Differentiation and Culture Method

for Pituitary Cells

A Dissertation Submitted to

the Graduate School of Life and Environmental Sciences,

the University of Tsukuba

in Partial Fulfillment of the Requirements

for the Degree of Doctor of Philosophy in Science

(Doctoral Program in Biological Sciences)

Yuko KOKUBU

Table of Contents

Ⅰ. Abstract

The pituitary gland is a center of the endocrine system and controls homeostasis in an organism by secreting various hormones. The pituitary organ consists of three different lobes, including the anterior, posterior, and intermediate lobes. Particularly, the anterior lobe consists of five different cell types and secretes six different hormones. This anterior lobe controls entire body via secreted hormones. Dysfunction of the anterior pituitary is known as hypopituitarism, which causes various abnormalities in the body, such as low height, infertility, hypothyroidism, reduced breast milk production, and adrenal dysfunction. This hypopituitarism is a rare intractable disease in Japan. Currently, most patients must take medicine or inject hormones every day to compensate for their pituitary dysfunction. Cell transplantation therapy may be useful for curing this disease in the future; however, primary pituitary cells are not a candidate as a source of cells for cell therapy because they are not expandable *in vitro*.

To develop new methods for the cell therapy of this organ, I attempted to directly reprogram the pituitary cells using mesenchymal stem cells (MSCs) by transfecting transcription factors. This screening identified 4 transcription factors as candidates for increasing the expression of anterior pituitary cell markers. These factors can induce the

expression of markers such as *Lhx3* and *Pit1* and thyroid stimulating hormone beta (*Tshb*). However, after introduction of the 4 factors, the cells did not proliferate and the morphological features were not similar to those of anterior pituitary cells. In addition, immunostaining of these cells to detect several markers revealed background staining because of severe cell damage. To overcome these limitations, further analysis including optimization of the medium conditions for pituitary cell culture is necessary.

For this purpose, I attempted to immortalize anterior pituitary cells by introducing the *TERT*, *E6*, and *E7* genes. Next, the culture conditions were optimized for these cells. The immortalized cells could proliferate and maintain morphological characteristics similar to those of primary pituitary cells under sphere culture conditions in DMEM/F12 medium supplemented with N2, B27, basic fibroblast growth factor (bFGF), and epidermal growth factor (EGF). These cell lines responded to protein kinase A (PKA) or protein kinase C (PKC) pathway activators and induced the expression of *Tshb* mRNA. Moreover, transplantation of the immortalized cell line into subcutaneous regions or the kidney capsules of mice further increased *Tshb* expression.

Taken together, in this research, I attempted to induce pituitary cells from MSCs using direct reprogramming method. As a result, I found 4 factors to induce the expression of pituitary markers. However, further analysis and optimization of culture conditions for

the progression of this research were still required. For this purpose, I established new immortalized cells and determined the suitable culture method for pituitary cells. The culture conditions determined in this study will be useful for *in vitro* research of the pituitary and for further analyzing direct reprogramming.