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学位の種類	博士（医学）
学位記番号	博甲第　８４１５　号
学位授与年月	平成　２９年　１２月　３１日
学位授与の要件	学位規則第４条第１項該当
審査研究科	人間総合科学研究科
学位論文題目	Function of the Small G Protein Arf6 in Lymphangiogenesis （リンパ管新生における低分子量 G タンパク質 Arf6 の機能）
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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 β1 integrin internalization assay. Western blotting was used to determine the expression level of Arf6 and β1 integrin in Arf6-knocked-down LECs. The level of activated β1 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 β1 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 β1 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 β1 integrin endocytosis in VEGF-C-stimulated LECs.
3) Appropriate cycling of Arf6 between activation and inactivation that are precisely regulated by Arf6-specifci 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-deficinet 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 β1 integrin from the plasma membrane and modulating the amount of active β1 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.