

Review

Morphology, Function, and Differentiation of Bone Cells

Hiroaki Nakamura

Department of Oral Histology, Matsumoto Dental University, Shiojiri, Japan

(Accepted for publication, March 1, 2007)

Abstract: Bone plays a pivotal role in storing calcium and phosphate in vertebrates. This tissue is maintained by the balance of bone formation and bone resorption. Osteoblast-lineage cells, consisting of osteoblasts, osteocytes and bone lining cells, are engaged in bone formation. Bone resorption is mediated by osteoclasts. Recent research revealed that receptor activator of NF- κ B (RANK)-RANK ligand (RANKL) mechanism is essential for the differentiating and activating osteoclasts. Osteoblast-lineage cells regulate bone resorption *via* the expression of RANKL and osteoprotegerin (OPG), a decoy receptor for RANKL. Additionally, osteoblast-lineage cells participate in degradation of bone matrix by secreting MMP-13. Thus, bone remodeling is achieved by the harmonized orchestration of osteoblast-lineage cells and osteoclast-lineage cells.

Key words: Bone, Cell-cell interaction, Osteoblasts, Osteoclasts

Introduction

Bone is not inert tissue but dynamically metabolized connective tissue throughout life^{1,2}. Old bone matrices are always replaced by newly formed matrices. This continual process, named bone remodeling, is important for maintaining bone volume and strength. Bone volume is maintained by the balance of bone resorption and bone formation. Bone cells consist of osteoblast-lineage cells^{3,4} and osteoclast-lineage cells⁵. Their differentiation and function are regulated by osteotropic hormones and cytokines. Recent research has revealed that osteoblast-lineage cells are not only involved in bone formation but also in bone resorption via supporting differentiation and activation of osteoclasts⁶. Hence, we need to re-consider the functional and morphological varieties of osteoblast-lineage cells. This review describes morphological characteristics of osteoblast-lineage and osteoclast-lineage cells and also discusses their function and differentiation.

Morphology and function of osteoblasts

Osteoblasts are engaged in bone formation. They are generally round in shape and line on the bone surfaces (Fig. 1A). Ultrastructural property of osteoblasts shows typical secretory characteristics, possessing well-developed rough endoplasmic reticulum with dilated cisterna^{1,2}. A large Golgi complex comprises multiple Golgi stacks, vesicles and vacuoles containing fibrillar structures which are considered to represent pro-collagen and proteoglycans (Fig. 1A). Newly formed bone matrix is not calcified immediately. Therefore, uncalcified matrix, named

osteoid, exists under the regulation of active bone-forming osteoblasts. Quantity of osteoid is closely related with bone-forming activity of osteoblasts. Much osteoid are seen under actively bone-forming osteoblasts.

After active bone formation, some osteoblasts become osteocytes buried in bone matrix. Others exist on quiescent bone surfaces and are called as bone lining cells. Bone lining cells show flattened shape and contain a few cell organelles. These morphological characteristics indicate that bone lining cells are hardly engaged in bone formation. In fact, little osteoid is seen under the bone lining cells.

Osteocytes are considered to be the terminal differentiation stage of osteoblasts. They are embedded in osteocytic lacunae and are most abundant cells in bone tissue. Osteocytic lacunae are connected by canaliculi containing their cytoplasmic processes. These canaliculi serve as pathway to supply nutrients and oxygen from blood capillary to osteocytes. Osteocytes possess extremely large surface area because of numerous cytoplasmic processes. Additionally, these processes contain well-developed bundles of actin filaments receivable of mechanical stress. It is conceivable that osteocytes are involved in bone metabolism by receiving and transducing mechanical stress. In fact, recent research revealed that osteocytes express stretch activated channel⁷ and shear-stress-responsive element⁸. However, mechanism of signal transduction and genes regulated by mechanical stress are not clarified yet.

Bone consists of 70% inorganic component, 20% organic component, and 10% water. Approximately 90% of organic content is type I collagen. Osteoblasts are responsible for the production of collagen and non-collagenous proteins including osteocalcin, bone sialoprotein, osteopontin, and osteonectin^{3,4}. They also

Correspondence to Dr. Hiroaki Nakamura, Department of Oral Histology, Matsumoto Dental University, 1780 Gohara Hirooka, Shiojiri, Nagano 399-0781, Japan. Tel.: +81-263-51-2042; Fax: +81-263-53-3456. E-mail address: nakam@po.mdu.ac.jp.

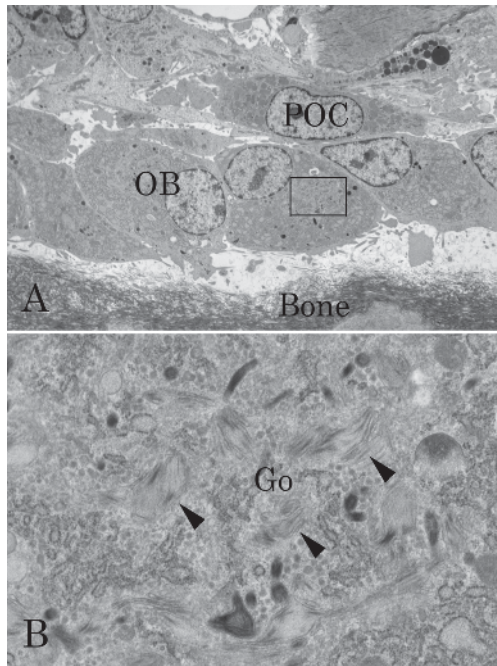


Fig. 1 Electron micrographs of osteoblasts. A: Round osteoblasts (OB) are seen on bone matrix (Bone). POC; preosteoclast. B: Electron micrograph, at a higher magnification, of square in A. Golgi apparatus (Go) of osteoblasts consists of cistern, vesicles, and vacuoles containing fibrillar structures (arrowhead).

synthesize and secrete proteoglycans such as decorin and biglycan. Since these glycoproteins and proteoglycans could bind calcium ion, they are considered to be involved in two functions: storing calcium ion for calcification and regulating growth of hydroxyapatite by preventing excess calcification. Osteoblasts also produce cytokines including insulin-like growth factor I, II, transforming growth factor β (TGF- β), and bone morphogenetic proteins (BMPs)³. These growth factors are stored in calcified bone matrix and play an important role in differentiation and function of osteoblasts. Thus, bone matrix acts as a storage site of growth factors in addition to calcium and phosphates.

Osteoblasts demonstrate intense alkaline phosphatase activity on their plasma membrane. This histochemical feature has been used for a marker of osteoblast-lineage cells. Recent research of tissue non-specific alkaline phosphatase (TNAP)-deficient mice revealed that TNAP acts as pyrophosphatase hydrolyzing pyrophosphate, inhibitor of calcification, and increases the concentration of inorganic phosphates required for calcification⁹. Although ALPase activity is intense in the basolateral plasma membrane of osteoblasts, their membrane towards osteoid and the plasma membrane of osteocytes hardly show ALPase activity. This histochemical evidence indicates that the distribution of ALPase does not always correlate with calcification sites. Moreover, calcification is not completely disturbed in TNAP-deficient mice. Thus, precise function of ALPase in osteoblasts remains controversial.

Cell-cell interaction among osteoblast-lineage cells is important for their differentiation and function. Arana-Chavez et al.¹⁰ reported three types of cell adhesion in osteoblasts at early developmental stage of calvaria by electron microscopy: focal tight junctions, adherens junctions, and gap junctions. Tight junctions are thought to be involved in maintaining cell polarity and preventing macromolecules to enter the intercellular spaces. Continuous tight junctions, also called a *zonula occludens*, are widely seen in epithelial cells. However, there is no continuous tight junction in osteoblasts. This evidence suggests that tight junctions in osteoblasts may not play a role in segregation of bone matrix from extracellular fluid. Gap junctions in osteoblast-lineage cells are mainly composed of connexin 43. Osteoblasts, bone lining cells, and osteocytes are connected by their cell processes through gap junctions. These junctions are involved in transport of ions and micromolecules among osteoblast-lineage cells. Thus, gap junctions are engaged in synchronized function of osteoblast-lineage cells to respond to various physiological signals. Adherens junctions are composed by cadherins. Major cadherins expressed in osteoblasts are N-cadherin and cadherin-11 (Ob-cadherin). In addition to mechanical function, adherens junctions are thought to be involved in signal transduction via cell-cell interaction because they are associated with β -catenin as well as tyrosin-kinases¹¹. Moreover, bone formation rate and bone volume are decreased in cadherin-11-deficient mice¹². These facts suggest that cell-cell adhesion via cadherins could contribute to regulation of differentiation, function and survival of osteoblasts.

Cell-matrix interaction between osteoblasts and bone matrix proteins, including type I collagen, non-collagenous proteins, and fibronectin, is also important for differentiation and function of osteoblasts. The interaction between β_1 integrins, $\alpha_1\beta_1$ and $\alpha_2\beta_1$, and type I collagen plays a key role in differentiation and function of osteoblasts via activation of mitogen-activated protein kinase (MAPK) signaling pathway¹³. Moreover, numerous cytoplasmic processes of osteoblasts extend into osteoid. The orientation of collagen fibers in lamellar bone alternates from layer to layer. This lamellar structure might be determined by cell-matrix adhesion between osteoblasts' process and collagen.

Recent works have revealed that osteoblast-lineage cells are involved in differentiation and activation of osteoclasts as well as bone formation. Macrophage colony stimulating factor (M-CSF) and receptor activator of nuclear factor (NF)- κ B ligand (RANKL) essential for osteoclastogenesis are expressed in osteoblast-lineage cells⁶. In addition, osteoprotegerin (OPG), decoy receptor for RANKL, is secreted by osteoblasts. Bone resorption by osteoclasts might be regulated by the balance of RANKL and OPG expressed in osteoblast-lineage cells.

On the other hand, osteoblasts and osteocytes secrete matrix metalloproteinase (MMP)-13, indicating that osteoblast-lineage cells are engaged in degradation of collagen¹⁴. Sakamoto and Sakamoto¹⁵ reported that osteoblasts and osteocytes participate

in bone resorption by secretion of collagenase. Indeed, osteoclasts form deeper resorption lacunae in living bone than those in devitalized bone. Fewer collagen fibrils in living bone suggest collagenase secretion by osteoblast-lineage cells¹⁶. Additionally, in situ hybridization studies have shown that mRNA of MMP-13 is expressed in osteoblast-lineage cells¹⁷. Immunolocalization of MMP-13 protein is detected on the bone surface of Howship's lacunae and osteocytes adjacent to osteoclasts, but hardly seen in actively bone-forming osteoblasts and osteoclasts¹⁴. Immunogold labeling for MMP-13 is detected in Golgi apparatus of osteocytes under osteoclasts and bone canaliculi, indicating that MMP-13 is secreted by osteocytes and translocated into Howship's lacunae through the lacunae-caliculi channel. Taken together, collagen fibrils may be fragmented by MMP-13 produced by osteoblast-lineage cells, and further degraded to low-molecules by MMP-9 secreted by osteoclasts¹⁸. These findings suggest that osteoblast-lineage cells participate in degradation of collagen during bone resorption in concert with osteoclasts. Furthermore, parathyroid hormone (PTH) regulates the MMP-13 promoter in osteoblast-lineage cells via activator protein (AP)-1 and Runx 2 binding sites^{19,20}. This mechanism might be partly involved in enhancement of bone resorption by PTH.

Differentiation of osteoblasts

Osteoblasts originate from mesenchymal stem cells (MSCs). MSCs could differentiate into chondrocytes, osteoblasts, myoblasts, and adipocytes⁴. Their differentiation is regulated by specific transcription factors. Sox 5, 6 and 9 regulate chondrocytic differentiation. Differentiation of adipocytes and myoblasts is determined by PPAR γ and Myo D, respectively. In case of osteoblast-lineage cells, Runx 2/Cbfa^{21,22} and Osterix/Sp7²³ are essential regulators. Runx 2-deficient mice can not develop bone tissue. Cleidocranial dysostosis showing abnormality in membranous ossification is caused by defect in Runx 2 gene. Although Runx 2 is found as transcriptional factor binding to osteocalcin promoter, it also contributes to the gene expression of osteopontin, bone sialoprotein, dentin sialoprotein, and TGF β receptor I.

Osteoblast-lineage cells show stepwise expressions of their specific markers including matrix proteins and ALPase in the process of their differentiation. Differentiation and function of osteoblast-lineage cells are regulated by hormones, including 1, 25(OH) $_2$ D $_3$, PTH, and estrogen, and cytokines such as BMPs. BMPs, BMP-2, BMP-4, and BMP-7, induce osteogenesis in vivo and in vitro²⁴. BMPs were discovered as osteo-inductive factors in decalcified bone and dentin by Urist et al.²⁵ BMPs prevent multipotential muscle satellite cells to differentiate into myoblasts and adipocytes, and, in turn, promote chondrocytic and osteoblastic differentiation. These phenomena are closely related with the somite derived from mesoderm. Somite contains multipotential cells to undergo myogenic, osteogenic, and

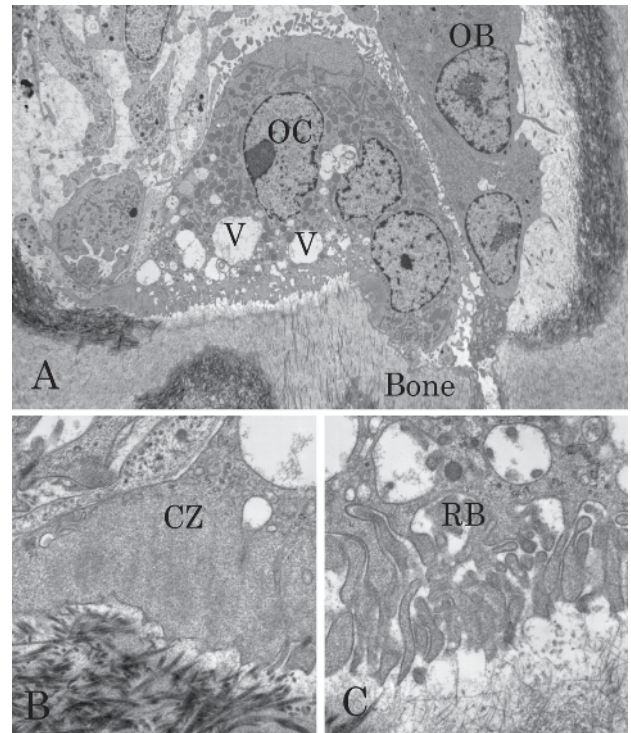


Fig. 2 Electron micrographs of an osteoclast.

A: A multinucleated osteoclast attaches bone surface (Bone). Numerous mitochondria and vacuoles (V) are seen in its cytoplasm.

B: Clear zone (CZ) contains networks of actin filaments.

C: Ruffled border (RB) shows finger-like cytoplasmic processes.

adipogenic differentiation. BMPs conduct endochondral ossification by inducing mesenchymal cells to differentiate into osteogenic cells.

In signal transduction pathway of BMPs, their specific receptor complex leads to phosphorylation of Smads (R-Smads) that form heterodimer with Smad4 and regulate gene expression. BMP activity is also regulated by inhibitory Smads (I-Smads) and antagonists including noggin, chordin and sclerostin⁴. Although BMPs promote differentiation of osteoblasts by preventing MyoD expression²⁶ and inducing Runx 2 expression, precise transcriptional mechanism has not been clarified yet.

Recent reports suggest that the canonical Wnt/ β -catenin pathway is involved in early development by promoting osteoprogenitor differentiation²⁷. Furthermore, cell-cell and cell-matrix interaction participate in functional and morphological changes of osteoblast-lineage cells. Numerous factors might be involved in the differentiation of osteoblasts.

Function and morphology of osteoclasts

Osteoclasts are multinucleated giant cells responsible for bone resorption. Active osteoclasts come in contact with calcified bone surface and exist within Howship's lacunae which are eroded by their own resorptive activity¹. Osteoclasts are generally distinguished from other bone cells by their large size and multiple nuclei (Fig 2A). Their ultrastructures show numerous

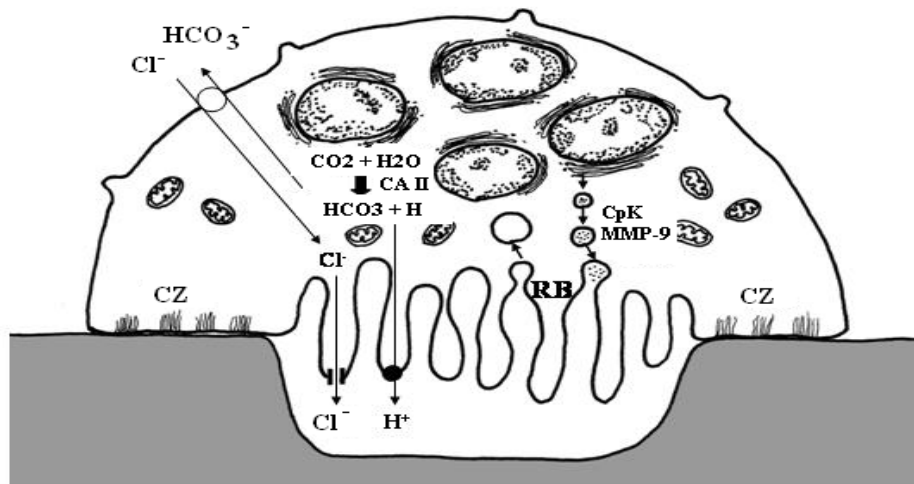


Fig. 3 Mechanism of osteoclast bone resorption.

Osteoclast secretes proton produced by carbonic anhydrase II (CA II) into Howship's lacuna through vacuolar type H⁺-ATPase. Chloride ions are also transported into the lacuna by chloride channel. The acidic environment dissolves hydroxyapatite in bone matrix. On the other hand, organic components such as collagen are degraded by cathepsin K (CpK) and matrix metalloproteinase (MMP) -9. RB:ruffled border, CZ; clear zone

mitochondria, endoplasmic reticulum and well-developed Golgi apparatus around nuclei. They also contain vesicles, lysosomes, tubular lysosomes and vacuoles^{1,2}. These structures indicate that osteoclasts are actively involved in energy production and protein synthesis, particularly production of lysosomal enzymes.

Active bone-resorbing osteoclasts show definite cell-polarity. Their plasma membrane is classified into three regions: clear zone, ruffled border, and basolateral plasma membrane.

Clear zone was named by clear appearance lacking cell organelle. This region shows a ring-like structure and consists of accumulated focal contacts. Cytoplasm of clear zone contains abundant actin filaments (Fig. 2B). Actin ring observed in *in vitro* osteoclasts by phalloidine staining correspond to this structure. Clear zone is engaged in attachment of osteoclast to bone matrix and isolation of bone-resorbing compartment from extracellular fluid. This compartment provides efficient condition for bone resorption. The attachment of osteoclast to the bone matrix is mediated by vitronectin receptor, $\alpha_5\beta_3$ integrin, in membrane of clear zone²⁸. One of the ligands is considered to be osteopontin in bone matrix. Cell-matrix interaction stimulates c-Src, a non-receptor-type tyrosine kinase, involving in maintenance of cell polarity and activity of osteoclasts. Soriano et al.²⁹ revealed that c-Src-deficient mice showed a phenotype of osteopetrosis. Despite numerous osteoclasts, they do not develop ruffled border, indicating that c-Src is essential for bone resorbing function of osteoclasts. c-Src appears to control cytoskeleton by acting with Pyk2, a focal adhesion kinase³⁰, and c-Cbl, a proto-oncogene³¹. Additionally, coated pits indicating receptor-mediated endocytosis are occasionally seen in clear zone. MT-MMP1 is also localized in clear zone³². These findings suggest that clear zone is also involved in endocytosis of bone matrix and migration of osteoclasts.

Prominent feature of osteoclast is ruffled border, the folding of the plasma membrane in the area facing bone matrix (Fig. 2C). Ruffled border is closely associated with bone resorption. Bone resorption is achieved by dissolution of mineral components consisting of hydroxyapatite and degradation of organic

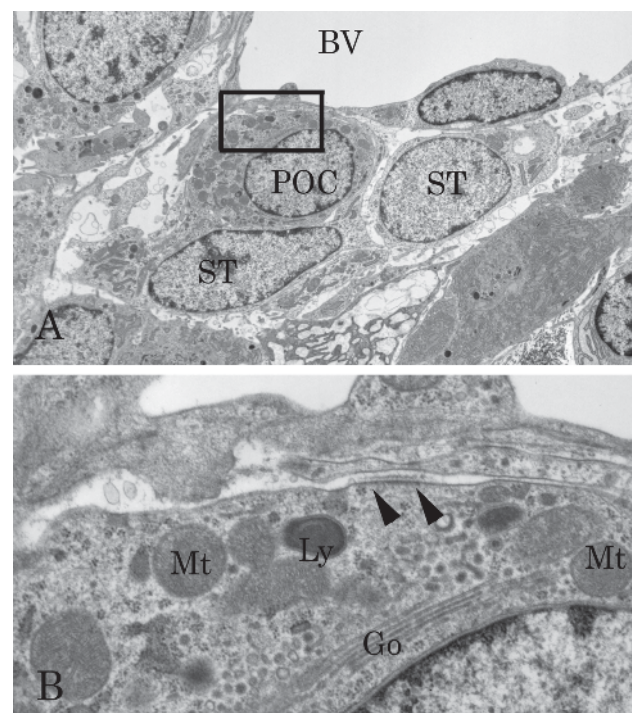


Fig. 4 Electron micrographs of a preosteoclast.

A: A preosteoclast is surrounded by stromal cells (ST). BV; blood vessel
B: Electron micrograph, at a higher magnification, of square in A. The preosteoclast possesses numerous mitochondria (Mt), the Golgi apparatus (Go) around a nucleus, and lysosomal structures (Ly). Adherent structures through extracellular matrices and close contact structures (arrows) are seen at the region between the preosteoclast and the stromal cell.

components of bone matrix. Carbonic anhydrase, converting CO_2 and H_2O into H^+ and HCO_3^- , in cytoplasm^{33,34} and vacuolar type H^+ -ATPase in ruffled border membrane are involved in acidification of Howship's lacuna³⁵ (Fig. 3). H^+ -ATPase is composed of 13 subunits, forming a large complex. Oc/oc mice showing osteopetrosis attribute to the abnormality of the subunit of H^+ -ATPase³⁶. Numerous mitochondria in osteoclasts are considered to provide ATP for H^+ transport. Thus, extracellular acidic environment (pH 4-5) under ruffled border leads to focal decalcification of hydroxyapatite in bone matrix. Moreover, ion balance in cytoplasm is maintained by chloride channel (ClC)-7. Importance of this channel is also clarified by ClC-7-deficient mice showing osteopetrosis³⁷.

In contrast, extracellular digestion of organic components is accomplished by lysosomal enzymes such as cathepsin K^{38,39} and MMP-9¹⁸. Cathepsin K belongs to a cysteine protease family and could degrade collagen fibers in acidic condition. Therefore, this protease mainly participates in degradation of native collagen in bone matrix. In fact, immunolocalization of cathepsin K is detected in Howship's lacunae. On the other hand, MMP-9 acts as a gelatinase for further digestion of segmented collagen fibrils (Fig. 3). Tartrate resistance acid phosphatase (TRAP) is widely used as a marker enzyme of osteoclasts and secreted in Howship's lacunae. Although this enzyme could dephosphorylate osteopontin⁴⁰, its precise role in bone resorption has not been clarified yet.

Basolateral plasma membrane of osteoclasts is thought to be responsible for receiving stimulation of calcitonin and cytokines. This area is also important for cell-cell interaction with osteoblast-lineage cells. Salo et al.⁴¹ divide basolateral membrane into the central region and the lateral region. They consider the former region is a functional secretory domain involved in transcytosis, exocytosis of degraded bone matrix^{41,42}.

Calcitonin is a hormone to inactivate osteoclasts. This causes destruction of actin filaments, loss of clear zone and retraction of osteoclast, followed by detachment from bone surface. These processes are also caused by dibutyryl cAMP and increase of cytoplasmic calcium, indicating that protein kinase A and C may regulate activity of osteoclasts through cytoskeletal reorganization^{43,44}. In addition, extracellular signal-regulated kinase (ERK) is also involved in disorder of cytoskeleton by calcitonin⁴⁵. Thus, it is conceivable that ERK participates in the maintenance of cell polarity of osteoclasts as well as their survival^{46,47}.

Differentiation of osteoclasts

It is widely accepted that osteoclasts originate from monocyte-macrophage lineage precursor cells. Because osteopetrotic animals cured by bone marrow transplantation or parabiosis are linked by a common crossing circulation with normal littermates¹. This evidence suggests that osteoclast precursor cells were carried through blood capillaries and resided in bone tissue. Osteoclast precursors or preosteoclasts show several resemblances in

morphological feature to osteoclasts. They possess numerous mitochondria, the Golgi apparatus around nucleus and lysosomal structures (Fig. 4). They also express TRAP, cathepsin K and calcitonin receptors. Osteoclast precursors demonstrate undifferentiated characteristics such as a large amount of free ribosome and a few rough endoplasmic reticulum. Nevertheless, it is difficult to identify undifferentiated osteoclast precursors by their morphological characteristics because sections only reveal a limited aspect of them.

Molecular mechanism of osteoclast differentiation and activation has been clarified. First finding was a discovery of macrophage colony stimulating factor (M-CSF) as a critical factor for osteoclast differentiation. Osteopetrotic (op/op) mice showing a marked reduction of osteoclasts in bone tissue were caused by a point mutation of the M-CSF gene. Their osteopetrotic phenotypes were cured by administration of recombinant M-CSF⁴⁸. Currently, M-CSF is required for proliferation of osteoclast precursors and differentiation and survival of osteoclasts. However, M-CSF is not enough to induce osteoclast differentiation *in vitro*. Osteoclasts appear in bone tissue of op/op mice according to their age. These results suggest that other factors would be also required for osteoclastogenesis.

Morphological findings have demonstrated that osteoclasts and preosteoclasts always come in contact with ALP-positive osteoblast-lineage cells. It had been suggested that cell-cell interaction between osteoblast-lineage cells and osteoclast precursors is essential for osteoclastogenesis^{49,50}. One of the most exciting findings was the discovery of RANK/RANKL system in osteoclast differentiation^{51,52}. RANKL, a member of the tumor necrosis factor (TNF) family, was originally reported to be expressed in activated T cells. RANKL, produced in osteoblast-lineage cells, participates in differentiation and activation of osteoclasts via binding to RANK, expressed in osteoclast progenitors and osteoclasts. The critical role of RANK/RANKL system was confirmed by mouse genetic studies. RANK- or RANKL-deficient mice show osteopetrosis attributing to the defect in osteoclastogenesis. Stimulators of osteoclastogenesis such as $1,25\text{-(OH)}_2\text{D}_3$, prostaglandin E_2 (PGE_2), interleukin (IL)-1, PTH and PTH related protein upregulate the expression of RANKL in osteoblast-lineage cells. Conversely, OPG, a soluble form of the TNF receptor, works as a decoy receptor for RANKL and inhibits osteoclastogenesis. OPG-deficient mice demonstrate severe osteoporosis associated with an increased number of osteoclasts⁵³. OPG expression is upregulated by estrogen, TGF- β and BMP. Thus, differentiation and activation of osteoclasts are controlled by the balance between RANKL and OPG in osteoblast-lineage cells. The effects of hormones and cytokines converge on RANKL and OPG⁵⁴.

Signaling of RANK/RANKL in osteoclast-lineage cells is mediated by TNF receptor-associated factor (TRAF). TRAF6 is associated with intracellular domain of RANK. TRAF6-deficient

mice with osteopetrosis revealed that this molecule is implicated in osteoclast differentiation as well as activation^{55, 56}). Although TRAF6 stimulates ERK, JNK, p38 and NF- κ B signalings, genes regulated by them had not been fully understood. Recently, microarray regarding RANKL-inducible genes revealed that NFATc1^{57, 58}) and dendritic cell-specific transmembrane protein (DC-STAMP) are involved in osteoclast differentiation. DC-STAMP-deficient mice demonstrate that mononuclear osteoclastic cells instead of multinucleated osteoclasts could resorb bone matrix⁵⁹). On the other hand, Fc γ R and DAP12 harboring immunoreceptor tyrosine-based activation motif (ITAM) cooperate with RANK to stimulate calcium signaling and activate NFATc1⁶⁰). Thus, signaling pathway involving in immunoresponse also regulates osteoclast differentiation. This new research area, referred to osteoimmunology, might unveil the mechanism of pathological bone resorption.

We reported cell attachment structures between osteoclast-lineage cells and osteoblast-lineage cells⁶¹). Extracellular matrix, including heparan sulfate proteoglycan and fibronectin, is involved in cell attachment. In addition to RANK/RANKL system, other regulatory mechanism such as cell-cell attachment might be important to determine the region where osteoclasts should differentiate because osteoclasts always appear in bone tissue. Future research will be necessary to understand a signaling pathway mediated by this cell-cell interaction.

Conclusion

Bone remodeling is performed by osteoblasts and osteoclasts. Their proliferation, differentiation and function are regulated by hormones such as parathyroid hormone (PTH), estrogen, 1,25(OH)₂D₃ and calcitonin as well as cytokines. In the case of osteoblasts, bone morphogenetic protein (BMP) is one of the most effective cytokines. On the other hand, osteoclastogenesis requires M-CSF and RANKL. It is no doubt that cell-cell interaction between osteoblast-lineage cells and osteoclast-lineage cells is essential for maintenance of bone volume. Recent bone research including discovery of Runx2 and RANK/RANKL system provide progress for understanding bone metabolism. However, it is not enough to explain the mechanism of bone remodeling. Interdisciplinary research including cell biology, biochemistry, physiology, morphology and etc. will be necessary to clarify the bone cell biology and develop therapy for bone diseases such as osteoporosis and periodontal disease.

References

1. Cormack D.H. Ham's Histology 9th Edition, J.B. Lippincott Company, Philadelphia, 1987
2. Baron R. Anatomy and ultrastructure of bone. In: Primer on the metabolic bone diseases and disorders of mineral metabolism, ed. by Favus M.J., Lippincott Williams & Wilkins, Philadelphia, 1999, pp3-10. Book
3. Lian JB, Stein GS, Canalis E, Robey PG, Boskey AL. Bone formation: osteoblast lineage cells, growth factors, matrix proteins and the mineralization process. In: Primer on the metabolic bone diseases and disorders of mineral metabolism, ed. by Favus M.J., Lippincott Williams & Wilkins, Philadelphia, 1999, pp14-29. Book
4. Aubin JE, Lian JB, Stein GS. Bone formation: maturation and functional activities of osteoblast lineage cells. In: Primer on the metabolic bone diseases and disorders of mineral metabolism, ed. by Favus M.J., American society for Bone and Mineral Research, Washington, 2006, pp20-29. Book
5. Ross FP. Osteoclast biology and bone resorption. In: Primer on the metabolic bone diseases and disorders of mineral metabolism, ed. by Favus M.J., American society for Bone and Mineral Research, Washington, 2006, pp30-35. Book
6. Suda T, Takahashi N, Udagawa N, Jimi E, Gillespie MT, Martin TJ. Modulation of osteoclast differentiation and function by the new members of the tumor necrosis factor receptor and ligand families. *Endocr Rev* 20:345-357, 1999
7. Ypey DL, Weidema AF, Hold KM, Van der Laarse A, Ravesloot JH, Van Der Plas A, Nijweide PJ. Voltage, calcium, and stretch activated ionic channels and intracellular calcium in bone cells. *J Bone Miner Res* 7(Suppl 2): S377-387, 1992
8. Resnick N, Collins T, Atkinson W, Bonthron DT, Dewey CF Jr, Gimbrone MA Jr. Platelet-derived growth factor B chain promoter contains a cis-acting fluid shear-stress-responsive element. *Proc Natl Acad Sci U S A* 90(10):4591-4595, 1993
9. Hessle L, Johnson KA, Anderson HC, Narisawa S, Sali A, Goding JW, Terkeltaub R, Millan JL. Tissue-nonspecific alkaline phosphatase and plasma cell membrane glycoprotein-1 are central antagonistic regulators of bone mineralization. *Proc Natl Acad Sci USA* 99(14):9445-9449, 2002
10. Arana-Chavez VE, Soares AM, Katchburian E.: Junctions between early developing osteoblasts of rat calvaria as revealed by freeze-fracture and ultrathin section electron microscopy. *Arch Histol Cytol* 58(3):285-292, 1995
11. Tsukita S, Oishi K, Akiyama T, Yamanashi Y, Yamamoto T, Tsukita S. Specific proto-oncogenic tyrosine kinases of src family are enriched in cell-to-cell adherens junctions where the level of tyrosine phosphorylation is elevated. *J Cell Biol* 113(4):867-879, 1991
12. Kawaguchi J, Azuma Y, Hoshi K, Kii I, Takeshita S, Ohta T, Ozawa H, Takeichi M, Chisaka O, Kudo A. Targeted disruption of cadherin-11 leads to a reduction in bone density in calvaria and long bone metaphyses. *J Bone Miner Res* 16(7):1265-1271, 2001
13. Takeuchi Y, Suzawa M, Kikuchi T, Nishida E, Fujita T, Matsumoto T. Differentiation and transforming growth factor-beta receptor down-regulation by collagen-alpha2beta1 integrin interaction is mediated by focal adhesion kinase and its downstream signals in murine osteoblastic cells. *J Biol*

- Chem 272(46):29309-29316, 1997
14. Nakamura H, Sato G, Hirata A, Yamamoto T. Immunolocalization of matrix metalloproteinase-13 on bone surface under osteoclasts in rat tibia. *Bone* 34(1):48-56, 2004
 15. Sakamoto S, Sakamoto M. Biochemical and immunohistochemical studies on collagenase in resorbing bone in tissue culture. A novel hypothesis for the mechanism of bone resorption. *J Periodontal Res* 17(5):523-526, 1982
 16. Shimizu H, Sakamoto M, Sakamoto S. Bone resorption by isolated osteoclasts in living versus devitalized bone: differences in mode and extent and the effects of human recombinant tissue inhibitor of metalloproteinases. *J Bone Miner Res* 5(4):411-418, 1990
 17. Fuller K, Chambers TJ. Localisation of mRNA for collagenase in osteocytic, bone surface and chondrocytic cells but not osteoclasts. *J Cell Sci* 108 (Pt 6):2221-2230, 1995
 18. Reponen P, Sahlberg C, Munaut C, Thesleff I, Tryggvason K. High expression of 92-kD type IV collagenase (gelatinase B) in the osteoclast lineage during mouse development. *J Cell Biol* 124(6):1091-1102, 1994
 19. Hess J, Porte D, Munz C, Angel P. AP-1 and Cbfa/runt physically interact and regulate parathyroid hormone-dependent MMP13 expression in osteoblasts through a new osteoblast-specific element 2/AP-1 composite element. *J Biol Chem* 276(23):20029-20038, 2001
 20. Porte D, Tuckermann J, Becker M, Baumann B, Teurich S, Higgins T, Owen MJ, Schorpp-Kistner M, Angel P. Both AP-1 and Cbfa1-like factors are required for the induction of interstitial collagenase by parathyroid hormone. *Oncogene* 18(3):667-678, 1999
 21. Komori T, Yagi H, Nomura S, Yamaguchi A, Sasaki K, Deguchi K, Shimizu Y, Bronson RT, Gao YH, Inada M, Sato M, Okamoto R, Kitamura Y, Yoshiki S, Kishimoto T. Targeted disruption of Cbfa1 results in a complete lack of bone formation owing to maturational arrest of osteoblasts. *Cell* 89(5):755-764, 1997
 22. Ducy P, Zhang R, Geoffroy V, Ridall AL, Karsenty G. Osf2/Cbfa1: a transcriptional activator of osteoblast differentiation. *Cell* 89(5):747-754, 1997
 23. Nakashima K, Zhou X, Kunkel G, Zhang Z, Deng JM, Behringer RR, de Crombrughe B. The novel zinc finger-containing transcription factor osterix is required for osteoblast differentiation and bone formation. *Cell* 108(1):17-29, 2002.
 24. Yamaguchi A, Katagiri T, Ikeda T, Wozney JM, Rosen V, Wang EA, Kahn AJ, Suda T, Yoshiki S. Recombinant human bone morphogenetic protein-2 stimulates osteoblastic maturation and inhibits myogenic differentiation in vitro. *J Cell Biol* 113(3):681-687, 1991
 25. Urist MR, Mikulski A, Lietze A. Solubilized and insolubilized bone morphogenetic protein. *Proc Natl Acad Sci USA* 76(4):1828-1832, 1979
 26. Katagiri T, Yamaguchi A, Komaki M, Abe E, Takahashi N, Ikeda T, Rosen V, Wozney JM, Fujisawa-Sehara A, Suda T. Bone morphogenetic protein-2 converts the differentiation pathway of C2C12 myoblasts into the osteoblast lineage. *J Cell Biol* 127(6 Pt 1):1755-1766, 1994
 27. Hill TP, Spater D, Taketo MM, Birchmeier W, Hartmann C. Canonical Wnt/beta-catenin signaling prevents osteoblasts from differentiating into chondrocytes. *Dev Cell* 8(5):727-738, 2005
 28. Davies J, Warwick J, Totty N, Philp R, Helfrich M, Horton M. The osteoclast functional antigen, implicated in the regulation of bone resorption, is biochemically related to the vitronectin receptor. *J Cell Biol* 109(4 Pt 1):1817-1826, 1989.
 29. Soriano P, Montgomery C, Geske R, Bradley A. Targeted disruption of the c-src proto-oncogene leads to osteopetrosis in mice. *Cell* 64(4):693-702, 1991
 30. Duong LT, Lakkakorpi PT, Nakamura I, Machwate M, Nagy RM, Rodan GA. PYK2 in osteoclasts is an adhesion kinase, localized in the sealing zone, activated by ligation of alpha(v)beta3 integrin, and phosphorylated by src kinase. *J Clin Invest* 102(5):881-892, 1998
 31. Tanaka S, Amling M, Neff L, Peyman A, Uhlmann E, Levy JB, Baron R. c-Cbl is downstream of c-Src in a signalling pathway necessary for bone resorption. *Nature*. 383(6600):52 8-531, 1996
 32. Irie K, Tsuruga E, Sakakura Y, Muto T, Yajima T. Immunohistochemical localization of membrane type 1-matrix metalloproteinase (MT1-MMP) in osteoclasts in vivo. *Tissue Cell* 33(5):478-482, 2001
 33. Anderson RE, Schraer H, Gay CV. Ultrastructural immunocytochemical localization of carbonic anhydrase in normal and calcitonin-treated chick osteoclasts. *Anat Rec* 204(1):9-20, 1982
 34. Gay CV, Mueller WJ. Carbonic anhydrase and osteoclasts: localization by labeled inhibitor autoradiography. *Science* 183(123):432-434, 1974
 35. Blair HC, Teitelbaum SL, Ghiselli R, Gluck S. Osteoclastic bone resorption by a polarized vacuolar proton pump. *Science* 245(4920):855-857, 1989
 36. Scimeca JC, Franchi A, Trojani C, Parrinello H, Grosgeorge J, Robert C, Jaillon O, Poirier C, Gaudray P, Carle GF. The gene encoding the mouse homologue of the human osteoclast-specific 116-kDa V-ATPase subunit bears a deletion in osteosclerotic (oc/oc) mutants. *Bone* 26(3):207-213, 2000
 37. Kornak U, Kasper D, Bosl MR, Kaiser E, Schweizer M, Schulz A, Friedrich W, Delling G, Jentsch TJ.: Loss of the ClC-7 chloride channel leads to osteopetrosis in mice and man. *Cell* 104(2):205-215, 2001
 38. Gelb BD, Shi GP, Chapman HA, Desnick RJ.: Pycnodysostosis, a lysosomal disease caused by cathepsin K deficiency. *Science* 273(5279):1236-1238, 1996
 39. Inaoka T, Bilbe G, Ishibashi O, Tezuka K, Kumegawa M, Kokubo T.: Molecular cloning of human cDNA for cathepsin

- K: novel cysteine proteinase predominantly expressed in bone. *Biochem Biophys Res Commun* 206(1):89-96, 1995
40. Ek-Rylander B, Flores M, Wendel M, Heinegard D, Andersson G. Dephosphorylation of osteopontin and bone sialoprotein by osteoclastic tartrate-resistant acid phosphatase. Modulation of osteoclast adhesion in vitro. *J Biol Chem* 269(21):14853-14856, 1994
41. Salo J, Lehenkari P, Mulari M, Metsikko K, Vaananen HK. Removal of osteoclast bone resorption products by transcytosis. *Science* 276(5310):270-273, 1997
42. Palokangas H, Mulari M, Vaananen HK. Endocytic pathway from the basal plasma membrane to the ruffled border membrane in bone-resorbing osteoclasts. *J Cell Sci* 110 (Pt 15):1767-1780, 1997
43. Nicholson GC, Livesey SA, Moseley JM, Martin TJ. Actions of calcitonin, parathyroid hormone, and prostaglandin E2 on cyclic AMP formation in chicken and rat osteoclasts. *J Cell Biochem* 31(3):229-241, 1986
44. Holloway WR, Collier FM, Herbst RE, Hodge JM, Nicholson GC. Complex shape changes in isolated rat osteoclasts: involvement of protein kinase C in the response to calcitonin. *Calcif Tissue Int* 61(4):306-312, 1997
45. Nakamura H, Nagaoka N, Hirata A, Inoue M, Ozawa H, Yamamoto T. Distribution of actin filaments, non-muscle myosin, M-Ras, and extracellular signal-regulated kinase (ERK) in osteoclasts after calcitonin administration. *Arch Histol Cytol* 68(2):143-150, 2005
46. Miyazaki T, Katagiri H, Kanegae Y, Takayanagi H, Sawada Y, Yamamoto A, Pando MP, Asano T, Verma IM, Oda H, Nakamura K, Tanaka S. Reciprocal role of ERK and NF-kappaB pathways in survival and activation of osteoclasts. *J Cell Biol* 148(2):333-342, 2000
47. Nakamura H, Hirata A, Tsuji T, Yamamoto T. Role of osteoclast extracellular signal-regulated kinase (ERK) in cell survival and maintenance of cell polarity. *J Bone Miner Res* 18(7):1198-1205, 2003
48. Kodama H, Yamasaki A, Nose M, Niida S, Ohgame Y, Abe M, Kumegawa M, Suda T. Congenital osteoclast deficiency in osteopetrotic (op/op) mice is cured by injections of macrophage colony-stimulating factor. *J Exp Med* 173(1):269-272, 1991
49. Takahashi N, Akatsu T, Udagawa N, Sasaki T, Yamaguchi A, Moseley JM, Martin TJ and Suda T. Osteoblastic cells are involved in osteoclast formation. *Endocrinology* 123: 2600-2602, 1988
50. Tanaka S, Nakamura I, Inoue J, Oda H, Nakamura K. Signal transduction pathways regulating osteoclast differentiation and function. *J Bone Miner Metab* 21(3):123-133, 2003
51. Lacey DL, Timms E, Tan HL, Kelley MJ, Dunstan CR, Burgess T, Elliott R, Colombero A, Elliott G, Scully S, Hsu H, Sullivan J, Hawkins N, Davy E, Capparelli C, Eli A, Qian YX, Kaufman S, Sarosi I, Shalhoub V, Senaldi G, Guo J, Delaney J, Boyle WJ. Osteoprotegerin ligand is a cytokine that regulates osteoclast differentiation and activation. *Cell* 93: 165-176, 1998
52. Yasuda H, Shima N, Nakagawa N, Yamaguchi K, Kinoshita M, Mochizuki S, Tomoyasu A, Yano K, Goto M, Murakami A, Tsuda E, Morinaga T, Higashio K, Udagawa N, Takahashi N and Suda T: Osteoclast differentiation factor is a ligand for osteoprotegerin/osteoclastogenesis-inhibitory factor and is identical to TRANCE/RANKL. *Proc Natl Acad Sci USA* 95: 3597-3602, 1998
53. Mizuno A, Amizuka N, Irie K, Murakami A, Fujise N, Kanno T, Sato Y, Nakagawa N, Yasuda H, Mochizuki S, Gomibuchi T, Yano K, Shima N, Washida N, Tsuda E, Morinaga T, Higashio K, Ozawa H. Severe osteoporosis in mice lacking osteoclastogenesis inhibitory factor/osteoprotegerin. *Biochem Biophys Res Commun* 247(3):610-615, 1998
54. Hofbauer LC, Khosla S, Dunstan CR, Lacey DL, Boyle WJ, Riggs BL. The roles of osteoprotegerin and osteoprotegerin ligand in the paracrine regulation of bone resorption. *J Bone Miner Res* 15(1):2-12, 2000
55. Naito A, Azuma S, Tanaka S, Miyazaki T, Takaki S, Takatsu K, Nakao K, Nakamura K, Katsuki M, Yamamoto T and Inoue J. Severe osteopetrosis, defective interleukin-1 signalling and lymph node organogenesis in TRAF6-deficient mice. *Genes Cells* 4:353-362, 1999
56. Lomaga MA, Yeh WC, Sarosi I, Duncan GS, Furlonger C, Ho A, Morony S, Capparelli C, Van G, Kaufman S, van der Heiden A, Itie A, Wakeham A, Khoo W, Sasaki T, Cao Z, Penninger JM, Paige CJ, Lacey DL, Dunstan CR, Boyle WJ, Goeddel DV and Mak TW. TRAF6 deficiency results in osteopetrosis and defective interleukin-1, CD40, and LPS signaling. *Genes Dev* 13:1015-1024, 1999
57. Takayanagi H, Kim S, Koga T, Nishina H, Isshiki M, Yoshida H, Saiura A, Isobe M, Yokochi T, Inoue J, Wagner EF, Mak TW, Kodama T, Taniguchi T. Induction and activation of the transcription factor NFATc1 (NFAT2) integrate RANKL signaling in terminal differentiation of osteoclasts. *Dev Cell* 3(6):889-901, 2002
58. Asagiri M, Takayanagi H. The molecular understanding of osteoclast differentiation. *Bone* 40(2):251-264, 2007
59. Kukita T, Wada N, Kukita A, Kakimoto T, Sandra F, Toh K, Nagata K, Iijima T, Horiuchi M, Matsusaki H, Hieshima K, Yoshie O, Nomiya H. RANKL-induced DC-STAMP is essential for osteoclastogenesis. *J Exp Med* 200(7):941-946, 2004
60. Koga T, Inui M, Inoue K, Kim S, Suematsu A, Kobayashi E, Iwata T, Ohnishi H, Matozaki T, Kodama T, Taniguchi T, Takayanagi H, Takai T. Costimulatory signals mediated by the ITAM motif cooperate with RANKL for bone homeostasis. *Nature* 428(6984):758-763, 2004
61. Nakamura H, Ozawa H. Immunohistochemical localization of heparan sulfate proteoglycan in rat tibiae. *J Bone Miner Res* 9(8):1289-1299, 1994