

Osteocytes-The Known and Unknown

Osteositler-Bilinenler ve Bilinmeyenler

Zeliha Hekimsoy

Celal Bayar University, Medical Faculty, Department of Internal Medicine, Division of Endocrinology and Metabolism, Manisa, Turkey

Abstract

Osteocytes are the most numerous, longest-living, and least studied cells of bone. In this review, osteocyte functions are discussed. *Turk Jem* 2008; 12: 23-7

Key words: Osteocyte, bone cells

Özet

Osteositler kemiğin en kalabalık, en uzun yaşayan ve en az çalışılmış hücreleridir. Bu değerlendirmede osteositlerin fonksiyonları tartışılmıştır. *Turk Jem* 2008; 12: 23-7

Anahtar kelimeler: Osteosit, kemik hücreleri

Bone contains both organic and inorganic components. The organic component consists of proteins and bone cells. The inorganic component is a specialized, calcium-poor form of apatite, resembling hydroxyapatite $[Ca_{10}(PO_4)_6(OH)_2]$, in which the hydroxyl residues are replaced by phosphate and carbonate ions (1).

There are three major types of bone cells: osteoblasts, osteoclasts, and osteocytes (Fig. 1). Osteocytes make up more than 90% to 95% of all bone cells in the adult skeleton whereas osteoblasts comprise less than 5% and osteoclasts less than 1%. Osteocytes are long-lived whereas osteoblasts last for weeks and osteoclasts for days. Osteoclasts and osteoblasts are defined by function. Osteoclasts are cells that resorb bone and osteoblasts are cells that make bone. Osteocytes are defined by location. Osteocytes are cells that are embedded in the mineralized bone matrix. In contrast to osteoblasts and osteoclasts which have functions that are well known, there is a lack of knowledge about the function of osteocytes. There are several reasons why much less is known concerning osteocyte function compared with osteoblast and osteoclast function. These reasons include the following: 1) the fact that it is difficult to isolate sufficient numbers of osteocytes from mineralized bone matrix, 2) it is difficult to maintain their differentiated function in vitro. 3) there is a lack of suitable cell lines, and 4) availability of osteo-

cyte-specific promoters for targeted transgenic approaches is lacking. The major known function of osteocytes is to translate signals related to mechanical strain into biochemical signals between osteocytes and cells on the bone surface. Osteocytes are metabolically active cells (2,3).

Osteocyte origin and differentiation

Osteocytes are derived from osteoblasts. Osteoblasts (bone forming cells) are of mesenchymal origin, secrete non-mineralized bone matrix (osteoid), and eventually become incorporated as osteocytes in mineralized bone matrix. After completion of bone formation, an osteoblast can have one of three different fates: 1) it can become embedded in its own osteoid and continue differentiation into an osteocyte, 2) transform into an inactive osteoblast and become a bone-lining cell, or 3) undergo programmed cell death (apoptosis) (Fig 2). The proportion of osteoblasts following each fate is not the same. It has been reported that 10% to 20% of osteoblasts differentiate into osteocytes (2). It is not clear why some of the osteoblasts are designated to become osteocytes. The cellular and molecular mechanisms that regulate this process are not fully understood. Similarly to caterpillars becoming butterflies within the cocoon

they weave about themselves, osteoblasts become osteocytes when entombed within the osteoid they synthesize (4).

There are six intermediate or transitional cell types with different morphological and molecular characteristics between differentiated osteoblasts and osteocytes (Fig. 3) (2).

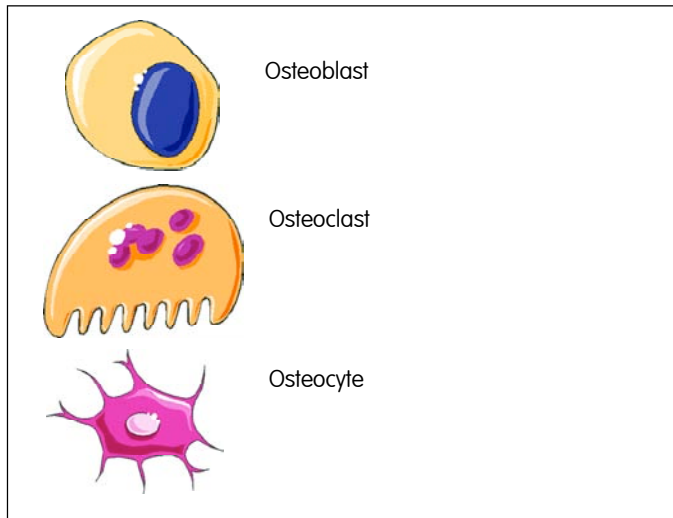


Figure 1. The major types of bone cells

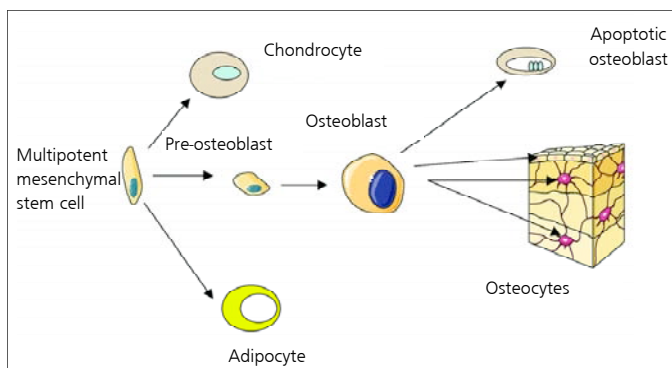


Figure 2. Osteoblast differentiation

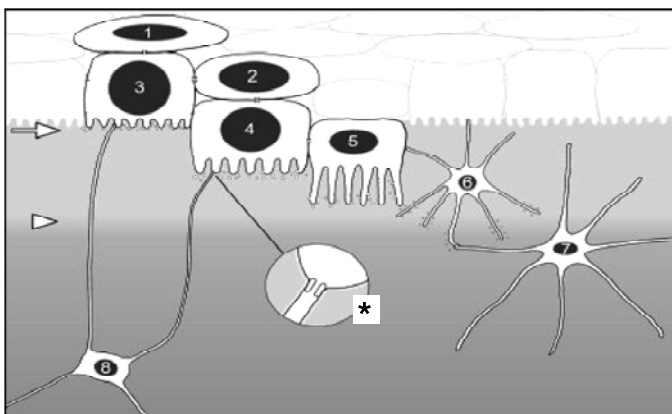


Figure 3. An osteoblast transformation into an osteocyte (2): 1) pre-osteoblast, 2) preosteoblastic osteoblast, 3) osteoblast, 4) osteoblastic osteocyte (type I preosteocyte), 5) osteoid-osteocyte (type II preosteocyte), 6) type III preosteocyte, 7) young osteocyte, and 8) old osteocyte *Gap junction between the cell process of an osteocyte and an embedding osteoblast. Gap junctions are present between all cells for direct communication

Osteocyte markers

There are a few markers for osteocytes. These markers range from low alkaline phosphatase to high casein kinase and osteocalcin expression. Franz-Odenaal et al. provided a list of molecular markers for the transformation of preosteoblast to osteocyte (2). E11 is the earliest marker that is specific for embedding osteocytes. The major function of E11 may be in the formation of dendritic processes. Other proteins that are highly expressed in osteocytes are CD 44, dentin matrix protein 1 (DMP 1), phosphate-regulating neutral endopeptidase on chromosome X (PHEX), osteoblast/osteocyte factor 45 (OF 45), which is also known as matrix extracellular phosphoglycoprotein (MEPE), and the SOST gene product sclerostin. DMP 1, PHEX, and MEPE play roles in the mineralization process and potentially in phosphate metabolism. The loss-of-function mutations in the PHEX gene results in X-linked hypophosphatemic rickets. DMP 1-null mice with deletions show mineralization defects, skeletal abnormalities, rickets, and elevated fibroblast growth factor (FGF) 23 (5). This suggests that DMP 1 and PHEX are interactive and essential for phosphate metabolism. The protein product of the SOST gene, sclerostin, is a bone morphogenetic protein (BMP) antagonist that decreases osteoblast activity and reduces the differentiation of osteoprogenitors. Sclerostin is also an antagonist of low-density lipoprotein receptor-related protein (LRP) 5, which is a co-receptor for the Wnt protein (a positive regulator of bone mass). The loss of the SOST gene product sclerostin leads to sclerosteosis which is characterized by high bone mass. It has also been proposed that the anabolic effects of parathyroid hormone (PTH) are through inhibition of SOST gene expression (6).

Lifecycle of an osteocyte

The transformation of an osteoblast to an entrapped osteocyte takes about 3 days. During this time an osteoblast produces a volume of extracellular matrix three times its own cellular volume. Cell polarity is maintained during the transformation from osteoblast to osteocyte so that the nucleus remains in proximity to the vasculature, but a shift in cell volume distribution takes place, changing the rounded, active osteoblast to a more stellate or dendritic-shaped osteocyte. This results in a 30% volume reduction in the nascent osteocyte cell body and a 70% volume reduction in the mature osteocyte cell body compared with the volume of the original osteoblast. During transformation of the osteoblast to the nascent osteocyte, cell processes first radiate toward the mineralizing matrix. These processes are thick and pseudopod-like. Once the mineralization front surrounds the cell, longer and thinner cell processes are observed on the vascular side of the cell. The mature osteocyte has more cell processes oriented toward vascularity than toward the mineralization front. In the nascent osteocyte, the structure of organelles is similar to that of osteoblasts, although the size and number of organelles are diminished. Osteocytes die as a consequence of senescence, degeneration/necrosis, apoptosis, and/or osteoclastic engulfment (Fig.4) (7). The osteocyte death rate increases with age from less than 1% at

birth up to 75% beyond the eighth decade. Osteocyte apoptosis can occur by immobilization, microdamage, lack of estrogen, elevated cytokines such as $\text{TNF-}\alpha$ as occurs in menopause, and treatment with glucocorticoids. Damaged osteocytes secrete receptor activator for nuclear factor κB ligand (RANKL) and macrophage colony-stimulating factor (M-CSF), activating osteoclastic cell formation (7).

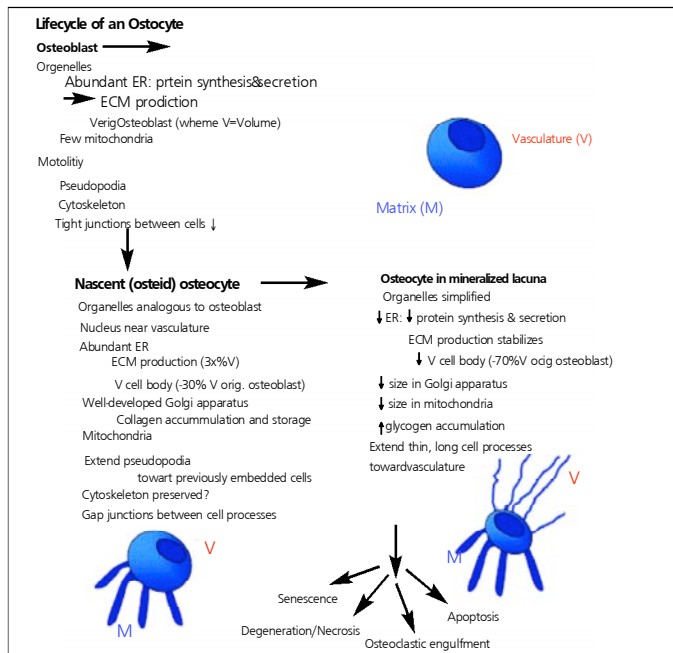


Figure 4. The life cycle of an osteocyte from nascence to death (7)

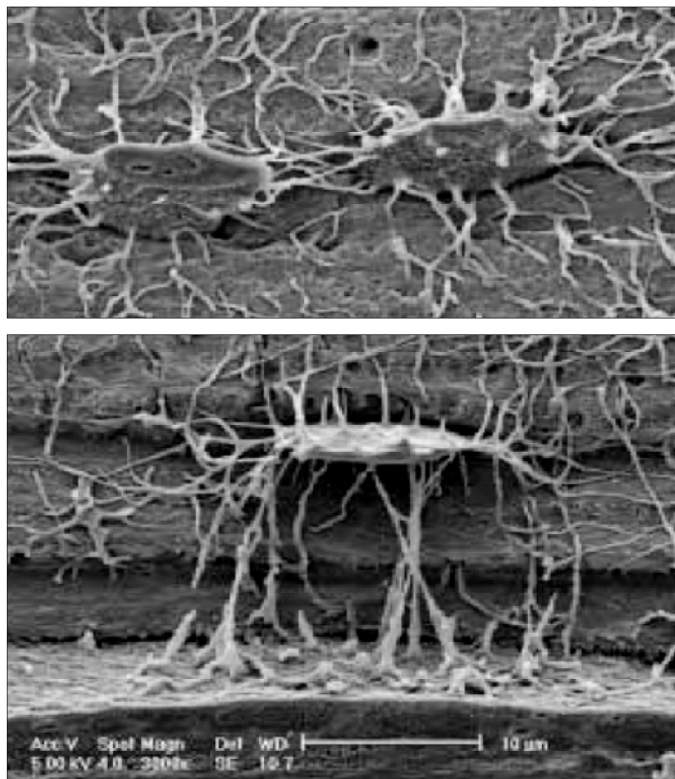


Figure 5. The osteocyte lacunocanalicular network (8)

Lacunocanalicular system and dendrite formation

Osteocytes are regularly dispersed throughout the mineralized matrix within "caves" called lacunes. There are about 20,000-80,000 cells per mm^3 bone tissue. Each osteocyte has on average 50 cytoplasmic dendritic processes. The cell processes or dendrites pass through the bone in thin "tunnels" called canaliculi. Osteocytes communicate with one another and with cells at the bone surface via a meshwork of these cell processes (Fig. 5) (8). The bone surface cells include the lining cells, which cover quiescent surfaces, cellular elements of the bone marrow, and the endothelial cells of the bone marrow vasculature. The cell processes of osteocytes are connected with each other via gap junctions, thereby allowing direct cell-to-cell coupling. Gap junctions are transmembrane channels which connect the cytoplasm of two adjacent cells and provide intercellular communication. These channels permit the passage of molecules with molecular weights approximately less than 1 kDa and they are formed by members of a family of proteins known as connexins (Cx). Three types of connexins, Cx43, Cx45, and Cx46, are expressed in bone tissue. Cx43 has been identified in all types of bone cells. Connexins have recently been shown to exist and function in the form of unapposed halves of gap junction channels called hemichannels. These hemichannels are localized at the cell surface, away from cell-cell junction regions, and provide communication between cells and extracellular matrix (Fig. 6) (9). The transformation of a plump polygonal osteoblast to a dendritic osteocyte is striking and dramatic, and requires extensive reorganization of the cytoskeleton. Osteocytogenesis and dendrite formation are an active process requiring cleavage of collagen and other matrix molecules. Osteocytes in mice null for the metalloproteinase MT1-MMP have significantly reduced number and length of dendritic processes. MT1-MMP is a membrane-anchored proteinase that can cleave collagens, fibrin, fibronectin, and other matrix molecules. Many molecules such as E11, metalloproteinase, DMP 1, CD 44, and Cx 43 play a role in the formation of dendritic processes.

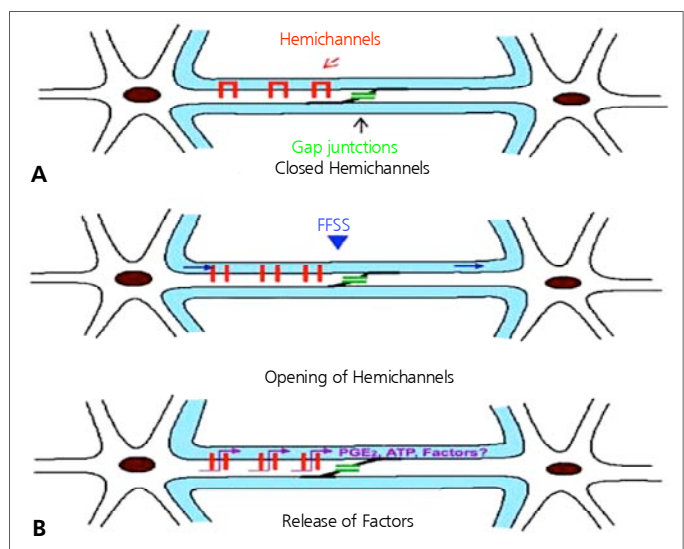


Figure 6. Gap junction channel and hemichannels. **A**. In the absence of mechanical stress hemichannels remain closed whereas gap junctions are kept open. **B**. Fluid flow shear stress induces the opening of hemichannels. PGE₂, ATP, and other responding physiological factors are released into canaliculi to mediate biological responses elicited by mechanical stress (9)

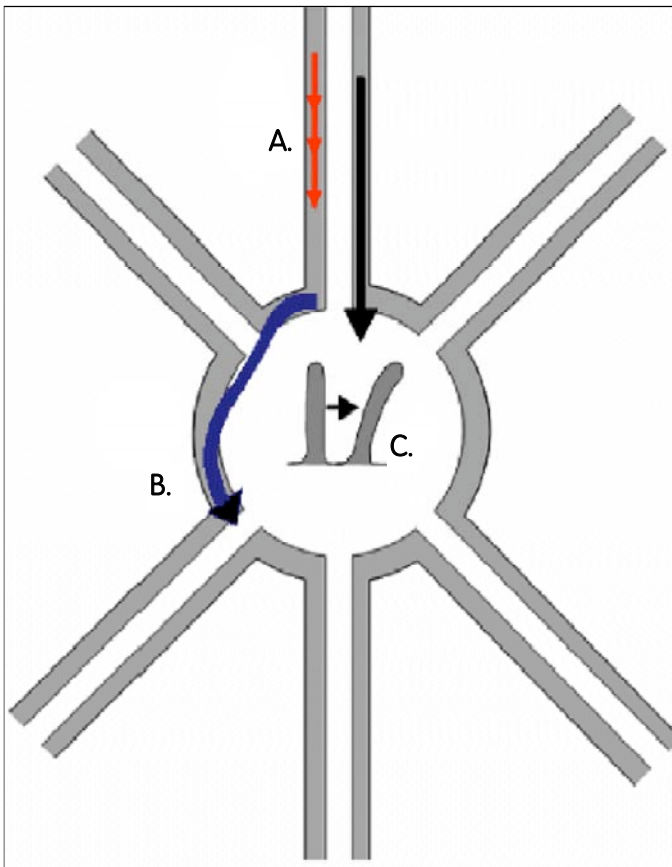


Figure 7. The potential ways that an osteocyte may sense fluid flow shear stress. FFSS affects dendrites (A), cell body (B), and cilia (C), causing deformation (3)

Osteocyte functions

Decades ago osteocytes were considered to be inactive placeholders in the bone matrix. Recent data, however, shows that osteocytes are not passive cells. On the contrary, they are metabolically active and multifunctional cells (10).

Osteocyte as a mechanosensor cell

Mechanical strain is a known key regulator of osteoblast and osteoclast activity. The skeleton is able to continually adapt to mechanical loading. In 1638 Galileo first documented that the shape of bones is related to loading. Julius Wolff in 1892 proposed that bone accommodates or responds to strain-high or too low levels of strain induce bone loss whereas physiological levels of strain maintain bone mass. A major question for decades has been how does bone respond to loading and unloading. It is thought that osteocytes are ideally located in bone to sense mechanical strain and translate that strain (or lack of) into biochemical signals to cells on the bone surface. Osteocytes are thought to send signals for both bone resorption and bone formation. With normal loading of the skeleton that maintains normal homeostasis, osteocytes send signals inhibiting osteoclast activity. The cells respond to load-induced strains by the production of molecules such as nitric oxide, prostaglandin E-2, insulin-like growth factor, ATP, and increased intracellular calcium. The canonical Wnt/b-catenin pathway activation and expression of

DMP 1, E11, and MEPE are increased in response to load. Upon immobilization, osteocytes send signals for bone resorption. Osteocytes produce RANKL and M-CSF, and support the generation of functional active osteoclasts. Sclerostin expression is increased in response to unloading and decreased in response to loading (10,11). Another major question is how does the osteocyte sense mechanical strain? It is thought that mechanical strain causes flow of interstitial fluid of bone between the canalicular wall and the cell membrane. Fluid flow imposes a shear stress on osteocytes that appears to deform the cells within their lacunae and the dendrites within their canaliculi. The cilia of the cells may also play a role in osteocyte mechanosensation (Fig. 7) (3,11). Fluid flow shear stress (FFSS) induces the opening of hemichannels. PGE₂, ATP, and other responding physiological factors are released into canaliculi to mediate biological responses elicited by mechanical stress (Fig.6) (3).

Osteocyte modification of their microenvironment

The osteocytes can modify their microenvironment in response to environmental factors. These cells may also be able to modify the diameter of their canaliculi. An increase of canalicular diameter would decrease FFSS and a decrease in diameter would increase shear stress (11).

Osteocytes as regulator of mineralization and mineral metabolism

Osteocytes play a role in bone mineralization and phosphate homeostasis. Several osteocyte specific markers such as DMP1, MEPE, and sclerostin regulate bone mineralization and formation. DMP1 is a promoter of mineralization and mineral homeostasis. MEPE is an inhibitor of mineralization. MEPE and SOST null mice have greater bone. There are three key molecules expressed in osteocytes that play a role in phosphate homeostasis. These molecules are DMP1, PHEX, and FGF 23. FGF 23 is a phosphate-depleting hormone that reduces the expression of the sodium-phosphate cotransporter at the brush-border membrane of renal tubular cells (thereby increasing phosphate excretion) and decreases the synthesis of the 1-hydroxylase enzyme which is necessary for vitamin D synthesis (thereby decreasing intestinal absorption of phosphate and calcium). Both DMP1 and PHEX appear to downregulate FGF23 expression, which in turn allows reabsorption of phosphate by the kidney. This provides sufficient circulating phosphate level for bone mineral content. In the absence of DMP1 or PHEX, FGF23 is increased in osteocytes which leads to phosphate excretion by the kidney. A decreased level of circulating phosphate leads to osteomalacia and rickets. The loss-of-function mutations in the PHEX gene results in X-linked hypophosphatemic rickets. DMP1 mutation leads to autosomal recessive hypophosphatemic rickets. Therefore, the osteocyte lacunocanalicular system could be viewed as an endocrine gland (5,11).

Although bone renewing seems like an osteoblast and osteoclast duet, it has been understood that other cells are involved in this cellular symphony of bone remodeling, especially osteocytes which are the most numerous, longest-living, and most important members of this orchestra. However, unanswered questions outnumber the answered ones. Answering these questions may light the way for new treatment methods in the future.

References

1. Rosenberg AE and Sanford IR. Bone. In: Mills SE ed. Histology for pathologists. 3th ed. Lippincott Williams & Wilkins 2007; p75-95.
2. Franz-Odenaal TA, Hall BK and Witten PE. Buried alive: how osteoblasts become osteocytes. *Developmental Dynamics* 2006; 235: 176-90.
3. Bonewald LF. Mechanosensation and transduction in osteocytes. *Bonekey Osteovision*. 2006; 3: 7-15.
4. Seeman E. Osteocytes-martyrs for integrity of bone strength. *Osteoporos Int* 2006; 17: 1443-8.
5. Feng JQ, Ward LM, Liu S, Lu Y, Xie Y, Yuan B, Yu X, Rauch F, Davis SJ, Zhang S, Rios H, Drezner MK, Quarles LD, Bonewald LF, White KE. Loss of DMP 1 causes rickets and osteomalacia and identifies a role for osteocytes in mineral metabolism. *Nat Genet* 2006; 38: 1310-5.
6. Bellido T. Downregulation of SOST/sclerostin by PTH: a novel mechanism of hormonal control of bone formation mediated by osteocytes. *J Musculoskelet Neuronal Interact*. 2006; 6: 358-9.
7. Knothe Tate ML, Adamson JR, Tami AE, Bauer TW. The osteocyte. *Int J Biochem Cell Biol* 2004; 36: 1-8.
8. Bonewald LF. Generation and function of osteocyte dendritic processes. *J Musculoskelet Neuronal Interact*. 2005; 5: 321-4.
9. Jiang JX, Siler-Jackson AJ and Bura S. Roles of gap junctions and hemichannels in bone cell functions and in signal transmission of mechanical stress. *Front Biosci* 2007; 12: 1450-62.
10. Kogianni G, Noble BS. The biology of osteocytes. *Curr Osteoporos Rep* 2007; 5: 81-6.
11. Bonewald LF. Osteocytes as dynamic multifunctional cells. *Ann N Y Acad Sci* 2007; 1116: 281-90.