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学位の種類	博士（医学）
学位記番号	博甲第　9713　号
学位授与年月	令和　2　年　9　月　25　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	TBC1D24 regulates formation of tubular recycling endosomes and promotes recycling of clathrin-independent cargo proteins （TBC1D24 はチューブ様リサイクリングエンドーソームの形成を制御し、クラスリン非依存性に取り込まれる膜タンパク質のリサイクリングを促進する）

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論文の内容の要旨
Abstract of thesis

In this doctoral dissertation, NGUYEN THI KIM NGUYEN describes the role of TBC1D24 in the formation of tubular recycling endosomes. The summary is as follows:

（**目的**　Purpose）

TBC1D24 was initially identified as a novel causative gene for familial infantile myoclonic epilepsy. TBC1D24 has a Tre2/Bub2/Cdc16 (TBC) domain, which is a common structure found in regulators of Rab small GTPases, and a TBC-Lysin motif (LysM)-Domain catalytic (TLDc) domain, which is suggested to be involved in oxidative stress resistance. Accumulating evidence supports that TBC1D24 is required for neuronal development and regulation of synaptic endocytic vesicle trafficking. TBC1D24 has been shown to bind Arf6, through which TBC1D24 regulates neuronal migration and maturation in a mouse model. Skywalker, the *Drosophila melanogaster* homologue of TBC1D24, exhibits *in vitro* GTPase-activating protein (GAP) activity toward Rab35 and regulates synaptic endocytic vesicle trafficking through Rab35. However, detailed functions of TBC1D24 in mammalian cells are still unclear. Plasma membrane proteins internalize into cells through either clathrin-mediated endocytosis (CME) or clathrin-independent endocytosis (CIE). Following endocytosis, cargo proteins are transported to lysosomes for degradation or recycled back to the plasma membrane. Since Arf6 and Rab proteins play pivotal roles in endocytosis and following intracellular membrane trafficking of plasma membrane proteins, the author aimed to clarify the role of TBC1D24 in the regulation of these processes.

（**対象と方法**　Materials and Methods）

To construct plasmids for Myc-TBC1D24 and FLAG-TBC1D24, TBC1D24 cDNA was amplified by RT-PCR from total RNA prepared from HeLa cells and inserted into pcDNA3 containing Myc-tag sequence for Myc-TBC1D24 and FLAG-tag sequence for FLAG- TBC1D24. The TBC1D24 gene was deleted in the HeLa cell line using the genomic editing technique CRISPR/Cas9. The trafficking of internalized endocytic cargo proteins in the cells was analyzed by the antibody uptake assay.

（**結果**　Results）

In this study, the author showed in HeLa cells that overexpression of TBC1D24 increases CIE cargo-containing tubular recycling endosomes (TREs) that are a hallmark of the CIE cargo trafficking pathway, while CRISPR/Cas9-based deletion of *TBC1D24* decreases this compartment. The deletion of TBC1D24 did not affect the internalization of CIE cargo into cells, suggesting that the decrease of TREs filled with CIE cargo proteins is not due to the reduction in incoming cargo proteins into cells. By employing GFP appended with the C-terminal 20 amino acids of H-Ras (GFP-Ras-C^{20}) as a marker for TREs, the author found that the deletion of *TBC1D24* impairs formation of TREs. The impairment of TRE formation in *TBC1D24*-deleted cells led to the delay of CIE cargo recycling back to the plasma membrane. The author also found that TBC1D24 binds to the small GTPase Rab22A, the key regulator of TRE formation as well as recycling of CIE cargo proteins. Knockdown of Rab22A reversed the effect of TBC1D24 overexpression on the trafficking of CIE cargo proteins, while the decrease of CIE cargo-specific TREs in *TBC1D24*-deleted cells was rescued by overexpression of Rab22A. These results suggest that TBC1D24 regulates TRE formation through Rab22A. Furthermore, the author found that deletion of TBC1D24 and knockdown of Rab22A both impairs cell proliferation. Based on the results obtained, the author proposes a novel function of TBC1D24 in the CIE cargo trafficking pathway: TBC1D24 promotes formation of CIE cargo-specific TREs through Rab22a, leading to facilitation of CIE cargo recycling. Through this function, TBC1D24 appears to control quantity and/or quality of the plasma membrane proteins at the cell surface, thereby contributing to the regulation of cellular functions, including cell proliferation.

（**考察**　Discussion）

TBC1D24 was initially identified as a novel epilepsy-related gene mutated in familial infantile myoclonic epilepsy, and since then TBC1D24 has been reported to be a causative gene in various diseases and syndromes. Although TBC1D24 has been suggested to be involved in the regulation of various cellular functions, including neuronal maturation, cell migration, and cancer cell proliferation and invasion, detailed physiological functions of TBC1D24 in the cell are not well understood. The author demonstrated that TBC1D24 regulates the formation of CIE cargo-specific TREs, thereby controlling the recycling of CIE cargo proteins. In addition, TBC1D24 binds to Rab22A, through which TBC1D24 regulates TRE formation. Furthermore, TBC1D24 regulates cell proliferation likely through controlling the components of the plasma membrane. These findings provide insights into the molecular mechanism of TRE-mediated CIE cargo recycling. The findings in this study suggest that TBC1D24 contributes to the control of the amount and localization of plasma membrane proteins, such as receptors, transporters and adhesion molecules, by facilitating the TRE-mediated recycling, thereby regulating various cellular functions. Therefore, the author suggests importance of identifying TBC1D24-dependent cargo proteins and investigate how TBC1D24 regulates cellular functions through those target proteins in order to elucidate the pathogenesis of TBC1D24-related disorders.

（批評　General Comments）

In the present study, the author proposed a novel function of TBC1D24 in the CIE cargo trafficking pathway: TBC1D24 promotes formation of CIE cargo-specific TREs through Rab22a, leading to facilitation of CIE cargo recycling. Through this function, TBC1D24 appears to control quantity and/or quality of the plasma membrane proteins at the cell surface, thereby contributing to the regulation of cellular functions, including cell proliferation. These findings provide a novel insight into the function of TBC1D24 in intracellular membrane trafficking.

（最終試験の結果　Assessment）

The final examination committee conducted a meeting as a final examination on 3 June, 2020. 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）

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