Immunohistochemical Analysis of Insular Carcinoma of the Thyroid Gland

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Background : Insular thyroid carcinoma (ITC) is a relatively infrequent thyroid carcinoma that has distinctive histologic features. ITC shows an aggressive clinical course and the predominant presence of an insular component, which has been reported to be an independent factor of a poor prognosis. We retrospectively examined clinical details of the nine ITC patients, which represented 9 years of experience with ITC, and investigated the expressions of variable neuroendocrine and other immunohistochemical markers associated with well-differentiated thyroid carcinomas. **Methods :** We adopted an immunohistochemical approach and studied the expressions of synaptophysin, chromogranin A, CD56, NSE, S-100, RET, PPARγ, calcitonin, galectin-3, and thyroglobulin in formalin-fixed, paraffin embedded tissue array slides of the 9 ITC patients, and investigated clinical features. Seven cases of follicular carcinoma and 4 cases of medullary carcinoma were also included as controls. **Results :** ITCs were positive for synaptophysin (44%, 4/9), CD56 (11%, 1/9), NSE (89%, 8/9), S100 (67%, 6/9), calcitonin (22%, 2/9), galectin-3 (78%, 7/9), and thyroglobulin (100%, 9/9), but completely negative for chromogranin A, RET, and PPARγ. **Conclusion :** ITCs express neuroendocrine markers in variable proportions and appear not to be associated with the oncoproteins of conventional thyroid carcinomas. Notably, its differential diagnosis from medullary carcinoma is required in cases showing focal calcitonin positivity.

Key Words : Insular carcinoma; Thyroid gland; Neuroendocrine markers

Although there has been some debate as to how to define poorly differentiated thyroid carcinoma (PDTC),[1] PDTC has been proposed to be an intermediate form between well differentiated thyroid carcinoma and undifferentiated (anaplastic) carcinoma based on its biological behavior and histology. Microscopically, PDTC is characterized by solid, trabecular, and insular growth subtypes, and of these, insular thyroid carcinoma (ITC) is a relatively infrequent, but well-characterized group. ITC was initially described in 1984 by Carcangiu *et al.* as a distinctive form of PDTC arising from follicular epithelial cells.[2] ITC typically shows well-defined large solid nests (insulae) and small round or oval follicles, which are comprised of relatively monotonous growths of small cells that show mitotic activity and necrosis. ITC tumor cells have hyperchromatic or vesicular nuclei with indistinct nucleoli and scanty cytoplasm. Although it is debated whether the insular component in well-differentiated thyroid carcinomas influences clinical outcome,[3,4] this histotype has been reported to be associated with a poorer outcome than well-differentiated thyroid carcinoma, and to be an independent predictor of a poor prognosis.[5-7]

Immunohistochemically, ITC shows positivity for thyroglobulin but negativity for calcitonin. Recently, neuroendocrine markers were reported to be expressed in several thyroid epithelial tumors such as papillary/follicular carcinoma, by immunohistochemistry, despite a lack of identifiable neurosecretary or endocrine granules by electron microscopy.[8] ITC has been reported to show distinct immunoexpressions of neuron-specific enolase (NSE), synaptophysin, S-100 protein, Leu-7, and myelin basic protein (MBP).[8,9] In addition, a case of PDTC was reported, that presented with elevated serum chromogranin A but a normal calcitonin concentration,[10] and showed intense staining for NSE and chromogranin A, but only local calcitonin positivity. These two reports suggest the occasional presence of aberrant neural or neuroendocrine differentiation in ITC and PDTC, but these features have been described only sporadically.

To more precisely evaluate the expression patterns of neuroendocrine markers and the other overall immunohistochemical features of ITC, a more intensive larger-scale investigation was re-

quired. Therefore, we collected data and examined the archived tissue samples of 9 patients with ITC who had been diagnosed during a period of 9 years.

MATERIALS AND METHODS

Clinicopathologic and histologic evaluations

We retrospectively studied 12 patients who underwent surgery for ITC at Seoul National University Hospital during the period from 1996 to 2004. Clinical and pathologic records and histologic slides were reviewed by two pathologists unaware of the clinical data. ITCs were diagnosed according to the previously described criteria,[1,2] which suggest that an insular growth pattern is present in at least 70% of the tumor area by microscopy. Two of the 12 cases were excluded due to a predominantly solid, trabecular growth, and one case that had been diagnosed as ITC without immunostaining for calcitonin was rediagnosed as medullary carcinoma. Nine ITC cases were characterized by predominant insular patterns surrounded by artificial clefts (Fig. 1). The uniform neoplastic cells were usually small with scant, pale, eosinophilic cytoplasm. Nuclei showed finely granular chromatin and indistinct nucleoli. Mitoses were usually observed at the rate of 1-10 per 10 high-power fields and small foci of necrosis were also occasionally observed. Seven and four patients with follicular or medullary thyroid carcinoma, respectively, were used as controls. Tissue microarray formalin-fixed paraffinized blocks (one 4 mm representative core) were used for hematoxylin and eosin (H&E) staining and immunos various markers.

The clinicopathologic data of the histochemistry for nine cases are summarized in Table 1. Mean age at onset was 49.1 years (range 16 to 75 years) without gender predominance. All patients underwent total thyroidectomy, and lymph node metastasis was identified in 5 patients at the time of lymph node dissection. Extrathyroidal extension was present in 7 cases (78%), and 4
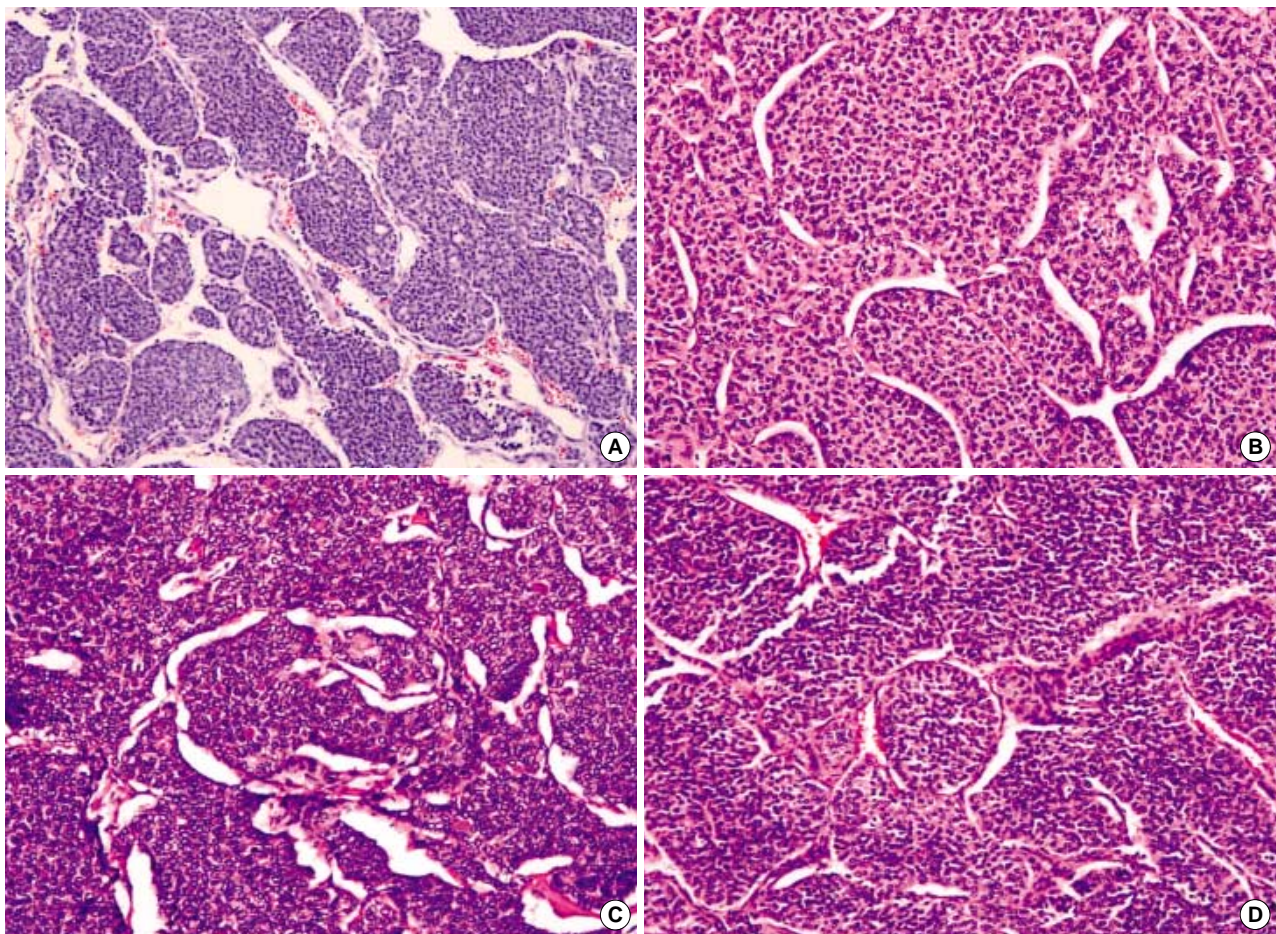
Fig. 1. The representative histologic patterns of ITCs (cases 1 (A), 3 (B), 6 (C) & 9 (D)) commonly show the presence of insulae surrounded by artificial clefts, which are comprised of a monotonous growth of small cells with hyperchromatic or vesicular nuclei with indistinct nucleoli.
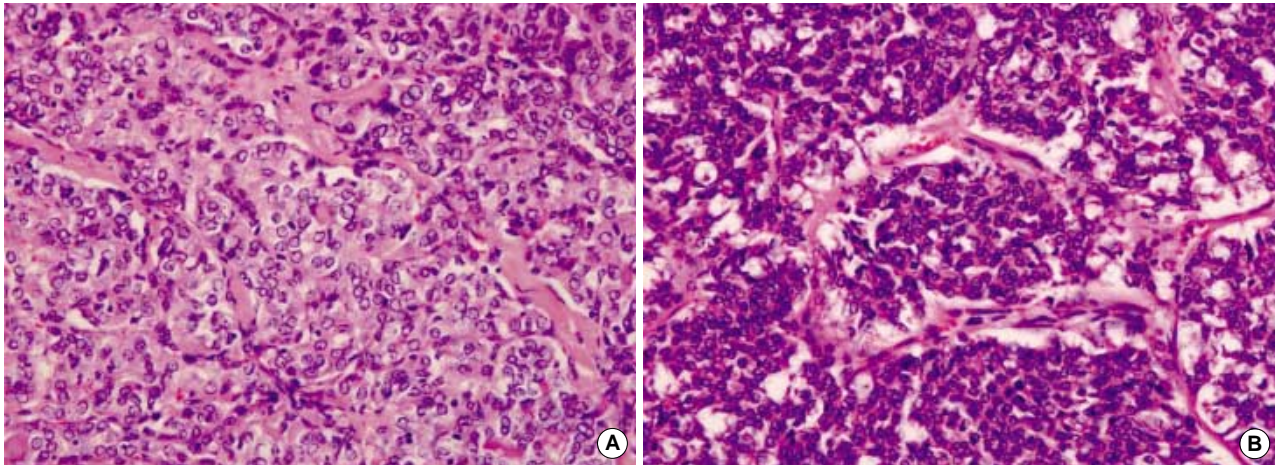
Fig. 2. In cases 5 (A) and 7 (B), the nuclear features of papillary carcinoma, including the empty appearance, overlapping nuclei, and nuclear grooves are observed.
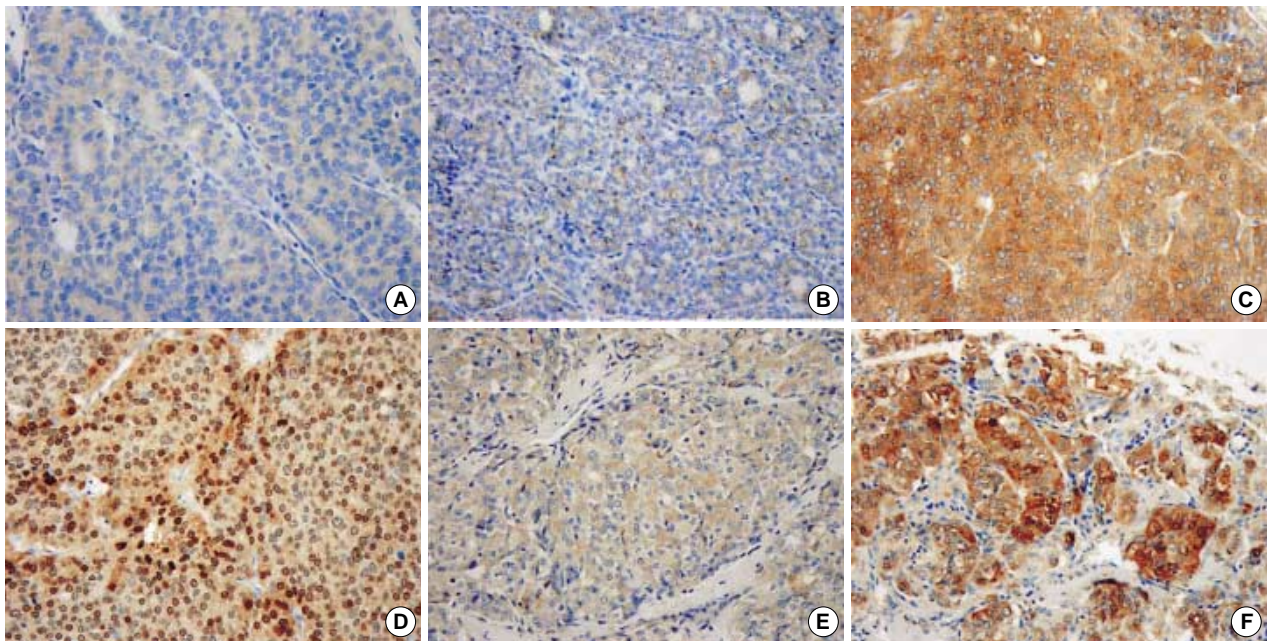

Fig. 3. Immunostaining of a case positive for synaptophysin (A), CD56 (B), NSE (C), S-100 (D), calcitonin (E), and galectin-3 (F). CD56 (B) and calcitonin (E) are focally positive, and synaptophysin (A), NSE (C), S-100 (D) and galectin-3 (F) show diffusely positive immunostaining in tumor cells. (A, B, C, D, E: ×400, F: ×200).

cases (44%) showed vascular invasion. Only two cases (patient Nos. 8 and 9) had coexisting tumors in the other lobe, both of which were well differentiated papillary carcinomas. Patients 5, 6, and 7 showed the following nuclear features; empty nuclei, nuclear grooves and nuclear overlapping (constituting 10-30% of the tumor area, Fig. 2). However, these features were absent in the two cases with papillary carcinoma. Three cases (patients 4, 5, and 8) were developed in a background of Hashimoto's thyroiditis, and patient 8 had coexisting papillary carcinoma with Hashimoto's thyroiditis. Mean follow-up duration was 45.3 months

(range 8 to 115 months) and patient 3 was lost during clinical follow-up. Laboratory investigations revealed normal serum calcitonin concentration in all patients, but serum chromogranin A testing was not performed.

Immunohistochemistry

Immunohistochemical analysis was performed on formalin-fixed, paraffin-embedded microarray slides using primary antibodies for synaptophysin, chromogranin A, CD56, NSE, S100,

Table 1. Clinical features of 9 cases of insular thyroid carcinoma

Patient No.	Gender/Age (M/F, years)	Tumor size (cm)	Extrathyroidal extension	Vascular invasion	Associated tumor	LN metastasis	Radioiodine treatment	Follow up (month)	Survival
1	M/16	1.8	N	N	N	Y	Y	8	AWD
2	M/75	5	Y	Y	N	N	N	21	NED
3	F/52	7	Y	N	N	N	N	27	-
4	F/28	3	N	N	N	N	N	115	NED
5	F/45	2	Y	N	N	Y	Y	N.A	AWD
6	M/56	5	Y	Y	N	N	N	103	NED
7	M/53	10	Y	N	N	Y	N	8	DOD
8	F/65	4	Y	Y	PC	Y	Y	61	AWD
9	M/52	6.5	Y	Y	PC	Y	Y	27	DOD

M, male; F, female; Y, yes; N, no; PC, papillary carcinoma; AWD, alive with disease; NED, no evidence of disease; DOD, death of disease.

Table 2. Antibodies used in the immunohistochemical stainings

Name	Manufacturer	Antigen retrieval	Dilution
PPARγ	Santa Cruz, CA, USA	microwave	1:100
RET	Santa Cruz, CA, USA	microwave	1:100
Neuron specific enolase (NSE)	DAKO, Denmark	none	1:300
Chromogranin A	Novocastra, UK	microwave	1:50
Synaptophysin	DAKO, Denmark	microwave	1:200
CD56	Zymed, USA	microwave	1:100
S100	DAKO, Denmark	none	1:500
Calcitonin	DAKO, Denmark	none	1:400
Thyroglobulin	DAKO, Denmark	none	1:1,000
Galectin-3	DAKO, Denmark	microwave	1:100

RET, PPARγ, calcitonin, galectin-3, and thyroglobulin (Table 1). Briefly, after deparaffinization, sections were rehydrated, washed, and subjected to microwave retrieval in citrate buffer if required. Sections were then immersed in 3% H_2O_2 to block endogenous peroxidase activity and incubated with the primary antibodies. Expressions were detected using peroxidase-labeled streptoavidin-biotin complex, according to the manufacturer's instructions. Sections were counterstained with Mayer's hematoxylin. Immunoreactivities were evaluated separately by two pathologists. Immunolabeling patterns fell into 3 categories: strongly positive (++), weakly positive (+), or negative (-) according to the intensity. The strongly positive (++) reactions observed were as follows; uniform intense nuclear (PPARγ), cytoplasmic (RET, chromogranin A, synaptophysin, NSE, thyroglobulin, galectin-3, calcitonin), uniform intense nuclear and cytoplasmic (S100), or cytoplasmic membrane (CD56) staining. Weakly positive (+) results showed the same pattern with interspersed negative cells. Tumors were considered negative if staining was completely absent or present in only a few scattered cells. The percentage of immunostained tumor cells was scored as follows; 1:1% to 15%; 2:16% to 75%; 3: >75%. Immunoreactivities were presented as; -, +, ++ and as 1, 2, 3, according to the intensity and the distribution, respectively.

RESULTS

Immunohistochemistry

Normal follicular epithelial cells and medullary carcinoma tumor cells were used as positive controls for thyroglobulin, synaptophysin, chromogranin A, and calcitonin. Follicular carcinoma

Table 3. Immunohistochemical results of a cases of insular thyroid carcinoma

Case No.	PPARγ	RET	Synap-tophysin	CD56	Chromogra-nin A	NSE	S-100	Calci-tonin	Thyroglob-ulin	Galectin-3
1	-	-	-	+, 1	-	+, 1	-	-	++, 2	-
2	-	-	+, 2	-	-	++, 2	+, 2	-	+, 2	+, 2
3	-	-	-	-	-	+, 2	+, 1	-	++, 2	+, 2
4	-	-	+, 1	-	-	+, 2	+, 1	-	++, 2	+, 1
5	-	-	+, 1	-	-	++, 2	++, 2	+, 1	++, 2	++, 2
6	-	-	-	-	-	+, 2	+, 1	+, 1	++, 2	+, 2
7	NA	NA	+, 2	-	-	NA	-	NA	NA	NA
8	-	-	-	-	-	++, 2	++, 2	-	++, 1	+, 2
9	-	-	-	-	-	+, 1	-	-	++, 1	+, 1

-, negative; +, weak postivie; ++, strong positive; 1, 1-15%; 2, 16-75%; 3, >75%; NA, not applicable.

tumor cells were used as a positive control for galectin-3 and PPARγ. Immunohistochemistry results are summarized in Table 3 and Fig. 3. Four (44%) ITC cases were weakly positive for synaptophysin in focal or relatively diffuse areas, but all 9 cases were negative for chromogranin A. Only one case showed focal weak positivity for CD56. S100 immunostaining was positive in relatively wide areas in three ITC cases and focally positive in three cases. Calcitonin was focally and weakly positive in two cases. On the other hand, all cases except case 7, which was not examined because of a shortage of tissue, were positive for NSE and thyroglobulin (100%). Galectin-3 immonopositivity (78%, 7/9) was also high. All ITC cases were negative for RET and PPARγ.

DISCUSSION

Of the neuroendocrine markers examined, NSE showed diffuse or strong positivity in most cases, but synaptophysin stained positively in four cases with a focal distribution. Notably, calcitonin was positive focally in two cases, one of which showed positive synaptophysin staining, and both of which were positive for NSE and S-100. Although we did not perform an electron microscopic examination, our results are consistent with those of previous reports in terms of positivity for NSE, synaptophysin, and S-100,[8,9] but not in terms of focal and weak calcitonin positivity, which is somewhat problematic considering needed to differentiate ITC from medullary carcinoma. Calcitonin is known to be positive in 80% of medullar carcinomas, and is expressed in only small and focal areas, but occasionally also in relatively diffuse areas. On the other hand, thyroglobulin is rarely expressed in medullary carcinoma despite some positive results.[11,12] Our two cases that were focally positive for calcitonin (cases 5 and 6), had the characteristic histologic features of ITC, i.e., well-formed insulae with artificial clefts, on which ITC was clearly differentiated from medullary carcinoma. Furthermore, both showed intense positivity for thyroglobulin and complete negativity for chromogranin A, which is a more sensitive marker of medullary carcinoma than calcitonin.[13] Considering previous reports on the identification of calcitonin in non-neuroendocrine thyroid tumors,[10,14] we conclude that ITCs express neuroendocrine markers, though weakly and in variable proportions. Therefore, the pattern of calcitonin immunostaining, the intensity of thyroglobulin immunoexpression, other ancillary studies such as congored staining and clinicopathologic correlations may be help ful in the differential diagnosis of medullary carcinoma and other thyroid carcinomas.

PAX8-PPARγ and RET/PTC gene rearrangements are recognized to represent initiating events in the carcinogeneses of thyroid follicular carcinoma and papillary carcinoma, respectively. Recently, immunostaining and real-time RT-PCR for PAX8-PPARγ fusion gene expression showed complete negativity in 13 cases of PDTC[15] without evidence of RET/PTC fusion gene rearrangement,[16-18] which is consistent with the present results. In addition, a large thyroid carcinoma containing mixtures of both well and poorly differentiated components was developed in RET/PTC3p53-/-mice, although RET/PTC3 expression was found to be attenuated in older mice.[19] These results support the disease-progression model of thyroid carcinoma, namely, that well-differentiated thyroid carcinomas associated with these gene arrangements do not progress to the dedifferentiated form, for example, to PDTC.

The coexistences of ITC with papillary carcinoma, or with the nuclear features of papillary carcinoma, or with Hashimoto's thyroiditis were not found to be related to RET immunoreactivity in the present study. However, in a previous comparative study, the frequency of RET activation was found to be variable, i.e., from 0% to 80%, even in typical papillary carcinoma.[20] Recently, RET/PTC expression was reported to be more common in insular carcinomas with histologic evidence of coexistent papillary carcinoma or only with the cardinal nuclear features of papillary carcinoma, which implies a possible evolution from papillary carcinoma.[20] Further more, it was suggested that PD carcinoma is a tumor of follicular lineage and that it is related to both follicular and papillary carcinoma, not exclusively limited to follicular carcinoma. However, this remains to be confirmed.

In summary, some ITCs expressed neuroendocrine markers and seemed not to be associated with the oncoproteins of conventional thyroid carcinomas by immunohistochemistry. In cases showing focal positivity for calcitonin, ITCs must be diagnosed with knowledge of their variable immunohistochemical expressions and clinicopathologic correlations.

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