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Osteoclast function and bone-resorbing activity: An overview

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ABSTRACT

Bone resorption is an important cellular function in skeletal development and remodeling of the adult skeleton. Most of the pathological bone disease conditions like osteoporosis reflect increased osteoclast activity; hence, increased bone resorption. Researchers have unraveled most of the intracellular mechanisms responsible for osteoclast bone-resorbing activity in last few decades. Therefore, understanding the fundamentals of osteoclast-induced bone resorption and the cytokines and other substances that modulate the osteoclast activity unequivocally provide insights into the development of drugs to ameliorate pathological bone diseases with enhanced bone resorption. The aim of this review is to examine the literature on osteoclast function and bone-resorbing activity.

1. Introduction

Osteoclasts (OCs) are the exclusive bone resorbing cells of hematopoietic origin. Osteoclastic bone resorption is indispensable during the development of bone (modeling) and in reshaping or replacement of bone during growth (remodeling) following injuries. Excessive OC activity, without a doubt, will give rise to enhanced bone resorption and osteopenia, whereas reduce activity will lead to imbalance in remodeling, favoring osteopetrosis. The importance of OC activity is clearly apparent in c-Src-deficient mice who developed severe osteopetrosis despite an abundance of OCs [1]. Therefore, the OC activity is as important as OC formation and differentiation. The significance of OC activity is further confirmed in mice lacking the tyrosine kinase, Syk. The skeletal phenotype of Syk deficiency shows impaired activity of the mature OCs but not differentiation [2]. Therefore, this review is an attempt to briefly look into the published literature on osteoclast function and bone-resorbing activity.

2. Osteoclast attachment, bone resorption and vesicular trafficking

Activity or the function of OCs is defined by varied morphological and functional characteristics. OC activation is initiated with matrix recognition, adhesion to the bone surface followed by cytoskeletal rearrangement. The cytoskeleton of the osteoclast is unique that it has the ability to polarize the OC onto the bone-cell interface to form a ruffled border with an isolated milieu of resorptive microenvironment and forms a gasket-like structure known as the actin ring or sealing zone. The sealing zone segregates the resorptive microenvironment from the general extracellular space [3]. In the cytosol, OCs synthesize lytic enzymes and secrete them to acidify the lacunae created underneath the OCs by exocytosis (Fig. 1). And then OCs internalize the degraded products by endocytosis. This vectorial trafficking of exocytic and endocytic vesicles is necessary for the effective bone resorption by OCs.

Bone resorption by OCs involves multi-step processes including adherence to bone, cytoskeletal reorganization, ruffled border formation, etc. Upon contact with the bone, the cytoskeleton of the OCs changes and cells become polarized under the influence of Rho family of regulatory GTPases [4]. The OCs form a distinct apical domain and a basolateral membrane domain. Importantly, the reorganization results in the formation of 'functional secretory domain' opposite the contact surface of the bone (Fig. 1). At the same time, cytosol-residing acidified vesicles are transported to the apical membrane to form the ruffled border, the main resorptive organelle of the OCs on the bone-apposing membrane. The formation of the ruffled border increases the surface area of OCs in contact with the bone.

SNARE proteins and Rab GTPases are also implicated in the vesicular trafficking in exocytosis. Whereas Rab3A and Rab3C are known to negatively regulate exocytosis, Rab3B and Rab3D do so positively. Attesting to this, disruption of Rab3D activity by using

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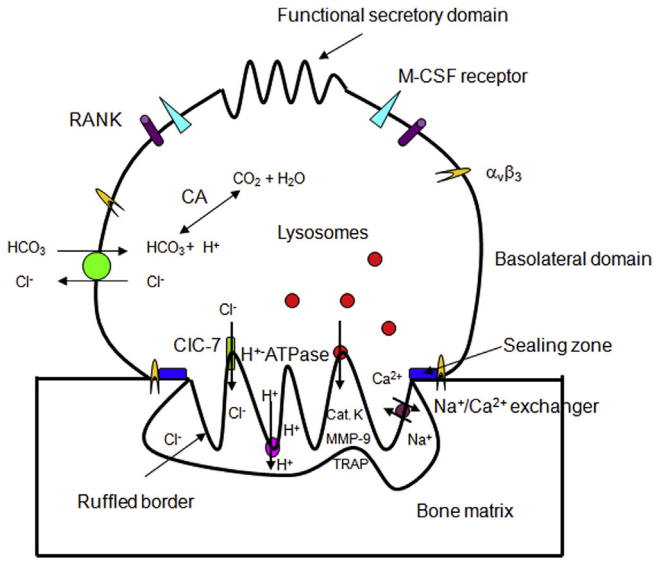

Fig. 1. Schematic view of a bone resorbing osteoclast. OCs acidify the extracellular vacuole by secreting protons by electrogenic H^+-transporting adenosine triphosphatase (v-ATPase) which transports protons provided by the enzyme carbonic anhydrase (CA). Na^+/Ca^{2+} exchanger exchanges extracellular Na^+s for intracellular Ca^{2+} during bone resorption. Intracellular alkalization is prevented by extrusion of Cl^- through ClC-7 chloride channel and chloride-bicarbonate exchanges at the basolateral membrane. Collagen is degraded by proteases released by lysosomes such as cathepsin K and metalloproteases (eg.MMP-9) including collagenase and gelatinase.

dominant-negative Rab3D and by targeted inactivation of expression of Rab3D have been shown to significantly retard the bone resorbing activity of OCs *in vitro* and *in vivo* [5]. In accord with the above observations, ultra-structural analysis reveals irregular ruffled borders in Rab3D$^{-/-}$ OCs. The lysosomal protein, synaptotagmin VII (Syt VII) is found in OCs in association with the protease cathepsin K and lysosome-associated membrane protein-2 (LAMP2) in lysosomes. Syt VII synchronizes OC function by regulating the secretion of cathepsin K in lysosomes. Furthermore, Syt VII along with SNARE proteins play a vital role in controlling the merging of lysosomes with the ruffled border membrane by bringing the two membranes together. Furthermore Syt VII$^{-/-}$ OCs fail to generate ruffled borders or resorb bone showing the vital role of Syt VII in ruffled border formation [6].

Autophagocytosis or autophagy is important for the degradation of dysfunctional cytoplasmic constituents in the autophagosome by binding to a lysosome. The role of autophagocytosis in ruffled border formation is unraveled very recently [7]. It has been shown that autophagy proteins Atg5, Atg7, and LC3 partnering with Rab7 are involved in ruffled border formation suggesting that autophagy proteins play a vital role in vectorial secretion of lysosomal constituents including cathepsin K.

OCs form a sealing zone consisting of certain contractile proteins with the aid of integrins such as $\alpha_v\beta_3$ surrounding the ruffled border. Sealing zone which is rich in filamentous actin (thus called the actin ring) allows OCs to attach to the bone while ensuring their continued ability to migrate. The Sealing zone separates the resorptive microenvironment/lacunae from the general extracellular space [8] and protects the adjacent cells from the acidic environment as the ruffled border resides within the sealing zone.

Transmission of extracellular signals by the integrins results in migration of acidifying vesicles containing proton pumps towards the bone-apposing surface. OCs acidify the extracellular vacuole, the area between the ruffled border and the bone lining by secreting protons with the help of vacuolar electrogenic H + -transporting adenosine triphosphatase (v-ATPase). This enzymatic pump transports protons into resorptive lacunae. Carbonic anhydrase (CA) forms protons (H^+) and HCO_3 from CO_2 and H_2O. The Na^+/Ca^{2+} exchanger NCX-1 in the OCs allow the electrogenic exchange of three extracellular Na^+s for one intracellular Ca^{2+} during bone resorption. To prevent the intracellular alkalization and thus hyperpolarization, the proton secretion is counterbalanced by the extrusion of Cl^- through the ClC-7 chloride channel and chloride-bicarbonate exchange at basolateral membrane. Thus, the generation of HCl in the isolated milieu creates a pH of 4.5. This results in the degradation of mineralized components thereby uncovering the organic matrix which consists mainly of type I collagen, to be degraded by proteases such as Cathepsin K. Furthermore, the OCs secrete several metalloproteases, including collagenase and gelatinase. The significance of the aforementioned key enzymes in the acidification process was observed in mice and humans where the mutations of V-ATPase [9], the ClC-7 chloride channel [10] and CA [11] cause osteopetrosis while loss of function of Cathepsin K leads to pyknodysostosiss, an autosomal recessive osteochondrodysplasia [12,13].

There is ample evidence to imply that during active resorption some of the extracellularly degraded products are endocytosed, and again degraded in secondary lysosomes while some of which are transcytosed and secreted via the secretory domain of the basolateral membrane into the OC microenvironment and paratrabecular sinusoids in the bone. This transcytotic route allows the OCs to excavate deep resorption channels into the compact bone and to remove large amounts of matrix-degradation products without losing their tight attachment to the bone. It also facilitates further processing of degradation material intracellularly while they move across the cells [8]. The transcytotic pathway is supported with thick bundles of microtubules which originate from the ruffled border and extend towards the secretory domain of the polarized osteoclasts. In addition, the degraded products might be secreted into the local environment during periods of relapse of the sealing zone, possibly induced by a calcium sensor that responds to the rise of extracellular Ca^{2+} in the resorptive milieu [14]. The functional cycle of OCs consists of episodes of attachment and bone resorption followed by detachment and movement to a new site for bone degradation [8]. Although the process of bone resorption is reasonably understood, less is known about the signals that arrest it. Sensing of high Ca^{2+} levels within the resorbed milieu by plasma membrane receptors could prompt the withdrawal of the OCs from the bone surface terminating their bone resorption activity which results in motile changes in the cells.

3. Podosomes

The sealing zone is composed of tightly packed dot-like attachment structures called podosomes. Podosomes bring about motility and bone resorption functions of the OCs. Podosomes have a core structure and are able to organize into large patterns in the OCs. The dynamic nature of the podosomes results in half-life turnover of these organelles every 2–12 min. The podosomal core consists of F-actin co-localized with actin-associated proteins such as α–actinin, fimbrin, cortactin, gelsolin (GSN)) and the actin-regulatory protein Wiskott-Aldrich syndrome protein (WASP), neuronl WASP (N-WASP) and Arp2/3. The core is surrounded by a ring structure of integrins and integrin-associated proteins including paxillin, talin and vinculin (Fig. 2). The core and ring are connected by bridging molecules including α-actinin, and both the core and the ring are surrounded by monomeric actin molecules [15]. Therefore, proteins that regulate actin assembly and dissembly are likely to play critical functional roles in the OCs by regulating podosome turnover [14].

Recently it has been demonstrated that conditional deletion of