Osteoblastogenesis and Role of Osteoblasts in Calcium Homeostasis and Remodeling of Bone

Kemik Yeniden Yapılanmasında ve Kalsiyum Metabolizmasında
Osteoblastlarım ve Osteoblastogenezisin Rolü

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Abstract

Bone remodeling is very important for repair of microfractures and fatigue damage and prevention of excessive aging and its consequences. Bone remodeling lasts for about 6-9 months. During this period osteoclasts resorb damaged bone and osteoblasts synthesize new bone. The lifespan of mature osteoclasts is about 15 days and for osteoblasts 3 months. Therefore, the time required for the remodeling of a given segment of bone is much longer than the lifespan of its cells which perform remodeling. A supply of new osteoblasts and osteoclasts are therefore needed for successful remodeling by the basic multicellular unit. The major event that triggers osteogenesis is the transition of mesenchymal stem cells into bone differentiating osteoblast cells. Osteoblast commitment and differentiation are controlled by complex activities. Many factors are involved in the regulation of osteoblastogenesis. Bone morphogenetic proteins and the Wnt glycoproteins play crucial roles in signaling osteoblast commitment and differentiation, and are the only known factors capable of initiating osteoblastogenesis from uncommitted progenitors. They can initiate commitment of mesenchymal cells to osteoblastic lineage. The initial cell division is asymmetric, giving rise to another stem cell and a committed osteoprogenitor. After commitment to the osteoblastic lineage, a preosteoblast cell gives rise to the transit-amplifying compartment. At this stage osteoprogenitor cells proliferate intensively. After this stage, the cells are more differentiated and give rise to preosteoblasts which express both STRO1, alkaline phosphatase, pyrophosphate, and type I collagen. Preosteoblasts are committed to the osteoblast lineage with extensive replicative capacity, but have no self-renewal capacity. Preosteoblasts form the intermediate stage of osteoblastogenesis. The mature osteoblasts express osteopontin, alkaline phosphatase, bone sialoprotein, and osteocalcin. This stage is responsible for the laying down of bone. Mature osteoblasts have limited replicative potential. About 65% of mature osteoblasts and a proportion of cells in the transient amplifying compartment terminate in apoptosis. Apoptosis is a critical determinant of osteoblast number in the basic multicellular unit. The terminal stage of the bone lineage is the post-mitotic osteocyte which is embedded within the advancing osteoid. A minor component of mature osteoblasts differentiate into lining cells of the bone. Lining cells line the quiescent bone with no remodeling activity. Bone morphogenetic proteins, Wnt glycoproteins, Hedgehog proteins, PPARγa ligands, and transcription factors such as Runx 2 and Osterix play important roles in these critical steps of osteoblastogenesis and bone remodelling. Turk Jem 2008; 12: 18-22

Key words: Osteoblasts, Bone morphogenetic proteins, Wnt/b-catenin pathway, PPARγa ligands

Özet


Anahtar kelimeler: Osteoblastlar, Kemik morfogenetik proteinler, Wnt/b-catenin yolu, PPAR gamma ligandlar

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Skeleton is a highly specialized and dynamic organ that undergoes continuous regeneration. During development and growth the skeleton is sculpted to achieve its shape and size by the removal of bone from one site and deposition at another site. This process is called remodeling. Once the skeleton has reached maturity, regeneration continues in the form of periodic replacement of old bone with new bone at the same location. This process is called remodeling. An adult skeleton regenerates by formation of temporary cellular structures that comprise groups of juxtaposed osteoclasts and osteoblasts and which periodically replace old bone with new bone. This remodeling is responsible for the complete regeneration of the adult skeleton every 10 years. Bone remodeling is very important for the repair of microfractures and fatigue damage and prevention of excessive aging and its consequences. Bone remodeling lasts about 6-9 months. During this time osteoclasts resorb the damaged bone and osteoblasts synthesize the new bone. The lifespan of mature osteoclasts is about 15 days and for osteoblasts it is 3 months. Therefore, the time required for the remodeling of a given segment of bone is much longer than the lifespan of the cells which carry out remodeling. A supply of new osteoblasts and osteoclasts are therefore needed for successful remodeling by the basic multicellular unit. Consequently, the balance between the supply of new cells and their lifespan are key determinants for both cell types in the basic multicellular unit at the site of remodeling and the work performed by each type of cell is critical for the maintenance of bone homeostasis (1-5).

Osteoblasts arise from common progenitors of chondrocytes, adipocytes, and muscle cells. These cells all arise from pluripotent mesenchymal stem cells which are derived from the mesodermal cells of the ventromedial somites at the end of the third week of embryonic development. Although not very well understood, differentiation from mesenchymal cells is a very well orchestrated process. Recent advances in molecular studies using gene targeting in mouse have enabled a better understanding of the multiple factors and signalling processes that control osteoblast differentiation at a molecular level. The major event that triggers osteogenesis is the transition of mesenchymal stem cells into bone differentiating osteoblast cells. Osteoblast commitment and differentiation are controlled by complex activities. There are many factors involved in the regulation of osteoblastogenesis. Bone morphogenetic proteins (BMPs) and the Wnt glycoproteins play crucial roles in signaling osteoblast commitment and differentiation. Bone morphogenetic proteins and the Wnt glycoproteins are the only known factors capable of initiating osteoblastogenesis from uncommitted progenitors. They can initiate commitment of mesenchymal cells to osteoblastic lineage. The initial cell division is asymmetric, giving rise to another stem cell and a committed osteoprogenitor. After commitment to the osteoblastic lineage, the osteoprogenitor cell gives rise to the transit-amplifying compartment. At this stage osteoprogenitor cells proliferate intensively. After this stage the cells are further differentiated and give rise to preosteoblasts which express STR01, alkaline phosphatase, pyrophosphate, and type 1 collagen. Preosteoblasts are committed to the osteoblast lineage with extensive replicative capacity, but no self-renewal capacity. Preosteoblasts form the intermediate stage of osteoblastogenesis. The mature osteoblasts express osteopontin, alkaline phosphatase, bone sialoprotein, and osteocalcin. This stage is responsible for the laying down of bone. Mature osteoblasts have limited replicative potential. About 65% of the mature osteoblasts and a proportion of cells in the transient-amplifying compartment terminate in apoptosis. Apoptosis is a critical determinant of the number of osteoblasts in the basic multicellular unit. However, apoptosis is a fleeting event that leaves no trace after the process has been completed, which makes the phenomenon difficult to recognize and quantify. Nevertheless, cells undergoing apoptosis briefly exhibit unique histologic features before they die. Among these features, fragmented DNA is frequently used to visualize apoptotic cells. The terminal stage of bone lineage is the post-mitotic osteocyte which is embedded within the advancing osteoid. Osteocytes secrete exclusively a protein named sclerostin, a Wnt antagonist, when bone is not actively remodeling. This protein prevents binding of Wnt to its frizzled receptor and thus inhibits osteoblastogenesis. Sclerostin normally exerts a restraining effect on osteoblast differentiation. Moreover, osteocytes may contribute to regulation of osteoblast number via alterations in sclerostin production. A minor component of mature osteoblasts differentiate into lining cells of the bone. These cells line the quiescent bone with no remodeling activity. They lie over unmineralized collagen which is about 1-2 mm thick. When a signal for remodeling arises, the lining cells synthesize collagenase to dissolve the unmineralized collagen to which osteoblasts cannot attach. Lining cells are the point of remodeling for the osteoclasts (1-3,6).

**Bone Morphogenetic Proteins**

Bone morphogenetic proteins are a group of phylogenetically conserved signaling molecules which were initially identified by their capacity to induce endochondral bone formation. BMPs were first cloned in 1988 by Wozney et al. At that time they cloned four cDNAs for human BMPs (BMP-1, BMP-2, BMP-3, and BMP-4). As of the present, at least 15 BMPs have been cloned, and with the exception of BMP-1, these BMPs belong to the transforming growth factor (TGF) superfamily. BMPs, which are expressed during skeletogenesis, are derived from mesenchymal cells. BMP-1, -4, and -6 are the most widely expressed isoforms in osteoblast cultures. BMP-3 is a negative regulator of bone formation. BMPs have two distinct receptors, BMPR-1 and BMPR-2. They are serine...

![Figure 1. Bone morphogenetic protein signaling through MAPK](image-url)
threonine receptors. BMPs transduce signals in mesenchymal cells and in cells of osteoblastic lineage by intracellular signal-transducing molecules of the TGF family named Smads. There are 8 different Smads which are classified into three subgroups. R-Smads (Smad 1/5, 8) consist of receptor-regulated Smads. Co-Smads (Smad 4) interact with phosphorylated R-Smads and form a complex which translocates to the nucleus and starts transcription of the target genes of BMPs. I-Smads are inhibitory Smads which block the activity of BMPs by binding to BMPR-1. After the BMP binds to its predimerized type I and II receptors (RI and RII), Smad 1 and 5 proteins are phosphorylated (pSmad) and associated with Smad 4, and then this complex translocates to the nucleus to regulate transcription of target genes by interacting with various transcription factors and transcriptional co-activators and co-repressors. Runx2 (it was known as “core binding factor α1”, Cbfa1) and R-Smads physically interact with each other upon activation of BMP signaling, and co-operatively regulate the transcription of target genes which leads to osteoblast differentiation of mesenchymal progenitors (Figure 1). Another pathway used by BMPs involves binding to their type II receptor, an intrinsic kinase that activates the type I receptor, the newly dimerized receptor complex activates the mitogen-activated protein kinase (MAPK) extracellular regulated kinase (ERK) pathway to regulate transcription. Extracellular antagonists bind bone morphogenetic protein or its receptor and prevents signal transduction (Figure 2) (7-10). BMPs induce the transcription of the most important transcription factor for osteoblastogenesis which is named Runx2. BMPs induce Runx2 expression in osteoblast progenitor cells through the action of R-Smads, and in turn, R-Smads interact with Runx2 and further induce osteoblastic differentiation. BMPs do not directly induce the expression of Runx2 in mesenchymal cells, but facilitate the expression of distal-less 5 (Dlx5), another transcription factor for the commitment of stem cells to osteoblastic lineage, in osteoblasts, and Dlx5 then induces expression of Runx2 in osteoprogenitor cells. BMPs also induce transcription of Runx2 by activating MAPK. This pathway also induces transcription of a homeobox-containing gene, Dlx5, and further differentiation of the cells to osteoblasts. Dlx5 also itself induces the transcription of a third transcription factor important for stem cell commitment to osteoblastic lineage, which is named osteoblast commitment factor, or Osterix, in the mesenchymal cells (6,10).

Action of BMPs on mesenchymal cells and osteoprogenitors are also influenced by many factors. Shh (Sonic hedgehog protein) and Ihh (Indian hedgehog protein) may act cooperatively with BMPs. They potentiate the effects of BMPs. On the other hand, Noggin and chordin inhibit BMP action by competing for binding to BMPRs in an extracellular region. Thus, among the BMP antagonists, noggin, chordin, follistatin, and gremlin act at the extracellular level and inhibit the actions of BMPs (Figure 3) (6,7,10).

**Wnt Glycoproteins**

Wnts are a family of secreted glycoproteins that have multiple inhibitors. They are ligands for the family of 7-membrane spanning frizzled (FZD) receptors. The Wnt family is involved in numerous aspects of cellular biology, ranging from cell fate determi-
tion, polarity, and differentiation to migration, proliferation, and function. Wnt proteins are divided into two classes. The first class activates the canonical Wnt signaling pathway, which involves the formation of a complex between Wnt proteins, FZD, and low-density lipoprotein (LDL) receptor-related protein-5 (LRP5) or (LRP6) receptors. The noncanonical Wnt5a class binds FZD proteins and activates heterotrimeric G proteins. Wnt/b-catenin or canonical pathway appears to be particularly important for bone biology (4,6,8-12).

Under basal conditions, b-catenin is phosphorylated by glycogen synthase kinase 3 (GSK-3), axin, and adenomatous polyposis coli (APC) tumor-suppressor protein and degraded in the proteasome. After Wnt binding to its receptor (frizzled) and co-receptors (low-density lipoprotein receptor–related proteins 5 and 6 [LRP5 and LRP6]), Dishevelled, an intracellular protein, is induced to degrade GSK-3. In addition, the cytoplasmic tails of LRP5 and LRP6 bind and anchor axin. These two events lead to the stabilization of b-catenin and its translocation to the nucleus, where it binds to T-cell factor 4 (TCF-4) or lymphoid enhancer binding factor 1 (LEF-1) to regulate transcription. Extracellular Wnt antagonists prevent Wnt signaling. Among these antagonists, Dickkopf-1 (Dkk-1) in association with Kremen and sclerostin bind LRP5 and LRP6. Soluble frizzled-related protein 1 (sFRP-1) binds Wnt and prevents its interaction with frizzled (6,8,9,13) (Figure 4).

Wnt signaling represents both a cell-autonomous mechanism for inducing osteoblastic differentiation and suppressing chondrocytic differentiation in early osteochondroprogenitors and a mechanism in fully differentiated osteoblasts for stimulating the production of osteoprotegerin, an inhibitor of osteoclast formation. Osteoprotegerin is a direct target gene of the b-catenin-T cell factor (TCF) complex in osteoblasts and Tcf1 is probably the relevant transcription factor required for osteoprotegerin regulation. Wnt signaling, that is, the b-catenin pathway, is required for osteoblast differentiation at the preosteoblast stage. Wnt/b-catenin signaling regulates bone development and accrual through different mechanisms at different stages of life (4,8,9,11).

**Hedgehog Protein**

Indian hedgehog (Ihh) protein is a protein produced by prehypertrophic chondrocytes and appears to act directly on perichondrially located osteoblast progenitors to specify the osteoblast precursors. Hedgehog signaling acts to initiate an osteogenic program of mesenchymal cells. Genetic manipulation of Smo (Smoothened), which encodes an obligatory component of hedgehog protein signalling pathway, has revealed that cells devoid of Smo, hence hedgehog signaling, fail to undergo osteoblast differentiation. Although Ihh signaling plays a crucial role in regulating the temporal and spatial program of early osteoblast commitment, Ihh does not play an ongoing role beyond this stage. The interaction between Ihh and Wnt signaling is probably complex. Ihh signaling is required for Wnt expression. Alternatively, the hedgehog and Wnt signaling pathways intersect intracellularly via common regulators like glycogen synthase kinase 3 and Suppressor of fused protein (6,9,10).

**PPARγ Ligands**

Pluripotent mesenchymal cells can also differentiate into adipocytes in the presence of PPARγ activation. Increased expression of PPARγ or its ligands like PGJ2 may suppress Runx2 and promote adipocyte differentiation at the expense of osteoblastogenesis in the bone marrow (Figure 5). This pathway is important during senescence. In aging there is increased expression of genes that favor differentiation of multipotent mesenchymal stem cells toward adipocytes. There is a reciprocal relationship between adipogenesis and osteoblastogenesis with advancing age (6).

**Transcription Factors That Regulate Osteoblastogenesis**

The master genes for osteoblast differentiation are Runx2 and Osterix. Commitment of mesenchymal cells to tissue-specific cell types is orchestrated by transcriptional regulators that serve as “master switches.” A central regulator of bone formation is the Runx2 transcription factor which fulfills its role as a master regulatory switch through unique properties for mediating the temporal activation and/or repression of cell growth and phenotypic genes as osteoblasts progress through stages of differentiation. Runx2 is expressed in early osteoprogenitors to induce a program of gene expression required for lineage determination and differentiation of mesenchymal cells. Runx2 is also required for osteoblast function beyond differentiation. Msx2 is an upstream protein of Runx2 which directly or indirectly regulates Runx2 expression. Bapx1 is another transcription factor upstream of Runx2 which activates Runx2 expression. Osterix is
a BMP inducible gene, which is downstream of Runx2. Osterix induces osteoblastic differentiation in bipotential osteochondroprogenitor cells. Osteocalcin, bone sialoprotein, and alkaline phosphatase expression are dependent on Osterix (6,9,10).

Formation of skeletal elements during embryogenesis and the dynamic remodeling of bone in the adult involve an exquisite interplay of developmental cues, signaling proteins, transcription factors, and their regulators that support differentiation of osteogenic lineage cells from the initial mesenchymal progenitor cell to the mature osteocyte in mineralized connective tissue. There are many unresolved issues that need to be investigated more extensively to understand normal bone homeostasis and thus the pathogenesis of various causes of bone loss and the development of osteoporosis.

References