論 文 要 旨

Relationship between cytokeratin staining patterns and clinico-pathological features in somatotropinomae.

成長ホルモン産生下垂体腺腫における サイトケラチン染色パターンと臨床病理学的所見 との相関に関する研究

Yuriz Bakhtiar

(ユーリズ バクティアル)

【序論および目的】

Somatotropinomae are classified as densely and sparsely granulated adenomae, which typically exhibit a perinuclear pattern (PP) and a dot pattern (DP) in cytokeratin (CK) immunostaining respectively. Some exhibit a mixed pattern (MP). We studied the relationship between these somatotropinoma subtypes and their clinico-pathological features.

【材料および方法】

The study population consisted of 141 Japanese acromegalic patients. We evaluated their clinical presentation and their response to provocation tests with TRH and LHRH and to suppression (octreotide) test. Tumour tissues were subjected to immunostaining for CAM-5.2, MIB-1, CD34, E-cadherin (CDH1) and p53 (TP53). In 43 cases (30 non-DP and 13 DP), we analysed gsp mutations (constitutively activating mutations of the G(s)α protein that is encoded by GNAS gene).

【結 果】

The 141 adenomae were categorised into three subtypes based on their CK staining patterns; 30 (21.3%) exhibited DP, 83 (58.9%) exhibited PP, and 28 (19.9%) exhibited MP. Compared with the other subtypes, DP adenomae were significantly larger, and their E-cadherin expression and response to TRH, LHRH and octreotide challenge were lower. The

postoperative cure rate tended to be lower in DP adenomae. Gsp mutations were detected in 25 of 43 cases examined (58.1%); 20 of the 30 non-DP (66.7%) and 5 of the 13 DP tumours (38.5%) were affected by the mutation.

【結論及び考察】

DP somatotropinomae exhibit characteristic features. Compared with the non-DP subtypes, DP adenomae manifested a larger tumour size, a lower incidence of abnormal responses to TRH and LHRH challenge, a poor response to octreotide test and a lower expression of E-cadherin. gsp mutation was not exclusive for non-DP somatotropinomae.

（Europian Journal of Endocrinology　Vol.163, No.4　2010 年　掲載）

論文審査の要旨

報告番号	総研第 170 号		学位申請者	Yuriz Bakhtiar (ユーリズ・バクティアル)
審査委員	主査	米澤 傑	学位	博士 (医学・歯学・学術)
	副査	乾 明夫	副査	夏越 祥次
	副査	橋口 照人	副査	西尾 善彦

Relationship between cytokeratin staining patterns and clinico-pathological features in somatotropinomae
(成長ホルモン産生下垂体腺腫におけるサイトケラチン染色パターンと臨床病理学的所見との相関に関する研究)

成長ホルモン産生下垂体腺腫は、その電顕所見に基づいて、densely granulated adenoma と sparsely granulated adenoma に分けられる。両者は、サイトケラチン免疫染色の perinuclear pattern (PP) と dot pattern (DP) に一致することが知られているが、両者の臨床的、病理学的な差異については充分に明らかになっているとは言い難い。今回、学位申請者らは自験の成長ホルモン産生下垂体腺腫 141 例を基に両者の臨床的、病理学的な相違を検討し、また、成長ホルモン産生下垂体腺腫の発生に深く関与している G(s) α protein 遺伝子の変異出現との関係を求めた。

対象は日本人の先端巨大症患者 141 例。臨床像 (性、年齢、腫瘍径、術後治癒の有無、腫瘍体積あたりの成長ホルモン分泌能等)、TRH, LHRH, octreotide に対する成長ホルモンの反応を評価した。また、全腫瘍検体を CAM-5.2, MIB-1, CD34, E-cadherin (CDH1), p53 (TP53) 等で免疫染色した。43 例については凍結組織 (-80℃) が保存されていたので、G(s) α 蛋白をエンコードする GNAS gene の mutation の有無を調べた。

その結果、本研究で以下の知見が明らかにされた。

1) 141 個の成長ホルモン産生下垂体腺腫は CAM-5.2 によるサイトケラチン免疫染色所見から DP 30 例 (21.3%)、 PP 83 例 (58.9%)、MP (mix pattern) 28 例 (19.9%) に分けることが出来た。2) PP 群と MP 群 (併せて non-DP 群と称す) に比較して DP 群は、腫瘍径が有意に大きく、E-cadherin の細胞膜上の発現が有意に少なく、 TRH、LHRH に対する異常反応が有意に少なく、octreotide に対する低下反応が有意に乏しかった。 3) DP 群は手術による根治率が低い傾向にあった。 4) GNAS gene の遺伝子変異は対象 43 例中 25 例 (58.1%) で認められた。non-DP 群では 30 例中 20 例 (66.7%) で、DP 群 13 例では 5 例 (38.5%) であった。

以上のように本研究は、成長ホルモン産生腺腫中約 2 割を占める DP 群 (sparsely granulated adenoma) が他のサブタイプとは明らかに異なる臨床病理像を呈することを明らかにした。また従来、G(s) α 蛋白の遺伝子変異は専ら densely granulated adenoma (non-DP 群) にのみ認められると言われてきたが、本研究では、頻度は少ないながら DP 群にも認められることを明らかにした点で非常に興味深い。よって本研究は学位論文として十分な価値を有するものと判定した。

最終試験の結果の要旨

報告番号	総研第170号		学位申請者	Yuriz Bakhtiar (ユーリズ バクティアル)
審査委員	主査	米澤 傑	学位	博士 (医学・歯学・学術)
	副査	乾 明夫	副査	夏越 祥次
	副査	橋口 照人	副査	西尾 善彦

主査および副査の５名は、平成24年1月30日、学位申請者 Yuriz Bakhtiar 君に面接し、学位申請論文の内容について説明を求めると共に、関連事項について試問を行った。具体的には、以下のような質疑応答がなされ、いずれについても満足すべき回答を得ることができた。

Q1) How could Dot Pattern or Non-dot pattern cytokeratin staining express in somatotropinoma?

A) It could be due to the loss of E-cadherin expressions in the surface of cells in dot pattern adenomas. Then this loss expression could inhibit desmosomal plaque and lead to cytokeratin collapse.

Q2) Is it right that 4 sites of gsp mutation are constitutively activating cAMP signal?

A) Yes it is.

Q3) Does the gsp mutation have an impact on tumor behavior, invasion or proliferation?

A) On this study, MIB-1 index did not correlate with gsp mutation type of the tumor, but the proliferation in early event of the tumorigenesis could be affected by the presence of *gsp* mutation. Some reports said that the constitutive activation of cAMP in gsp mutated cell could activate or inhibit the proliferation signal. And it was hypothesized that variation of counteracting relaxation of the Gs α expression from the paternal allele in gsp mutated cells may induce different features of adenoma progression in this tumor.

Q4) Why did you choose this sequence of gsp mutation?

A) Because the mutation site of Gs α protein in pituitary adenoma cells has been reported to mostly locate in codon 201 and 227.

Q5) In cytokeratin staining or gsp gene analysis, which is the best predictor for the prognosis?

A) In this study, the gsp mutation correlates well to the endocrine features. But the prognosis was not different between two cytokeratin types of somatotropinoma. In clinical setting, non dot pattern of cytokeratin staining may predict the response of somatostatin-analogue treatment because non dot pattern adenoma revealed more frequently good response to the octreotide test.

Q6) Is it acceptable that the gsp mutation is the major mutation in somatotropinoma?

A) Yes, it is the major protein harboring mutation among G-proteins of somatotropinoma.

Q7) Where do cytokeratin express in the sparsely granulated adenoma?

A) Cytokeratin in sparsely granulated adenoma was expressed within fibrous body as a dot.

Q8) What factor could affect the location of the cytokeratin pattern in dot pattern adenoma?

A) As one of factors involving cell-cell adhesion, I think loss of E-cadherin expression in dot pattern adenoma play a main role in cytokeratin collapse.

最終試験の結果の要旨

Q9) Could you explain the result of Ki67 immunostaining in your study?

A) Ki67 immunostaining was expressed as an MIB-1index in our study. And, we could not find its correlation with both cytokeratin type or gsp mutation types adenoma.

Q10) Could you explain the result on the differences between TRH test response and cytokeratin or gsp mutation?

A) Higher incidence of abnormal TRH response in not dot pattern adenoma may be due to the higher expression of TRH-receptor mRNA in densely granulated adenoma.

And higher incidence of abnormal TRH response in mutation type tumor may be due to the presence of ectopic TRH receptor which has been noted to be induced by increased cAMP signaling or high intracellular cAMP protein level.

Q11) Why did you choose the CAM5.2 for the cytokeratin staining?

A) Because CAM 5.2 is commonly cited as reacting with cytokeratin 8 & 18. This type of antibody can detect a low-molecular-weight keratin and seems to be most appropriate for the detection of cytokeratin in pituitary adenomas, because the endocrine cells of the human pituitary have been reported to express mostly cytokeratin 8 and 18.

Q12) Is there any correlation between E-cadherin and tumor behavior or other proliferation factor?

A) So far from our data, there is a correlation between lower expression of E-cadherin and bigger tumor size.

Q13) Which is better in prognosis between Dot pattern and Non Dot pattern?

A) Cure rate in our study is better in non-Dot pattern adenoma but insignificant because small residual tumor with lower GH producing index may be judged as "cured" using a commonly used criteria of cure.

Q14) Is it right that the small tumor is non dot pattern?

A) In this study it was true that the non dot pattern is smaller than dot pattern adenoma, but it cannot be assumed that all of small tumors are non dot pattern. Because when we divide the tumor into micro and macroadenomas, there were no differences of incidence between dot pattern and non –dot pattern.

Q15) Is there any literature that explains the Epithelial-mesenchymal transition process in pituitary adenoma?

A) No, there is not, as far as my understanding.

Q16) Can you establish the new pathological criteria of tumor from your study?

A) In several studies said that cytokeratin pattern could be a good surrogate of densely and sparsely granulated adenoma, but establishing of mix pattern cytokeratin type and gsp mutation type somatotropinoma needs further discussion of its pathogenesis.

Q17) Preoperatively, could you predict the type of tumor by watching the clinical characteristic of patients?

A) Yes, we can predict in some cases. For example a big adenoma in a young age patient with clinically no response to TRH test could be predicted as dot pattern adenoma.

Q18) Are there any reports on gene expression concerning E-cadherin regression?

A) So far, I just only know a report on loss expression of E-cadherin correlating with lower expression of β-catenin.

Q19) Are there any reports about the cAMP level or protein A-kinase level in E-cadherin gene regression?

A) As far as I know, there were no reports on those.

最終試験の結果の要旨

Q20) Regarding other sites of G protein gene, is there any reports on other mutation site on *gsp* mutation (-) adenoma?

A) In GNAS1 gene of pituitary adenoma, I know that mutation of Gs protein is only in codon 201 and 227

Q21) Are there any relation between somatostatin response and dopamine analog response?

A) It is difficult to answer whether there is any correlation or not because our available data is limited to 39 cases in which only somatostatin analog (octreotide) test was conducted.

Q22) Does the Dot patterns which tend to have higher proliferation rate respond well to radiotherapy?

A) In general agreement as standard treatment for acromegaly, medical and radiotherapy should be reserved as the second line therapy after surgical failure. We can suppose that higher proliferation neoplasms respond well to radiotherapy. But, we didn't have the evidence whether dot pattern respond well to radiotherapy because of paucity of cases those underwent radiation treatment.

Q23) Why did you classify both mix pattern adenoma and perinuclear adenoma in one group of Non dot pattern adenoma?

A) In the table 3 and 4 of our study, we found special characteristic in pure dot pattern adenoma comparing to mix pattern adenoma and perinuclear adenoma, therefore we include mix pattern and perinuclear pattern as non dot pattern adenoma.

Q24) Can you distinguish the intensity of GH staining pattern in both type of tumor (Dot pattern and Non dot pattern)?

A) No I could not, because it was diffusely stained in both types in my inspection.

Q25) Is there any co-expression of GH staining and cytokeratin staining?

A) I am sorry I do not know, because I did not do the co-expression analysis of both staining.

Q26) Do you have any data on the expression of E-cadherin staining in atypical pituitary adenoma?

A) Yes I have, because one patient of our series with atypical adenoma characterized with high MIB-1 index, high p53, and dot pattern of cytokeratin also had lower expression of E-cadherin.

Q27) Do you have any data on other types of cytokeratin staining expression?

A) Until now, I am sorry, I don't have.

Q28) Is there any report of other cytokeratin staining on pituitary adenoma?

A) So far as I know, yes there is. They analyzed the different expression of cytokeratin 7 and cytokeratin 20 in different type of pituitary adenoma and its comparison with CAM 5.2. They asserted that the cytokeratin 20 was expressed mostly in corticotrophs and sparsely granulated growth hormone adenomas.

以上の結果から、５名の審査委員は申請者が大学院博士課程修了者としての学力・識見を有しているものと認め、博士（医学）の学位を与えるに足る資格を有するものと認定した。