

氏 名	<small>とふりざる ひん ありむでいん</small> Tofrizal Bin Alimuddin
学 位 の 種 類	博士（医学）
学 位 記 番 号	甲第 526 号
学位授与年月日	平成 29 年 3 月 21 日
学位授与の要件	自治医科大学学位規定第 4 条第 2 項該当
学 位 論 文 名	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
論 文 審 査 委 員	(委員長) 教 授 矢 田 俊 彦 (委 員) 教 授 田 中 亨 准教授 黒 須 洋

論文内容の要旨

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 craniopharyngioma 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 (α SMA) 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, α SMA, 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, α SMA (+), 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, α SMA (+), CK (+), and *RGS5* (+), 2) pericytes, 3) myofibroblasts, which

were located in the middle of fibrous matrix, α SMA (+), CK (-), and *RGS5* (+), and 4) fibroblasts, which were α SMA (-), 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 *TIMPs* (*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.

論文審査の結果の要旨

申請者は、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研究を行った。細胞外マトリックス分子としてコラーゲンに注目し、その産生と維持に関わるコラーゲン産生細胞およびTIMP (tissue inhibitor of metalloproteinase)発現細胞の発現、分布の変化をヒト下垂体腺腫で調べた。組織は虎ノ門病院より提供された。

申請者は、ヒト下垂体において、4種類のコラーゲン産生細胞と各々の発現分子を同定した。さらに、ヒト下垂体腺腫では、このコラーゲン産生細胞の種類と数、および、TIMP サブタイプ (TIMP1, TIMP2, TIMP3, TIMP4) 発現細胞が変化し、これらの変化の大きさが下垂体腺腫の線維化の程度と関連することを見出した。

ヒト下垂体腺腫は稀であり、その組織学的特徴はよくわかっていない。本研究において、下垂体腺腫の線維化と関連する細胞の種類、その増減、その分布局在の変化を見出したことには一定の新規性があり、ヒト下垂体腺腫の組織学的理解に一定の貢献をするものであり、病理学的な意義が認められる。本研究の一部は国際学術雑誌に受理されている。

審査会では、研究目的や仮説の提示が不十分であること、患者の臨床背景の記載がないこと、染色像のネガティブ対照像がないこと、統計解析結果の表示の不足、文章や英語表記の多くの誤りなど、数多くの指摘があった。これに対し、申請者は指摘内容を理解し、限られた時間の中で誠実に改定を行った。

以上より、申請者は学位授与に相応しいと審査員全員で判断した。

最終試験の結果の要旨

申請者は、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研究を発表した。細胞外マトリックス分子としてコラーゲンに注目し、その産生と維持に関わるコラーゲン産生細胞およびTIMP(tissue inhibitor of metalloproteinase)発現細胞の発現、分布の変化をヒト下垂体腺腫で調べた。

申請者は、ヒト下垂体において、4種類のコラーゲン産生細胞と各々の発現分子を同定した。さらに、ヒト下垂体腺腫では、このコラーゲン産生細胞の種類と数、および、TIMP サブタイプ (TIMP1, TIMP2, TIMP3, TIMP4) 発現細胞が変化し、これらの変化の大きさが下垂体腺腫の線維化の程度と関連することを見出した。申請者は、これらの結果を発表した。英語による発表は解りやすいものであった。

審査員から、研究目的や仮説の提示、患者の臨床背景の記載、染色像のネガティブ対照像の表示、統計解析結果の表示などに関して数多くの指摘があったが、申請者はこれに真摯に答えるとともに、指摘内容を理解し、限られた時間の中で誠実に論文の改定を行った。

ヒト下垂体腺腫は稀であり、その組織学的特徴はよくわかっていない。本研究において、下垂体腺腫の線維化と関連する細胞の種類、その増減、その分布局在の変化を見出したことには一定の新規性があり、ヒト下垂体腺腫の組織学的理解に一定の貢献をするものであり、病理学的な意義が認められる。

以上より、申請者は学位授与に相応しいと審査員全員で判断した。