RET/PTC and CK19 Expression in Papillary Carcinoma and Its Clinicopathologic Correlation

Eunah Shin

Department of Medical Science
The Graduate School, Yonsei University

RET/PTC and CK19 Expression in Papillary Carcinoma and Its Clinicopathologic Correlation

Directed by Professor Woo Ick Yang

Thesis submitted to the Department of Medical Science, the Graduate School of Yonsei University, in partial fulfillment of the requirements for the Master's Degree

Eunah Shin

December, 2002

This certifies that the Master's thesis by Eunah Shin has been approved

Thesis Supervisor, Woo Ick Yang

Supervisory Committee

Supervisory Committee

The Graduate School
Yonsei University

December, 2002

감사의 글

이 논문을 완성하기까지 세심하게 지도해주시고 힘이 되어주신 양우익 선생님께 진심으로 감사를 드립니다. 관심을 가지고 조언해주시고 도와주신 홍순원 선생님과 박정수 선생님께도 감사드립니다.

어려울 때에 아낌없이 배려해주시고 위로해주신 김태승 선생님을 비롯한 병리학교실의 모든 선생님들께 마음으로부터 깊은 감사를 드립니다. 또한 힘든 시기에 불평함 없이 기꺼이 도와주고 희생을 마다하지 않은 임범진 의국장 선생님과 혜령, 자승, 그리고 효섭에게 너무나도 고마운 마음을 전합니다.

훌륭한 기술과 창의력으로 실험이 가능케 해주신 정형재 기사님께 진심으로 감사드립니다.

늘 든든한 울타리가 되어주시고 지켜봐 주시는 부모님과 시부모님, 그리고 저의 가장 큰 후원자이며 언제나 사랑을 아끼지 않는 남편에게 감사한 마음을 전합니다.

그리고 무엇보다도 부족한 며느리를 데리고 있어주시며 사랑과 너그러움으로 덮어주시는 어머님과 제게 가장 귀한 선물인 아들 진원이, 그리고 뱃속의 아기에게 이 논문을 바칩니다.

저 자 씀

Table of Contents

List of Tables

List of Figures

Abstract

RET/PTC and CK19 Expression in Papillary Carcinoma and Its Clinicopathologic Correlation

Eunah Shin

Department of Medicine

The Graduate School, Yonsei University

(Directed by Professor Woo Ick Yang)

Papillary thyroid carcinoma (PTC) is the most common malignancy arising in the thyroid, with an excellent long-term survival rates. Its diagnosis has rested on the characteristic nuclear features, but problem arises with unequivocal cytologic features that are present focally or multifocally. Although controversial, CK19 has been reported to be a useful ancillary tool for diagnosing papillary carcinoma of the thyroid.

Recently, the rearrangement of RET proto-oncogene has been reported to be the most common genetic change in the development of PTC. However, its prevalence has been reported variably and its correlation with clinical outcome has been controversial.

To evaluate the expression rate of RET/PTC rearrangement and CK19 in papillary thyroid carcinoma in a Korean population and its clinicopathologic correlation, we studied 115 papillary thyroid carcinomas in 3mm-core tissue microarray based immunohistochemical

analysis.

The prevalence of Ret protein expression was 62.6% (72 out of 115 cases) and the CK19 immunoreactivity was 80.9% (93 out of 115 cases) in papillary thyroid carcinomas, which were in accord with those in Western countries. There was no statistically significant associations between the Ret positivity and CK19 immunoreactivity, although the percent agreement of the two was relatively high. The clinicopathological variables, i.e. age, sex, size of the tumor, regional lymph node metastasis, perithyroidal extension, and multiplicity, did not correlate with the expression of Ret.

In conclusion, the prevalence of Ret protein expression and its clinicopathological implications in a Korean population are not different from those reported in previous studies. However, its detection via immunohistochemistry can be a useful diagnostic tool for diagnosing papillary thyroid carcinoma in conjunction with CK19.

Key words: thyroid carcinoma, Ret protein, cytokeratin 19, Korea, immunohistochemistry, tissue microarray

RET/PTC and CK19 Expression in Papillary Thyroid Carcinoma and Its Clinicopathologic Correlation

<Directed by Professor Woo Ick Yang>

Department of Medical Science
The Graduate School of Yonsei University

Eunah Shin

I. INTRODUCTION

Papillary thyroid carcinoma (PTC) is the most common malignancy arising in the thyroid, with an excellent long-term survival rates of more than 90%.[1] Its pathogenesis is associated with previous exposure to ionizing radiation, both external and internal.[2] Several genes have been implicated in the carcinogenesis of PTC, including TP53, p21, met, trk and ras.[3,4] Recently, however, the rearrangement of RET proto-oncogene, which is normally expressed in neural crest-derived tissues but not in thyroid follicular cells, has been reported to be the most common genetic change in the development of PTC.[5] The RET proto-oncogene maps to the long arm of chromosome 10 and encodes a cell membrane-bound receptor tyrosine kinase.[6] When the tyrosine kinase encoding domain of the RET proto-oncogene undergoes fusion with the 5'-terminal region of another gene that is constitutively expressed, it results in activation of an oncogene designated RET/PTC. As a consequence, the products of the chimeric RET/PTC oncogenes

lead to the relocation of the ret tyrosine kinase domain from the membrane to the cytoplasm and display constitutive tyrosine-kinase activity by autophosphorylation.[7-11]

Such rearrangements of the RET proto-oncogene have only been found in thyroid gland tumors of the papillary histotype. However, its prevalence has been reported variably in different geographical regions,[12-16] and its correlation with clinical outcome has been controversial.[2,7,17-19]

Identification of a patient subgroup with potential for developing aggressive disease is important, for the appropriate use of adjuvant therapy is harbored on such identification. The problem arises when patients lacking the previously established poor prognostic factors, i.e. age greater than 40, tumor size greater than 5cm, multicentricity, blood vessel invasion, extrathyroidal extension, distant metastasis, and aneuploidy, or the histological subtype develop aggressive disease. Thus, the study of oncogene and tumor suppressor gene expression may be useful in identifying additional reliable prognostic factors.

Recent studies of cytokeratin expression in thyroid neoplasms have demonstrated that various low and high molecular-weight cytokeratins in general are expressed differentially in papillary thyroid carcinoma.[20,21] In particular, cytokeratin (CK) 19 is reported to be limited to papillary carcinomas, thus favoring a diagnosis of papillary carcinoma in all its variant patterns.[22] Sahoo et al. has reported that immunoreactivity for CK19 is not specific for PTC, although the extent and intensity of staining are significantly greater in PTC than in follicular adenoma.[23]

However, most of the previous studies have proposed CK19 to be a useful immunohistochemical marker to distinguish PTC from other benign and malignant follicular lesions.[21,24,25]

The purpose of this current study is first to evaluate the expression rate of Ret protein in a large series of classic papillary thyroid carcinomas in a Korean population using immunohistochemistry in tissue microarray and explore the possibility of Ret protein expression as a reliable prognostic factor. Secondly, this study is intended to clarify a more specific role of Ret antibody as an ancillary tool for diagnosing papillary carcinoma in conjunction with CK19.

II. MATERIALS AND METHODS

1. Patient Selection

The surgical pathology files of Yonsei University Severance Hospital in the year 2001 were searched for 'papillary carcinoma' in thyroid. After review of the search results, a consecutive series of 115 classic papillary thyroid carcinomas, with all variants including follicular variant excluded, were selected for the study. Clinical data were obtained from the medical records.

2. Histopathology, Tissue microarray construction, and Immunohistochemistry

The slides of 115 cases were reviewed with special attention to the diagnostic nuclear features of papillary thyroid carcinoma, i.e. nuclear grooves, intranuclear inclusions and nuclear clearing[1], and papillary structures to identify representative areas of the specimen for tissue microarray. From these defined areas core biopsies were taken with a precision instrument. Tissue cores with a diameter of 3mm from each specimen were punched and arrayed on a recipient paraffin block. Four-㎛ sections of these tissue array blocks were cut and used for immunohistochemical analysis (Figure 1). Normal thyroid tissue distant from the i) tumor area, ii) adenomatous nodule, and iii) inflammatory cell infiltrates were obtained from each specimen and arrayed. These arrayed normal tissues served as baseline controls.

Sections from tissue arrays were deparaffinized in xylene, rehydrated

in graded alcohols, and processed using the labeled streptavidin - biotin - peroxidase method. Briefly, sections were submitted to antigen retrieval for 15 minutes in 44% formic acid for Ret and in 0.4% pepsin for CK19, both at room temperature. Slides were subsequently incubated in 10% normal blocking serum for 30 minutes. They were then incubated overnight at 4℃ in appropriately diluted primary antibody. Rabbit polyclonal antibody Ret (1:200; Santa Cruz Biotechnology, Santa Cruz, California, USA) and mouse monoclonal antibody CK19 (1:75; Biomeda, Hayward, California, USA) were used. After washing with Tris buffer, sections were incubated with biotin-labelled secondary antibodies and then with streptavidin - horseradish peroxidase using the DAKO LSAB kit (DAKO, Carpinteria, California, USA) at room temperature for 30 minutes for each step. Nova red (Vector Laboratory, Burlingame, California, USA) was used as the chromogen and hematoxylin as the nuclear counterstain. This procedure was performed for all antibodies under study.

The sections were also stained with antibodies to thyroglobulin (1:500, DAKO, Glostrup, Denmark), thyroid transcription factor-1 (1:100, DAKO, Glostrup, Denmark), and calcitonin (1:200, DAKO, Glostrup, Denmark) to validate the follicular cell origin of each case.

Diffuse cytoplasmic staining of more than 30% of the tumor cells was defined as positive immunoreactivity for Ret and CK19.

Informations on regional lymph node metastasis, perithyroidal extension of the tumor, multiplicity at the time of diagnosis, and size of the tumor were assessed from the surgical pathology reports.

3. Statistical Analysis

Summary statistics were obtained using established methods.[26] Associations between Ret immunoreactivity and each variable, i.e. lymph node metastasis, perithyroidal extension and multiplicity, were evaluated using the chi-square test. Kappa statistics was performed for the evaluation of percent agreement, the extent to which Ret immunoreactivity and CK19 immunoreactivity concur, in classic papillary carcinoma.

In all statistical analyses, a two-tailed p value $\leq$ 0.05 was considered statistically significant. All analyses were performed using SPSS for Windows statistical software (Version 10.0).

III. RESULT

1. Clinicopathological data

The male to female sex ratio of the patient group was 1:10.5, which was similar in both Ret positive and negative groups. The average age at surgery was 44.5 years, with a range from 19 - 76 years (Table 1). Twenty-nine patients had multifocal disease, and 84 patients had single tumors. Information on the multiplicity of the tumor at the time of the surgery was not available in two patients. The average tumor size was 1.8cm with a range from 0.5 - 6.0cm. The size of the tumor was not mentioned in the surgical pathology reports in 6 patients. Of the remaining 109 tumors, 37 cases were less than 1cm, 68 were in the range of 1 - 4cm, and only 4 cases were more than 4cm (Table 2). Eighty-five patients underwent regional lymph node dissection, of which 63 had lymph node metastasis. Perithyroidal extension was present in 84 cases and 29 cases had tumors confined to the thyroid parenchyma (Table 2). The presence of perithyroidal extension was not evaluable in two cases, for perithyroidal soft tissue was not included within the specimen.

2. RET protein expression by immunohistochemistry and clinicopathologic correlation

A total of 72 cases (62.6%) of 115 papillary thyroid carcinomas expressed Ret protein. The immunohistochemical detection of Ret protein was consistently observed in the cytoplasm of the tumor cells in

more than 30% of the tissue core for each case. Ret protein expression was absent in the normal thyroid tissues (Figure 3).

The Ret protein expression rate was not different in those groups with and without regional lymph node metastasis, perithyroidal extension, and multifocal disease (Table 2). Furthermore, age and sex of the patient group (Table 1), and the size of the tumor did not influence the expression of Ret (Table 2).

3. CK19 and Ret protein expression

Ninety-three papillary carcinomas (80.9%) showed diffuse cytoplasmic staining of CK19 (Table 3) (Figure 4). As with the Ret protein expression, CK19 immunoreactivity also had no statistically significant correlation with the aforementioned clinicopathological variables. Of the 115 cases, 7 cases were in lack of normal thyroid tissue in the specimen and 12 out of the 108 matched normal thyroid tissue showed CK19 immunoreactivity in the follicular epithelial cells.

Of the 115 papillary carcinomas, 62 (53.9%) showed positivity for both CK19 and Ret, and twelve (10.4%) showed negative results for both CK19 and Ret. The percent agreement of the two antibodies in classic papillary carcinomas was relatively high, however, it had no statistical significance (κ=0.16, p=0.06) (Table 3).

Table 1. Clinical data and their correlation with Ret protein expression in papillary carcinoma

		Ret		p-value
		Positive	Negative	
Age (yr)				
Mean	44.5±13.4	45.1±13.2	43.6±13.8	
Range	19 - 76	20 - 68	19 -76	p=0.55
≤40	43	24(55.8%)	19(16.5%)	
>40	72	48(66.7%)	24(33.3%)	
Sex				
Female	105	66(62.9%)	39(37.1%)	p=1.00
Male	10	6(60.0%)	4(40.0%)	

Table 2. Clinicopathologic variables and correlation with Ret protein expression in papillary carcinoma

	Ret		p-value	Total
	Positive	Negative		
LN metastasis			1.00	
Yes	40(63.5%)	23(36.5%)		63(74.1%)
No	14(63.6%)	8(36.4%)		22((25.9%)
Perithyroidal extension			1.00	
Yes	52(61.9%)	32(38.1%)		84(74.3%)
No	18(62.1%)	11(37.9%)		29(25.7%)
Multiplicity			0.50	
Yes	20(69.0%)	9(31.0%)		29(25.7%)
No	50(59.5%)	34(40.5%)		84(74.3%)
Size (cm)				
Mean	1.7±1.1	2.0±1.1	0.31	1.8±1.1
≤1cm	25(67.6%)	12(32.4%)		37(33.9%)
1-4cm	40(58.8%)	28(41.2%)		68(62.4%)
〉4cm	3(75.0%)	1(25.0%)		4(3.7%)

Table 3. Percent agreement of Ret and CK19 immunoreactivity in papillary carcinoma

	CK19		Total
	Positive	Negative	
Ret			
Positive	62 (53.9%)	10 (8.7%)	72
Negative	31 (27.0%)	12 (10.4%)	43
Total	93	22	

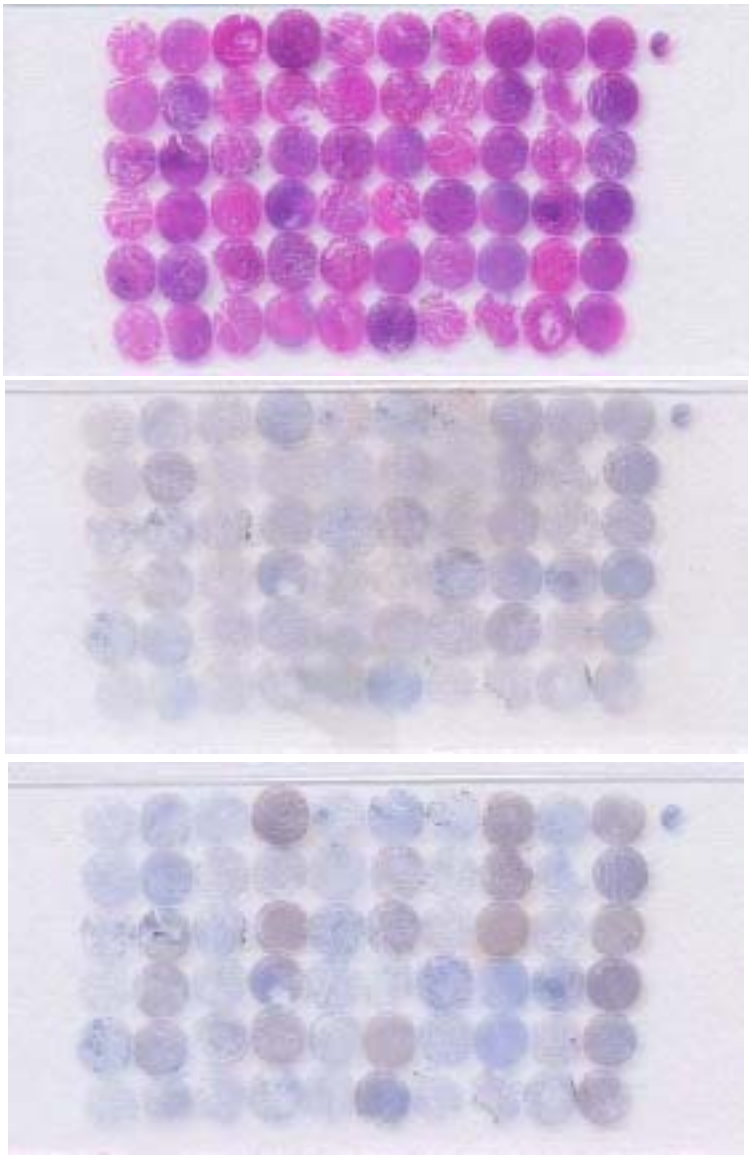

Figure 1. ***Top:*** 4-μm section of tissue microarray stained with H&E, showing 3mm cores of papillary thyroid carcinomas and matched normal thyroid tissues.

Center: Immunohistochemical stain for Ret antibody.

Bottom: Immunohistochemical stain for CK19.

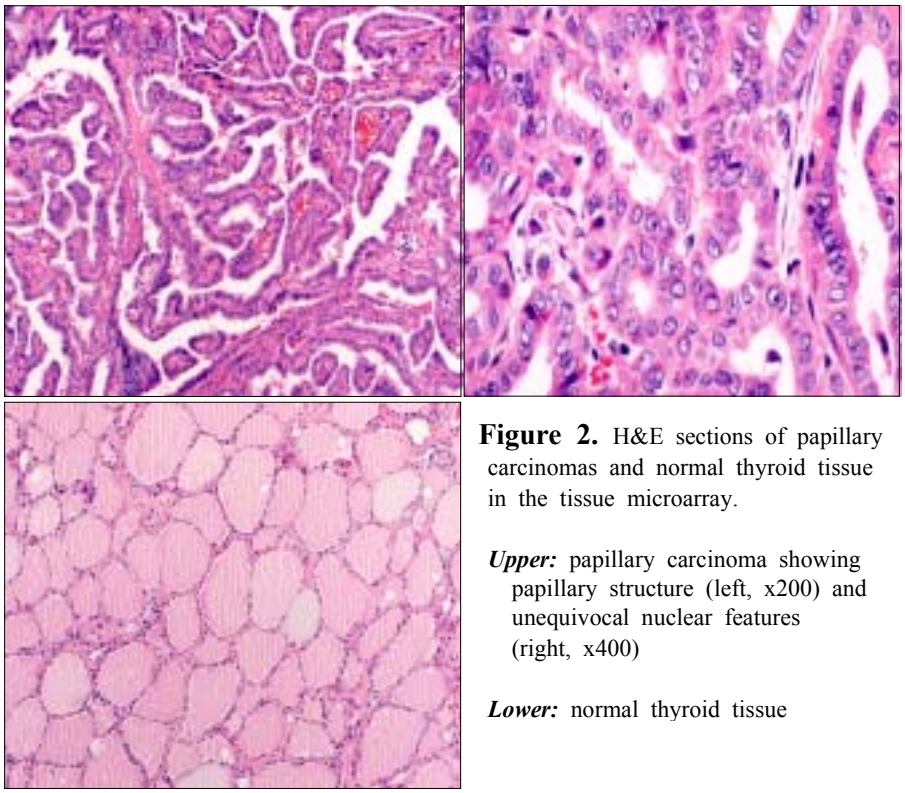

Figure 2. H&E sections of papillary carcinomas and normal thyroid tissue in the tissue microarray.

Upper: papillary carcinoma showing papillary structure (left, x200) and unequivocal nuclear features (right, x400)

Lower: normal thyroid tissue

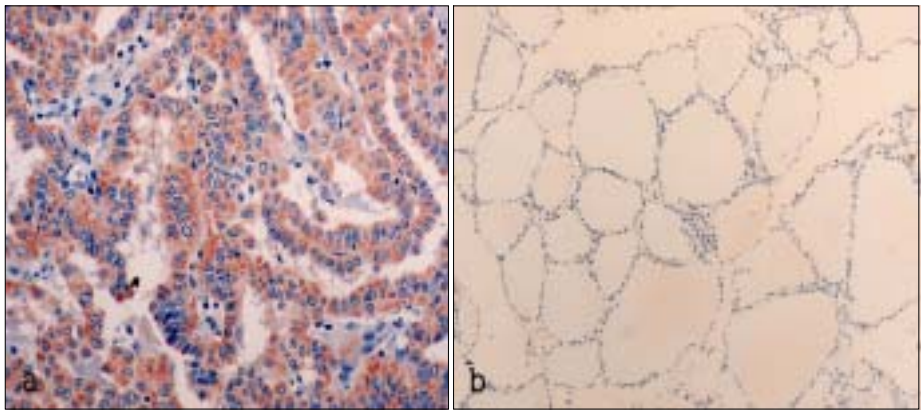

Figure 3. a) Diffuse cytoplasmic staining of the Ret antibody in the tumor tissue(x200) b) no immunoreactivity in the matched normal thyroid tissue(x100)

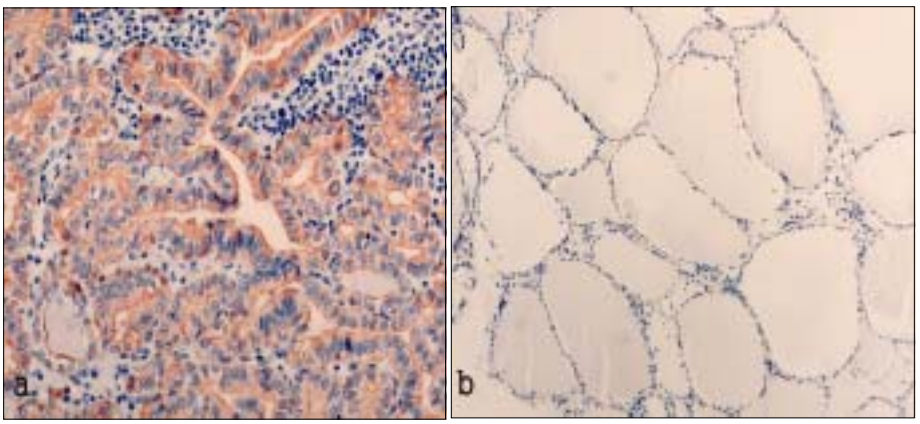

Figure 4. a) Diffuse cytoplasmic staining of the tumor cells for CK19(x200) b) no immunoreactivity in the matched normal thyroid tissue(x100)

IV. DISCUSSION

The RET proto-oncogene has been identified to be responsible for the inherited cancer syndrome MEN 2, and also involved in the molecular pathogenesis of sporadic medullary thyroid carcinomas, papillary thyroid carcinomas, and Hirschsprung's disease.[27]

In the pathogenesis of papillary thyroid carcinoma (PTC), RET activation has been hypothesized to constitute a specific genetic event and to account for the characteristic nuclear changes of PTC.[28] Sugg et al. identified RET/PTC rearrangements in the majority of occult papillary microcarcinomas and lower percentage of rearrangements in clinically manifest tumors, suggesting a significant role of RET/PTC rearrangements in the initiation of papillary carcinoma. However, the exact role of RET/PTC in the development of papillary carcinoma remains unclear and the prevalence of RET/PTC rearrangement is ill-defined, varying widely from 2.5-34.5% among patient series from different geographical regions.[12-16] The existence of geographic variability is shown by comparison of the prevalence of RET/PTC activation in different studies of sporadic papillary carcinomas, all of which have used reverse transcriptase polymerase chain reaction.[14,29,30,36] Santoro et al. found 11% in the French samples, 33% in the Italian samples, and 17% in the U.S. tumors.[7] These discordant results may be due in part to the different methods and sensitivity of the techniques used, as well as the influence of environmental factors such as ionizing radiation exposure. There have been two published data in Korea prior

to this current study. Park et al., in 1998, found no RET/PTC-1, -2, or -3 rearrangement in 24 cases of papillary carcinoma by RT-PCR in a Korean population[31], whereas Chung et al. reported a prevalence of 12.9% in Korean papillary thyroid carcinomas.[32] The latter authors found four out of thirty-one papillary thyroid carcinomas to be positive for RET/PTC rearrangement by RT-PCR, two of which were positive for RET/PTC-2 and the remaining two were positive for RET/PTC-3, and concluded that the prevalence of RET/PTC rearrangement in Korean population is not different from that of other Western countries.

Although the method of detection was different, the frequency of RET/PTC expression in our study was higher than in those two studies. By immunohistochemistry, we found 72 cases out of 115 classic papillary thyroid carcinomas (62.6%) to be positive for Ret protein expression, which is also in accord with previous results in Western countries employing immunohistochemistry as the method of detection.[33]

The immunohistochemical analysis on 3mm-core tissue microarray in our study can be reliably used to overcome the problem of tumor heterogeneity in protein expression throughout the entire tumor specimen. According to the recently published data on validation of tissue microarrays for immunohistochemical profiling of cancer specimens by Hoos et al.[34], the concordance of data between full tissue sections and tissue microarray was the highest with triplicate 0.6mm cores (96 - 98%) when compared with one and two 0.6mm tissue cores. However, even with the one core analysis, the nonconcordance rate was only 9.4 - 11.4%.[34] When the larger size of our tissue core

and the relatively smaller size of papillary carcinoma in general are taken into consideration, the validity of our immunohistochemical analysis on tissue microarray need not be in doubt.

Immunohistochemical staining using antibodies against the RET tyrosine kinase domain for the detection of RET/PTC in papillary carcinomas is based on the assumption that since Ret is not expressed in thyroid follicular cells lacking RET/PTC activation, negative RET-TK immunoreactivity is consistent with lack of RET/PTC rearrangement.[35,36] Furthermore, a good correlation has been shown between immunohistochemical reactivity for RET-TK and RT-PCR.[17,18,36] However, the discrepancy in results between groups employing RT-PCR/in situ hybridization and those using immunohistochemistry may be explained by the existence of wild-type RET oncogene. In addition to RET/PTC 1, 2, and 3 and their variants, it is probable that wild-type RET oncogene activation plays a role in the development of PTC. The fact that positive immunoreactivity for RET oncogene not only detects the product of RET/PTC oncogenes, but also the products of wild-type RET may account for the high rate of Ret protein immunoreactivity.

Many attempts have been made to correlate the presence of RET/PTC activation with the clinical parameters of human papillary thyroid carcinomas. Our results demonstrate that the Ret protein expression is not influenced by any of the clinicopathologic parameters, i.e. age, sex, size of the tumor, multiplicity, lymph node metastasis, and perithyroidal extension. Jhiang et al proposed that RET/PTC may be

associated with distant metastatic disease, as 2 of 4 (50%) tumors that had distant metastases expressed RET/PTC rearrangements compared to 4 of 32 (12%) tumors that did not metastasize.[15] Miki et al. found a higher incidence of RET/PTC expression in cases with extrathyroidal invasion than in those without invasion by immunohistochemistry (68% vs. 20%, $p<0.01$), suggesting that RET/PTC may be related to the local invasion of papillary carcinomas.[19] However, other investigators have shown that RET/PTC is more frequently detected in smaller, slow growing, and less aggressive PCs, proposing that RET/PTC may serve as a marker for favorable prognosis.[37,38] It has also been suggested that RET/PTC positivity correlates with early lymph node spread but lower metastatic potential.[29,39,40] This discrepancy may be explained by the differences in screening methods, sample preparation, genetic background of patients, and environmental factors. Thus, it is difficult to compare reports that have studied different patient populations and used different approaches to detect RET/PTC rearrangements. Also, it is yet to be determined whether the clinical behavior of human PC is affected by differences in RET/PTC expression levels, and/or the different forms of RET/PTC. It is conceivable that signaling pathways perturbed by RET/PTC activation in thyroid cells could be overcome by other factors, if RET/PTC expression occurs at a low level.[41]

Although papillary thyroid carcinomas have the propensity for regional lymph node metastasis, most of the patients have an excellent long-term survival rates. Such excellent prognosis rests on the appropriate treatment at the right time, which makes it imperative for

the pathologists to render an accurate diagnosis. However, identification of the cytologic features that are diagnostic of papillary carcinoma can be controversial, especially when these features are present focally or multifocally rather than diffusely throughout the lesion, or when the tumors assume non-papillary architecture, i.e. follicular variant of the papillary carcinoma.

Immunohistochemical investigation of papillary thyroid neoplasm had detected many markers for papillary thyroid carcinomas that have varying degrees of sensitivity and specificity for this neoplasm. The markers that appear to yield a high sensitivity and specificity include high molecular weight cytokeratin (AE3)[40], HBME, CD57 and CD15.[42-45] CK19 and high molecular weight cytokeratin were reported to be reactive in most papillary thyroid carcinomas.[20,21,24] The findings of our study showed 80.9% positivity in the 115 classic papillary thyroid carcinomas, which are in corroboration of previous reports that CK19 immunohistochemistry is a useful ancillary tool for diagnosing papillary carcinoma of the thyroid.[23,46,47] Cheung et al. have reported that CK19 and Ret antibody comprise an excellent diagnostic panel for papillary thyroid carcinoma along with HBME-1.[33] However, they did not show the percent agreement of the two antibodies in immunoreactivity. We have found 62 (53.9%) out of 115 papillary carcinomas to be positive for both Ret and CK19, implicating a relatively high percent agreement of the two antibodies in diagnosing papillary carcinoma. However, it was statistically not significant ($p=0.06$, $\kappa=0.16$), due in part to comparatively high percentage of

positive immunoreactivity for CK19.

Although none of the clinicopathological parameters showed any correlation with Ret protein expression in our cohort of 115 classic papillary thyroid carcinomas, RET/PTC oncogene activation is a marker for papillary thyroid carcinoma with potentially useful applications to the diagnosis of clinically suspicious thyroid lesions with questionable cytologic features of papillary carcinoma as well as to the follow-up of patients. Larger patient numbers and longer follow-up times may provide additional information in future studies.

V. CONCLUSION

RET protein expression and CK19 immunoreactivity were analyzed in 115 classic papillary thyroid carcinomas in Korea via tissue microarray.

1. The prevalence of RET protein expression detected by immunohistochemistry was 62.6% (72 out of 115 cases), which was in accord with that in Western countries.

2. The clinicopathological variables, i.e. age, sex, size of the tumor, regional lymph node metastasis, perithyroidal extension, and multiplicity, did not correlate with the expression of RET.

3. CK19 immunoreactivity was 80.9% (93 out of 115 cases) in papillary thyroid carcinomas, which was slightly higher but similar to that in Western countries.

4. The percent agreement of Ret positivity and CK19 immunoreactivity was relatively high, but statistically not significant.

In conclusion, the prevalence of RET protein expression and its clinicopathological implications in a Korean population are not different from those reported in previous studies. However, its detection via

immunohistochemistry can be a useful diagnostic tool for diagnosing papillary thyroid carcinoma in conjunction with CK19.

REFERENCE

0). Rosai J, Carcangiu ML, DeLellis RA. Tumors of the Thyroid Gland. Atlas of Tumor Pathology, Third Series, Fascicle 5. Washington, DC: Armed Forces Institute of Pathology; 1992:65-121.

0). Kjellman P, Learoyd DL, Messina M, Weber G, Hoog A, Wallin G, et al. Expression of the RET proto-oncogene in papillary thyroid carcinoma and its correlation with clinical outcome. Br J Surg 2001;88(4):557-63.

0). Belfiore A, Gangemi P, Costantino A, Russo G, Santonocito G, Ippolito O, et al. Negative/low expression of the Met/hepatocyte growth factor receptor identifies papillary thyroid carcinomas with high risk of distant metastases. J Clin Endocrinol Metab 1997;82:2322-28.

0). Learoyd DL, Twigg SM, Zedenius JV, Robinson BG. Molecular genetics of endocrine tumours. J Pediatr Endocrinol Metab 1998;11:195-228.

0). Basolo F, Molinaro E, Agate L, Pinchera A, Pollina L, Chiappetta G, et al. RET protein expression has no prognostic impact on the long-term outcome of papillary thyroid carcinoma. Eur J Endocrinol 2001;145:599-604.

0). Pierotti MA, Bongarzone I, Borrello MG, Greco A, Pilotti S, Sozzi G. Cytogenetics and molecular genetics of carcinomas arising from thyroid epithelial follicular cells. Genes Chromosomes Cancer 1996;16:1-14.

0). Santoro M, Carlomagno F, Hay ID, Hermann MA, Grieco M, Melillo R, et al. Ret oncogene activation in thyroid neoplasms is restricted to the papillary cancer subtype. J Clin Invest 1992;89:1517-22.

0). Cho JY, Sagartz JE, Capen CC, Mazzaferri EL, Jhiang SM. Early cellular abnormalities induced by RET/PTC1 oncogene in thyroid-targeted transgenic mice. Oncogene 1999;18:3659-65.

0). Grieco M, Santoro M, Berlingieri MT, Melillo RM, Donghi R, Bongarzone I, et al. PTC is a novel rearranged form of the ret proto-oncogene and is frequently detected in vivo in human thyroid papillary carcinomas. Cell 1990;60:557-63.

0). Pierotti MA, Santoro M, Jenkins RB, Sozzi G, Bongarzone I, Grieco M, et al. Characterization of an inversion on the long arm of chromosome 10 juxtaposing D10S170 and RET and creating the oncogenic sequence RET/PTC. Proc Natl Acad Sci USA 1992;89:1616-20.

0). Bongarzone I, Butti MG, Coronelli S, Borrello MG, Santoro M, Mondellini P, et al. Frequent activation of ret proto-oncogene by fusion with a new activating gene in papillary thyroid carcinomas. Cancer Res 1994;54:2979-85.

0). Lee CH, Hsu LS, Chi CW, Chen GD, Yang AH, Chen JY. High frequency of rearrangement of the RET proto-oncogene (RET/PTC) in Chinese papillary thyroid carciomas. J Clin Endocrinol Metab 1998;83:1629-32.

0). Wajjwalku W, Nakamura S, Hasegawa Y, Miyazaki K, Satoh Y, Funahashi H, et al. Low frequency of rearrangements of the ret and trk proto-oncogenes in Japanese thyroid papillary carcinomas. Jpn J Cancer Res 1992;83:671-75.

0). Ishizaka Y, Kobayashi S, Ushijima T, Hirohashi S, Sugimura T, Nagao M. Detection of retTPC/PTC transcripts in thyroid adenomas and adenomatous goiter by an RT-PCR method. Oncogene 1991;6:1667-72.

0). Jhiang SM, Caruso DR, Gilmore E, Ishizaka Y, Tahira T, Nagao M, et al. Detection of the PTC/retTPC oncogene in human thyroid cancers. Oncogene 1992;7:1331-37

0). Zou M, Shi Y, Farid NR. Low rate of ret proto-oncogene activation (PTC/retTPC) in papillary thyroid carcinomas from Saudi Arabia. Cancer 1994;73:176-80.

0). Viglietto G, Chiappetta G, Martinez-Tello FJ, Fukunaga FH, Tallini G, Rigopoulou D, et al. RET/PTC oncogene activation is an early event in thyroid carcinogenesis. Oncogene 1995;11:1207-10.

0). Sugg SL, Zheng L, Rosen IB, Freeman JL, Ezzat S, Asa SL. ret/PTC-1, -2, and -3 oncogene rearrangements in human thyroid carcinomas: implications for metastatic potential?. J Clin Endocrinol Metab 1996;81:3360-65.

0). Miki H, Kitaichi M, Masuda E, Komaki K, Yamamoto Y, Monden Y. ret/PTC expression may be associated with local invasion of thyroid papillary carcinoma. J Surg Oncol 1999;71(2):76-81.

20. Raphael SJ, McKeown-Eyssen G, Asa SL. High-molecular weight cytokeratin and cytokeratin 19 in the diagnosis of thyroid tumors. Mod Pathol 1994;7(3):295-300.

21. Schelfhout LJDM, Van Muijen GNP, Fleuren GJ. Expression of keratin 19 distinguishes papillary thyroid carcinoma from follicular thyroid adenoma. Am J Clin Pathol. 1989;11:654-58.

22. Beesley MF, McLaren KM. Cytokeratin 19 and galectin-3 immunohistochemistry in the differential diagnosis of solitary thyroid nodules. Histopathology 2002;41:236-43.

23. Sahoo S, Hoda SA, Rosai J, DeLellis RA. Cytokeratin 19 immunoreactivity in the diagnosis of papillary thyroid carcinoma: a note of caution. Am J Clin Pathol 2001;116:696-702.

24. Miettinen M, Kovatich AJ, Karkkainene P. Keratin subsets in papillary and follicular thyroid lesion: a paraffin section analysis with diagnostic implications. Virchows Arch 1997;431:407-13.

25. Baloch ZW, Abraham S, Roberts S, LiVolsi VA. Differential expression of cytokeratins in follicular variant of papillary carcinoma: an immunohistochemical study and its diagnostic utility. Hum Pathol 1999;30:1166-71

26. Altman DG. Practical Statistics for Medical Research. London: Chapman & Hall: 1996.

27. Komminoth P. The RET proto-oncogene in medullary and papillary thyroid carcinoma. Molecular features, pathophysiology and clinical implications. Virchows Arch 1997;431(1):1-9.

28. Fischer AH, Bond JA, Taysavang P, Battles OE, Wynford-Thomas D. Papillary thyroid carcinoma oncogene (RET/PTC) alters the nuclear envelope and chromatin structure. Am J Pathol 1998;153:1443-50.

29. Sugg SL, Ezzati S, Rosen IB, Freeman JL, Asa SL. Distinct multiple RET/PTC gene rearrangements in multifocal papillary thyroid neoplasia. J Clin Endocrinol Metab 1998;83:4116-22.

30. Motomura T, Nikiforov YE, Namba H, Ashizawa K, Nagataki S, Yamashita S, et al. RET rearrangements in Japanese pediatric and adult papillary thyroid cancers. Thyroid 1998;8:485-89.

31. Park KY, Koh JM, Kim YI, Park HJ, Gong G, Hong SJ, et al. Prevalences of Gs alpha, ras, p53 mutations and ret/PTC rearrangement in differentiated thyroid tumors in a Korean population. Clin Endocrinol 1998;49(3):317-23.

32. Chung JH, Hahm JR, Min YK, Lee MS, Lee MK, Kim KW, et al. Detection of RET/PTC oncogene rearrangements in Korean papillary thyroid carcinomas. Thyroid 1999;9(12):1237-43.

33. Cheung CC, Ezzat S, Freeman JL, Rosen IB, Asa SL. Immunohistochemical diagnosis of papillary thyroid carcinoma. Mod Pathol 2001;14(4):338-42.

34. Hoos A, Urist MJ, Stojadinovic A, Mastorides S, Dudas M, Kuo D, et al. Validation of tissue microarrays for immunohistochemical profiling of cancer specimens using the example of human fibroblastic tumors. Am J Pathol 2001;158:1245-51.

35. Chiappetta G, Hui P, Santoro M. RET/PTC oncogene activation and assessment of clonality in thyroid nodules with minimal cytologic evidence of papillary carcinoma. Lab Invest 2001;81:75A

36. Tallini G, Asa SL. RET oncogene activation in papillary thyroid carcinoma. Adv Anat Pathol 2001;8(6):345-54.

37. Soares P, Fonseca E, Wynford-Thomas D, Sobrinho-Simoes M. Sporadic ret-rearranged papillary carcinoma of the thyroid: a subset of slow growing, less aggressive thyroid neoplasms? J Pathol 1998;185:71-78

38. Tallini G, Santoro M, Helie M, Carlomagno F, Salvatore G, Chiappetta G, et al. RET/PTC oncogene activation defines a subset of papillary thyroid carcinomas lacking evidence of progression to poorly differentiated or undifferentiated tumor phenotypes. Clin Cancer Res 1998;4:287-94.

39. Mayr B, Goretzki P, Ruschoff J, Dralle H. Ret/PTC-1, -2, and -3 oncogene rearrangements in human thyroid carcinomas: implications for metastatic potential [Letter]? J Clin Endocrinol Metab 1997;82:1306-07.

40. Mai KT, YazdiHM, Perkins DG, Commons AS. Immunohistochemical study of papillary thyroid carcinoma and possible papillary thyroid carcinoma-related benign thyroid nodules. Pathol Res Pract 2000;196:533-40.

41. Jhiang SM. The RET proto-oncogene in human cancers. Oncogene 2000;19(49):5590-97.

42. van Hoeven KH, Kovatich AJ, Miettinen M. Immunocytochemical evaluation of HBME-1, CA19-9, and CD-15 (Leu-M1) in fine-needle aspirates of thyroid nodules. Diagn Cytopathol 1998;18:93-97.

43. Sack MJ, Astengo-Osuna C, Lin BT, Battifora H, LiVolsi VA. HBME-1 immunostaining in thyroid fine-needle aspiration: a useful marker in the diagnosis of carcinoma. Mod Pathol 1997;10:668-74.

44. Miettinen M, Karkkainen P. Differential reactivity of HBME-1 and CD15 antibodies in benign and malignant thyroid tumours. Preferential reactivity with malignant tumours. Virchows Arch 1996;429:213-19

45. Ostrowski ML, Brown RW, Wheeler TM, Green LK, Schaffner DL. Leu-7 immunoreactivity in cytologic specimens of thyroid lesion, with emphasis on follicular neoplasms. Diagn Cytopathol 1995;12:297-302.

46. Nasser SM, Pitman MB, Pilch BZ, Faquin WC. Fine-needle aspiration biopsy of papillary thyroid carcinoma: diagnostic utility of cytokeratin 19 immunostaining. Cancer 2000;90:307-11.

47. Beesley MF, McLaren KM. Cytokeratin 19 and galectin-3 immunohistochemistry in the differential diagnosis of solitary thyroid nodules. Histopathology 2002;41:236-43.

국문요약

갑상선 유두암에서 RET/PTC 와 CK19의 발현 및 임상적 의의

갑상선 유두암은 갑상선 종양 중 가장 흔한 것으로서 그 예후가 매우 좋은 것으로 알려져 있다. 갑상선 유두암은 그에 특이한 세포학적 형태, 특히 핵의 모양이 진단적인 것으로 되어왔으나 그러한 세포형태가 애매하거나 부분적으로만 존재할 경우에 진단에 어려움이 생기게 된다. 이러한 문제에 대한 해결책의 일환으로 여러 가지 면역화학적 표지자들이 연구되어왔으며 이 중에서도 그 특이성에 대하여 아직 논란은 있지만 CK19가 갑상선 유두암의 진단에 가장 유용하다는 보고들이 알려져 있다.

최근 갑상선 유두암의 발생에 관여하는 유전자 단계의 변화 중 가장 흔한 것으로 RET 원종양유전자의 재배열이 보고되었으나 이의 빈도는 지역마다 차이가 있는 것으로 보고되어 왔으며 임상적 예후와의 관련성도 보고마다 많은 차이가 있다. 한국인의 갑상선 유두암에서 RET/PTC 재배열의 빈도 역시 보고마다 차이가 있으며 임상적 예후와의 관련성은 아직 보고된 바가 없는데 이를 위하여 저자는 3㎜-core tissue microarray를 사용하여 115개의 한국인 갑상선 유두암에서 Ret과 CK19의 면역화학적 분석을 통하여 그 발현 빈도와 임상적 변수와의 연관성을 연구하였다.

115개의 갑상선 유두암 중 62.6%인 72개에서 Ret에 대하여 양성이었고 80.9%인 93개에서 CK19에 양성반응을 보였으며 이러한 빈도는 서방에서의 면역화학적 연구결과와 일치하는 것이었다. Ret과 CK19의 일치도는 비교적 높은 비율을 보였으나 통계학적으로 의의는 없는 것이었다. 또한 환자의 나이나 성별, 종양의 크기, 주변

임파선으로의 전이 여부, 주위조직으로의 침윤 여부, 그리고 종양의 다발성등과 Ret의 발현과는 연관성이 없는 것으로 나타났다.

결론적으로 한국인의 갑상선 유두암에서 Ret 단백의 발현 빈도와 임상적 변수와의 연관성은 지금까지의 보고와 큰 차이가 없으나 Ret에 대한 면역화학적 염색은 CK19와 함께 갑상선 유두암의 진단에 있어 유용하게 사용될 수 있을 것으로 사료된다.

핵심되는 말: 갑상선 유두암, Ret단백, CK19, tissue microarray, 한국인, 면역조직화학 염색