박 사 학 위 논 문

PTTG Expression in Non-functioning Pituitary Adenomas: Correlation with Tumor Invasiveness

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이 논문을 박사학위 논문으로 제출함

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금 수 정

Table of Contents

List of Tables

List of Figures

1. Introduction

Pituitary adenoma is a common neuroendocrine tumor that accounts for approximately 17% of all primary intracranial neoplasms (1). Most of these tumors are benign but some show aggressive patterns, such as invasion into surrounding structures. In contrast to the functioning pituitary adenomas, which are usually quickly detected due to symptoms of excess hormone secretion, detection of a non-functioning pituitary adenoma (NFPA) is relatively delayed. Therefore, NFPAs are usually found as macroadenomas (1–4 cm) or giant adenomas (> 4 cm) with suprasellar extension, which tend to invade the cavernous sinus. In addition, the effectiveness of hormone control therapy for NFPA is limited, and surgical removal is the only effective treatment (2,3). Based on these aspects, it is clinically important to identify the prognostic markers of NFPA.

In the 2004 World Health Organization (WHO) classification, three subcategories were proposed for the classification of primary pituitary tumors: typical, atypical, and carcinoma (4). Atypical pituitary adenoma was diagnosed based on histopathological features, including a high Ki-67 proliferation index (> 3%), p53 expression, and a high mitotic count. However, as the WHO classification was revised in 2017, the term “atypical pituitary adenoma” is no longer recommended, based on studies reporting that this subtype does not reflect prognosis (5-7). Instead, histological or radiological invasiveness status has emerged as an important factor for predicting prognosis, which was also introduced in the 2017 WHO classification (5,8). In general, two factors are mainly used to evaluate the invasiveness status of pituitary adenoma; cavernous sinus invasion (CSI) and suprasellar extension (SSE). It is widely

known that CSI is directly associated with prognosis (9). On the other hand, there are many reports that SSE alone lacks prognostic value (10-12). However, several grading systems combining SSE and CSI have shown significant prognostic value and are widely used currently (13).

p53 was generally used as a biomarker for aggressive pituitary adenoma until the term “atypical pituitary adenoma” was accepted (14). However, as studies asserting that p53 is not suitable as a prognostic marker have been continuously published, there are currently no biomarkers of aggressive pituitary adenomas with proven validity (9,15,16). Only a few candidate proteins are currently being studied (17). Among many candidates of the prognostic biomarkers, this study focused on three proteins: pituitary tumor transforming gene (PTTG), paired-like homeodomain 2 (PITX2), and galectin-3.

PTTG is a multifunctional oncogene expressed in various organs, including the pituitary gland. PTTG participates in tumorigenesis via various mechanisms such as mitosis, DNA repair, and angiogenesis (18-20). In addition, PTTG is known to induce tumor aggressiveness by being involved in epithelial-to-mesenchymal transition (EMT), and several studies have reported that PTTG causes E-cadherin loss in the intermediate process (19,21,22). Also, there are several studies on PTTG expression and cell proliferation, and their results are conflicting (23). Although some studies have evaluated the association between PTTG expression and the invasiveness of pituitary adenomas, only a few studies conducted to date have focused on NFPA (18,24).

PITX2 is a member of the paired-like homeobox transcription factor family, which is necessary for the development of various organs. It regulates the expression of cell cycle regulators, such as cyclin D1, cyclin D2, and c-Myc (25,26). Recent studies have demonstrated a

relationship between PITX2 expression and aggressive behavior of various tumors, including ovarian cancer, colorectal cancer, thyroid cancer, lung adenocarcinoma, and esophageal squamous cell carcinoma (27-31). Additionally, a few studies have reported the correlation between PITX2 and the invasiveness of NFPAs (32,33).

Galectin-3, a β-galactoside-binding protein, binds to carbohydrates on the cell surface, and is involved in various biological processes, including cell growth, angiogenesis, cell adhesion, apoptosis, and tumor progression (34,35). These functions of the galectin-3 have been identified in various organs, such as the thyroid gland, colon, liver, brain, and pituitary gland (36-39). Although many previous studies have demonstrated that pituitary adenoma show galectin-3 overexpression, only a few studies have investigated a large number of NFPA-limited patients (37,38). In addition, few studies have focused on tumor invasiveness (40,41).

In this study, we aimed to determine the correlation among PTTG, PITX2, and galectin-3, and the various clinicopathologic characteristics of the NFPAs, including tumor invasiveness status. Moreover, we evaluated the relationships among PTTG, E-cadherin, and Ki-67 in NFPA. This study is significant because it focuses on NFPA, and to the best of our knowledge, it is the first study to investigate PTTG, PITX2, and galectin-3 expression in Korean patients with pituitary adenoma.

2. Materials and Methods

2.1. Patients:

This study was approved by the Institutional Review Board (IRB) of Keimyung University Dongsan Medical Center (DSMC 2021-02-036). The archived specimens of 124 patients with NFPA obtained from 2000 to 2019 by surgical resection, including the trans-sphenoidal approach, at Keimyung University Dongsan Hospital (Daegu, Korea) were analyzed in this study. A pathological diagnosis was made based on hematoxylin and eosin staining and immunohistochemical staining for six pituitary hormones (growth hormone, thyroid-stimulating hormone, prolactin, follicle-stimulating hormone, luteinizing hormone, and adrenocorticotropic hormone) in the surgical specimens. Clinical confirmation that these adenomas did not exhibit excess hormone secretion was based on checking the patients' serum hormone levels and the absence of hormone-related symptoms. Cases showing positive immunohistochemical staining but not associated with excess hormone secretion were diagnosed as NFPAs. Sufficient and adequate tissue for the construction of microarrays was available for 87 of the 124 cases.

Clinical data, including age, sex, symptoms, recurrence, and survival status, were obtained from a retrospective chart review.

2.2. Radiographic Analysis:

Tumor size and invasion status were evaluated using magnetic resonance imaging (MRI). Tumor size was recorded as the longest

diameter. Cavernous sinus invasion (CSI) was evaluated based on the criteria presented by Cottier (42). The samples were then classified into three groups: group I, neither suprasellar extension (SSE) nor CSI; group II, only one of SSE or CSI; and group III, both SSE and CSI. We considered that a higher group level indicated a more aggressive tumor.

2.3. Construction of Tissue Microarrays (TMA):

Four TMA blocks were constructed from archived formalin-fixed paraffin blocks of the 87 samples with sufficient tumor cells. After checking the tumor cell-rich area on the hematoxylin and eosin slides, a 3-millimeter-diameter core was collected from each sample and then arranged on premade recipient paraffin blocks (UB06-3, UNITMA, Seoul, Korea).

2.4. Immunohistochemistry:

Information on the primary antibodies used for immunohistochemistry is presented in Table 1. Sections (5 μm in thickness) were obtained from the four tissue microarray blocks described above. Immunohistochemical staining for PTTG, E-cadherin, Ki-67, PITX2, and galectin-3 was performed using an automated slide-processing system (BenchMark XT, Ventana Medical System, Tucson, AZ, USA). The cut sections were pretreated with Cell Conditioner 1 (CC1, Cat#950-124, Ventana Medical System, Tucson, AZ, USA) for 40 min. The sections were then incubated with diluted primary antibodies for 32 min (Table 1). The OptiView DAB Detection Kit (Cat#760-700, Ventana Medical System, Tucson, AZ, USA) was used for chromogenic detection.

Protein expression was evaluated by scoring the intensity of each stain on a scale of 0 (negative), 1 (weak), 2 (moderate), and 3 (strong). Since the proportion of each stain is uniform in most of the tumors, the expression level was determined only by the intensity of the stain. For Ki-67 staining, the stained cells were counted using a computer-assisted image analyzer (GenASIs HiPath, Applied Spectral Imaging Inc., Carlsbad, CA, USA).

2.5. Statistical Analysis:

Chi-squared test was used to determine the difference between expression groups of PTTG, PITX2, galectin-3, and E-cadherin, associated with clinical characteristics (e.g., SSE, CSI, sex, and recurrence). Linear-by-linear association was used to evaluate the correlation of the three invasiveness groups with PTTG, PITX2, and galectin-3 expression. Independent T-test was used to analyze the relationships among the three candidate biomarkers and clinical factors, including age, tumor size, and Ki-67 index. Analysis of variance (ANOVA) test was used to find a difference between the three invasiveness groups associated with age and tumor size. Ordinal logistic regression model was used to compare the influence of individual factors, including PTTG, PITX2, galectin-3, E-cadherin, Ki-67, age, sex, and tumor size, on NFPA invasiveness. All analyses were performed using IBM SPSS Statistics for Windows, version 25 (IBM Corp., Armonk, NY, USA). Statistical significance was set at $p < 0.05$.

Table 1. Information of the Antibodies Used for Immunohistochemical Staining

Antibody	Manufacturer and product number	Positive control	Dilution
PTTG (securin)	ThermoFisher, #34-1500	Testis	1:200
E-cadherin	Dako, #M3612	Large intestine	1:400
Ki-67	Abcam, #ab16667	Large intestine	1:200
PITX2	Abcam, #ab32832	Large intestine	1:50
Galectin-3	ThermoFisher, #18-0393	Large intestine	1:200

PTTG: pituitary tumor transforming gene; PITX2: paired-like homeodomain 2

3. Results

3.1. Patient Characteristics:

The characteristics of the 87 patients with NFPA are shown in Table 2. The median age was 54 years (range, 16–83 years). Among these 87 patients, 48 (55.2%) were men and 39 (44.8%) were women. The mean tumor size (± SD) was 28.3 ± 12.5 mm and there were two cases with missing tumor size data.

Overall, 77 (88.5%) patients showed SSE and 33 (37.9%) showed CSI on MRI (Table 2). Among the 87 patients, 7 (8.0%) were in group I, 50 (57.5%) were in group II (SSE only: 47, CSI only: 3), and 30 (34.5%) were in group III (Figure 1).

Thirty-five (40.2%) patients underwent post-operative radiotherapy. During each follow-up period (mean 66 months, range 0.5 - 209 months), tumor recurrence was observed in 16 patients (18.4%), and non-disease-related death was observed in one patient. Disease-related death was not observed.

3.2. Expression of PTTG, PITX2, Galectin-3, and Their Relationship with Invasiveness of NFPA:

The individual staining patterns and scores of PTTG, PITX2, and galectin-3 are shown in Figure 2. Staining of PTTG was observed in the cytoplasmic and paranuclear patterns. In PTTG staining, a score of 0 was considered negative expression, scores 1 and 2 were considered

low expression, and a score of 3 was considered high expression. Staining of PITX2 and galectin-3 showed both nuclear and cytoplasmic patterns. In PITX2 and galectin-3 stains, score 0 was considered negative, score 1 was considered low expression, and scores 2 and 3 were considered high expression. Ten (11.5%) specimens were negative for PTTG, and 52 (59.8%) and 25 (28.7%) specimens showed low and high PTTG expression, respectively. In PITX2 staining, 21 (24.1%) of the specimens were negative, 30 (34.5%) showed low expression, and 36 (41.4%) showed high expression. In galectin-3 staining, 37 (42.5%) were negative, 27 (31.0%) showed low expression, and 23 (26.4%) showed high expression.

PTTG expression was significantly correlated with NFPA invasiveness. In the high PTTG expression group, SSE and CSI are observed more frequently than those in the negative or low PTTG expression group ($p < 0.05$, Table 3). Similarly, the higher invasiveness group levels showed higher PTTG expression, and the lower invasiveness group showed lower PTTG expression ($p < 0.05$, Table 4, Figure 4). There was no association between PITX2 or galectin-3 expression and invasiveness (Table 4). PTTG, PITX2 and galectin-3 did not show any relationship with other clinical characteristics, such as age, sex, tumor size, and recurrence. Even in the group without post-operative radiotherapy, there was no correlation between PTTG expression and recurrence (Table 3).

3.3. Relationships of E-cadherin Loss and Ki-67 Index Associated with PTTG Expression:

The individual staining patterns of E-cadherin, and Ki-67 are shown in Figure 3. The E-cadherin was stained in a membranous pattern. A score of 0 was considered negative, and scores of 1 to 3 were considered positive for E-cadherin staining. Forty-eight (55.2%) of the specimens were negative and 39 (44.8%) showed positive E-cadherin expression. In the high PTTG expression group, 11 (44%) showed negative E-cadherin expression. In negative or low PTTG expression group, 37 (60%) showed negative E-cadherin expression (Table 3). There was no significant correlation between the expression of E-cadherin and PTTG.

Ki-67 staining showed nuclear staining, and the overall average index (± SD) was 1.4 ± 1.4%. The average Ki-67 index in the high PTTG expression group was 1.8 ± 2.5%, which was higher than that observed in the negative or low PTTG expression group (1.3 ± 1.1%) (Table 3). Ki-67 expression tended to be associated with PTTG expression. However, this result was not statistically significant ($p > 0.05$).

3.4. Other Clinical Factors Associated with Aggressive Features of NFPA:

The tumor invasiveness was significantly correlated with the tumor size; the higher the level of the invasion group, the larger the tumor size ($p < 0.001$, Table 4 and Figure 5A). In addition, tumor size was associated with the recurrence of NFPA (Figure 5B). As the size of the tumor increased, the NFPA tended to recur. However, post-operative radiotherapy was not considered in this study.

3.5. Multivariate Analysis for Invasiveness of NFPA:

In multivariate analysis using ordinal logistic regression, PTTG expression and tumor size were statistically associated with tumor invasiveness group levels (p <0.05) (Table 5). The other variables including PITX2, galectin-3, E-cadherin, Ki-67, age, and sex did not show any relationships with tumor invasiveness groups. In addition, the PTTG expression and the tumor size were statistically independent of each other or other variables, including PITX2, galectin-3, E-cadherin, Ki-67, age, and sex (Table 5).

Table 2. Clinical Characteristics of Patients with NFPA

Clinical data	Number of patients (%)
Age (yr)	
Median (range)	54 (16-83)
Sex	
Male	48 (55.2)
Female	39 (44.8)
Tumor size (mm)*	
Mean ± SD	28.3 ± 12.5
Post-operative radiotherapy	
Yes	35 (40.2)
No	52 (59.8)
Recurrence	
Present	16 (18.4)
Absent	71 (81.6)
Suprasellar extension (SSE)	
Present	77 (88.5)
Absent	10 (11.5)
Cavernous sinus invasion (CSI)	
Present	33 (37.9)
Absent	54 (62.1)

SD: Standard deviation

* Unknown tumor size in 2 cases.

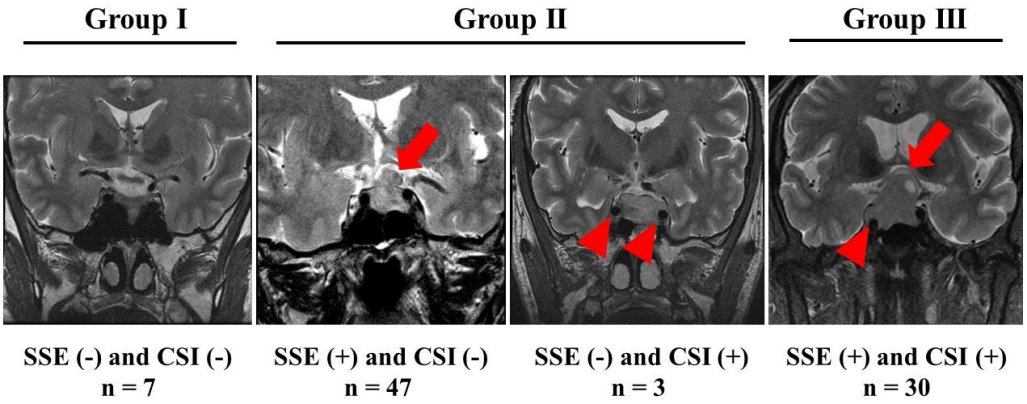

Figure 1. Invasiveness groups of NFPA based on suprasellar extension (SSE) and cavernous sinus invasion (CSI) on MRI. The representative MRI images of SSE (arrow) and CSI (arrowhead) are shown. NFPAs are classified into three groups based on the MRI findings: Group I, neither SSE nor CSI; Group II, only one of SSE or CSI; Group III, both SSE and CSI.

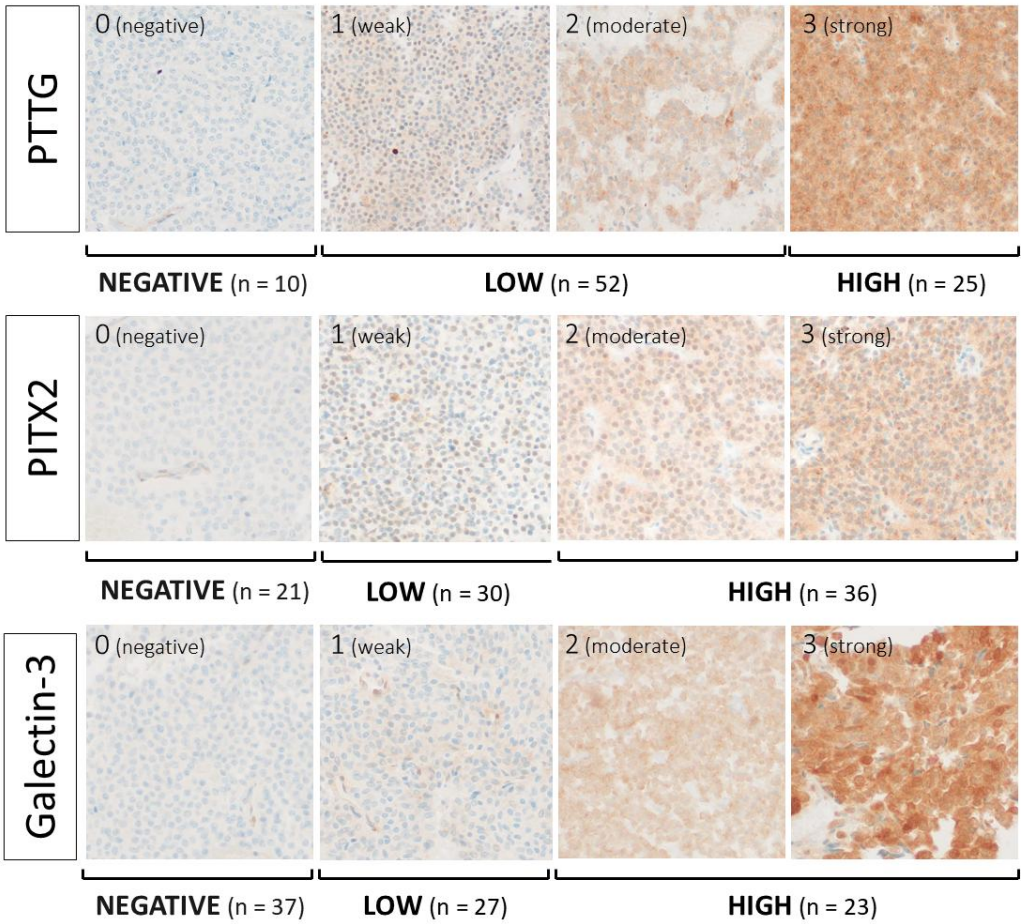

Figure 2. Scoring of immunohistochemical staining for PTTG, PITX2, and galectin-3. The intensity of immunohistochemical stains is scored from 0 (negative) to 3 (strong). PTTG: score 0 is regarded as negative, scores 1 and 2 are regarded as low, and score 3 is regarded as high expression (x 200). PITX2 and galectin-3: score 0 is regarded as negative, score 1 is regarded as low, and scores 2 and 3 are regarded as high expression (x 200).

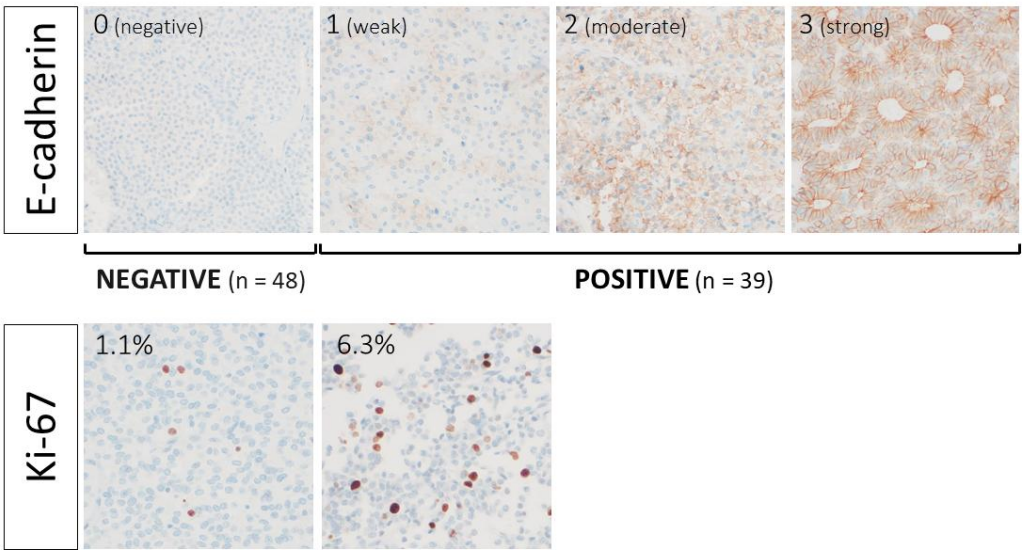

Figure 3. Scoring of immunohistochemical stainings for E-cadherin and Ki-67. E-cadherin: score 0 is regarded as negative, and scores 1 to 3 are regarded as positive expression (x 200). Ki-67 index is measured by image analyzer and two samples of the stain are shown (x 200).

Table 3. Clinicopathologic Characteristics and PTTG Expression

Characteristics	N (%)		*p*-value
	Negative or low PTTG (n = 62)	High PTTG (n = 25)	
Age (yr)			
Median (range)	53 (31-83)	55 (16-83)	0.656
Sex			
Male	37 (59.7)	11 (44.0)	0.183
Female	25 (40.3)	14 (56.0)	
Invasiveness status			
Suprasellar extension			
Present	52 (83.9)	25 (100.0)	0.033*
Absent	10 (16.1)	0 (0.0)	
Cavernous sinus invasion			
Present	19 (30.6)	14 (56.0)	0.027*
Absent	43 (69.4)	11 (44.0)	
Tumor size (mm)**			
Mean ± SD	29.0 ± 13.5	26.5 ± 9.2	0.612
Recurrence			
Present	13 (21.0)	3 (12.0)	0.329
Absent	49 (79.0)	22 (88.0)	
Present (N-PRT)	11 (28.9)	0 (0.0)	0.103
Absent (N-PRT)	27 (71.1)	14 (100.0)	
E-cadherin			
Positive (score 1-3)	25 (40.3)	14 (56.0)	0.183
Negative (score 0)	37 (59.7)	11 (44.0)	
Ki-67 index (%)			
Mean ± SD	1.3 ± 1.1	1.8 ± 2.5	0.389

SD: Standard deviation; N-PRT: No post-operative radiotherapy

* Statistically significant ($p < 0.05$).

** Unknown tumor size in 2 cases with low PTTG expression.

Table 4. Clinicopathologic Characteristics and Tumor Invasiveness Group

Characteristics	Tumor invasiveness group			*p*-value
	I (n = 7)	II (n = 50)	III (n = 30)	
Age (yr)				
Median (range)	50 (42-71)	55 (16-83)	54.5 (31-83)	0.605
Sex (%)				
Male	3 (42.9)	28 (56.0)	17 (56.7)	0.790
Female	4 (57.1)	22 (44.0)	13 (43.3)	
Tumor size (mm)*				
Mean ± SD	17.2 ± 5.1	25.9 ± 9.2	35.3 ± 15.0	< 0.001**
PTTG (%)				
High	0 (0.0)	11 (22.0)	14 (46.7)	0.003**
Low or negative	7 (100.0)	39 (78.0)	16 (53.3)	
PITX2 (%)				
High	3 (42.9)	19 (38.0)	14 (46.7)	0.745
Low or negative	4 (57.1)	31 (62.0)	16 (53.3)	
Galectin-3 (%)				
High	1 (14.3)	15 (30.0)	7 (23.3)	0.605
Low or negative	6 (85.7)	35 (70.0)	23 (76.7)	
E-cadherin (%)				
Positive	3 (42.9)	23 (46.0)	13 (43.3)	0.989
Negative	4 (57.1)	27 (54.0)	17 (56.7)	
Ki-67 index (%)				
Mean ± SD	1.5 ± 0.8	1.6 ± 2.0	1.2 ± 1.1	0.681

SD: Standard deviation

* Unknown tumor size in 2 cases in group III

** Statistically significant ($p < 0.05$).

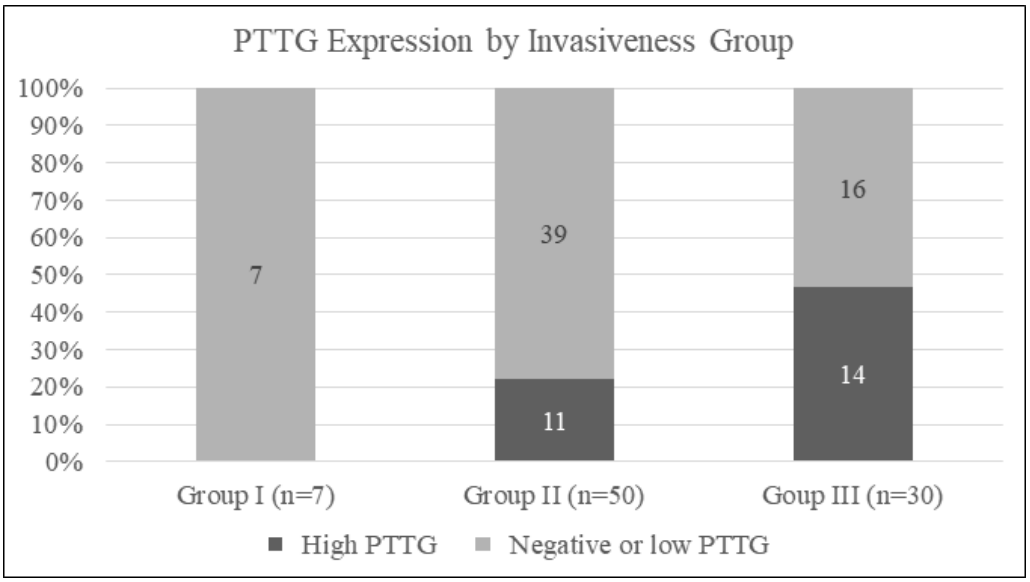

Figure 4. Stacked column chart showing the ratio of PTTG expression in each invasiveness group. As the level of the invasiveness group increases, the proportion of high PTTG expression tends to increase.

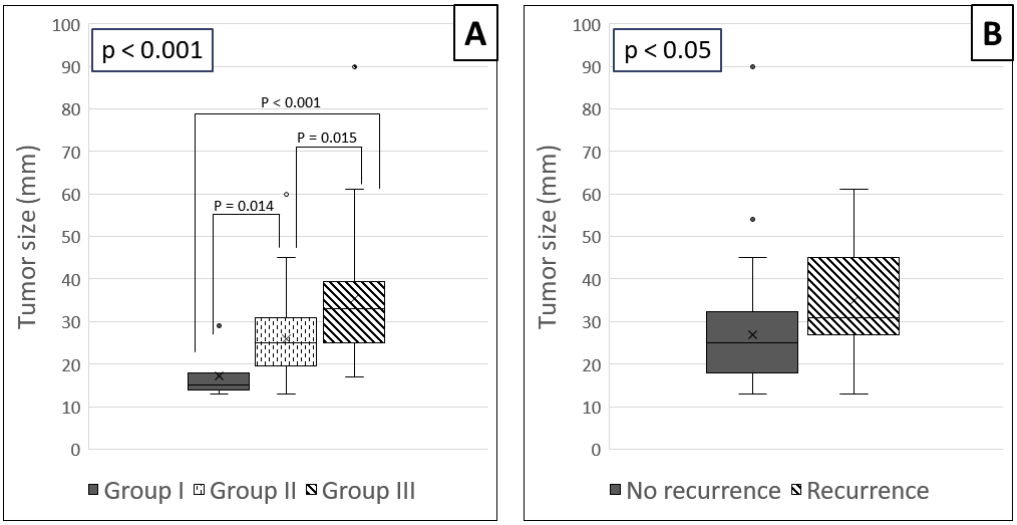

Figure 5. Box plots of distribution of the tumor size by invasion group and recurrence. Larger tumor size is associated with higher invasiveness (A) and higher recurrence rate (B).

Table 5. Multivariate Analysis (Ordinal Logistic Regression Test) of Invasiveness Groups Associated with Clinicopathologic Variables

Variables	Estimate	*p*-value	95% Confidence interval	
			Lower	Upper
Age	0.030	0.180	-0.014	0.074
Sex	-0.085	0.876	-1.152	0.983
Tumor size	0.13	< 0.001*	0.069	0.191
PTTG	2.518	0.001*	1.086	3.950
PITX2	-0.320	0.391	-1.052	0.411
Galectin-3	0.195	0.575	-0.485	0.875
E-cadherin	0.482	0.498	-0.914	1.879
Ki-67 index	0.347	0.696	-1.398	2.093

* Statistically significant ($p < 0.05$).

4. Discussion

Although several studies have shown PTTG overexpression in pituitary adenoma and in tumors of other organs, a limited number of studies have investigated the relationship between PTTG expression and tumor invasiveness (18,43,44). A meta-analysis of these studies concluded that there was a significant relationship between PTTG expression and the invasiveness of pituitary adenoma (45). However, most of these studies were limited to a specific subtype of functioning pituitary adenoma or did not distinguish between functioning and non-functioning adenomas.

In this study, we performed an analysis limited to NFPA, and demonstrated that PTTG expression was significantly correlated with the invasiveness of NFPA. Only a few previous studies have explored PTTG expression and invasiveness status limited to NFPA (18,24,43). One of these study revealed that PTTG expression and the invasiveness status of NFPA had an important correlation (24). Also, another study showed PTTG overexpression in both invasive NFPAs and invasive functioning pituitary adenomas (18). The results of the present study support these two studies. In contrast, there is a previous study that did not identify a correlation between PTTG expression and invasiveness in NFPA, but did reveal the correlation in functioning pituitary adenoma (43). However, the number of the NFPA samples in their study was 30 which was smaller than that of studies which confirmed the significant correlation between PTTG and NFPA invasiveness, including our study: the respective NFPA sample numbers were 92, 56, and 87 (18,24). It is reasonable to assume that PTTG expression is correlated with NFPA invasiveness like other organ tumors.

Several studies have revealed a correlation between PTTG expression and recurrence of pituitary adenomas (24,46,47). One of these studies did not report the factor of post-operative radiotherapy (46). Also, other studies excluded the patients who underwent post-operative radiotherapy (24,47). The present study did not reveal a correlation between PTTG expression and recurrence. We also analyzed PTTG expression and recurrence in a group of patients who did not undergo post-operative radiotherapy, but no meaningful results were obtained contrary to the results of previous studies.

A few studies have demonstrated that PITX2 expression is increased in pituitary adenomas (32,33). Moreover, these studies highlighted a correlation between PITX2 overexpression and the aggressiveness of NFPA (32,33). However, the results of the present study did not show a relationship between PITX2 expression and NFPA invasiveness. These contradictory results are probably due to the small number of cases in our study. However, since few studies have been conducted on this subject to date, more data should be accumulated to establish the relationship between the PITX2 expression and NFPA invasiveness.

In pituitary adenoma, galectin-3 expression is increased in functioning pituitary adenomas, especially in functioning corticotroph adenomas, but not in NFPAs (37,41). In addition, there are several studies on galectin-3 and the aggressive behavior of pituitary adenoma, which demonstrated that galectin-3 expression is positively correlated with the aggressiveness of pituitary adenomas, but not that of NFPAs (40,41). In the present study with only NFPAs, except for functioning adenomas, the intensity of galectin-3 staining was varied and there was no correlation with the invasiveness of NFPAs. Although we noted a high intensity of galectin-3 staining in our study, the validity of this result should be reassessed because we did not compare the intensity in

NFPAs with that in functioning adenomas.

As the PTTG was the only protein that showed an association with the invasiveness of NFPAs in this study, further investigation was performed on the relationship among PTTG, E-cadherin, and Ki-67. Based on the previous studies, which showed an association between PTTG and E-cadherin loss in the process of EMT in other organs (e.g., head and neck, esophagus, ovary, and breast), we hypothesized that E-cadherin is correlated with PTTG expression in NFPAs, and this is the first study on PTTG and E-cadherin in pituitary adenoma (19,21,22). However, we could not identify the relationship between PTTG expression and E-cadherin loss in NFPAs. This may be the result of a small number of cases, but it may also be the result of PTTG not being the main causative factor for E-cadherin loss. There are several epigenetic mechanisms that induce E-cadherin loss other than PTTG, including some microRNAs (e.g., miR-192, miR-200, miR-205, etc.) and hypermethylation of promoter of *CDH1*, a gene encoding E-cadherin (48, 49). The results of our study suggest that the factors such as the microRNAs and hypermethylation of *CDH1* promoter may play a greater role than PTTG in inducing E-cadherin loss in pituitary adenomas.

There are some previous studies on PTTG expression and cell proliferation, but the results are conflicting (50-53). Some of these studies revealed that PTTG promoted cell proliferation (50,51). However, other studies revealed that there was no correlation between PTTG and cell proliferation, which is consistent with the results of the present study (52,53). In terms of its ability to inhibit chromatid separation during mitosis, PTTG is expected to inhibit cell proliferation, but its ability to induce angiogenesis or disrupt the DNA repair system may promote cell proliferation. Since there are contradictory views in many studies, the relationship between PTTG expression and cell proliferation

needs to be further studied.

Additionally, we determined that the invasiveness status and recurrence of NFPAs were significantly correlated with tumor size. According to many studies, pituitary adenoma shows aggressive behavior, as the size of the tumor increases, which is consistent with the results of our study (54). However, with the respect to tumor recurrence, previous studies have shown that tumor recurrence is not influenced by tumor size, which contradicts the results of our study (55). Although the results of our study revealed the correlation between tumor size and recurrence, it should be noted that the factor of post-operative radiotherapy was not considered.

In conclusion, we demonstrated here that PTTG has the potential to be a predictive marker for the invasiveness of NFPAs. Although there are many previous studies that have explored the relationship between PTTG expression and tumors including pituitary adenoma, the present study is meaningful as we elucidate the role of PTTG, particularly in NFPAs. Furthermore, we provide evidence for the development of PTTG-targeting agents and reference for studies on the correlation between PTTG and various tumors, which have been continuously reported.

5. Summary

PTTG, PITX2, and galectin-3 are emerging proteins that have potential as predictive biomarkers for various tumors. However, only a few studies have investigated NFPAs, which usually show more aggressive behavior than functioning adenomas. The present study evaluated PTTG, PITX2, and galectin-3 as predictive biomarkers for invasive NFPA, by determining the correlation between their expression and NFPA invasiveness. NFPA samples were classified into three groups based on the MRI findings of suprasellar extension and cavernous sinus invasion. Immunohistochemical staining of PTTG, PITX2, and galectin-3 was performed using tissue microarrays of the samples. Stains for E-cadherin, and Ki-67 staining were also performed to investigate their relationship with PTTG. In result, PTTG showed a significant correlation with NFPA invasiveness. PITX2 and galectin-3 did not show any association with the invasiveness of NFPA, and there was no relationship among PTTG, E-cadherin, and Ki-67 index. In conclusion, PTTG has the potential to serve as a predictive biomarker for the invasiveness of NFPA. Furthermore, these results may serve as an evidence for the development of PTTG-targeting therapeutic agents.

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PTTG Expression in Non-functioning Pituitary Adenomas: Correlation with Tumor Invasiveness

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(Abstract)

Pituitary tumor transforming gene (PTTG), paired-like homeodomain 2 (PITX2), and galectin-3 have been widely studied as predictive biomarkers for various tumors, and these proteins are involved in tumorigenesis and tumor progression. We evaluated PTTG, PITX2, and galectin-3 as predictive biomarkers for invasive non-functional pituitary adenomas (NFPAs) by determining the relationship between the expression of these three proteins and the invasiveness of NFPAs. We also investigated whether PTTG, E-cadherin, and Ki-67, which are known to be related to each other, show a correlation with NFPA features. We evaluated the invasiveness of 87 NFPAs by classifying them into three groups based on magnetic resonance imaging findings of

suprasellar extension and cavernous sinus invasion. Immunohistochemical staining for PTTG, PITX2, galectin-3, E-cadherin, and Ki-67 was performed on tissue microarrays. The results showed that PTTG expression was significantly correlated with the invasiveness of NFPAs, while PITX2 and galectin-3 did not reveal any relationship with invasiveness of NFPAs. Moreover, there was no association between PTTG, E-cadherin, and Ki-67 expression. In conclusion, PTTG has the potential to serve as a predictive biomarker for invasive NFPA. Furthermore, this study may serve as a reference for the development of PTTG-targeting therapeutic agents.

비기능뇌하수체샘종에서의 PTTG의 발현: 종양의 침습성과의 상관관계

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(초록)

Pituitary tumor transforming gene (PTTG), paired-like homeodomain 2 (PITX2), 그리고 galectin-3는 다양한 종양에서의 예측성 생체표지자로서 활발하게 연구되고 있다. 이 단백질들은 각자의 생물학적 기능으로서 종양형성과 종양진행에 관여한다. 본 연구는 이 세 가지 단백질의 발현과 비기능뇌하수체샘종의 침습 정도의 관련성을 밝힘으로써 비기능뇌하수체샘종의 예측성 생체표지자로서 PTTG, PITX2, 그리고 galectin-3를 평가하였다. 또한, 서로 관련이 있다고 알려진 PTTG, E-cadherin, 그리고 Ki-67이 비기능뇌하수체샘종에서 실제로 상관성 보이는지 알아보고자 하였다. 우리는 87개의 비기능뇌하수체샘종을 자기공명영상에서의 안장위 확장(suprasellar extension)과 해면정맥굴 침습(cavernous sinus invasion) 유무를 바탕으로 3개의 그룹으로 분류하여 평가하였다. 이후 조직 미세배열을 이용하여 PTTG, PITX2, galectin-3, E-cadherin, 그리고 Ki-67에 대한 면역조직화학

염색을 시행하였다. 연구 결과, PTTG 발현은 종양의 침습성과 유의한 상관관계를 보였으나 PITX2와 galectin-3는 상관성을 보이지 않았다. 또한 PTTG, E-cadherin, 그리고 Ki-67 사이의 관련성도 관찰되지 않았다. 결론적으로, PTTG는 비기능뇌하수체샘종에서 침습 정도를 예측할 수 있는 생체표지자로서의 가능성을 보였다. 더 나아가 본 연구의 결과는 PTTG가 추후의 표적 치료 약물 개발의 대상이 될 수 있음을 시사한다.