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Usefulness of Galectin-3, Cytokeratin 19, *p*53, and Ki-67 for the Differential Diagnosis of Thyroid Tumors

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Background : The expressions of galectin-3, cytokeratin 19, *p*53, and Ki-67 in papillary carcinoma (PC), follicular carcinoma (FC), follicular adenoma (FA), and nodular hyperplasia (NH) are characteristic for the differential diagnosis between benign and malignant thyroid tumors. **Methods :** The expressions of the four markers were evaluated in PC (n=37), FC (n=12), FA (n=22), and NH (n=23) by immunohistochemical staining. **Results :** Statistical analyses revealed that galectin-3 was significantly expressed in the malignant tumor cells of PC and FC, while CK19 was expressed only in PC. **Conclusion :** These results show that galectin-3 is useful in differential diagnosis between malignant and benign thyroid lesions, especially between FC and FA in the patients over 20 years old, and indicate that CK19 is valuable in differentiating between follicular variant of PC and FC and between PC and papillary area of nodular hyperplasia.

Key Words : Galectin-3; Cytokeratin 19; Thyroid gland

Thyroid cancers account for 3.7% of all the cancers in Koreans. 84% of these tumors are papillary carcinomas, including the follicular variant, and 5.5% are follicular carcinomas. The most commonly performed diagnostic procedures are fine needle aspiration (FNA) cytology and core needle biopsy. Nevertheless, the follicular carcinoma and follicular adenoma cannot be easily distinguished except when there are findings of capsular or vascular invasion.[1]

Many researchers have searched for markers to differentiate between the two lesions, and they have proposed that galectin-3 is a useful candidate.[2-12] Galectin-3 is a 31-kDa β-galactoside-binding lectin.[13] It is predominantly expressed in the cytoplasm, but it is also expressed in the nucleus and at the cell surface of epithelial cells and immune cells.[12] However, several authors have insisted that galectin-3 does not reliably distinguish benign from malignant thyroid neoplasms.[13-15]

In addition to galectin-3, the proposed markers for differentiating benign from malignant thyroid lesions are cytokeratin-19 (i.e., CK19),[3,5,12] *p*53,[16-18,22] Ki-67,[2,3,5,18-20,23] and bcl-2.[18,19,21] CK19 is the lowest molecular weight cytokeratin and it is found on a diverse range of normal epithelia and also tumors.[12] Some authors have insisted that its expression in the thyroid gland is limited to papillary carcinomas and it's believed that it is useful in differentiating the follicular variant of papillary carcinoma from follicular carcinoma. *p*53 is a well known tumor suppressor gene, and its mutation is the most common genetic alteration in human tumors.[24] Ki-67 is an antigen that corresponds to a nuclear nonhistone protein that's expressed by cells in the proliferative phases.[25]

RET/PTC rearrangements have been observed in childhood papillary carcinomas, but these are not common in adults suffering with sporadic papillary carcinoma.[26,27] Rosenbaum *et al*.[28] observed BRAF mutations in four of the 20 papillary carcinomas from young patients as compared to 50 of 65 papillary carcinomas from adult patients (20% vs 77%, respectively). The age range of the young patients was from 10 to 17 years and that of the adult patients was more than 20 years. These genetic alterations were considered to be a function of age.

We assessed the expressions of galectin-3, CK19, *p*53, and Ki-67 in papillary carcinoma (PC), follicular carcinoma (FC), follicular adenoma (FA), and nodular hyperplasia (NH) to evaluate their utility in the differential diagnosis between benign and malignant thyroid lesions. We also compared the expression level of the markers according to patients' age of PC.

MATERIALS AND METHODS

Specimens

A total of 94 formalin fixed, paraffin-embedded thyroid specimens were obtained from the Department of Pathology at Chungnam National University Hospital. All of the patients had been operated on between 1998 and 2005. There were cases of PC (n=37), FC (n=12), FA (n=22), and NH (n=23). 27 cases of PC were ≥20 years old (PC20), and 10 cases were <20 years old (PC19). Five of the PC20 cases were follicular variants of PC. Ten of the FC cases (83%) were of the minimally invasive type.

Immunohistochemical stains and evaluation

Monoclonal antibodies to galectin-3, CK19, *p*53 and Ki-67 were applied according to the manufacturer's guidelines. For galectin-3, mouse monoclonal antibody clone 9C4 (Novocastra, Newcastle, UK) was used at a dilution of 1:150. For CK19, monoclonal antibody clone BA-17 (Dako, Glostrup, Denmark) was used at a dilution of 1:70. For *p*53, mouse monoclonal antibody clone DO-7 (Dako, Glostrup, Denmark) was used at a dilution of 1:70. For Ki-67, mouse monoclonal antibody clone MIB-1 (Zymed, San Francisco, CA, USA) was used at a dilution of 1: 150. Antigen retrieval for CK19 was performed via a microwave oven for 10 min with citrate buffer at pH6. Antigen retrieval for galectin-3, *p*53 and Ki-67 was performed by autoclaving for 10 min with citrate buffer at pH6. Incubation was done overnight at 4℃.

Multiple microscopic fields were examined and the results were expressed in a semiquantitative fashion according to the estimated percentage of positive tumor cells: 0, stained in less than 5% of the tumor cells; 1, stained in 5% to 30% of the tumor cells; 2, stained in more than 30% of the tumor cells. A score of 0 was considered as "negative" and scores of 1 and 2 were considered "positive".

Table 1. Results of immunohistochemical stainings

Diagnosis (No. of cases)	Score of positivity			No. of cases (%)	
	0	1	2	N	P
Expression of galectin-3					
PC20 (n=27)	8	1	18	8 (29.6)	19 (70.4)
FC (n=12)	5	2	5	5 (41.7)	7 (58.3)
FA (n=22)	21	1	0	21 (95.5)	1 (4.5)
NH (n=23)	21	2	0	21 (91.3)	2 (8.7)
PC19 (n=10)	8	2	0	8 (80.0)	2 (20.0)
Expression of cytokeratin 19					
PC20 (n=27)	5	2	20	5 (18.5)	22 (81.5)
FC (n=12)	11	0	1	11 (91.7)	1 (8.3)
FA (n=22)	19	2	1	19 (86.4)	3 (13.6)
NH (n=23)	21	1	1	21 (91.3)	2 (8.7)
PC19 (n=10)	0	0	10	0 (0.0)	10 (100.0)
Expression of *p*53					
PC20 (n=27)	21	4	2	21 (77.8)	6 (22.2)
FC (n=12)	10	2	0	10 (83.3)	2 (16.7)
FA (n=22)	20	2	0	20 (90.9)	2 (9.1)
NH (n=23)	18	5	0	18 (78.3)	5 (21.7)
PC19 (n=10)	9	1	0	9 (90.0)	1 (10.0)
Expression of Ki-67					
PC20 (n=27)	26	1	0	26 (96.3)	1 (3.7)
FC (n=12)	12	0	0	12 (100.0)	0 (0.0)
FA (n=22)	20	2	0	20 (90.9)	2 (9.1)
NH (n=23)	23	0	0	23 (100.0)	0 (0.0)
PC19 (n=10)	8	2	0	8 (80.0)	2 (20.0)

PC20, papillary carcinoma (age ≥20 years old); FC, follicular carcinoma; FA, follicular adenoma; NH, nodular hyperplasia; PC19, papillary carcinoma (age <20); N, negative; P, positive.

Table 2. Results of statistical analyses

Comparison	p-value	
	Unpaired T-test	χ^2 or Fisher's exact test
galectin-3 expression		
PC20 vs FC	0.175	0.713
PC20 vs FA	0.000	0.000
PC20 vs NH	0.000	0.000
FC vs FA	0.005	0.001
FC vs NH	0.007	0.003
CK19 expression		
PC20 vs FC	0.000	0.000
PC20 vs FA	0.000	0.000
PC20 vs NH	0.000	0.000
FC vs FA	0.937	1.000
FC vs NH	0.840	1.000
*p*53 expression		
PC20 vs FC	0.503	1.000
PC20 vs FA	0.130	0.269
PC20 vs NH	0.603	1.000
FC vs FA	0.527	0.602
FC vs NH	0.731	1.000
Ki-67 expression		
PC20 vs FC	0.512	1.000
PC20 vs FA	0.445	0.581
PC20 vs NH	0.361	1.000
FC vs FA	0.162	0.529
FC vs NH	identical	identical
PC20 vs PC19		
CK19	0.008	0.295
Galectin-3	0.000	0.009
*p*53	0.212	0.647
Ki-67	0.265	0.172

PC20, papillary carcinoma (age ≥20 years old); FC, follicular carcinoma; FA, follicular adenoma; NH, nodular hyperplasia; PC19, papillary carcinoma (age <20).

Statistical analysis

The expressions of the four markers were compared between the PC20 and FC, between the PC20 and FA, between the PC20 and NH, between the FC and FA, between the PC20 and PC19, and between the FC and NH using unpaired T-tests. Each lesion was then divided into two groups, the "negative" and "positive", and χ^2 tests or Fisher's exact tests were also performed in the same way.

RESULTS

The immunohistochemical staining results are summarized in Table 1. The statistical analysis results are also summarized in Table 2.

Galectin-3 expression

Galectin-3 was expressed in 19 of the 27 cases (70.4%) of PC20 (Fig. 1B), in 7 of the 12 cases (58.3%) of FC (Fig. 2B), in

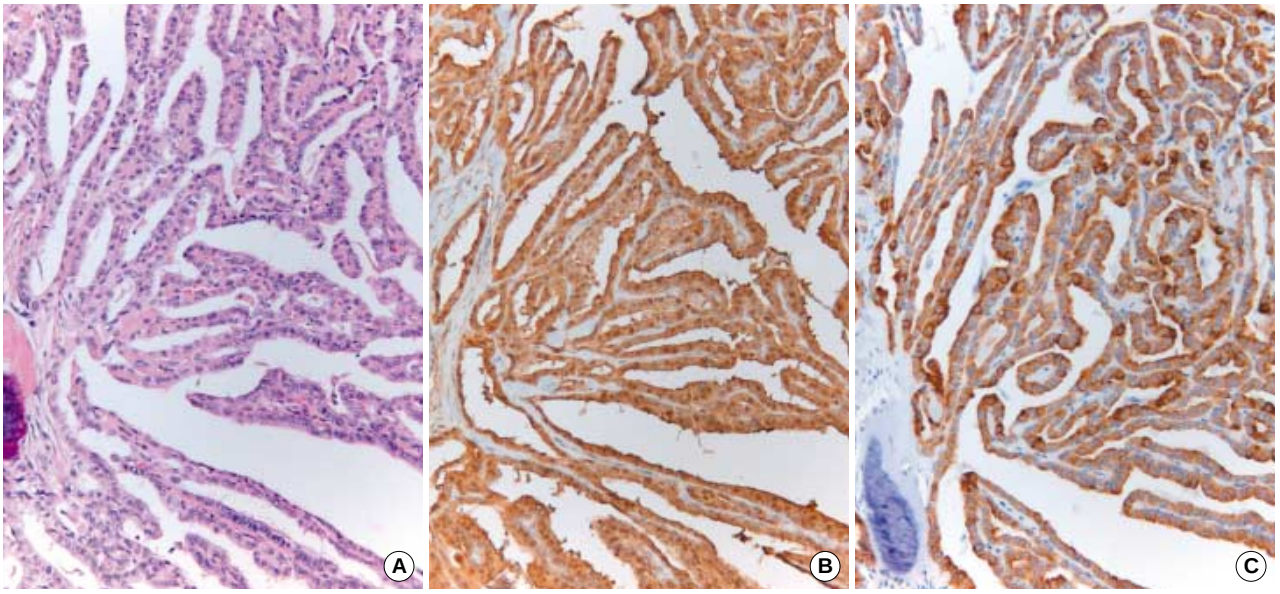

Fig. 1. Papillary carcinoma (age >= 20) (A) showing strong expressions of galectin-3 (B) and CK19 (C).

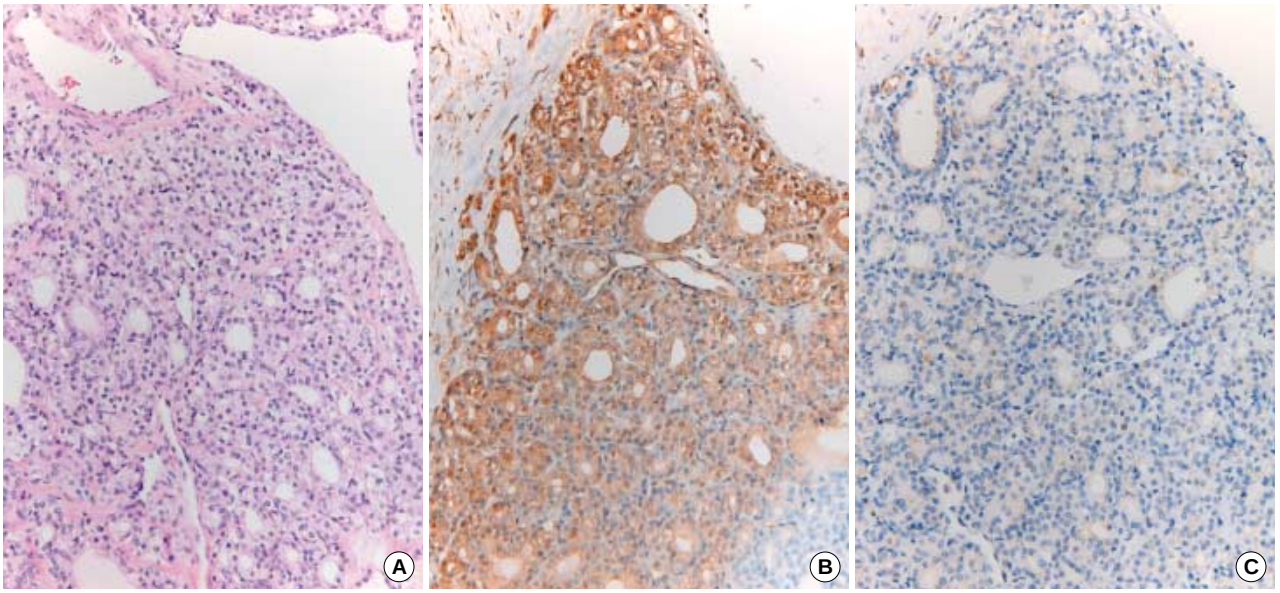

Fig. 2. Follicular carcinoma (A) showing strong expression of galectin-3 (B) and no expression of CK19 (C).

1 of the 22 cases (4.5%) of FA (Fig. 3B), and in 2 of the 23 cases (8.7%) of NH (Fig. 4B). The unpaired T-tests were significant between the benign and malignant cases, for PC20 versus FA, for PC20 versus NH, for FC versus FA and for FC versus NH, but they were not significant between PC20 and FC. The results of χ^2 or Fisher's exact tests were same as those of the unpaired T-tests.

Expression of CK19

CK19 was expressed in 22 of the 27 cases (81.5%) of PC20 (Fig. 1C), in 1 of the 12 cases (8.3%) of FC (Fig. 2C), in 3 of the 22 cases (13.6%) of FA (Fig. 3C), and in 2 of the 23 cases (8.7%) of NH (Fig. 4C). The results of the unpaired T-tests and χ^2 or Fisher's exact tests revealed that CK19 was significantly expressed only in the PC20.

Expression of *p*53

*p*53 was expressed in 6 of the 27 cases (22.2%) of PC20, in 2 of the 12 cases (16.7%) of FC, in 2 of the 22 cases (9.1%) of FA,

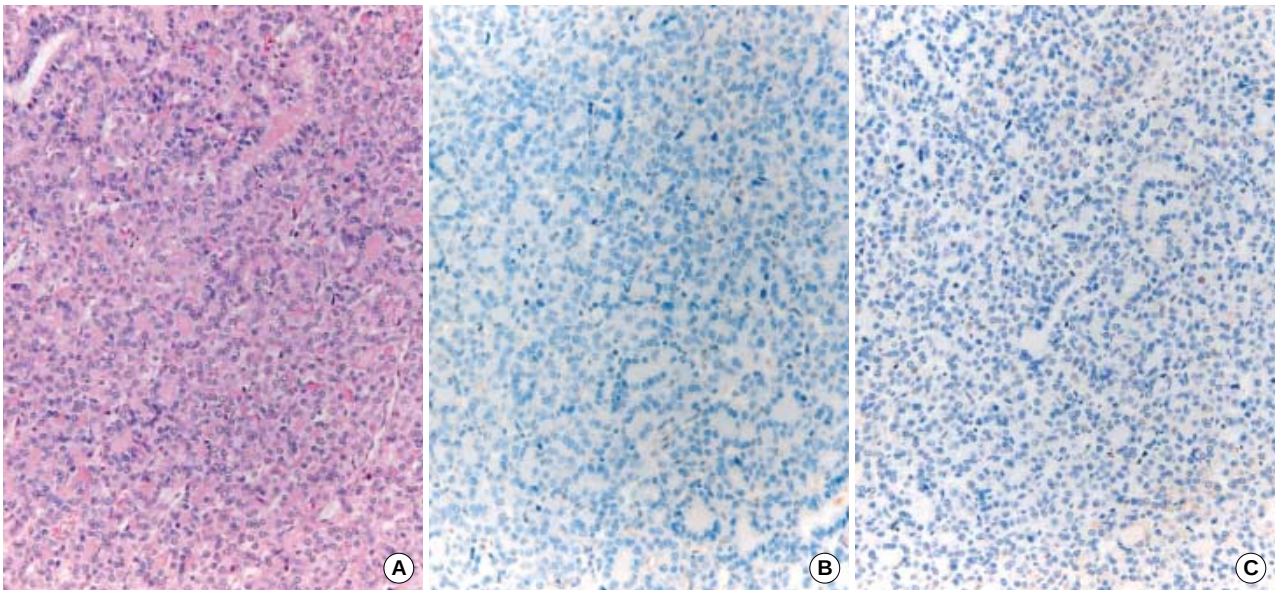

Fig. 3. Follicular adenoma (A) showing negativity for galectin-3 (B) and CK19 (C).

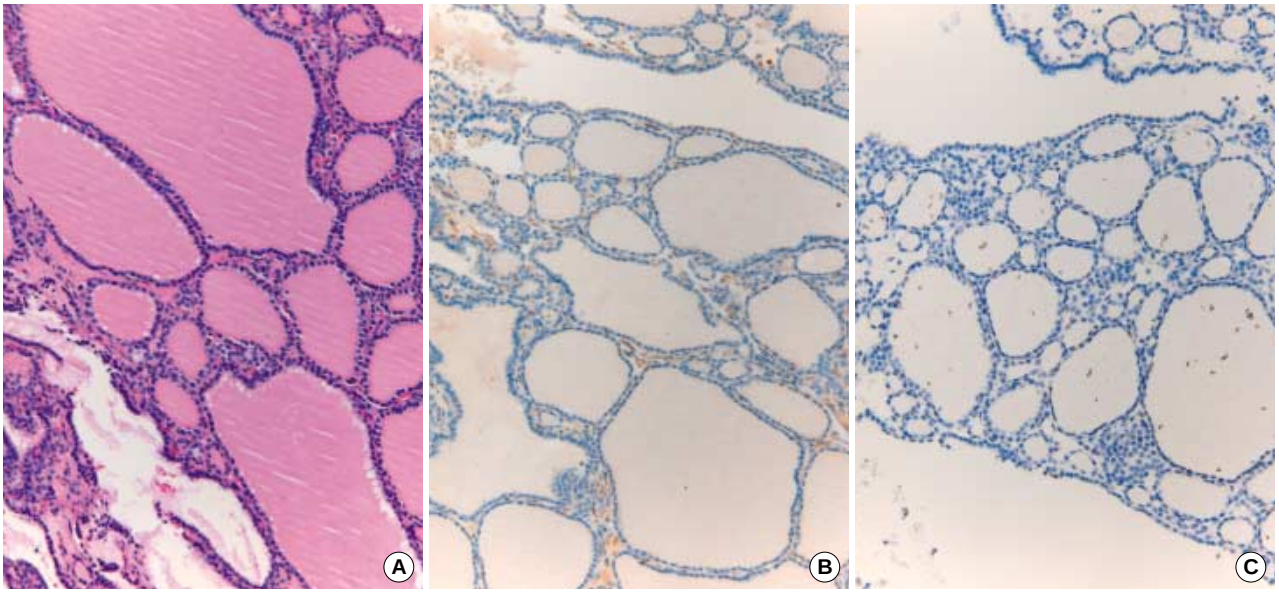

Fig. 4. Nodular hyperplasia (A) showing negativity for galectin-3 (B) and CK19 (C).

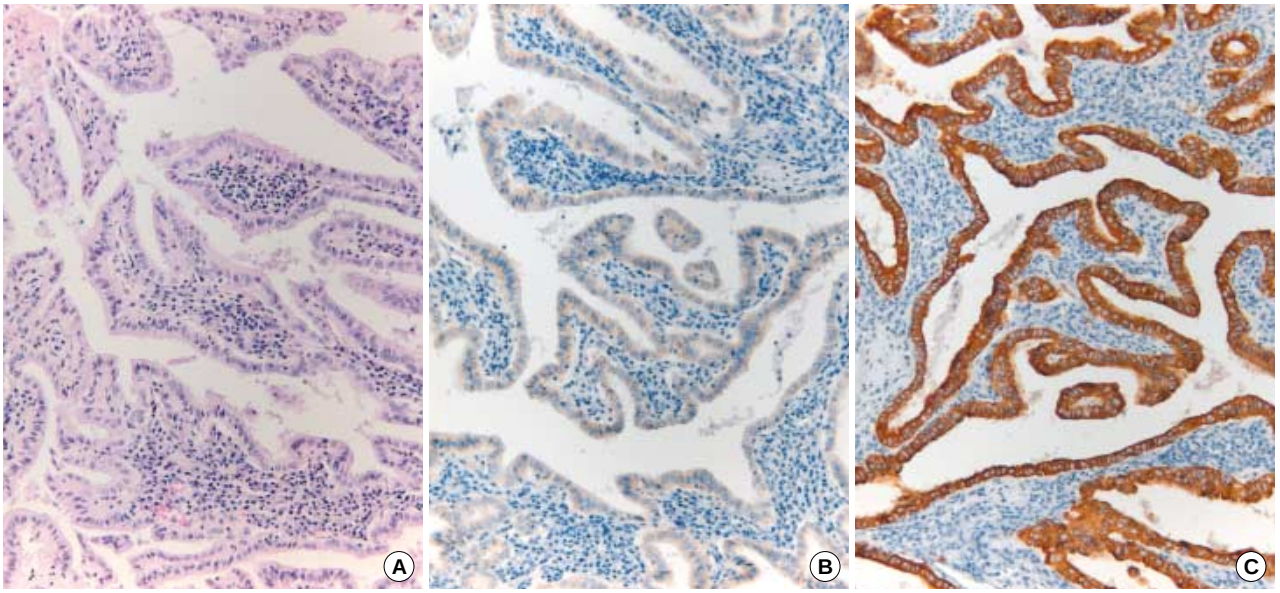

Fig. 5. Papillary carcinoma (age <20) (A) showing weak expression of galectin-3 (B) and strong expression of CK19 (C).

and in 5 of the 23 cases (21.7%) of NH. The results of the unpaired T-tests and χ^2 or Fisher's exact tests were not significant for any of the compared pairs.

Expression of Ki-67

Ki-67 was expressed in 1 of 27 cases (3.7%) of PC20, in 0 of 12 cases (0.0%) of FC, in 2 of 22 cases (9.1%) of FA, and in 0 of 23 cases (0.0%) of NH. The results of unpaired T-tests and χ^2 or Fisher's exact tests were not significant for any of the compared pairs.

Comparison of immunohistochemical stains between PC20 and PC19

The expression of galectin-3 was the only significant difference between the two groups on both the unpaired T-test and χ^2 or Fisher's exact test (Fig. 1B and Fig. 5B). Galectin-3 was expressed in two of 10 cases (20.0%) of PC19 in contrast to 19 of 27 cases (70.4%) of PC20. In addition, it was only weakly expressed in the two positive cases of PC19; this is in contrast to the positive cases of PC20.

DISCUSSION

The exact function of galectin-3 is not known, but it has been implicated in many biological processes, including cell growth, adhesion, inflammation and apoptosis.[12] Galectin-3 has been reported to be overexpressed in malignant thyroid lesions and it's been reported by many authors to be useful in differentiating between follicular carcinoma and follicular adenoma.[10,12] In our cases, the galectin-3 expression was prominent in the PC20 and follicular carcinoma, but it was negative or focally positive in the benign lesions such as follicular adenoma and nodular hyperplasia. Statistical analyses proved that the differences of galectin-3 expression between the benign and malignant lesions were significant. However, the galectin-3 expression of the PC20 was not significantly different from that of the follicular carcinoma. These findings disprove the insistence of Mehrotra *et al*.[13] In their study, there were more positive cases than negative or equivocal cases for the benign lesions as well as for the malignant lesions. In practice, one of the most difficult problems is the differential diagnosis between follicular carcinoma and follicular adenoma. Both the unpaired T-test and the χ^2 or Fisher's exact test suggest that evaluating galectin-3 is meaningful in such situations. Yet the galectin-3 expression was absent or focal for the cases of papillary carcinoma for which the patients were less than 20 years old (PC19). So evaluating galectin-3 expression might be valuable only for the patients who are over 20 years old. The possible explanation for this phenomenon might be that the gene encoding galectin-3 is scheduled for activation after the age of 20.

Beesley and McLaren[12] have reported that all the cases of papillary carcinoma (n=26) showed moderate or strong positivity to CK19, and most cases of benign lesions and follicular carcino-

ma were weakly stained or negative. In our cases, 81.5% of the cases of PC20 and 100% of the PC19 cases showed moderate to strong and diffuse staining for CK19. The expressions of CK19 were observed on the plasma membranes. We think that the negativity observed for the five cases of PC20 may be due to technical errors. Only a few cases of follicular carcinoma, follicular adenoma and nodular hyperplasia were positive to CK19. This finding is consistent with that of Beesley and McLaren. So in our opinion, CK19 is also useful for differentiating between the follicular variant of papillary carcinoma and follicular carcinoma, and between papillary carcinoma and the papillary area of nodular hyperplasia. The differences in CK19 expression that were seen in the PC and follicular neoplasm suggest that the cell type is transformed from the endocrine cell type to the nonendocrine cell type in PC.

*p*53 is a tumor suppressor gene that is located on chromosome 17p13.1. The major function of the *p*53 protein is cell cycle arrest and initiation of apoptosis in response to DNA damage. A little over 50% of the human tumors contain mutations in this gene.[24] Nasir *et al*.[17] reported that 90% of cases of follicular carcinoma exhibited a strong nuclear *p*53 expression, but *p*53 staining was weakly expressed in only 15% of the cases of follicular adenoma. So they concluded that immunohistochemical detection of *p*53 might be useful in the differential diagnosis of follicular carcinoma from follicular adenoma. In our cases, only 16.7% of the cases of follicular carcinoma and 9.1% of the cases of follicular adenoma showed a moderate to strong expression of *p*53. The statistical analyses were also not significant.

Ki-67 is a nuclear protein that's expressed by cells in the G1, G2, M and S proliferative phases.[25] It is well known that there is a good correlation between Ki-67 staining and the mitotic count. Muller-Hocker[18] and Augustynowicz *et al*.[20] reported that proliferative activity, as measured by Ki-67, was much higher in oncocytic carcinoma than in oncocytic adenoma. In our cases, 100% of the cases of follicular carcinoma and 90.9% of the cases of follicular adenoma showed negative or weak staining for Ki-67. The statistical analyses were also not significant.

In conclusion, galectin-3 is a candidate marker for the differential diagnosis between malignant and benign thyroid lesions, and especially between follicular carcinoma and follicular adenoma in the patients over 20 years old. Further, CK19 is valuable in differentiating between the follicular variant of papillary carcinoma and follicular carcinoma, and between papillary carcinoma and the papillary area of nodular hyperplasia. *p*53 and Ki-67 immunohistochemical stainings are not significantly helpful in differentiating between papillary carcinoma and nodular hyperplasia.

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