Review

Insight into the Critical Roles of Rab11 GTPase in Regulating Osteoclastogenesis through Modulating the Lysosome-Mediated Turnover of the Cell Surface Receptors

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Insight into the Critical Roles of Rab11 GTPase in Regulating Osteoclastogenesis through Modulating the Lysosome-Mediated Turnover of the Cell Surface Receptors

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Abstract: Osteoclasts (OCs) are the multinucleated, bone-resorbing giant cells originally differentiated from a monocyte/macrophage lineage. OC differentiation and maturation, also called osteoclastogenesis, are strictly regulated by a variety of signaling pathways primarily induced by the interactions of two prerequisite cytokines, macrophage colony-stimulating factor (M-CSF) and the receptor activator of nuclear factor-κB ligand (RANKL) to their respective surface receptors, c-fms and RANK, in OC precursors (pre-OCs). Rab11 has emerged as the spatiotemporal regulators of intracellular vesicular transport in the endosomal recycling system; however, how it regulates osteoclastogenesis is incompletely understood. OC-triggered bone resorption is best characterized to be immensely dependent upon lysosomal function, the intracellular acidic organelles containing more than 50 acid hydrolases secreted into bone matrix microenvironment (BME). On the contrary, it is little known about the lysosomal function on modulating the turnover of c-fms and RANK surface receptors via Rab GTPase-mediated vesicular transport, thereby dictating osteoclastogenesis. In this review, I briefly describe the mechanism underlying lysosome-induced osteoclastogenesis via the Rab11-mediated modulation of the surface receptors in OCs.

Keywords: c-fms; RANK; Rab11; lysosomes and osteoclastogenesis

1. Introduction

Osteoclasts (OCs) that are the multinucleated cells differentiated and formed by the fusion of hematopoietic cells of the monocyte/macrophage lineage are responsible for bone tissue destruction, which is essential for bone remodeling and morphogenesis during development [1,2]. OC differentiation and maturation, also referred to as osteoclastogenesis, is primarily induced by binding of receptor activator of nuclear factor Kappa-B ligand (RANKL) to the cell surface receptor, RANK [3,4]. In principle, this interaction mechanistically stimulates six signaling cascades critical for promoting osteoclastogenesis including (i) nuclear factor of activated T cells cytoplasmic-1 (NFATc-1); (ii) nuclear factor kappa B (NF-κB); (iii) phosphatidylinositol 3-kinase (PI3K/Akt); (iv) Jun N-terminal kinase (JNK); (v) extracellular signal-regulated kinase (ERK); and (vi) p38 mitogen-activated protein kinase (MAPK) [4-8], thereby leading to secretion of the lysosomal enzymes such as, for instance, the tartrate-resistant acid phosphatase (TRAP), Cathepsin K (CTSK), and the matrix metalloproteinase (MMP9), through the ruffle borders into the mineralized bone extracellular matrix (ECM) for bone destruction [9,10]. In addition, binding of the monocyte/macrophage colony stimulating factor (M-CSF), to its specific receptors, c-fms receptors, is also essential for survival and differentiation of pre-mature to mature OCs [11,12].

In order to acidify bone ECM and resorb bone as well, mature OCs firstly attach to bone surface, resulting in establishing a sealing zone (actin ring) required for creating the ruffled borders [13], and secondly secreting lysosomes through the ruffled borders [13]. Interestingly, the proton (H^+) pumps and chloride (Cl^-) channels are highly expressed in the ruffled borders so as to acidify the resorption lacuna [10,13]; moreover, the lysosomal proteins are abundantly founded at the ruffled borders, suggesting that lysosomes are the major sources for secreting the bone-resorbing components into

bone ECM [10]. Specifically, lysosomes break up hydroxyapatite (the mineral component of the bone ECM), the decarboxylation, and osteocalcin (a bone-derived hormone), i.e. Besides, lysosomes synthesize and secrete specific proteases into the resorption lacuna to destruct the organic ECM constituents, mainly comprising the type I collagen (90%) [10]. Generally, lysosomal enzymes required for OC-mediated bone degradation could be functionally branched into 2 classes consisting of (i) the enzymes such as the vacuolar-type H^+ATPases (V-ATPases) and chloride (Cl^-) channels responsible for acidifying the resorption lacuna and (ii) the proteases such as Cathepsin K (CTSK), the matrix metalloproteinases (MMPs, noticeably MMP9 and MMP13) and tartrate acid-resistant phosphatase (TRAP or ACP5) necessitating the degradation of the bone ECM [9].

The network of intracellular vesicle trafficking amongst subcellular compartments is evolutionarily conserved in eukaryotic cells. Rab GTPases, the largest subfamily of small GTPases (approx. 70 members) in human genome [14], specifically localize to integral membrane of intracellular organelles, and are best characterized to be crucial for delivering cargos to accurate destination via the vesicular delivery system, including budding, motility, docking and fusion of vesicles, cooperatively coupled to receptor signaling pathways [15-17]. Dynamic modification of Rab GTPases between inactive (GDP-bound) and active (GTP-bound) forms are catalyzed by specific enzymes. More particularly, guanine exchange factors (GEFs) catalyze conversion of GDP-bound to GTP-bound form to switch on various cellular signals whereas GTPase activating proteins (GAPs) inactivate Rab GTPases via catalyzing GTP hydrolysis of GTP-bound form [15]. Rab11 family, consisting of three members Rab11a, Rab11b and Rab11c/Rab25, accumulated to perinuclear recycling endosomes and post-Golgi vesicles, is cardinal for regulating recycling of endocytosed cargos via the endosomal membrane recycling system. Rab11 deficiency resulted in accumulation of recycling carriers comprising endocytosed transferrin and transferrin receptor beneath the cell surface [18]. Besides, Rab11 connected with recycling carriers moved along microtubules to the cell periphery, and directly regulated vesicle exocytosis at the cell surface in concert with the exocyst [19]. Rab11a is expressed ubiquitously whereas Rab11b is exclusively abundance in the brain, heart and testes. Structurally, Rab11b shares 89% amino acid sequence homology with Rab11a. Their structural differences are based on their C-terminal regions [20]. The active (GTP-bound) state of Rab11 is able to recruit its effector proteins such as Rab11-family of interacting proteins (Rab11FIPs) [21], motor proteins, such as myosin Va/b [22], the octameric exocyst tethering complex subunit Sec15 (also known as EXOC6 in mammals) [23], and heat shock proteins 90 (HSP90) [24,25]. Previous reports have elaborately highlighted the critical roles of Rab GTPases in regulating OC-mediated bone resorption [26,27] and autophagy [26] though still questionably; therefore, it will not be detailed here. In this review, I endeavor to discuss a novel role of Rab11 on modulating the turnover of cell surface receptors through a lysosome-dependent mechanism, therefore contributing to maintain bone remodeling and homeostasis.

2. Cell surface receptors (c-fms and RANK) and osteoclastogenesis

2.1. Colony-stimulating factor 1 receptor (c-fms)

c-fms, also known as CSF1R, is a receptor tyrosine kinase interacting either with macrophage factor (M-CSF) or with IL-34 ligand to activate downstream signaling cascades prerequisite for the survival, function, proliferation and differentiation of myeloid lineage cells, thereby inducing osteoclastogenesis [28]. Consistently, the expression level of c-fms is limited in pre-OCs as compared to this in mature OCs. M-CSF- or IL-34-induced c-fms-mediated signaling activation results in the elevated expression level of receptor activator of NF-κB ligand (RANKL), which is crucial for inducing OC differentiation, maturation and activity. Besides, the M-CSF/c-fms signaling activation also induces the expression of receptor activator of NF-κB (RANK), a specific receptor for RANKL [29]. Genetically, *Csf1r* gene comprises 21 introns and 22 exons, and located on human chromosome 5 (5q32) [30] and in a syntonic region on mouse chromosome 18 (18D) [31]. To date, a plenty of transcription factors required for the transcriptional activation of Csf1r have been identified such as Ets (the E26 transformation-specific family of transcription factors), PU.1, ATF, C/EBP, RUX, AP-1,

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IRF, STAT, KLF, REL, and FUS/TLS [32]. c-fms receptor, of which conformation structure is highly conserved in human and mouse, principally consists of two distinct regions: (1) the extracellular domain and (2) the intracellular cytoplasmic domain [33,34]. Of which, the extracellular domain contains immunoglobulin (Ig)-like domains directly binding to their specific ligands, a linker region, and a single-pass trans-membrane helix [11]. In the absence of ligands, c-fms receptor is abundantly present in an inactive auto-inhibitory state. The binding of M-CSF or IL-34 results in c-fms receptor dimerization and subsequent auto-phosphorylation of the dimer in *trans* on selected tyrosine residues. Specifically, it was reported that six tyrosine residues, comprising Y559, Y697, Y706, Y721, Y807 and Y974, positioned in the c-fms cytoplasmic domain, and two tyrosine residues, positioned in an oncogenic form of c-fms receptor, were phosphorylated, and therefore being as the binding platforms for SH2 and/or PTB domain-containing proteins, which activate the downstream signaling events [35,36].

c-fms receptor-induced signaling pathways are crucial for differentiating pre-OCs into mature OCs. *Csf1r*-silencing mice or *op*/*op* mice exhibit a significant reduction of OCs, disorganized matrix, reduced mineralization, weakened long bones and abnormal OBs [37,38]. During *in vitro* osteoclastogenesis, c-fms receptor-induced signaling pathways lead to a remarkable increase in the expression of RANK receptors in pre-OCs. In addition to its vital role in proliferating and differentiating pre-OCs into mature OCs, c-fms-mediated signaling also regulates OC motility and cytoskeletal reorganization via Tyr559 phosphorylation-induced c-Src-dependent manner [39]. Furthermore, crosstalk between c-fms and other specific molecules such as integrin β3 and DAP12 contributes to regulate cytoskeletal reorganization and adhesion of mature OCs. Integrin β3 is induced by RANKL-mediated stimulation and binds to extracellular matrix proteins such as vitronectin, osteoponin, and bone sialoprotein [40,41]. Indeed, it was revealed that integrin β3-deficient mice exhibited remarkably dysfunctional OCs and simultaneously developed an osteoclerotic phenotype [41], and enhancement of M-CSF/c-fms-mediated signaling rescued the defectively functional OCs in integrin β3-deficient mice [41]. Besides, it was shown that DAP12 that is an adaptor molecule containing an immunoreceptor tyrosine-based activation motif (ITAM) was indispensable for osteoclastogenesis. Activation of DAP12-mediated c-fms receptor triggered ITAM phosphorylation mediated by Src family kinases, thereby contributing to regulate cytoskeletal reorganization in OCs [42].

2.2. RANK receptors

As above-mentioned, the binding of RANKL to its receptor RANK, which is encoded by *Tnfrsf11a*, leads to generate downstream RANKL-RANK signaling cascades, which trigger pre-OCs to differentiate into mature OCs [4]. RANKL is expressed in two distinct forms including (1) a membrane-bound form expressed in cell surface of osteoblasts (OBs) and a soluble form. The membrane-bound RANKL is cleaved into the soluble form by metalloproteinases such as matrix metalloproteinase (MMP)-14 [43,44]. Although both forms function as agonistic ligands for RANK receptor, the membrane-bound RANKL is believed to be more efficient. Mice with the genetic deletions of *Tnfsf11* or *Tnfsf11a* exhibit severe osteopetrosis accompanied by defective tooth eruption owing to the mortality of their OCs [43,45]. On the other hand, RANKL-RANK signaling is also suppressed by *Tnfsf11b*- encoded osteoprotegerin, which is a soluble decoy receptor for RANKL preventing RANKL from binding to RANK receptor [4]. RANK is a member of the tumor necrosis factor receptor (TNFR) superfamily that lacks intrinsic enzymatic activity required for directly activating its downstream signaling molecules. Consequently, following the RANKL-RANK interaction, RANK transduces intracellular signals by recruiting adaptor molecules such as TNFR-associated factors (TRAFs), which then trimerize cytosolic tail of RANK receptor, and activate MAPKs, NF-κB, activator protein-1 (AP-1) and finally nuclear factor of activated T-cells cytosolic 1 (NFATc1), resulting in the commitment of monocyte/macrophage precursor cells to the OC lineage and the activation of mature OCs [7]. In the late stage of OC differentiation, the osteoclastogenic genes required for bone resorption are transcribed by NFATc1 [7]. More particularly, activation of TRAF6 leads to upregulate the NF-κB transcriptional activity by enhancing IκB kinase (IKK) complex that is

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mediated by either atypical protein kinase C (PKC) or TGFβ-activated kinase 1 (TAK1)-dependent phosphorylation [46]. The interaction of scaffolding protein p62 with TRAF6 and aPKCs brings out the establishment of a multimeric protein complex that regulates IKKβ [47]. In addition, TRAF6 also forms complexes with TAK1 and the adaptor proteins TAK1-binding protein 1 (TAB1) and TAB2, which enables TAK1 to phosphorylate IKK complex and subsequently activate NF-κB downstream signaling cascades [48]. It is best characterized that NF-κB signaling is indispensable for osteoclastogenesis. That is, NF-κB p50/p52 double-knockout mice exhibit considerable osteopetrosis owing to the deterioration in OC formation [49]. AP-1 transcription factor comprising Fos (c-Fos, FosB, Fra-1, and Fra-2), Jun (c-Jun, JunB, and JunD), and ATF (ATFa, ATF, ATF2, ATF4, and B-ATF) family members is also activated via the induction of c-Fos by the adaptor proteins [50]. c-Fos knockout mice and transgenic mice that overexpress dominant negative c-Jun exhibit severe osteopetrosis, indicating that the transcription factors, NF-κB and AP-1, are crucial for activating the downstream targets of RANKL signaling pathway at the early stage of OC differentiation [7]. Furthermore, the recruitment of adaptor proteins activates MAPKs such as c-Jun N-terminal kinase (JNK), p38, and extracellular signal-regulated kinase, and Akt/PKB [51].

3. Rab11, lysosomes and osteoclastogenesis

3.1. Lysosomes

As mentioned above, mature OCs are responsible for resorbing the bone extracellular matrix (ECM). Bone destruction highly depends upon lysosomes, membrane-enclosed organelles containing an array of approx. 50 hydrolases and are capable of breaking down all types of biological polymers including nucleic acids, carbohydrates and lipids [52]. Several published articles including ours thoroughly described the functional roles of lysosomes on in mediating OC-induced bone resorption [10,52,53]; therefore, it should not be mentioned in this review.

3.2. The axis of c-fms and RANK receptors-Rab GTPases-Lysosomes

As mentioned above, osteoclastogenesis is essentially regulated by RANK/c-fms-mediated signaling pathways, following the binding of specific ligands to these receptors. Besides, it is well-known that the ligand-induced stimulation of c-fms/RANK receptors promotes lysosomal function that is crucial for bone resorption; however, little evidence unravels how lysosomes provide a feedback to diminish the turnover of these receptors on cell surface to debilitate osteoclastogenesis and bone-resorbing activity thereof, thereby stabilizing the resting state and maintaining bone homeostasis. In our previous studies, it was obvious that Rab11a and Rab11b seemed not to be involved in the regulation of the expression of c-fms and RANK receptors via the endosomal membrane recycling system, but via the endosomal-lysosomal system in OCs [54,55]. To date, two endosomal recycling pathways described are fast and slow recycling routes. Of which, transferrin (TfR) receptor is a representative candidate recycled to cell surface via the slow recycling pathways; however, we demonstrated that Rab11a-mediated transport route of c-fms and RANK surface receptors is distinct from that of TfR receptor [55]. Instead of it, it was interestingly noted that Rab11a and Rab11b upregulated during OC differentiation caused size-based enlargement of early and late endosomes and enhanced lysosomal activity, prompting us that Rab11a and Rab11b are involved in cargo internalization into early and late endosomes, and mediate the vesicular transport of internalized cargos to lysosomes via the axis of early endosomes-late endosomes-lysosomes in OCs. Later, inhibiting lysosomal activity by a specific inhibitor, chloroquine (CLQ), we revealed that lysosomes play a crucial role in degrading these receptors in OCs, and Rab11a and Rab11b accelerate the delivery of internalized cargo to lysosomes for their proteolysis [54,55]. Additionally, later we revealed Rab34 also participated in regulating the expression of c-fms and RANK receptors; however, it is not clear whether Rab11 and Rab34 regulate vesicular transport of these receptors independently or independently [56] to lysosomes via this axis (Fig. 1). From these observations, we propose a regulatory mechanism that lysosomes play a bi-directional role in (1) regulating bone resorption via secreting specific proteases such as TRAP, Cathepsin K and MMP9 into BME [24,25], and (2) reversing

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osteoclastogenesis via abolishing the surface expression of c-fms and RANK receptors in OCs, thereby balancing bone remodeling.

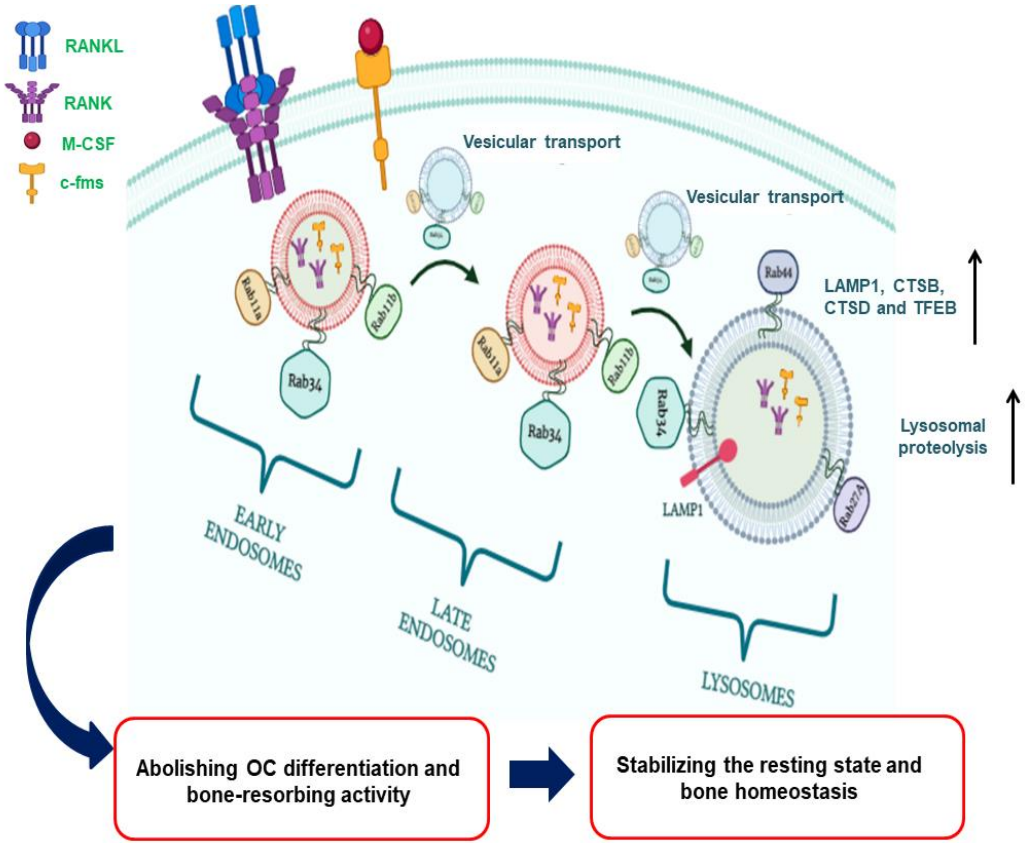

Figure 1. The graphical abstract for the role of Rab11a and Rab11b in regulating lysosomal degradation of c-fms and RANK surface receptor through facilitating the vesicular transport of these receptors to lysosomes via the axis of early endosomes (EEs)- late endosomes (LEs)- lysosomes (L) in OCs, following the binding of ligands M-CSF and RANKL to c-fms and RANK receptors, respectively. It triggers an elevation of lysosomal enzymatic activity via increasing the expression of LAMP1, CTSB and CTSD, thereby debilitating the secretion of lysosomal proteases such as TRAF, CTSK and MMP9 into BME, abolishing the bone-resorbing activity of OCs, and eventually stabilizing the resting state and maintaining bone homeostasis. .

4. Discussion

In this review, we briefly described a novel effect of lysosomes on degrading the cell surface receptors (c-fms and RANK receptors), and Rab11 play a housekeeping role in facilitating lysosome-induced degradation of these receptors in OCs, thereby stabilizing osteoclastogenesis and bone remodeling.

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