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審査研究科	人間総合科学研究科
学位論文題目	Characterization of a bicistronic knock-in reporter mouse model for investigating the role of *Cables2 in vivo* （*in vivo* における *Cables2* の役割を研究するためのバイシストロニックノックイン・レポーターマウスモデルの特性）

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論文の内容の要旨
Abstract of thesis

In this doctoral dissertation, Ammar Shaker Hamed Hasan describes generation and characterization of bicistronic Cables2 knock-in reporter mice that express Cables2 tagged with a 3×FLAG epitope as well as a fluorescent reporter tdTomato. The thesis examines the expression patterns and protein-protein interactions of Cables2 *in vivo* and its functional roles in the ovary. The content is summarized as follows:

（目的　Purpose）

The CDK5 and Abl enzyme substrate (Cables) family comprises two members, Cables1 and Cables2. Both proteins have a cyclin box-like domain at the C-terminus, which is highly homologous as it presents 78% amino acid identity. Cables family members interact physically and associate with cyclin-dependent kinase 3 (Cdk3), Cdk5, and c-Abl. Although Cables family members share a similar protein structure, the role of Cables2 *in vivo* remains unknown, largely due to lack of suitable antibodies against mouse Cables2 and absence of a *Cables2* mouse model. In this study, the author created and characterized a novel knock-in mouse that promotes our understanding of the expression pattern, protein interaction network, and *in vivo* functions of Cables2. The author generated bicistronic *Cables2* knock-in reporter mice that expressed Cables2 tagged with a 3×FLAG epitope as well as a fluorescent reporter tdTomato inserted downstream of Cables2 via 2A peptide sequence.

（対象と方法 Materials and Methods）

Cables2-3×FLAG-2A-tdTomato mouse, hereafter referred to as *Cables2*^{Tom} mouse, was generated

by modifying the *Cables2* gene in embryonic stem cells (ES cells) using the CRISPR/Cas9 system. The author knocked-in 3xflag, 2A, and tdTomato immediately before the stop codon of *Cables2*. Expression of *Cables2* RNA was determined by RT-PCR and RT-qPCR. Cables2 in mouse organs and tissues was visualized by observing tdTomato fluorescent signal in fixed samples and was also evaluated by Western blotting. Cables2 protein-protein interactions with Cdk5 in *Cables2*^{Tom} mice was evaluated by immunoprecipitation. Serum progesterone concentrations in pregnant *Cables2*^{Tom} mice were measured by ELISA.

（結果　Results）

First, the author confirmed targeted gene insertion and homologous recombination of ES cells clones and evaluated random integration in these clones. RT-PCR analysis detected *tdTomato* expression in the brain, lung, kidney, spleen, colon, testis, and ovary from *Cables2*^{Tom} mice but not from wild-type mice. RT-qPCR analysis and fluorescent signal was higher in the brain, testis and ovary from *Cables2*^{Tom} mice comparing to wild-type mice. Interestingly, unique expression pattern was observed in corpus luteum of the ovary. By using anti-FLAG antibody, western blot showed FLAG-tagged Cables2 in the brain, testis and ovary from *Cables2*^{Tom} mice but not wild-type mice. Immunoprecipitation analysis of the cell extracts derived from the brain and testis in *Cables2*^{Tom} revealed interaction of Cables2 with Cdk5.

The author then used *Cables2*^{Tom} mice to investigate whether Cables2 is one of the molecules involved in the luteinization process. At gestation day 15.5, RT-qPCR analysis revealed that *Cables2* mRNA expression in the ovary was increased by ~4 fold in *Cables2*^{Tom} mice as compared with wild-type mice. Strong tdTomato signals were observed in the corpus luteum of pregnant mice. Measuring the number of viable fetuses *Cables2*^{Tom} mice obtained by inbreeding was significantly less than that in wild-type mice. Furthermore, *Cables2*^{Tom} mice showed significantly lesser ovary weight than wild-type mice at gestation day 15.5. Surprisingly, although pregnant *Cables2*^{Tom} mice that overexpress *Cables2* mRNA in organs including the ovary showed reductions in the litter size and ovary weight as compared with pregnant wild-type mice, serum progesterone concentrations were significantly higher in pregnant *Cables2*^{Tom} mice.

（考察　Discussion）

The author analyzed the derived knock-in mice by examining the expression patterns of FLAG-tagged Cables2 and its mRNA to reveal expression patterns of Cables2 in several tissues during development. These results suggest that *Cables2*^{Tom} mouse is a useful tool for further studies on the *in vivo* functions of Cables2. Moreover, the localization of expressed Cables2 in the ovary suggests an important role for this protein in the ovary, especially with regards to hormonal regulation. These results strongly imply that Cables2 is a functional molecule involved in progesterone biosynthesis process and regulation of pregnancy.

審査の結果の要旨
Abstract of assessment result

（批評　General Comments）

In this thesis, the author demonstrates that bicistronic *Cables2* knock-in reporter mouse is a useful model for the comprehensive analysis of *in vivo* functions of Cables2, particularly in the ovary. The extensive characterization of the knock-in mouse using RT-qPCR, immunochemistry, and immunoprecipitation revealed that the expression of Cables2 in the ovary is strongest at the stage of corpus luteum. Together with the analysis of protein-protein interactions, the author showed a possible mechanism for Cables2 functions in the ovary. The characterization of knock-in mice and the new findings on tissue- and development-specific expression patterns of Cables2 provide a solid foundation for future mechanistic studies of Cables2 functions during pregnancy.

（最終試験の結果　Assessment）

The final examination committee conducted a meeting as a final examination on December 23, 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.