Diagnostic utility of thyroid transcription factors Pax8 and TTF-2 (FoxE1) in thyroid epithelial neoplasms

Daisuke Nonaka¹, Yunjia Tang¹, Luis Chiriboga¹, Michael Rivera² and Ronald Ghossein²

¹Department of Pathology, New York University School of Medicine, New York, NY, USA and ²Department of Pathology, Memorial Sloan-Kettering Cancer Center, New York, NY, USA

Thyroid-specific transcription factors, Pax8, TTF-1, and TTF-2, are crucial for thyroid organogenesis and differentiation. Compared with TTF-1, the other two markers have scarcely been investigated in surgical pathology. The goal of this study is to evaluate the expressions of these markers in thyroid tumors of the full spectrum of differentiation, with special emphasis on anaplastic carcinomas. A total of 94 cases of thyroid neoplasms were studied: 17 papillary carcinomas, 18 follicular adenomas, 16 follicular carcinomas, 7 poorly differentiated carcinomas, 28 anaplastic carcinomas, and 8 medullary carcinomas. Immunostains for these three markers were performed. The antibodies to Pax8 and TTF-2 were also applied on 147 lung carcinomas as well as a variety of normal tissues and malignant tumors. All three markers were seen in papillary carcinomas, follicular adenomas and carcinomas, and poorly differentiated carcinomas in a diffuse manner, whereas their expressions in medullary carcinomas were variable. Pax8 was expressed in 79% of anaplastic carcinomas to a variable extent, whereas TTF-1 and TTF-2 were seen only in 18 and 7% of anaplastic carcinomas, respectively. TTF-2 was negative in all other neoplastic and non-neoplastic tissues including those of the lung. Pax8 was expressed in renal tubules, fallopian tubes, ovarian inclusion cysts, and lymphoid follicles as well as renal carcinoma, nephroblastoma, seminoma, and ovarian carcinoma, but not in normal tissue and carcinomas of the lung. Pax8 is a useful marker for the diagnosis of anaplastic carcinomas, particularly when the differential diagnosis includes pulmonary carcinoma. In differentiated thyroid neoplasms, no significant difference in expression was seen in all the three transcription factors.

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TTF-1 is one of the most commonly used markers in diagnostic pathology. It has shown a very high sensitivity and specificity in the diagnosis of tumors of pulmonary and thyroid lineage. In the vast majority of thyroid tumors, a diagnosis can be confidently rendered based on routine histologic examination. Immunohistochemistry study is, however, needed in some instances. The latter situations include anaplastic thyroid carcinoma and its discrimination from metastatic poorly differentiated tumors to the thyroid gland and neck, especially lung carcinomas. TTF-1 is one of the three most important transcription factors for thyroid gland organogenesis. In contrast to TTF-1, the other two

transcription factors, Pax8 and TTF-2 (FoxE1), have been rarely studied for diagnostic purposes.¹ We, hereby, describe expressions of the three thyroid-related transcription factors on the full spectrum of primary thyroid epithelial neoplasms, and discuss the utility of the markers from a diagnostic perspective, with special emphasis on their expressions in anaplastic carcinomas.

Materials and methods

Thyroid Epithelial Neoplasms

Case selection

A total of 94 cases of primary thyroid neoplasms were retrieved from the archives of the Department of Pathology of New York University Medical Center and Memorial Sloan-Kettering Cancer Center. The cases include 17 cases of papillary carcinoma, 18 cases of follicular adenoma, 16 cases of follicular carcinoma, 7 cases of poorly differentiated (insular)

Correspondence: Dr D Nonaka, MD, Department of Pathology, New York University School of Medicine, TCH-461, 560 First Avenue, New York, NY 10016, USA.
 E-mail: daisuke.nonaka@med.nyu.edu
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carcinoma, 28 cases of anaplastic carcinoma, 8 cases of medullary carcinoma, and 2 cases of C-cell hyperplasia. Hematoxylin and eosin (H&E) stained slides of all the cases were reviewed, and the histologic diagnosis was based on the most recent WHO classification of tumors of endocrine organs.² Anaplastic carcinomas were further subdivided into morphologic variants based on the predominant pattern.³

Tissue microarray construction

Sixty-nine of the 94 cases of thyroid epithelial neoplasms were used for tissue microarray construction using formalin-fixed, paraffin-embedded tissue. Tissue microarrays were assembled using a Manual Tissue Arrayer I (Beecher Instruments, Sun Prairie, WI, USA) as described previously.^{4,5} A representative area of each case was identified on the conventional sections, and at least three cylinders per tissue were arrayed using a punch biopsy (needles with a diameter of 0.6 mm).

Whole tissue sections for immunostaining

Thirteen out of the 69 cases including all 3 anaplastic carcinomas and 8 medullary carcinomas, in addition to the tissue microarray sections, were selected and examined using conventional whole sections. A further 25 cases of anaplastic carcinomas and 2 cases of C-cell hyperplasia were examined only on the conventional whole tissue sections.

Non-Thyroid Malignant Tumors and Normal Tissues

Tissue microarrays were also assembled as follows: (1) 147 cases of primary lung carcinoma, including 114 cases of adenocarcinoma, 29 cases of squamous cell carcinoma, and 4 cases of large cell carcinoma; (2) 95 malignant tumors from 14 different organs, including esophagus (squamous cell carcinoma), stomach (adenocarcinoma), colon (adenocarcinoma), liver (hepatocellular carcinoma), pancreas (ductal carcinoma), kidney (clear cell carcinoma, nephroblastoma), breast (invasive ductal carcinoma, invasive lobular carcinoma), ovary (serous papillary carcinoma, endometrioid carcinoma, clear cell carcinoma), prostate gland (adenocarcinoma), urinary bladder (urothelial carcinoma), skin (squamous cell carcinoma), testis (seminoma), lymph node (diffuse large B-cell lymphoma), and soft tissue (rhabdomyosarcoma); and (3) 53 cores of normal tissue from 17 organs, including esophagus, stomach, colon, prostate, lung, liver, kidney, breast, uterine cervix, ovary, fallopian tube, urinary bladder, skin, pancreas, testis, tongue, and placenta.

Immunohistochemistry

Immunohistochemical studies were performed using the following antibodies: rabbit anti-human Pax8 (polyclonal, Protein Tech Group Inc., Chicago,

IL, USA), mouse anti-human TTF-1 (clone 8G7G3/1, Neomarkers, Fremont, CA, USA), and goat anti-human TTF-2 (FoxE1) (polyclonal, Abcam Inc., Cambridge, MA, USA). TTF-2 and Pax8 staining was performed on all the above-mentioned tissues, that is all the thyroid tumors, tissue microarrays of (1), (2), and (3), whereas TTF-1 staining was only applied on all the thyroid tumors.

The sections were deparaffinized in xylene (three changes), rehydrated through graded alcohols (three changes 100% ethanol, three changes 95% ethanol) and rinsed in distilled water. Heat-induced epitope retrieval was performed in a 1200-W microwave oven at 90% power in 10 mM citrate buffer pH 6.0. Pax8 and TTF-2 were retrieved for 10 min and TTF-1 was retrieved for 15 min. Sections were allowed to cool for 30 min and then rinsed in distilled water. Antibody incubations and detection were carried out at 37°C on a NEXes instrument (Ventana Medical Systems, Tucson, Arizona) using Ventana's reagent buffer and detection kits unless otherwise noted. Endogenous peroxidase activity was blocked with hydrogen peroxide. Antibodies against TTF-1 and TTF-2 were diluted 1:50 and Pax8 was diluted 1:25. All antibodies were incubated overnight at room temperature. TTF-2 was detected using a biotinylated horse anti-goat (Vector Laboratories, Burlingame, CA, USA) diluted 1:100 and incubated for 30 min. TTF1 and Pax8 were detected with Ventana's biotinylated goat anti-mouse secondary. After secondary antibody application, streptavidin-horse-radish peroxidase conjugate was applied. The complex was visualized with 3,3 diaminobenzidine and enhanced with copper sulfate. Slides were washed in distilled water, counterstained with hematoxylin, dehydrated and mounted with permanent media. Appropriate positive and negative controls were included with the study sections.

Two pathologists with special interest in lung (DN) and thyroid gland (RG, DN) scored the immunostains. The extent of nuclear staining was estimated and graded as follows: 1+, 1–25%; 2+, 25–50%; 3+, 50–75%; 4+, ≥75%.

Results

Clinicopathologic Features

Basic clinicopathologic findings are listed in Table 1. All cases of papillary carcinomas were of the conventional type except for one case of a follicular variant (Figure 1a). Eighteen cases of follicular adenoma included 10 cases of oncocyctic variant (Hürthle cell adenoma), and 16 cases of follicular carcinoma (Figure 1e) included 3 cases of oncocyctic variant (Hürthle cell carcinoma).

Most of the anaplastic carcinomas contained more than one morphologic pattern, and they were subdivided based on the predominant pattern. They constituted five cases of squamoid type, eight cases of squamous cell type (squamous cell carcinoma),

Table 1 Clinical characteristics

	PCA	FA	FCA	ICA	ACA	MCA
Total number of cases	17	18	16	7	28	8
Age (years) (median)	18–83 (47)	27–82 (52)	27–69 (47.5)	25–85 (63)	32–90 (67)	16–75 (46.5)
Sex	6M:11F	3M:15F	7M:9F	3M:4F	12M:16F	3M:5F
Tumor size (cm) (median)	0.5–8 (1.5)	1.1–4.5 (2.0)	1.2–5.5 (2.5)	2–5.5 (3.5)	1.8–9.5 (5.5)	0.5–3 (1.0)

PCA, papillary carcinoma; FA, follicular adenoma; FCA, follicular carcinoma; ICA, poorly differentiated carcinoma; ACA, anaplastic carcinoma; MCA, medullary carcinoma.

Figure 1 Papillary carcinoma: (a) H&E stain, (b) Pax8, (c) TTF-1, (d) TTF-2. Follicular carcinoma: (e) H&E stain, (f) Pax8, (g) TTF-1, (h) TTF-2. Poorly differentiated carcinoma: (i) H&E stain, (j) Pax8, (k) TTF-1, (l) TTF-2. Anaplastic carcinoma: (m) H&E stain, (n) Pax8, (o) TTF-1, (p) TTF-2. Medullary carcinoma: (q) H&E stain, (r) Pax8, (s) TTF-1, (t) TTF-2.

six cases of spindle cell sarcomatoid type including one case of hemangiopericytoma-like morphology, two cases of giant cell type (Figure 1m), five cases of solid epithelioid type, and two cases of rhabdoid type.

Immunohistochemical Features

The results of the immunohistochemistry study on all types of thyroid tumors are presented in Table 2A. Further analysis of morphologic subtypes of anaplastic carcinomas is listed in Table 2B. Pax8 was diffusely and strongly expressed in a nuclear pattern in all papillary carcinomas (Figure 1b), follicular adenomas, follicular carcinomas (Figure 1f), and poorly differentiated carcinomas (Figure 1j). Seventy-nine percent of anaplastic carcinomas showed a positive reaction to a variable extent and intensity (Figure 1n). The expression was focal in 75% of the medullary carcinomas (Figure 1r). Pax8 was expressed in thyroid follicular cells of normal thyroid glands in a diffuse manner, and expressed in a few C-cells in thyroid C-cell hyperplasia. In other tumors, Pax8 was expressed in nephroblastoma and ovarian serous carcinoma in a diffuse and strong manner, and clear cell renal carcinoma and seminoma in a focal reaction (Table 3). Pax8, however, was not detected in lung carcinomas, and tumors of any other organs and sites. In normal tissues, Pax8

was expressed in renal tubules, epithelial cells of the fallopian tube, and ovarian inclusion cysts as well as lymphoid follicles of lymph nodes and tonsils, but not in lung parenchyma including alveolar pneumocytes and bronchiolar epithelial cells.

Diffuse and strong TTF-1 nuclear staining was seen in all papillary carcinomas (Figure 1c), follicular adenomas, follicular carcinomas (Figure 1g), and poorly differentiated carcinomas (Figure 1k), whereas its expression in medullary carcinomas was variable (Figure 1s). TTF-1 was generally negative in anaplastic carcinomas (Figure 1o), with only 18% of the cases being focally positive.

TTF-2 expression was generally diffuse and strong in all the papillary carcinomas (Figure 1d), follicular adenomas, follicular carcinomas (Figure 1h), and poorly differentiated carcinomas (Figure 1l). Seventy-five percent of the medullary carcinomas showed only focal nuclear expression (Figure 1t). A majority of anaplastic carcinomas were non-reactive to TTF-2 (Figure 1p), with only two cases (7%) of the cases showing focal (1+, 1–25%) nuclear staining. TTF-2 was expressed in thyroid follicular cells of normal thyroid glands in a diffuse manner, and expressed in a few C cells in thyroid C-cell hyperplasia. TTF-2 was negative in all other neoplastic and non-neoplastic tissues including those of the lung.

Table 2A Immunohistochemistry study results for thyroid epithelial neoplasms

	Pax8					TTF-1					TTF-2							
	0	1+	2+	3+	4+	0	1+	2+	3+	4+	0	1+	2+	3+	4+			
Papillary carcinoma	0	0	0	0	17	17/17 (100%)	0	0	0	0	17	17/17 (100%)	0	0	0	5	12	17/17 (100%)
Follicular adenoma	0	0	0	1	17	18/18 (100%)	0	0	2	0	16	18/18 (100%)	0	0	2	2	14	18/18 (100%)
Follicular carcinoma	0	0	0	3	13	16/16 (100%)	0	0	0	1	15	16/16 (100%)	0	0	0	4	12	16/16 (100%)
Poorly differentiated carcinoma	0	0	0	0	7	7/7 (100%)	0	0	0	1	6	7/7 (100%)	0	0	0	1	6	7/7 (100%)
Anaplastic carcinoma	6	4	5	5	8	22/28 (79%)	23	4	1	0	0	5/28 (18%)	26	2	0	0	0	2/28 (7%)
Medullary carcinoma	2	5	1	0	0	6/8 (75%)	1	3	2	0	2	7/8 (88%)	2	5	1	0	0	6/8 (75%)

0 indicates no staining; 1+, 1–25% tumor cells reactive; 2+, 25–50% tumor cells reactive; 3+, 50–75% tumor cells reactive; 4+, ≥75% tumor cells reactive.

Table 2B Immunohistochemistry study results for anaplastic carcinomas

	<i>Pax8</i>						<i>TTF-1</i>						<i>TTF-2</i>					
	0	1+	2+	3+	4+		0	1+	2+	3+	4+		0	1+	2+	3+	4+	
Squamoid	1	1	1	1	1	4/5 (80%)	4	1	0	0	0	1/5 (20%)	0	0	0	0	0	0/5 (0%)
Squamous cell	0	1	2	2	3	8/8 (100%)	8	0	0	0	0	0/8 (0%)	8	0	0	0	0	0/8 (0%)
Spindle	3	0	1	2	0	3/6 (50%)	5	1	0	0	0	1/6 (17%)	5	1	0	0	0	1/6 (17%)
Giant cell	0	0	0	0	2	2/2 (100%)	1	1	0	0	0	1/2 (50%)	1	1	0	0	0	1/2 (50%)
Epithelioid	2	2	1	0	0	3/5 (60%)	3	1	1	0	0	2/5 (40%)	5	0	0	0	0	0/5 (0%)
Rhabdoid	0	0	0	0	2	2/2 (100%)	2	0	0	0	0	0/2 (0%)	2	0	0	0	0	0/2 (0%)

0 indicates no staining; 1+, 1–25% tumor cells reactive; 2+, 25–50% tumor cells reactive; 3+, 50–75% tumor cells reactive; 4+, ≥75% tumor cells reactive.

Table 3 Immunohistochemistry study results for non-thyroid neoplasms

	PAX8						TTF-2					
	0	1+	2+	3+	4+		0	1+	2+	3+	4+	
<i>Lung</i>												
Adenocarcinoma	114	0	0	0	0	0/114 (0%)	114	0	0	0	0	0/114 (0%)
Squamous cell carcinoma	29	0	0	0	0	0/29 (0%)	29	0	0	0	0	0/29 (0%)
Large cell carcinoma	4	0	0	0	0	0/4 (0%)	4	0	0	0	0	0/4 (0%)
<i>Esophagus</i>												
Squamous cell carcinoma	6	0	0	0	0	0/6 (0%)	6	0	0	0	0	0/6 (0%)
<i>Stomach</i>												
Adenocarcinoma	5	0	0	0	0	0/5 (0%)	5	0	0	0	0	0/5 (0%)
<i>Colon</i>												
Adenocarcinoma	8	0	0	0	0	0/8 (0%)	8	0	0	0	0	0/8 (0%)
<i>Pancreas</i>												
Adenocarcinoma	5	0	0	0	0	0/5 (0%)	5	0	0	0	0	0/5 (0%)
<i>Liver</i>												
Hepatocellular carcinoma	6	0	0	0	0	0/6 (0%)	6	0	0	0	0	0/6 (0%)
<i>Kidney</i>												
Nephroblastoma	0	0	0	2	0	2/2 (100%)	2	0	0	0	0	0/? (0%)
Renal cell carcinoma	0	0	2	3	0	5/5 (100%)	5	0	0	0	0	0/5 (0%)
<i>Breast</i>												
Invasive ductal carcinoma	10	0	0	0	0	0/10 (0%)	10	0	0	0	0	0/10 (0%)
Invasive lobular carcinoma	10	0	0	0	0	0/10 (0%)	10	0	0	0	0	0/10 (0%)
<i>Ovary</i>												
Serous papillary carcinoma	0	0	0	0	10	10/10 (100%)	10	0	0	0	0	0/10 (0%)
Endometrioid carcinoma	1	0	0	1	1	2/3 (67%)	3	0	0	0	0	0/3 (0%)
Clear cell carcinoma	0	0	0	0	2	2/2 (100%)	2	0	0	0	0	0/2 (0%)
<i>Prostate</i>												
Adenocarcinoma	6	0	0	0	0	0/6 (0%)	6	0	0	0	0	0/6 (0%)
<i>Urinary bladder</i>												
Urothelial carcinoma	5	0	0	0	0	0/5 (0%)	5	0	0	0	0	0/5 (0%)
<i>Skin</i>												
Squamous cell carcinoma	4	0	0	0	0	0/4 (0%)	4	0	0	0	0	0/4 (0%)
<i>Testis</i>												
Seminoma	1	1	1	0	0	2/3 (67%)	3	0	0	0	0	0/3 (0%)
<i>Lymph node</i>												
Diffuse large B-cell lymphoma	3	0	0	0	0	0/3 (0%)	3	0	0	0	0	0/3 (0%)
<i>Soft tissue</i>												
Rhabdomyosarcoma	2	0	0	0	0	0/2 (0%)	2	0	0	0	0	0/2 (0%)

0 indicates no staining; 1+, 1–25% tumor cells reactive; 2+, 25–50% tumor cells reactive; 3+, 50–75% tumor cells reactive; 4+, ≥75% tumor cells reactive.

Discussion

Primary thyroid and lung carcinomas are common neoplasms, accounting for approximately 3 and 12% of all malignancies, respectively.⁶ Thyroid carcinoma is the most common type of endocrine malignancy, accounting for 90% of all endocrine malignant tumors. It is, therefore, not uncommon that the same patients suffer from both neoplasms, synchronously or metachronously. The lung is the

most common distant metastatic site for thyroid carcinomas (49%), followed by bone (25%) and brain (10%).⁷ The latter two organs, bone and brain, are also common metastatic destinations for lung carcinomas, and metastases to them are found in 30 and 45% of patients with metastasis to distant organs, respectively.⁸ Although rare, lung carcinoma can metastasize to the thyroid gland, with the reported incidence of clinically detected cases ranging from 0.05 to 3.1%.^{9–11} Metastatic thyroid

carcinoma in the lung may grow slowly, remain solely for an extended period of time, and may, therefore, simulate a primary pulmonary neoplasm.¹² Some lung and thyroid carcinomas may have similar morphologic features. For example, features typical of conventional papillary carcinoma such as papillary architecture, psammoma bodies, and nuclear pseudoinclusions and grooves can be seen in non-mucinous bronchioloalveolar and papillary types of lung adenocarcinoma. Anaplastic spindle and giant cells are found in both pleomorphic carcinoma of the lung and anaplastic thyroid carcinoma.^{8,13} In addition, both thyroid and lung carcinomas generally share TTF-1 immunoreactivity. Indeed, TTF-1 is positive in 75% of lung adenocarcinomas,¹⁴ and almost all cases of well-differentiated thyroid neoplasms of follicular origin.¹⁵ Thorough routine microscopic examination, along with adequate clinical and radiographic information, generally would allow a definitive diagnosis in such a setting. Immunohistochemistry study can further solidify the diagnosis. Well-differentiated thyroid carcinomas of follicular origin always express thyroglobulin, whereas well-differentiated lung adenocarcinomas express surfactant proteins such as surfactant apoprotein A in approximately 50% of cases.^{14,16} A minority of differentiated tumors and a majority of poorly and undifferentiated carcinomas may cause a diagnostic problem due to the lack of differentiation markers. In fact, thyroglobulin may be identified in less than half of poorly differentiated thyroid carcinomas.¹⁷ Furthermore, thyroglobulin and surfactant protein are completely negative in all anaplastic thyroid carcinomas and pulmonary pleomorphic carcinomas.^{18–20} Even the transcription factor TTF-1 is expressed only in 5.7%²¹ of anaplastic thyroid carcinomas, and 55% of lung pleomorphic carcinomas.²⁰

The diagnosis of anaplastic carcinomas, which is the least differentiated thyroid epithelial neoplasm, is conventionally based, similarly to undifferentiated carcinomas of other organs such as upper aerodigestive tract and uterus, on: (1) a presence of differentiated thyroid carcinoma component—usually papillary carcinomas but also follicular carcinomas and poorly differentiated carcinomas—which can be found in 50–89% of well-sampled cases; (2) involvement of the bulk of the tumor in the thyroid gland; (3) exclusion of undifferentiated tumor by metastasis or direct invasion from a neighboring organ, such as larynx. Immunohistochemistry has not been reliable in identifying the origin of the organ due to a lack of thyroglobulin and TTF-1 expression as mentioned earlier.

Transcription factors are proteins that control the first step of gene expression, and their role is to orchestrate the complex pathways of cellular growth and differentiation.²² Some of these molecules are tissue/organ-specific, and they are frequently expressed at an early stage of embryogenesis where they regulate specific differentiation pathways.

These features make them particularly helpful for determining cell lineage of tumors. Among transcription factors, the most commonly used and certainly the most useful marker in diagnostic pathology is TTF-1. In thyroid organogenesis, in addition to *Ttf-1*, two more transcription factors are crucially involved: *Pax8* and *Ttf-2* (*Foxe1*).

The development of the thyroid gland and its normal migration in the embryonic stage, and furthermore, the maintenance of the differentiated state throughout its life is dependent on the interplay between transcription factors, particularly *Pax8*, *Ttf-1*, and *Ttf-2*.²³ These transcription factors act to maintain the thyroid-differentiated phenotype.^{24,25} Among the three transcription factors, the *Pax8* gene is the first to be expressed in the median thyroid anlage and laterally in the fourth pharyngeal arch, followed by the *Ttf-1* gene, and then *Ttf-2*.²³ The importance of these three transcription factors is evidenced by congenital thyroid agenesis, which results from mutations in any of the transcription factors.^{26–32}

We found consistent nuclear expressions of Pax8, TTF-1, and TTF-2 in well-differentiated neoplasms of follicular cell origin, that is, papillary carcinomas, follicular adenomas, follicular carcinomas, and poorly differentiated carcinomas. Seventy-nine percent of anaplastic carcinomas variably showed Pax8 reaction, whereas TTF-1 and TTF-2 were expressed in only 18 and 7% of anaplastic carcinomas, respectively. In light of the chronological emergence of these transcription factors in thyroid organogenesis, that is, *Pax8* at first, followed by *Ttf-1* and *Ttf-2*, the latter finding is no surprise, since it is generally observed that more mature differentiation markers become lost at an early stage of tumor progression, and *Pax8* is the earliest transcription factor in thyroid embryogenesis, that is, the marker found at the most immature stage of the thyroid anlage.³³ In fact, loss of thyroid-specific proteins and differentiation has been described as a common process in thyroid carcinogenesis.^{1,34,35}

Medullary carcinomas expressed all three markers to a variable extent in most cases in our study. TTF-1 generally showed more extensive staining than Pax8 and TTF-2. This finding is interesting, since C cell, which medullary carcinoma originates from, is of ultimobranchial body derivation. *Ttf-1* is expressed in the ultimobranchial body, and it was proposed that *Ttf-1* may regulate the calcitonin gene in the maintenance of calcium homeostasis in C cells.^{36,37} *Pax8* expression was also demonstrated in the ultimobranchial body, but its role in C cells remains largely unclear.²³ A relation of *Ttf-2* to the ultimobranchial body has not been reported to our knowledge. Pax8 and TTF-2 were expressed in C cells of C-cell hyperplasia. Given that all of the three transcription factors are expressed in medullary carcinomas and C-cell hyperplasia, it can be speculated that they may play some role not only in follicular cells but also in C-cell development.

Previous studies of Pax8 expression in thyroid neoplasms showed discrepant results. By immunohistochemistry, this molecule was shown to be expressed in 33–100% of follicular adenomas, 38–67% of follicular carcinomas, 31–78% of papillary carcinomas, 0% of anaplastic carcinomas, and 0% of medullary carcinomas tested.^{1,24,38} Sequeira *et al*³⁹ reported TTF-2 expression by *in situ* hybridization in: 15/21 (71%) cases of follicular adenomas, 8/18 (44%) cases of follicular carcinomas, 11/17 cases (65%) of papillary carcinomas, and 0/2 cases (0%) of anaplastic carcinomas. Our results are clearly at variance with previous reports on the subject. The reason for this disagreement is not clear. It may be attributed to a difference in antibodies given that the Pax8 antibody used in the current series was not utilized in previous studies. The reliability of our marker is evident, because we tested it not only on the thyroid neoplasms but also on a variety of normal tissues and common malignant neoplasms. In contrast to the results of Pax8 and TTF-2 staining, our finding of TTF-1 expression in thyroid epithelial neoplasms is in keeping with reported results.^{15,21,40}

TTF-2 was not expressed in the non-thyroid normal tissues and tumors tested, whereas Pax8 was expressed in B lymphocytes, the renal tubule, epithelial cells of the fallopian tube and ovarian surface inclusion cyst, and tumors of metanephric and gonadal derivation, such as clear renal cell carcinomas, ovarian carcinomas, and seminomas. The latter findings are in keeping with those reported previously.^{41,42}

In summary, although expressed in several tumors, it is evident that Pax8 would serve as a useful marker in the following situations: (1) a solitary or even multiple carcinomas in the lung where primary lung and metastasis from the thyroid gland are considered as an origin (Figure 2a); (2) a metastatic carcinoma in other organs such as bone and brain where lung and thyroid gland are primary sites under consideration; (3) an undifferentiated neoplasm (Figure 2b) of the neck, which involves thyroid gland as well as adjacent organs, such as larynx, trachea, and bronchus; (4) a carcinoma in the thyroid gland or more commonly in the neck where the differential diagnoses include primary thyroid carcinoma and metastatic carcinoma from the lung. This distinction is important since the chemotherapeutic regimens are different, that is, adriamycin for anaplastic carcinoma, and platinum-based chemotherapy for lung carcinoma.^{43,44} The diagnostic dilemma is exemplified by cases where there is uncertainty as to whether the undifferentiated tumor is thyroid based or not. We have seen a case of lung carcinoma mistaken for an anaplastic thyroid carcinoma, because the pathologist was led to believe that the tumor was in the thyroid, while it was simply a lymph node metastasis from a large cell carcinoma of pulmonary origin. Pax8 would have helped significantly in such a context.

Figure 2 (a) Pax8-positive metastatic anaplastic thyroid carcinoma involving the lung parenchyma. Note that lung tissue is negative for Pax8. (b) Anaplastic/pleomorphic giant cells demonstrating strong nuclear Pax8 expression.

For differentiated thyroid tumors, TTF-2 is as specific a marker as thyroglobulin, and is more specific than Pax8 and TTF-1, but it has no greater utility in anaplastic carcinomas than thyroglobulin does.

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