의학박사 학위논문

부갑상선 선종, 비정형 부갑상선 선종, 부갑상선 암에서 면역조직염색법에 따른 서로 다른 단백질의 발현 양상

Differential protein expression of parathyroid adenoma, atypical parathyroid adenoma, and parathyroid carcinoma by immunohistochemical staining

울 산 대 학 교 대 학 원

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이 논문을 의학박사 학위 논문으로 제출함

2022 년　2 월

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국문요약

부갑상선암은 드문 암종의 일부이나, 진행된 케이스에서 높은 합병증 발생률과 사망률을 나타내어 부갑상선 선종이나 비정형 부갑상선 선종 등과의 감별진단이 중요하다. 이 연구의 목적은 부갑상선암에서 대표적으로 발현하는 유전자 변이를 확인하고 이에 따른 단백질 발현의 변화가 부갑상선 선종, 비정형 부갑상선 선종, 부갑상선암에서 면역조직염색법에 따라 어떠한 차이를 보이는지에 대해 알아보고자 하였다.

2000 년부터 2020 년까지 본원에서 수술을 시행한 부갑상선암으로 진단된 환자들이 연구에 포함되었다. 이 부갑상선암 환자와 비교하기 위해 부갑상선 선종 환자와 비정형 부갑상선 환자들을 선정하였으며 크기의 차이가 5mm 미만을 위주로 선별하였다. 대표적인 유전자 변이는 기존의 문헌 분석을 통해 선정하였으며 총 12 개의 유전자 변이가 선택되었으며 각 유전자 변이에 따른 항체를 통해 면역조직염색법을 진행하여 그 발현의 정도를 분석하였다.

10 명의 부갑상선 선종, 13 명의 비정형 부갑상선 선종, 8 명의 부갑상선암 환자가 선정되어 분석되었다. 총 12 개의 유전자 변이 중 통계학적으로 유의한 결과를 보였던 유전자는 총 6 개로 CDC73, Ki-67, Galectin-3, PGP9.5, NF1, E-cadherin 이었다. CDC73 은 부갑상선 선종, 비정형 부갑상선 선종, 부갑상선 암으로 갈수록 점차 발현도가 감소하는 소견을 보였고 통계적으로 유의하였다. Ki-67 과 Galectin-3, PGP9.5 는 비정형 부갑상선 선종, 부갑상선 암으로 갈수록 점차 발현도가 증가하는 소견을 보였으나 부갑상선 선종과 비정형 부갑상선 선종간의 차이는 유의하지 않았고 부갑상선 암에서 유의하게 증가하여 있었으며 이 차이는 선종과 부갑상선암을 비교하였을 때 유의하였다. 반면에 NF1 과 E-cadherin 은 부갑상선암에서 선종과 비교하였을 때 유의하게 감소한 소견을 보였으나 연속적인 감소추세는 보여주지 못하였다. 6 개의 변이 중 단독으로 가장 검정력이 좋았던 변이는 Ki-67 이었으며 4%에서 가장 높은 민감도와 특이도를 보였다.

CDC73 에 대한 면역조직염색법으로 분석한 단백질 발현의 차이는 부갑상선 선종에서 부갑상선 암으로 갈수록 연속적으로 감소하였다. CDC73, Ki-67, Galectin-3, PGP9.5, NF1, E-cadherin 은 부갑상선암을 진단하는데 유용할 것으로 생각되며 Ki-67 은 단독으로 가장 설명력이 높은 변이었으며 4% 이상일 때 부갑상선암으로 진단할 수 있을 것으로 생각된다.

차 례

표 차례

그림 차례

Introduction

Parathyroid carcinoma is a rare endocrine malignancy accounting for less than 1% of all cases of hyperparathyroidism. The estimated incidence is 0.005% of all cancers and it may be increasing (1). Despite the very low incidence, parathyroid carcinoma has a high morbidity and mortality in advanced cases, so it is very important to distinguish between parathyroid adenoma from parathyroid carcinoma (2-4). However, most parathyroid carcinomas produce parathyroid hormone and present as primary hyperparathyroidism, because of these characteristics, it is difficult to distinguish parathyroid carcinoma clinically from benign parathyroid adenomas or hyperplasia (4). Extremely high level of serum calcium and parathyroid hormone (PTH) are associated with carcinoma, but these are not absolute predictors (5).

Histopathological diagnosis is essential for identifying parathyroid carcinoma, finding histological features of malignancy such as invasive growth, capsular invasion, vascular invasion, abnormal mitoses and nuclear monotomy (6). Accurate histopathological diagnosis can be difficult in some cases because the presence of atypical parathyroid adenoma. Atypical parathyroid adenomas represent a group of intermediate form of parathyroid neoplasms of uncertain malignant potential which show some atypical histological features often seen in carcinomas that make it difficult to draw out differential diagnosis with parathyroid carcinomas (7).

Immunohistochemistry staining can be helpful in the diagnosis of parathyroid tissue and tumors in difficult cases. Many immunohistochemical markers continue to be studied for diagnostic and prognostic utility for parathyroid carcinoma (8). However, due to the rarity of carcinoma cases, few studies have analyzed immunohistochemical markers associated with adenoma, atypical adenoma, and parathyroid carcinoma simultaneously (9-12).

The purpose of this study was to investigate the difference in the protein expression of representative parathyroid gene mutations in parathyroid adenoma, atypical parathyroid adenoma, and parathyroid carcinoma through immunohistochemical staining and to find out the clinical implication.

Method

Patients and specimens

All cases of parathyroid carcinoma who underwent surgery at Asan Medical Center from 2000 to 2020 were investigated and collected. Parathyroid adenoma and atypical parathyroid adenomas cases smaller than 5cm in size were selected among patients who underwent surgery from 2017 to 2020. The specimens were fixed in formalin and processed using the usual technique with paraffin embedding and hematoxylin and eosin staining. The histological diagnosis of parathyroid carcinoma had been made by an expert pathologist (D.E.S) and all cases were reviewed based on previously studied data and 2017 WHO classification (13-15). Clinical information was collected retrospectively from electronic medical record.

Antibody selection for immunohistochemical staining based on previous studies

Representative markers for parathyroid carcinoma were selected for immunohistochemical staining through comparing NGS results of 2 studies (16, 17). At first, CDC73, TP53, MEN1, PTEN, NF1, TERT and PIK3CA were selected based on the previous studies that the genes reported to be frequently mutated in parathyroid carcinoma. Additionally, 5 markers (Ki-67, PGP9.5, Galectin-3, RB, and E-cadherin) were included in this study. Ki-67, PGP9.5, and Galectin-3 had been reported in previous studies and the 3 markers were useful to diagnose parathyroid cancer (9, 12, 18). RB also has been extensively studied in parathyroid tumors (19-22). E-cadherin is a marker associated with thyroid cancer and have not yet been used in the diagnosis of parathyroid cancer, which were available at our institution. Finally, 12 markers were selected and proceeded to staining. Characteristics of antibodies used against the immunogens from 12 markers are summarized in **Table 1**.

Immunohistochemical staining

Tumor tissues obtained during surgery for routine diagnostic pathologic examinations were used for immunohistochemistry studies of the anti-antibody. Formalin fixed, paraffin-embedded tissue sections were immunohistochemically stained for anti-antibody using a OptiView DAB IHC Detection Kit on BenchMark XT automatic immunostaining device (Ventana Medical Systems, Tucson, AZ, USA) according to the manufacturer's instructions. Four-micrometer-thick sections, obtained with a microtome, were transferred onto silanized charged slides and allowed to dry for 10min at room temperature, followed by 20min in an incubator at 65°C. Sections were performed by heat-induced epitope retrieval (HIER) method using Cell Conditioning 1(CC1) buffer for 32min and incubated for 16min with antibodies in the autoimmunostainer. Antigen-antibody reactions were visualized using Ventana OptiView DAB IHC Detection Kit (Optiview HQ Linker 8min, Optiview HRP Multimer 8min, Optiview H2O2/DAB 8min, Optiview Copper 4min). Counterstaining was performed by using Ventana Hematoxylin II for 12minutes and Ventana Bluing reagent for 4minutes. Finally, all slides are removed from the stainer, dehydrated, and coverslipped for microscopic examination.

Full immunohistochemistry staining for 12 markers was performed in the entire patients. All patients were classified in two ways. First, they were classified into 3 groups, such as parathyroid adenoma, atypical parathyroid adenoma, and parathyroid carcinoma group. After comparing the 3 groups, all patients were reclassified into 2 groups such as parathyroid adenoma and parathyroid carcinoma to distinguish parathyroid carcinoma from all other parathyroid lesions. All immunogens except Ki-67 were counted for each immunogen’s overall proportion of positive nuclear or cytoplasmic staining. Ki-67 was counted as a percentage of positive nuclear-staining tumor cells per 1000 tumor cells in hot spot area by a manual count. After comparing the expression ratio of the antibodies, it was analyzed as categorical value by dividing positive expression or loss of expression of the antibodies. Positive expression of P53 was categorized as 0, 1, 2, and 3, each corresponding to less than 5%, 33%, 66%, and more than 66%. Loss of E-cadherin and RB were defined as less than 10%, loss of CDC73 was defined as 5%. Positive expression of Galectin-3, PGP9.5 were defined as more than 30% and more than 50% respectively. Ki-67 index over 4% was considered as highly proliferative.

Statistical analysis

Continuous variables presented as mean ± SD and compared using Student's t-test after confirming a normal distribution. If normal distribution was not confirmed, Kruskal-Wallis test was used. Categorical variables are presented as numbers and percentages and compared by Fisher's exact test. $P < 0.05$ was considered statistically significant, and $P < 0.017$ was considered statistically significant when groups compared by Kruskal-Wallis test. Diagnostic performance of markers were compared using receiver-operating characteristic (ROC) analysis with calculating area under curve (AUC). All statistical analysis were conducted using SPSS version 21.0 for windows (SPSS inc., Chicago, USA).

Table 1. Detailed information of 12 antibodies used

Antibody	Manufacturer	Species	Clone	Dilution
CDC73	Abcam	Rabbit	EPR19927	1:200
Ki-67	Dako	Mouse	MIB1	1:200
PGP9.5	Abcam	Rabbit	EPR4118	1:500
Galectin-3	Novocastra	Mouse	9C4	1:200
E-cadherin	Cell Marque	Rabbit	EP700Y	1:1500
NF1	Abcam	Rabbit	Polyclonal	1:200
PIK3CA	Abcam	Rabbit	SP139	1:100
TERT	Abcam	Rabbit	Polyclonal	1:200
MEN1	Abcam	Rabbit	EPR3986	1:250
RB	Abcam	Rabbit	EPR17512	1:500
P53	Dako	Mouse	DO-7	1:1000
PTEN	Cell signaling	Rabbit	138G6	1:100

Result

8 cases of parathyroid carcinoma, 13 cases of atypical parathyroid adenoma and 10 controls with a benign parathyroid adenoma were collected for this study. Of the 8 parathyroid cancer patients, 4 died from uncontrolled hypercalcemia, 4 are still under follow-up at outpatient clinic. Preoperative calcium level, and PTH level tended to be higher in the parathyroid carcinoma group than other groups (**Table 2**). Among the 12 markers, only 6 markers represented statistical significance.

In comparison between the 3 groups, CDC73 presented serial decrease of staining percentage in adenoma (78.0%), atypical adenoma (51.5%), and carcinoma (17.9%) and the differences were statistically significant between parathyroid adenoma versus atypical parathyroid adenoma and atypical parathyroid adenoma versus parathyroid carcinoma (**Fig. 1**). Ki-67 and Galectin-3 showed serial increase of staining percentage in 3 groups comparing, however, the change was not statistically significant when comparing between parathyroid adenoma and atypical parathyroid adenoma (**Fig. 2**). PGP9.5 staining was significantly increased in parathyroid carcinoma group, but it did not show serial change in 3 group comparing (**Fig. 3**).

E-cadherin, and NF1 did not show difference in 3 group comparing, however, presented difference in comparison between the 2 groups, adenoma and carcinoma. E-cadherin and NF1 loss was found in parathyroid carcinoma group, positive number of E-cadherin was significantly decreased in parathyroid carcinoma group (**Fig. 4**). NF1 also presented statistically decreased staining percentage in parathyroid carcinoma, but the difference was small (88.5% in parathyroid adenoma, 63.8% in parathyroid carcinoma). Representative examples of the staining seen in each case of carcinoma, atypical adenoma, and a benign adenoma are shown in **Fig. 5**.

Immunohistochemical staining results associated with parathyroid carcinoma were seen in 5/8 CDC73, 8/8 Ki-67, 5/8 Galectin-3, 5/8 PGP9.5, 6/8 E-cadherin, and 3/8 NF1 (**Table 3)**. Among the 6 markers, Ki-67 was the single most descriptive marker (AUC=0.959), and its cutoff value was 3.8% with sensitivity 100.0% and specificity 91.3%. The results were analyzed using different combinations

shown in **Table 4**. Ki-67 was excluded from the analysis because it showed a sensitivity of 100% in combination with any other marker. 8 of the 8 carcinoma cases had at least one immunohistochemical result associated with parathyroid carcinoma. Combination of Galectin-3 and/or E-cadherin in immunohistochemical staining suggested carcinoma in 8/8 cases and presented 100.0% sensitivity than other combinations.

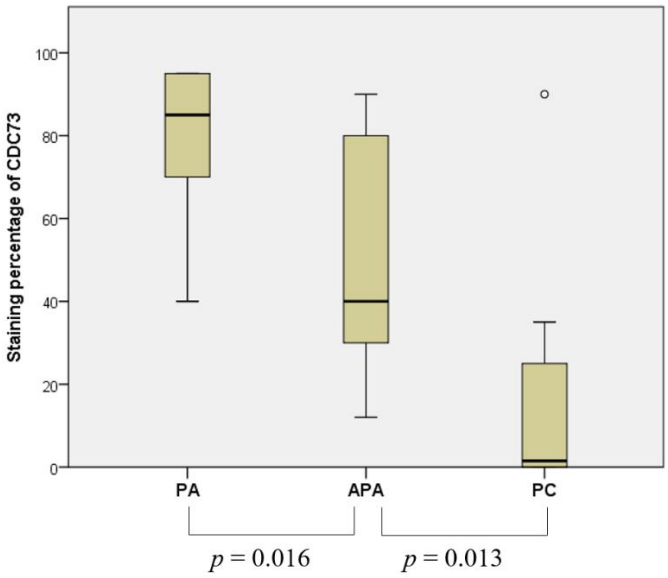

Fig. 1 Staining percentage of CDC73 in each group, parathyroid adenoma, atypical parathyroid adenoma, and parathyroid carcinoma.

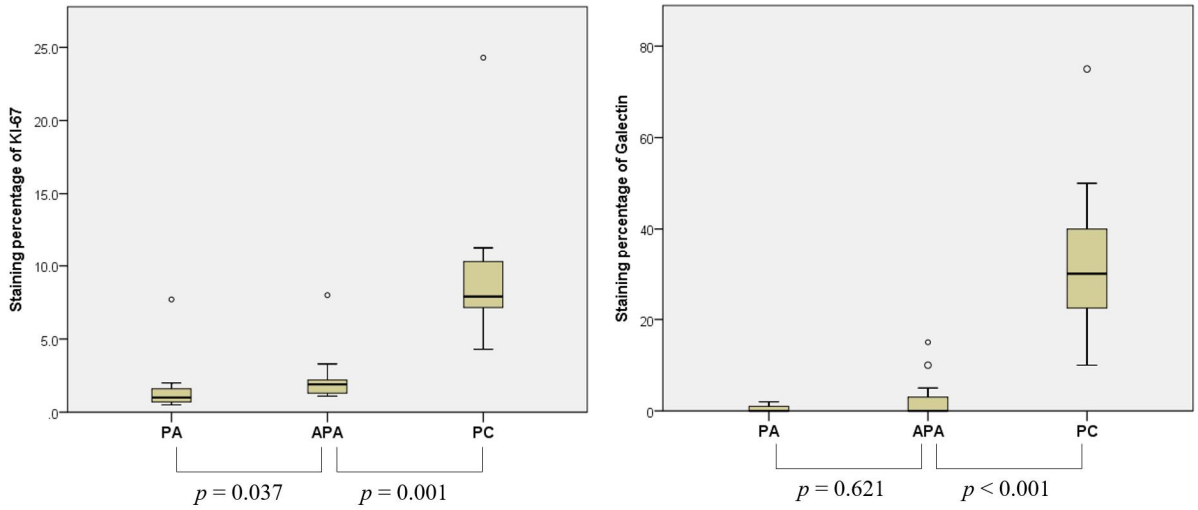

Fig. 2 Ki-67 and Galectin-3 presented serial increase in 3 group comparing

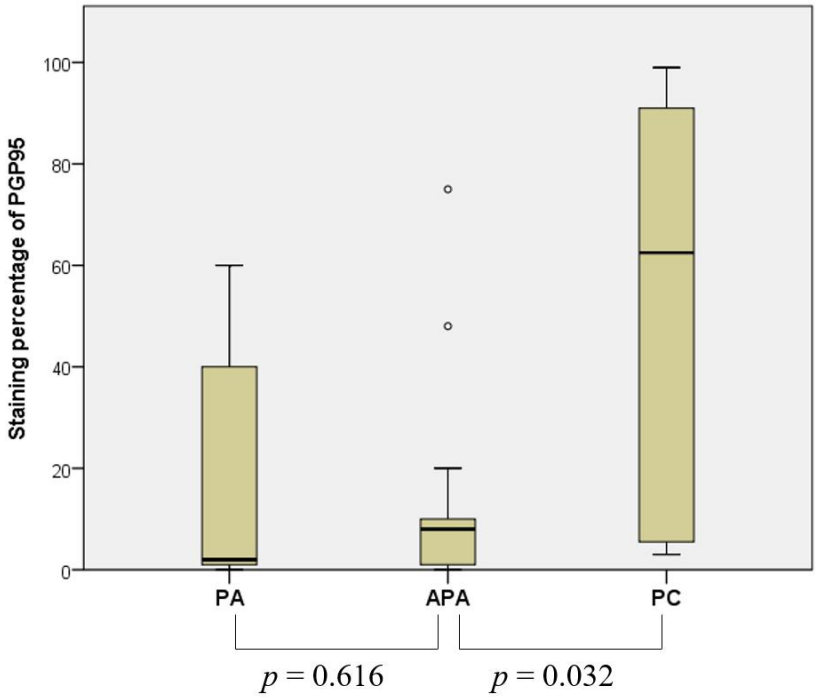

Fig. 3 PGP9.5 staining showed marked increase in parathyroid carcinoma group

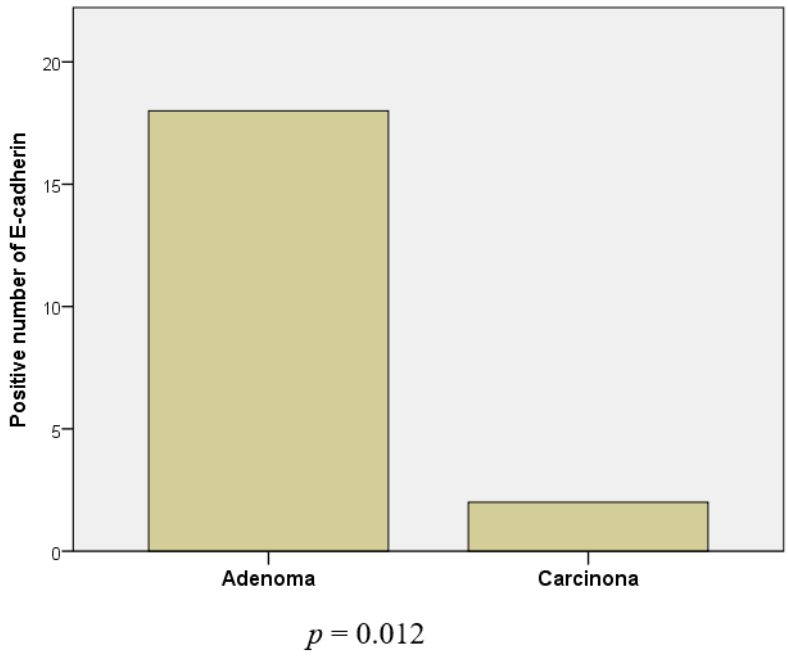

Fig. 4 Immunohistochemical staining of E-cadherin in 2 group comparing

Table 2. Clinical characteristics of parathyroid neoplasm patients

	PA (n=10)	APA (n=13)	PC (n=8)	p-value
	N (%) or Mean (range)	N (%) or Mean (range)	N (%) or Mean (range)	
Calcium (mg/dL)	11.8 (9.7-16.7)	11.6 (10.3-13.9)	14.7 (12.7-17.3)	<0.001
PTH (pg/mL)	313.4 (111.0-803.0)	372.5 (167.0-1090.0)	1374.5 (417.0-2281.0)	<0.001
Age (years)	51.9 (31-72)	58.7 (23-75)	45.2 (23-80)	0.082
Size (cm)	2.3 (1.2-4.8)	2.5 (1.1-4.2)	3.4 (1.5-4.5)	0.064
Female sex	6 (60.0%)	6 (45.2%)	5 (62.5%)	0.707

PA: Parathyroid adenoma, APA: Atypical parathyroid adenoma, PC: Parathyroid carcinoma

Table 3. Immunohistochemistry results of 6 markers in parathyroid carcinoma patients

Immunostain	Abnormal result suggesting parathyroid carcinoma (n = 8) Number (percentage)
CDC73	5 (62.5%)
Ki-67[a]	8 (100.0%)
Galectin-3	5 (62.5%)
PGP9.5	5 (62.5%)
E-cadherin	6 (75.0%)
NF1	3 (37.5%)

[a]Abnormal result suggesting parathyroid carcinoma in Ki-67 was 7 when high proliferative index was set to 5%

Table 4. Combination of immunohistochemistry results without Ki-67

Immunostain	Abnormal result suggesting parathyroid carcinoma (n = 8) Numbers (percentage)
Any one stain abnormal	8 (100.0%)
Any two stains abnormal	5 (62.5%)
Any three stains abnormal	5 (62.5%)
Any four stains abnormal	3 (37.5%)
Any five stains abnormal	3 (37.5%)
CDC73 AND Galectin-3	4 (50.0%)
CDC73 AND PGP9.5	5 (62.5%)
CDC73 AND E-cadherin	4 (50.0%)
CDC73 AND NF1	3 (37.5%)
CDC73 AND/OR Galectin-3	7 (87.5%)
CDC73 AND/OR E-cadherin	6 (75.0%)
PGP9.5 AND/OR Galectin-3	7 (87.5%)
PGP9.5 AND/OR E-cadherin	6 (75.0%)
Galectin-3 AND/OR E-cadherin	8 (100.0%)

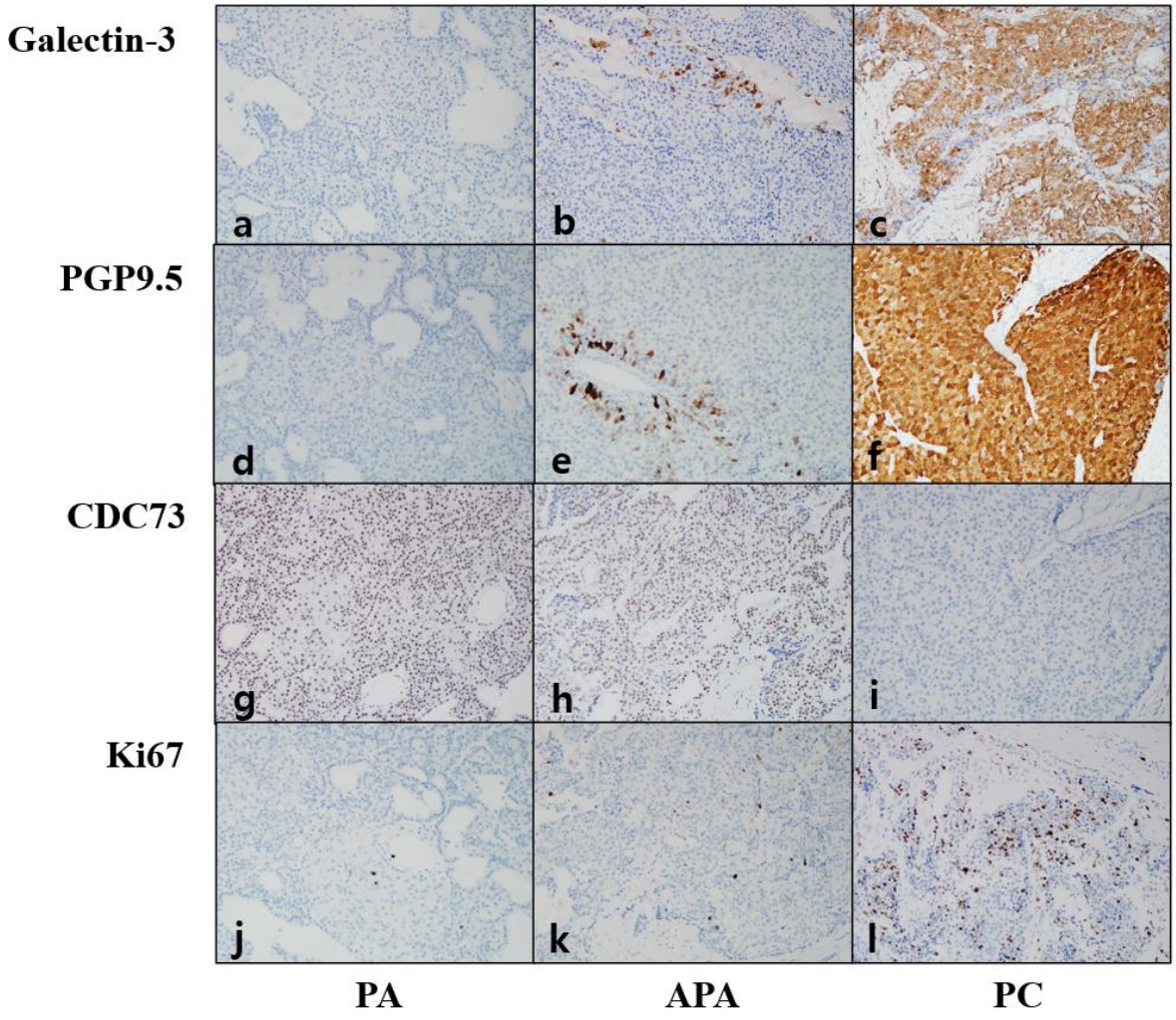

Fig. 5 Representative immunohistochemical stains of parathyroid adenoma (PA), atypical parathyroid adenoma (APA), and parathyroid carcinoma (PC) for Galectin-3, PGP5.9, CDC73, and Ki-67

Discussion

In this study, 6 markers of 12 markers presented statistically significant difference in protein expression, especially for CDC73, showed serial decreased immunohistochemical staining along the sequence of parathyroid adenoma, atypical parathyroid adenoma and parathyroid carcinoma. Ki-67 was the best individual test, had a sensitivity of 100% and specificity of 91% and its cutoff value was 3.8%.

Serial decrease of CDC73 was observed in this study and there was significant difference between atypical parathyroid adenoma and parathyroid carcinoma group. Parafibromin, a protein product of CDC73, appears to involve regulation of gene expression and inhibition of cell proliferation (23). Mutation of the CDC73 tumor suppressor gene that previously known as HRPT2 gene, is the most studied marker in parathyroid carcinoma and has been recognized to play a central role in the etiology of parathyroid carcinoma. Cetani et al. investigated CDC73 mutations in 7 parathyroid canrcinoma patients and 35 parathyroid adenoma patients, and reported that mutations were found in 4 of 7 parathyroid carcinoma patients (24). Kim et al. evaluated the usefulness of parafibromin mutation in distinguishing parathyroid carcinoma from adenoma., in 8 patients with parathyroid cancer, 3 were weakly stained and 3 reported negative (25). In meta-analysis performed by Pyo et al., to determine the diagnostic and prognosis implication of CDC73 immunohistochemical staining for parathyroid carcinoma, reported the rate of parafibromin loss in parathyroid carcinoma was 0.522, in atypical parathyroid adenoma was 0.291 and in parathyroid adenoma was 0.291 repectively. They also reported that parafibromin loss in parathyroid carcinoma correlated worse disease-free survival (hazard ratio: 2.832) (26). In this study, CDC73 presented significantly decreased staining percentage in parathyroid carcinoma group, and the result is similar to other studies. Serial decrease of CDC73 immunohistochemical staining was also observed and similar to previous studies, but the detailed etiology is unknown.

Ki-67 is a nuclear protein that is associated with cellular proliferation (27). Ki-67 is widely used for diagnosis several types of cancer or tumor prognosis. There have been attempts to apply ki-67 in

parathyroid carcinoma to evaluate proliferative activity. In a study of 22 histologically normal parathyroid glands, 33 hyperplasias, 43 adenomas, and 17 carcinomas, Ki-67 was significantly higher in carcinomas than in adenomas and hyperplasia (28). In the study of Bergero et al., they evaluated Galectin-3. Ki-67, p27, and bcl2 presenting differential expression between 26 parathyroid carcinomas and 30 adenomas. Ki-67 was 6.7% (range 1–38%) in carcinomas and 1.9% (0.5–13%) adenomas, and showed 42.3% of sensitivity and 93.3% of specificity when the cutoff value of Ki-67 index was 6%. (19) Fernandez-Ranvier et al evaluated multiple gene expressions such as CDC73, Galectin-3, Ki-67, RB, p27 and MDM-27 to distinguish parathyroid carcinoma from benign parathyroid lesions. They considered Ki-67 over 5% as highly proliferative index cutoff and reported sensitivity for 60% and specificity for 93%, even higher sensitivity (80%) in metastatic parathyroid carcinoma lesion (9). On the other hand, according to Wang et al.'s analysis of 15 parathyroid carcinoma patients, only 27% of the parathyroid carcinoma patients represented Ki-67 index over 5% (18). In this study, Ki-67 presented a high proliferative index over in all parathyroid carcinoma cases with none of the parathyroid adenoma or atypical parathyroid adenoma cases showed KI-67 index over 4%. As in other studies, when the index was 5% or higher, 7 out of 8 parathyroid carcinoma patients could be diagnosed, and when the index was 4%, all parathyroid carcinoma patients were included. Also, Ki-67 was the single most descriptive marker (AUC=0.959) among the 6 markers, in cutoff value of 3.8% with high sensitivity (100.0%) and high specificity (91.3%).

Galectins are a class of proteins that bind specifically to β-galactoside sugar and have a broad variety of functions including mediation of cell–cell interactions, cell–matrix adhesion and transmembrane signalling. Especially for endocrine tumors, Galactin-3 is important in the diagnosis of follicular thyroid malignancies and in tumor development (12, 19). It has been reported that the expression of Galectin-3 is increased in parathyroid cancer, and the same results have been consistently reported in previous studies (9, 18, 19). According to Bekir et al, a study of Galectin-3 expression in atypical parathyroid adenoma, they reported galactin-3 overexpression in both carcinomas and atypical adenomas (29). However, in this study, Galectin-3 was overexpressed in parathyroid carcinoma, but not in atypical

parathyroid adenoma group. In addition, overexpression of Galectin-3 in atypical parathyroid adenoma was not distinguishable to parathyroid adenoma group ($p = 0.621$).

Protein gene product 9.5 (PGP 9.5), also known as ubiquitin carboxyl-terminal hydrolase-1 (UCH-L1), is a 27-kDa protein originally isolated from whole brain extracts and expressed in neuroendocrine and nerve tissues (30, 31). Previous studies applied PGP9.5 to immunohistochemical markers for parathyroid carcinoma. Howell et al, compared PGP9.5 with parafibromin, reported a slightly superior sensitivity and similar high specificity to that of parafibromin (32). Truran et al, introduced diagnostic panel for diagnosing parathyroid carcinoma including PGP9.5 (12). According to our study, PGP was overexpressed in parathyroid carcinoma group, but not in parathyroid adenoma or atypical parathyroid adenoma group.

E-cadherin is a protein encoded by CDH1 gene, present their function as calcium-dependent cell to cell adhesion glycoprotein. Only one study investigated the association of E-cadherin with parathyroid neoplasm, Schneider et al, reported parathyroid tumors suspicious for atypical parathyroid adenoma are characterized by a strong membranous E-cadherin staining in contrast to parathyroid carcinoma (33). Similar result was found in our study, loss of E-cadherin staining was observed in parathyroid carcinoma cases. However, the difference was not significant when comparing parathyroid adenoma and atypical parathyroid adenoma.

NF1 is a gene codes for neurofibromin, a GTPase-activating protein that negatively regulates RAS/MAPK pathway activity by accelerating the hydrolysis of Ras-bound GTP (34, 35). Mutations in NF1 can alter cellular growth control, and neural development, resulting in neurofibromatosis type 1 (34, 35). Genomic profiling of parathyroid carcinoma, conducted by Kang et al, reported genomic alterations suggesting potential benefit from matched targeted therapy were identified in 11 patients and most frequently found in PTEN (25%), NF1 (12.5%), KDR (12.5%), PIK3CA (12.5%), and TSC2 (12.5%) (16). NF1 presented difference comparing whole patient in 2 groups, adenoma and carcinoma group, but the difference was small so further analysis is needed.

Many immunohistochemical markers have been studied, alone or in combination with other markers in the evaluation of parathyroid lesions. Bergero et al. (2005) studied the combination of Galectin-3, Ki-67, p27, and bcl2, and reported the best sensistivity (96.2%) and specificity (90%) for 2 or more markers in a panel were Galectin-3 and Ki-67 (19). Fernandez-Ranvier et al. (2009) evaluated the combination of parafibromin, Galectin-3, Ki-67, Rb, p27, and mdm-2, and found a combination of parafibromin loss, Rb loss, and Galectin-3 overexpression were generally able to identify carcinomas (9). In the year of 2014, Truran et al suggested a panel including parafibromin, Galectin-3, PGP9.5, and Ki-67. They reported that the panel has a sensitivity of 79 % and specificity of 100 %, better than any single immunohistochemical marker, in the diagnosis of suspected parathyroid carcinoma (12). In our study, most combinations showed lower discriminative power than Ki-67 except the combination of Galectin-3 and E-cadherin. Parathyroid carcinoma cases that were not detected in Galectin-3 was detected in E-cadherin, showed a complementary relationship. However, there were many ambiguous cases close to 30%, the cutoff value of Galectin-3, so it would be better to check Ki-67index additionally as much as possible. Ki-67 is commonly used to assess many other carcinomas and Galectin-3 is used to diagnose follicular thyroid malignancies, it would be easy to apply in most histopathology laboratories.

This study suggests the usefulness of 6 markers for diagnosis of parathyroid carcinoma, and NF1 was not had been reported. Serial change of CDC73 could be a clue to the development of parathyroid carcinoma and further studies are needed to find out detailed process of parathyroid carcinogenesis. Combination of 6 markers for diagnosis of parathyroid carcinoma will be valuable when assessing ambiguous cases, especially the combination of galetin-3 and E-cadherin would be helpful. If not all the markers are available, Ki-67 could be used for single descriptive marker for diagnosis of carcinoma when the index is over 4%.

This study has several limitations. First, the number of parathyroid carcinoma cases was not enough to calculate differences based on normal distribution so statistical power is low. In addition, among

parathyroid cancer patients, recurrent lesions in areas other than the parathyroid gland were also included in the analysis, so the results could be heterogenous. However, it is significant because the results of this study are similar to the previous studies, and additional studies will be able to clarify this difference. Finally, the expert pathologist could not be blinded to the status of patients' disease because the pathologist participated in the selection of parathyroid tumors for this study. Although the pathologist is a high level of expertise on endocrine tumors and there is a time gap between the H&E diagnosis and the immunohistochemical diagnosis, the bias still exists due to not blinding the pathologist. Thus, a larger dataset and adequate study design to blind pathologist are needed for further investigations.

Conclusion

Immunohistochemistry staining for CDC73 presented serial change from parathyroid adenoma to parathyroid carcinoma. CDC73, Ki-67, Galectin-3, PGP9.5, E-cadherin, and NF1 can be useful markers for diagnosing parathyroid cancer, and Ki-67 was the most descriptive marker and an index of over 4% is suggestive for diagnosing parathyroid carcinoma.

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Abstract

Objective: Parathyroid carcinoma is an uncommon malignancy, however, has a high morbidity and mortality in advanced cases. It is important to distinguish between parathyroid adenoma and atypical parathyroid adenoma from parathyroid carcinoma. The purpose of this study was to investigate the difference in the protein expression of representative parathyroid gene mutations in parathyroid adenoma, atypical parathyroid adenoma, and parathyroid carcinoma through immunohistochemical staining and to find out the clinical implication.

Methods: All cases of parathyroid carcinoma who underwent surgery at out institution from 2000 to 2020 were collected retrospectively. Controls with parathyroid adenoma and atypical parathyroid adenoma were included for comparison. 12 representative genes which occurring in the parathyroid gland were selected for immunohistochemistry staining and analyzed.

Results: There were 8 cases of PC, 13 cases of atypical parathyroid adenoma, and 10 cases of parathyroid adenoma. Of the 12 markers, 6 markers (CDC73, Ki-67, Galectin-3, PGP9.5, NF1, E-cadherin) presented significant results. According to the immunohistochemical results, CDC73 presented serial decrease of staining percentage in adenoma (78.0%), atypical adenoma (51.5%), and carcinoma (17.9%). Ki-67, and Galectin-3, on the other hand, presented serial increase of staining percentage but statistically not significant. Additionally, NF1, and E-cadherin showed loss of staining in carcinoma cases. Combination of Galectin-3 and/or E-cadherin in immunohistochemical staining suggested carcinoma in 8/8 cases and presented 100.0%. Ki-67 was the single most descriptive marker (AUC=0.959), and its cutoff value was 3.8% with sensitivity 100.0% and specificity 91.3%

Conclusion: Immunohistochemistry staining for CDC73 presented serial change from parathyroid adenoma to parathyroid carcinoma. CDC73, Ki-67, Galectin, PGP9.5, E-cadherin, and NF1 can be useful markers for diagnosing parathyroid cancer, and Ki-67 was the most descriptive marker and an index of over 4% is suggestive for diagnosing parathyroid carcinoma.