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COMMENTARY

The cell biology of osteoclast function

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SUMMARY

Osteoclasts are multinucleated cells responsible for bone resorption. They have developed an efficient machinery for dissolving crystalline hydroxyapatite and degrading organic bone matrix rich in collagen fibers. When initiating bone resorption, osteoclasts become polarized, and three distinct membrane domains appear: a ruffled border, a sealing zone and a functional secretory domain. Simultaneously, the cytoskeleton undergoes extensive re-organisation. During this process, the actin cytoskeleton forms an attachment ring at the sealing zone, the membrane domain that anchors the resorbing cell to bone matrix. The ruffled border appears inside the sealing zone, and has several characteristics of late endosomal membrane. Extensive vesicle transport to the ruffled border delivers hydrochloric acid and proteases to an area between the ruffled border and the bone surface called the resorption lacuna. In this extracellular compartment, crystalline hydroxyapatite is dissolved by acid, and a mixture of proteases degrades the organic matrix. The degradation products of collagen and other matrix components are endocytosed, transported through the cell and exocytosed through a functional secretory domain. This transcytotic route allows osteoclasts to remove large amounts of matrix-degradation products without losing their tight attachment to underlying bone. It also facilitates further processing of the degradation products intracellularly during the passage through the cell.

Key words: Bone, Osteoclast, Cell biology

INTRODUCTION

Bone resorption is necessary for many skeletal processes. It is an obligatory event during bone growth, tooth eruption and fracture healing, and is also necessary for the maintenance of an appropriate level of blood calcium. In the adult human skeleton, continuous physiological remodelling of bone is exclusively dependent on bone resorption. In several human diseases (e.g. malignant hypercalcemia and postmenopausal osteoporosis) enhanced bone resorption is the key pathophysiological event, and therapies for these diseases are currently based on its inhibition. In contrast, some rare genetic disorders are manifested as decreased resorption and lead to osteopetrosis.

Osteoclasts are multinuclear cells derived from hematopoietic stem cells (Suda et al., 1992). Their differentiation pathway is common to that of macrophages and dendritic cells. Thus a promyeloid precursor can differentiate into either an osteoclast, a macrophage or a dendritic cell, depending on whether it is exposed to receptor activator of NF-κB ligand (RANKL; also called tumor necrosis factor-related activation-induced cytokine (TRANCE), osteoprotegerin ligand (OPGL) or osteoclast differentiation factor (ODF)) macrophage colony-stimulating factor (M-CSF) or granulocyte-macrophage colony-stimulating factor (GM-CSF), respectively (Kong et al., 1999; Nutt et al., 1999; Rolink et al., 1999; Suda et al., 1999). Simonet et al. (1997) found that several cells and tissues produce a soluble factor, osteoprotegerin (OPG), that strongly inhibits osteoclast formation in vitro and in vivo. More recently, several groups demonstrated that bone marrow stromal cells and osteoblasts produce membrane bound and soluble RANKL/TRANCE/OPGL/ODF, an important positive regulator of osteoclast formation (Lacey et al., 1998). The inhibitory effect of OPG on the osteoclast differentiation is due to the fact that it can prevent the binding of RANKL to its receptor, RANK (Hsu et al., 1999). Major breakthroughs in osteoclast differentiation have thus been made, and some excellent reviews on this topic has been written recently (Suda et al., 1999; Roodman, 1999). Here, we do not discuss any further details of the osteoclast differentiation and its regulation but encourage readers to familiarise themselves with the above-mentioned articles.

Osteoclasts have developed efficient and unique machinery for dissolving mineral and degrading organic bone matrix. Our understanding of the cellular and molecular processes that are utilized by these professional hard-tissue destroyers has also significantly increased during the past decade. Here, we discuss the recent progress in the understanding of the cell biology of osteoclasts function and activation. Furthermore, we suggest that these cells represent good model systems for addressing some general questions in cell biology.

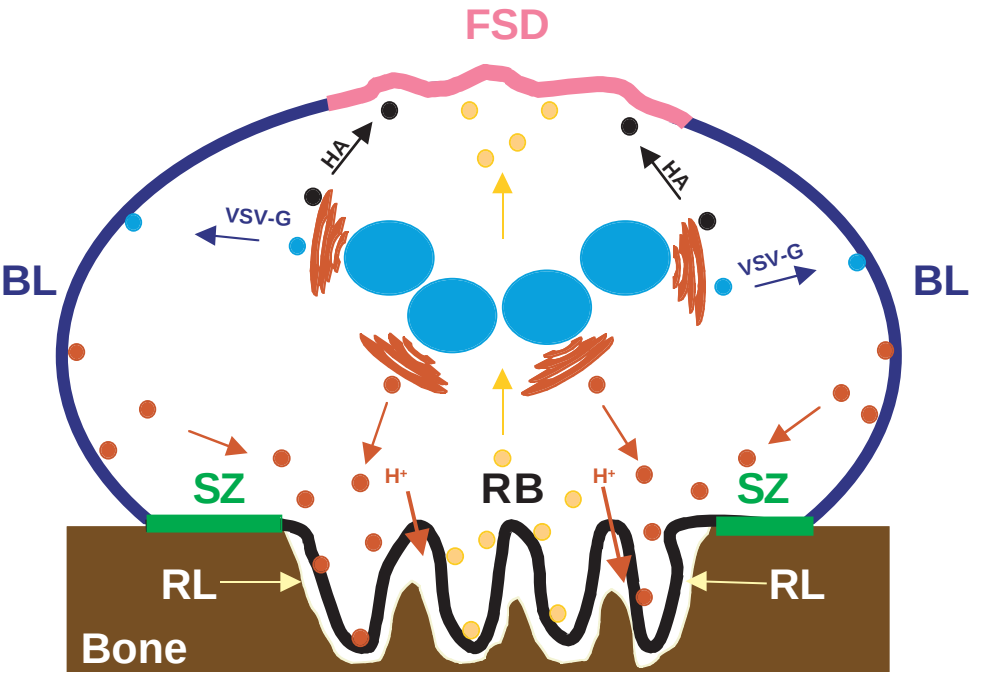

Fig. 1. Schematic view of a bone-resorbing osteoclast. Extensive vesicular trafficking involving several specific membrane domains is a hallmark of actively resorbing cells. BL, basolateral domain (blue); FSD, functional secretory domain (red); SZ, sealing zone (green); RB, ruffled border (black). Brown vesicles illustrate vesicular pathways from the trans-Golgi network and the basolateral membrane to RB, and yellow vesicles illustrate the transcytotic route from the RB to the FSD. Vesicular pathways from the trans-Golgi network to the apical (black vesicles) and basolateral (blue vesicles) membrane domains are shown (for references see text). HA, haemagglutinin; VSV-G, vesicular stomatitis virus G protein. RL, resorption lacuna (white).

RESORPTION CYCLE

The development of an in vitro bone resorption model using isolated primary osteoclasts and mineralized bone or dentin matrix as a substrate almost twenty years ago provided an excellent system for detailed cell biological studies of bone resorption (Boyde et al., 1984; Chambers et al., 1984). Although this model has several limitations in attempts to study the whole physiological cascade of bone resorption, it provides an excellent tool for detailed studies of the cellular mechanisms involved in the destruction of mineralized bone matrix. The sequence of cellular events needed for bone resorption is called the resorption cycle (see Väänänen, 1996). Resorption requires cellular activities: migration of the osteoclast to the resorption site, its attachment to bone, polarization and formation of new membrane domains, dissolution of hydroxyapatite, degradation of organic matrix, removal of degradation products from the resorption lacuna, and finally either apoptosis of the osteoclasts or their return to the non-resorbing stage. The term resorption cycle covers neither the differentiation pathway nor the cellular activities needed for the fusion of mononuclear precursors to form the multinuclear mature osteoclast. It should not be mistaken for the more widely used term remodelling cycle, which is used to describe the bone remodelling at the tissue level that involves the activities of several different cell types.

OSTEOCLASTS ATTACH TO BONE MATRIX THROUGH THE SEALING ZONE

After migration of the osteoclast to a resorption site, a specific membrane domain, the sealing zone, forms under the osteoclast. The plasma membrane attaches tightly to the bone matrix and seals the resorption site from its surroundings (Väänänen and Horton, 1995; see Fig. 1). The molecular interactions between the plasma membrane and the bone matrix at the sealing zone are still unknown. Several lines of evidence have shown, however, that integrins play an important role in early phases of the resorption cycle. At least four different integrins are expressed in osteoclasts: αvβ3, αvβ5, α2β1 and αvβ1 (Nesbitt et al., 1993). The role of αvβ3 has received much attention, because antibodies against αvβ3, as well as RGD-containing peptides such as echistatin and kistrin, are effective inhibitors of bone resorption both in vitro and in vivo (Horton et al., 1991; Lakkakorpi et al., 1991; Fisher et al., 1993). αvβ3 is highly expressed in osteoclasts and is found both at the plasma membrane and in various intracellular vacuoles. However, the precise function(s) of αvβ3 in resorbing osteoclasts remains unknown; the integrin could play a role both in adhesion and migration of osteoclasts and in endocytosis of resorption products. The latter possibility is supported by the observation that high amounts of αvβ3 are present at the ruffled border and by recent data from receptor-binding assays showing that denatured type I collagen has a high affinity for αvβ3 (Helfrich et al., 1996).

Some authors have suggested that αvβ3 integrin also mediates the attachment of the sealing zone to the bone matrix (Reinholt et al., 1990; Nakamura et al., 1996; Holt and Marchall, 1998). However, early in vitro and in vivo studies, although clearly demonstrating the functional importance of αvβ3 in bone resorption and its presence in the focal adhesions of migrating osteoclasts, failed to demonstrate that it is present at the sealing zone (Lakkakorpi et al., 1991). In addition, studies with labelled echistatin, which is a much smaller molecule than immunoglobulins and should not have any difficulty penetrating to its ligand failed to localise vitronectin receptors at the sealing zone (Masarachia et al., 1998). Another recent study has illustrated that the sealing zone might have common features with the epithelial zonula-adherens-type junction, given that tight sealing could be prevented by a hexapeptide containing the cell adhesion recognition sequence of cadherins (Ilvesaro et al., 1998). In addition, pan-cadherin antibodies recognised sealing

zone membrane, which suggests that some member(s) of the cadherin family are important for the tight attachment of osteoclasts to the bone matrix. However, the specific molecules responsible for the cell-extracellular matrix junction at the sealing zone of resorbing osteoclasts remain to be elucidated.

RESORBING OSTEOCLASTS ARE POLARIZED

Previous ultrastructural studies indicated that resorbing osteoclasts (in contrast to non-resorbing osteoclasts) are highly polarized cells (Fig. 1). Current data suggest that resorbing osteoclasts contain not only the sealing zone but also at least three other specialized membrane domains: a ruffled border, a functional secretory domain and a basolateral membrane.

As the osteoclast prepares to resorb bone, it attaches to the bone matrix through the sealing zone and forms another specific membrane domain, the ruffled border. The ruffled border is a resorbing organelle, and it is formed by fusion of intracellular acidic vesicles with the region of plasma membrane facing the bone (Blair et al., 1989; Väänänen et al., 1990). During this fusion process much internal membrane is transferred, and forms long, finger-like projections that penetrate the bone matrix. The characteristics of the ruffled border do not match those of any other plasma membrane domain described. Although facing the extracellular matrix, it has several features that are typical of late endosomal membranes. Several late endosomal markers, such as Rab7, V-type H-ATPase and lgp110, but not lgp120, are densely concentrated at the ruffled border (Palokangas et al., 1997).

We have shown that after infection of resorbing osteoclasts with different viruses, neither apically nor basolaterally targeted viral proteins are found at the ruffled border. Instead, VSV G-protein and hemagglutinin of influenza A, as well as receptors for both viruses, associate with the non-bone-facing surface (Salo et al., 1996). Interestingly, these experiments also revealed distinct and complementary distributions of viral proteins at the basal membrane of the resorbing osteoclasts. Hemagglutinin of influenza A was targeted to the central area of the basolateral domain, and VSV G-protein was targeted exclusively to the peripheral membrane areas (see Fig. 1). These results suggest that the basolateral domain of the resorbing osteoclast is divided into two distinct domains and that the centrally located domain is an equivalent to the apical membrane of epithelial cells (Simons et al., 1992). So far, no evident structural barrier, such as that present in the epithelial cells and neurones, between these two plasma membrane domains has been demonstrated. Thus, on the basis of earlier morphological studies, it was concluded that the basal membrane represents homogeneous membrane area. The presence of the apical domain (also known as the functional secretory domain) in this unexpected site raises the question of its functional significance. More-recent results from two different laboratories indicate that this novel membrane domain might function as a site for exocytosis of resorbed and transcytosed matrix-degradation products (Nesbitt and Horton, 1997; Salo et al., 1997).

BONE MATRIX IS DEGRADED IN THE RESORPTION LACUNA

The main physiological function of osteoclasts is to degrade mineralized bone matrix. This involves dissolution of crystalline hydroxyapatite and proteolytic cleavage of the organic matrix, which is rich in collagen. Before proteolytic enzymes can reach and degrade collageneous bone matrix, tightly packed hydroxyapatite crystals must be dissolved. It is now generally accepted that the dissolution of mineral occurs by targeted secretion of HCl through the ruffled border into the resorption lacuna (Blair et al., 1989; Väänänen et al., 1990). This is an extracellular space between the ruffled border membrane and the bone matrix, and is sealed from the extracellular fluid by the sealing zone.

The low pH in the resorption lacuna is achieved by the action of ATP-consuming vacuolar proton pumps both at the ruffled border membrane and in intracellular vacuoles. Acridine Orange-accumulation experiments have revealed that acidic extracellular compartments lie beneath the resorbing cells (Baron et al., 1985) and also that there is a high density of acidic intracellular compartments inside non-resorbing osteoclasts (Palokangas et al., 1997). Concomitant with the appearance of the ruffled border, the number of intracellular acidic compartments promptly decreases as the vesicles containing proton pumps are transported to the ruffled border. Although direct kinetic evidence is still lacking, because it is extremely difficult to obtain, it seems obvious that the resorption lacuna is further acidified by direct secretion of protons through the ruffled border. The osteoclast proton pump is sensitive to bafilomycin A1, which also effectively inhibits bone resorption both in vitro (Sundquist et al., 1990) and in vivo (Sundquist and Marks, 1994). The recent finding that vacuolar ATPase at the ruffled border contains cell-specific subunits has further encouraged development of resorption inhibitors that inhibit the osteoclast proton pump (van Hille et al., 1995; Hernando et al., 1995; Li et al., 1996). Protons for the proton pump are produced by cytoplasmic carbonic anhydrase II, high levels of which are synthesized in osteoclasts (Gay and Mueller, 1974). Excess cytoplasmic bicarbonate is removed via the chloride-bicarbonate exchanger located in the basolateral membrane (Hall and Chambers, 1989). Correspondingly, there is a high number of chloride channels in the ruffled border, which allows a flow of chloride anions into the resorption lacuna to maintain electroneutrality (Schlesinger et al., 1997).

After solubilization of the mineral phase, several proteolytic enzymes degrade the organic bone matrix, although the detailed sequence of events at the resorption lacuna is still obscure. Two major classes of proteolytic enzymes, lysosomal cysteine proteinases and matrix metalloproteinases (MMPs), have been studied most extensively. The high levels both of expression of MMP-9 (gelatinase B) and cathepsin K and of their secretion into the resorption lacuna suggest that these enzymes play a central role in the resorption process (Drake et al., 1996; Tezuka et al., 1994; Wucherpfennig et al., 1994). The role of cathepsin K is further supported by the fact that patients who possess a mutation in the gene that encodes cathepsin K display pycnodystosis (Johnson et al., 1996). In contrast, MMP-9-knockout mice have only transient disturbances of bone resorption (Vu et al., 1998).

DEGRADATION PRODUCTS ARE REMOVED BY TRANSCYTOSIS

After matrix degradation, the degradation products are removed

from the resorption lacuna through a transcytotic vesicular pathway from the ruffled border to the functional secretory domain, where they are liberated into the extracellular space (Nesbitt and Horton, 1997; Salo et al., 1997). Quantitative data are still missing, but clearly large amounts of degraded extracellular material must be transported through the resorbing cell, given that the volume of the resorption pit can easily exceed the volume of the entire cell. The extent to which the degradation of collagen and other matrix components is extracellular and the extent to which this takes place in intracellular transcytotic compartments are not known. Recent results have suggested that tartrate-resistant acid phosphatase (TRAP), a widely used osteoclast marker, is localised in the transcytotic vesicles of resorbing osteoclasts, and that it can generate highly destructive reactive oxygen species able to destroy collagen (Halleen et al., 1999). This activity, together with the co-localisation of TRAP and collagen fragments in transcytotic vesicles, suggests that TRAP functions in further destruction of matrix-degradation products in the transcytotic vesicles. The observed mild osteopetrosis in TRAP-knockout mice supports this hypothesis (Hayman et al., 1996).

The transcytotic pathway may offer an interesting model for studies of the vesicular route from late endosomal compartments to the cell surface. A similar transport route also operates during antigen processing in antigen-presenting cells, such as macrophages. Macrophages and osteoclasts have a common differentiation pathway, which further suggests that the transcytotic pathway of osteoclasts is analogous to the antigen presentation pathway. Several interesting questions regarding the transcytotic route remain open: for example, what is the mechanism of endocytosis of partially degraded matrix components at the ruffled border membrane? The characteristics of transcytotic vesicles remain to be defined, as does the extent to which matrix degradation occurs intracellularly during transcytosis. Furthermore, the signalling events involved in the transcytosis remain to be clarified.

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