論　　文　　概　　要 (Thesis Abstract)

論 文 題 目 Title	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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Abstract

Introduction and 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. *Cables1* plays a key regulatory role in human intestinal tumor progression, endometrial hyperplasia, and oocyte development. Furthermore, Cables1 is a mediator for progesterone-induced differentiation of endometrial epithelial cells. *CABLES1* is up-regulated by progesterone, but down-regulated by estrogen. Further, Cables1 is associated with protecting p63 from protein degradation and maintaining p21/Cip1 stability. In addition, Cables1 induces p53- and p73-mediated apoptosis, while binding to 14-3-3 supresses the apoptosis-inducing function of Cables1. *In vitro,* Cables2 induces apoptotic cell death in both a p53-dependent and a p53-independent manner. *Cables2* mRNA is found to be widely expressed in adult mouse tissues by northern blot analysis. Although Cables family members share a similar protein structure, the role of Cables2 *in vivo* remains unknown, largely due to a lack of suitable antibodies against mouse Cables2 and absence of a *Cables2* mouse model.

In this present study, I created and characterized a novel knock-in mouse that can boost our understanding of Cables2 expressing cells, protein interaction network, and functions *in vivo*. I generated bicistronic *Cables2* knock-in reporter mice that expressed Cables2 tagged with 3×FLAG and 2A-mediated fluorescent reporter tdTomato. Hereafter, *Cables2-3×FLAG-2A-tdTomato* mouse is referred to as $Cables2^{Tom}$.

Materials and Methods

Cables2^{Tom} mouse was generated by modifying *Cables2* gene in embryonic stem cells (ES cells) using the CRISPR/Cas9 system. I knocked-in 3xflag, 2A, and tdTomato just before stop codon of *Cables2*. To investigated whether the *Cables2*^{Tom} mouse can provide a valuable tool in studying Cables2, I subjected it to several different analyses. Expression of *Cables2* RNA was determined by RT-PCR and RT-qPCR. Visualizing of Cables2 in mouse organs and tissues was examined by observing tdTomato fluorescent signal in fixed samples. Expression of Cables2 protein was evaluate by Western blotting. Cables2 protein-protein interactions with Cdk5 in *Cables2*^{Tom} mice was evaluated by IP. Furthermore, serum progesterone concentrations in pregnant *Cables2*^{Tom} mice were measured by ELISA.

Results and Discussion

First, I confirmed targeted gene insertion and homologous recombination of ES cells clones and I evaluated random integration in these clones. Then I characterized *Cables2*^{Tom} mice by subjecting the knock-in mice to several analyses. RT-PCR analysis detected *tdTomato* band in the brain, lung, kidney, spleen, colon, testis, and ovary from *Cables2*^{Tom} mice but not from wild-type mice. RT-qPCR analysis showed significant increase of *Cables2* expression level in the brain and testis from *Cables2*^{Tom} mice comparing to wild-type mice. 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 band in the brain, testis and ovary from *Cables2*^{Tom} mice but not wild-type mice. Immunoprecipitation analysis using the brain and testis in *Cables2*^{Tom}

revealed interaction of Cables2 with Cdk5. These results suggest that *Cables2*^{Tom} mouse is a useful tool to study Cables2 *in vivo*.

Finally, to progress in understanding the functional roles of Cables2 in the corpus luteum, I investigated whether Cables2 is one of the molecules involved in the luteinization process by using *Cables2*^{Tom} mice. The corpus luteum is an endocrine structure that secretes progesterone hormone which prepares the endometrium for possible implantation. I confirmed whether *Cables2*^{Tom} mouse is an effective overexpression model for investigating *Cables2* in the ovary. At gestation day 15.5, RT-qPCR analysis revealed that *Cables2* mRNA expression in the ovary was increased at approximately 4 times in *Cables2*^{Tom} mice compared with wild-type mice. Strong tdTomato signals were observed in the corpus luteum of pregnancy. Measuring the number of viable fetuses *Cables2*^{Tom} mice obtained by inbreeding was significantly less than that in wild-type. Further, *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 less in litter size and ovary weight than pregnant wild-type mice, serum progesterone concentrations were significantly higher in pregnant *Cables2*^{Tom} mice. These results imply that Cables2 is a functional molecule involved in progesterone biosynthesis process.

Conclusion

Collectively, I demonstrate that our bicistronic *Cables2* knock-in reporter mouse is a useful model for the comprehensive analysis of *in vivo* Cables2 function, particularly in the corpus luteum.