



Function of the Small G Protein Arf6 in Lymphangiogenesis

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論文の内容の要旨
Abstract of thesis

(目的 Purpose)

The small G protein ADP-ribosylation factor 6 (Arf6) plays pivotal roles in a wide variety of cellular events such as endocytosis, exocytosis, and actin cytoskeleton reorganization. However, the physiological functions of Arf6 at the whole animal level have not been thoroughly understood. The purpose of this study is to clarify physiological functions of Arf6 in lymphatic endothelium in mice.

(対象と方法 Materials and Methods)

The applicant generated and analyzed lymphatic endothelial cell (LEC)-specific *Arf6* conditional knockout (LEC-*Arf6* cKO) mice. Whole-mount immunofluorescence staining of embryonic dorsal skin was performed to visualize the lymphatic vessel network on the skin of embryos. Transplantation of B16 melanoma cells was further conducted in adult LEC-*Arf6* cKO mice to study tumor lymphangiogenesis. In addition, human LECs (hLECs) were utilized to investigate the cellular mechanism of Arf6 in LECs by transfecting Arf6 siRNA, followed by *in vitro* capillary tube formation assay, cell proliferation and migration assays, and $\beta 1$ integrin internalization assay. Western blotting was used to determine the expression level of Arf6 and $\beta 1$ integrin in Arf6-knocked-down LECs. The level of activated $\beta 1$ integrin and focal adhesion formation in Arf6-knocked-down LECs were analyzed by immunofluorescence staining and confocal microscopy.

(結果 Results)

The applicant showed that Arf6 regulated developmental and tumor lymphangiogenesis in mice.

LEC-*Arf6* cKO mouse embryos exhibited severe skin edema and impairment in the formation of lymphatic vessel network at the mid-gestation stage. Furthermore, the applicant found that knockdown of *Arf6* in hLECs inhibited *in vitro* capillary tube formation and directed cell migration induced by vascular endothelial growth factor-C (VEGF-C), and *Arf6* mediated VEGF-C induced cell migration through the internalization of $\beta 1$ integrin. Finally, the applicant found that LEC-*Arf6* cKO mice transplanted with B16 melanoma cells attenuated tumor lymphangiogenesis and progression.

(考察 Discussion)

- 1) The molecular mechanism for VEGF-dependent *Arf6*-mediated $\beta 1$ integrin recycling in LECs still remains unclear. However, activation of *Arf6* through *Arf6* GEFs in LECs in response to VEGF-C stimulation could be essential for integrin recycling.
- 2) Two lipid-metabolizing enzymes, phosphatidylinositol 4-phosphate 5-kinase and phospholipase D1 may be the cellular signaling downstream of the activated *Arf6* that couples to $\beta 1$ integrin endocytosis in VEGF-C-stimulated LECs.
- 3) Appropriate cycling of *Arf6* between activation and inactivation that are precisely regulated by *Arf6*-specific GEFs and GAPs, respectively, may be essential for the development of dermal lymphatic vascular network.
- 4) Ablation of *Arf6* from both venous and non-venous mesenchymal cells in *Arf6*-deficient embryos may induce lymphedema at earlier stage than in LEC-*Arf6* cKO.

(結論 Conclusion)

These results provide evidence that *Arf6* in LECs plays a crucial role in physiological and pathological lymphangiogenesis.

審査の結果の要旨 Abstract of assessment result

(批評 General Comments)

Mr. Lin clearly demonstrated by using LEC-specific *Arf6* knockout mice that *Arf6* was essential for dermal lymph vessel development *in vivo*. Mechanistically, the applicant proposed that *Arf6* was essential for VEGF-C-induced tube formation and directed migration of LECs by regulating internalization of $\beta 1$ integrin from the plasma membrane and modulating the amount of active $\beta 1$ integrin. These findings are novel and provide new insight into the mechanism of lymphangiogenesis *in vivo*. Furthermore, the applicant showed that deletion of *Arf6* in LECs suppressed tumor growth and tumor angiogenesis, suggesting that inhibition of *Arf6* could serve as a novel strategy to treat subsets of tumors *in vivo*.

(最終試験の結果 Assessment)

The final examination committee conducted a meeting as a final examination on October 25, 2017. The applicant provided an overview of dissertation, addressed questions and comments raised during Q&A session. All of the committee members reached a final decision that the applicant has passed the final examination.

(結論 Conclusion)

Therefore, the final examination committee approved that the applicant is qualified to be awarded Doctor of Philosophy in Medical Sciences.