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Review

Osteoclast lineage and function

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ABSTRACT

Osteoclasts are members of the monocyte/macrophage lineage and are formed by cellular fusions from their mononuclear precursors. Their differentiation is regulated by a number of other cells and their products, especially by RANKL and M-CSF. The resorbing osteoclasts are polarized and show specific plasma membrane domains. Polarization and bone resorption need a continuous membrane trafficking and modulation of the cytoskeleton. The most characteristic feature of osteoclasts is their unique capacity to dissolve crystalline hydroxyapatite by targeted secretion of HCl into the extracellular resorption lacuna. Organic matrix is degraded by enzymes like cathepsin K and the degradation products are transcytosed through the cell for secretion. Dissolution of hydroxyapatite releases large amounts of soluble calcium, phosphate and bicarbonate. Removal of these ions apparently involves the vesicular pathways and direct ion transport via different ion exchangers, channels and pumps. Detailed molecular knowledge of osteoclast differentiation and function has helped us to identify several target molecules and develop specific treatments to inhibit pathological bone resorption in various skeletal diseases.

Mammalian bone is under a continuous remodeling. Bone remodeling is needed to remove old bone and stress-induced microcracks in order to insure biomechanical stability and to regulate mineral homeostasis of the whole organism. Osteoclasts are cells that initiate bone remodeling and perform the actual removal of old bone matrix. Bone resorption involves both dissolution of bone mineral as well as degradation of organic bone matrix. Osteoclasts are highly specialized cells that can perform both of these functions, and many of the molecular mechanisms that are needed for resorption have recently been clarified.

An important role of osteoclasts has become evident also in various skeletal diseases, and specific inhibition of osteoclast function has become a major strategy to treat osteoporosis and many other metabolic bone diseases.

Osteoclasts are members of the monocyte/macrophage lineage and are formed by multiple cellular fusions from their mononuclear precursors. In a way they can be considered as terminally differentiated cells of this family since RANKL and M-CSF can induce their differentiation from myeloid precursors as well as from well differentiated tissue macrophages, and apparently also from various intermediate stages of the monocyte/macrophage lineage. However, until now we do not have firm evidence that multinucleated mature osteoclasts can be induced to a cellular fission and subsequent re-differentiation to other specific phenotypes of tissue macrophages. Fully differentiated human osteoclasts are large cells, approximately with five to eight nuclei and reveal also many other cell specific phenotypic features. The most characteristic functional feature of osteoclasts is their unique capacity to dissolve bone mineral, which is mainly crystalline hydroxyapatite. In order to finalize bone resorption after mineral dissolution they also perform an enzymatic degradation of organic bone matrix. In addition, they may have some, although not yet firmly established, regulatory functions towards other bone cells and cell lineages present in a bone marrow environment.

Although many of the molecular mechanisms responsible for osteoclast specific actions have already been clarified, many of them still remain uncharacterized. The differentiation and function of osteoclasts thus remains to be a fruitful area of research to develop new diagnostic and therapeutic tools for bone diseases. In this review we will first discuss the osteoclastic lineage and then, in more detail, the cellular and molecular mechanisms which osteoclasts are using to perform bone resorption.

Osteoclast lineage

It is now well established that osteoclasts are members of the monocyte/macrophage lineage and are formed via multiple cellular fusions from their mononuclear precursors. The early work of Walker et al. [1] in parabiotic animals indicated that circulating blood contains cells that have capacity to form resorbing osteoclasts in osteopetrotic animals. Later *in vitro* studies, especially from Burger et al. [2] with fetal bone rudiments and bone marrow-derived cells suggested that osteoclasts are derived from macrophage precursors of colony-forming unit-macrophage (CFU-M lineage, Fig. 1). Thus osteoclasts share a common haematopoietic

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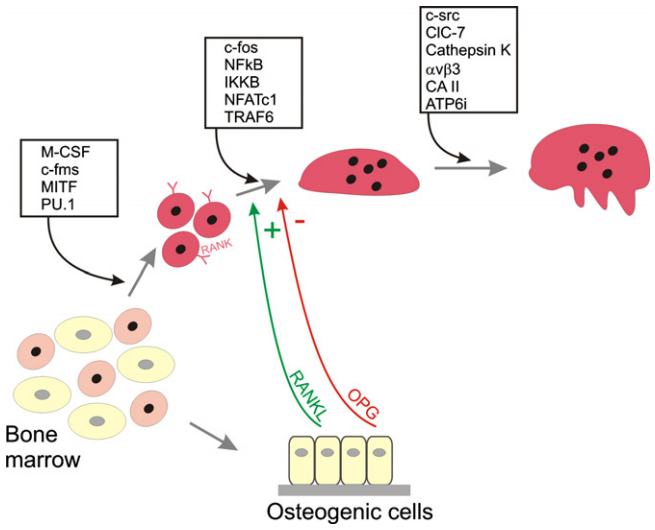

Fig. 1. Osteoclasts differentiate from myeloid stem cells via macrophage-colony-forming units (CFU-M). The exact point at which definitive commitment into osteoclasts along the differentiation pathway occurs is currently unknown. The mononuclear precursors fuse to form terminally differentiated multinuclear osteoclasts. For details and references see the text.

origin with antigen presenting dendritic cells and different tissue macrophages, like alveolar macrophages and Kupfer cells in the liver. It is not known yet, however, if all circulating monocytes are able to differentiate towards the osteoclast lineage, or if this capacity is present only in specific sub-populations of circulating monocytes. However, both an unfractioned monocyte population or various purified sub-populations can be induced to osteoclasts by ligand of receptor activator of nuclear factor-kappaB (RANKL) and macrophage-colony stimulating factor (M-CSF) (details; see elsewhere in this volume). Transgenic mice with a genetic mutation in one of the members of RANK/RANKL/OPG pathway have elegantly demonstrated the importance of these gene products for osteoclast differentiation and function [3–5]. A capacity of different monocyte sub-populations, isolated from the human peripheral blood (including CD14+, CD11b+, CD61+, CD15+ and CD169+ monocytes) to form bone-resorbing osteoclasts has been shown to be clearly different [6]. Interestingly, Ciraci et al. [7] recently demonstrated that the CD34+ human haematopoietic stem/progenitor cell line, MUTZ-3, differentiated to functional osteoclasts in the presence of RANKL and M-CSF. On the other hand, some studies have indicated that even well differentiated alveolar macrophages can be induced to fuse and form bone-resorbing osteoclasts [8]. These and other results suggest that RANKL and M-CSF could induce several intermediate stages of differentiation, from haematopoietic stem cells/precursors to tissue macrophages, to differentiate to osteoclasts. Details and properties of these different intermediate steps remains to be clarified, but even the present data confirm that the combination of RANKL and M-CSF have a remarkable power to direct haematopoietic cells to osteoclasts at different stages of their differentiation pathway. It should be mentioned, however, that not all multinucleated cells with a macrophage-like phenotype are able to resorb bone.

A typical phenotypic feature of osteoclasts is their multinuclearity and ability to resorb bone. While resorption is unique to mature osteoclasts multinuclearity is also a common phenomenon among other macrophage-type cells. Detailed discussion of the molecular mechanism of cellular fusion is beyond this review but from the point of view of osteoclast biology it is interesting that CD200 is expressed in osteoblasts and its receptor, CD200 receptor, is expressed in macrophages and osteoclasts, suggesting that the CD200-CD200R axis could be a regulator of the formation of osteoclasts [9].

Normal human osteoclasts that are found at resorption sites usually contain from five to eight nuclei. Although it has been demonstrated *in vitro* that even mononuclear osteoclasts are able to resorb, the formation of large multinucleated cells is often seen when rapid and effective bone resorption is needed, for example in bones of egg-laying birds. We do not exactly know what is the mean age of osteoclast but there are a lot of indirect evidence to conclude that after starting the resorption they have a limited life-span and finally die via apoptosis.

Specific membrane domains and extensive vesicular trafficking are hallmarks of resorbing osteoclasts

In histological sections from normal mammalian bone one can very seldom see multinucleated osteoclasts that are not in the immediate vicinity of mineralized matrix. As a matter of fact most of them are attached to mineralized matrix and reveal clear morphological signs of cellular polarity. This suggests that osteoclasts spend most of their lifetime resorbing and both the preliminary stages as well as post-resorption time must be relatively short compared to the actual resorption stage. From *in vitro* time lapse video studies we know that osteoclast can form more than one resorption site but it is not known if this can occur also *in vivo*. *In vitro* bone resorption assay, where isolated osteoclasts are cultured on bone, has allowed a detailed analysis of the osteoclast behavior during the resorption cycle and revealed an extensive re-organization of the cytoskeleton and polarization of plasma membrane during different phases of the resorption cycle [10,11].

Non-resorbing osteoclasts do not reveal clear morphological or other signs of plasma membrane polarization. However, when osteoclasts are resorbing four distinct membrane domains can be detected both using morphological criteria as well as molecular markers [12]. Sealing zone membrane attaches the resorbing cell to the mineralized extracellular matrix and circulates the ruffled border membrane, which is the actual resorbing organ (Fig. 2). Basolateral or non-bone facing plasma membrane is also divided into two distinct domains since a specific membrane domain, called functional secretory domain (FSD), is formed in the center of the basolateral membrane [13].

The extensive membrane polarization is tightly linked to the re-organization of microfilaments and microtubules and is also associated to new targeting of vesicular trafficking pathways [14]. When cultured osteoclasts migrate on bone surface they do not show distinct plasma membrane polarization other than formation of integrin mediated focal adhesions in bone facing plasma membrane. In the beginning of the resorption cycle one can see a dense accumulation of podosomal focal adhesions with a simultaneous new arrangements of actin microfilaments [15]. It is obvious now that sealing zone formation is not only a simple accumulation of podosomes to form a dense belt of actin called actin ring, but also includes other spatial re-arrangements of actin microfilaments [16–18]. Luxenburg et al. [17] demonstrated recently that a lot of new actin is recruited and a new type of microfilament arrangement is seen, namely fibers that interconnect directly different podosomal actin cores. Thus the sealing zone is clearly not only a simple structure of clustered podosomes, but in addition of high density of podosomes, reveals a high degree of F-actin mediated inter-connectivity between podosomal structures. The re-organization of actin microfilaments is obviously connected to changes in activity of actin associated proteins and involves a lot integrin mediated signaling events (see Teti et al. in this issue).

The role of avβ3-integrin in the formation of sealing zone has been debated a lot during recent years. On the basis of our light