学 位 論 文 の 要 旨

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学 位 論 文 名　Adrenocorticotropic Tumor Cells Transplanted into Mouse Embryos Affected Pancreatic Histogenesis

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論 文 内 容 の 要 旨

INTRODUCTION

There are wide individual variations in the size of organs and the total number of structural and functional units in the organ, such as β cells in the pancreas and nephrons in the kidney. This wide variation is produced during the histogenetic period, i.e., from mid-gestation to the early postnatal period, depending on the organ. During histogenesis, organ-specific cells are differentiated and organized to form structural units, the number of these units increases, and eventually organ-specific functions emerge. The total number of units, which is attained at the end of histogenesis, determines the total functional capacity, including the functional reserve of the organ, and thus may be related to predispositions to postnatal organ-based diseases. We previously reported that tissues and organs in mouse embryos express melanocortin type 2 and type 5 receptors (MC2R and MC5R) that are for adrenocorticotropic hormone (ACTH), and that ACTH-producing AtT20 pituitary tumor cells transplanted into mouse embryos affected the histogenesis of the adrenal gland and testis. Glucocorticoid has long been known to affect the histogenesis of organs such as the lung, liver, and intestine. In the present study, therefore, we confirmed the MCR expression in the pancreas and applied the same AtT20 cell-transplantation

procedure to observe the effects of ACTH and/or secondarily induced glucocorticoid on the histogenesis of the pancreas.

MATERIALS AND METHODS

AtT20 cells, which secrete ACTH continuously, were injected subcutaneously into mouse embryos at embryonic day (E) 12.5, and the embryos were allowed to develop *exo utero* till E18.5 (AtT20 group), when the embryos were removed and the blood was collected immediately. Upon measurement of the embryo's body weight (BW) and crown-rump length (CRL), the organs were dissected and prepared for histological examination. The serum ACTH level of each E18.5 embryo was analyzed by enzyme immunoassay. Expression of MC2R and MC5R was examined by immunohistochemistry at E14.5. Using serial sections of the E18.5 pancreas, we stereologically measured the volumes of the whole pancreas, endocrine and exocrine parts, and counted total cell numbers and numbers of mitotic or pyknotic cells of the whole pancreas, endocrine and exocrine cells, and glucagon-immunopositive α cells and insulin-immunopositive β cells in the endocrine part. We further counted the number of cells immunopositive for Ngn3, a bHLH transcription factor and one of the gene regulatory factors in pancreatic development, in the pancreas at E14.5, when the differentiation of the endocrine pancreas has already started and Ngn3 is expressed in the cells that are committed to endocrine lineage.

RESULTS AND DISCUSSION

Serum ACTH levels increased significantly at E18.5 in the AtT20 group (23.74 ± 6.19 ng/ml vs. control group, 0.48 ± 0.40 ng/ml, $p<0.05$). BW tended to be greater in AtT20-transplanted embryos, while there was no difference in CRL between the groups. MC2R, but not MC5R, was found to be expressed moderately in the embryonic pancreas, i.e., epithelia of the pancreatic duct and developing gland that include both exocrine and endocrine lineage cells. At E18.5, the volumes standardized by BW of the whole and exocrine pancreas tended to be greater in AtT20-transplanted embryos but did not differ significantly from the control ($p=0.081$ for the whole, $p=0.091$ for exocrine), while that of the endocrine pancreas significantly

increased in AtT20-transplanted embryos (0.155 ± 0.033 mm^3 vs. control group, 0.098 ± 0.017 mm^3, $p<0.01$) . The total cell numbers of endocrine pancreas standardized by BW also significantly increased in the AtT20 group ($2.91 \pm 0.56 \times 10^5$ vs. control group, $1.18 \pm 0.43 \times 10^5$, $p<0.01$). The ratio of the number of pyknotic cells to the number of total endocrine cells was significantly lower in AtT20 transplanted embryos at E18.5 (0.0003 ± 0.0001 vs. control group, 0.0023 ± 0.0019, $p<0.05$), whereas the mitotic indices did not differ between the groups, suggesting that the increase in the endocrine pancreas was due to decreased cell death and not to increased cell proliferation. In the endocrine pancreas at E18.5, cell numbers standardized by BW of α cells and β cells did not differ between the AtT20 and control groups, while the numbers of non-α, non-β cells significantly increased (AtT20 group, 2.43×10^5 cells vs. control group, 0.59×10^5 cells, $p<0.05$). The numbers of Ngn3-immunopositive cells were not significantly different between the AtT20 and control groups at E14.5, suggesting that ACTH and/or glucocorticoid did not affect the differentiation of endocrine precursors via Ngn3 upregulation at this stage.

The present study demonstrated that excessive ACTH from transplanted AtT20 tumor cells and/or glucocorticoid induced by ACTH secondarily from the adrenal gland significantly affected the histogenesis of the pancreas in mouse embryos. These effects appeared basically to prevent cell death rather than to promote cell proliferation or the modulation of cell lineage determination in pancreatic endocrine cells.

CONCLUSION

The present results suggest that the high level of ACTH and/or glucocorticoid, both of which are among the critical molecules in the neuro-immuno-endocrine network, affects the histogenesis of the pancreas, and thus evidently illustrated that the neuro-immuno-endocrine network indeed acts to modulate organ size, i.e., the total functional capacity of the organs during histogenesis. This suggests that detailed studies should be conducted to clarify this network's significance in the organ histogenesis and predisposition to postnatal diseases.