論　文　要　旨

学 位 論 文(要約)

表　　題　ヒト下垂体腺腫における細胞外マトリックスの分子形態学的解析

Molecular morphological analysis of extracellular matrix in the human pituitary adenoma

申 請 者 氏 名　Tofrizal Bin Alimuddin

担当指導教員氏名　屋代　隆　教授

所　　属　自治医科大学大学院医学研究科
人間生物学系　専攻
生体構造医学　分野
機能細胞学

使用文字数　994　字

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１　研究目的

The purpose of the study was to find out the mechanism of extracellular matrix deposition in terms of production and maintenance of collagen in human anterior pituitary and pituitary adenomas. This experiment was aimed to identify and characterize collagen-producing cells and the tissue inhibitors of metalloproteinases (TIMPs)-expressing cells in human anterior pituitary and pituitary adenomas.

２　研究方法

Human pituitary adenomas were obtained during surgery at Toranomon Hospital. Normal part of pituitary tissue from craniopharingioma lesion was used as control tissue. Tissues were selected through serial histological sampling techniques as HE staining, azan staining and *in situ* hybridization on β-actin and GAPDH. 99 samples that consist of control tissues (4 samples), GH-producing adenomas (25 samples), mammosomatotroph adenoma (1 sample), PRL-producing adenomas (3 samples), TSH-producing adenomas (19 samples), plurihormonal adenoma (1 sample), ACTH-producing adenomas (3 samples), and null cell adenomas (47 samples) were analyzed. Azan staining sections were also used to measure the proportion of fibrous matrix in the tissues. To identify collagen-producing cells, *in situ* hybridization of *COL1A1* and *COL3A1* was performed. Immunohistochemistry for α-smooth muscle actin (αSMA) or cytokeratin (CK) was performed to characterize collagen-producing cells. To identify TIMP-expressing cells, *in situ* hybridization of *TIMP1*, *TIMP2*, *TIMP3*, and *TIMP4* was performed. Immunohistochemistry for pituitary hormones, S-100 protein, αSMA, and CK or lectin histochemistry was performed to characterize TIMP-expressing cells.

３　研究成果

In situ hybridization showed that collagen-producing cells in control human anterior pituitary were low in number. Immunohistochemistry revealed that collagen-producing cells in control tissue of human pituitary were pericytes, which were located closely to capillary, αSMA (+), CK (-), and *RGS5* (+). In pituitary adenomas, there were 4 types of collagen-producing cells. 1) myoepithelial-like cells, which were located next to epithelial cells, αSMA (+), CK (+), and *RGS5* (+), 2) pericytes, 3) myofibroblasts, which were located in the middle of fibrous matrix, αSMA (+), CK (-), and *RGS5* (+), and 4) fibroblasts, which were αSMA (-), CK (-), and *RGS5* (-). There were changes in number and types of collagen-producing cells in pituitary adenomas, and these changes were correlated to fibrous matrix deposition.

All *TIMP*s (*TIMP1*, *TIMP2*, *TIMP3*, and *TIMP4*) were expressed in human pituitary gland with different

pattern. *TIMP1* and *TIMP2* were expressed in parenchymal cells and stromal cells. *TIMP3* were expressed in stromal cells, and *TIMP4* were exclusively expressed in parenchymal cells. Among all *TIMPs*, *TIMP3* was expressed in distinct pattern with high number of cells and strong intensity. Double-staining of *in situ* hybridization and immunohistochemistry showed that *TIMP1* and *TIMP2* were expressed in somatotrophs, lactotrophs, thyrotrophs, folliculostellate cells, pericytes, myoepithelial-like cells, and endothelial cells. TIMP3-expressing cells included pericytes, myoepithelial-like cells, and endothelial cells. TIMP4-expressing cells were somatotrophs, lactotrophs, thyrotrophs and folliculostellate cells. There were changes in TIMPs expression in pituitary adenomas both in cell number and number of cell types. The expression of *TIMP3* was mostly correlated to the fibrous matrix deposition in pituitary adenomas.

4　考察

In this study, I first identified the collagen-producing cells in human pituitary and pituitary adenomas. Although pericytes produce collagen in the control pituitary, pituitary adenomas exhibited variation in the number and types of collagen-producing cells. The collagen-producing cells in adenomas included pericytes, myofibroblasts, myoepithelial-like cells, and fibroblasts. Myofibroblasts are known to derive from various cells, including pericytes, endothelial cells, fibroblasts, and epithelial cells. Thus these cells might have differentiated into myofibroblasts in pituitary adenomas. In addition, myoepithelial-like cells have unique characteristics, resembling those of myoepithelial cells in exocrine glands. No previous study has identified myoepithelial-like cells in the pituitary gland. Further study is needed in order to determine the origin of these cells in pituitary adenomas. The number of collagen-producing cells was associated with fibrous matrix deposition in adenomas, which indicates that collagen turnover rate was altered in these adenomas.

On the other hand, all *TIMPs* (*TIMP1-4*) mRNA were expressed in control pituitary tissue. The first experiment showed a very low number of collagen-producing cells in human anterior pituitary, despite clear ECM deposition. The high expression of *TIMP3* helps to explain this discrepancy and suggest that the rate of collagen turnover in human pituitary is low. In pituitary adenomas, *TIMP3* mRNA expression exhibited the most consistent correlation with fibrous matrix deposition. Myoepithelial-like cells expressed *TIMP3* in control pituitary and adenomas, but only found to produce collagen in adenomas. These findings suggest that the characteristics of myoepithelial-like cells can change during tumorigenesis. A future study should attempt to clarify the characteristics of myoepithelial-like cells in the gland.

This study revealed the alteration of both number and types of collagen-producing cells as well as TIMP-expressing cells in pituitary adenomas, and these alterations were associated with fibrous matrix deposition. Adenomas with low number or types of collagen-producing cells as well as TIMP3-expressing cells had less fibrous matrix deposition. In contrast, adenomas with greater number or types of collagen-producing cells or TIMP3-expressing cells exhibited greater fibrous matrix deposition. Interestingly, most TSH-producing adenomas had more diverse types of TIMP3-expressing cells and high fibrous matrix deposition. The greater number and types of cells are consistent with the results of a previous study that reported fibrosis and tumors with a clinically hard consistency, which are characteristics of TSH-producing adenomas.

５　結論

The results of this study showed that there were alterations in collagen-producing cells and the TIMP-expressing cells in pituitary adenomas, and the changes of number as well as types of collagen-producing cells and TIMPs-expressing cells were associated with pituitary adenomas fibrosis. These data suggest that fibrous matrix deposition in human anterior pituitary is controlled by both production and maintenance. This study helps to lay the foundation for future research on anterior pituitary function and disease.