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授与した学位	博　士
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学位授与の要件	医歯薬学総合研究科生体制御科学専攻 （学位規則第４条第１項該当）
学位論文の題目	The Inhibitory Role of Rab11b in Osteoclastogenesis through Triggering Lysosome-Induced Degradation of c-Fms and RANK Surface Receptors (破骨細胞形成・分化における Rab11b の抑制的役割)
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学位論文内容の要旨

Rab family of small GTPases that belong to Ras superfamily play a pivotal role in regulating the vesicular trafficking pathways that are responsible for a vast array of cellular cargos across membrane organelles. Rab11b, one of Rab11 subfamily isoforms that abundantly localizes to recycling endosomes, was identified to govern the vesicular traffic of cell surface receptors via the endocytic recycling pathway. In osteoclasts (OCs), Rab11b was required for regulating the dynamics of ruffled borders and OC motility; nonetheless, the specific mechanisms underlying Rab11b-mediated regulation of such OC traits are unclear. Hence, in this study, I purposely tested whether Rab11b was involved in regulating the vesicular traffic of the colony stimulating factor 1 receptor (c-Fms) and the receptor activator of nuclear factor kappa-B receptor (RANK) surface receptors in OCs.

At the cellular levels, the RANK ligand (RANKL)-induced OC formation was assessed by the tartrate-resistant acid phosphatase staining. At the molecular levels, reverse transcription polymerase chain reaction (RT-PCR) assay was applied to evaluate the Rab11b mRNA levels; besides, some OC markers including c-fos, nuclear factor of activated T cells 1 (NFATc-1), cathepsin K (CTSK) and Rab11b were examined by Western blots. The results suggested that Rab11b was strongly up-regulated at the late-stage of OC differentiation.

To evaluate the regulatory effects of Rab11b on osteoclastogenesis, gain-and loss-of Rab11b expression experiments were performed. The specific small interfering RNA-mediated knockdown of endogenous Rab11b strongly enhanced OC formation and the expression levels of OC markers including c-Fms, RANK, NFATc-1 and CTSK whereas Rab11b overexpression significantly abolished these OC traits. These results indicated that Rab11b may act as a negative regulator of osteoclastogenesis.

By immunocytochemistry, the subcellular localization of Rab11b was identified in both OC precursors (without RANKL stimulation) and OCs. The results showed that Rab11 localized to early endosomes (EEs), late endosomes (LEs), but not lysosomes (Ls) in both OC precursors and OCs. Together, it was suggested that Rab11b may regulate the turnovers of c-Fms and RANK surface receptors via the axis of EEs-LEs-Ls in OCs.

To confirm the hypothesis above, two specific lysosomal and proteasomal inhibitors, MG132 and chloroquine, were used. By RT-PCR and Western blot, the results showed that lysosomes, but not proteosomes, regulated the turnovers of these receptors in OCs. Importantly, by cell surface biotinylation, it was demonstrated that Rab11b silencing markedly augmented the surface levels of these receptors while Rab11b overexpression declined those of these receptors in OCs.

In conclusion, Rab11b, up-regulated at the late stage of OC differentiation, may negatively regulate osteoclastogenesis via directing the vesicular transport of c-Fms and RANK surface receptors to Ls via the axis EEs-LEs-Ls, subsequently promoting the lysosomal proteolysis of these receptors in OCs. The lysosomal degradation of these receptors triggers the alleviation of the osteoclastogenic signaling pathways, thereby switching off to the resting state.

論文審査結果の要旨

Osteoclasts (OCs), the multinucleated cells differentiated from monocytic/macrophagic cells, play a critical role in bone resorption. OC differentiation is directly induced by a series of the specific events initialized the up-regulation of colony stimulating factor 1 (c-Fms) receptor and the receptor activator of nuclear factor kappa-B (RANK) receptor, which lead to the activation of the activated T cells cytoplasmic-1 (NFATc-1) upstream signaling pathways, essentially required for osteoclastogenesis and bone resorption. However, the specific mechanisms of how the turnovers of c-Fms and RANK surface receptors in OCs are regulated remain obscure. In this study, I sought to clarify the possible pathway controlling the abundance of these surface receptors in OCs.

Interestingly, it is well-known that Rab GTPases belonging to Ras superfamily have emerged as the spatiotemporal regulators of vesicular traffic of intracellular cargos including cell surface receptors. The Rab11 subfamily is composed of three closely related isoforms, Rab11a, Rab11b and Rab11c (also known as Rab25). Among these isoforms, Rab11b localizes to recycling endosomes may regulate the recycling of cell surface receptors to cell surface, dynamics of ruffled borders and OC motility. However, in-depth understanding of how Rab11b-mediated regulation of osteoclastogenesis through controlling the surface levels of OC receptors is still disputable. Consequently, (1) the physiological functions of Rab11b on the regulation of osteoclastogenesis and (2) whether Rab11b was involved in modulating the abundance of c-Fms and RANK surface receptors in OCs were clarified in this study.

In this study, it was revealed that Rab11b was strongly up-regulated at the late stage of RANK ligand-induced OC differentiation. Rab11b silencing strongly enhanced OC formation, bone-resorbing activity and the expression levels of OC markers such as c-Fms, RANK, Cathepsin K and NFATc-1 while Rab11b overexpression reversed all of these osteoclastogenic traits, suggesting that Rab11b was a negative regulator of osteoclastogenesis. Besides, it was observed that Rab11b localized to early endosomes (EEs) and late endosomes (LEs), but not lysosomes (Ls), and Rab11b overexpression enlarged EEs and LEs in OCs. Upon blockade of the proteosomal activity by MG132 or the lysosomal activity by chloroquine, it was shown that lysosomes exclusively served as the proteolytic pools of c-Fms and RANK proteins in OCs. More crucially, silencing Rab11b augmented the surface levels of c-Fms and RANK receptors while Rab11b overexpression alleviated those of these surface receptors in OCs.

Together, these results suggested that Rab11b may negatively regulate osteoclastogenesis by facilitating the vesicular transport of c-Fms and RANK surface receptors to Ls via the axis of EEs-LEs-Ls, subsequently promoting the lysosomal proteolysis of these surface receptors in OCs. Lysosomal proteolysis of these surface receptors triggers the alleviation of the osteoclastogenic signals, thereby abolishing osteoclastogenesis. Finally, my results also suggest a model of how Rab11b-mediated osteoclastogenesis is switched off to the resting state through diminishing the surface levels of c-Fms and RANK receptors, which is prerequisite for the maintenance of bone homeostasis.

The scientific significance of this study is to further provide one of the insights of the inhibitory mechanisms of osteoclastogenesis. The entire content of this study has already been published in the International Journal of Molecular Sciences (2020) after peer-reviewed. Therefore, the thesis defense committee hereby accepted this article as a doctoral dissertation.