of MSCs to adipocytes and osteoblasts has been specially implicated in pathological conditions of abnormal bone remodeling.[4–6] For example, increased marrow fat content has been observed in osteoporosis patients, the most common bone remodeling disorder worldwide.[7,8] Actually, the increase in bone marrow adiposity has been observed in most bone loss conditions, including aging,[8,9] and various pathological conditions.[10–17] Therefore, modulating lineage commitment of MSCs could provide effective therapeutic regime for related bone diseases.

The lineage commitment of MSCs to adipocytes and osteoblasts definitely warrants further detailed studies, not only because they share a common precursor, but also for the critical roles they play in the bone marrow microenvironment. Investigations in these directions will undoubtedly offer insights into various metabolic and hematological abnormalities during conditions such as obesity, osteoporosis, cancer, and aging. Here, we will review the signaling mechanisms involved in adipogenesis and osteogenesis and discuss the factors that determine the lineage commitment of MSCs.

## Mesenchymal Stem Cells

Friedenstein *et al.*[18] first discovered mesenchymal stem cells as spindle-shaped, adherent, non-hematopoietic stem cells in

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**Abbreviations:** MSCs, mesenchymal stem cells; FGF, fibroblast growth factor; TGFβ, transforming growth factor-β; BMP, bone morphogenic protein; Wnt, wingless-type MMTV integration site; Hh, Hedgehog; Runx2, runt-related gene 2; PPARγ, peroxisome proliferator-activated receptor-γ; ALP, alkaline phosphatase; OPN, osteopontin; ROS, reactive oxygen species
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bone marrow. Decades of studies have offered significant in-depth understanding of these cells. MSCs can be easily obtained from many tissues, such as bone marrow, umbilical cord, placenta, fat, lung, liver, and skin.[19,20] The most intensely studied MSCs are those derived from adult bone marrow. In fact, in bone marrow, MSCs are a minimal fraction of nucleated cells, representing 0.001–0.01% of nucleated cells.[21,22] They are typically isolated from whole bone marrow aspiration after removing the non-adherent cells. The adherent mononuclear layer of bone marrow is often cultured in DMEM supplemented with 10% fetal bovine serum and basic fibroblast growth factor (bFGF).[23] When a lag phase is broken, the enriched MSCs will proliferate rapidly, reaching confluence at time intervals related to plating density and origins, but often in less than 5 days.[24] After expansion and serial passaging, the enriched MSCs are usually heterogeneous, though in most culture, more than 95% are MSCs. Individual MSC clones can be obtained through seeding cells in 96-well plates by limited dilution.[25] These homogenous MSC clones can be picked and expanded for studies. As no specific markers have been identified, the purified MSCs are characterized by a combination of positive markers (for human: Sca-1, CD44, CD71, CD73, CD90, and CD105;[26,27] for murine: Sca-1, CD44, CD105, and CD140a[28]) and negative markers, such as the hematopoietic and endothelial markers (CD45, CD34, CD19, CD11b, CD11c, CD79a, and CD31), costimulatory molecules (CD80, CD86, and CD40), and MHC molecules (negative for class II and low for class I).[21,29,30] Another important criterion for defining MSCs is their multipotency. MSCs have been confirmed to be induced to differentiate into mature cells of several cell lineages of other types of tissue, such as cartilage, bone, tendon, ligament, and adipose tissue.[21,22] In most laboratories, the differentiation into adipocytes, chondrocytes, and osteoblasts has been used to define MSCs,[30] though *in vivo* bone formation has been urged to be adapted as gold standard for MSC designation (Figure 1).

## Molecular Regulation of the Adipo-Osteogenic Differentiation of MSCs

**Signaling pathways in adipo-osteogenic differentiation of MSCs.** The differentiation of MSCs is a two-step process, lineage commitment (from MSCs to lineage-specific progenitors) and maturation (from progenitors to specific cell types). Intensive studies in recent decades have demonstrated that a number of critical signaling pathways are involved in regulating the lineage commitment of MSCs, including transforming growth factor-beta (TGF$\beta$)/bone morphogenic protein (BMP) signaling, wingless-type MMTV integration site (Wnt) signaling, Hedgehogs (Hh), Notch, and fibroblast growth factors (FGFs). As these pathways are well-established, we only briefly review their roles in MSC differentiation (Figure 2).

*TGF$\beta$/BMPs family:* The TGF$\beta$ superfamily consists of more than 30 members, which are widely involved in regulating cell proliferation, cell differentiation, and embryonic development.[31] The TGF$\beta$ superfamily is divided into three subtypes: TGF$\beta$1, TGF$\beta$2, and TGF$\beta$3 and BMPs belong to TGF$\beta$1 family.[32] Different members exert various functions, being dose dependent for some of them,[33] in MSC differentiation.[34] For example, BMP4 alone can promote

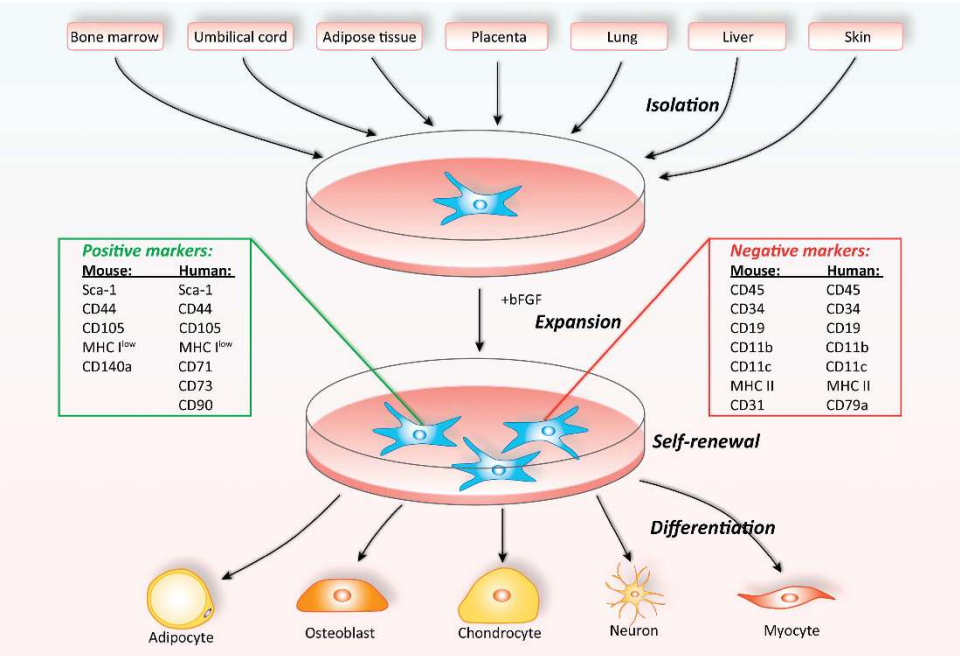


**Figure 1** Isolation, expansion, and differentiation of MSCs. MSCs can be isolated from various tissues of either human or mouse. This minor population of cells can be isolated, expanded, and enriched after serial passages *in vitro*. A combination of positive and negative markers can be used to determine the purity of MSCs. In addition to self-renewal, these multipotent MSCs can also undergo differentiation in culture. One of the gold standards for defining MSCs is their differentiation ability to cell lineages such as adipocytes and osteoblasts

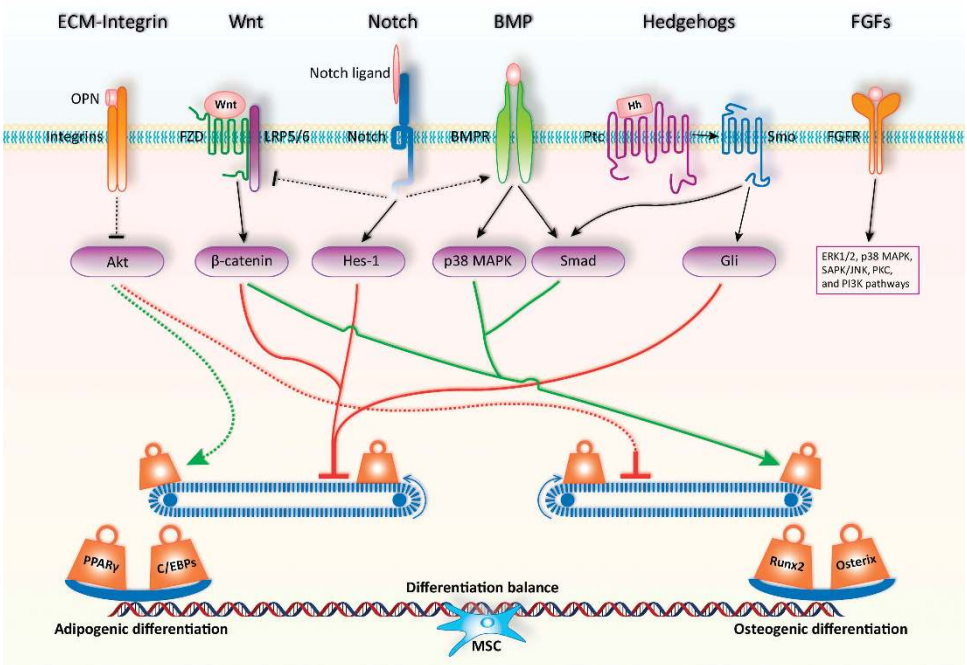


**Figure 2** Signaling pathways and key transcription factors in regulating the adipo-osteogenic differentiation of MSCs. The fine balance of adipogenic and osteogenic differentiation of MSCs is achieved by the actions of critical signaling pathways and key transcription factors. MSCs exist in specific microenvironments or niches, which is composed of various extracellular matrix components, growth factors, cytokines, and chemokines. Upon interaction with MSCs, these components activate or inhibit the lineage commitment of MSCs. In addition, the initiated cellular signaling pathways can also interfere each other to form a fine regulatory network. Ultimately, this signaling network maintains a delicate differentiation balance through regulating key transcription factors such as PPAR$\gamma$ and C/EBPs or Runx2 and Osterix for adipogenesis or osteogenesis respectively. OPN, osteopontin; FZD, Frizzled receptor; Hh, Hedgehog; Ptc, Patched; Smo, Smoothened

adipogenic differentiation of MSCs,[35] while BMP2 needs to work together with rosiglitazone to induce adipogenic differentiation.[36] Furthermore, low dose of BMP2 promotes C3H10T1/2 to differentiate into adipocytes. However, high dose of BMP2 accelerates osteogenic and chondrogenic differentiation of C3H10T1/2.[33] The TGF$\beta$/BMPs signaling pathway has been generally recognized to have dual roles in regulating adipogenic and osteogenic differentiation of MSCs.[34] By binding to their transmembrane serine-threonine kinase receptors (type I and type II), TGF$\beta$/BMPs activate canonical Smad-dependent pathways (TGF$\beta$/BMP ligands, receptors, and Smads) and non-canonical Smad-independent signaling pathway (e.g., p38 mitogen-activated protein kinase (MAPK) pathway).[37] Upon TGF$\beta$/BMPs stimulation, the expression of runt-related gene 2 (Runx2/Cbfa1)[32] and peroxisome proliferator-activated receptor-$\gamma$ (PPAR$\gamma$)[34] can be regulated by either the Smad or the p38 MAPK pathway. The altered expression level of lineage-specific transcription factors directly control MSC differentiation. Therefore, the composition and concentration of cytokines in the microenvironment of the MSC niche are critical for MSC lineage commitment.

*Wnt:* The Wnt family consists of a large number of secreted glycoproteins, which function in either paracrine or autocrine manner.[38] As a highly conserved signaling pathway during the evolution of multicellular organisms, Wnt signaling is involved in many critical biological processes, including development,[38] metabolism,[39] and maintenance of stem cells.[40] Through binding to the 7-transmembrane domain-spanning Frizzled receptor (FZD) and LRP5/6 coreceptors, Wnt ligands stabilize $\beta$-catenin via preventing its phosphorylation.[41] Unphosphorylated $\beta$-catenin translocates into the nucleus and regulates target genes expression.[38] Increasing evidence suggests that Wnt signaling may have an important role in regulating MSC differentiation.[42,43] The activation of Wnt signaling has been reported to facilitate osteogenic differentiation[44] and inhibit adipogenic differentiation of MSCs.[43] Wnt3a has been specifically shown to stimulate osteogenic differentiation through activation of TAZ by PP1A-mediated dephosphorylation.[45] Most recently, it has been demonstrated that YAP/TAZ could mediate alternative Wnt signaling-induced osteogenesis.[44] Animal studies showed that activation of Wnt signaling by overexpression of Wnt10b or supplementation of lithium could increase the thickness of trabecular bone.[46] Accordingly, deficiency of Wnt10b leads to decrease in bone density.[47] Aging-associated increase in adipocytes is also thought to be related to the reduction of Wnt10b.[46,47] In addition, the loss of $\beta$-catenin in the mesenchyme of the developing mouse uterus was found to be a switch to adipogenesis in the myometrium.[48] These studies provide strong evidence for the role of Wnt signaling in regulating the balance between adipogenic and osteogenic differentiation of MSCs.

*Notch:* The Notch signaling pathway involves Notch, Notch ligand (Delta/Serrate/LAG-2, DSL protein), and CBF1/Su (H)/Lag-1 (CSL, DNA binding protein).[49] Both Notch and Notch ligands are single transmembrane proteins, which involve cell–cell communication to regulate various cell differentiation processes. Like a double-edged sword, Notch showed an inhibitory role and an absolute necessary role in adipogenic

differentiation, as demonstrated by studies of the 3T3-L1 model. The expression of PPAR$\gamma$ and C/EBP$\alpha$ was blocked by exposure to Notch ligand jagged1 or overexpression of the Notch target gene Hes-1 in 3T3-L1 cells. Surprisingly, the adipogenic differentiation capability can be reduced in these cells by knockdown of Hes-1 using siRNA.[50] Recently, it has been demonstrated that blocking Notch signaling promotes autophagy-mediated adipogenic differentiation of MSCs via the PTEN-PI3K/AKT/mTOR pathway.[51] Besides its role in adipogenic differentiation, Notch signaling has also been shown to suppress osteogenic differentiation via inhibiting Wnt/$\beta$-catenin signaling.[37] However, other studies showed that Notch signaling could also promote osteogenic differentiation through cross-talk with BMP2 signaling.[52] Therefore, Notch signaling pathway regulates both adipogenesis and osteogenesis of MSCs in a complex manner through direct targeting related genes or interacting with other signaling pathways.

*Hedgehogs:* Hedgehogs are secreted proteins consisting of three orthologs: Sonic Hedgehog (SHh), Indian Hedgehog (IHh), and Desert Hedgehog (DHh). Hedgehog precursor is cleaved to produce an active 19 kD N-terminal fragment, which binds to membrane proteins, Patched (Ptc) and Smoothened (Smo). With the ligation of Hedgehog, Smo is released, resulting in activation of the transcription factor Cubitus Interruptus in fly (vertebrate orthologs Gli1, Gli2, and Gli3) to regulate the expression of Hedgehog targeted genes.[37] The components of Hedgehog signaling pathway such as SHh, IHh, and DHh as well as Gli are highly expressed in MSCs. During adipogenic differentiation of MSCs, Hedgehog signaling is downregulated due to the decreased expression of Gli. Consistent with this observation, activation of Hedgehog signaling blocked adipogenic differentiation by inhibiting PPAR$\gamma$ and C/EBP$\alpha$ expression and lipid accumulation in 3T3-L1and C3H10T1/2 cells. In addition, inhibition of Gli could promote adipogenic differentiation.[53] Regarding osteogenic differentiation, the Hedgehog pathway has a positive role.[54–56] Furthermore, the cross-talk between Hedgehog signal and BMP signal has also been shown to promote osteogenic differentiation through modulating Smad.[57] In conclusion, these studies clearly demonstrate that the Hedgehog signaling pathway is pro-osteogenic and anti-adipogenic.

*Other signaling molecules involved in MSC differentiation:* Several other signaling pathways have also been implicated in regulating adipogenic and osteogenic differentiation of MSCs, including FGFs, PDGF, EGF, and IGF.[58–60] Their roles in MSC differentiation mainly exert through regulating signaling pathways we discussed previously, such as Wnt and TGF$\beta$/BMP pathways.

FGFs have been implicated in both adipogenesis and osteogenesis.[61] The FGF family consists of 23 structurally related members that are ubiquitously expressed in almost all tissue types. After the binding of FGF, FGF receptors dimerize and set off the downstream signaling cascade. The FGF receptor signaling cascade has been shown to involve ERK1/2, p38 MAPK, SAPK/JNK, PKC, and PI3K pathways,[61,62] which all have been shown to play important roles in regulating MSC differentiation. FGF members exert different effects on adipogenic and osteogenic differentiation of MSCs. For example, the osteogenic transcription factor Runx2 can be upregulated by FGF2, FGF4, and FGF8.[63] In addition, FGF2 could induce alkaline phosphatase activity in rat bone marrow precursors,[64] and promote mineralization during the late phase of osteogenic differentiation, together with FGF9 and FGF18. In terms of adipogenic differentiation, FGF1, FGF2, and FGF10 have been shown to possess strong adipogenic effect under the adipogenic condition.[65–67] Accumulating evidence has clearly shown that FGF2 exerts dual roles in regulating adipogenic and osteogenic differentiation. This is probably due to the interactions between FGF-initiated signaling pathways and other differentiation-related signaling pathways.

It is important to emphasize that the signaling pathways discussed above do not function in isolation. The lineage commitment of MSCs is determined by a network of various signaling pathways (Figure 2) that can be activated simultaneously by stimuli in specific microenvironment. For example, BMP2 signaling can interact with Wnt pathway through $\beta$-catenin and N-cadherin,[68] which may explain the dual roles of BMP2 in the adipogenic and osteogenic differentiation of MSCs.

*microRNAs:* Compared with well-known molecular signaling pathways, microRNAs involved in the lineage commitment of MSCs have just been caught on. It is found that various microRNAs are related to the regulation of differentiation of MSCs. Some of them have roles in lineage commitment while others are critical for terminal differentiation.[69–71] Here, we summarize the microRNAs involved in lineage determination of MSCs.

Huang *et al.*[72] demonstrated that miR-204 promoted adipogenic differentiation of MSCs via targeting Runx2, an osteogenic transcription factor. Overexpression of miR-204 and its human homolog miR-211 suppressed osteogenic differentiation and enhanced adipogenic differentiation. By targeting Osterix, another important osteogenic transcription factor, miR-637 promoted adipogenesis while inhibited osteogenesis.[73] miR-27b was reported to inhibit adipogenesis by blunting PPAR$\gamma$ and C/EBP$\alpha$, key adipogenic transcription factors.[74] In addition, miR-21 has been suggested as a negative regulator of TGF$\beta$ signaling. Overexpression of miR-21 can restore the inhibition effect of TGF$\beta$ on adipogenic differentiation of MSCs. Further study showed that miR-21 was transiently upregulated after adipogenic differentiation along with the decreased TGF$\beta$R2 expression. miR-21 blocked the TGF$\beta$ signaling via inhibiting the phosphorylation of Smad3. Therefore, miR-21 might have a negative role in osteogenic differentiation via inhibiting TGF$\beta$ signaling.[75,76]

Besides controlling the balance of adipo-osteogenic differentiation in MSCs, there are some other microRNAs that exert a parallel effect on adipogenic and osteogenic differentiation. The expression of miR-335, high level in quiescent human MSCs (hMSCs), decreased during osteogenesis. However, overexpression of miR-335 inhibited both osteogenic and adipogenic differentiation ability of hMSCs. Further studies showed that miR-335 regulated the differentiation of hMSCs through direct targeting Runx2.[70] Similarly, miR-138 has also been reported to inhibit both adipogenic and osteogenic differentiation of MSCs.[77,78] The regulation of MSC differentiation by microRNAs has recently been reviewed

**Table 1** Role of microRNAs[a] in the regulation of MSC differentiation

| MicroRNA | Protein encoded by target gene(s) | Effect | Reference |
|---|---|---|---|
| miR-20a | TGFBR2, KDM6B | ↑Adipogenesis | 142 |
| miR-26a | Smad1 | ↓Osteogenesis (ADSCs) | 143 |
| | GSK3$\beta$ | ↑Osteogenesis (BMSCs) | |
| | Tob1 | ↑Osteogenesis (BMSCs) | 144 |
| miR-30e | LRP6 | ↑Adipogenesis ↓Osteogenesis | 71 |
| | IGF2 | ↓Osteogenesis | 145 |
| miR-140 | [b]NEAT1 (lncRNA) | ↑Adipogenesis | 146 |
| miR-153 | BMPR2 | ↓Osteogenesis | 147 |
| miR-188 | HDAC9, RICTOR | ↓Osteogenesis ↑Adipogenesis | 148 |
| miR-194 | COUP-TFII | ↑Osteogenesis ↑Adipogenesis | 69 |
| miR-199a-5p | N/A | ↑Osteogenesis | 149 |
| miR-216a | c-Cbl | ↑Osteogenesis | 150 |
| miR-223 | FGFR2 | ↑Adipogenesis ↓Osteogenesis | 151 |
| miR-320 | Runx2 | ↑Adipogenesis ↓Osteogenesis | 152 |
| miR-375 | N/A | ↑Adipogenesis | 153 |
| miR-455-3p | Runx2 | ↑Chondrogenesis | 154 |

Abbreviations: ADSCs, adipose tissue-derived stem cells; BMSCs, bone marrow-derived mesenchymal stem cells; N/A, not available
[a]MicroRNAs reported in last 2 years; refer to these excellent reviews for more (Fang *et al.*,[80] Hamam *et al.*,[81] and Lian *et al.*[79])
[b]Target of microRNA-140 is a long non-coding RNA (lncRNA)

elsewhere. The bone homeostasis controlled by microRNAs was summarized recently.[79] In addition, detailed lists of target genes of various microRNAs and related signaling pathways during osteogenic and adipogenic differentiation have also been published.[80,81] Interestingly, during the last 2 years, along with the understanding of the MSC biology, remarkable progress has been accomplished in this exciting field. Hereby, we present a summary of latest identified microRNAs with the capacity to regulate MSC differentiation (Table 1).

**Transcription factors involved in osteogenic and adipogenic differentiation of MSCs.** Transcription factors that help to initiate and promote the differentiation process are direct or indirect targets of various signaling pathways. Multiple transcription factors have been demonstrated to be critical for the differentiation of MSCs to adipocytes or osteoblasts. The PPAR$\gamma$ and C/EBPs are involved in adipogenic differentiation of MSCs,[82–85] while Runx2 and Osterix are required for osteogenic differentiation.[68] Here, we provide detailed descriptions of transcriptional cascades for adipogenic and osteogenic differentiation of MSCs.

During adipogenic differentiation, the level of cyclic AMP is elevated in the adipogenic condition, which results in phosphorylation of cyclic AMP response element-binding protein (CREB).[86] The phosphorylated CREB induces the expression of C/EBP$\beta$, a member of the C/EBP family. The other two C/EBP family members (C/EBP$\alpha$ and C/EBP$\delta$) have also been implicated in adipogenic differentiation of MSCs.[83] C/EBP$\beta$ and C/EBP$\delta$ are rapidly (within 4 h) upregulated following the induction of adipogenic differentiation; however, C/EBP$\beta$ is inactive and unable to bind to DNA. C/EBP$\beta$ requires activation through phosphorylation on Thr188 by MAP kinase and on Thr179 or Ser184 by GSK3$\beta$. Then, the transcription of PPAR$\gamma$ and C/EBP$\alpha$ is activated after the binding of C/EBP$\beta$ to regulatory elements in their proximal promoters.[86] Once expressed, C/EBP$\alpha$ maintains the continuous expression of both PPAR$\gamma$ and C/EBP$\alpha$ through binding to their respective C/EBP regulatory elements. PPAR$\gamma$ and C/EBP$\alpha$ work together to regulate large group of genes that induce the adipocyte phenotype.[87] Unlike the down-regulation of C/EBP$\beta$ in later stage of differentiation process, the expression of PPAR$\gamma$ and C/EBP$\alpha$ maintains a high level through the entire differentiation process and continue the expression throughout the whole life of adipocytes.

Runx2 and Osterix are considered as master transcription factors in regulating osteogenic differentiation of MSCs.[88,89] During osteoblast differentiation, most signaling pathways investigated so far are targeted at Runx2.[88] Upregulation of Runx2 in MSCs promotes their differentiation potential into immature osteoblasts, while inhibits their lineage commitment to the adipocytes.[90] In addition, Runx2 has been shown to be required for the induction of major bone matrix genes in immature osteoblasts, while unnecessary for the maintenance of these genes in mature osteoblasts.[91] Indeed, one recent study has demonstrated that the feedforward regulation between Runx2 and Glut1 (a glucose transporter) facilitates the initiation of osteoblast differentiation.[92] On the other hand, Osterix and $\beta$-catenin are required for the maturation of osteoblasts,[89,90] while Runx2 is decreased during the maturation process.[93] Although great progress has been made in the past few years, further studies are required for better understanding of the transcriptional network regulating osteogenic differentiation compared with the well-established transcriptional cascade during adipogenic differentiation.

## Regulators Controlling the Balance Between Adipogenic and Osteogenic Differentiation of MSCs

Meunier *et al.*[7] reported that there's a replacement of cell populations of the bone marrow by adipose tissue in osteoporosis patients. The balance between adipocytes and osteoblasts in bone marrow has attracted significant attention ever since. As the prevalence of obesity and osteoporosis increases in the past few years, the commitment of MSCs has been intensely studied. Accumulating information clearly shows that the lineage commitment of MSCs is directed by a multitude of cues. Here, we will discuss the cues controlling the balance between adipogenic and osteogenic differentiation of MSCs, including chemical, physical, and biological factors (Figure 3).

**Chemical factors.** The stemness of freshly isolated MSCs is determined using well-established assays in differentiation medium containing several chemicals. For adipogenic differentiation, MSCs are usually cultured in medium supplemented with isobutylmethylxanthine (IBMX), indomethacin, dexamethasone (Dex), and insulin.[21,25] IBMX and Dex are important for the initiation of adipogenic differentiation. It is

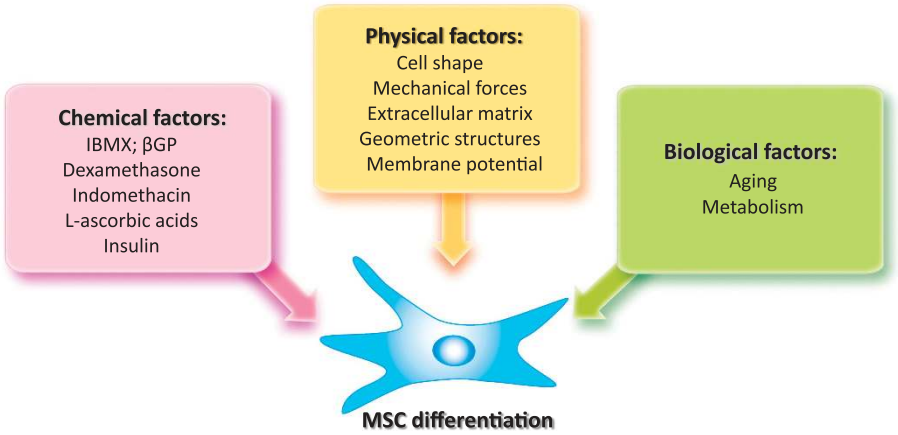


**Figure 3** Multiple factors control MSC differentiation. The lineage commitment of MSCs can be regulated by three major cues, including chemical, physical, and biological factors. Chemical factors have been proven to be important in directing adipogenesis and osteogenesis of MSCs *in vitro* through regulating key transcription factors during MSC differentiation. *In vivo*, the differentiation of MSCs can also be altered by physical factors in the stem cell niche. Investigations into the regulation of MSC differentiation commitment by cell shape, external mechanical forces, extracellular matrix or geometric structures have provided very useful information for stem cell-based bone tissue regeneration/engineering. Meanwhile, tilted differentiation balance of MSCs is also observed during aging or other pathological processes, arguing for the roles of biological factors in lineage commitment of MSCs. Taken together, these three types of factors likely work closely and cooperate with each other to regulate MSC differentiation. IBMX, isobutylmethylxanthine; $\beta$GP, $\beta$-glycerophosphate

reported that IBMX can inhibit phosphodiesterases, which causes an elevation of intracellular cAMP.[94] Elevated cAMP then leads to the alteration in transcription factors through protein kinase A. At the same time, IBMX can directly induce C/EBP$\beta$ expression as well. Similarly, Dex activates C/EBP$\delta$ expression through binding to intracellular glucocorticoid receptor.[83] Indomethacin is a well-known inhibitor of COX1/2 though its adipogenic activity is not due to the inhibition of COX, but the activation of PPAR$\gamma$.[95] Insulin functions to promote the uptake of glucose for the synthesis of triglycerides in adipocytes.

To differentiate into osteoblasts, MSCs are usually cultured in osteogenic medium containing Dex, L-ascorbic acid (AA), and $\beta$-glycerophosphate ($\beta$GP).[21,25] To analyze the effects of these chemicals on the differentiation of MSCs, Coelho and Fernandes[96] cultured MSCs in standard medium supplemented with AA, $\beta$GP, or Dex alone, or two combinations: AA+$\beta$GP and AA+$\beta$GP+Dex. AA was found to initiate the formation of a collagenous extracellular matrix (ECM), which further led to the upregulation of alkaline phosphatase (ALP) and osteocalcin. Similar observation was also made previously by another group.[97] Dex, however, promoted the cell proliferation,[98] which resulted in the induction of ALP activity[99] and mineral deposition.[96] $\beta$GP, on the other hand, was hydrolyzed by ALP, and thus provided high level of phosphate ions for mineral deposition of ECM.[96]

The differentiation of MSCs is driven by several biological processes, such as proliferation, morphological changes, expression of lineage-specific markers, lipid accumulation, and mineral deposition. The chemicals mentioned above undertake the work mutually, cooperate closely, and regulate the MSC differentiation interactively.

**Physical factors.** *In vivo*, MSCs are not in isolation, but physically interact with components in the microenvironment. For several decades, physical factors including cell shape, external mechanical forces, ECM, and geometric structures have been implicated in stem cell fate decision (Figure 4). MSCs exist in almost all adult tissues and have been isolated from a variety of tissues, such as muscle, umbilical cord, bone marrow, brain, and amniotic fluid.[19,20] Thus, MSCs physically exist in diverse microenvironment. Engler *et al.*[100] documented that ECM controls the lineage commitment of naive MSCs. Matrices mimicking the brain physical force support neurogenic differentiation. Stiffer matrices promote myogenic differentiation, while rigid matrices support osteogenic differentiation. It was found that non-muscle myosin II is the key mechano-transducer of this ECM physical property-dependent control of MSCs fate decision.

Integrins are transmembrane receptors mediating cell–matrix and cell–cell interactions. Integrins are a family of transmembrane heterodimer adhesion molecules that transduce signals to and from the cytoplasm across the plasma membrane. Ligands binding to integrins lead to its activation, which results in phosphorylation of focal adhesion kinase (FAK) and followed by activation of a series of signaling proteins including phosphatidylinositol 3-kinase (PI3K), MAPK ERK1/2, protein kinase C (PKC), and GTPases of the Rho family.[101] FAK-mediated activation of ERK1/2 and p38 has been reported to phosphorylate and activate Runx2, resulting in increased osteogenic differentiation of MC3T3-E1 cells.[102] Pref-1/DLK1, a known inhibitor of adipocyte differentiation, was reported to be involved in skeletal malformations, growth retardation, and obesity during development.[103] Initially, it was believed to regulate adipogenic differentiation via Notch signaling. Recent study has proved that Pref-1 interacts with fibronectin to inhibit adipogenesis.[104,105] Fibronectin is an important component of the ECM, which interacts with various integrin receptors and results in suppression of known transcription factors of adipogenesis.[106]

Integrins can be sensors of mechanical forces through transducing mechanical signals to the actin cytoskeleton.[107] It

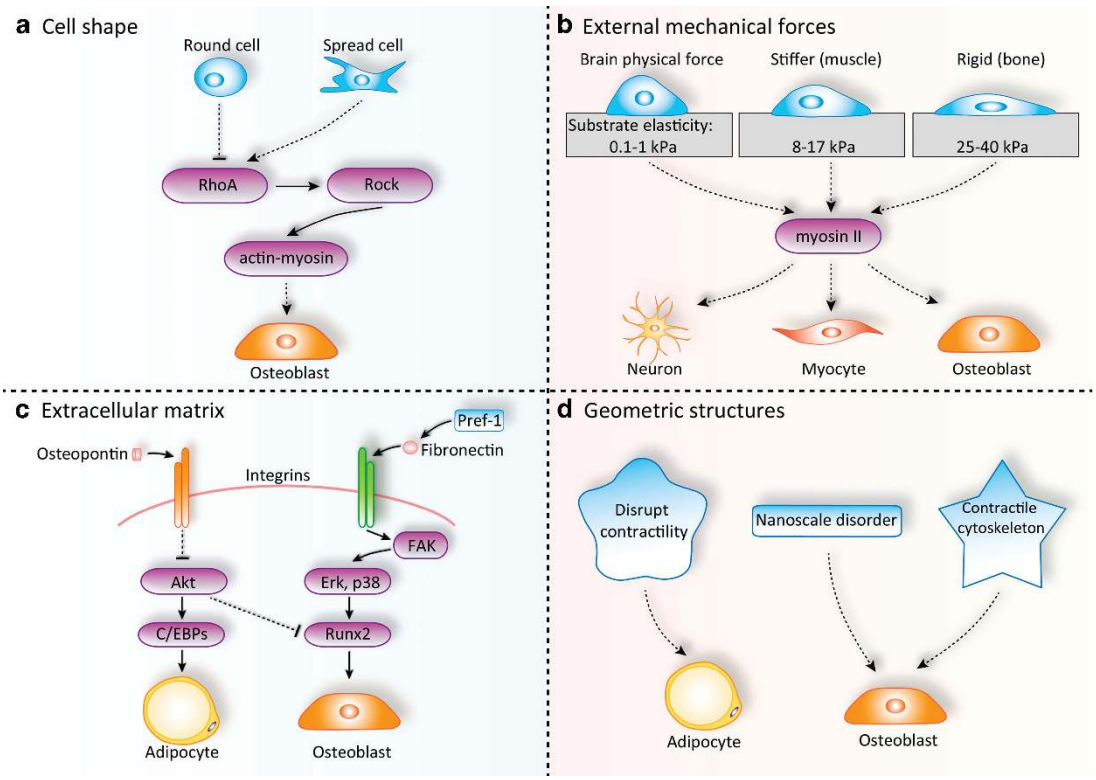


**Figure 4** Physical factors regulating lineage commitment of MSCs. MSCs physically interact with various components in the tissue microenvironment *in vivo*. The physical factors including cell shape, external mechanical forces, extracellular matrix, and geometric structures are involved in stem cell fate decision. By regulating RhoA-ROCK signaling pathway, spread cells tend to differentiate into osteoblasts while round cells tend to become adipocytes (**a**). Different physical forces can also direct MSCs to differentiate into different lineages via controlling myosin II activity (**b**). Meanwhile, components of extracellular matrix, such as osteopontin and fibronectin, can regulate the adipo-osteogenic balance of MSCs through binding to integrin receptors (**c**). In addition, geometric cues such as nanoscale changes can also effectively dictate the differentiation of MSCs (**d**)

has been shown that mechanical forces could facilitate osteogenic differentiation and inhibit adipogenic differentiation of MSCs.[108] Interestingly, modulation of actin using depolymerizing drugs cytochalasin D or latrunculin A, and stabilizing drug jasplakinolide during mechanical loading, was demonstrated to regulate ERK and AKT-mediated signal transduction and mechanical force-induced MSC differentiation.[107] Meanwhile, the role of mTORC2 in mechanically induced signaling transduction and MSC differentiation was also investigated. It was found that Fyn, a Src family kinase, mediated the mechanical activation of mTORC2 and phosphorylation of FAK, an enhancer for mTORC2 activation. This mechanically induced Fyn/FAK/mTORC2 signaling pathway decreases adipogenic differentiation of MSCs via enhancing $\beta$-catenin signaling and regulates cytoskeleton by activating RhoA.[109] In addition, a recent study showed that mTORC2 is also involved in cytoskeleton reconstruction. Deficiency of mTORC2 in MSCs has been found to abolish strain-induced cytoskeletal reorganization, and impair osteogenic differentiation while facilitate adipogenic differentiation of these cells.[110] These studies provide important information for the understanding of exercise therapy regimens in treating osteogenesis and adipogenesis related diseases, such as osteoporosis.

Recently, osteopontin (OPN) has been demonstrated to inhibit adipogenic differentiation and promote osteogenic differentiation in MSCs through interacting with integrin $\alpha v\beta 1$ and regulating C/EBPs expression.[111] Blockade of OPN by neutralizing antibody or siRNA knockdown of OPN promotes robust adipogenic differentiation, while inhibiting osteogenic differentiation. Its role in MSC differentiation is further verified in a hydroxyapatite-tricalcium phosphate-based implantation model *in vivo*. Although the OPN-deficient mice develop normally, these mice show an increase ratio of both subcutaneous and visceral fat tissue to body weight.[111] It indicates that OPN has a critical role in regulating the balance between adipogenesis and osteogenesis during the development.

Geometric cues showed dramatic effects on MSC lineage commitment. Nanoscale disorders have been shown to stimulate MSC differentiation into osteoblasts in the absence of osteogenic inducers.[112] In addition, by culturing geometrically patterned MSCs in medium containing both adipogenic and osteogenic chemical inducers, it has been shown that geometric cues of native contractile cytoskeleton were osteogenic, while those disrupting contractility were adipogenic.[113]

A micro-patterning technique was developed to study the effect of geometric cues on MSC differentiation. By using this technology, researchers were able to precisely monitor MSC attachment by depositing specific proteins (cell resistant) on substrate and control the cell culture substrates geometrically.[114] Therefore, the interaction between MSCs and patterned substrates can be specifically analyzed

through applying this technique. Chen *et al.*[115] reported that geometrically patterned substrates controlled the cell growth and viability of endothelial cells. Recently, McBeath and Chen used this technique to control the cell shape and found that spread cells tend to differentiated into osteoblasts whereas round cells tend to differentiate into adipocytes. This cell shape-controlled lineage commitment was exerted through activating the RhoA-ROCK signaling, which was activated by actin-myosin-generated tension. Moreover, as the culture density determines the spreading degree of cells,[116] it might be a potential explanation for the different requirement of cell density during the differentiation of MSCs into adipocytes, osteoblasts, or chondrocytes.[21]

To better mimic the cell biology properties of physical factors *in vivo*, three-dimensional culture systems have been constructed. This is a millstone for the cell biology moving from *in vitro* to *in vivo*. These three-dimensional culture systems could better imitate the *in vivo* microenvironment, so that scientists are able to control the cell shape artificially in three dimensions.[117] Usually, three-dimensional systems are built relying on poly (ethylene glycol)-based hydrogels. In hydrogels, the cells are more rounded than those cultured in standard two-dimensional systems. Recently, it was reported that the shape of MSCs could be modulated dynamically through creating photodegradable poly (ethylene glycol)-based hydrogels.[118] This system makes it possible to study the dynamic physical interactions between ECM and MSCs as well as the effect of these dynamic interactions on MSC differentiation.

In addition to the physical contact with ECM, the membrane potential also has important roles in controlling the differentiation of MSCs. Interestingly, depolarization suppresses the adipogenic and osteogenic differentiation of MSCs while hyper-polarization promotes osteogenic differentiation.[119] Moreover, uniaxial mechanical tension and fluid flow-induced shear stress have been shown to significantly increase alkaline phosphatase activity and the expression of osteogenic genes in MSCs.[120,121] Therefore, in order to better understand the role of physical factors in the differentiation of MSCs, models better mimicking the *in vivo* situations are awaiting to be developed.

### Other biological factors

*Aging:* It has been known for a long time that bone loss during aging and some pathological processes are accompanied by increased bone marrow adiposity due to the shift of differentiation balance between osteoblasts and adipocytes of bone marrow mesenchymal stem cells.[7–9] However, detailed mechanisms underlying this balance shift are poorly understood. Sun *et al.*[122] had examined the effects of aging on osteogenic differentiation of MSCs by using proteomics analysis. Several molecules associated with this age-related loss of osteogenic potential were identified in MSCs. Chloride intracellular channel 1 (CLIC1) and prohibitin were found to be decreased in aged MSCs, while LIM and SH3 domain protein 1 (LASP1) and annexin V were increased. As aging progressing, reactive oxygen species (ROS) and oxidative stress have been shown to be increased and to play important roles in age-related bone loss and adipo-osteogenic differentiation through forkhead homeobox type O (FOXO), Wnt, and PPARγ.[123–125]

PPARγ, as the central transcription factor in adipogenic differentiation, suppresses osteoblast differentiation. Moerman *et al.*[9] demonstrated that the expression of PPARγ was increased in aged bone marrow MSCs by unknown PPARγ activators. The increased PPARγ expression promoted adipogenesis and inhibited osteogenesis of bone marrow MSCs in old animals. Rosiglitazone, an activator of PPARγ for type II diabetes therapy, was reported to cause side effects on bone metabolism, such as osteoporosis. It was also found to induce ROS accumulation specifically in osteoblasts resulting in PPARγ-dependent apoptosis.[126] Interestingly, adipocytes were protected from rosiglitazone-induced ROS-related apoptosis. Therefore, aging alters the elaborate balance system between osteogenic differentiation and adipogenic differentiation in MSCs.

*Metabolism:* Accumulative evidence shows that altered metabolic processes, such as mitochondrial metabolism,[127] oxidative stress,[128] and glucose uptake,[92] have been implicated to affect MSC differentiation. An increase in mitochondrial metabolism and ROS generation is a key property of MSCs undergoing adipogenic differentiation.[129,130] However, it is unknown whether this increase is a causal factor or a consequence of adipogenic differentiation. It has been demonstrated that mitochondrial-targeted antioxidants could decreased the adipogenic differentiation of MSCs, while exogenous hydrogen peroxide could restore it. In addition, it has been showed that ROS generated by mitochondrial complex III is essential for the activation of adipogenic transcription factors.[130] These results implicate that the increased mitochondrial metabolism is an early causal factor for adipogenesis. Indeed, increased mitochondrial metabolism has been shown to be prerequisite of adipogenic differentiation demonstrated by specific blocking the mitochondrial respiratory pathways.[127] On the other hand, hypoxia signaling that shifts metabolism from oxidative to glycolysis has been shown to inhibit both osteogenic[131–133] and adipogenic[134] differentiation of MSCs. However, it has also been demonstrated that hypoxia pretreatment of human adipose tissue MSCs could facilitate both adipogenic and osteogenic differentiation under normoxic condition.[135] In addition, there is another report shows that the osteogenic and adipogenic differentiation of MSCs is not affected by either hypoxia or normoxic conditions.[136] There are several possibilities for these contradictory findings: (1) variation in the standards of hypoxia and normoxia; (2) differences in culture time under hypoxia (short-term, long-term, or transient); and (3) different regimes of hypoxia and normoxic culture conditions, such as pretreatment with hypoxia for a while then transfer into normoxia conditions for differentiation assay, or first normoxic conditions then transfer into hypoxic conditions. Although considerable progress have been made in deciphering the role of metabolism in regulating MSC differentiation, criteria should be put forward to standardize the experiment system and reasonable care should be taken when performing a direct extrapolation of *in vitro* findings to the situations *in vivo*.[137]

### Reciprocality Between Adipogenesis and Osteogenesis

Over decades of study, it is more and more clear that the adipogenesis and osteogenesis of MSCs are competing and reciprocal. For example, the BMP signaling pathway has a dual role in regulating the adipogenic and osteogenic differentiation of MSCs. BMP4 subjects MSCs to adipogenic differentiation.[35] Interestingly, BMP2 promotes osteogenic differentiation at high concentrations while favors adipogenic differentiation at low concentrations.[33]

Usually, adipose tissue is recognized as an organ of energy storage. Recently, accumulating studies have identified adipose tissue as an active endocrine organ because of the secretion of various active molecules (adipokines), such as leptin, adiponectin, IL-6, and TNF-α.[138] Similarly, bones have also been recognized as endocrine organs besides their role in supporting the body. They secrete a variety of active cytokines (osteokines), including osteopontin, osteocalcin, and osteoprotegerin.[139] These adipokines and osteokines have key roles in bone and fat metabolism reciprocally.

It has been reported that PKA stimulators can promote adipogenesis and inhibit osteogenesis through leptin expression and secretion.[140] This effect of PKA stimulators on MSCs differentiation can be blocked by adding leptin exogenously. In addition, leptin can restore skeletal ossification in IBMX-treated developing zebrafish. Recently, it has been confirmed that overexpression of leptin in MSCs upregulates osteocalcin expression and promotes ALP activity. Cbf*α*1 and Cbf*β*, key osteogenic transcription factors, were also upregulated in those MSCs.[141] In summary, adipogenesis and osteogenesis are reciprocally regulated processes of MSC differentiation. They modulate each other through secreting various active adipokines and osteokines.

### Conclusions and Future Directions

Investigations from various groups in different systems have demonstrated that biological, chemical, and physical cues can exert their effects via a batch of signaling pathways on the balance between adipogenesis and osteogenesis of MSCs by affecting initiation, commitment, and differentiation. These signals finally converge at a tightly controlled cascade of transcription events, including C/EBPs and PPAR*γ* for adipogenesis and Runx2 and TAZ for osteogenesis.[86] The chemical cues provide us a well-established system to identify and study the commitment of MSCs. The physical cues, especially those from the ECM, promise MSCs a future in biomaterial-based regenerative medicine. The critical roles of biological factors, including various types of cytokines and microRNAs, provide us a better understanding of pathophysiology control of MSC differentiation. These remarkable advances in understanding the fate decision of MSCs are still preliminary and more intense studies including 'MSC omics' are urgently needed to fully understand the mechanisms underlying the balance between adipogenic and osteogenic differentiation of MSCs. These new data will be of great value to identify the pathogenic causes of fat and bone marrow related diseases, to develop novel therapies for these diseases, and to better clinical application of MSCs in tissue engineering and regenerative medicine.

### Conflict of Interest

The authors declare no conflict of interest.

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