TISSUE ENGINEERING: Part B
Volume 20, Number 1, 2014

DOI: 10.1089/ten.teb.2013.0204

The Roles of Bone Morphogenetic Proteins and Their Signaling in the Osteogenesis of Adipose-Derived Stem Cells

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Large-size bone defects can severely compromise both aesthetics and musculoskeletal functions. Adipose-derived stem cells (ASCs)-based bone tissue engineering has recently become a promising treatment strategy for the above situation. As robust osteoinductive cytokines, bone morphogenetic proteins (BMPs) are commonly used to promote the osteogenesis of ASCs. In this process, BMP signaling plays a pivotal role. However, it remains ambiguous how the pleiotrophic BMPs are involved in the commitment of ASCs along osteogenesis instead of other lineages, such as adipogenesis. BMP receptor type-IB, extracellular signal-regulated kinase, and Wnt5a appear to be the main switches controlling the *in vitro* osteogenic commitment of ASCs. Tumor necrosis factor-alpha, an acute inflammatory cytokine, is reported to play an important role in mediating osteogenic commitment of ASCs *in vivo*. In addition, various active agents and methods have been used to enhance and accelerate the osteogenesis of ASCs through promoting BMP signaling. In this review, we summarize the current knowledge on the roles of BMPs and their signaling in the osteogenesis of ASCs *in vitro* and *in vivo*.

Introduction

Large-size bone defects, resulting from congenital nonunion, trauma, inflammation, and osteosarcoma resection, can severely compromise aesthetics and musculoskeletal functions. In the clinic, the autograft is regarded as the gold-standard treatment since it can simultaneously provide osteoconductive scaffolds, osteoinductive cytokines, and osteogenic cells. However, the use of autografts is limited by their low availability, donor-site pain, and morbidity.[1] Therefore, various alternative grafts and techniques are being developed to facilitate osseous restoration in the clinic.

Adipose-derived stem cells (ASCs)-based bone tissue engineering appears to be very promising for the osseous restoration of large-size bone defects. ASCs can undergo rapid and efficient osteogenic differentiation both *in vitro* and *in vivo*.[2–4] These properties confer human ASCs a promising application potential in the clinic. Several members of the bone morphogenetic protein (BMP) family are robust osteoinductive agents[2,5,6] and have been shown to enhance and accelerate the osteogenesis of ASCs. Further, BMP signaling pathways also mediate the promoting effects of many drugs on the osteogenesis of ASCs. In this review, we summarize the current knowledge of the roles of BMPs and their signaling pathways in the osteogenesis of ASCs.

Adipose-Derived Stem Cells

The development of mesenchymal stem cell-based techniques has become a major research objective in the field of bone tissue engineering. Bone marrow was originally considered to be the main source of mesenchymal stem cells for such a purpose.[7] However, the clinical use of bone marrow-derived stem cells (BMSCs) is associated with several disadvantages, such as donor-site pain and low cell output, which has led to continuous efforts to search for alternative sources of mesenchymal stem cells.

In 2001, Zuk *et al.*, for the first time, demonstrated that human adipose tissue contains multipotent stem cells that can differentiate along different lineages, such as bone, cartilage, fat, and muscle.[8] In 2004, the International Fat Applied Technology Society adopted "adipose-derived stem cells" (ASCs) as the official nomenclature for these multipotent stem cells harvested from adipose tissue.[9] Unfortunately, hitherto, there are still no definitive surface markers for identifying ASCs, since marker expression changes during

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in vitro culture. In general, cells that express CD44, CD90, and CD105 and lack the hematopoietic lineage markers–CD34, CD45, and CD117 are recognized as ASCs.[10,11] ASCs have several advantages over BMSCs: they are more easily accessible, have a 500-fold higher yield efficiency, and are associated with lower donor-site morbidity.[12] In addition, the osteogenic capacity of ASCs is less affected by aging than that of BMSCs.[13] Due to these properties, ASCs have become an attractive source of seed cells for bone tissue engineering.

BMPs and Their Signaling

The BMP family belongs to the superfamily of transforming growth factor-beta (TGF-β). The discovery of BMPs in the pioneering work by Urist in 1965[14] was a landmark in the development of bone tissue engineering. The classical role for BMPs was considered to be the induction of (ectopic) cartilage and bone formation.[14] Several isotypes of BMPs have been demonstrated to play paramount roles in the osteogenic differentiation of various mesenchymal stem cells.[2,15–17] Particularly, BMP-2 and BMP-7 have already been approved for clinical use in the United States, Europe, and Australia.[18] Owing to continuous efforts over the last 50 years, BMPs are now recognized as a group of metabologens that constitute pivotal morphogenetic signals and orchestrate tissue architecture throughout the body.[19] Consequently, it has been suggested to change their name from "bone morphogenetic proteins" to "body morphogenetic proteins."[18]

BMPs play pleiotropic roles in promoting the differentiation of pluripotent stem cells along different lineages, for example, in osteogenesis,[2] adipogenesis,[20] and chondrogenesis.[21] The cellular and therapeutic effects of BMPs are mediated by their downstream signaling pathways that are initiated by binding of BMPs to transmembrane serine/threonine kinase receptors. Subsequently, they trigger specific intracellular signaling pathways that control the transcription of specific target genes.[22] Two types of BMP receptors exist: type I and type II. Type I receptors include activin receptor type-IA (ACTR-IA), BMP receptor type-IA (BMPR-IA), and BMP receptor type-IB (BMPR-IB). The type II receptors include BMP receptor type-II (BMPR-II), activin receptor type IIA (ACTR-IIA), and activin receptor type IIB (ACTR-IIB).[23]

BMPs can trigger two main downstream signaling pathways through binding to different receptor complexes: Smad-dependent and Smad-independent signaling pathways.[22] Activated BMP receptors phosphorylate Smad1/5/8, which assembles into a complex with Smad4 and translocates to the nucleus, regulating the transcription of target genes, such as *Runt-related transcription factor 2 (RUNX2)* and *osterix*. In addition to Smad-dependent signaling, a series of Smad-independent downstream signaling pathways, including mitogen-activated protein kinase (MAPK) pathways such as p38, c-Jun N-terminal kinase (JNK), and extracellular signal-regulated kinase (ERK), are also activated. These pathways have essential roles in BMP-induced osteogenic events.[24] On the other hand, BMP signaling is also crucial in inducing adipogenesis of pluripotent stem cells. Both Smad-dependent and Smad-independent signaling pathways participate in BMP-induced adipogenesis.[20,25,26] Two key adipogenic transcription factors (CCAAT/enhancer binding protein [C/EBP] and peroxisome proliferator-activated receptor gamma [PPAR-γ]) can be subsequently upregulated by BMP signaling, which then leads to the specific activation of adipocyte-associated genes.[25,26]

The Roles of BMPs in the Osteogenesis of ASCs *In Vitro* and *In Vivo*

BMPs, as a group of pleiotrophic cytokines, play dynamic and pivotal roles in the osteogenic differentiation of ASCs.[2,5,6] For the induction of osteogenesis of ASCs, BMPs are introduced through three ways: the administration of exogenous BMPs, gene technology, and the induction of endogenous BMPs.

Administration of exogenous BMPs

The ability of exogenous BMPs to promote *in vitro* osteogenic differentiation of ASCs is highly dependent on several factors, such as BMP type, concentration, differentiation medium, and administration time point.

BMP-2, BMP-6, and BMP-14 are highly associated with the osteogenic differentiation of ASCs *in vitro*.[2,27,28] In contrast, BMP-7 is thought to contribute to both chondrogenesis[29] and osteogenesis[30] of ASCs. From the view of dose-effect, BMP-6 and BMP-14 exhibited a significantly higher efficiency than BMP-2.[27,28] These results suggest that different types of BMPs induce the osteogenic differentiation of ASCs through distinct regulatory mechanisms. In addition, BMP-14 may simultaneously increase the expression of vascular epithelium growth factor (VEGF)—an angiogenic factor,[28] which might contribute to more pronounced bone regeneration.

Concentration is one of the main modulating mechanisms for the effect of BMPs.[31] This is also true for their effects on ASCs. For example, the transfection of BMP-4 significantly enhances the osteogenesis of ASCs,[32,33] whereas BMP-4 at an extremely low concentration range (0.01–0.1 ng/mL) can increase the survival rate of ASCs and maintain their stemness and multipotency.[34] Analogously, BMP-2 at a very high concentration range (300–500 ng/ml) can directly induce the osteogenic differentiation *in vitro* without the need for additional active agents.[35,36] However, at an intermediate concentration range (5–200 ng/mL), the effects of BMP-2 remain ambiguous. BMP-2 appears to induce the osteogenic differentiation of ASCs only in the presence of osteogenic medium. It seems that the active agents, such as ascorbic acid and β-glycerophosphate in osteogenic medium, play crucial roles in committing the osteogenic differentiation of ASCs. In the presence of osteogenic medium, 50 ng/mL BMP-2 appears to be the minimum dose to obtain a significant difference in extracellular mineralization[37]; the optimal effect is obtained at approximately 100 ng/mL.[6,37] The amount and type of active agents in osteogenic medium also influence the efficacy of the osteogenic differentiation of ASCs. For example, dexamethasone was one of the main components for the osteogenic differentiation of ASCs in the traditional osteogenic medium. However, a lack of efficacy and clinical biosafety may limit its clinical application.[38–40] Vitamin D_3 is a good alternative to dexamethasone.[38] In addition, ascorbic acid induces collagen matrix formation, while β-glycerophosphate provides an organic phosphate source that supports mineral deposition during osteogenic differentiation. These latter two components form hydroxyapatite (HA)-containing mineral within the collagen matrix.[41]

Interestingly, administration time point has been recently shown to significantly influence the effects of BMPs on the osteogenic differentiation of ASCs. When vitamin D_3 is constantly treated, BMP-2 is more efficacious to induce the osteogenesis of ASCs when treated for the last 7 days (8–14 days) than the first 7 days (1–7 days).[42] It seems plausible that specific compositions in these media determine the differentiation direction of ASCs, and then BMPs can significantly both enhance and accelerate the process.

The possibility of coadministering BMP-2 with other cytokines to achieve synergistic effects has also been investigated. Retinoic acid (RA) can simultaneously promote the osteogenic differentiation and inhibit the adipogenic differentiation of murine ASCs. Coapplication of RA and BMP-2 is reported to synergistically promote the osteogenic differentiation of murine ASCs *in vitro*.[43] In addition, combined treatment with BMP-2 and vitamin D_3 can synergistically induce the osteogenic differentiation of human ASCs *in vitro*.[42]

Traditionally, *in vitro* pretreatment with osteogenic medium is necessary to commit the osteogenic differentiation of ASCs before transplantation *in vivo*. However, this method is not ideal because the long-term culture *in vitro* will increase the risks of contamination and the possibility of change in biological behavior of cells, and significantly compromise the application potential to some urgent clinical cases.[44] Therefore, *in vivo* osteogenic induction in ASCs by BMPs has recently become an important research focus for ASCs-based bone tissue engineering. BMPs can be administrated by a simple subcutaneous injection for 1–3 days[44] or preintegration into scaffolds.[45] The latter has the advantage in aspects of cost-effectiveness and clinician-friendliness.

However, to exert their optimal osteoinductive efficacy, BMPs need to be gradually delivered to the target site, at a low level and in a sustained manner, instead of in a single high-dose burst.[1] Therefore, many efforts have been performed to develop slow-delivery systems to significantly enhance the efficiency of BMPs in promoting the osteogenic differentiation of ASCs *in vitro* and *in vivo*. ASCs and the BMP-slow-delivery scaffolds synergistically enhance the osteogenic events. Composite scaffolds that comprise of organic and inorganic phases are especially promising for inducing osteogenesis of ASCs. For example, a HA/β-tricalcium phosphate (β-TCP) scaffold that can facilitate the sustained release of BMP-2 over a 20-day period has been shown to significantly augment the osteogenic differentiation of ASCs *in vitro*.[46] In addition, many other scaffolds with similar properties have shown promise in this type of application, for example, a poly (DL-lactic-coglycolic acid) (PLGA) scaffold with a fibrin/hyaluronic acid coating[47] and a gelatin/β-TCP scaffold.[48] These slow-release systems can stimulate osteogenic differentiation, extracellular matrix deposition, maturation, and mineralization of ASCs.[49] Further, besides the slowly released BMP-2, the scaffold biomaterial itself may also play a role in the osteogenic differentiation of ASCs *in vitro*.[48] These benefits can be attributed to the interaction between BMP-2 and β-TCP: BMP-2 can increase the dissolution of β-TCP, while β-TCP can resorb BMP-2 from media and provide Ca^{2+} and PO_4^{3-} that are needed for bone mineralization.[45]

Consistent with the findings of *in vitro* studies, a controlled-released system is thought to maximize the promoting effect of BMPs on the *in vivo* osteogenesis of ASCs. For example, a PLGA/HA composite scaffold is capable of releasing BMP-2 over a 4-week period *in vitro* and thereby stimulates bone regeneration following transplantation of undifferentiated human ASCs *in vivo*.[49] This method avoids an *in vitro* culture period, and thus maximally favors the application potential of ASCs in clinic. The contribution of the transplanted human ASCs to newly formed bone is corroborated by the presence of human nuclear antigen-positive cells and the expression of specific human osteogenic proteins in the area of new bone formation in nude mice models.[2,44,49,50]

Gene technology

Over the past decade, BMPs and their signaling have also been introduced using gene technologies to induce the osteogenic differentiation of ASCs. These gene technologies include gene transfection of BMPs and the key BMP signaling components, and gene knockdown of the BMP antagonists (Fig. 1). Human ASCs transfected with *BMP-2* have been shown to significantly promote bone formation in many animal models, including ectopic bone induction in mice,[51–53] critical-size bone defects of rats,[54,55] and spine fusion in rats.[56] Further, cotransfection of *RUNX2* or *VEGF* with *BMP-2* is shown to enhance bone regeneration and accelerate the healing of segmental defects more effectively than *BMP-2* alone *in vivo*.[57,58] In addition to *BMP-2*, significant bone formation can also be achieved by transfection of ASCs with *BMP-4*,[32,33] *BMP-6*,[5] or *BMP-7*.[59,60]

On the other hand, the gene knockdown of *noggin*, one of the BMP antagonists, significantly upregulates BMP signaling and enhances BMP-2-induced osteogenic differentiation of ASCs *in vitro* and *in vivo*.[2,61] Interestingly, *noggin* knockdown also increases angiogenesis, which is essential for bone formation.[61] Moreover, simultaneous overexpression of *BMP-2* and repression of *noggin* synergistically enhance the osteogenesis of ASCs.[62]

The induction of endogenous BMPs

Many active agents can upregulate endogenous BMPs (Fig. 2), which at least partially accounts for their promoting effect on the osteogenesis of ASCs. The expression of endogenous BMPs, such as BMP-2, BMP-4, and BMP-6, can be detected in osteogenic medium.[3,37,38] The expression level of endogenous BMPs is also adopted as an important parameter to evaluate the potency of an osteogenic medium.[3,38] The types of endogenous BMPs are also dependent on the types of active reagents in the osteogenic medium. For example, endogenous BMP-6 can be induced by both dexamethasone and vitamin D_3, whereas BMP-2 is only detected in the presence of vitamin D_3.[38] These results suggest that the different active agents in osteogenic medium induce the osteogenic differentiation of ASCs through regulating BMPs and their signaling pathways.

In 1999, Mundy *et al.*, for the first time, reported that statins could effectively stimulate bone formation in rodents, both *in vitro* and *in vivo*.[63] Many subsequent studies confirmed that statins exert their osteoinductive effects through promoting BMP-2 expression.[64] Among statins, simvastatin, an inhibitor of the competitive 3-hydroxy-3-methyl coenzyme A reductase, is considered to be the most potent

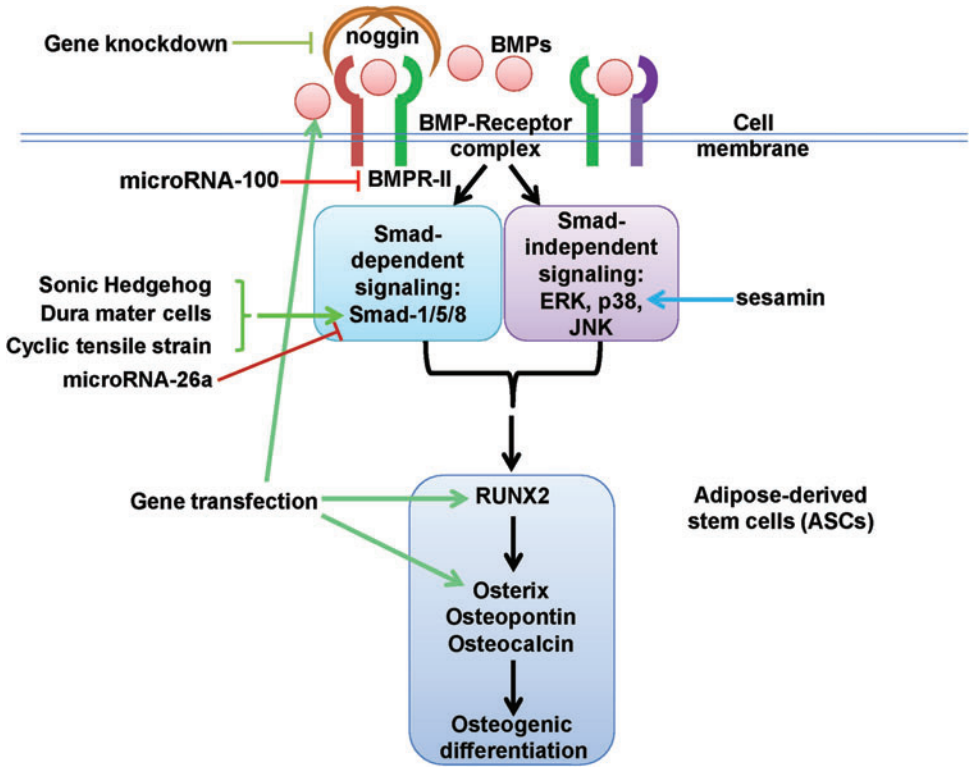

FIG. 1. Schematic diagram depicting the modulation of osteogenesis of ASCs by different active agents through modulating BMP signaling pathways. ASCs, adipose-derived stem cells; BMP, bone morphogenetic protein; BMPR-II: BMP receptor type-II; ERK, extracellular signal-regulated kinase; JNK, Jun N-terminal kinase; RUNX2, Runt-related transcription factor 2; →, promotion; ⊣, inhibition. Color images available online at www.liebertpub.com/teb

inducer for the osteogenic differentiation of mesenchymal stem cells. We recently reported that simvastatin can enhance the osteogenesis of human ASCs *in vitro* and *in vivo* by significantly increasing the expression of mRNA encoding BMP-2, RUNX2, VEGF, and fibroblast growth factor-2.[3] The upregulated BMP-2 seems to be one of the major mechanisms for the promoting effect of simvastatin on the osteogenesis of human ASCs.[3] In ASCs, endogenous BMPs can also be induced by TGF-β1,[65] sonic hedgehog (shh),[66] inhibitor of β-catenin and TCF-4 (ICAT),[67] and sesamin.[68] These active agents may significantly contribute to a rapid significant progress of the ASC-based bone tissue engineering.

In addition to cytokines and active agents, mechanical loading, such as cyclic tensile strain, can modulate the osteogenic differentiation of ASCs via BMP-2 signaling.[69] This mechanism may also account for the promotion of ASC osteogenesis by the application of an electromagnetic field.[70]

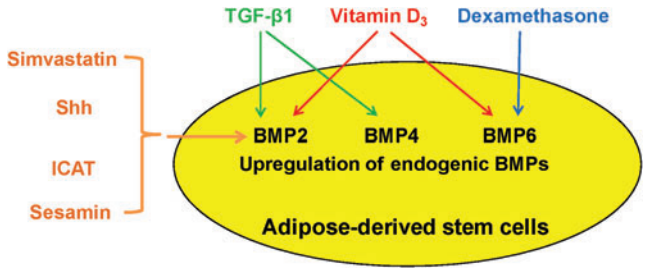

FIG. 2. Schematic diagram depicting the induction of endogeneous BMPs by different active agents for their promoting effects on the osteogenesis of ASCs. ICAT, inhibitor of β-catenin and TCF-4; Shh, sonic hedgehog; TGF-β1, transforming growth factor-β1. Color images available online at www.liebertpub.com/teb

The Roles of BMP Signaling Pathway in the Osteogenesis of ASCs

The osteogenic differentiation of multipotent ASCs involves two main steps: commitment of ASCs along the osteogenic lineage and promotion of osteogenic differentiation. BMP signaling is repeatedly shown to play crucial but ambiguous roles in the osteogenesis of ASCs.[57,62]

The roles of BMP signaling in the commitment of ASCs to osteogenic differentiation

Multipotent mesenchymal stem cells express small amounts of both adipogenic factors, such as C/EBP and PPAR-γ, and osteogenic factors, such as RUNX2 and osterix. Factors of one lineage repress factors of the other lineage, thereby maintaining the undifferentiated state.[71] Generally, the eventual fate of mesenchymal stem cells is considered to be controlled by the antagonistic balance between RUNX2 and PPAR-γ. For example, the activation of PPAR-γ can not only promote the adipogenesis, but also suppress the osteogenesis both by downregulating the expression and interfering with the transactivation ability of RUNX2.[72] $RUNX2^{-/-}$ cells show enhanced adipocyte development,[72] whereas $PPAR\text{-}\gamma^{-/-}$ cells fail to differentiate into adipocytes, but spontaneously differentiate into osteoblasts.[73]

Unlike BMSCs, ASCs show a native tendency to differentiate into adipogenic cells.[33] For a single ASC, differentiation toward osteogenic or adipogenic lineages is mutually exclusive and antagonistic.[74] Osteogenic commitment of ASCs requires both suppression of the adipogenic differentiation and enhancement of osteogenic differentiation. Interestingly, BMPs have been reported to promote both osteogenic and adipogenic differentiation of mesenchymal stem cells. This raises the question of whether BMPs can

commit a certain differentiation direction of ASCs or not. It seemed that, in the most commonly used concentration range (5–250 ng/mL) and without additional osteogenic agents, BMPs cannot significantly commit ASCs to either osteogenesis or adipogenesis. One hypothesis is that BMP alone can activate and induce equivalent levels of osteogenic and adipogenic signaling. Both signaling antagonize each other through different signaling levels. The mutual suppression and inhibition between these two signaling result in a noncommitment stage.

The balance between osteogenic and adipogenic signaling can be disequilibrated by the addition of osteogenic agents, such as osteogenic medium and RA. The disequilibration may be mediated, at least partially, by three main switches: endogenous Wnt5a and ERK and changing the ratio of BMPR-IB/BMPR-IA (Fig. 3).

BMPR-IA and BMPR-IB exhibited versatile and divergent effects on the process of osteogenesis both *in vitro* and *in vivo*.[75–77] Their exact functions are highly dependent on factors including cell type and differentiation stage.[75] For mouse ASCs, signaling through BMPR-IB played a more significant role in osteogenic differentiation, whereas signaling through BMPR-IA seemed to be more relatively relevant to adipogenesis.[43] Data from 2T3 mouse calvarial stem cells corroborated the distinct functions of these two BMP receptors.[77] Therefore, the type of receptor and level of expression on stem cells can be a switch for their commitment. It is also important to note that RA signaling is indispensable for osteogenic differentiation in mouse ASCs,[43] although this is not the case for human ASCs.[78] Consequently, precautions must be taken when extrapolating data from mouse ASCs to humans.

ERK also appears to be a key switch of adipogenic and osteogenic differentiation of mesenchymal stem cells.[79] Consistent with this finding, ERK is also important for the adipogenic and osteogenic commitment in ASCs.[68,80–83] Inhibition of ERK by PD98059 blocks the expression of osteogenic differentiation-related proteins in a dose-dependent manner and switches ASCs to adipogenic differentiation.[83] Further, ERK is indispensable for the effects of sesamin,[81] akermanite,[68] wedelolactone,[82] and oncostatin M[80] in promoting the osteogenic differentiation and inhibiting adipogenic differentiation of ASCs. However, how ERK is modulated during osteogenesis of ASCs remains to be elucidated.

Wnt signaling also has an important role in the osteogenic commitment of mesenchymal stem cells (Fig. 3). Wnt5a, a noncanonical Wnt ligand, promotes the osteogenesis by repressing PPAR-γ transactivation through CaMKII-TAK1/TAB2-NLK signaling cascade and the subsequent activation of the histone methyltransferase, SETDB1 (SET domain bifurcated 1).[84,85] SETDB1 leads to the formation of a corepressor complex that inactivates PPAR-γ function through histone H3-K9 methylation. Thus, noncanonical Wnt5a has emerged as a fate determinant of mesenchymal stem cells through shifting from adipogenesis to osteogenesis.[84,85] Exogenous Wnt5a induces osteogenic differentiation and downregulates PPAR-γ expression in human ASCs.[86] However, the interaction between endogenous Wnt and BMP signaling during the osteogenesis of ASCs needs further elucidation.

In contrast with their ambiguous effects *in vitro*, BMPs have a more definite effect on promoting the *in vivo*

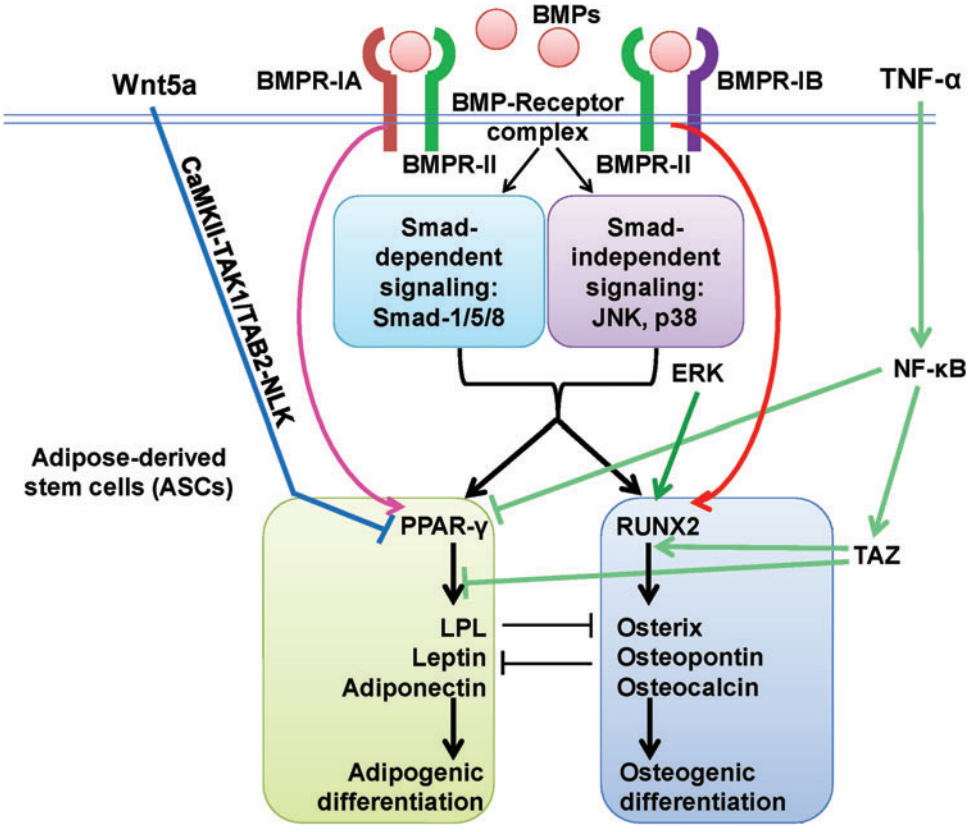

FIG. 3. Schematic diagram depicting the signaling pathways of BMPs in ASCs and the main switches for the osteogenic commitment of ASCs. BMPR-IA, BMP receptor type-IA; BMPR-IB, BMP receptor type-IB; BMPR-II, BMP receptor type-II; CaMK-II, calcium/calmodulin-dependent kinase II; ERK, extracellular signal-regulated kinase; LPL, lipoprotein lipase; NF-κB, nuclear factor κB; NLK, Nemo-like kinase; PPAR-γ, peroxisome proliferator-activated receptor gamma; RUNX2, Runt-related transcription factor 2; TAB2, TGF-β-activated kinase 1/MAP3K7-binding protein 2; TAK1, transforming growth factor β-activated kinase-1; TAZ, transcriptional coactivator with PDZ-binding motif; TNF-α, tumor necrosis factor-alpha. →, promotion; ⊣, inhibition. Color images available online at www.liebertpub.com/teb

osteogenesis of ASCs. Endogenous cytokines that are elevated during acute inflammation may also significantly facilitate the osteogenic commitment of ASCs *in vivo* (Fig. 3). Tumor necrosis factor-alpha (TNF-α) is one of the main cytokines responsible for acute inflammation.[87] TNF-α activates nuclear factor-κB (NF-κB), which inhibits the transactivation of PPAR-γ through a physical association.[85] NF-κB activation also leads to upregulation of TAZ (transcriptional coactivator with PDZ-binding motif), a coactivator of RUNX2-dependent transcription, and suppression of PPAR-γ-dependent transcription.[88] Through these three pathways, TNF-α can help to commit the osteogenic differentiation of ASCs in *in vivo* microenvironments. The role of TNF-α in their osteogenic differentiation is supported by the finding that ASCs can be used to heal acute, but not chronic, calvarial defects in nude mice.[89] TNF-α that occurs during acute inflammation may help to commit the osteogenesis of ASCs, leading to a much more definite effect of exogenous BMPs in inducing the osteogenesis of ASCs *in vivo* than that *in vitro*.

The roles of BMP signaling in the promotion of osteogenic differentiation of ASCs

BMP signaling plays a crucial role in the osteogenesis of ASCs. The upregulation of BMP signaling significantly enhances and accelerates the process. This is supported by the fact that the osteogenesis of ASCs positively correlates to the expression levels of BMPR-II, Smad-1/5, RUNX2, and osterix.[4,90–94]

Different active agents may modulate the osteogenesis of ASCs by targeting different genes and proteins for BMP signaling pathways (Fig. 1). For example, microRNA-26a inhibits osteogenic differentiation of human ASCs via targeting the Smad1 transcription factor.[95] In addition, Shh promotes bone repair by ASCs, which is associated with upregulation of phospho-Smad1/5/8.[66] Simvastatin promotion of ASC osteogenesis[3] may be possibly mediated by the activation of Ras/Smad/ERK/BMP-2 pathway.[96] Moreover, sesamin can stimulate the osteogenic differentiation of human ASCs with the upregulation of BMP-2, and further exert its effect via p38 and ERK1/2 MAPK signaling pathways.[68]

Conclusion

BMPs and their signaling pathways play paramount roles in the osteogenic differentiation of ASCs *in vitro* and *in vivo*. Various cytokines and gene technologies have been developed to promote the osteogenesis of ASCs through promoting BMPs-induced osteogenic signaling and suppressing adipogenesis. BMPs-ASCs-based bone tissue engineering is very promising for the repair of large-size bone defects.

Acknowledgments

This study was supported by a grant from the National Natural Science Foundation of China (2011/81170937 to Prof. Dr. Y. Zhou), and the Program for New Century Excellent Talents in University from Ministry of Education of China (NCET-11-0026).

Disclosure Statement

The authors indicate no potential conflicts of interest.

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Received: March 31, 2013
Accepted: June 10, 2013
Online Publication Date: August 7, 2013