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Review

Vesicular trafficking in osteoclasts

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ABSTRACT

Bone-resorbing osteoclasts are highly dependent on vesicular trafficking pathways that are regulated by Rab GTPases. In particular, polarised transport of acidic vesicles of the endocytic/lysosomal pathway is required for formation of the ruffled border, the resorptive organelle of the osteoclast. The breakdown products of resorption are then transported through the osteoclast by transcytosis, enabling their excretion. In this review, we summarise these trafficking routes, highlight the emerging evidence that the bone disease osteopetrosis results from defects in vesicular trafficking in osteoclasts, and outline the similarities between the endocytic/lysosomal compartment in osteoclasts and secretory lysosomes in other cell types.

Contents

1. Membrane domains in osteoclasts

Osteoclasts are large, multinucleated, specialised bone-resorbing cells that form as a result of the fusion of mononuclear precursors from the haematopoietic lineage. When activated to resorb, these cells become polarised and reorganise their membrane into four distinct and unique membrane domains that are essential for this function to be carried out, namely the sealing zone (SZ), the ruffled border (RB), the basolateral domain (BD) and the functional secretory domain (FSD) [1] (Fig. 1). The first step in polarisation involves the rearrangement of the actin cytoskeleton to form an F-actin-rich ring, which forms the SZ that mediates the tight attachment of the osteoclast to the extracellular matrix. The SZ isolates an area of membrane that subsequently develops into the RB, the site where bone resorption actually takes place.

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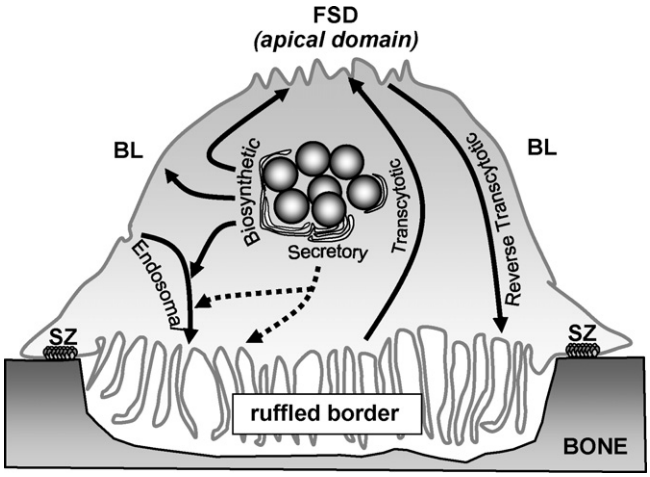

Fig. 1. Vesicular trafficking pathways in osteoclasts. The ruffled border is formed as a consequence of trafficking of vesicles in the endosomal pathway, and therefore has characteristics of a late endosomal membrane. A distinct biosynthetic pathway may also contribute to the formation of the ruffled border. Degradation products from the resorption process are removed from the resorption lacuna by a transcytotic pathway and released at the FSD, which exhibits characteristics of an apical membrane. Recently, a reverse pathway from the FSD to the ruffled border has been identified. FSD, functional secretory domain; BL, basolateral domain; SZ, sealing zone.

This is a highly convoluted membrane that forms as a result of vectorial vesicular transport, which also serves to deliver the proteins involved in the resorption process. These are principally the vacuolar type H^+-ATPase (V-ATPase), which acidifies the resorption space beneath the RB, enabling dissolution of the mineral component of bone, and cathepsin K, which degrades collagen, the predominant organic component of bone. The SZ and the RB constitute the membrane that is directly apposed to the bone surface. The plasma membrane facing away from the bone surface is also composed of two separate domains, which have similarities to the basolateral and apical domains seen in other polarised cells such as epithelial cells. This has been elegantly demonstrated by studies of the trafficking of viral proteins in osteoclasts [2]. Vesicular stomatitis virus G-protein (VSV-G), which is delivered to the basolateral membrane in polarised epithelial cells, localises only to the lateral part of the basolateral surface in resorbing osteoclasts. By contrast, influenza haemagglutinin, which is delivered to the apical membrane in epithelial cells, is trafficked exclusively to the central region of the basolateral surface, indicating that this specific domain represents the functional equivalent of the epithelial cell apical domain. This membrane domain also possesses microvilli, clearly distinguishing it from the smooth peripheral basolateral membrane [1,3], and based on its known function in the release of bone resorption products, it is now commonly referred to as the functional secretory domain (FSD) [2].

Surprisingly, neither VSV-G nor influenza haemagglutinin localise to the RB, indicating that this membrane is unlike conventional plasma membranes. In support of this, it was found that the RB accumulated proteins associated with the endosomal/lysosomal pathway, such as LAMP1, LAMP2 and lgp110 [4], and indeed the V-ATPase [5], indicating this membrane is more akin to an intracellular lysosomal membrane than a plasma membrane [2]. By contrast, markers of early endosomes, such as EEA1, are absent from the RB, and are restricted to vesicles within the cytoplasm of osteoclasts [6].

The maintenance of these membrane domains is dependent on tightly regulated vesicular transport processes (Fig. 1), which will now be discussed in more detail below with respect to the two most important functional domains, the ruffled border and the FSD.

2. Vesicular trafficking to the ruffled border

The RB is the resorptive organelle of the osteoclast. The enormous surface area of this membrane is clearly indicative of extensive vesicular trafficking and fusion, and provides a large surface for the release of acid and proteases into the resorption lacuna. As outlined above, trafficking of late endosomes/lysosomes is crucial for formation of the RB and for osteoclastic resorption. In this respect, osteoclasts closely resemble other cell types that contain "secretory lysosomes" (also often referred to as lysosome-related organelles), such as melanocytes and platelets (see Section 8). In osteoclasts, as in other cell types, late endosomes and lysosomes are acidified by the action of a V-ATPase [7], which pumps protons generated by carbonic anhydrase II into the lumen of the vesicle. In parallel, the chloride channel ClC-7, which is highly expressed in osteoclasts, transports chloride ions into the lumen as a counter ion to maintain electroneutrality of the vesicles [8]. Delivery of the late endosomes/lysosomes to the RB membrane therefore serves to directly acidify the resorption lacuna, as well as to insert the V-ATPase and ClC-7 into this membrane, enabling further acidification of the extracellular compartment (Fig. 2). Cathepsin K is also most likely delivered to the resorption lacuna via endosomes/lysosomes [9]. This cysteine protease is specifically expressed in osteoclasts and is most active at degrading type I collagen, the major organic component of bone, under the acidic conditions of the resorption lacuna [10]. MMP-9 may also play a role in bone resorption, although its importance appears to be restricted to osteoclasts at specific skeletal sites [11].

The origin of the endocytic vesicles responsible for formation and maintenance of the RB appears to be the basolateral membrane of the osteoclast, since numerous endocytic markers, such as HRP and iron-loaded transferrin, have been shown to be internalised and delivered to the RB via this route [12]. This trafficking is dependent on microtubules [13], although the vesicles are probably transferred to actin microfilaments prior to fusion [12], which occurs at the peripheral region of the RB close to the SZ, where V-ATPase and cathepsin K are localised [12]. It is likely that PI3K also plays an important role in this fusion process, since treatment of cells with the PI3K inhibitor wortmannin causes the accumulation of V-ATPase-expressing vesicles adjacent to the rudimentary RB [14].

It is clear that vesicular transport from the biosynthetic pathway is also necessary for the formation of the RB [4], since blockade of this pathway disrupts this membrane domain [15,16]. This is not surprising, given that this route is essential for the delivery of newly synthesised proteins to the lysosomal compartment, and indeed essential for the biogenesis of lysosomes [17]. However, it remains unclear whether there is a distinct biosynthetic vesicular trafficking route to the RB that is completely independent of the endosomal/lysosomal pathway (Fig. 1).

Tartrate-resistant acid phosphatase (TRAP) is a secreted enzyme that is highly and selectively expressed in osteoclasts, and as a result is routinely used as a marker of osteoclasts *in vitro*, and of bone resorption *in vivo*. Although the precise function of this phosphatase remains unclear, it is known to localise to the lumen of intracellular vesicles and there is some evidence indicating that it is released into the resorption space at the RB [18–20]. Interestingly however, other studies indicate that TRAP does not colocalise with endosomal markers [21] or acidic vesicles in osteoclasts [22], suggesting that it is a component of a vesicular population distinct from late endosomes/lysosomes, most likely transcytotic vesicles [21] (see Section 3 below).