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Review

New functions of lysosomes in bone cells

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ABSTRACT

Background: Lysosomes are intracellular acidic organelles that contain approximately 50 hydrolases and 25 species of integral membrane proteins. Although lysosome-like specific compartments, termed lysosome-related organelles (LROs), are found in osteoclasts, their functions in these cells and lysosomal functions in osteoblasts remain to be elucidated.

Highlight: Recently, we found that expression of RAB27A is markedly increased during osteoclastic differentiation. RAB27A deficiency causes multinucleation and giant cell formation, characterized by abnormal transport of cell surface receptors and LROs into osteoclasts. Furthermore, we have shown that transcription factor EB (TFEB), a master regulator of lysosomal biogenesis, regulates osteoblastic differentiation. Overexpression of TFEB in preosteoblastic MC3T3-E1 cells enhances osteoblastic differentiation via decreased expression of activating transcription factor 4 (ATF4) and CCAAT/enhancer-binding protein homologous protein (CHOP). These results indicate that the expression of ATF4 and CHOP is essential for differentiation into osteoblasts.

Conclusion: RAB27A participates in bone resorption by LROs in osteoclasts. In addition, lysosomal biogenesis modulated by TFEB is necessary for osteoblastic differentiation.

Contents

1. Introduction

Lysosomes are acidic membrane-bound organelles that contain approximately 50 hydrolases, including proteases, glycosidases, lipases, nucleases, phospholipases, phosphatases, and sulfatases [1–3]. Lysosomes contain approximately 25 species of integral membrane proteins. The most enriched membrane proteins are lysosome-associated membrane protein 1 (LAMP1), LAMP2, lysosome integral membrane protein 1 (LIMP1), LIMP2, and CD63 [3]. Lysosomes continuously digest macromolecules via three major pathways: endocytosis, phagocytosis, and autophagy. Defective lysosomal functions cause neurodegenerative diseases, immune-system-related diseases, infectious diseases, cancer, cell differentiation, and so-called lysosomal storage diseases [4,5]. Although lysosomes are present ubiquitously in mammalian cells, there are cell type-specific compartments, termed lysosome-related

Abbreviations: (BMM), bone marrow macrophage; (LROs), lysosome-related organelles; (M-CSF), macrophage-colony stimulating factor; (siRNA), small interfering RNA; (V-ATPase), vacuolar-type H^+-ATPase.

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organelles (LROs), which are roughly similar to lysosomes, but morphologically and functionally different from them [6,7]. Examples of LROs include melanosomes in melanocytes [8], lytic granules in lymphocytes [9,10], and delta granules in platelets [11]. In addition to these examples, osteoclasts contain LROs surrounded by the ruffled-border membrane [12,13]. So far, studies on LRO functions in osteoclasts have been scarce as compared to studies on LRO functions in other cell types. Moreover, the function of lysosomes in osteoblasts remains unclear. In this review, we compare and discuss lysosomal functions in osteoclasts and osteoblasts.

2. LROs in osteoclasts

Osteoclasts are the bone-resorbing cells that function using LROs [6,7,14]. Osteoclastic LROs contain certain specific components, the most abundant of which are cathepsin K and tartrate-resistant acid phosphatase (TRAP). Cathepsin K was initially identified as a lysosomal cysteine protease that is predominantly expressed in osteoclasts [15]. Because cathepsin K has a powerful collagenolytic activity, it is predominantly involved in the degradation of extracellular-matrix components such as collagens, lamin, tenascin, and osteonectin [16,17]. Cathepsin K-deficient mice show osteopetrosis characterized by reduced osteoclastic bone resorption [18]. Mutations in human cathepsin K cause an autosomal recessive disease termed pycnodysostosis, a specific type of osteopetrosis, characterized by osteosclerosis with increased bone fragility [19,20]. TRAP (also known as ACP5) is a metalloenzyme that is abundant in osteoclasts although this enzyme is also expressed in some organs and tissues such as the spleen, liver, gastrointestinal tract lining, lung, thymus, and skin [21]. TRAP is also useful as a biomarker of bone resorption; its serum levels are measured for the clinical diagnosis of metabolic and bone diseases [22,23]. TRAP-deficient mice show early onset of the osteopetrotic bone phenotype because their osteoclasts are less active in bone degradation [24].

In addition to these specific soluble enzymes, several lysosomal membrane proteins are known to be osteoclast specific. Vacuolar-type H^+-ATPases (V-ATPases), which help to maintain acidic pH of lysosomes, are proton pumps composed of 13 different subunits [25]. Among these, the a3 subunit is known to be osteoclast specific. Expression of a3 is approximately 100-fold greater in osteoclasts than in other cell types [26]. Indeed, mice deficient in this subunit have severe osteopetrosis [27]. In humans, mutation of the gene encoding the a3 subunit of V-ATPase results in autosomal recessive osteopetrosis caused by impaired osteoclast-mediated bone resorption [28]. The complex of voltage-gated chloride channel 7 (CLC7) and osteopetrosis-associated transmembrane protein 1 (OSTM1) has been reported as an osteoclast-specific protein complex [29]. CLC7 is a $2Cl^-/1\,H^+$ ion exchanger, and OSTM1 is a lysosomal protein important for the stability of the CLC7–OSTM1 complex [29]. Any defect in the gene of either CLC7 or OSTM1 causes severe malignant infantile osteopetrosis [30–32]. Thus, it is likely that all mutant animals deficient in osteoclast-specific lysosomal proteins have osteopetrosis.

Osteoclastic LROs have unique segregation systems for their proteins. Localization of several cathepsins is indeed distinct in osteoclasts, whereas it is similar among other cell types. Our immunocytochemical studies have suggested that cathepsins B, E, and K are present in the ruffled-border membrane and nearby vacuoles, whereas cathepsin D is localized to vacuoles and lysosomes that are detached from the ruffled-border membrane [33–36]. In agreement with our findings, osteoclasts from mice deficient in GlcNAc-1-phosphotransferase α/β-subunits [these animals have a defect in the mannose 6-phosphate (Man-6-P)-dependent pathway] show enhanced secretion of cathepsin K and TRAP but normal targeting of cathepsin D [37]. In addition to lysosomal enzymes, lysosomal membrane proteins are also segregated in osteoclasts. For example, V-ATPase is present along the actin ring of osteoclasts, whereas cathepsin K is localized to the center of the actin ring as a punctate structure [38]. These findings suggest that LROs in osteoclasts have specific segregation systems.

3. RAB27A and osteoclasts

To identify a gene that regulates membrane trafficking of LROs during osteoclastic differentiation, we have conducted a DNA microarray analysis. During the analysis, we observed a marked increase in the expression of RAB27A during osteoclastic differentiation [39]. RAB27A, a member of the RAB family of small GTPases, is exclusively localized to secretory granules in various secretory cells, including endocrine and exocrine cells and various leukocytes [40]. Mutation of RAB27A in humans causes type 2 Griscelli syndrome, which is characterized by hypopigmentation of the skin and eyes and immunodeficiency [9,41]. Nonetheless, the involvement of RAB27A in LROs of osteoclasts has yet to be elucidated. Therefore, we further analyzed the functions of RAB27A using a small interfering RNA (siRNA)-mediated knockdown in a murine macrophagic RAW-D cell line or bone marrow macrophages (BMMs) derived from *ashen* mice, which carry a mutation in Rab27a. Osteoclasts derived from either RAB27A knockdown RAW-D cells or *ashen* mice were found to show multinucleation and giant cell formation as key phenotypes when compared with control cells [39]. The *ashen* mouse osteoclasts showed abnormal actin ring formation, aberrant subcellular localization of LAMP2 and cathepsin K, and impaired resorption activity [39]. In addition to the lysosomal systems, trafficking of cell surface receptors was abnormal in the ashen mouse osteoclasts. The surface amount of c-fms, a macrophage-colony stimulating factor (M-CSF) receptor, was slightly greater in ashen mouse BMMs than in wild-type BMMs. Additionally, downregulation of receptor activator of nuclear factor κ B (RANK; a RANK ligand [RANKL] receptor) was delayed. In fact, although cellular RANK in wild-type BMMs was gradually degraded, RANK in ashen mouse BMMs was found to be upregulated [39]. As a result, upon stimulation with M-CSF and RANKL, phosphorylation levels of extracellular signal-regulated kinase (Erk), proto-oncogene tyrosine-protein kinase (Src), and p38 were slightly enhanced in ashen mouse BMMs in comparison with wild-type BMMs [39]. Thus, Rab27A deficiency causes abnormal transport of cell surface receptors thereby modulating multinucleation and LROs in osteoclasts. As shown in Fig. 1, RAB27A deficiency in osteoclasts causes abnormal transport of cell surface receptor called RANK, eventually enhancing signaling that drives osteoclastic differentiation. Nevertheless, RAB27A deficiency leads to impaired transport of LROs for bone resorption.

Given that the phenotype of RAB27A-deficient osteoclasts is unique, this phenotype is different from that of osteoclasts deficient for other RABs. For example, RAB3 is known to modulate exocytosis in secretory cells. Osteoclasts from mice deficient in Rab3D—the Rab3 isoform predominantly expressed in osteoclasts—show an abnormal ruffled-border membrane [42]. In addition, a knockdown of RAB7—a key regulator of biogenesis of late endosomes and lysosomes—disrupts polarization of ruffled-border formation and decreases bone resorption by osteoclasts [43]. Thus, our results indicate that RAB27A plays a unique role in membrane trafficking of osteoclasts as compared with the roles of other RAB proteins.

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