

Regulation of bone development and maintenance by Runx2

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1. ABSTRACT

Runx2 and Sp7/Osterix determine the osteoblast lineage from mesenchymal stem cells with canonical Wnt signaling. In the process of osteoblast differentiation, these factors and canonical Wnt signaling molecules inhibit mesenchymal cells from differentiating into chondrocytes and adipocytes. After the commitment to osteoblast lineage, Runx2 maintains the osteoblasts in an immature stage, during which immature bone forms with randomly and loosely packed collagen fibrils and low mineralization. Runx2 must be suppressed for immature osteoblasts to become fully mature osteoblasts, which form mature bone with regularly and densely packed collagen fibrils and high mineralization. During the early stage of osteoblast differentiation, Runx2 regulates the expression of major bone matrix protein genes. However, Runx2 is not essential for the maintenance of the expression of the major bone matrix protein genes in mature osteoblasts. Estrogen and parathyroid hormone (PTH) enhance Runx2 expression and activity through anabolic effects, however, estrogen negatively regulates Runx2 in osteoclastogenesis. Runx2 is also involved in the catabolic effect of PTH through the induction of Tnfrsf11. Thus, Runx2 regulates bone development, bone maturation, and bone maintenance through the regulation of osteoblast differentiation and function.

2. INTRODUCTION

Runx2 (runt-related transcription factor 2) is a transcription factor and a member of the Runx family, which consists of Runx1, Runx2, and Runx3 (1). These transcription factors form heterodimers with Cbfb and acquire DNA binding capacity through recognition of the consensus sequence, PyGPyGGTPy. Runx1 is essential for hematopoietic stem cell differentiation and is involved in acute myeloid leukemia. Runx2 is essential for osteoblast differentiation, and a haploinsufficiency of *Runx2* causes cleidocranial dysplasia characterized by hypoplastic clavicles, open fontanelles, supernumerary teeth, and short stature. Runx3 plays important roles in the growth regulation of gastric epithelial cells and in neurogenesis, and is related to gastric cancer. In addition, Runx1 and Runx3 are required for thymocyte development. Further, Runx2 and Runx3 are essential for chondrocyte differentiation during the late stage, which is required for endochondral ossification. Therefore, all of the Runx family transcription factors play important roles in the diverse tissues, although some redundancies are observed. In this review, we will focus on the roles of Runx2 in bone development, bone maturation, and bone maintenance.

3. EARLY OSTEOBLAST DIFFERENTIATION

Mesenchymal stem cells differentiate into chondrocytes, osteoblasts, muscle cells, adipocytes, and

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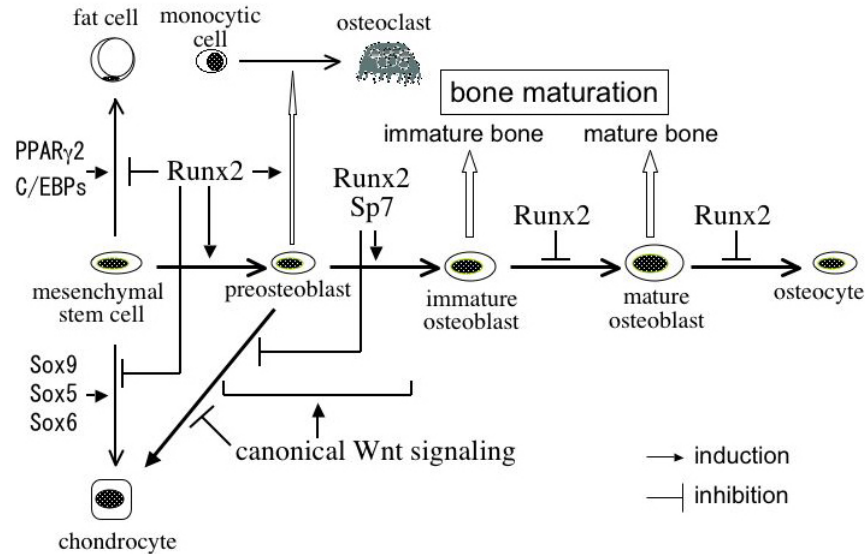


Figure 1. Regulation of osteoblast differentiation by transcription factors. Runx2, Sp7, and canonical Wnt signaling induce the differentiation of mesenchymal stem cells to immature osteoblasts, PPAR γ 2 and the C/EBP family (C/EBP β , C/EBP δ , and C/EBP α) induce the differentiation of mesenchymal stem cells to adipocytes, and Sox9, Sox5, and Sox6 induce the differentiation of mesenchymal stem cells to chondrocytes. In the process of osteoblast differentiation, Runx2 inhibits the differentiation of mesenchymal cells into adipocytes, and Runx2, Sp7, and canonical Wnt signaling inhibit the differentiation of mesenchymal cells into chondrocytes. Runx2 inhibits osteoblast maturation and the transition of osteoblasts to osteocytes. Bone maturation proceeds according to the osteoblast maturation. Runx2 is also involved in osteoclastogenesis through the induction of *Tnfrsf11* expression and inhibition of *Tnfrsf11b* expression, mainly during the preosteoblast stage.

tendon cells. Mice deficient in *Runx2* lack osteoblasts (2, 3). Sp7/Osterix belongs to the Sp family of transcription factors, and *Sp7*-deficient mice also lack osteoblasts (4). In *Sp7*-deficient mice, *Runx2* is expressed in the perichondrial cells surrounding hypertrophic chondrocytes, and these cells differentiated into chondrocytes. Recently, several groups reported that *Ctnnb1*/ β -catenin depletion in osteoblast progenitors using *Prx1* promoter *cre* transgenic mice or *Twist2/Dermo1 cre* knock-in mice and *Ctnnb1* depletion in preosteoblasts using *Sp7* promoter *cre* transgenic mice result in a complete block of osteoblast differentiation (5-8). In these conditional knockout mice, *Runx2* is expressed in the perichondrial cells surrounding hypertrophic chondrocytes, and these cells differentiate into chondrocytes. *Sp7* is not detected in perichondrial cells whose *Ctnnb1* had been depleted at the osteoblast progenitor stage, whereas *Sp7* is detected in the perichondrial cells whose *Ctnnb1* had been depleted at preosteoblast stage. This result indicates that *Ctnnb1* is required for not only *Sp7* expression, but also osteoblast differentiation after the expression of *Sp7*. Therefore, Runx2 is the first transcription factor required for the determination of the osteoblast lineage, while Sp7 and canonical Wnt signaling further direct the fate of the mesenchymal cells to osteoblasts, blocking their differentiation into chondrocytes (Figure 1).

Fgf18-deficient mice show a delay in osteoblast differentiation in calvaria and long bones (9, 10). Recently, Runx2 was shown to form a complex with Lef1 or Tcf4 and to bind the *Fgf18* promoter and induce *Fgf18* expression (11). Therefore, Runx2 regulates osteoblast

differentiation, at least in part, through the induction of *Fgf18*.

4. LATE OSTEOBLAST DIFFERENTIATION AND BONE MATURATION

After commitment to the osteoblast lineage, the committed cells become immature osteoblasts that express *Spp1/osteopontin* and upregulated *Col1a1*. The immature osteoblasts form woven bone, in which collagen fibrils run to all directions with low mineralization. These immature osteoblasts express high levels of *Runx2*. Early mature osteoblasts express *Spp1*, *Bglap2/osteocalcin*, and *Runx2*, but the expression of *Spp1* and *Runx2* is downregulated and the expression of *Bglap2* is upregulated during osteoblast maturation. The mature osteoblasts form lamellar bone, in which collagen fibrils are densely packed and highly organized with high mineralization (12).

Bone is composed of compact bone and cancellous bone. In long bones, the shaft (cortical bone) consists of compact bone, while the inside of the shaft (trabecular bone), which is a three-dimensional lattice of branching bony spicules, consists of cancellous bone. Compact bone is mature bone, because it is composed of densely packed, highly organized collagen fibrils with high mineralization, and is relatively resistant to osteolysis. In contrast, cancellous bone is less mature because it is composed of loosely organized collagen fibrils with low mineralization. Cancellous bone is easily resorbed and plays an important role in calcium homeostasis (13).

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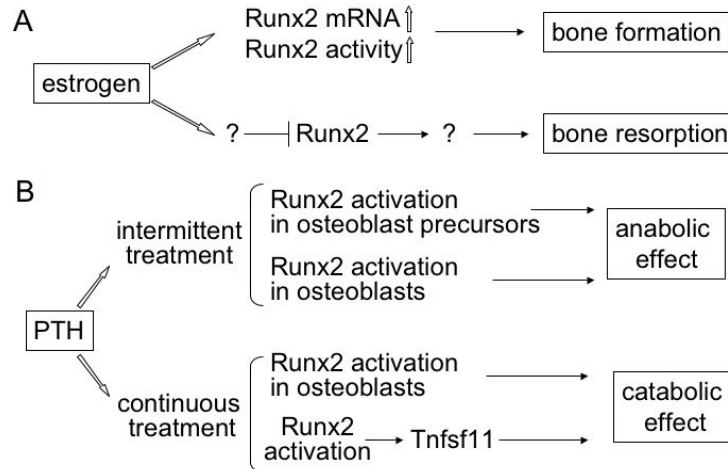


Figure 2. Involvement of Runx2 in the effects of estrogen and PTH. (A) Involvement of Runx2 in the effects of estrogen. Estrogen increases Runx2 expression and activity, leading to the enhancement of bone formation. Estrogen inhibits Runx2-dependent osteoclastogenesis probably through the induction of undetermined factors that interact with Runx2. However, the molecule that is regulated by Runx2 in the osteoclastogenesis is unknown. (B) Involvement of Runx2 in the effects of PTH. Intermittent treatment with PTH will cause the anabolic effect partly through the activation of Runx2 in osteoblast precursors and osteoblasts, whereas continuous treatment with PTH will cause the catabolic effect partly through the continuous activation of Runx2 in osteoblasts, which inhibits osteoblast maturation, and Runx2-dependent *Tnfsf11* induction.

The overexpression of *Runx2* using the mouse 2.3 kb *Colla1* promoter, which directs the transgene expression in immature osteoblasts, mature osteoblasts, and early osteocytes, inhibits osteoblast maturation, and woven bone is continuously formed by immature osteoblasts throughout the life of the animal (14, 15). Since woven bone is easily resorbed, the transgenic mice show severe osteopenia and suffer from multiple fractures. Interestingly, the number of osteocytes is drastically reduced in the transgenic mice, however, the reason is unclear since the transition from immature osteoblasts to osteocytes occurs in other transgenic mice that we generated (unpublished observation). In contrast, trabecular bone of the transgenic mice, which overexpress dominant-negative form of *Runx2* (dn-*Runx2*) using the same *Colla1* promoter, increases due to less bone resorption (12). In the trabecular bone of dn-*Runx2* transgenic mice, collagen fibrils are more densely packed and more highly organized compared with wild-type mice, and the mineralization is greater than that of wild-type mice. Together with the findings of *Runx2* expression in osteoblasts during the maturation, these results indicate that Runx2 is involved in the formation of immature bone by supplying immature osteoblasts and inhibiting osteoblast maturation (Figure 1). In addition, these results suggest that Runx2 needs to be suppressed for osteoblast maturation and the formation of mature bone. Recently, the continuous activation of canonical Wnt signaling was reported to inhibit osteoblast maturation (8).

5. REGULATION OF BONE MATRIX PROTEIN GENES BY RUNX2

Many recent *in vitro* studies demonstrated that Runx2 is a positive regulator that up-regulates the

expression of or activates the promoters of genes related to bone matrix proteins, including *Colla1*, *Colla2*, *Spp1*, *Ibsp/BSP*, *Bglap2*, *Fnl1/fibronectin*, *Mmp13*, and *Tnfrsf11b/Opg* (16-21). In the osteoblasts of *Colla1* promoter *Runx2* transgenic mice, however, the expression of *Colla1*, alkaline phosphatase (*Akp2*), *Bglap2*, and *Mmp13*, all of which normally increase during osteoblast maturation, are reduced (14, 15). Further, dn-Runx2 fails to reduce *Colla1*, *Spp1*, and *Bglap2* in mature osteoblasts *in vitro* and *in vivo* (12). Therefore, Runx2 regulates the expression of major bone matrix genes during the early stage of osteoblast differentiation, but Runx2 is not essential for the maintenance of these gene expressions in mature osteoblasts. These activities are compatible with the expression of Runx2 in osteoblasts, which is down-regulated in mature osteoblasts, during bone development (12).

6. RUNX2 AND ESTROGEN

Postmenopausal bone loss results from the inability of osteoblastic activity to match the increase in osteoclastic bone resorption induced by estrogen deficiency. Estrogen or selective estrogen receptor modulators (SERMs) induce *Runx2* mRNA or enhance *Runx2* promoter activity (22, 23), and estrogen inhibits *Il7* expression, which suppresses *Runx2* promoter activity and bone formation (24). The presence of both an estrogen response element (ERE) and a *Runx2* binding site synergistically enhances transcription (25). Further, Runx2 directly interacts with estrogen receptor α (ER α) in an estrogen-dependent manner, and the interaction enhances Runx2 activity, but inhibits the stimulatory effect of ER α on gene expression through consensus ERE (26). These findings indicate that estrogen positively regulates Runx2 function (Figure 2). In accordance with these findings, the

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treatment with high-dose estrogen induces *Runx2*-expressing bone marrow cells in wild-type mice and induces less bone formation in *Runx2*^{+/-} mice (27, 28). In the regulation of estrogen activity in osteoblasts, however, Runx2 seems to suppress *Esr1/ERα* promoter activity in the physiological condition (29). Therefore, it is likely that estrogen enhances Runx2 function, but Runx2 inhibits *Esr1* transcription.

In dn-*Runx2* transgenic mice under the control of 2.3 kb *Colla1* promoter, trabecular bone is conserved after ovariectomy, and neither bone resorption nor bone formation is enhanced (12). These findings show that the trabecular bone of dn-*Runx2* transgenic mice is relatively resistant to bone resorption and that Runx2 is involved in the enhancement of bone resorption after ovariectomy. Runx2 has been shown to induce osteoclastogenesis by enhancing *Tnfrsf11/Rankl* expression and inhibiting *Tnfrsf11b/Opg* expression (12, 15, 30-32). However, we could not detect evidence that Runx2 regulated *Tnfrsf11* and *Tnfrsf11b* in either *Runx2* or dn-*Runx2* transgenic mice. These findings suggest that Runx2 regulates *Tnfrsf11* and *Tnfrsf11b* expression only during the differentiation stage of mesenchymal cells. Thus, estrogen regulates Runx2 function negatively in osteoclastogenesis, but the molecular mechanisms through which Runx2 mediates the enhancement of osteoclastogenesis in the estrogen-depleted state need to be further investigated. Since estrogen enhances Runx2 function in osteoblasts, however, estrogen may negatively regulate Runx2 function in osteoclastogenesis through the regulation of other transcription factors or cofactors that interact with Runx2 (Figure 2).

7. RUNX2 AND PARATHYROID HORMONE (PTH)

Runx2 plays an important role in the regulation of PTH-dependent *Mmp13* expression through the interaction with AP-1 (33-35). PTH stimulates transactivation of Runx2 in a protein kinase A (PKA)-dependent manner (34). PTH inhibits or induces osteoblast differentiation *in vitro* depending on the duration of PTH treatment (36, 37). Long term treatment with PTH inhibits osteoblast differentiation and reduces Runx2 mRNA and protein levels. However, PTH treatment for a limited duration *in vitro* enhances osteoblast differentiation and Runx2 activity. Further, the intermittent administration of PTH increases Runx2 protein *in vivo* (38). The difference may be caused by the fact that PTH negatively regulates Runx2 protein through a ubiquitin/proteasome-dependent mechanism (39). Although Runx2 is involved in a PTH-induced anti-apoptotic effect through the induction of *Bcl2*, Smurf1-mediated proteasomal degradation of Runx2, the process of which is enhanced by PTH, also limits the anti-apoptotic effect of PTH (40, 41).

The anabolic effect of PTH is partly reduced, but not completely abolished in dn-*Runx2* transgenic mice under the control of mouse 2.3 kb *Colla1* promoter (12), while the anabolic effect of PTH is abolished in *Runx2* transgenic mice under the control of rat 2.3 kb *Colla1* promoter (42). These findings indicate that Runx2 is partly

involved in the anabolic effect of PTH in osteoblasts, but that continuous upregulation of Runx2 in osteoblasts abolishes the anabolic effect of PTH. Therefore, PTH exerts its anabolic effect through osteoblasts as well as osteoblast precursors, and intermittent upregulation of Runx2 in osteoblasts is required for the anabolic effect of PTH. It will be due to the fact that the constant activation or upregulation of Runx2 results in the maturational inhibition of osteoblasts leading to the formation of immature bone that is easily resorbed (14, 15, 43) (Figure 2). As PTH treatment activates Runx2 but enhances Runx2 degradation, however, Runx2 protein levels need to be carefully compared *in vivo* between continuous and intermittent treatments of PTH. Further, levels of the activated Runx2 protein in osteoblast precursors and osteoblasts have to be evaluated carefully, because continuous activation of Runx2 in osteoblast precursors increases osteoblast number but the continuous activation of Runx2 in osteoblasts inhibits osteoblast maturation favoring the catabolic effect of PTH. Finally, Runx2 is also involved in the catabolic effect of PTH, because Runx2 and CREB binding sites in a distant transcriptional enhancer of *Tnfrsf11* are required for PTH-induced *Tnfrsf11* expression (32) (Figure 2).

8. PERSPECTIVE

Bone maturation proceeds through the process of osteoblast differentiation. Bone maturation depends, at least in part, on bone matrix protein gene expression in osteoblasts, which is regulated in a manner dependent on the maturational level of osteoblasts. However, determining why woven bone and lamellar bone are formed by immature and mature osteoblasts, respectively, is insufficient. Further, the molecular process for the transition of osteoblasts to osteocytes is completely unknown. Moreover, the molecular basis of the anabolic actions of estrogen and PTH is not completely understood. The elucidation of these processes is required for the ability to improve bone quality, as well as increase bone mass. Combined morphological and molecular analyses *in vivo* are needed to reveal these issues, and, most likely, Runx2 will be revealed as a key player in the regulation of these processes.

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