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Regulation of Bone Metabolism

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Abstract

Bone is formed through the processes of endochondral and intramembranous ossification. In endochondral ossification primary mesenchymal cells differentiate to chondrocytes and then are progressively substituted by bone, while in intramembranous ossification mesenchymal stem cells (MSCs) differentiate directly into osteoblasts to form bone. The steps of osteogenic proliferation, differentiation, and bone homeostasis are controlled by various markers and signaling pathways. Bone needs to be remodeled to maintain integrity with osteoblasts, which are bone-forming cells, and osteoclasts, which are bone-degrading cells.
In this review we considered the major factors and signaling pathways in bone formation; these include fibroblast growth factors (FGFs), bone morphogenetic proteins (BMPs), wingless-type (Wnt) genes, runt-related transcription factor 2 (RUNX2) and osteoblast-specific transcription factor (osterix or OSX).

Keywords: BMP, FGF, Osteogenesis, OSX, RUNS2, Wnt

Introduction

Bone is formed through the processes of endochondral and intramembranous ossification (1). In each process mesenchymal progenitors condense and initiate developmental programs that include chondrogenesis and osteoblastogenesis (2).

During endochondral ossification, mesenchymal cells differentiate into chondrocytes, which form the cartilage growth plate. The cartilage growth plate is then gradually replaced by bone (3). Most bones in the human skeleton are made through endochondral ossification (4). These include the long, short, and irregular bones (5). Flat bones, including those of the skull, facial bones, and pelvis are made by intramembranous ossification (4-6). In this process mesenchymal stem cells (MSCs) differentiate directly into osteoblasts to organized bone (4).

In both processes, osteoblastic bone formation is identical. The synthesis of bone matrix initiates with the construction of type 1 collagen via osteoblasts. Most extracellular matrix protein of bone is type 1 collagen, which supplies strength and elasticity of bone, and scaffolding for the deposition of other matrix components such as hydroxyapatite (7).

Bone homeostasis is controlled by various signaling pathways (8). The main pathways that participate in osteoblast differentiation include members of the fibroblast growth factor (FGF) and bone morphogenic protein (BMP) families and the Wnt signaling pathway (8, 9). Correspondingly, two important transcription factors, runt-related transcription factor 2 (RUNX2) and osteoblast-specific transcription factor (osterix or OSX), are expressed in osteoblasts, both of which are essential and sufficient for osteoblast differentiation (9, 10). Some studies reported that RUNX2 and OSX are not sufficient for osteoblast maturation (11); however, it is clear that RUNX2 regulate osteogenesis (12).

Bone must be constantly replaced to preserve its strength and integrity. Bone remodeling is organized by two conflicting activities; these are bone formation by osteoblasts, which produce the organic bone matrix, and bone resorption by osteoclasts, which dissolve bone mineral and extracellular matrix (7, 13). Osteogenesis and angiogenesis are two closely-associated processes involved in bone growth, remodeling, and repair (6). Osteoclasts activate angiogenesis in vitro via expression of proangiogenic

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factors, including vascular endothelial growth factor A (VEGF-A) (14). Additionally, VEGF works with receptor activator of nuclear factor kappa-B ligand (RANKL) to promote osteoclastogenesis (15). Deckers et al. demonstrated that osteoclast activity is not vital for angiogenesis because angiogenesis occurs in the absence of osteoclast activity (16). However, Cackowski et al. reported that osteoclasts could increase angiogenesis and that inhibition of osteoclast activity with osteoprotegerin (OPG) decreased angiogenesis, and enhancement of osteoclast activity with parathormone (PTH) increased angiogenesis (17).

Role of Wnt signaling pathway in bone formation

Wnt ligands are a group of 19 secreted glycol-proteins that activate their cell surface receptors to stimulate specific intracellular signaling cascades leading to gene expression (18). Wnt ligands have crucial roles in the development and homeostasis of various organs and bone metabolism via both canonical (β-catenin-dependent) and non-canonical (β-catenin-independent) signaling pathways (11, 19, 20).

Wnt signaling has revealed both pro-osteogenic and anti-adipogenic activities in canonical and non-canonical pathways (11).

In canonical Wnt pathways such as Wnt1, Wnt3a, and Wnt8, Wnt proteins bind to cell surface receptors that are members of the frizzled (Frz) protein family, and one of the low-density lipoprotein (LDL) receptor-related proteins (LRP-5 or LRP-6), which trigger intracellular disheveled (DSH) (11, 18, 21), leading to inactivation of a cytoplasmic protein complex that typically catalyzes β-catenin phosphorylation and the subsequent destruction of β-catenin. Therefore, canonical Wnt signaling enhances the stabilization of cytosolic β-catenin and translocation into the nucleus where it binds transcription factors that include members of the T-cell factor (Tcf)/lymphoid enhancer-binding factor (Lef-1) family and upregulates the transcription of specific target genes (18, 21).

Tcf/Lef-1 are critical for proper osteogenesis. Lef-1+/- mice exhibited significant low bone volume through the early years of life while Tcf-/- mice demonstrated increased bone resorption, consequently leading to a low bone mass phenotype (18).

In the non-canonical Wnt signaling pathway, a parallel transmembrane complex communicates between Wnt, Frz, DSH, and Ror2, and then stimulates G protein and a signal cascade resulting in the release of intra-cellular calcium from the endoplasmic reticulum (11, 18). The non-canonical Wnt calcium-dependent pathway is vital in embryonic growth, cell migration, and cancer regression (18); nevertheless, the data regarding non-canonical Wnt pathways in bone metabolism is limited.

The role of the canonical Wnt signaling pathway in bone mass has been described (19). It has been shown that β-catenin stimulates the differentiation of MSCs from osteoblastic precursor cells into more mature osteoblasts, while suppressing differentiation into adipogenic and chondrogenic lineages (18).

Studies on loss-of-function mutations in the LRP-5 gene discovered that LRP-5 be situated as a co-receptor of Wnt/β-catenin and positively controlled bone volume in humans and mice (20). Some loss-of-function mutations in LRP-5 are related by autosomal recessive osteoporosis-pseudoglioma (OPPG) syndrome, which displays slight bone volume, clarifying the main role of the Wnt/β-catenin pathway in bone formation (11, 22).

Korvala et al. provides additional evidence of the role of LRP-5 in patients with primary osteoporosis by two heterozygous missense mutations, L1149Q and G1185R. All LRP-5 mutations associated with primary osteoporosis in this study are situated in the LRP-5 coding regions 5 (23). Jacobsen et al. determined that increased LRP-5-mediated signaling is an effective procedure for improving bone properties in a mouse model of osteogenesis imperfecta (24). Inhibiting the canonical Wnt signaling through endochondral ossification resulted in a low level of β-catenin protein (25). The activation of canonical Wnt signaling induces OSX expression, which promotes osteoblast differentiation (19).

Kobayashi et al. showed that Wnt5a non-canonical Wnt enhanced osteoclast formation (19) by enhancing the expression of Rank in osteoclast precursors (26). In contrast, the main role of canonical Wnt signaling in osteoblasts is to suppress RANKL and enhance OPG expression, which together are proposed to prevent osteoclast formation (22). For example, Wnt16 repressed osteoclast formation via inhibition of RANKL-induced osteoclastogenesis, and consequently, Wnt signaling molecules involved in bone formation and resorption (19, 26).

Interestingly, Wnt5a, a classical non-canonical Wnt, was described as a key element of BMP2-mediated osteogenic differentiation. Others have shown that BMPs can downregulate Wnt signaling in osteogenic differentiation via sclerostin and Dkk-1 (18). Dkk1 and sclerostin are expressed and secreted by osteoblasts, and cooperatively obstruct Wnt/β-catenin signaling through binding to LRP5 and LRP6 (27). Disturbance of Dkk1 or sclerostin improve the ability of Wnts to protect β-catenin and increase osteogenesis (28).

Role of BMPs in bone formation

Bone morphogenic proteins are members of the transforming growth factors-β (TGF-β) superfamily (4). The role of BMPs was first observed in the mid-1960s when it was established that they could induce ectopic bone formation (29). Bone morphogenic proteins are extracellular cytokines originally isolated from bone extract (11) and are produced in nearly all skeletal cells (9). Bone morphogenic proteins have various roles in the development and regulation of bone formation (4). One role is to stimulate early osteoprogenitors to instigate their differentiation to pre-osteoblastic cells (7). Although this family name indicates that all members are bone inducers, some BMPs, such as BMP3 and BMP13, inhibit bone formation (11, 29). Some BMPs that stimulate osteoblastic differentiation in MSCs in vitro and in vivo are BMP2, BMP6, BMP7, and BMP9 (4). Recombinant BMP2 and BMP7 are approved by the FDA for the regeneration of the bone in spinal fusion surgery and other orthopedic applications (11). Bone morphogenic proteins in pre-osteoblastic cell lines increase alkaline phosphatase (ALP) activity, as well as osteocalcin and osteopontin (7). Recently, it was shown that BMP2 can induce activation of ALP, an early marker of osteoblast differentiation (30).

Bone morphogenic protein 9, a major osteogenic BMP, is also known as growth differentiation factor 2 or GDF2 (4). Xiang et al. showed that ALP activities induced by BMP9 were higher than those influenced by BMP2, suggesting that BMP9 had greater osteogenic stimulating potential than BMP2. In calcium salt sedimentation experiments, sedimentation was greater with BMP9 than with BMP2, indicating that BMP9 is likely a more efficient osteo-inductive growth factor than BMP2 (31).

Bone morphogenic proteins also normalize cartilage development, which is naturally related to bone formation (29). The BMP signaling pathway is necessary for endochondral bone formation, as severe chondrodysplasia was observed in mice lacking BMP signaling elements (3). Bone morphogenic protein 5, expressed during endochondral ossification, induces the condensation of mesenchymal cells to chondrocytes (7). Bones of BMP5 mutant mice are weaker and shorter than those of normal mice (29). Disturbances in BMP signaling cause a range of skeletal anomalies (4, 9). Loss of BMP2 alone or BMP2 and BMP4 result in a severe chondrodysplastic phenotype, although loss of BMP4 alone demonstrated only a slight effect on cartilage development. These results indicated that BMP2, but not BMP4, is essential for endochondral ossification (4). Some studies reported that BMP4 acts synergistically with VEGF to support bone healing; furthermore, it has been shown that VEGF cooperates synergistically with BMP2 to induce bone formation (32).

Bone morphogenic protein 2 also regulates expression of the critical osteogenic regulator RUNX2 (4). Phan et al. indicated that BMP2-induced expression of OSX in osteoprogenitor (MC3T3) cells was RUNX2 independent, even though OSX has been proposed to be downstream of RUNX2 (7). Bone morphogenic protein signaling through the activation of heterodimeric Smad proteins regulates RUNX2 expression (13). Smad proteins are intracellular signaling molecules that are activated by TGF-β super family ligands (33) and recognized in growth plate cartilage (34). Smad6 seems to play a role in blocking BMP signaling, whereas Smad7 blocks both TGF-β and BMPs, and Smad7 is involved in inhibiting TGF-β-dependent signaling (33, 34). Smad6 is required for inhibition of endochondral ossification (29). Loss of Smad6 results in increased activity of both proliferative and hypertrophic chondrocytes and is associated with increased collagen production (3). Horiki et al. reported that overexpression of Smad6 has no significant effect on chondrocyte proliferation, but significantly disturbs chondrocyte hypertrophy, which can result in postnatal dwarfism with osteopenia (34).

Bone morphogenic protein 5/14 double mutants have extra defects in bone compared to single mutants in mouse, proposing possible synergistic function of BMP5 and BMP14 (29). Bone morphogenic proteins 12, 13, and 14 are essential in standard bone and joint formation. Bone morphogenic protein 12 is proposed to function in the structural integrity of bone and may act as a negative regulator of chondrogenesis. Bone morphogenic protein 13 knockout mice have enhanced coronal suture fusion, representing an inhibitory role of BMP13 in osteogenic differentiation (29).

Therefore, BMPs play important roles in both intramembranous and endochondral ossification; moreover, they normalize the entire evolution of ectopic skeletal formation (7).

Role of FGFs in bone formation

Fibroblast growth factors are pleiotropic growth factors that normalize cell proliferation, migration, and differentiation in various organs, including bone (35). The FGF family comprises 22 genes encoding structurally related proteins (36). The FGF family comprises three subfamilies known as canonical, hormone-like, and intracellular. The roles of canonical and hormone-like FGFs have been characterized in bone differentiation, but the roles of intracellular FGFs have not been studied in bone (35). Four diverse FGF receptors (FGFRs) have been identified. A typical FGFR involves an extracellular domain and an intracellular divided tyrosine kinase domain. Fibroblast growth factor ligands bind to their respective FGFR extracellular domains and induce the phosphorylation of tyrosine residues in their intracellular domains (37).

Fibroblast growth factor ligands such as FGF2, 3, 4, 9, and 18 are involved in normal skeletal growth (9). Bone formation is inhibited in FGF2-deficient mice (36). Fibroblast growth factor 2 is expressed in osteoblast-lineage cells (35) and enhances RUNX2 phosphorylation and functional activity (9).

Fei et al. showed that addition of exogenous FGF2 moderately protected the low osteoblast mineralization in FGF2-/- bone marrow stromal cells (BMSCs), which is consistent with the increased β-catenin accumulation seen in the nucleus; FGF2-mediated osteoblast mineralization is dependent on β-catenin function (38). Fibroblast growth factor 4 can stimulate BMSC proliferation in vitro and forcefully stimulate RUNX2 expression in osteoblast cells including MC3T3-E1 and murine pre-myoblast C2C12 cells (37).

Some evidence also exists for FGF8 and FGF17 expression in bone formation (36).

Moon et al. showed that FGF8 is necessary for limb axis formation; elimination of FGF8 from the apical ectodermal ridge (AER) had more severe effects on formation of proximal than distal elements (39).

Interestingly, Lu et al. showed that FGF9 prevents the osteogenic differentiation of BMSCs in vitro, contradicting previous reports that FGF9 is necessary for growth plate development and osteogenesis. Mice lacking FGF9 display low chondrocyte proliferation, late chondrocyte hypertrophy, and deficiencies in skeletal vascularization resulting in abnormalities in osteogenesis (40). Similarly, it was reported that FGF9 plays a role during the development of skeletal vascularization (32). These contradictory results suggest that FGF9 may promote the chondrogenesis of mesenchymal stem cells while preventing osteogenesis in vitro (40).

Wallner et al. showed in type2 diabetes mellitus (T2DM) that angiogenesis, osteogenesis, and bone remodeling are reduced, and VEGFA, which facilitates osteogenesis and angiogenesis during skeletal development, is upregulated in FGF9-enriched environments (41).

Fibroblast growth factor 18 also regulates chondrocyte and osteoblast differentiation and bone development (42, 43). Fibroblast growth factor 18 protein was found in the perichondrium loci that overlap with loci of osteoblast differentiation and increase β-catenin levels (44). Fibroblast growth factor 18-null mice have delayed calvarial ossification, indicating a requirement for FGF18 in intramembranous bone formation (36).

Fibroblast growth factor 18 is a downstream target of canonical Wnt signaling that leads to osteoblast differentiation. Fibroblast growth factor 18 is induced following inhibition of glycogen synthase kinase 3 (GSK3) in canonical Wnt signaling. In addition, RUNX2 is necessary

for stimulation of FGF18 expression through Wnt. Activation of FGF18 expression by RUNX2 in Wnt signaling may promote early osteoblast differentiation but suppress later processes. Reinhold et al. showed that FGF18 can suppress osteoblast differentiation in cultured metatarsals (44).

Fibroblast growth factor 23 is a bone-derived phosphaturic hormone and a member of the FGF19 subfamily, which is expressed in osteoblasts/osteocytes in response to phosphate and vitamin D (35, 45, 46). Fibroblast growth factor 23 has a crucial role in balancing mineral ions such as phosphate. Furthermore, FGF23 is a major factor for bone homeostasis and mineralization. Fibroblast growth factor 23 itself is an inhibitor of mineralization but its mechanism of action is not yet known. 1, 25-dihydroxyvitamin differentiate (1, 25-(OH) 2D) can induce FGF23 expression in osteocytes. Parathyroid hormone (PTH) might similarly normalize FGF23 levels (46). The main functions of PTH are to control plasma calcium and bone formation, and prevent osteoblast and osteocyte apoptosis (13). Reduced mineralization detected partially in FGF23-/-mice is a result of increased circulatory 1, 25 (OH) 2D3 and subsequent increases in bony pyrophosphate (PPi) and osteopontin (OPN) concentrations (45). These findings indicate secondary effects of elevated 1,25(OH)2D as a result of loss of FGF23 effects on the kidney more than direct effects of FGF23 effects on organs. Fibroblast growth factor 23 seems to work as a vitamin D counter-regulatory hormone (47). Osteopontin, a well-known inhibitor of bone mineralization, may play a key role in imperfect mineralization of bone in FGF23-/-mice. Despite high mineral ion levels in FGF23-/-mice sera, severe defects in skeletal mineralization are observed. The reason for this finding is not yet known (46). Fibroblast growth factor receptors are related to normal skeletal development. Mutation of FGFRs has revealed a key role of FGF signaling in bone endochondral and intramembranous ossification (36).

Fibroblast growth factor receptor 1 is expressed in osteoblast and osteocytes. Studies in humans and mice demonstrate that FGFR1 plays a major role in bone formation. Fibroblast growth factor receptor 1 stimulates the differentiation of mesenchymal progenitors to osteoblasts, conversely, inactivation of FGFR1 in differentiated osteoblasts in mice increases bone mass, possibly due to a reduction of osteoclast activity in FGFR1-deficient mice (37).

The lack of FGFR2 signaling during endochondral and intramembranous bone formation results in bent bone dysplasia-FGFR2 type. These skeletal defects result, at least in part, from deficient FGFR2 signaling, which is found through loss-of-function studies in mice (48).

Liu et al. demonstrated that the FGFR2C342Y mutation may increase primary osteoblast differentiation; nevertheless, this mutation can prevent expression of tissue non-specific alkaline phosphatase and mineralization in more differentiated cells. Furthermore, the FGFR2C342Y mutation causes autonomous abnormalities in osteoblast differentiation (49).

FGFR3 is expressed in mature osteoblasts, osteocytes, and proliferating chondrocytes of the epiphyseal growth plate (37). Fibroblast growth receptor 3-deficient mice display skeletal overgrowth (36). Deng et al. revealed overgrowth of axial and appendicular skeleton in these mice (50). It seems that FGFR3 activation is responsible for negative regulation of bone elongation because FGFR3-null mice have increased long bone length (51). Cool et al. showed that FGFR4 is highly expressed in osteogenic stem cells that are undergoing proliferation and differentiation and during intramembranous ossification.

The Key Osteogenic Transcription Factor, Runx2

Runt-related transcription factor 2, also known as core binding factor alpha1 (cbfa1), is a key transcriptional regulator for osteoblast cell fate determination (8, 13, 25).

Runt-related transcription factor 2 expression is initially seen in osteochondroprogenitor cells at the beginning of skeletal development. Levels of RUNX2 gradually increase in subsequent stages of osteoblast differentiation, with maximum expression detected in the mature osteoblast (9). Studies showed that RUNX2 binds to the osteocalcin (OCN) promoter and expresses in osteochondral progenitors to stimulate osteoblastic

differentiation at the early stage, and prevent osteoblastic differentiation at the late stage (53).

In endochondral ossification, after developing cartilage, RUNX2 expression is up-regulated in the perichondrium by the canonical Wnt signaling to form endochondral bone (25). Runt-related transcription factor 2-knockout mice display no intramembranous or endochondral ossification and a complete lack of bone formation. Consequently, RUNX2 acts as a vital regulator of both intramembranous and endochondral bone formation (8, 9). In addition, RUNX2-/- mice completely lose their ability to differentiate mesenchymal cells to osteoblasts and die shortly after birth (7, 11).Similarly, RUNX2 haplo-insufficiency is the cause of cleidocranial dysplasia in humans; this disease is characterized by defective bone formation (9).

Expression of RUNX2 in non-osteoblastic fibroblasts was sufficient to induce expression of osteoblastic markers such as type1 collagen, bone sialoprotein, osteocalcin, and osteopontin (8); for example, RUNX2 expression in an osteochondral progenitor suppressed chondrocyte differentiation to enhance osteoblast differentiation (25). Conversely, suppression of RUNX2 prevented the differentiation of mesenchymal cells to osteoblasts (9).

Runt-related transcription factor 2 has been found to cooperate with Smad1 and Smad5 to increase the expression of some osteoblast-specific genes (10). Runt-related transcription factor 2 acts downstream of BMP-2/Smad signaling (54). Furthermore, RUNX2 is an essential transcription factor for osteoblast differentiation, and its expression can be-induced by both BMP-2 and BMP-7 (55).

Celli et al. showed that RUNX2 plays an important role in both basal and BMP-2-mediated OSX expression in C3H10T1/2 cells. Both Smad ubiquitin regulatory factor 1 (Smurf1) and acute myelogenous leukemia translocation (AML/ETO) -overexpressing cells expressed OSX in the presence of BMP-2. Alkaline phosphatase expression was also inhibited by blocking RUNX22 activity with AML/ETO or Smurf1 (54).

Inactivation of the AKT pathway also leads to a downregulation of RUNX2; additionally, AKT has a positive role in insulin-like growth factor-1(IGF-1) -induced expression of RUNX2 (10).

Fujita et al. showed that RUNX2 is not involved in IGF-1 induction, however IGF-1 signaling has a major role in RUNX2-dependent osteoblastic differentiation of the osteoprogenitor in mouse MC3T3-E1 cells. This group also showed that PI3K-Akt signaling plays a key role in RUNX2 DNA binding and transcriptional activation via RUNX2 (56).

Insulin-like growth factor-1 also controls specific hormone receptor and cytokine genes that are vital in postnatal development, differentiation, and angiogenesis. It has been shown that IGF-1 increases bone sialoprotein and osteocalcin (OC) gene expression in an in vivo rat model of bone formation. Bone sialoprotein and OC are RUNX2 target genes in MC3T3-E1 cells, suggesting that IGF-1 may control the expression of these genes through RUNX2 activation (57).

One of the remarkable notes from the IGF-1 knockout is the necessity of IGF-1 for parathyroid hormone (PTH) activity in bone. In the absence of IGF-1, PTH had no positive effect on the rate of bone formation in mice. RUNX2 activity is also regulated through other transcription factors, such as signal transducer and activator of transcription 1 (Stat1), which prevents the transcription activity of RUNX2 (10). Kim et al. studied RUNX2 nuclear localization in primary osteoblasts from wild-type and Stat1-/- mice. Runt-related transcription factor 2 nuclear translocation was more prominent in Stat1-/- osteoblasts than in wild-type osteoblasts, indicating that RUNX2 nuclear localization is regulated via the transcriptionally latent form of Stat1 in the cytoplasm (59).

Hairy/enhancer-of-split related with YRPW motif protein 1 (Hey1) prevents bone matrix mineralization by osteoblasts by controlling RUNX2 activity (30). Sharff et al. suggested that Hey1 might increase osteogenic differentiation and prevent chondrogenic differentiation. Runt-related transcription factor 2 might be regulated via BMP9 in a parallel pathway and RUNX2 may act downstream of Hey1 in BMP9-induced osteogenic differentiation. This group also demonstrated that Hey1 is a major mediator of BMP9-induced osteogenic differentiation of

MSCs; moreover, both Hey1 and RUNX2 are involved in BMP9 osteogenic signaling. Hey1 is induced by BMP2 in RUNX2-deficient progenitors, which cannot differentiate into osteoblasts, suggesting that Hey1 may act upstream of RUNX2 during BMP-stimulated osteoblast differentiation (55).

Young et al. reported that RUNX2 is organized in multiple distinct sub-nuclear foci during interphase and is localized on chromosomes during all mitotic stages; moreover, they detected mitotic association of endogenous RUNX2 with chromosomes in multiple cell lines, including normal calvarial osteoblasts and osteosarcomas (60).

The Key Osteogenic Transcription Factor, Osterix

The other transcription factor essential for osteoblast differentiation and bone formation during embryonic development is osterix (OSX) (9, 61). Osterix and Runx2 are expressed in the perichondrial cells that eventually differentiate into osteoblasts (62). Day et al. reported that cell destiny at the initial differentiation stage is not yet established, and early RUNX2 expression preserves some cell fate and requires OSX to ensure full differentiation to osteoblasts (25).

Inactivation of OSX in mice after birth causes multiple skeletal phenotypes including deficiency of new bone formation, deficiency of resorption of mineralized cartilage, and failings in osteocyte maturation and function (63). While these knockout mice expressed RUNX2, correct function of RUNX2 requires OSX expression (7). Similar to RUNX2, forced OSX expression in non-bone cells promoted expression of both early and late phase gene markers in osteoblasts (9). Lee et al., in histological analyses, showed that transplantation of OSX-overexpressing BMSCs increased trabecular bone mass. They also reported that OSX expression is increased by BMP-2 treatment (64). Celil et al. studied whether OSX expression in mesenchymal progenitor cells required RUNX2 in the absence or presence of the BMP-2 stimuli and suggested that BMP-2- and IGF-1 intramembranous ossification induces OSX up-regulation. Additionally, RUNX2 was necessary, but not sufficient, for the BMP-2-mediated OSX induction. Among various growth factors implicated in bone formation, BMP-2 and IGF-1 up-regulated OSX expression during early osteoblast differentiation (54).

Zhou et al. showed that OSX in mice has multiple crucial roles in postnatal bone development and homeostasis. First, inactivation of OSX during and after the main postnatal growth period blocked osteoblast differentiation and new bone formation. This study showed that a particular transcription factor that is crucial for embryonic skeletal development is equally necessary for osteoblast differentiation and new bone formation postnatally (61). Furthermore, Baek et al. showed that OSX is required for pre-osteoblast differentiation into fully functioning osteoblasts, or for osteoblasts to continue their function, and for subsequent bone formation and mineralization (65).

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