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Expression of CK-19, cErbB2, galectin-3, and p53 in papillary thyroid carcinomas

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Aim: To determine the immunohistochemical expression of CK-19, cErbB2, galectin-3, and p53 in papillary thyroid carcinomas (PTC).

Materials and methods: In this study immunohistochemistry was performed on 23 papillary carcinomas (PCs) and 20 papillary microcarcinomas (PMCs). The extents of staining and intensity were scored semiquantitatively.

Results: Of the PC cases 56.5% and of the PMC cases 15.0% were stained strongly by cErbB2. The extent of 2+ staining by galectin-3 was 47.8% in PC, whereas it was only 10.0% in PMC (P=0.003). All PMC cases were stained by CK-19 and 21 cases of PC expressed positivity in varying extent of staining. No nuclear immunostaining was detected by p53 in PC and PMC. However, 65.2% of PC and 55.0% of PMC cases showed cytoplasmic reactivity.

Conclusion: Both PC and PMC cases showed similar staining extent and intensity by CK-19. This may conclusively help in identifying the papillary carcinoma cases. The majority of PCs stained diffusely and strongly by galectin-3 and expressed strong reaction by cErbB2, which might suggest a relation with the size of the tumor as PMCs stained less diffusely and strongly. As no nuclear staining was detected by p53 in 43 papillary carcinomas, we support the idea that p53 expression is a late event in thyroid carcinogenesis.

Key words: Papillary thyroid carcinoma, papillary thyroid microcarcinoma, CK-19, cErbB2, galectin-3, p53

Papiller tiroid karsinomlarında CK-19, cErb B2, galectin-3 ve p53 ekspresyonu

Amaç: Bu çalışmada papiller tiroid karsinomlarında CK-19, cErbB2, galectin-3 ve p53 ekspresyonu araştırıldı.

Yöntem ve gereç: Çalışmaya dahil edilen 23 papiller karsinom (PK) ve 20 papiller mikrokarsinom (PMK) olgusuna immünhistokimya uygulandı. Boyanma yaygınlığı ve yoğunluğu semikantitatif olarak değerlendirildi.

Bulgular: PK olgularının % 56,5' i ve PMK olgularının % 15,0' i cErbB2 ile güçlü immünreaksiyon gösterdi. Galectin-3 ile PK olgularının % 47,8' inde boyanma yaygınlığı skoru 2+ iken, PMK olgularında bu oran % 10,0 idi (P = 0,003). PMK olgularının tamamı ile PK olgularının 21' inde değişken yaygınlıkta CK-19 ekspresyonu gözlendi. p53 ile nükleer boyanma saptanmazken PK olgularının % 65,2'sinde ve PMK olgularının % 55,0'inde sitoplazmik immünreaksiyon gözlendi.

Sonuç: Hem PK hem de PMK olgularının benzer yaygınlık ve yoğunlukta CK-19 ekspresyonu göstermesi, papiller karsinomun saptanmasında bu markırın yardımcı olabileceğini düşündürür. PK olgularının çoğunluğunda yaygın ve güçlü galectin-3 ekspresyonu, cErbB2 ile bu olguların güçlü immünreaksiyon göstermesi ve PMK olgularında daha az yaygın olan zayıf boyanma, bu boyanmaların tümör boyutu ile ilişkili olabileceğini akla getirir. Toplam 43 papiller karsinomun hiçbirinde p53 ile nükleer immünreaksiyon gözlenmemesi, p53'ün tiroid karsinogenezisinde daha ileri basamaklarda rol aldığı görüşünü desteklemektedir.

Anahtar sözcükler: Papiller tiroid karsinomu, papiller tiroid mikrokarsinomu CK-19, cErbB2, galectin-3, p53

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Introduction

Papillary thyroid carcinoma is the most prevalent endocrine cancer. The diagnosis of papillary thyroid carcinoma is based on nuclear features. However, identification of these features is controversial. Previous studies have advocated various antibodies to aid in the differential diagnosis but there is little agreement on their utility.

CK-19 is a sensitive marker of papillary thyroid carcinoma. Focal or nonspecific CK-19 staining may be found in benign thyroid lesions but diffuse and strong positivity is characteristic for PC (1,2) and a negative result is helpful in excluding PC (3).

cErbB2 is known to be expressed in several human neoplasms but the prognostic significance varies in different tumor types. Overexpression of cErbB2 is easily identifiable by immunohistochemistry in papillary thyroid carcinomas (4,5).

Galectin-3 is a useful thyroid malignancy marker. It is a beta-galactosyl-binding lectin and involved in regulating cell-cell and cell-matrix interactions. Galectin-3 is expressed in normal breast epithelial cells, inflammatory cells, and various malignant cells. It is suggested that galectin-3 expression be of value in discriminating benign and malignant thyroid lesions (6,7).

The p53 gene is a protein with many regulatory functions involving cell cycle progression, DNA repair, and programmed cell death. The p53 gene mutations are the most prevalent genetic alterations in human tumors and have been found in over 15% of thyroid neoplasms. The p53 gene mutations promote more aggressive cancers (8).

The purpose of our study was to determine the expression of CK-19, cErbB2, and galectin-3 in papillary thyroid carcinomas. Besides, we tried to assess the potential staining of those tumors by p53.

Materials and methods

Materials were collected from surgically removed thyroid lobes of 43 patients who were subjected to thyroidectomy between 2003 and 2006 at Numune Training and Research Hospital, Ankara-Turkey. Histopathological classification was performed by the Department of Pathology at the same hospital, and in this study the lesions included were 23 papillary carcinomas (PCs) and 20 papillary microcarcinomas (PMCs). Papillary carcinoma group included 14 conventional PCs and 9 follicular variant PCs. All the tissues were routinely formalin fixed and paraffin embedded.

Immunohistochemistry was performed on 5 μm thick sections using the labeled streptavidin-biotin peroxidase complex system (LSAB2). Heat-induced antigen retrieval was carried out for CK-19 (clone A53-3/A2.26, Neomarkers), cErbB2 (clone SP3, Neomarkers), galectin-3 (clone 9c4, Neomarkers) and p53 (clone DO-7, DakoCytomation) (Dako's Target Retrieval solution, pH 6, steaming for 20'), and sections were incubated with primary antibodies for 30'-60' at room temperature. After this step, all sections were blocked for endogenous biotin through incubating by biotin solution for 20' (Dako's biotin peroxidase). Positive controls were the sections of skin for CK-19, small bowel for galectin-3, breast cancer for cErbB2, and colon adenocarcinoma for p53.

Semiquantitative evaluations were made on the basis of intensity and extent of staining for CK-19, cErbB2, and galectin-3. The extent of staining by CK-19, cErbB2, and galectin-3 were calculated according to the percentage of positive cells as no staining:0; < 25% stained cells:1+; 25% to 75% stained cells:2+; >75% stained cells:3+, whereas the intensity of staining by CK-19, cErbB2, and galectin-3 was evaluated as no staining:0; faint:1+; and, strong:2+. Nuclear staining for p53 was evaluated.

Chi-square test was used to determine statistical significance with P value for comparing the extent and intensity of those markers between 2 groups.

Results

In the majority of PCs we found strong and diffuse expression of CK-19. Six of 14 conventional PC (42.9%) and 6 of 9 follicular variant PC (66.7%) cases stained diffusely by CK-19. In 11 of 14 conventional PCs (78.6%) and 6 of 9 follicular variant PCs (66.7%), CK-19 expression was strong. Between 2 groups, the difference was not statistically significant in both the extent and intensity of CK-19 expression (P>0,05). All PMC cases were stained by CK-19 (35.0% 1+, 25.0% 2+, 40.0% 3+) and 21 cases of PCs (17.4% 1+, 21.7% 2+, 52.2% 3+) expressed positivity. CK-19 expression was strong in 73.9% of PCs and 65.0% of PMCs and the difference was statistically not significant (P=0.140) (Tables 1A, 1B) (Figures 1A and 1B).

Except 4 PCs and 7 PMCs, cErbB2 was positive in varying extents. Of the PC cases 56.5% and of the PMC cases 15.0% were stained strongly by cErbB2. Although by means of staining intensity of cErbB2, the difference in PC and PMC cases was found statistically significant (P = 0.019), but the results in the extent of staining were not found significant (P = 0.195) (Tables 2A and 2B) (Figures 2A,2B, and 2C). In 7 of 14 conventional PCs (50.0%) and 3 of 9 follicular variant PCs (33.3%) the expression of cErbB2 scored as 3+. cErbB2 expression was strong in 9 of 14 conventional PCs (64.3%) and in 4 of 9 follicular variant PCs (44.4%). The difference between 2 groups was not statistically significant (P > 0.05).

Of the PC cases, 47.8% was scored as 2+ while only 10.0% was scored as 2+ in PMC by galectin-3 (P = 0.003). Strong immunoreaction for galectin-3 was obtained in 52.2% of PCs and 15.0% of PMCs (P = 0.006) (Tables 3A and 3B) (Figures 3A and 3B). Three of 23 PC and 11 of 20 PMC cases were not stained by galectin-3. Among PC cases, galectin-3 negative ones were all conventional. Of the conventional PC group 6 cases (42.9%), and of the follicular variant group (11.1%) 1 case expressed galectin-3 diffusely (3+). Staining intensity by galectin-3 was strong in 8 of 14 conventional PCs (57.1%) and in 3 of 9 follicular variant PCs (33.3%). The differences were not statistically significant (P > 0.05).

Table 1. A) Extent of staining by cytokeratin-19.

	Extent	PC	PMC
CK- 19	0	2(8.7%)	0
	1+	4(17.4%)	7(35.0%)
	2+	5(21.7%)	5(25.0%)
	3+	12(52.2%)	8(40.0%)

P > 0.05

Table 1. B) Intensity of staining by cytokeratin-19.

	Intensity	PC	PMC
CK- 19	0	2(8.7%)	0
	1+	4(17.4%)	7(35.0%)
	2+	17(73.9%)	13(65.0%)

P > 0.05

Neither PCs, nor PMCs expressed nuclear p53. Cytoplasmic reactions with p53 in 15 of PC cases (10 of 14 conventional PCs and 5 of 9 follicular variant PCs) and 11 of PMC cases were observed. Also in 2 PCs there was cytoplasmic p53 expression in the peritumoral thyroid tissue (Figures 4A and 4B).

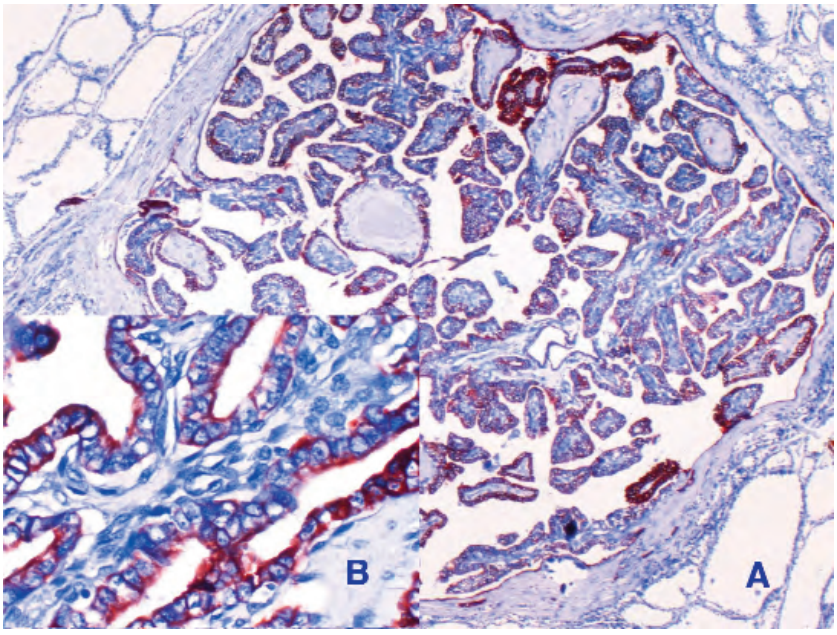

Figure 1. A) 3+ staining extention of CK-19 in PMC (×40); B) 2+ staining intensity of CK-19 in PMC (×200).

Table 2. A) Extent of staining by cErbB2.

	Extent	PC	PMC
cErbB2	0	4(17.4%)	7(35.0%)
	1+	4(17.4%)	5(25.0%)
	2+	5(21.7%)	5(25.0%)
	3+	10(43.5%)	3(15.0%)

P > 0.05

Table 2. B) Intensity of staining by cErbB2.

	Intensity	PC	PMC
cErbB2	0	4(17.4%)	7(35.0%)
	1+	6(26.1%)	10(50.0%)
	2+	13(56.5%)	3(15.0%)

P = 0.019

Table 3. A) Extent of staining by galectin-3.

	Extent	PC	PMC
Galectin-3	0	3(13.0%)	11(55.0%)
	1+	3(13.0%)	5(25.0%)
	2+	11(47.8%)	2(10.0%)
	3+	6(26.1%)	2(10.0%)

P = 0.003

Table 3. B) Intensity of staining by galectin-3.

	Intensity	PC	PMC
Galectin-3	0	3(13.0%)	11(55.0%)
	1+	8(34.8%)	6(30.0%)
	2+	12(52.2%)	3(15.0%)

P = 0.006

Discussion

In this study we evaluated the expressions of CK-19, cErbB2, galectin-3, and p53 in 23 PCs and 20 PMCs.

In the majority of PCs, we found strong and diffuse expression of CK-19. In PMCs 40.0% stained 3+ and 65.0% reacted strongly. Focal CK-19 staining may be found in benign lesions but diffuse positivity is characteristic of papillary carcinomas (1). Our results are in concordance with the previous studies related to CK-19 expression in PCs (1-3).

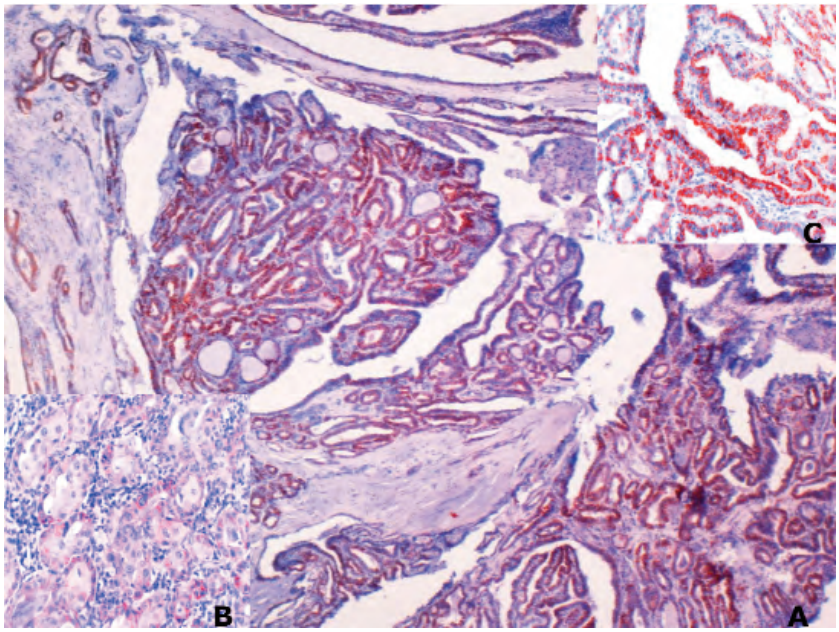

Figure 2. A) 3+ staining extention of cErb B2 in PC (×40); B) 1+ staining intensity of cErb B2 in PC (x200). C. 2+ staining intensity of cErbB2 in PC (×200).

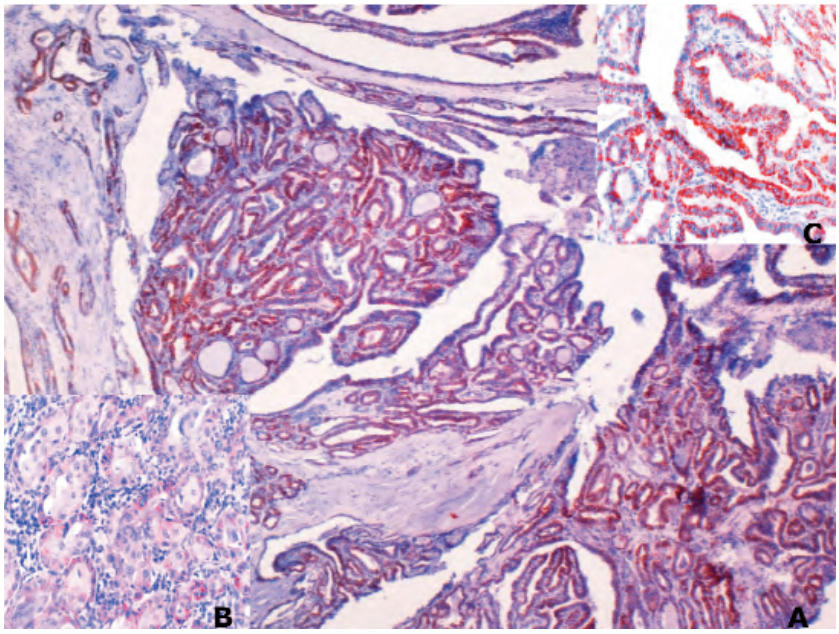

Figure 3. A) 3+ staining extention of galectin-3 in PC (×40); B) 2+ staining intensity of galectin-3 in PC (×200).

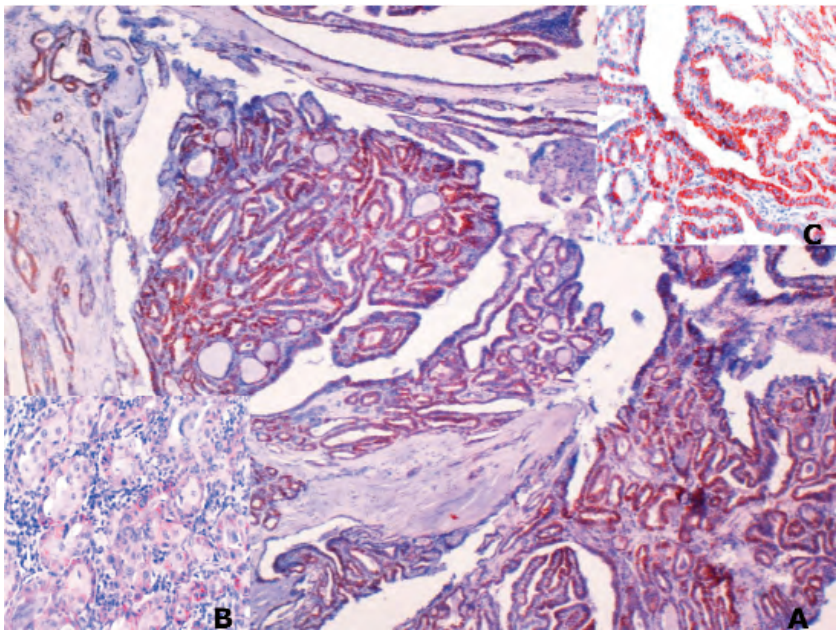

Figure 4. A;B) Cytoplasmic expression of p53 in PC (×40;×200).

The role of cErbB2 oncogenes in the pathogenesis of thyroid tumors remains unclear. In a large series, Lemoine did not detect cErbB2 abnormalities in thyroid tumors (9). Most other investigators found cErbB2 levels to be significantly elevated in papillary thyroid cancer (4,5,10,11,12). It is reported that the detection of cErbB2 overexpression by immunohistochemistry in papillary thyroid carcinomas suggests that the detection of cErbB2 may possibly be involved in proliferative responses and

differentiation of follicular cells (4). In a study conducted on differentiated thyroid carcinomas by Ensinger et al., it was found that cErbB2 overexpression has been detected in both papillary and follicular carcinomas (5). In our study the majority of PCs and PMCs were stained by cErbB2. The extent of staining by cErbB2 was 3+ in 43.5% of PCs and in 15.0% of PMCs. Intensity of staining by cErbB2 was strong in 13 PCs (56.5%) and in 3 PMCs (15.0%). The result was statistically significant. This may indicate the importance of the size of the tumor and cErbB2 oncoprotein may play a role in disease progression.

Galectin-3 immunostaining is recognized to be highly sensitive and accurate to identify cancer (6). It is known that, in conjunction with the other markers, galectin-3 may serve as a useful tool in the diagnosis of thyroid cancer (7). In the present study the extent of staining by galectin-3 in PCs was significantly higher than in PMCs (P = 0.003) and strong immunoreactivity was obtained in 12 PCs (52.2%) while only 3 PMCs (15.0%) showed strong positivity. This might reflect a relation between the expression of galectin-3 and the size of the tumor. On the other hand, in a study on 63 PMC cases, the expression of galectin-3 was reported to be 80.9% (13). We found the expression of galectin-3 in 45.0% of PMC's in varying extents. The presence of galectin-3 in clinically silent PMCs may indicate some other role of galectin-3 in thyroid tumor biology.

Though expected, no nuclear immunostaining was detected by p53 in PCs and PMCs. However, 62.2% of PC and 55.0% PMC cases showed cytoplasmic staining. Nuclear p53 expression is known to have a relation with tumor size, extrathyroidal extension, and survival (8). Cytoplasmic p53 staining was obtained together with p21 and BAX expressions in a study and it was concluded that this could be responsible for the tumor biology (14). Our results might support the idea that p53 is a late event in thyroid carcinogenesis.

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