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Review

Intrathyroid epithelial thymoma (ITET) and carcinoma showing thymus-like differentiation (CASTLE). CD5-positive neoplasms mimicking squamous cell carcinoma of the thyroid

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Summary. Carcinoma of possible thymic epithelial origin may occur within the thyroid gland, which was first reported by Miyauchi et al. as intrathyroid epithelial thymoma (ITET). ITET is a rare tumor comprising about 0.08% of all primary thyroid malignancies. It is a low-grade thyroid carcinoma with squamous cell differentiation whose overall survival rate was found to be 71%. Lymph node metastasis at surgery was found in 40% and hematogenous metastases developed in bones, liver and lungs. This tumor grows within the thyroid gland and invades into the thyroid parenchyma as well as into the extrathyroid structures. It is a well-circumscribed solid tumor with a sharp tumor border, but is not capsulated. After fixation, the cut surface of the tumor is gray-white in color and is a solid tumor with lobulation. Tumor calcification was not detected in our 15 cases. The tumor cells show solid sheets of growth with occasional keratinization without follicular or papillary structures. Lymphocytic infiltration in the stroma is one of the most characteristic features of this tumor. The tumor cells are polygonal epithelial cells with distinct nucleoli and ill-defined cell border. Positive immunoreactivity for CD5 is a key feature to differentiate it from undifferentiated carcinoma, poorly differentiated carcinoma, medullary (C cell) carcinoma and high-grade squamous cell carcinoma (so-called primary squamous cell carcinoma) of the thyroid. Negative immunoreactivity for calcitonin, TTF1 and thyroglobulin, and positive immunoreactivity for p63 and KIT are also helpful for differential diagnosis. Nuclear atypia is mild and mitoses are less frequent, with an intermediate proliferation index (MIB-1 labeling index is usually less than 20%), which are also helpful to differentiate it from high-grade primary squamous cell carcinoma of the thyroid. The tumors in our 15 cases demonstrate 3 histological subtypes: keratinizing squamous cell carcinoma type, non-keratinizing basaloid cell carcinoma (lymphoepithelioma-like) type and neuroendocrine carcinoma type, which correspond to subtypes of the mediastinal thymic carcinomas.

Key words: Thyroid carcinoma, CD5, Squamous cell, Lymphoepithelioma, Neuroendocrine

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Introduction

In the recent tumor classifications of thyroid glands, several types of malignancy were separated from undifferentiated carcinoma (UC) as new tumor entities because of their better prognosis than UC. These findings led us to the discovery of new histological types of thyroid neoplasms with different cell origins. The first one in the history of thyroid pathology was C cell carcinoma of the thyroid, which was described by Hazard et al. as medullary (solid) carcinoma in 1959 (Hazard et al., 1959). This tumor entity was extensively studied for its C cell nature (Williams, 1966; Hazard, 1977) and familial background (Sipple, 1961; Williams, 1965; Steiner et al., 1968). It is now defined as a

carcinoma of the thyroid gland showing C cell differentiation (Williams, 1966; Kakudo et al., 1979; Harach and Bergholm, 1988; Lertprasertsuke et al., 1989; Harach, 1997). The germ-line point mutation of RET proto-oncogene was first identified in patients with the familial form of this cancer (Mulligan et al., 1993). The second one is primary malignant lymphoma of the thyroid gland, which was formally called small cell type UC. Because of its good response to chemotherapy and its lymphocyte nature, as clarified by electron microscopy and immunohistochemical studies, small cell type UC has been replaced with malignant lymphoma in recent classification (Cameron et al., 1975; Mambo and Irwin, 1984; Tobler et al., 1984). The third one is a carcinoma of the thyroid gland showing thymic epithelial differentiation, so-called intrathyroid epithelial thymoma (ITET)/carcinoma showing thymus-like differentiation (CASTLE). It was first reported by Miyauchi et al. and named ITET in 1985, which was separated from so-called primary squamous cell carcinoma (PSCC) of the thyroid and UC with squamous cell component because the prognosis of the former is better and the pathologic characteristics differ (Miyauchi et al., 1985). This epithelial tumor and others of possible thymic epithelial origin were reclassified into three types by Chan and Rosai in 1991 (Table 1) and each of them was listed as a new tumor entity in the WHO classification of thyroid tumors in 2004 (Cheuk et al., 2004).

This paper deals with 15 cases of ITET/CASTLE in our experience and from our consultation cases, and contains a detailed description of their histopathologic features, which should be useful for pathologists in differential diagnosis.

Discovery of ITET/CASTLE

Thymic-type epithelial tumors may occur within the thyroid gland and are believed to arise from the thymic remnants or branchial pouch (solid cell nest) in the thyroid gland (Miyauchi et al., 1985, 1989; Asa et al., 1988; Kakudo et al., 1988; Chan and Rosai, 1991). This tumor entity was first introduced by Miyauchi et al. as a tumor entity distinct from so-called primary squamous cell carcinoma (PSCC) of the thyroid because of its better prognosis and different gross appearance (Miyauchi et al., 1985). Miyauchi et al. pointed out the characteristic features, which included 1) location in the middle to lower pole of the thyroid lobe, 2) lobulation at cut surface, 3) thick fibrous bands dividing the tumor nests, 4) lymphocyte-rich stroma, 5) rare or infrequent mitoses and 6) lack of association with UC or well-differentiated follicular cell tumors. Regarding its histogenesis, they speculated that it has a thymic epithelial origin, on the basis of morphological similarity to mediastinal thymic tumors under HE observation (Miyauchi et al., 1985, 1989). Kakudo et al. reported the first autopsy case that developed hematogenous metastases in the lungs, liver and bones, in addition to the pleura and lymph nodes, and they emphasized that it was a subtype of squamous cell carcinoma of the thyroid. They were the first to point out a direct continuity from the cervical thymic tissue to the primary ITET/CASTLE tumor mass (Kakudo et al., 1988). This tumor and thyroid carcinoma with thymic differentiation were further classified into three subgroups by Chan and Rosai as follows: 1) ectopic thymoma, 2) spindle cell tumor with thymus-like differentiation (SETTLE) and 3) carcinoma showing thymus-like differentiation (CASTLE), depending on variable differentiation toward thymus epithelium (Table 1) (Chan and Rosai, 1991). They postulated that this family of tumors arises either from ectopic thymus or remnants of branchial pouches that retain the potential to differentiate along the thymic line. Berezowsky et al. (1996) and Dorfman et al. (1998a) added CD5 immunoreactivity of ITET/CASTLE as evidence of its thymic differentiation. Ito et al. (2007) confirmed its better prognosis than PSCC and UC in their 25 cases, collected from the member institutes of the Japanese Society of Thyroid Surgery in 2007. The 10-year cause-specific survival (CSS) rate was first reported as 82% (Ito et al., 2007). Synonyms of CASTLE include intrathyroid epithelial thymoma (ITET) by Miyauchi et al., primary thyroid thymoma by Asa et al., and lymphoepithelioma-like carcinoma by Shek et al. (Miyauchi et al., 1985; Asa et al., 1988; Shek et al., 1996). From our experience, ITET/CASTLE may be more common in adult patients than SETTLE and ectopic thymoma, and this paper deals only with ITET/CASTLE.

Patient series and clinical features of ITET/CASTLE

The pathology reports and histological slides of 9,582 primary malignant tumors of the thyroid gland surgically treated at Kuma Hospital, Kobe, Japan, between 1983 and 2007 were reviewed and eight cases of ITET/CASTLE were collected for this study (Kakudo et al., 2009). Additionally, one autopsy case previously reported by one of the authors, two archival cases with only HE sections and four consultation cases from other sources, giving a total of 15 cases (Table 2), were analyzed in this study. T-1 and I-1 have each been reported as single case report (Kakudo et al., 1988; Kusada et al., 2005), and cases from Kuma Hospital

Table 1. Classification of thyroid tumors of thymic differentiation by Chan and Rosai.

1) Extrathyroid tumor	
1	Ectopic hamartomatous thymoma
2) Intrathyroid tumor	
1	Ectopic thymoma
2	Carcinoma showing thymus-like differentiation (CASTLE)
3	Spindle cell tumor with thymus-like differentiation (SETTLE)

were analyzed in several previous publications (Miyauchi et al, 1985, 1989; Dorfman et al., 1998a,b; Ito et al., 2006, 2007; Reinmann et al, 2006).

In our series of 15 cases of ITET/CASTLE, the average age at presentation was 49.9 (25-73) years old and the male/female ratio was 7:8 and was nearly equal. The overall survival rate of 14 cases was 71.4% (10/14 cases), as shown in Table 2, and the 10-year overall survival rate by the Kaplan-Meier method was 74%, as shown in Fig. 1. Paraneoplastic syndrome, such as myasthenia gravis, was not recorded in our 15 cases and it has not been reported in the literature to date.

Incidence of ITET/CASTLE

ITET/CASTLE is a rare thyroid carcinoma in our experience, comprising eight cases (0.083%) within 9,582 primary malignant tumors of the thyroid gland surgically treated at Kuma Hospital, Kobe, Japan, between 1983 and 2007 (Kakudo et al., 2009). Chow et al. from Hong Kong reported a frequency of 0.15% (3/2,033 patients) in their Chinese patients (Chow et al., 2007). Cappeli et al. and Yamazaki et al. stated in 2008 that approximately thirty cases of ITET/CASTLE had been reported in the English literature, and Ito et al. collected 25 cases of ITET/CASTLE in 2007 from a nationwide survey by the Japanese Society of Thyroid Surgery (Ito et al., 2007; Cappeli et al., 2008; Yamazaki et al., 2008).

When HE sections were reviewed in the undifferentiated thyroid carcinoma research consortium of Japan by Sugitani et al., one case of ITET/CASTLE was identified in 65 cases of UC that survived more than 1 year (personal communication). This observation may indicate that a small number of ITET/CASTLE patients might be misplaced in the UC group, which could be one of the explanations why a few patients in the UC series were reported to survive for an exceptionally long period with the disease. This is true in some case reports on ITET/CASTLE, in which the previous histological diagnosis had been UC or PSCC (Asa et al., 1988; Mizukami et al., 1995; Kusada et al., 2005).

Gross appearance and invasive pattern of ITET/CASTLE

The tumor size of ITET/CASTLE at surgery ranged from 2.2 to 8.0 cm (average: 4.2 cm) (Table 2). The cut surface was gray-white after fixation (Fig. 2). No co-existing nodular lesions were found in our series, partly because our patients are all Japanese and Japan belongs to the iodine-sufficient and non-endemic goiter area geographically. The tumor was usually well demarcated but not capsulated (Fig. 2). A solid tumor with lobulation and no calcification was found in our 15 cases. The sharp border between the tumor and thyroid parenchyma (Fig. 2) was a very characteristic feature differing from widely invasive follicular carcinoma, poorly differentiated carcinoma (PDC), UC or so-called PSCC of the thyroid. It invades into the thyroid parenchyma as well as surrounding anatomic structures. The close vicinity of the tumor mass to the cervical thymus was recorded in two cases (Kakudo et al., 1988) and this rare observation was separately confirmed by Tai et al. (2003).

Table 2. Clinical and prognostic features of 15 cases of ITET/CASTLE.

Case*	age	gender	tumor size (cm)	LN involvement	outcome**
K-1	51	F	4x3.5	no	ANED: 25 years
K-2	47	F	4x2.9	no	ANED: 24 years
K-3	65	F	4.1x3.0	yes	ANED: 13 years
K-4	52	M	4.3x3.3	yes	D: 8 years
K-5	29	F	5.8x4.2	yes	ANED: 18 years
K-6	73	M	2.7x1.5	no	ANED: 3 months and lost
K-7	47	F	5.9x4.3	no	ANED: 6 years
K-8	44	M	2.8x2.3	no	ANED: 4 years
K-9	49	F	2.8x1.9	no	ANED: 3 years
K-10	25	F	2.2x2.1	no	ANED: 1 year
NC-1	48	M	3.5x2.9	no	ANED: 3 years
T-1#	59	M	4x4	yes	D: 17 months
I-1##	68	M	8x5	yes	D: 11 months
N-1	53	M	3x2.5	yes	ANED: 3 years
W-1	38	F	4x3	no	DUD: 18 years
Average	49.9	/	4.2	yes 40%	survival rate: 71.4%

*: K: Kuma Hospital, Kobe, Japan, NC: Niigata Cancer Center, T: Tokai University, I: Inazawa Hospital, N: Nagasaki University, W: Wakayama Medical University, LN involvement: Lymph node involvement at the primary thyroid surgery, **: ANED: Alive with no evidence of disease, D: Died with disease, DUD: Died due to unknown cause without tumor recurrence, #: Autopsy case report by Kakudo K, J. Surg. Oncol., 38:187, 1988, ##: Case report by Kusada N. Thyroid, 15:1383, 2005

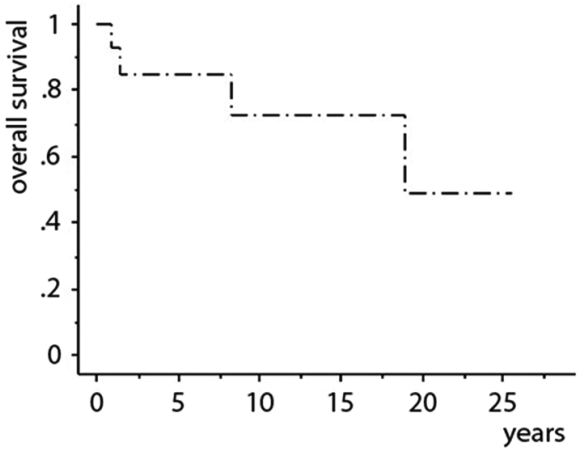

Fig. 1. Overall survival rates of 14 cases of ITET/CASTLE were analyzed by the Kaplan-Meier method. The overall survival rate at 10 years was 74%.

Histological characteristics of ITET/CASTLE (Table 3)

ITET/CASTLE develops a lobular architecture separated by fibrous bands and consisting of solid sheets of polygonal cells to spindle cells with a single cell keratinization or whorl formation (Fig. 3). The tumor nests are divided by fibrous stroma, which contain various numbers of small lymphocytes of both T cell and B cell lineages (Kakudo et al., 1988; Mizukami et al., 1995). Under low magnification, this tumor looks blue in HE sections because of abundant lymphocyte infiltration into the stroma and high cellularity of the tumor nests (Figs. 4, 5). This tumor shows expansive growth with a sharp tumor border (Fig. 4), but it is invasive to the thyroid parenchyma (Fig. 5) as well as to the extrathyroid structure. ITET/CASTLE is basically a squamous cell carcinoma (Figs. 3-6) with various numbers of keratinizing cells. Keratinizing squamous cell carcinoma type (Fig. 3) was found in 11 of our 15 cases, and four cases show negligible keratinization (lymphoepithelioma-like or basaloid cell type) (Figs. 4-6) under HE examination. A rosette-like arrangement was reported by Yamazaki et al. (Yamazaki et al., 2008) and occasional glandular structures may be seen in a part of the tumor. No follicle structures with colloid or papillary arrangements suggesting follicular cell differentiation were found in our 15 cases. The tumor

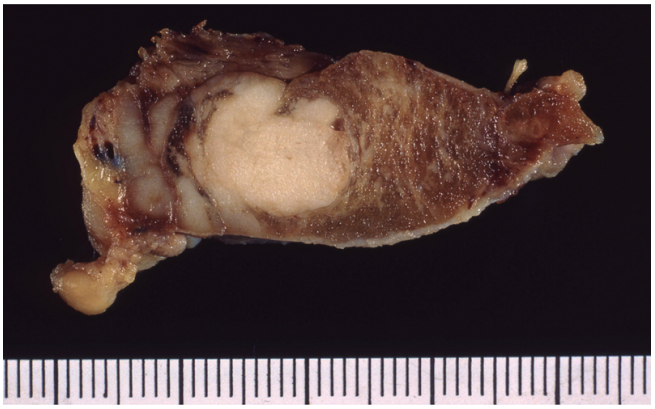

Fig. 2. Cut surface of ITET/CASTLE after fixation. The tumor was located in the lower (left side of the figure) pole of the thyroid lobe. It was gray-white in color and solid with lobulation. No calcification, necrosis or cystic change was found in the tumor mass. The upper pole was on the right side of the figure, and the cervical thymus (yellow tissue mass) on the left was next to the tumor mass. Note no co-existing nodular lesions in the thyroid parenchyma.

Table 3. Histopathologic characteristics of ITET/CASTLE useful for differential diagnosis.

No.	Differential diagnosis
1	Expansive growth pattern (sharp tumor border and pushing margin)
2	Lymphoid stroma
3	Squamous cell differentiation with keratinization and basaloid cell appearance
4	Positive immunoreactivity for CD5 and p63
5	Negative immunoreactivity for thyroglobulin, TTF-1 and calcitonin
6	Low-grade histology (infrequent mitoses and rare necrosis)
7	Infrequent MIB-1 labeling index (usually less than 20%)

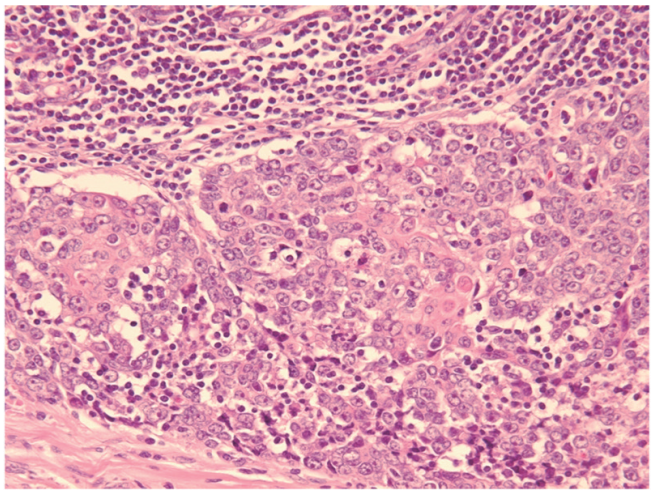

Fig. 3. Eosinophilic cytoplasm in polygonal or spindle-shaped tumor cells in ITET/CASTLE suggesting keratinization. Note many small lymphocytes in the stroma. Hematoxylin and eosin, x 20

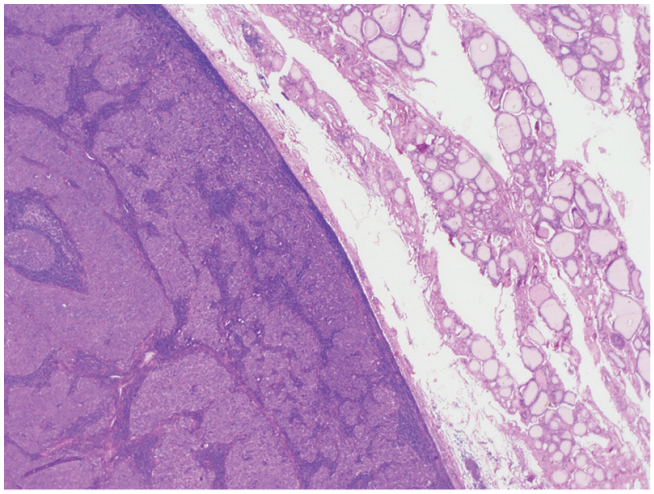

Fig. 4. A well-demarcated thyroid tumor (left) showing expansive growth toward thyroid parenchyma (right). This growth pattern was different from those of the other aggressive thyroid carcinomas, such as undifferentiated carcinoma, primary squamous cell carcinoma and widely invasive follicular carcinoma of the thyroid. The tumor mass was blue at this low magnification because of stromal lymphocytes and high cellularity of tumor nests. Hematoxylin and eosin, x 2

cells have large and distinct nuclei and they have ill-defined cell border and poorly developed intercellular bridges (Figs. 3, 6), which are more evident in plastic sections (Fig. 7). The nuclear characteristics of papillary thyroid carcinoma, such as nuclear inclusions and grooves, are not characteristic features in ITET/CASTLE. The histological grade is relatively low compared with that of so-called PSCC; as a result, the nuclear atypia is mild, mitoses are rare and tumor necrosis is usually absent. The MIB-1 labeling index is about 10-30% (usually 10-20%), which is different from UC or PSSC whose MIB-1 labeling index is usually more than 50%.

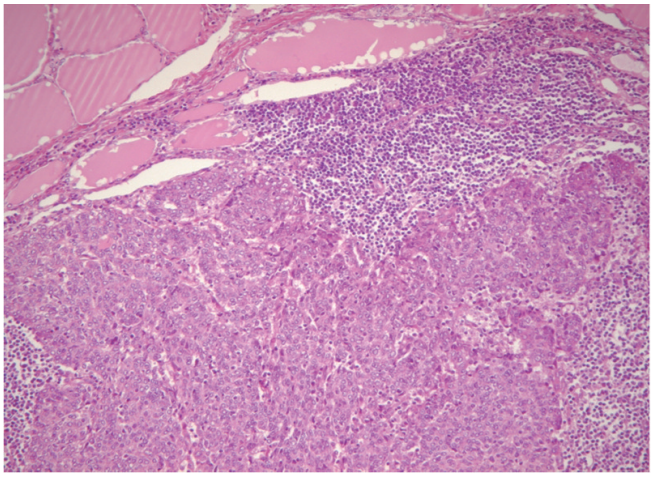

Fig. 5. Invasive front of ITET/CASTLE into thyroid parenchyma. Note many small lymphocytes in the stroma. Hematoxylin and eosin, x 4

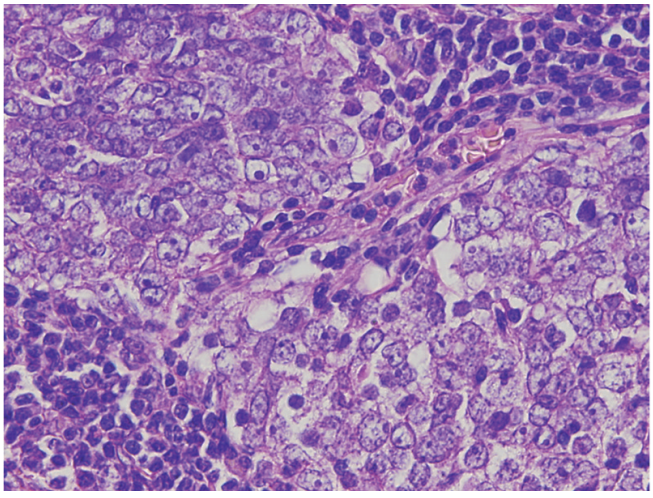

Fig. 6. Lymphoepithelioma type ITET/CASTLE showed relatively narrow tumor cytoplasma and ill-defined cell border. Note lymphocytic infiltration in the stroma. Hematoxylin and eosin, x 40

Immunohistochemical characteristics of ITET/CASTLE

Ten cases were available for immunohistochemical study. Formalin-fixed paraffin-embedded tissue blocks were sectioned and used for immunohistochemistry studies. The examined antigens were CD5 (4C7, Nichirei Bioscience, Tokyo, Japan), pancytokeratin (AE1/AE3, DakoCytomation, Tokyo, Japan), p53 (DO-7, DakoCytomation, Tokyo, Japan), p63 (4A4, DakoCytomation, Tokyo, Japan), thyroglobulin (DAK-Tg6, DakoCytomation, Tokyo, Japan), thyroid transcription factor 1 (TTF-1, which is also named as Homeobox Protein NKX2-1) (8G7G3/1, Dako Cytomation, Tokyo, Japan), neuron-specific enolase (NSE) (BBS/NC/VI-H14, DakoCytomation, Tokyo, Japan), synaptophysin (rabbit polyclonal lot 071, DakoCytomation, Tokyo, Japan), chromogranin A (LK2H10, Novocastra, Newcastle upon Tyne, UK), Ki67 (MIB-1, DakoCytomation, Tokyo, Japan) and carcinoembryonic antigen (CEA) (COL-1, Nichirei Bioscience, Tokyo, Japan).

The tumor cells were positive for CD5 (Fig. 8), pancytokeratin, p53 and p63 (Fig. 9) in all 10 cases examined and negative for thyroglobulin, TTF-1 and calcitonin. Archival tissue blocks stored for more than 20 years demonstrated the CD5 positivity immunohistochemically (Fig. 10), which confirmed good antigen preservation of CD5 even in formalin-fixed autopsy tissue samples.

CD5 is a membrane-bound protein and a member of the scavenger receptor cysteine-rich superfamily. It is immunohistochemically positive in most T lymphocytes

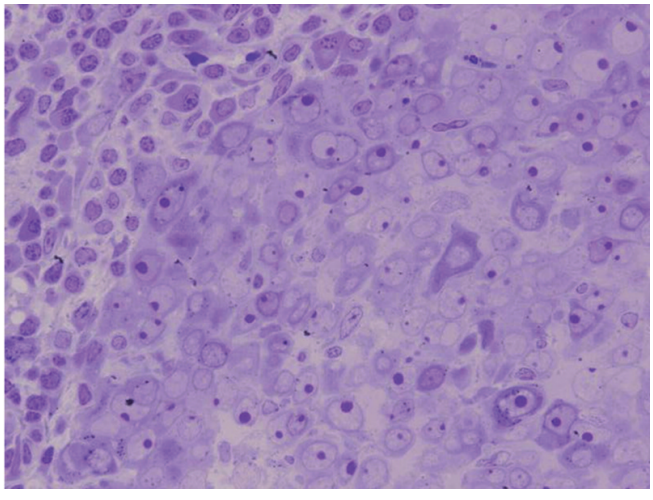

Fig. 7. Toluidine blue plastic section of lymphoepithelioma type. The tumor cells were polygonal and compactly arranged each other. The nuclear characteristic, a large nucleolus in dispersed chromatin, was evident in most of the tumor cells. Stromal lymphocytes and plasma cells are seen in the upper left. Toluidine blue, x 40

and in T cell neoplasms and thymic carcinomas. Positive CD5 immunoreactivity was first reported in ITET/CASTLE by Berezowski et al. and these researchers concluded that this appeared to support the thymic derivation of ITET/CASTLE (Berezowski et al., 1996). Dorfman et al. reported CD5 positivity in five cases of ITET/CASTLE and concluded that this was new evidence in support of the thymic origin of this thyroid carcinoma (Dorfman et al., 1998a). Ito et al. showed that CD5 immunopositivity had excellent sensitivity (82.4%) and high specificity (100%) for the diagnosis of ITET/CASTLE (Ito et al., 2007). CD5 is now a key immunohistochemical marker to confirm the histopathologic diagnosis of ITET/CASTLE (Tables 3, 4).

Ito et al. investigated S100A9 expression in 23 cases of ITET/CASTLE, 26 cases of PSCC and 19 cases of UC with a squamous cell component, as well as eight thymomas and 12 normal thymic tissues (Ito et al., 2006). All thymomas and thymic tissues showed a small number of S100A9-positive cells in a scattered pattern. Twenty-one of 23 ITET/CASTLEs showed the same pattern as thymomas or thymic tissues, while 14 PSCCs and 15 UCs with squamous cell component showed diffuse and laminated positivity of S100A9. It was concluded that the positive predictive value and specificity of S100A9 for diagnosis of ITET/CASTLE were 91.3% and 93.9%, respectively, and sensitivity was 75.0% (Ito et al., 2006).

Calretinin (a mesothelial marker) has been reported to be positive in ITET/CASTLE by Cappelli et al. Their 73-year-old male patient with thyroid mass developed pleural implantation, so they had to rule out mesothelioma; as a result, they incidentally found it was positive in ITET/CASTLE. They confirmed the positive results for CD5 as well as negative results for other mesothelial markers, WT1 and cytokeratin 5/6 (Cappelli et al., 2008).

KIT, a tyrosine kinase receptor protein encoded by the proto-oncogene c-kit, is overexpressed in several tumors including gastrointestinal stromal tumors and thymic squamous cell carcinomas. Pan et al. reported KIT- and CD5-positive staining in eight cases of ITET/CASTLE (Pan et al., 2012). Immunohistochemical examinations for bcl-2 and mcl-1, antiapoptosis proto-oncogenes, were carried out by Dorfman et al., who found five cases of ITET/CASTLE that were immunoreactive for bcl-2 and mcl-1. To determine whether ITET/CASTLE and solid cell nests of thyroid are derived from similar embryologic origins/lines of differentiation, and for better differentiation of ITET/CASTLE from other thyroid neoplasms, Reinmann et al. (2006) examined p63, high-molecular-weight keratin and CEA in addition to CD5. It was concluded that all these markers help distinguish

Table 4. Immunohistochemical profiles of ITET/CASTLE.

Positive Markers	CD5, p63, KIT, high-molecular-weight keratin, p53, bcl-2, mcl-1, S100A9, CEA, calretinin, MIB-1 (ki-67) labeling index: 10-30% (usually less than 20%)
Negative Markers	Thyroglobulin, TTF1, calcitonin

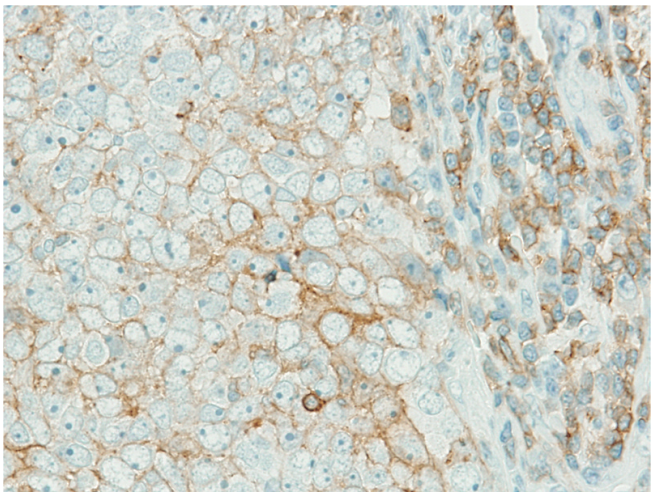

Fig. 8. CD5 immunoreactivity was predominantly observed in the membranous pattern of the tumor cells, as well as mature lymphocytes of the T cell lineage. CD5, methyl green nuclear counterstain, x 40

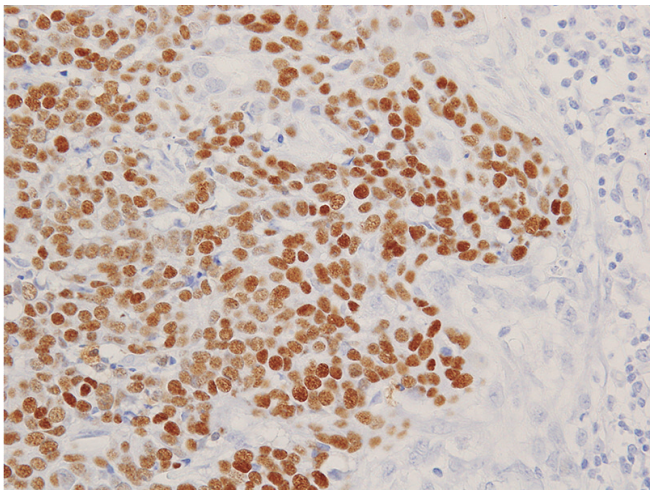

Fig. 9. Most nuclei of ITET/CASTLE were positively stained for p63 immunohistochemically. p63, hematoxylin nuclear counterstain, x 20

ITET/CASTLE from other thyroid tumors, which expands the range of useful immunohistochemical markers diagnostic for ITET/CASTLE and related tumors, as shown in Table 4.

Few positive cells exhibited CEA or neuroendocrine markers immunohistochemically, such as NSE (Fig. 11), synaptophysin and chromogranin A, in the tumor nests, which may indicate stem cell features toward neuroendocrine differentiation.

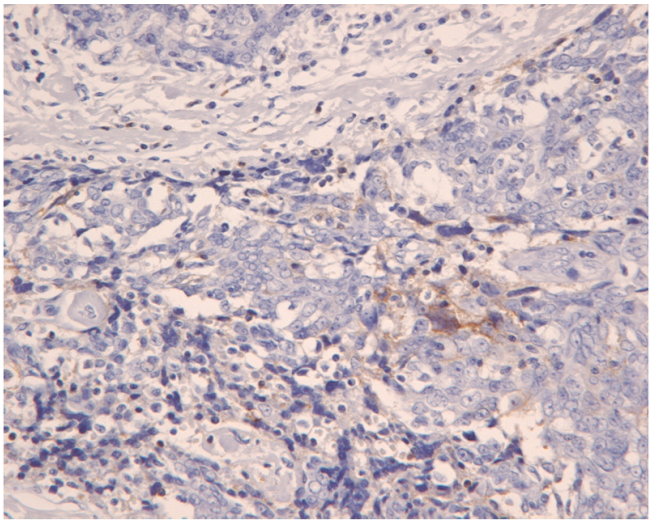

Fig. 10. CD5 positivity was well preserved in most of the tumor cells in a membranous pattern, even in autopsy samples stored for more than 20 years. CD5, hematoxylin nuclear counterstain, x 20

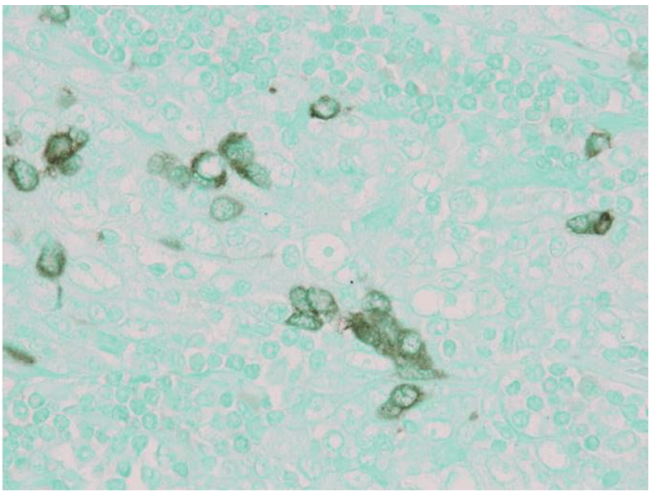

Fig. 11. Positive cells for neuron-specific enolase that were scattered or in small clusters were evidence for neuroendocrine differentiation in ITET/CASTLE immunohistochemically. Neuron-specific enolase, methyl green nuclear counterstain, x 40

Subclassification of ITET/CASTLE and neuroendocrine differentiation

The tumors in our 15 cases demonstrated three histological subtypes as follows: keratinizing squamous cell carcinoma type (11 cases) and non-keratinizing basaloid cell (lymphoepithelioma-like) type (four cases); one case of keratinizing squamous cell carcinoma type also showed extensive immunoreactivity for NSE, synaptophysin and chromogranin A in addition to CD5, p63 and KIT.

A case summary of neuroendocrine carcinoma type ITET/CASTLE, as a consultation case from Niigata Cancer Center, Japan, is as follows:

A 48-year-old male patient presented with a low-echoic solid tumor mass, measuring 3.5x2.9x2.5 cm, at the lower pole of the left lobe of the thyroid. He was alive with no evidence of disease three years after lobectomy with lymph node dissection. Keratinizing squamous cell carcinoma type ITET/CASTLE (Fig. 12) was found in the lower pole of the left lobe of the thyroid. No lymph node metastasis was found at surgery. The patient developed no paraneoplastic syndrome, including myasthenia gravis or any endocrine symptoms clinically. Serum calcitonin level was not elevated.

Immunohistochemical study for CD5 demonstrated marked immunoreactivity, as shown in Fig. 13, at the cell membrane of both tumor cells and lymphocytes, and those for chromogranin A, synaptophysin and NSE (Fig. 14) showed diffuse positivity in more than 50% of the tumor cells. We confirmed negative immunoreactivity for calcitonin in the tumor cells in order to rule out medullary (C cell) carcinoma. A summary of the immunohistochemistry in this case is as follows: positive

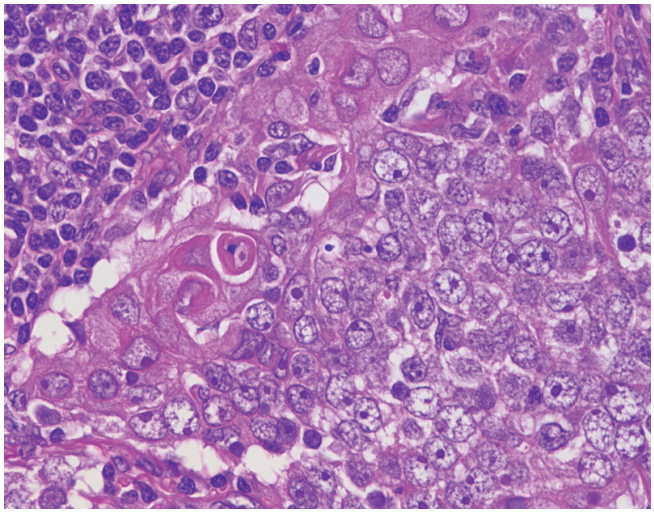

Fig. 12. ITET/CASTLE with neuroendocrine differentiation showed eosinophilic keratinization in the left field and basaloid cell features in the right lower field. Note lymphocyte-rich stroma in the left upper field. Hematoxylin and eosin, x 40

reaction was found for CD5, p63, p53, AE1/AE3, NSE, CEA, synaptophysin and chromogranin A. Negative staining was confirmed in TTF1, thyroglobulin and calcitonin.

Neuroendocrine differentiation in ITET/CASTLE poses a difficult problem in the differential diagnosis with medullary (C cell) carcinoma, paraganglioma and (primary or secondary) neuroendocrine carcinoma of the thyroid. In the WHO classification, medullary (C cell) carcinoma is defined as a malignant tumor showing C cell differentiation, in which calcitonin production is the key feature. Under this definition, thyroid carcinoma with neuroendocrine differentiation should be medullary (C cell) carcinoma when calcitonin production is evident. As a result, thyroid carcinoma without calcitonin production should not be medullary (C cell) carcinoma and it should be ITET/CASTLE when both CD5 and any neuroendocrine markers are evident. There have been few reports in the literature on neuroendocrine differentiation in ITET/CASTLE. Yamazaki et al. reported a 62-year-old male patient with a thyroid mass involving the trachea and the esophagus. They interpreted this thyroid tumor as ITET/CASTLE because of CD5 positivity and found neuroendocrine differentiation with chromogranin A and synaptophysin immunohistochemically (Yamazaki et al., 2008).

In an aggresive form of medullary (C cell) carcinoma, calcitonin production may be reduced due to dedifferentiation of C cell nature. In our group, it was proposed that an aggressive variant of medullary (C cell) carcinoma with low calcitonin productivity be called poorly differentiated medullary (C cell) carcinoma of thyroid, because of poor calcitonin positivity immunohisto-chemically and poorly developed cytoplasmic organelle ultrastructurally (Kakudo et al., 1987). Schmid and Ensinger studied 142 cases of medullary (C cell) carcinoma and found four atypical cases in terms of calcitonin productivity, three cases with very few calcitonin-positive cells and one completely lacking calcitonin immunoreactivity at both mRNA and protein levels (Schmid and Ensinger, 1998). They stated in their discussion that neuroendocrine differentiation had yet to be attributed to thyroid tumors of putative thymic origin (Schmid and Ensinger, 1998). This conclusion may be due simply to the fact that their publication occurred before CD5 was proved to be the key immunohisto-chemical diagnostic marker for thymic tumors; they also stated that there was currently no specific marker available to prove the putative thymic origin (Schmid and Ensinger, 1998). Neuroendocrine markers were also reported to be positively stained in hyalinizing trabecular adenomas of the thyroid immunohistochemically (Katoh et al., 1989) and Shikama et al. (2003) reported a 56-year-old female with typical morphology of hyalinizing trabecular adenoma, in which they demonstrated a mixture of endocrine cells positively stained for somatostatin and chromogranin A.

These observations do not conflict with the hypothesis that ITET/CASTLE is a carcinoma with thymic epithelial differentiation, because thymic carcinomas in the mediastinum also have several histologic subtypes, including squamous cell carcinoma type, lymphoepithelioma type and neuroendocrine carcinoma (carcinoid) type according to the WHO classification of thymic tumors (Ströbel et al., 2005; Suster, 2006). Yamazaki et al. stated in their discussion that the expression of neuroendocrine markers in ITET/CASTLE also supported the idea that this thyroid carcinoma is of thymic origin because neuroendocrine markers have been reported to be positive in thymic

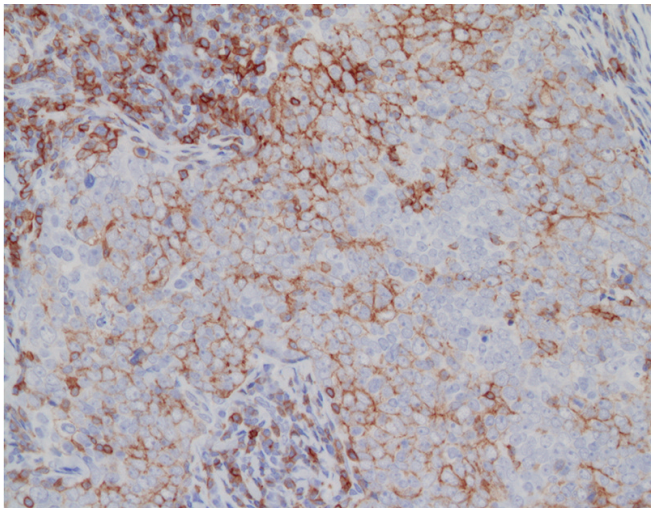

Fig. 13. ITET/CASTLE with neuroendocrine differentiation showed CD5 immunoreactivity in tumor cells as well as mature lymphocytes of the T cell lineage. CD5, hematoxylin nuclear counterstain, x 20

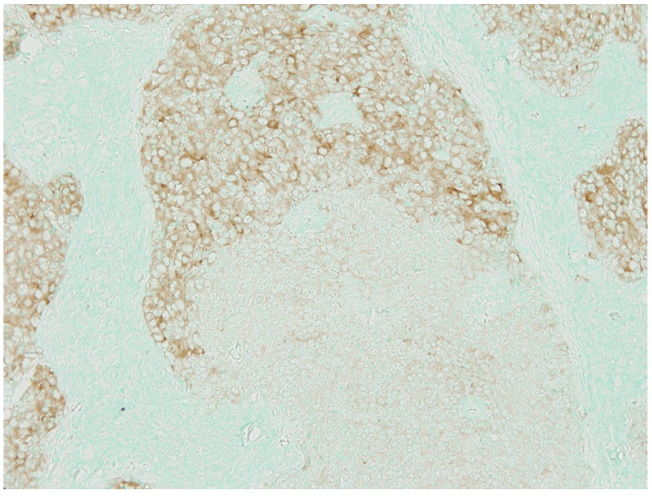

Fig. 14. Neuron-specific enolase immunostaining was shown in most of the tumor cells of ITET/CASTLE with neuroendocrine differentiation. Neuron-specific enolase, methyl green nuclear counterstain, x 20

carcinoma in focal or dispersed distribution (Hishima et al., 1998; Lauriola et al., 1998; Yamazaki et al., 2008).

Cytology of ITET/CASTLE

Preoperative diagnosis by aspiration cytology clearly demonstrated malignancy, but it is usually not possible to identify this tumor type (Fig. 15). There were numerous dissociated and loosely cohesive epithelial cells and they were usually polygonal or spindle-shaped with relatively abundant dense cytoplasm (Fig. 15). Orange-red keratinizing cells were rarely demonstrated in Papanicolau staining, but malignant cells with dense cyanophilic cytoplasm, suggesting keratinization, were often found (Fig. 15). The nuclei had an irregular nuclear contour, granular chromatin and distinct large nucleoli, differing from those of papillary thyroid carcinoma. There were usually no nuclear grooves and intranuclear cytoplasmic invaginations in their nuclei. The cytological features closely resemble those in metastatic head and neck carcinomas (Ng et al., 1996). Differential diagnosis on cytological examination usually includes UC, PSCC, metastatic carcinoma from other organs (such as head and neck, lung, esophagus and oral cavity) and medullary (C cell) carcinoma (Table 5).

Hirokawa et al. from our group summarized the characteristic cytological features of ITET/CASTLE. They were 1) hypercellularity, 2) large cell clusters without papillary or follicular pattern, 3) round or spindle-shaped tumor cells with distinct nucleoli and cell border, 4) few keratinized cells and intracytoplasmic lumina and 5) lymphocytic background (Hirokawa et al., 2012). Again, CD5 is the key immunohisto-chemical marker in cytology to support the diagnosis of ITET/CASTLE, which was reported by Cappelli et al. (2008) for the aggressive form of this carcinoma.

Ultrastructure of ITET/CASTLE

Small fragments of tumor tissue from cases K3 and K10 were fixed in 2% glutaraldehyde in phosphate-buffered saline and post-fixed in 1% osmium tetraoxide solution. They were embedded in epoxy resin after dehydration in graded ethanol. Ultrathin sections were viewed with JEM-1220 (JEOL, Tokyo, Japan) at 80 KV after uranyl and lead staining.

An electron microscopic examination of two cases showed immature squamous cells that had many cytoplasmic processes with poorly developed small desmosome junctions between the two cell membranes (Fig. 16). The nucleus had prominent nucleoli and dispersed chromatin (Fig. 16). The tumor cells had intermediate filaments arranged diffusely in the cytoplasm and bundles of dense tonofilaments together with well-developed mitochondria and rough endoplasmic reticulum in their cytoplasm. There were a small number of membrane-bound electron-dense granules present, but they were probably of lysosome type and neurosecretory granules were not identified.

Electron microscopic features on this type of tumor were first described by Asa et al. in 1988 in the English literature and later by Miyauchi et al. in 1989 in Japanese, followed by Shek et al. in 1996. There were two ultrastructural characteristics: 1) many intercellular junctions of desmosome type on the cell membrane and

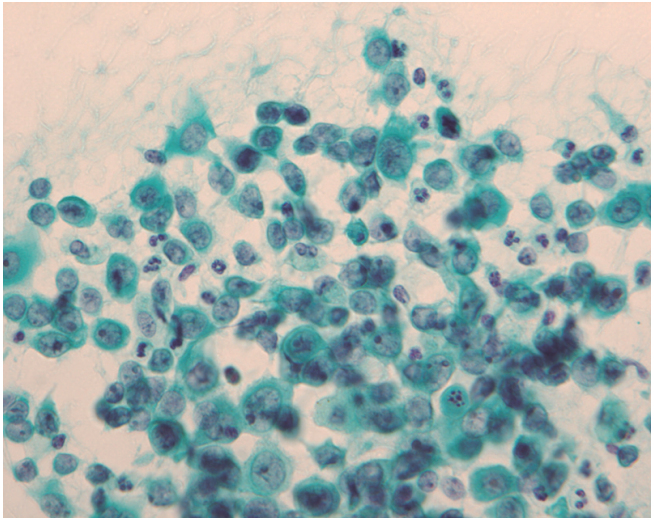

Fig. 15. Dissociated malignant epithelial cells from ITET/CASTLE were shown. They had irregularly shaped nuclei and dense cyanophilic cytoplasm indicating keratinization. Papanicolau stain, x 40

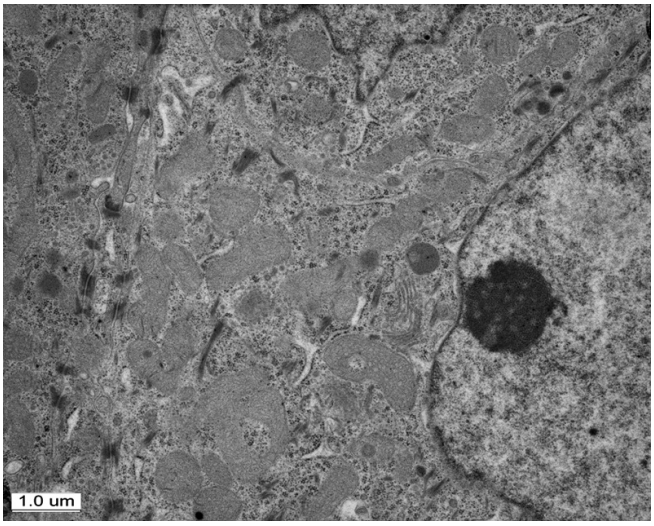

Fig. 16. Under electron microscopy, ITET/CASTLE more clearly demonstrated keratinizing epithelial cell nature. There were many small desmosome structures on the cell membrane in the left field and bundles of fine filaments in the cytoplasm. The nucleus on the right had a large nucleolus and dispersed chromatin. The bar indicated one micron. Uranyl and lead stain.

2) few tonofilament-type fibers in bundles or diffusely occupied cytoplasm in most of the tumor cells. Differentiation toward squamous cells was evident in terms of keratinization, both ultrastructurally and light-microscopically. These features may correspond to the light microscopic findings: 1) cytoplasmic keratinization, 2) rare intercellular bridges and 3) ill-defined cell borders in ITET/CASTLE. No gland (follicle) formation, cilia or neuroendocrine differentiation was detected in our electron microscopic study of two cases of the lymphoepithelioma type of ITET/CASTLE.

In earlier study from our group, the solid cell nest of the thyroid was examined with electron microscopy and their ultrastructural features were reported as squamous metaplasia (Kakudo et al., 1977). The main cells of the solid cell nest had many desmosome junctions and tonofilaments, which were observations similar to those in ITET/CASTLE.

Invasion, metastasis, recurrence and prognosis

Concerning its clinical behavior, most cases of ITET/CASTLE are slow growing and low-grade malignancy compared with PSCC or UC, as described by Miyauchi et al. in their original paper (Miyauchi et al., 1985). However, several patients with a more aggressive clinical course have been reported by several authors (Kakudo et al., 1988, Mizukami et al., 1995; Shek et al., 1996; Dominguez-Malagon et al., 2001; Kusada et al., 2005; Cappelli et al., 2008).

In an autopsy of a patient with ITET/CASTLE, hematogenous metastases were found in the lungs, pleura, liver and bone (Kakudo et al., 1988). A review of the literature by Roka et al. in 2004 revealed that six patients had local, three had distant and two had local and distant recurrence in 15 cases with unknown or involved lymph node (Roka et al., 2004). Ito et al. found that tumor invasion into extrathyroid structures was identified in 15 of 25 cases (Ito et al., 2007). Lymph node metastasis at surgery was found in nine of 18 cases (Ito et al., 2007). Curative surgery was performed in 22 cases and recurrence was found in six cases, including two cases showing loco-regional, one in the mediastinum, two in the bones, two in the liver and three in the lungs (Ito et al., 2007).

Ito et al. reported that the 5- and 10-year CSS rates were 90% and 82%, respectively, and they emphasized that these were better than those in PSCC and UC (Ito et al., 2007). The overall survival rate was also analyzed in our 14 cases by the Kaplan-Meier method and the 10-year overall survival rate was 74% (Fig. 1), which supported Miyauchi's original observation and Ito's prognostic analyses (Miyauchi et al., 1985; Ito et al., 2007).

Treatment of ITET/CASTLE

Patients with ITET/CASTLE have excellent outcomes after curative resection and postoperative radiotherapy compared with patients with UC and PSCC. Even patients with local recurrent disease benefited from salvage surgery with subsequent operation and/or radiotherapy (Sun et al., 2011); however, several case reports presented more aggressive and fatal cases with tumor recurrence and metastasis (Kakudo et al., 1988; Mizukami et al., 1995; Shek et al., 1996; Dominguez-Malagon et al., 2001; Kusada et al., 2005; Cappelli et al., 2008). There were few reports of this carcinoma treated with several different combinations of chemotherapy (Kakudo et al., 1988; Chow et al., 2007; Cappelli et al., 2008; Sun et al., 2011) and a well-accepted chemotherapy regimen for this carcinoma has not been established. Chow et al. stated that total thyroidectomy, combined with chemotherapy and radiotherapy, was effective for local control and symptom relief. In cases of inoperable disease or advanced local disease, it can be employed in combination with surgery (Chow et al., 2007). Roka et al. (2004) commented that radiotherapy seemed to be indicated when lymph nodes were tumor-positive and that it can be effective for recurrent tumors and Ito et al.

Table 5. List of tumors in differential diagnosis of ITET/CASTLE.

No.	Differential diagnosis
1	Metastatic (secondary) carcinoma to the thyroid, squamous cell carcinoma, lymphoepithelioma, neuroendocrine carcinoma or undifferentiated carcinoma types. (Possible primary sites include the lung, esophagus, naso-pharynx and oral cavity)
2	Undifferentiated carcinoma of the thyroid with squamous cell component
3	Primary squamous cell carcinoma of the thyroid
4	Poorly differentiated carcinoma of the thyroid
5	Follicular carcinoma of the thyroid, widely invasive type
6	Papillary carcinoma of the thyroid, solid variant
7	Papillary carcinoma of the thyroid with squamous metaplasia
8	Medullary (C cell) carcinoma of the thyroid (poorly differentiated or atypical)
9	Small cell carcinoma of the thyroid

(2007) reported a good response to radiation therapy in terms of recurrence. Owing to the rarity of this type of carcinoma, no widely accepted or well-established treatment guidelines have been published.

Histogenesis of ITET/CASTLE

Harach and Vujanic (1993) examined 58 fetal thyroid glands from autopsy and found thymus tissue in three cases. They concluded that their findings would support a IV-V pharyngeal pouch origin for some accessory thymic tissue and would provide an explanation of the histogenesis of ITET/CASTLE. The cervical thymus close vicinity of ITET/CASTLE tumor mass was reported in a few cases of ITET/CASTLE (Kakudo et al., 1988; Tai et al., 2003) and this rare observation would also provide an explanation of the histogenesis of this carcinoma.

Positive CD5 immunoreactivity was first reported in one case of ITET/CASTLE by Berezowski; this researcher concluded that this appeared to support the thymic derivation of this tumor because CD5 is a membrane-bound glycoprotein observed in thymic tumors and T lymphocytes (Berezowski et al., 1996). Dorfman et al. examined CD5 staining in 5 cases of ITET/CASTLE and concluded that the new evidence supported its thymic origin (Dorfman et al., 1998a). It has been reported that the expressions of p63, CEA and high-molecular-weight keratin in ITET/CASTLE are evidence of thymic epithelial origin and are useful diagnostic markers to distinguish ITET/CASTLE from other thyroid neoplasms (Reimann et al., 2006) (Table 5).

Solid cell nest and branchial pouch

Squamous cells in the thyroid appear in a variety of conditions, including benign follicular adenomas, hyperplastic adenomatous nodules, malignant neoplasms, inflammatory diseases and embryonic remnants. Solid cell nests of the thyroid are embryonic remnants derived from the third branchial pouch and sometimes create difficulty in differential diagnosis from the sinus structures, squamous metaplasia of follicular cells, microscopic squamous cell carcinomas, papillary microcarcinomas, intrathyroid parathyroid tissue, small medullary (C cell) carcinoma and C cell carcinoma in situ (C cell hyperplasia). Reis-Filho et al. reported that the main cells of solid cell nests are strongly positive for p63, a p53 homologue that is consistently expressed in basal/stem cells of stratified epithelia, and concluded that this was evidence of a stem cell nature (Reis-Filho et al., 2003). Ozaki et al. from our group analyzed the embryonic development of the thyroid gland using mice with various combinations of NKX2-1 (TTF-1) and p63 wild-type, heterozygous and null alleles. They found that the ultimobranchial body remained as a cystic vesicular structure in mice carrying the NKX2-1 (TTF-1)-null allele (Ozaki et al., 2011). The structure and p63 expression pattern of the ultimobranchial body cyst strikingly resembled those of the solid cell nest (Ozaki et al., 2011). They concluded that these results showed that, in the absence of NKX2-1 (TTF-1), the ultimobranchial body becomes cystic independent of p63, which is likely to be the origin of solid cell nests (Ozaki et al., 2011). Their data may provide a good explanation for why the solid cell nest is the only normal thyroid epithelium that is negative for TTF-1 (NKX2-1).

Reinmann et al. (2006) compared seven cases of ITET/CASTLE and the solid cell nest of thyroid for p63, CD5, high molecular weight keratin and CEA staining, and they found that the two lesions had essentially the same staining characteristics for these four antigens. They concluded that ITET/CASTLE is probably of thymic origin, and may arise from the branchial pouch remnants, the thyroid solid cell nests (Reinmann et al., 2006). Preto et al. examined the telomerase expression and proliferative activity of the solid cell nests and concluded that the main cells of the solid cell nests apparently harbor the minimal properties of a stem cell phenotype and may thus represent a pool of stem cells of the adult thyroid (Preto et al., 2004). Burstein et al and Cameselle-Teijeiro et al postulated that even papillary thyroid carcinoma could occur from the solid cell nest (Burstein et al., 2004; Cameselle-Teijeiro et al., 2009). Burstein et al proposed that p63-positive embryonal remnants, rather than mature follicular cells, are the cells of origin of a subset of papillary thyroid carcinomas and these p63-positive pluripotent cells may undergo thyroid follicular epithelial differentiation, and may undergo oncogenic change leading to papillary carcinoma. (Burstein et al., 2004; Cameselle-Teijeiro et al., 2009)

From our group, Bai et al reported an encapsulated thyroid neoplasm showing squamous cell differentiation, in which we identified intermediate cells between follicular cells and basaloid squamous cells (Bai et al., 2011). The intermediate cells had intermediate immunoreactivity between follicular and basaloid squamous cells (Bai et al., 2011). This transformation phenomenon observed in this case may indicate that basaloid squamous cell differentiation may occur in follicular cell neoplasm in view of the multipotential/stem cell nature of the tumor cells rather than this tumor being derived directly from the solid cell nest.

There are several types of thyroid carcinoma that have been claimed to be of solid cell nest origin: mucoepidermoid carcinoma of the thyroid (Katoh et al., 1990), sclerosing mucoepidermoid carcinomas with eosinophilia (Hunt et al., 2004) and small cell carcinoma of the thyroid (Cruz et al., 2011). ITET/CASTLE together with mucoepidermoid carcinoma and small cell carcinoma of the thyroid may share a similar histogenesis, that is, solid cell nest origin.

Branchiogenic carcinoma is a controversial entity and it is a cystic squamous cell carcinoma believed to arise from the second branchial pouch. Khafif et al. reviewed 67 cases from the literature in 1989 and

reported that only eight cases fulfilled their criteria, after 5-year follow-up without evidence of primary carcinoma elsewhere, and the majority of branchiogenic carcinomas were in fact cystic metastases in the lymph node (Khafif et al., 1989). Baranchiogenic carcinoma is a cystic type squamous cell carcinoma in the neck and the gross appearance is different from the ITET/CASTLE.

Etiological consideration of ITET/CASTLE

To the best of our knowledge, no oncogene abnormalities have been found and no array results have been published on ITET/CASTLE. Veits et al. evaluated four ITET/CASTLEs for chromosomal imbalances using the comparative genomic hybridization (CGH) method. They found that the most frequent gains were on chromosomal arm 1q (3/4), and losses were most frequently detected on 6p (4/4), 6q (3/4) and 16q (3/4). These CGH data show that ITET/CASTLE is characterized by chromosomal imbalances similar to those found in thymomas, and that thymic carcinomas indicate a similar sequence in terms of tumor development (Veits et al., 2011).

Epstein-Barr (EB) virus is usually positive in lymphoepithelioma of the upper aerodigestive tract and Shek et al. (1996), using the in situ hybridization method, studied EB virus in lymphoepithelioma-like carcinoma of the thyroid. Dominguez-Malagon et al. (2001), using both immunohistochemistry and polymerase chain reaction, examined EB virus in a case of ITET/CASTLE. Both studies could not detect any evidence of EB virus in their lymphoepithelioma-type thyroid carcinomas. In situ hybridization for EB virus-encoded RNA (EBER) was applied in eight cases of ITET/CASTLE by Liu et al., including both lymphoepithelial type and squamous cell carcinoma type, and all cases were reported to be negative (Liu et al., 2011). We examined two cases of lymphoepithelial type and one neuroendocrine carcinoma type by an in situ hybridization method for EBER, and negative results were confirmed (unpublished data).

Differential diagnosis of ITET/CASTLE

Differential diagnosis of ITET/CASTLE includes UC, PDC, medullary (C cell) carcinoma and high-grade PSCC in addition to secondary carcinoma from other sites (Table 5). Positive immunohistochemical findings for CD5 and stromal lymphocytes are key features to differentiate it from UC, PDC, medullary (C cell) carcinoma and PSCC (Tables 3, 4). CD5 is a reliable marker for ITET/CASTLE because morphology alone may not be sufficient for a conclusive diagnosis of ITET/CASTLE. UC, PDC, medullary (C cell) carcinoma and PSCC are all negative for CD5. Ito et al. reported negative results of CD5 immunoreactivity in three (17.6%) of 17 cases of ITET/CASTLE (Ito et al., 2007). This might have been due to poor antigen preservation or an inappropriate cut-off point in the immunohistochemical evaluation, and CD5-negative results were reported to occur even in mediastinal thymic carcinomas. Five (66%) out of nine cases of poorly differentiated thymic carcinoma were CD5-negative in Berezowski's study (Berezowski et al., 1996) and eight (33%) of 24 cases of thymic carcinomas were negative in Dorfman's study (Dorfman et al., 1998a).

ITET/CASTLE shows a solid and expansive growth pattern with lymphocyte-rich stroma, while PSCC of the thyroid usually exhibits invasive growth, high-grade histology (frequent mitoses, more than 50% MIB-1 labeling index and necrosis) and frequent granulocytic infiltration. Nuclear atypia of ITET/CASTLE is usually mild and mitoses are less frequent with an intermediate proliferation index (MIB-1 labeling index between 10% and 30%). All of these findings, together with immunohistochemical demonstration of CD5 positivity, are helpful to differentiate it from biologically aggressive PSCC of the thyroid (Table 3).

Conclusion

ITET/CASTLE is a low-grade and CD5-positive thyroid carcinoma; its prognosis is better than that of UC or PSCC. The overall survival rate at 10 years was found to be 74%. Positive immunoreactivity for CD5 is a key feature to differentiate it from UC, PDC, medullary (C cell) carcinoma, PSCC of the thyroid and metastatic thyroid carcinomas. Negative immunoreactivity for calcitonin, TTF1 and thyroglobulin and positive immunoreactivity for p63 and KIT are also helpful for differential diagnosis. In our 15 cases, there were three histological subtypes: keratinizing squamous cell carcinoma type, non-keratinizing basaloid carcinoma (lymphoepithelioma-like) type and neuroendocrine carcinoma type, which are comparable with the mediastinal thymic carcinomas.

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