論 文 の 内 容 の 要 旨

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論文題目

Functional Analysis of Ubiquitin C-Terminal Hydrolase-1 in Pituitary Gland Cells

（下垂体細胞におけるユビキチン C 末端加水分解酵素１型(UCH-L1)の機能解析に関する研究）

General Background

Protein degradation is one of essential intracellular activities to maintain normal cellular functions. Firstly, the small 76-amino acid protein ubiquitin can be attached covalently to its substrate proteins. Subsequently, the protein substrate is tagged by a polyubiquitin chain which is recognized by 26S proteasome and result in the degradation. The degradation of proteins mediated by ubiquitin-proteasome system (UPS) plays important roles in a wide variety of cellular processes, which include intracellular protein degradations, cell cycle control, transcriptional regulation, stress responses as well as apoptosis. Deubiquitinating enzymes (DUBs) are a group of more than sixty known proteases that regulate ubiquitins by cleaving ubiquitin-protein bonds.

DUBs are mainly divided into ubiquitin-specific proteases (UBPs) and ubiquitin C-terminal hydrolases (UCHs). Ubiquitin C-terminal hydrolase-L1 (UCH-L1) is one of UCHs, highly and selectively expressed in neurons, reproductive tissues. In addition, it has been clarified that a variety of tumors also express UCH-L1. UCH-L1 is considered as a unique enzyme since it carries a hydrolase activity in monomer, and a ligase activity in the form of dimer.

The pituitary gland is a vital component in the hypothalamic-pituitary-gonadal (HPG) axis, which is critical to reproductive and immune systems. The anterior lobe of pituitary gland is composed primarily of five specific hormone-producing cells and a kind of non-hormone producing folliculostellate (FS) cells. Although the expression of UCH-L1 in the anterior pituitary gland was reported, the detailed expression pattern and the role of UCH-L1 in the anterior pituitary gland remain unknown. In the present study, I attempted to examine the function of UCH-L1 in the anterior pituitary gland in detail.

Chapter 1

The ubiquitin-proteasome system (UPS) plays a fundamental role in regulating various biological activities. UCH-L1 is a deubiquitinating enzyme, belonging to the UPS. To date, it has been reported that UCH-L1 is highly and restrictedly expressed in the neural and reproductive tissues and plays significant roles in these organs. Although the expression of UCH-L1 in the anterior pituitary gland has been reported, the detailed localization and the role of UCH-L1 remain obscure. In this chapter, I detected UCH-L1 protein exclusively in hormone-producing cells, but not non-hormone producing folliculostellate cells in the anterior pituitary lobe. In addition, the cytoplasmic expression of UCH-L1 varied and was limited to gonadotropes and mammotropes. To investigate the role of UCH-L1 in the anterior pituitary cells, I performed a comparative analysis using wild-type and genetically UCH-L1-deficient *gad* mice. The numbers of gonadotropes and mammotropes in *gad* mice were obviously smaller than those in wild-type mice, although there was not difference in the number of other hormone-producing cells between wild-type and *gad* mice. The result suggests a close involvement of UCH-L1 in hormone production and/or development/maintenance of gonadotropes and mammotropes.

Chapter 2

In chapter 1, significant decreases in the numbers of gonadotropes and mammotropes

were observed in UCH-L1-deficient *gad* mice. UCH-L1 is an important protein in both testis and ovary. I had a special interest on the role of UCH-L1 in gonadotropes, because they are critical to reproduction. However, the anterior pituitary gland in mice is quite small and gonadotropes account for approximately 10% of anterior pituitary cell population. So, it is not easy to prepare a lot of murine gonadotropes to examine the role of UCH-L1. In this chapter, I chose gonadotropes cell lines αT3-1 cells and LβT-2 cells to examine the role of UCH-L1. Firstly, the morphologies of cultured cells and localizations of UCH-L1 were examined. I next examined the expression levels of UCH-L1 in both cell lines by RT-PCR and Western blotting. Apoptosis is a crucial cellular mechanism to maintain cell populations in tissues homeostatically. Several reports have demonstrated the involvement of UCH-L1 in apoptosis of neuron cells and germ cells. I hypothesized the decrease in gonadotropes in *gad* mice could be resulted from apoptosis. To examine whether UCH-L1 is involved in apoptosis of gonadotropes, a UCH-L1 specific inhibitor LDN-57444 was used. The result showed that UCH-L1 inhibitor upregulated apoptosis of LβT-2 cells, suggesting that the deficiency of UCH-L1 results in apoptosis of gonadotropes in *gad* mice.

Chapter 3

To study the effects of a given gene, vector-mediated overexpression and siRNA-induced knockdown methods are frequently used, because protein expression level can be transiently up- or down-regulated. In this chapter, I used these technologies to examine whether the expression of gonadotropin genes would be affected by either upregulation or downregulation of UCH-L1 protein. In an attempt to do this, pcDNA 3.1 vector carrying full-length sequence of UCH-L1 coding region followed with a FLAG tag sequence or UCH-L1 specific siRNA was transfected into LβT-2 cells. As a result, the overexpression of UCH-L1 did not influence the transcriptions of all three subunits of gonadotropin genes, suggesting that sufficient UCH-L1 proteins had functioned in the cells. On the other hand, the inhibition of UCH-L1 protein expression by specific siRNA did not impact on mRNA expressions of each subunit of gonadotropin genes as well. These results suggest that the level of UCH-L1 does not affect transcriptional expressions of gonadotropin genes in LβT2 cells. It might not be true that UCH-L1 is necessary in hormone production in gonadotropes in mice.