【歯学系（Dentistry）】

指導教授氏名 Instructing Professor Commissioned Professor	指導役割 Role
Kuniaki Okamoto 印	Supervising entire research for dissertation
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学位論文要旨
Dissertation Abstract

岡山大学大学院医歯薬学総合研究科

専攻分野 Department	Dental Pharmacology	身分 大学院生	氏名 Name	Tran Tien Manh

論文題名
Title of Doctoral Dissertation

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）

論文内容の要旨（2000字程度）Dissertation Abstract (approx. 800 words)

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.