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学位論文題目	Elucidation of physiological functions of the small G protein Arf6 and the Arf6 GAP ACAP3 in neurite outgrowth (低分子量 G タンパク質 Arf6 と Arf6 GAP ACAP3 の神経突起伸長における生理機能の解明)

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論文の要旨
Abstract of thesis

< Purpose >

The small G protein ADP-ribosylation factor 6 (Arf6) plays critical roles in membrane dynamics-based cellular functions including reorganization of actin filament and membrane trafficking, which are essential cellular processes for cancer cell progression and neural development. Arf6, as well as other small G proteins, functions as a molecular switch, cycling between GTP-bound active and GDP-bound inactive forms (GTP/GDP cycle of Arf6). This GTP/GDP cycle of Arf6 is precisely regulated by guanine nucleotides exchange factors (GEFs), which facilitate exchange of GDP for GTP on Arf6, and GTPase activating proteins (GAPs) that stimulate GAP activity of Arf6 to hydrolyze GTP on Arf6 to GDP. In a physiological setting, cycling between inactive and active forms of Arf6 seems to be required for appropriately regulating cellular events, suggesting that inactivation of Arf6 by GAPs as well as activation by GEFs could play important roles in the signal transduction to exert cell functions through Arf6. Although ACAP3 (ArfGAP with coiled-coil, ankyrin repeat and pleckstrin homology domains 3) belongs to the ACAP family of Arf GAPs, its specificity to Arf isoforms and physiological functions remain elusive. In the present study, the applicant aimed to investigate physiological roles of ACAP3 and specificity of its GAP activity to Arf isoforms.

< Materials and methods >

1) Examination of tissue distribution of ACAP3 using mice tissues.
2) Analysis of physiological roles of ACAP3 in primary cultured mouse hippocampal neurons.
3) Identification of Arf(s) regulated by ACAP3 *in vitro* GTP-Arfs pull-down assay.
4) Further analyses for molecular mechanisms through which ACAP3 regulates neurite outgrowth in primary cultured mouse hippocampal neurons.

< Results >

1) **ACAP3 is abundantly expressed in brain**

To obtain the information for tissues in which ACAP3 functions, tissue distribution of ACAP3 in P56 adult mice was analyzed. Notably, extremely higher expression of ACAP3 protein was observed in the brain compared with other tissues. In addition, *in situ* hybridization analysis in the brain revealed evident expression of *ACAP3* mRNA in the cortex, hippocampus and cerebellum; in particular, the signal in the hippocampus was very strong.

2) **ACAP3 is involved in neurite outgrowth of mouse hippocampal neurons**

Since *ACAP3* mRNA was highly expressed in the hippocampus, it is plausible that ACAP3 functions in hippocampal neurons. In primary cultured mouse hippocampal neurons, ACAP3 localized especially at the tip of the growth cone, which is leading process of extending neurite of neurons. This observation raises a possibility that ACAP3 is involved in neurite outgrowth. To address this possibility, ACAP3 in hippocampal neurons was efficiently knocked down with shRNA. Knockdown of ACAP3 in cultured hippocampal neurons markedly decreased total neurite length, supporting the possibility described above that ACAP3 is involved in neurite outgrowth.

3) **ACAP3 regulates neurite outgrowth through its GAP activity specific to Arf6**

To investigate whether regulation of neurite outgrowth by ACAP3 requires its GAP activity, the applicant conducted rescue experiments of neurite outgrowth suppressed by ACAP3 knockdown with shRNA-resistant wild type and the GAP activity deficient mutant R446Q of ACAP3. The neurite outgrowth inhibited by ACAP3 knockdown was rescued by ectopically expressed wild type of ACAP3, but not by its GAP activity-deficient mutant. Thus, ACAP3 regulates neurite outgrowth through its GAP activity. In addition, it was found that GAP activity of ACAP3 is specific to Arf6, and neurite outgrowth suppressed by ACAP3 knockdown was rescued by ectopic expression of a fast cycle mutant of Arf6 that spontaneously exchanges guanine nucleotide on Arf6, but not by that of wild type, GTP- or GDP-locked mutant of Arf6, demonstrating that GTP/GDP cycle of Arf6, which precisely regulated by ACAP3 is required for neurite outgrowth of hippocampal neurons.

< Discussion >

In the present study, the applicant has demonstrated that ACAP3 regulates neurite outgrowth through its GAP activity specific to Arf6 in mouse hippocampal neurons, and cycling of Arf6 regulated by ACAP3 in concert with undefined Arf6 GEF(s) at the growth cone is absolutely required for the neurite outgrowth of hippocampal neurons. Furthermore, results obtained in this study suggest that ACAP3 regulates neurite outgrowth by controlling appropriate levels of membrane phospholipid and membrane trafficking at the growth cone. Collectively, this study provides insight into the regulatory mechanisms of neurite outgrowth and physiological significance of the Arf6-specific GAP ACAP3.

審査の要旨
Abstract of assessment result

【批評 Review】

In the present study, the applicant has demonstrated the physiological significance of ACAP3 and Arf6 in neurite outgrowth of hippocampal neurons. Furthermore, this study suggests the significance of GTP/GDP cycling of Arf6 regulated by ACAP3 for precisely modulating the levels of membrane phospholipid, which may control the membrane trafficking at the growth cone in neurons. Thus, findings in this study will surely have impact in the field and will benefit for understanding of the molecular mechanisms of neurite outgrowth.

【最終試験の結果 Result】

The final examination committee conducted a meeting as a final examination on 13 January, 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 Human Biology.