

# Application of Immunohistochemistry to Thyroid Neoplasms

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● **Context.**—Thyroid lesions with nodular architecture and follicular pattern of growth often pose difficulties in accurate diagnosis during the assessment of cytologic and histologic specimens. The diagnosis of follicular neoplasm on cytology or of follicular tumor of uncertain malignant potential on histology is likely to cause confusion among clinicians and delay effective management of these lesions. Occasionally, thyroid tumors represent unusual or metastatic lesions and their accurate diagnosis requires immunohistochemical confirmation.

**Objective.**—To review the literature on the applications

Thyroid cancer is the most common endocrine malignancy, and more than 95% of thyroid carcinomas originate from follicular epithelial cells.<sup>1</sup> The incidence of thyroid carcinomas derived from follicular cells varies worldwide depending on dietary iodine intake, but in most countries it has increased during the past few decades and in North America it is one of the most rapidly increasing cancers, representing a major cause of morbidity in premenopausal women.<sup>2,3</sup> Medullary carcinomas that originate from parafollicular C cells, which are involved in the production of calcitonin, are rare, representing only about 3% of thyroid tumors. Most follicular cell-derived carcinomas are well-differentiated malignancies that can be effectively treated by surgical resection with or without radioactive iodine ablation. They tend to occur more often in females and in patients at a younger age. A subset comprising undifferentiated carcinomas that usually affect older patients behave very aggressively, and for these tumors there is no effective management at the present time. In addition, there is a third category of poorly differentiated carcinomas with an intermediate biology between well-differentiated and undifferentiated thyroid carcinomas.

Papillary thyroid carcinoma (PTC) is the most common of the well-differentiated carcinomas (85%) and is characterized by distinctive nuclear features.<sup>1,4</sup> Metastasis to

of immunohistochemistry in the differential diagnosis of thyroid tumors.

**Data Sources.**—Relevant articles indexed in PubMed (National Library of Medicine) between 1976 and 2006.

**Conclusions.**—Our review supports the use of ancillary techniques involving a panel of antibodies suitable for immunohistochemistry and molecular analysis in the assessment of thyroid nodules. These tools can improve diagnostic accuracy when combined with standard morphologic criteria.

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regional lymph nodes may occur in up to 50% of cases of PTC. Variants of papillary carcinoma include the classical subtypes with papillary architecture; the follicular variant PTC that lacks papillae but retains classical nuclear atypia; oncocyctic tumors that can have either papillary or follicular architecture associated with the nuclear features of PTC and oncocyctic cytoplasm; and tall-cell, solid, and cribriform types. Follicular thyroid carcinoma (FTC) is less frequent (10%) and is composed of follicles with variable size lined by well-differentiated epithelial cells lacking the characteristic nuclear features of papillary carcinoma. Because follicular carcinomas are able to disseminate hematogenously, distant metastasis occurs in up to 20% of cases. Variants of FTC include oncocyctic and clear cell subtypes.<sup>4</sup>

Most thyroid tumors can be readily diagnosed using histopathologic criteria, which allow the pathologist to differentiate benign from malignant lesions and guarantee an accurate classification for the majority of the variants of carcinomas derived from follicular epithelial cells. However, in most cases, the pathologist is confronted with thyroid lesions in which the distinction between benign and malignant can be quite subtle. The decision favoring one or another has clinical consequences and implies different modalities of treatment. On one hand, there is the need to avoid excessive treatment and psychological discomfort to the patient. On the other hand, patients with potentially aggressive disease need to be guaranteed effective management at the initial stages of disease when it is still curable. For this reason, the approach to these challenging tumors should include ancillary techniques, immunohistochemistry and molecular profiling, that can improve the standard morphologic assessment both in surgical specimens and in cytology samples obtained by fine-needle aspiration.<sup>5–8</sup>

Genetic studies have identified a process of cumulative

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molecular events involved in thyroid tumor initiation and progression, resulting in genomic instability and the capacity for independent cellular growth, invasion, and metastasis.<sup>9</sup> It seems unrealistic to expect a single tool, in the form of a magic biomarker, to be able to effectively resolve the diagnostic dilemmas in thyroid pathology. Each marker differentially expressed in tumorous and nontumorous tissues represents a snapshot of the molecular events succeeding in the tissue environment. The amount of information a single marker offers is often insufficient to understand tumor biology or to render accurate diagnosis. The use of combined immunohistochemical markers as a panel seems to be an alternative to aid some of the diagnostic challenges in surgical pathology and cytopathology of thyroid specimens.<sup>7,10,11</sup> Most important, genomic and proteomic technologic approaches are being developed to introduce molecular signatures capable of separating benign from malignant thyroid tumors and, in the last group, to distinguish tumors with indolent and aggressive behavior.<sup>12-14</sup>

### WHEN SHOULD ANCILLARY TECHNIQUES BE APPLIED?

Nodular tumors exhibiting a predominantly follicular architecture are the most common type of lesion of the thyroid. In most cases, a diagnosis can be readily assessed without difficulty based on histologic and clinical evidence. For instance, hyperplastic nodules are usually associated with nodular goiter and can be promptly recognized by their variability of follicular size and degenerative changes including fibrosis, hemorrhage, and cyst formation.<sup>15,16</sup> Follicular adenomas, usually presenting as single nodules, are separated from the normal thyroid parenchyma by an intact fibrous capsule; they usually exhibit a predominance of microfollicles or macrofollicles and lack vascular invasion.<sup>4</sup> Hyperplastic nodules (also called adenomatoid nodules) with an exclusive microfollicular or macrofollicular architecture and adenomas with incomplete or disrupted capsules can pose difficulties in diagnosis.<sup>15</sup> In addition, atypical follicular adenomas comprise a group of noninvasive lesions with increased cellularity, nuclear atypia, and/or mitotic activity, in which tumor necrosis and infarction can often be demonstrated.<sup>17</sup> Another controversial entity, the so-called hyalinizing trabecular adenoma (HTA), shares morphologic features with PTC.

Examples of malignant follicular patterned lesions of the thyroid that may impose assessment difficulties to the diagnosis include encapsulated or minimally invasive follicular carcinoma, clear cell carcinomas that can mimic metastatic carcinomas, follicular and oncocytic variants of papillary carcinoma, follicular variant of medullary carcinoma, and the rare mixed follicular-medullary tumors.<sup>15</sup> Traditionally, the diagnosis of follicular carcinoma relies on demonstrating capsular and/or vascular invasion.<sup>4</sup> The interpretation of what constitutes capsular and vascular invasion may vary among pathologists.<sup>15,18,19</sup> The term *well-differentiated tumor of undetermined malignant potential* has been used by some authors to designate follicular carcinomas presenting only minimal capsular invasion.<sup>20</sup> The basis of the nomenclature relies on the fact that this tumor category shows indolent behavior. In contrast, follicular carcinomas presenting vascular invasion with or without capsular invasion tend to recur and spread to distant organs more often and have been called *grossly encapsulated*

*angioinvasive follicular carcinoma*.<sup>15,17</sup> Clear cell tumors can be diagnosed as follicular adenomas or follicular carcinomas, but even minor atypical features should prompt the consideration of metastasis from renal, lung, or adrenal carcinoma.<sup>21</sup> Follicular variant of papillary thyroid carcinoma (FVPTC) can be difficult to diagnose when tumor cells lack all of the obvious nuclear features of papillary carcinoma.<sup>15,22</sup> Even more problematic is the interpretation of unifocal or multifocal nuclear changes of papillary carcinoma in nodules with a predominantly bland cytology.<sup>15,23</sup> Observer variation in the diagnosis of FVPTC has been demonstrated even by experienced pathologists.<sup>24</sup> The most important histologic features used to identify FVPTC include the presence of cytoplasmic invaginations, abundant nuclear grooves, and ground glass nuclei.<sup>24</sup> Some authors recommend the use of strict criteria when evaluating a potential FVPTC and propose a combination of major and minor histologic features. Among the latter are the presence of abortive papillae, elongated or irregularly shaped follicles, dark-staining colloid, rare nuclear pseudoinclusions, and multinucleated histiocytes in the follicular lumens.<sup>25</sup> The diagnosis of oncocytic follicular variant of papillary carcinoma remains controversial. Most of these lesions present irregular follicles with hypereosinophilic colloid and the nuclear features of papillary carcinoma, which can be often obscured by hyperchromasia, and prominent nucleoli that characterize oncocytic change.<sup>26</sup> True follicular differentiation may occur in medullary carcinomas of the thyroid gland and mixed follicular-medullary tumors.<sup>4</sup> Nuclear features suggestive of neuroendocrine differentiation and cytoplasmic granularity are the most relevant morphologic findings, but complementary immunohistochemical analysis is required to confirm the diagnosis.<sup>15</sup>

Fine-needle aspiration cytology (FNAC) has greatly improved the clinical management of thyroid nodules. However, FNA has inherent limitations related not only to inadequate sampling but also, most importantly, to its inability to distinguish between benign and malignant follicular lesions in the absence of nuclear features of papillary carcinoma. The indeterminate diagnosis of *follicular neoplasm* encompasses a number of heterogeneous thyroid lesions including cellular adenomatoid nodule, follicular adenoma, and follicular carcinoma.<sup>7,11,27</sup> Additionally, the interpretation of FVPTC cytology can be difficult when prominent classic nuclear features of PTC are absent. In such cases, a preoperative diagnosis of "follicular lesion suggestive of PTC" results in conservative surgical assessment until a definitive diagnosis can determine the appropriate treatment.

Several immunohistochemical markers using different antibodies, alone or combined in panels, have been postulated to improve diagnostic accuracy of follicular-pattern thyroid lesions.<sup>5,10</sup> They belong to different categories and are involved in cell adhesion (galectin-3, E-cadherin, fibronectin [FN]), receptor signaling (RET), gene transcription control (thyroid transcription factor 1 [TTF-1]), secretion (thyroglobulin [TG], calcitonin, carcinoembryonic antigen [CEA]), cell cycle regulation (p27, cyclin D1), and cellular structure (cytokeratin [CK] 19). They are detected in different cellular compartments such as membrane and/or cytoplasm (Hector Battifora mesothelial (cell) 1 [HBME-1],  $\beta$ -catenin) and nucleus (p53).

## RET

The *RET* proto-oncogene (*c-RET*) encodes a tyrosine-kinase receptor protein whose ligands belong to the family of glial cell line–derived neurotropic factors. In conjunction with membrane-bound, ligand-binding glial cell line–derived neurotropic factor receptors, *RET* operates as an intracellular signal-transducing element.<sup>28</sup> *RET/PTC* is a rearranged version of *RET*.<sup>29</sup> Somatic rearrangements of *RET* were identified in PTC before *RET* was recognized as the susceptibility gene for multiple endocrine neoplasia type 2. In multiple endocrine neoplasia type 2, point mutations result in constitutive activation of *RET*, resulting in medullary thyroid carcinomas; there is no role for immunohistochemistry in this setting. In contrast, *RET* rearrangements are considered specific for PTC, and they occur when *RET* undergoes a translocation fusing its tyrosine kinase domain to a variety of other 5' elements, thereby removing the promoter, the extracellular ligand-binding domain, and the membrane-anchoring domain of *RET*.<sup>28,30,31</sup> *RET/PTC-1*, *-2*, and *-3*, and a number of other less common rearrangements resulting in *RET* oncogene activation, play a key role in the pathogenesis of PTC.<sup>30–32</sup> The constitutive activation of the tyrosine domain in the carboxyl-terminal end of *RET/PTC3* induces signaling pathways within thyrocytes and causes cellular transformation in transgenic mice.<sup>33,34</sup> *RET/PTC* is also considered to lead to the characteristic nuclear features of PTC through alteration of the nuclear envelope and chromatin structure.<sup>35</sup> There is a wide variation in the reported frequency of *RET* activation, which is related to the sensitivity of the detection techniques and exposure to ionizing radiation.<sup>36</sup> *RET/PTC-1* and *RET/PTC-3* are the most common types of *RET* rearrangements reported in non–radiation-induced PTC (40% and 15% of cases, respectively).<sup>36–38</sup>

The identification of *RET/PTC* rearrangements has increased the ability to diagnose PTC. The utility of aberrant *RET* expression by rearrangement as a diagnostic marker in borderline thyroid lesions and preoperative evaluation of thyroid aspirates is supported by its high specificity for PTC.<sup>6,8,39</sup> *RET* immunostaining has been shown to be useful in the assessment of thyroid lesions with incomplete and/or focal features of PTC in which positive staining has been demonstrated in more than 50% of cases in close parallel with the morphologic features suggestive of PTC.<sup>39</sup> In the same study, no *RET/PTC-1* or *RET/PTC-3* rearrangements were detected by reverse transcription–polymerase chain reaction (RT-PCR) in the tumor areas lacking the cytologic alterations. *RET* protein detection by immunohistochemistry has also been reported in the so-called HTAs, and this finding was confirmed by detection of *RET/PTC-1* rearrangements by RT-PCR, suggesting that HTA may represent a variant of PTC.<sup>40,41</sup> Similarly, it has been shown that oncocytic tumors that exhibit nuclear features of PTC also harbor *RET/PTC* rearrangements.<sup>42,43</sup> *RET* protein–positive immunostaining can also be demonstrated in the oncocytic cells of this subset of tumors, supporting the revised classification system for oncocytic tumors that recognizes an oncocytic follicular variant of PTC.<sup>4,42</sup>

*RET/PTC* oncogene product immunostaining has been reported as a useful adjunct when used in combination with other antibodies including CK19, galectin-3, and HBME-1 in the assessment of thyroid specimens and aspirates.<sup>6,27,39,44</sup>

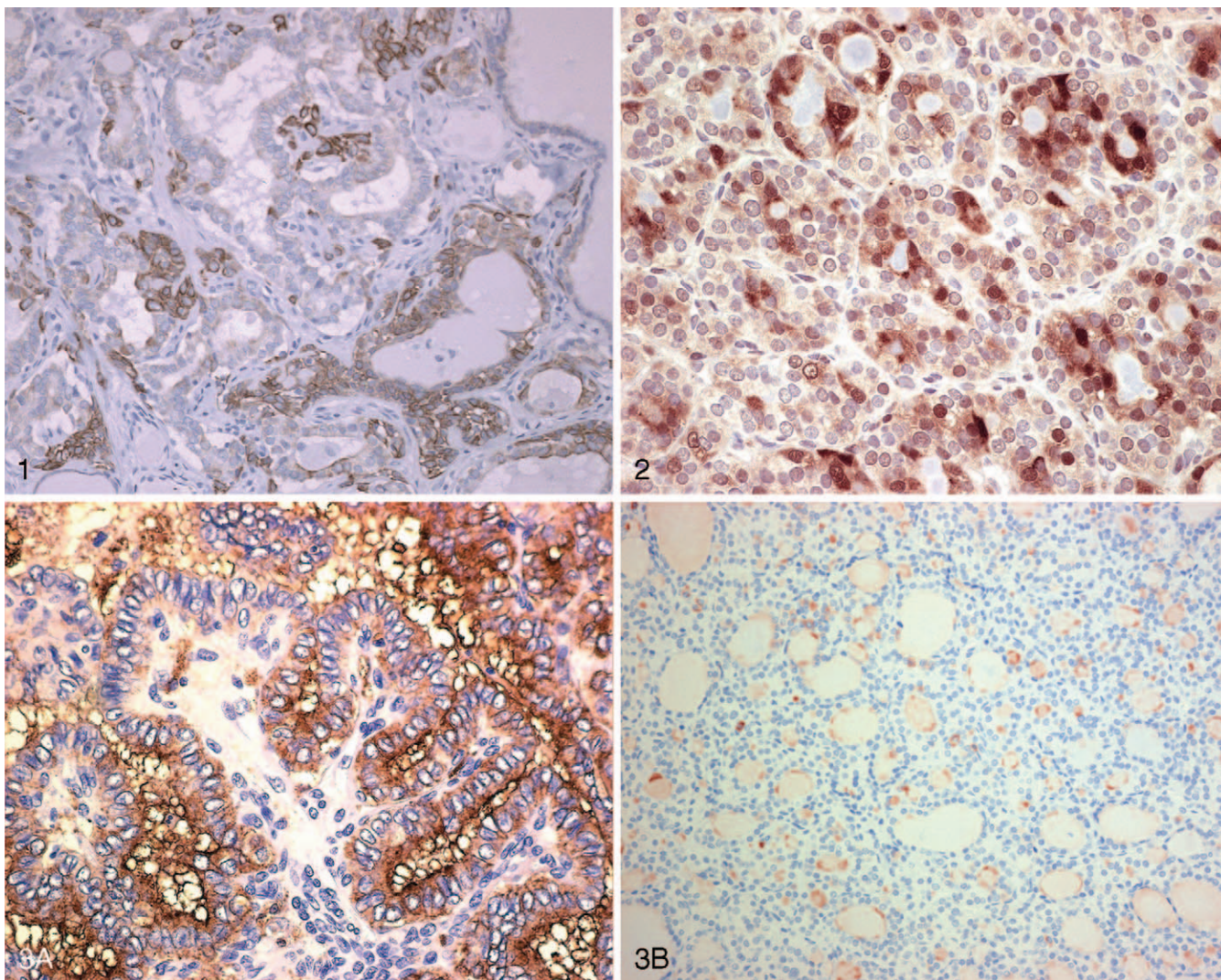
Although *RET* expression is a valuable diagnostic tool for PTC, it has no value as a predictive marker. A relatively low prevalence of positive *RET* staining has been reported in poorly differentiated and anaplastic thyroid carcinomas.<sup>36,45</sup> This is consistent with the biology of the rearrangements that rely on promoters of genes expressed by differentiated thyroid follicular cells; dedifferentiation results in down-regulation of expression and loss of *RET/PTC* gene expression by RT-PCR as well as protein expression by immunohistochemistry.

Although close correlation between RT-PCR detection of *RET/PTC* and immunohistochemical detection of *RET* protein product has been reported, these results were highly dependent on the availability of appropriate antisera; however, availability has been inconsistent, and therefore molecular testing appears to be superior to immunohistochemistry to identify these rearrangements.<sup>10,44,46</sup> Currently a number of monoclonal antibodies directed against the *RET* carboxy-terminus are commercially available, but most fail to reproduce the diffuse cytoplasmic positivity observed with previous polyclonal antisera that are no longer commercially available.<sup>44,47</sup>

## CYTOKERATIN 19

Different subtypes of keratin filaments are grouped according to molecular weight. High-molecular-weight CKs (CK1, CK4, CK10, and CK13) are detected in stratified squamous epithelium. Simple or glandular epithelium expresses CK7, CK8, CK18, and CK19. The thyroid gland has been extensively studied with various antibodies to CKs in an attempt to identify differential expression patterns in normal parenchyma, benign nodules, and malignant tumors. Papillary carcinomas have been shown to express strong and diffuse immunoreactivity for CK7, CK18, and CK19 in 80% to 100% of cases.<sup>45,48</sup> High-molecular-weight CK using the antibody 34βE12 has also been demonstrated in cases of PTC.<sup>49,50</sup> Similar but less intense staining patterns are seen in follicular variant PTC using CK17 and CK20 antibodies.<sup>51</sup> The expression of CK7, CK18, and CK19 has been shown to be less frequent in cases of poorly differentiated carcinomas (60%, 60%, and 40%, respectively).<sup>48</sup> Squamoid and giant cell–solid epithelioid variants of anaplastic thyroid carcinoma frequently express CK7, CK8, and CK18.<sup>52</sup> In contrast, these keratins and CK19 are rarely detected in the spindle cell sarcomatoid variant of anaplastic thyroid carcinoma.

Because CK19 detection in follicular adenomas and follicular carcinomas is often less intense and more focal than in PTC, this keratin has become one of the most commonly used to investigate thyroid lesions.<sup>44,45,51,53</sup> Several authors emphasize the importance of the distribution and intensity of CK19 staining as the most critical aspects of accurate interpretation.<sup>26,45,53,54</sup> Normal thyroid follicular epithelium is often negative, although focal staining for CK19 is usually identified in the compressed thyroid parenchyma surrounding nodules and in follicular cells within lymphocytic thyroiditis.<sup>51</sup> This pattern of staining is consistent with the intense pattern of staining seen in reactive follicular epithelium within thyroid nodules around the site of degeneration, especially at the site of a previous needle biopsy. However, the finely dispersed positivity seen in the cells of PTC is distinctive (Figure 1). Although this feature is usually diffuse throughout the lesion, focal staining for CK19 does not rule out a diag-



**Figure 1.** Immunostaining for cytokeratin 19 in a follicular lesion of thyroid demonstrates a diffuse cytoplasmic reaction in tumor cells. The intensity increases in invasive cells compared with those lining follicles. The staining is not dark and intense as is focally seen in reactive epithelium. This feature is characteristic of a subset of papillary thyroid carcinomas (original magnification  $\times 300$ ).

**Figure 2.** Galectin-3 is overexpressed in thyroid malignancies, where it is localized to both cytoplasm and nuclei (original magnification  $\times 400$ ).

**Figure 3.** A, Papillary and follicular carcinomas exhibit strong staining for Hectort Battifora mesothelial (cell) 1 (HBME-1) that can be intensely positive with diffuse cytoplasmic reaction and focal apical intensification of the reaction (original magnification  $\times 400$ ). B, In some lesions the staining for HBME-1 can be more subtle with predominant apical reactivity that can be misinterpreted as nonspecific reaction of colloid (original magnification  $\times 200$ ).

nosis of PTC, particularly in nodules with nuclear features of PTC that are seen focally.<sup>53</sup>

CK7 and CK18 have been detected in a high percentage of so-called HTAs.<sup>50</sup> Variable expression of CK19 (50%–100%) in HTA has been interpreted by some investigators as proof that these tumors are benign, whereas others use this as evidence that they should be classified as a variant of papillary carcinoma.<sup>50,55</sup> Medullary carcinomas have been reported to express strong positive staining for CK7 and CK18 in 77% of cases and only focal staining for CK19 in 69% of lesions.<sup>48</sup>

CK19 has also been considered by many investigators to be a useful ancillary tool for the diagnosis of papillary carcinoma in FNAC, especially in cytologically suggestive but indeterminate cases.<sup>7,56,57</sup> The reported sensitivity and specificity using CK19 as a single marker is as high as 92% and 97%, respectively.<sup>57</sup> In addition, stronger reactivity

has been reported in methanol-fixed thin-layer preparations. The use of CK19 immunolocalization in cell block preparation of thyroid aspirates has also been reported to aid in accurate diagnosis of malignancy in cytologically equivocal cases of PTC.<sup>56</sup> A panel of markers including CK19 and galectin-3 was reported as reaching 100% of both specificity and sensitivity in the management of thyroid lesions with a cytologic diagnosis of follicular oncocyctic tumors.<sup>7</sup> Given the well-known positivity of CK19 in reactive atypia and chronic lymphocytic thyroiditis that can mimic PTC, the use of CK19 on cytologic specimens should be interpreted with great caution, and many diagnosticians, the authors included, do not endorse this application.

### GALECTIN-3

Galectin-3 (31-kd molecular weight) is one of the members of a family of non-integrin  $\beta$ -galactoside-binding lec-

**Table 1. Immunohistochemical Detection of Galectin-3 in Thyroid Surgical Specimens\***

	Fernandez et al <sup>58</sup>	Herrmann et al <sup>60</sup>	Kovacs et al <sup>61</sup>	Weber et al <sup>65</sup>	Prasad et al <sup>67</sup>	Oestreicher-Kedem et al <sup>68</sup>	Cvejic et al <sup>70</sup>	Bartolazzi et al <sup>11</sup>	Saggiorato et al <sup>7</sup>
Normal	0 (0)	...	...	...	0 (0)	...	...	0/75 (0)	...
CLT	...	...	7/7 (100)	...	...	...	...	2/4 (50)	...
NH	0 (0)	0 (0)	...	...	16/29 (55)	...	...	0/50 (0)	...
FA	0 (0)	3/8 (37)	4/19 (21)	4/13 (31)	2/21 (9)	7/15 (47)	...	5/132 (4)	3/50 (6)
PTC	18/18 (100)	22/34 (64)	19/20 (95)	22/24 (92)	63/67 (94)	15/18 (83)	169/202 (84)	195/201 (97)	39/39 (100)
FTC	4/8 (50)	2/3 (67)	7/10 (70)	4/9 (44)	4/6 (67)	7/11 (64)	...	54/57 (95)	16/19 (84)
OVPFTC	...	5/8 (62)	...	...	...	...	...	13/13 (100)	16/17 (94)
PDTC	2/3 (67)	...	...	...	...	...	...	13/20 (65)	...
ATC	5/5 (100)	...	...	...	4/4 (100)	...	...	18/20 (90)	...

\* Values are number/total number (percent). Ellipses indicate not addressed in this article; CLT, chronic lymphocytic thyroiditis; NH, nodular hyperplasia; FA, follicular adenoma; PTC, papillary thyroid carcinoma; FTC, follicular thyroid carcinoma; OVPFTC, oncocytic variant of papillary and follicular thyroid carcinoma; PDTC, poorly differentiated thyroid carcinoma; and ATC, anaplastic thyroid carcinoma.

tins that have related amino acid sequences in the carbohydrate binding site. Galectin-3 has affinity for CEA, immunoglobulin (Ig) E, laminin, and other mucins. It is expressed by human macrophages and neutrophils, mast cells, and Langerhans cells. Galectin-3 is involved in several biologic and pathologic processes including cell cycle and apoptosis, cell-cell and cell-matrix interaction, adhesion, and migration. It is also believed to have a role in inflammation and cell damage repair, neoplastic transformation, and metastasis.<sup>58-61</sup> Galectin-3 is down-regulated in colorectal and breast cancer.<sup>62,63</sup> In the thyroid, several reports have shown that galectin-3 is overexpressed in malignant tumors (Figure 2).<sup>58-61,64-70</sup> Some of the reported frequencies of galectin-3 detection in different thyroid lesions are summarized in Table 1.

Galectin-3 shows strong diffuse cytoplasmic staining in most cases of PTC, including the classical and follicular variant.<sup>11,58,60,61,65,67,70</sup> In addition, solid variant PTC was reported to stain for galectin-3 in 23 of 46 cases.<sup>70</sup> Forty-five percent to 95% (mean, 65%) of follicular carcinomas with minimal and extensive invasion express galectin-3.<sup>58,60,61,65,67,68</sup> Most poorly differentiated and anaplastic thyroid carcinomas also demonstrate galectin-3 by immunohistochemistry.<sup>11,58,71</sup> Sporadic medullary carcinomas have shown variable expression of galectin-3 ranging from 45% to 80% of cases.<sup>11,58,61,64</sup> Galectin-3 is less often detected in follicular adenomas (0%–37.5%; mean, 21.84%).<sup>11,58,60,61,65,67,68</sup> Hyperplastic nodules, nodular goiters, and normal follicular epithelium usually show absence of galectin-3.<sup>11,58,60</sup> One study reported a high number of galectin-3–positive hyperplastic nodules (16/29).<sup>67</sup> Interesting, galectin-3 expression level significantly increases with the degree of vascular or capsular invasion of follicular tumors.<sup>69</sup> In addition, galectin-3 has been studied in follicular-patterned lesions with uncertain malignant potential in which it was detected in 60% to 85% of cases.<sup>11,66</sup>

Positive staining is often identified in follicular epithelium affected by chronic lymphocytic thyroiditis.<sup>11,60,61</sup> Oncocytic lesions including follicular adenoma and oncocytic variant of PTC and FTC express galectin-3 in 0% to 83% (mean, 32.4%) and 65% to 100% (mean, 85.6%), respectively.<sup>7,11,68</sup>

Although FNAC is a quick, inexpensive, safe, and effective tool for the management of thyroid nodules, it has well-known limitations in separating benign follicular lesions from well-differentiated carcinomas. Galectin-3 detection by immunohistochemistry has been used to improve diagnostic accuracy in preoperative evaluation of thyroid nodules.<sup>7,11,27,72,73</sup> In a large multicenter study with

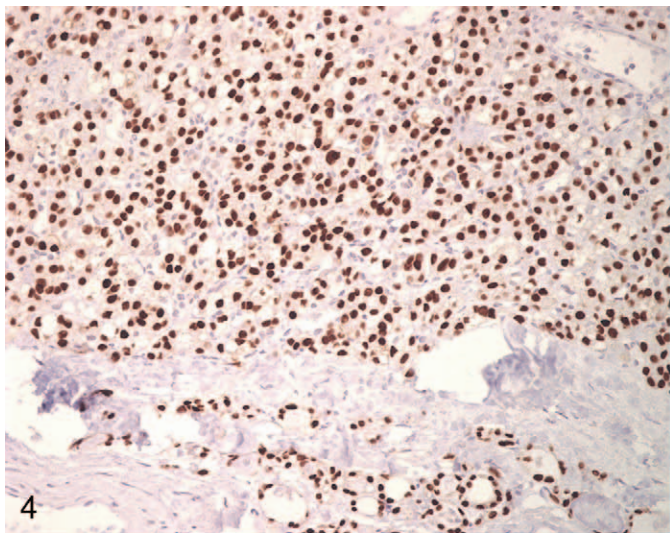
226 specimens of thyroid nodules obtained preoperatively by ultrasound-guided FNA (188 benign lesions and 34 carcinomas), the sensitivity, specificity, positive predictive value, and diagnostic accuracy of galectin-3 immunodetection were 100%, 98%, 92%, and 99%, respectively.<sup>11</sup> In addition, in another study of 125 thyroid aspirates (50 follicular adenomas, 33 follicular carcinomas, and 42 papillary carcinomas) the sensitivity, specificity, positive predictive value, and diagnostic accuracy of galectin-3 as a single marker in discriminating benign from malignant lesions were 92%, 94%, 95.8%, and 92.8%, respectively.<sup>33</sup> In contrast, a lower specificity (52%) was reported in a smaller series in which 11 of 44 follicular adenomas (and 31/35 follicular carcinomas) exhibited positive staining for galectin-3.<sup>73</sup>

It is evident from these data that galectin-3 may be a helpful tool for classical PTC, but it cannot be considered a diagnostic marker of malignancy. Caution has to be taken when interpreting positive results in the absence of unequivocal morphologic features of PTC because of the possibility of false positives. False-negative results in unconventional forms of PTC should also be considered when evaluating unconventional forms of PTC.

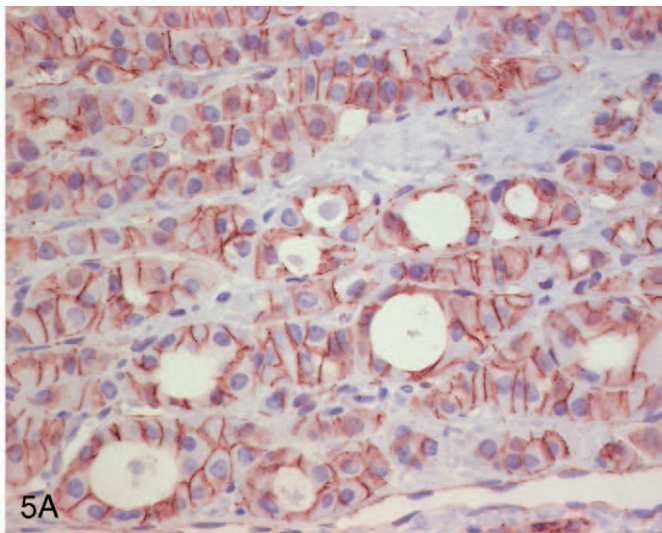
### HBME-1

HBME-1 is a monoclonal antibody that recognizes an unknown antigen in the microvilli of mesothelioma cells, normal tracheal epithelium, and adenocarcinoma of the lung, pancreas, and breast.<sup>74,75</sup> HBME-1 has also been reported by several investigators to be a useful marker of malignancy in thyroid nodules.<sup>5,7,10,27,45,49,66,67,75</sup> Overall in the thyroid, HBME-1 stains mostly follicular-derived malignant tumors, including well-differentiated and poorly differentiated carcinomas, with a variable sensitivity and specificity in different series. Interestingly, membranous and apical-colloidal immunoreactivity for HBME-1 (Figure 3, A and B) have been reported in follicular carcinomas with *RAS* mutations, either minimally or widely invasive.<sup>76</sup> In the same study, follicular carcinomas with PAX8–peroxisome proliferator-activated receptor  $\gamma$  (PPAR $\gamma$ ) rearrangements showed immunoreactivity for galectin-3 but not for HBME-1.<sup>76</sup> A summary of the reported detection of HBME-1 in thyroid nodules by immunohistochemistry is presented in Table 2.

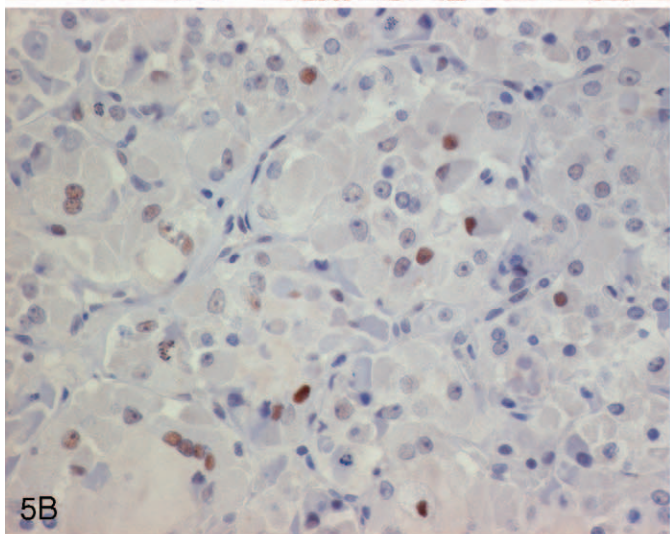
Most papillary carcinomas show diffuse positive staining for HBME-1 (55%–100%; mean, 88%).<sup>7,45,49,66,67,75</sup> One study has reported a sensitivity of 70% and 45% for the classical and follicular variant of PTC, respectively.<sup>45</sup> HBME-1 detection in follicular carcinomas in different



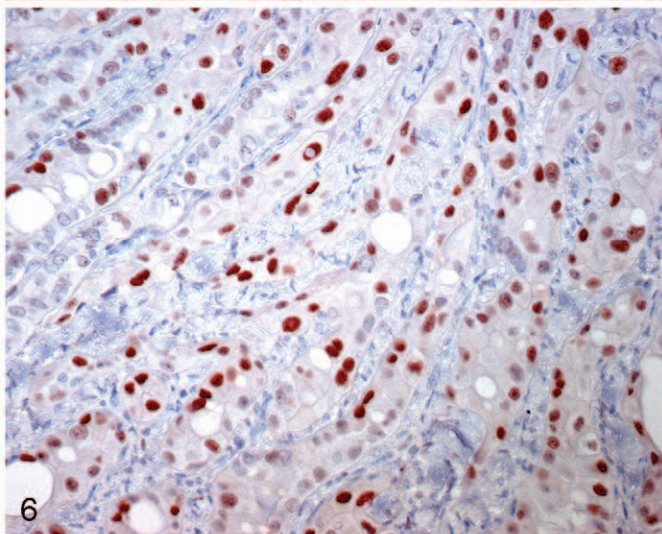
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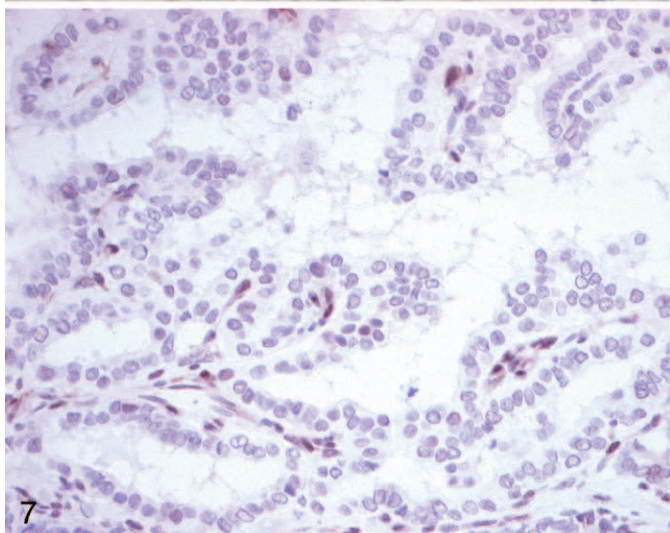
5A



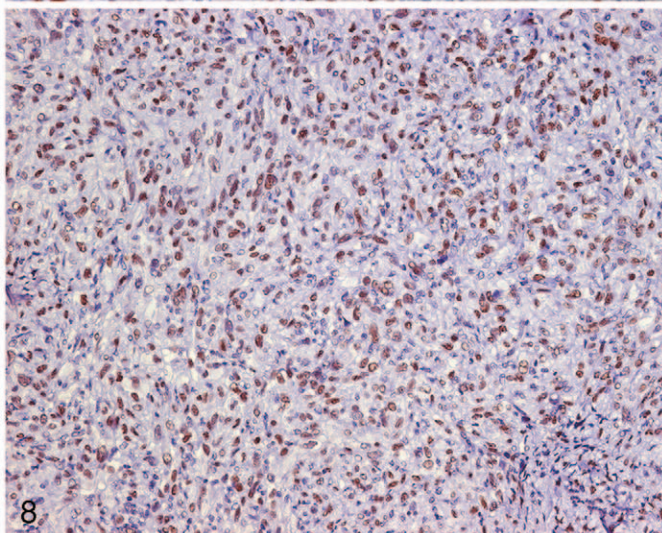
5B



6



7



8

**Figure 4.** Nuclear reactivity for peroxisome proliferator-activated receptor  $\gamma$  is almost undetectable in most thyroid follicular epithelial cells. In this invasive malignancy that harbors a  $t(2;3)(q13;p25)$  rearrangement, there is marked up-regulation of the protein product that can be identified by immunostaining (original magnification  $\times 300$ ).

**Figure 5.** Well-differentiated thyroid cells exhibit a discrete membranous staining pattern of  $\beta$ -catenin (A), whereas poorly differentiated malignancies that demonstrate dyscohesive growth lose the membrane staining pattern and instead demonstrate nuclear translocation of that protein (B) (original magnification  $\times 400$ ).

**Figure 6.** A papillary carcinoma that exhibits abundant nuclear reactivity for cyclin D1 is likely to be an aggressive tumor with great potential for metastatic spread (original magnification  $\times 400$ ).

**Table 2. Immunohistochemical Detection of Hector Battifora Mesothelial (Cell) 1 (HBME-1) in Thyroid Surgical Specimens\***

	Cheung et al <sup>45</sup>	Mase et al <sup>75</sup>	Prasad et al <sup>67</sup>	Choi et al <sup>49</sup>	Papotti et al <sup>66</sup>	Saggiolato et al <sup>7</sup>	Nikiforova et al <sup>76</sup>
Normal	...	...	0/59 (0)	...	...	...	...
NH	0/35 (0)	8/62 (12)	1/29 (3)	...	...	...	...
FA	0/35 (0)	17/62 (27)	2/21 (10)	...	1/15 (6.6)	2/50 (4)	3/23 (13)
PTC	76/138 (55)	35/36 (97.2)	57/67 (85)	65/67 (97)	14/14 (100)	37/39 (94.8)	...
FTC	2/4 (50)	33/39 (84.6)	3/6 (50)	30/30 (100)	...	17/19 (89.5)	11/33 (34)
OVPFTC	2/7 (29)	...	1/8 (13)	4/6 (66.6)	...	6/17 (39)	...
PDTC	4/6 (67)	...	...	11/12 (91.6)	...	...	...
ATC	1/2 (50)	0/2 (0)	...	2/10 (20)	...	...	...

\* Values are number/total number (percent). Ellipses indicate not addressed in this article; NH, nodular hyperplasia; FA, follicular adenoma; PTC, papillary thyroid carcinoma; FTC, follicular thyroid carcinoma; OVPFTC, oncocytic variant of papillary and follicular thyroid carcinoma; PDTC, poorly differentiated thyroid carcinoma; and ATC, anaplastic thyroid carcinoma.

studies has varied between 50% and 100% (mean, 75%).<sup>7,45,49,67,75</sup> Small series of oncocytic variant of papillary and follicular carcinoma have demonstrated positive HBME-1 staining in 13% to 66% of cases.<sup>7,45,49,67</sup> Poorly differentiated and anaplastic carcinomas also often express HBME-1 (67%–91% and 0%–50%, respectively).<sup>45,49,75</sup> A few studies have shown HBME-1 staining in follicular tumors of uncertain malignant potential that had either questionable vascular/capsular invasion or incomplete nuclear features of PTC, in 29% to 66% of cases.<sup>66,67</sup> Many of the reports in which HBME-1 was also studied in normal and hyperplastic thyroid demonstrated absence of this marker.<sup>45,67</sup> However, HBME-1 has been detected in cases of adenomatous goiter (3%–12%), and follicular adenomas (0%–27%).<sup>7,45,66,67,75</sup> Given the high specificity of this marker for malignancy in many series, and the known observer variability in the diagnosis of nodular goiter and adenoma, one wonders if these cases with HBME-1 immunoreactivity would have been considered malignant by some experts.

HBME-1 detection has been considered by some authors as a useful adjunct in the assessment of thyroid lesions by FNAC.<sup>7,27</sup> The sensitivity, specificity, positive predictive value, and diagnostic accuracy of HBME-1 as a single marker, in discriminating benign from malignant lesions, is 80%, 96%, 96.7%, and 86.4%, respectively.<sup>7</sup> In the same study, HBME-1 was shown to be less accurate for the evaluation of samples with oncocytic cytology.

## CYCLO-OXYGENASE 2

Cyclo-oxygenase (COX) or prostaglandin H synthase is involved in the formation of prostaglandins from arachidonic acid. COX-2 expression is induced by cytokines, hormones, inflammatory mediators, and mitogens.<sup>77</sup> Several reports have shown COX-2 up-regulation in human cancers, including tumors of the lung, prostate, breast, colon, and pancreas.<sup>78–80</sup> COX-2 promotes tumorigenesis by stimulation of cell growth, by induction of angiogenesis, and as an inhibitor of apoptosis. Recently, COX-2 has been detected in the thyroid, although the expression varies widely in tumors.<sup>81–86</sup> Papillary and follicular carcinomas of the thyroid show a significantly higher perinuclear cytoplasmic immunoreactivity, compared with normal follicular cells and follicular adenomas.<sup>83,84</sup> The sensitivity for PTC and FTC in different series has ranged between 70% to 90% and 26% to 93%, respectively.<sup>81,82,85,86</sup> In one study, a significantly higher percentage of papillary microcarcinomas expressed COX-2 in comparison with all PTC.<sup>82</sup> In another study, COX-2 expression was reduced in tumors associated with older age, larger size, advanced stage, satellite tumors, and scirrhous or trabecular patterns of growth.<sup>81</sup> These findings may indicate up-regulation of COX-2 in early phases of tumor progression. COX-2 has also been reported in poorly differentiated and anaplastic carcinomas and medullary carcinomas but was not detected in nonneoplastic C cells.<sup>81,82,87</sup> Follicular adenomas variably express COX-2 (0%–28%), and it seems to be absent in hyperplastic nodules.<sup>81,82,85,88</sup> Follicular cells in cases of Hashimoto thyroiditis stain positively for COX-2, and weak immunoreactivity has been reported in up to 17% of normal thyroid tissue samples.<sup>82,89</sup> Thus, COX-2 appears to be a novel biologic marker that can shed insight into tumorigenic mechanisms, but it does not seem to be valuable as a diagnostic marker. Its potential for prognostication remains to be proven because loss in a differentiated tumor may have significance.

**PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR  $\gamma$**

PAX8-PPAR $\gamma$ 1 rearrangements were initially reported in a subset of angioinvasive follicular carcinomas as a result of the translocation t(2;3)(q13;p25), which leads to fusion of the DNA-binding domains of the thyroid transcription factor PAX8 to domains A to F of the PPAR $\gamma$ 1.<sup>90</sup> In the same study, PAX8-PPAR $\gamma$ 1 messenger RNA (mRNA) and protein were absent in hyperplastic nodules, follicular adenomas, and papillary carcinomas, suggesting that PAX8-PPAR $\gamma$ 1 might be useful in the assessment of FTC. Since then, several studies have confirmed the presence of PAX8-PPAR $\gamma$ 1 expression by RT-PCR and strong nuclear staining by immunohistochemistry (Figure 4) in 53% to 69% of follicular carcinomas, as well as in a small number of papillary carcinomas, and its absence in nodular hyperplasia.<sup>91–94</sup> Some studies have confirmed an association with vascular invasion.<sup>76,95</sup> However, PAX8-PPAR $\gamma$ 1

**Figure 7.** Papillary carcinomas usually have weak but detectable p27 nuclear reactivity. Loss of this staining, with retained stromal positivity as an internal control, indicates a lesion with a higher likelihood of lymph node metastasis (original magnification  $\times 400$ ).

**Figure 8.** Anaplastic thyroid carcinoma has a high incidence of p53 mutation that is reflected in the presence of diffuse nuclear positivity because of stabilization of the inactive protein (original magnification  $\times 200$ ).

mRNA and protein have been demonstrated in 8% to 27% of follicular adenomas, suggesting that this marker might not be suitable to separate follicular adenoma from follicular carcinoma.<sup>91–94</sup> In addition, moderate to strong immunoreactivity for PPAR $\gamma$  has been detected in nonlesional surrounding tissue associated with chronic lymphocytic thyroiditis.<sup>93</sup> Thus, the value of this marker remains unproven. However, the data accumulating suggest that, in known malignancies, the presence of PPAR $\gamma$  up-regulation may predict a worse prognosis.

### E-CADHERIN

Cadherins are cell-cell adhesion molecules involved in the morphogenesis of developing tissues and maintenance of adult solid tissues. Cadherins have an intracellular domain that binds to catenins. Loss of cell-cell adhesion is a major cancer hallmark that correlates with loss of differentiation and aggressive tumor behavior. Normal thyroid follicular cells express uniformly high levels of E-cadherin mRNA and have a strong cell surface pattern of staining.<sup>96</sup> E-cadherin staining is variably reduced in well-differentiated thyroid carcinomas and frequently absent in poorly differentiated and anaplastic carcinomas.<sup>97–99</sup> Loss of E-cadherin expression is an adverse prognostic factor in differentiated thyroid carcinomas.<sup>96,97</sup>

### $\beta$ -CATENIN

$\beta$ -Catenin is a 94-kd protein that forms cytoplasmic/membranous-bound complexes with E-cadherin and is involved in the assembly of the zonula adherens.<sup>98</sup> When activated, it translocates to the nucleus where it promotes tumor growth through activation of the *wnt*-signaling pathway.<sup>5</sup> Strong membranous immunoreactivity with minimal  $\beta$ -catenin cytoplasmic staining is observed in normal follicular cells.<sup>45,100</sup> Thyroid tumors express different patterns of immunoreactivity, including strong to weak membranous positivity reported in follicular adenomas and well-differentiated thyroid carcinomas (Figure 5, A) and loss of membranous staining with nuclear and cytoplasmic staining in poorly differentiated and anaplastic carcinomas (Figure 5, B). Along with E-cadherin, loss of membrane  $\beta$ -catenin immunostaining is an indicator of loss of differentiation and adverse prognosis. Aberrant nuclear immunoreactivity of  $\beta$ -catenin is associated with stabilizing *CTNNB1* exon 3 mutations that are found almost exclusively in poorly differentiated and anaplastic carcinomas.<sup>100</sup> The cribriform-morular variant of PTC, which is pathognomonic of familial adenomatous polyposis-associated thyroid carcinoma, has been reported to demonstrate cytoplasmic and nuclear accumulation of  $\beta$ -catenin and *CTNNB1* exon 3 mutations.<sup>101,102</sup>

### FIBRONECTIN-1

Fibronectins are multifunctional adhesive glycoproteins found in the extracellular matrix and body fluids. They have affinity for collagen, fibrin, heparin, and cell surfaces and are involved in various biologic processes including cell adhesion, migration, and tumor progression. Plasma FN is produced by adult hepatocytes. Oncofetal FNs are cellular isoforms containing the extracellular domain A or B and III connecting segments. Oncofetal FNs are highly expressed in fetal and neoplastic tissues, including thyroid follicular cell-derived tumors.<sup>67,103–106</sup> Oncofetal FN has been proposed as a potential useful adjunct for preoperative diagnosis of thyroid nodules, based on findings of

initial reports demonstrating a restricted expression of oncofetal FN mRNA in papillary and anaplastic carcinomas, compared with normal thyroid tissues, hyperplastic lesions, follicular adenomas, and carcinomas, using an RT-PCR based approach.<sup>106–109</sup> However, other groups have identified oncofetal FN in chronic lymphocytic thyroiditis, follicular adenomas, and follicular and medullary carcinomas.<sup>67,105</sup>

Fibronectin emerged as a potential marker of thyroid carcinoma in microarray studies in which it was reported to be up-regulated compared with normal tissue.<sup>110–112</sup> An immunohistochemical panel consisting of FN, galectin-3, and HBME-1 has been reported to be effective in the diagnosis of follicular cell-derived thyroid tumors.<sup>67</sup> However, FN expression is reduced in more aggressive cancers and in the invasive components of differentiated carcinomas.<sup>104,113</sup> In fact, modulation to up-regulate FN may represent a therapeutic target to prevent invasion and metastasis in thyroid carcinoma.<sup>114</sup>

Fibronectin immunoreactivity is also observed in extracellular fibrosis in goiters; cytoplasmic and membranous staining is seen in reactive follicular cells associated with hemorrhage and fibrin deposition.<sup>67</sup> These findings may explain false-positive fine-needle aspirates examined by molecular-based studies because of contamination with fibroblasts and reactive follicular cells.<sup>115</sup>

### CD44v

CD44, also known as extracellular matrix receptor III, hyaluronate receptor, and heparan sulfate proteoglycan, is a family of immunologically related integral membrane glycoproteins that bind hyaluronic acid. CD44 mediates cell-cell and cell-matrix interactions through its affinity for hyaluronic acid and possibly also through its affinity for other ligands, such as osteopontin, collagens, and matrix metalloproteinases. Adhesion with hyaluronic acid plays an important role in cell migration, tumor growth, and progression. CD44 family members are highly polymorphic because of numerous alternative splicing and post-translational modification events. Isoforms sharing CD44 variant exon 6 are known to confer tumor invasiveness and metastatic potential in malignant rat cell lines.<sup>116–118</sup> CD44v6 has been comparatively assessed on thyroid lesions by immunohistochemistry in surgical specimens and fine-needle aspirates.<sup>11,73,119</sup> Intense membrane staining has been demonstrated in benign lesions, including hyperplastic nodules (40%) and follicular adenomas (30%–43%).<sup>11,73</sup> Well-differentiated papillary and follicular carcinomas show the highest immunoreactivity for CD44v6 (75%–90% and 90%–100%, respectively).<sup>11,73,119</sup> It has also been detected in poorly differentiated and anaplastic carcinomas, oncocytic variant follicular and papillary carcinomas, and medullary carcinoma.<sup>11</sup> These findings suggest that CD44v6 may be associated with deregulated follicular proliferation, rather than malignant transformation, and should not be used as a single marker to discriminate benign from malignant thyroid lesions.

### THYROID PEROXIDASE

Thyroid peroxidase (TPO) is a thyroid-specific enzyme involved in the synthesis of thyroid hormone. Gene suppression and mutations of the *TPO* gene were reported in some differentiated thyroid carcinomas.<sup>120,121</sup> Reduced TPO immunoreactivity has been proposed by some investigators as a marker to distinguish benign from malignant

thyroid tumors at preoperative assessment of thyroid nodules by FNAC.<sup>120,122–125</sup> In these studies, the absence of TPO immunoreactivity in more than 80% of cells could accurately demonstrate malignancy with sensitivities between 97% and 100% and specificities ranging from 68% to 90%. More recently, these numbers have been confirmed by other investigators as applicable to papillary but not to follicular carcinomas.<sup>65,126,127</sup> The reason for this discrepancy is a high overlap of TPO immunoreactivity patterns between follicular adenomas and carcinomas. In addition, preserved immunoreactivity for TPO, as occurring in follicular carcinomas, does not rule out malignancy. The use of TPO in combination with other immunohistochemical markers has been reported as useful for the diagnosis of papillary carcinoma in surgical thyroid specimens.<sup>65</sup>

### Cbp/p300-INTERACTING TRANSACTIVATOR 1

Cbp/p300-interacting transactivator 1 (CITED1), which is also known as melanocyte-specific protein 1, is a nuclear protein involved in coregulation of transcription factors. CITED1 has been detected in the nucleus and cytoplasm of melanocytes, epithelial breast cells, and testicular germ cells. CITED1 gene overexpression has been demonstrated in papillary carcinomas of the thyroid by complementary DNA microarray analysis and RT-PCR.<sup>110–112</sup> In addition, immunohistochemical studies have shown CITED1 reactivity in 87% to 93% of papillary carcinomas (all subtypes), 0% to 50% of follicular carcinomas, 10% to 16% of follicular adenomas, and 24% of nodular goiters.<sup>67,111,112,128</sup> These findings indicate that it lacks specificity as a diagnostic modality, but some authors maintain that when used in combination with other antibodies including HBME-1, galectin-3, CK19, and FN, CITED1 may be helpful in the diagnosis of papillary carcinoma.<sup>67,128</sup>

### CYCLIN D1

The *CCND1* gene located on chromosome 11q23 encodes a nuclear protein that forms complexes with cyclin-dependent kinases 4 and 6, resulting in phosphorylation and inactivation of the retinoblastoma protein, allowing cell cycle progression from G<sub>1</sub> to S phase. Normal thyroid follicular cells do not show immunoreactivity for cyclin D1. Cyclin D1 is a target for  $\beta$ -catenin, and cyclin D1 overexpression is associated with  $\beta$ -catenin alterations in thyroid cancer progression.<sup>129,130</sup>

Increased nuclear expression of cyclin D1 mRNA and protein has been demonstrated in benign and malignant tumors of the thyroid (Figure 6).<sup>131–133</sup> Cyclin D1 overexpression in thyroid papillary microcarcinomas was found to be significantly higher in tumors larger than 5 mm.<sup>130</sup> Malignant tumors exhibit the highest immunoreactivity for cyclin D1, particularly papillary carcinomas, although follicular adenomas have been reported to demonstrate increased expression for cyclin D1.<sup>132,133</sup> Again, these data must be interpreted with caution because of the known interobserver variability in the classification of adenomas and carcinomas.

A significant correlation between cyclin D1 overexpression and the presence of regional lymph node metastasis has been observed in patients with papillary carcinomas, suggesting that immunodetection of cyclin D1 by immunohistochemistry could be a useful prognostic tool to distinguish indolent from metastatic papillary carcinoma.<sup>131,134</sup>

### p27

The tumor suppressor gene *p27/kip1* located on chromosome 12p13 encodes a nuclear cyclin-dependent kinase I that inhibits the formation of cyclin D1/cyclin-dependent kinase complexes during G<sub>0</sub> and early G<sub>1</sub> phases of the cell cycle, thus preventing entry to S phase through activation of retinoblastoma protein. Normal thyroid follicular epithelium shows strong nuclear immunoreactivity for p27.<sup>135,136</sup> In contrast, thyroid tumors have been shown to exhibit decreased reactivity for p27, with significant differences of p27 expression between follicular adenomas and follicular-derived carcinomas.<sup>135</sup> In addition, some investigators have reported a higher immunoreactivity for p27 in the follicular variant of papillary carcinoma, in comparison with classical PTC.<sup>136</sup> In another study, p27 detection by immunohistochemistry was useful in distinguishing papillary hyperplasia of Graves disease from papillary carcinoma.<sup>137</sup>

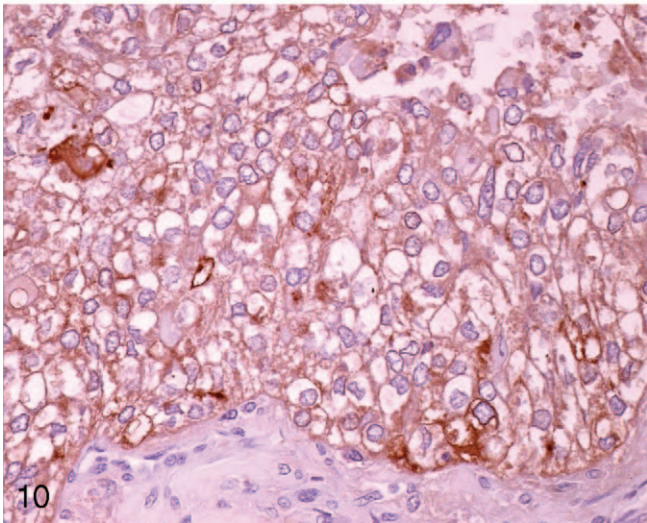
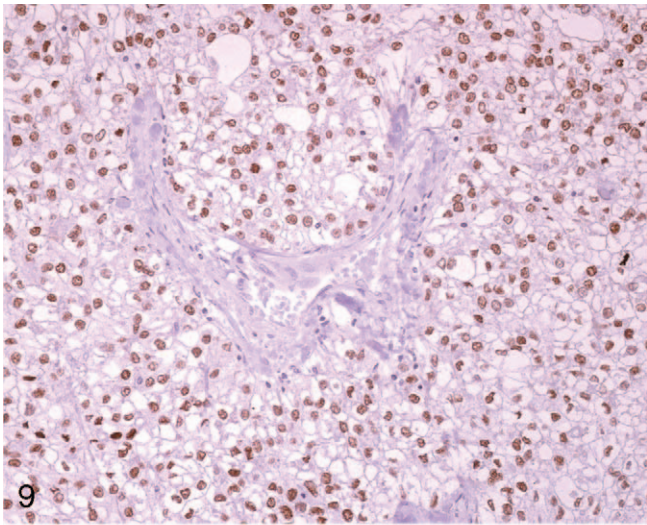
Additionally, metastasizing papillary carcinomas show significant loss of immunoreactivity for p27 compared with those that are not associated with regional lymph node metastasis (Figure 7), providing another potential tool to distinguish indolent from metastatic PTCs.<sup>138,139</sup>

### p53

Mutations of *TP53* represent late genetic events in thyroid carcinogenesis. As a result, accumulation of p53 can be detected by immunohistochemistry most often in anaplastic (Figure 8) and poorly differentiated thyroid carcinomas.<sup>140</sup> Rarely, it can be seen in well-differentiated papillary and follicular carcinomas as well as in medullary carcinomas.<sup>141</sup> Positive immunoreactivity for p53 is an independent prognostic factor for overall survival of patients with thyroid cancer.<sup>142,143</sup>

### THYROID TRANSCRIPTION FACTOR 1

TTF-1 was identified in 1989 as a nuclear tissue-specific protein with DNA-binding activity that interacted with the *TG* gene in the rat.<sup>144</sup> TTF-1 regulates gene expression in the thyroid, lungs, and diencephalon during embryogenesis. Importantly, TTF-1 together with PAX8 control the expression of *TG*, *TPO*, thyrotropin receptor and the sodium/iodide symporter, calcitonin, and major histocompatibility complex class I genes in the thyroid.<sup>144–146</sup> In the lung, TTF-1 regulates the expression of surfactant proteins A, B, and C and Clara cell secretory protein genes.<sup>147–150</sup> Because retained TTF-1 expression is highly specific for thyroid and lung tumors, it has been widely used to discern the primary site of tumor origin in patients with metastatic disease of unknown origin.<sup>151–154</sup> TTF-1 immunoreactivity is detected in pulmonary tumors, including neuroendocrine tumors, and rarely in small cell carcinomas from other sites.<sup>153–156</sup> In the thyroid, nuclear reactivity for TTF-1 is present in follicular cell-derived benign and malignant lesions (Figure 9) and medullary carcinomas.<sup>157–159</sup> Poorly differentiated carcinomas often show decreased and focal staining for TTF-1, and most anaplastic carcinomas lack TTF-1 reactivity. When used in combination with *TG*, TTF-1 is an effective marker for thyroid origin.<sup>158</sup> Lack of TTF-1 immunoreactivity in a thyroid tumor should prompt investigation of other differential diagnoses, including parathyroid tumors, paragangliomas, and metastatic lesions.



**Figure 9.** A clear cell tumor in thyroid can be a primary tumor, an intrathyroidal parathyroid lesion, or a metastasis from lung, kidney, adrenal, or other sites. The identification of strong nuclear thyroid transcription factor 1 indicates either a primary thyroid neoplasm or spread from a lung primary malignancy. In contrast, loss of this immunoreaction should prompt investigation of an alternative primary site (original magnification  $\times 200$ ).

**Figure 10.** The identification of cytoplasmic thyroglobulin is the proof of thyroid origin in a clear cell tumor (original magnification  $\times 400$ ).

### THYROGLOBULIN

Thyroglobulin is the primary product synthesized in the thyroid, and the macromolecular precursor of the iodinated thyroid hormones thyroxine ( $T_4$ ) and triiodothyronine ( $T_3$ ). TG gene expression is coordinately regulated by TTF-1, TTF-2, and PAX8.<sup>160</sup> Thyroglobulin is a reliable tumor marker that can be detected in the serum of patients with residual disease after ablation of the thyroid by surgery and radioiodine and relapse after disease-free interval. Compared with TTF-1, TG is also a highly sensitive histogenetic marker for follicular cell origin (Figure 10), although patchy staining pattern, particularly in the less well-differentiated tumors, may produce less reliable results in small biopsies.<sup>158,161,162</sup> Thyroglobulin is not produced by medullary carcinomas<sup>10</sup>; however, interpretation must be cautious because TG is well known to diffuse through local tissues, resulting in artefactual staining that

can hamper the diagnosis of medullary carcinoma. Interpretation of immunoreactivity in these lesions must recognize the geographic nature of diffusion. Similarly, TG positivity in perithyroidal lymph nodes is not a reliable marker of metastasis without confirmation of CK and/or TTF-1 staining.

### CALCITONIN

Medullary thyroid carcinoma (MTC) and C cells stain positively for calcitonin, a secreted protein produced by parafollicular C cells, which causes a rapid but short-lived drop in the level of calcium and phosphate in blood by promoting the incorporation of those ions in the bones. Calcitonin (Figure 11, A), together with CEA, chromogranin, synaptophysin, and calcitonin gene-related peptide are the most useful immunohistochemical markers for the diagnosis of MTC, especially when facing histologic subtypes such as the follicular, papillary, or encapsulated variants that can pose diagnostic difficulties with follicular cell-derived carcinomas and paraganglioma.<sup>21,163–166</sup> Calcitonin is also a diagnostic marker to confirm C-cell hyperplasia (Figure 11, B), which is usually associated with familial medullary carcinoma.<sup>4,21,167</sup>

Although calcitonin immunoreactivity is highly specific for MTC, the staining pattern may be variable; although usually diffuse, it may be focal, with 25% or less of cells exhibiting cytoplasmic reactivity.<sup>168</sup> Absence of calcitonin reactivity may occur in up to 5% of MTC cases.<sup>10</sup> Alternatively, calcitonin and calcitonin gene-related peptide mRNAs can be demonstrated by in situ hybridization in cases in which conventional immunohistochemistry is not able to detect these markers.<sup>169,170</sup>

### CARCINOEMBRYONIC ANTIGEN

Staining for CEA in thyroid has had historical difficulties because of nonspecific reaction with CEA-like substances. The advent of monoclonal antibodies has obviated the problems, and the use of monoclonal CEA for thyroid diagnosis is highly recommended in specific circumstances. Using these specific antibodies, thyroid follicular cells and tumor derived from them are negative. Thus, the finding of CEA immunoreactivity in a thyroid tumor should prompt the diagnosis of another entity, usually MTC but also other lesions such as thymic-derived lesions or metastatic carcinoma.

Diffuse cytoplasmic staining for CEA can be demonstrated in hyperplastic and neoplastic C cells by immunohistochemistry (Figure 12).<sup>171–173</sup> Carcinoembryonic antigen is a reliable marker for the diagnosis of MTC, with a higher sensitivity than calcitonin. Reports of CEA immunoreactivity in a small number of papillary and follicular carcinomas are almost certainly because of technical or diagnostic error.<sup>21,174</sup> Calcitonin is lost with dedifferentiation of MTC, whereas CEA expression is retained by these lesions; therefore, CEA is particularly helpful in the assessment of medullary carcinomas that lack or present only focal reactivity for calcitonin and should be used in combination with other markers including TG, chromogranin, and synaptophysin.<sup>10,21</sup>

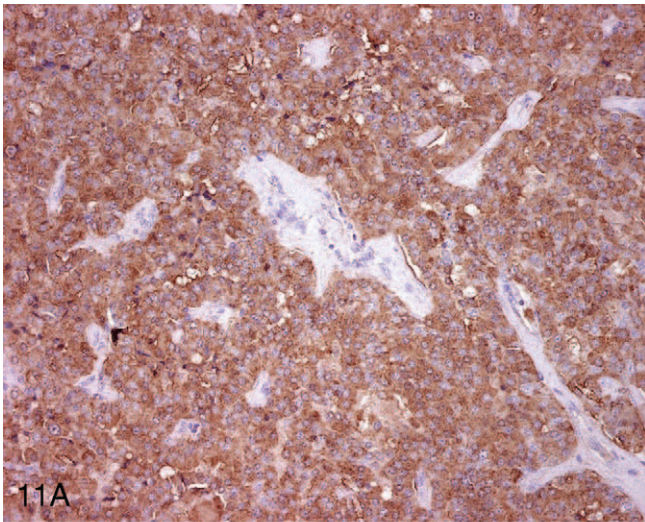
In addition, a wide number of immunohistochemical markers that indicate neuroendocrine differentiation have been shown to be present in medullary carcinoma, including serotonin, somatostatin, adrenocorticotrophic hormone, gastrin-releasing peptide, and others.<sup>175–180</sup>

## CONCLUSIONS

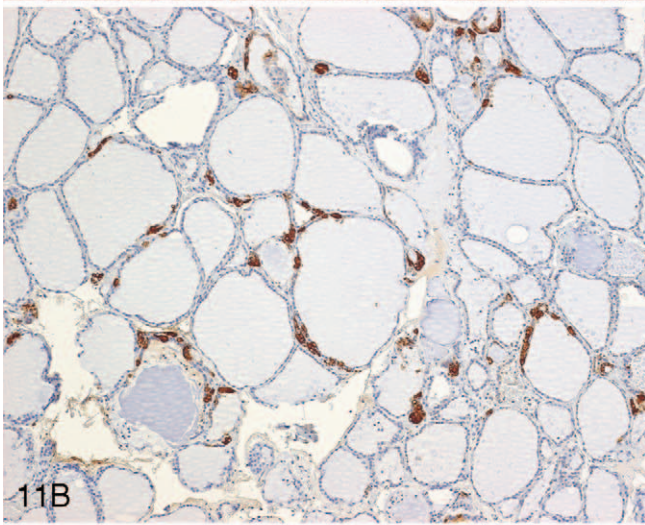
Most of the neoplastic lesions originated from the thyroid gland are diagnosed based on well-characterized histologic features. However, there is a subset of tumors with follicular architecture that lack unequivocal features of malignancy, thus posing difficulties in the distinction of benign and malignant conditions. Some tumors in the thyroid are not derived from follicular thyroid epithelium. In such cases, the use of ancillary techniques including immunohistochemistry and molecular analysis can significantly improve diagnosis. However, a single marker is usually suboptimal in terms of sensitivity and specificity. Panels of 2 or more antibodies usually are more effective and can improve diagnostic accuracy in fine-needle aspirates and paraffin-embedded tissue. Moreover, molecular profiling of tumors is a promising technologic approach that may unravel important novel biomarkers to be incorporated in the management of thyroid lesions.

## References

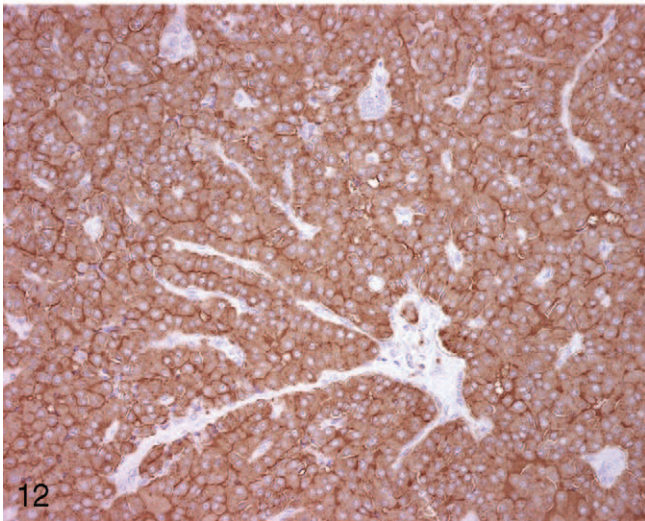
1. Hundahl SA, Fleming ID, Fremgen AM, et al. A National Cancer Data Base report on 53,856 cases of thyroid carcinoma treated in the U.S., 1985–1995. *Cancer*. 1998;83:2638–2648.
2. Liu S, Semenciw R, Ugnat AM, et al. Increasing thyroid cancer incidence in Canada, 1970–1996: time trends and age-period-cohort effects. *Br J Cancer*. 2001;85:1335–1339.
3. Parkin DM, Bray F, Ferlay J, et al. Global cancer statistics, 2002. *CA Cancer J Clin*. 2005;55:74–108.
4. De Lellis RA, Williams ED. Tumors of thyroid and parathyroid. In: DeLellis RA, Lloyd RV, Heitz PU, Eng C, eds. *Pathology and Genetics of Tumours of Endocrine Organs*. Lyon, France: IARC Press; 2004:49–133. *World Health Organization Classification of Tumours*; vol 8.
5. Rezk S, Khan A. Role of immunohistochemistry in the diagnosis and progression of follicular epithelium-derived thyroid carcinoma. *Appl Immunohistochem Mol Morphol*. 2005;13:256–264.
6. Cheung CC, Carydis B, Ezzat S, et al. Analysis of ret/PTC gene rearrangements refines the fine needle aspiration diagnosis of thyroid cancer. *J Clin Endocrinol Metab*. 2001;86:2187–2190.
7. Saggiorato E, De PR, Volante M, et al. Characterization of thyroid “follicular neoplasms” in fine-needle aspiration cytological specimens using a panel of immunohistochemical markers: a proposal for clinical application. *Endocr Relat Cancer*. 2005;12:305–317.
8. Salvatore G, Giannini R, Faviana P, et al. Analysis of BRAF point mutation and RET/PTC rearrangement refines the fine-needle aspiration diagnosis of papillary thyroid carcinoma. *J Clin Endocrinol Metab*. 2004;89:5175–5180.
9. Kondo T, Ezzat S, Asa SL. Pathogenetic mechanisms in thyroid follicular-cell neoplasia. *Nat Rev Cancer*. 2006;6:292–306.
10. Erickson LA, Lloyd RV. Practical markers used in the diagnosis of endocrine tumors. *Adv Anat Pathol*. 2004;11:175–189.
11. Bartolazzi A, Gasbarri A, Papotti M, et al. Application of an immunodiagnostic method for improving preoperative diagnosis of nodular thyroid lesions. *Lancet*. 2001;357:1644–1650.
12. Barden CB, Shister KW, Zhu B, et al. Classification of follicular thyroid tumors by molecular signature: results of gene profiling. *Clin Cancer Res*. 2003;9:1792–1800.
13. Finley DJ, Zhu B, Barden CB, et al. Discrimination of benign and malignant thyroid nodules by molecular profiling. *Ann Surg*. 2004;240:425–436.
14. Giordano TJ, Kuick R, Thomas DG, et al. Molecular classification of papillary thyroid carcinoma: distinct BRAF, RAS, and RET/PTC mutation-specific gene expression profiles discovered by DNA microarray analysis. *Oncogene*. 2005;24:6646–6656.
15. Baloch ZW, LiVolsi VA. Follicular-patterned lesions of the thyroid: the bane of the pathologist. *Am J Clin Pathol*. 2002;117:143–150.
16. Suster S. Thyroid tumors with a follicular growth pattern: problems in differential diagnosis. *Arch Pathol Lab Med*. 2006;130:984–988.
17. LiVolsi VA, Baloch ZW. Follicular neoplasms of the thyroid: view, biases, and experiences. *Adv Anat Pathol*. 2004;11:279–287.
18. Heffess CS, Thompson LD. Minimally invasive follicular thyroid carcinoma. *Endocr Pathol*. 2001;12:417–422.
19. Thompson LD, Wieneke JA, Paal E, et al. A clinicopathologic study of minimally invasive follicular carcinoma of the thyroid gland with a review of the English literature. *Cancer*. 2001;91:505–524.
20. Williams ED. Two proposals regarding the terminology of thyroid tumors. *Int J Surg Pathol*. 2000;8:181–183.
21. Rosai J, Carcangiu ML, DeLellis RA. *Tumors of the Thyroid Gland*. Washington, DC: Armed Forces Institute of Pathology; 1992. *Atlas of Tumor Pathology*; 3rd series, fascicle 5.
22. Castro MR, Gharib H. Continuing controversies in the management of thyroid nodules. *Ann Intern Med*. 2005;142:926–931.



11A



11B



12

**Figure 11.** Calcitonin immunoreactivity identifies C cells in the thyroid. Its presence in a thyroid tumor confirms the diagnosis of medullary thyroid carcinoma (A) (original magnification  $\times 200$ ). It also identifies the increased number of C cells in C-cell hyperplasia (B), a feature that indicates genetic predisposition to medullary thyroid carcinoma (original magnification  $\times 200$ ).

**Figure 12.** As medullary thyroid carcinoma dedifferentiates it can lose calcitonin positivity, but the presence of carcinoembryonic antigen reactivity confirms that diagnosis (original magnification  $\times 200$ ).

23. Rosai J, Kuhn E, Carcangiu ML. Pitfalls in thyroid tumour pathology. *Histopathology*. 2006;49:107–120.
24. Lloyd RV, Erickson LA, Casey MB, et al. Observer variation in the diagnosis of follicular variant of papillary thyroid carcinoma. *Am J Surg Pathol*. 2004;28:1336–1340.
25. Chan JK. Strict criteria should be applied in the diagnosis of encapsulated follicular variant of papillary thyroid carcinoma. *Am J Clin Pathol*. 2002;117:16–18.
26. Asa SL. My approach to oncocytic tumours of the thyroid. *J Clin Pathol*. 2004;57:225–232.
27. Rossi ED, Raffaelli M, Minimo C, et al. Immunocytochemical evaluation of thyroid neoplasms on thin-layer smears from fine-needle aspiration biopsies. *Cancer*. 2005;105:87–95.
28. Tallini G, Asa SL. RET oncogene activation in papillary thyroid carcinoma. *Adv Anat Pathol*. 2001;8:345–354.
29. Jing S, Wen D, Yu Y, et al. GDNF-induced activation of the ret protein tyrosine kinase is mediated by GDNFR- $\alpha$ , a novel receptor for GDNF. *Cell*. 1996;85:1113–1124.
30. Fenton CL, Lukes Y, Nicholson D, Dinauer CA, Francis GL, Tuttle RM. The ret/PTC mutations are common in sporadic papillary thyroid carcinoma of children and young adults. *J Clin Endocrinol Metab*. 2000;85:1170–1175.
31. Bounacer A, Wicker R, Caillou B, et al. High prevalence of activating ret proto-oncogene rearrangements, in thyroid tumors from patients who had received external radiation. *Oncogene*. 1997;15:1263–1273.
32. Asa SL. The role of immunohistochemical markers in the diagnosis of follicular-patterned lesions of the thyroid. *Endocr Pathol*. 2005;16:295–309.
33. Sagartz JE, Jhiang SM, Tong Q, et al. Thyroid-stimulating hormone promotes growth of thyroid carcinomas in transgenic mice with targeted expression of the ret/PTC1 oncogene. *Lab Invest*. 1997;76:307–318.
34. Tallini G, Costa J. Unraveling the pathogenesis of thyroid tumors using transgenic mice. *Lab Invest*. 1997;76:301–305.
35. Fischer AH, Bond JA, Taysavang P, et al. Papillary thyroid carcinoma oncogene (RET/PTC) alters the nuclear envelope and chromatin structure. *Am J Pathol*. 1998;153:1443–1450.
36. Santoro M, Papotti M, Chiappetta G, et al. RET activation and clinicopathologic features in poorly differentiated thyroid tumors. *J Clin Endocrinol Metab*. 2002;87:370–379.
37. Elisei R, Romei C, Viorntsova T, et al. RET/PTC rearrangements in thyroid nodules: studies in irradiated and not irradiated, malignant and benign thyroid lesions in children and adults. *J Clin Endocrinol Metab*. 2001;86:3211–3216.
38. Rhoden KJ, Johnson C, Brandao G, et al. Real-time quantitative RT-PCR identifies distinct c-RET, RET/PTC1 and RET/PTC3 expression patterns in papillary thyroid carcinoma. *Lab Invest*. 2004;84:1557–1570.
39. Fusco A, Chiappetta G, Hui P, et al. Assessment of RET/PTC oncogene activation and clonality in thyroid nodules with incomplete morphological evidence of papillary carcinoma: a search for the early precursors of papillary cancer. *Am J Pathol*. 2002;160:2157–2167.
40. Papotti M, Volante M, Giuliano A, et al. RET/PTC activation in hyalinizing trabecular tumors of the thyroid. *Am J Surg Pathol*. 2000;24:1615–1621.
41. Cheung CC, Boerner SL, MacMillan CM, et al. Hyalinizing trabecular tumor of the thyroid: a variant of papillary carcinoma proved by molecular genetics. *Am J Surg Pathol*. 2000;24:1622–1626.
42. Cheung CC, Ezzat S, Ramyar L, et al. Molecular basis of Hurthle cell papillary thyroid carcinoma. *J Clin Endocrinol Metab*. 2000;85:878–882.
43. Belchetz G, Cheung CC, Freeman JL, et al. Hurthle cell tumors: using molecular techniques to define a novel classification system. *Arch Otolaryngol Head Neck Surg*. 2002;128:237–240.
44. Cerilli LA, Mills SE, Rumpel CA, et al. Interpretation of RET immunostaining in follicular lesions of the thyroid. *Am J Clin Pathol*. 2002;118:186–193.
45. Cheung CC, Ezzat S, Freeman JL, et al. Immunohistochemical diagnosis of papillary thyroid carcinoma. *Mod Pathol*. 2001;14:338–342.
46. Shin E, Hong SW, Kim SH, et al. Expression of down stream molecules of RET (p-ERK, p-p38 MAPK, p-JNK and p-AKT) in papillary thyroid carcinomas. *Yonsei Med J*. 2004;45:306–313.
47. Sugg SL, Ezzat S, Rosen IB, et al. Distinct multiple RET/PTC gene rearrangements in multifocal papillary thyroid neoplasia. *J Clin Endocrinol Metab*. 1998;83:4116–4122.
48. Lam KY, Lui MC, Lo CY. Cytokeratin expression profiles in thyroid carcinomas. *Eur J Surg Oncol*. 2001;27:631–635.
49. Choi YL, Kim MK, Suh JW, et al. Immunorexpression of HBME-1, high molecular weight cytokeratin, cytokeratin 19, thyroid transcription factor-1, and E-cadherin in thyroid carcinomas. *J Korean Med Sci*. 2005;20:853–859.
50. Hirokawa M, Carney JA, Ohtsuki Y. Hyalinizing trabecular adenoma and papillary carcinoma of the thyroid gland express different cytokeratin patterns. *Am J Surg Pathol*. 2000;24:877–881.
51. Baloch ZW, Abraham S, Roberts S, et al. Differential expression of cytokeratins in follicular variant of papillary carcinoma: an immunohistochemical study and its diagnostic utility. *Hum Pathol*. 1999;30:1166–1171.
52. Miettinen M, Franssila KO. Variable expression of keratins and nearly uniform lack of thyroid transcription factor 1 in thyroid anaplastic carcinoma. *Hum Pathol*. 2000;31:1139–1145.
53. Sahoo S, Hoda SA, Rosai J, et al. Cytokeratin 19 immunoreactivity in the diagnosis of papillary thyroid carcinoma: a note of caution. *Am J Clin Pathol*. 2001;116:696–702.
54. Asa SL, Cheung CC. The mind's eye. *Am J Clin Pathol*. 2001;116:635–636.
55. Fonseca E, Nesland JM, Sobrinho-Simoes M. Expression of stratified epithelial-type cytokeratins in hyalinizing trabecular adenomas supports their relationship with papillary carcinomas of the thyroid. *Histopathology*. 1997;31:330–335.
56. Khurana KK, Truong LD, LiVolsi VA, et al. Cytokeratin 19 immunolocalization in cell block preparation of thyroid aspirates: an adjunct to fine-needle aspiration diagnosis of papillary thyroid carcinoma. *Arch Pathol Lab Med*. 2003;127:579–583.
57. Nasser SM, Pitman MB, Pilch BZ, et al. Fine-needle aspiration biopsy of papillary thyroid carcinoma: diagnostic utility of cytokeratin 19 immunostaining. *Cancer*. 2000;90:307–311.
58. Fernandez PL, Merino MJ, Gomez M, et al. Galectin-3 and laminin expression in neoplastic and non-neoplastic thyroid tissue. *J Pathol*. 1997;181:80–86.
59. Kawachi K, Matsushita Y, Yonezawa S, et al. Galectin-3 expression in various thyroid neoplasms and its possible role in metastasis formation. *Hum Pathol*. 2000;31:428–433.
60. Herrmann ME, LiVolsi VA, Pasha TL, et al. Immunohistochemical expression of galectin-3 in benign and malignant thyroid lesions. *Arch Pathol Lab Med*. 2002;126:710–713.
61. Kovacs RB, Foldes J, Winkler G, et al. The investigation of galectin-3 in diseases of the thyroid gland. *Eur J Endocrinol*. 2003;149:449–453.
62. Honjo Y, Nangia-Makker P, Inohara H, et al. Down-regulation of galectin-3 suppresses tumorigenicity of human breast carcinoma cells. *Clin Cancer Res*. 2001;7:661–668.
63. Sanjuan X, Fernandez PL, Castells A, et al. Differential expression of galectin 3 and galectin 1 in colorectal cancer progression. *Gastroenterology*. 1997;113:1906–1915.
64. Cvejic D, Savin S, Golubovic S, et al. Galectin-3 and carcinoembryonic antigen expression in medullary thyroid carcinoma: possible relation to tumour progression. *Histopathology*. 2000;37:530–535.
65. Weber KB, Shroyer KR, Heinz DE, et al. The use of a combination of galectin-3 and thyroid peroxidase for the diagnosis and prognosis of thyroid cancer. *Am J Clin Pathol*. 2004;122:524–531.
66. Papotti M, Rodriguez J, De Pampa R, Bartolazzi A, Rosai J. Galectin-3 and HBME-1 expression in well-differentiated thyroid tumors with follicular architecture of uncertain malignant potential. *Mod Pathol*. 2005;18:541–546.
67. Prasad ML, Pellegata NS, Huang Y, et al. Galectin-3, fibronectin-1, CITED-1, HBME1 and cytokeratin-19 immunohistochemistry is useful for the differential diagnosis of thyroid tumors. *Mod Pathol*. 2005;18:48–57.
68. Oestreicher-Kedem Y, Halpern M, Roizman P, et al. Diagnostic value of galectin-3 as a marker for malignancy in follicular patterned thyroid lesions. *Head Neck*. 2004;26:960–966.
69. Ito Y, Yoshida H, Tomoda C, et al. Galectin-3 expression in follicular tumours: an immunohistochemical study of its use as a marker of follicular carcinoma. *Pathology*. 2005;37:296–298.
70. Cvejic DS, Savin SB, Petrovic IM, et al. Galectin-3 expression in papillary thyroid carcinoma: relation to histomorphologic growth pattern, lymph node metastasis, extrathyroid invasion, and tumor size. *Head Neck*. 2005;27:1049–1055.
71. Miettinen M, Kovatich AJ, Karkkainen P. Keratin subsets in papillary and follicular thyroid lesions: a paraffin section analysis with diagnostic implications. *Virchows Arch*. 1997;431:407–413.
72. Gasbarri A, Martegani MP, Del PF, et al. Galectin-3 and CD44v6 isoforms in the preoperative evaluation of thyroid nodules. *J Clin Oncol*. 1999;17:3494–3502.
73. Maruta J, Hashimoto H, Yamashita H, et al. Immunostaining of galectin-3 and CD44v6 using fine-needle aspiration for distinguishing follicular carcinoma from adenoma. *Diagn Cytopathol*. 2004;31:392–396.
74. Sheibani K, Esteban JM, Bailey A, et al. Immunopathologic and molecular studies as an aid to the diagnosis of malignant mesothelioma. *Hum Pathol*. 1992;23:107–116.
75. Mase T, Funahashi H, Koshikawa T, et al. HBME-1 immunostaining in thyroid tumors especially in follicular neoplasm. *Endocr J*. 2003;50:173–177.
76. Nikiforova MN, Lynch RA, Biddinger PW, et al. RAS point mutations and PAX8-PPAR gamma rearrangement in thyroid tumors: evidence for distinct molecular pathways in thyroid follicular carcinoma. *J Clin Endocrinol Metab*. 2003;88:2318–2326.
77. Pairet M, Engelhardt G. Distinct isoforms (COX-1 and COX-2) of cyclooxygenase: possible physiological and therapeutic implications. *Fundam Clin Pharmacol*. 1996;10:1–17.
78. Soslow RA, Dannenberg AJ, Rush D, et al. COX-2 is expressed in human pulmonary, colonic, and mammary tumors. *Cancer*. 2000;89:2637–2645.
79. Yoshimura R, Sano H, Masuda C, et al. Expression of cyclooxygenase-2 in prostate carcinoma. *Cancer*. 2000;89:589–596.
80. Okami J, Yamamoto H, Fujiwara Y, et al. Overexpression of cyclooxygenase-2 in carcinoma of the pancreas. *Clin Cancer Res*. 1999;5:2018–2024.
81. Ito Y, Yoshida H, Nakano K, et al. Cyclooxygenase-2 expression in thyroid neoplasms. *Histopathology*. 2003;42:492–497.
82. Garcia-Gonzalez M, Abdulkader I, Boquete AV, et al. Cyclooxygenase-2 in normal, hyperplastic and neoplastic follicular cells of the human thyroid gland. *Virchows Arch*. 2005;447:12–17.
83. Lee HM, Baek SK, Kwon SY, et al. Cyclooxygenase 1 and 2 expressions in the human thyroid gland. *Eur Arch Otorhinolaryngol*. 2006;263:199–204.
84. Kajita S, Ruebel KH, Casey MB, et al. Role of COX-2, thromboxane A2

synthase, and prostaglandin I<sub>2</sub> synthase in papillary thyroid carcinoma growth. *Mod Pathol*. 2005;18:221–227.

85. Haynik DM, Prayson RA. Immunohistochemical expression of cyclooxygenase 2 in follicular carcinomas of the thyroid. *Arch Pathol Lab Med*. 2005;129:736–741.

86. Lo CY, Lam KY, Leung PP, et al. High prevalence of cyclooxygenase 2 expression in papillary thyroid carcinoma. *Eur J Endocrinol*. 2005;152:545–550.

87. Bell CD, Vidal S, Kovacs K, et al. An immunohistochemical survey of nine cases of medullary carcinoma of thyroid including reactivity for Cox-1 and Cox-2 enzymes. *Endocr Pathol*. 2002;13:331–340.

88. Bounacer A, Wicker R, Caillou B, et al. High prevalence of activating ret proto-oncogene rearrangements in thyroid tumours from patients who had received external radiation. *Oncogene*. 1997;15:1263–1273.

89. Cornetta AJ, Russell JP, Cunnane M, et al. Cyclooxygenase-2 expression in human thyroid carcinoma and Hashimoto's thyroiditis. *Laryngoscope*. 2002;112:238–242.

90. Kroll TG, Sarraf P, Pecciarini L, et al. PAX8-PPAR $\gamma$ 1 fusion oncogene in human thyroid carcinoma [corrected]. *Science*. 2000;289:1357–1360.

91. Nikiforova MN, Biddinger PW, Caudill CM, Kroll TG, Nikiforov YE. PAX8-PPAR $\gamma$  rearrangement in thyroid tumors: RT-PCR and immunohistochemical analyses. *Am J Surg Pathol*. 2002;26:1016–1023.

92. Marques AR, Espadilha C, Catarino AL, et al. Expression of PAX8-PPAR $\gamma$ 1 rearrangements in both follicular thyroid carcinomas and adenomas. *J Clin Endocrinol Metab*. 2002;87:3947–3952.

93. Gustafson KS, LiVolsi VA, Furth EE, Pasha TL, Putt ME, Baloch ZW. Peroxisome proliferator-activated receptor  $\gamma$  expression in follicular-patterned thyroid lesions: caveats for the use of immunohistochemical studies. *Am J Clin Pathol*. 2003;120:175–181.

94. Sahin M, Allard BL, Yates M, et al. PPAR $\gamma$  staining as a surrogate for PAX8/PPAR $\gamma$  fusion oncogene expression in follicular neoplasms: clinicopathological correlation and histopathological diagnostic value. *J Clin Endocrinol Metab*. 2005;90:463–468.

95. Castro P, Rebocho AP, Soares RJ, et al. PAX8-PPAR $\gamma$  rearrangement is frequently detected in the follicular variant of papillary thyroid carcinoma. *J Clin Endocrinol Metab*. 2006;91:213–220.

96. Scheumman GF, Hoang-Vu C, Cetin Y, et al. Clinical significance of E-cadherin as a prognostic marker in thyroid carcinomas. *J Clin Endocrinol Metab*. 1995;80:2168–2172.

97. von Wasielewski R, Rhein A, Werner M, et al. Immunohistochemical detection of E-cadherin in differentiated thyroid carcinomas correlates with clinical outcome. *Cancer Res*. 1997;57:2501–2507.

98. Cerrato A, Fulciniti F, Avallone A, et al. Beta- and gamma-catenin expression in thyroid carcinomas. *J Pathol*. 1998;185:267–272.

99. Rocha AS, Soares P, Fonseca E, et al. E-cadherin loss rather than beta-catenin alterations is a common feature of poorly differentiated thyroid carcinomas. *Histopathology*. 2003;42:580–587.

100. Garcia-Rostan G, Camp RL, Herrero A, et al. Beta-catenin dysregulation in thyroid neoplasms: down-regulation, aberrant nuclear expression, and CTNNB1 exon 3 mutations are markers for aggressive tumor phenotypes and poor prognosis. *Am J Pathol*. 2001;158:987–996.

101. Xu B, Yoshimoto K, Miyauchi A, et al. Cribriform-morular variant of papillary thyroid carcinoma: a pathological and molecular genetic study with evidence of frequent somatic mutations in exon 3 of the beta-catenin gene. *J Pathol*. 2003;199:58–67.

102. Lee S, Hong SW, Shin SJ, et al. Papillary thyroid carcinoma associated with familial adenomatous polyposis: molecular analysis of pathogenesis in a family and review of the literature. *Endocr J*. 2004;51:317–323.

103. Sugeno A, Usuda N, Adachi W, et al. Immunohistochemical studies on the localization of fibronectin in human thyroid neoplastic tissues. *Endocrinol Jpn*. 1988;35:111–120.

104. Ryu S, Jimi S, Takebayashi S. Thyroid carcinoma distinctively expresses intracellular fibronectin in vivo. *Cancer Lett*. 1997;121:189–193.

105. Scarpino S, Stoppacciaro A, Pellegrini C, et al. Expression of EDVA/EDB isoforms of fibronectin in papillary carcinoma of the thyroid. *J Pathol*. 1999;188:163–167.

106. Takano T, Miyauchi A, Yokozawa T, et al. Preoperative diagnosis of thyroid papillary and anaplastic carcinomas by real-time quantitative reverse transcription-polymerase chain reaction of oncofetal fibronectin messenger RNA. *Cancer Res*. 1999;59:4542–4545.

107. Takano T, Matsuzuka F, Sumizaki H, et al. Rapid detection of specific messenger RNAs in thyroid carcinomas by reverse transcription-PCR with degenerate primers: specific expression of oncofetal fibronectin messenger RNA in papillary carcinoma. *Cancer Res*. 1997;57:3792–3797.

108. Takano T, Matsuzuka F, Miyauchi A, et al. Restricted expression of oncofetal fibronectin mRNA in thyroid papillary and anaplastic carcinoma: an in situ hybridization study. *Br J Cancer*. 1998;78:221–224.

109. Takano T, Miyauchi A, Yokozawa T, et al. Accurate and objective preoperative diagnosis of thyroid papillary carcinomas by reverse transcription-PCR detection of oncofetal fibronectin messenger RNA in fine-needle aspiration biopsies. *Cancer Res*. 1998;58:4913–4917.

110. Aldred MA, Huang Y, Liyanarachchi S, et al. Papillary and follicular thyroid carcinomas show distinctly different microarray expression profiles and can be distinguished by a minimum of five genes. *J Clin Oncol*. 2004;22:3531–3539.

111. Huang Y, Prasad M, Lemon WJ, et al. Gene expression in papillary thyroid

carcinoma reveals highly consistent profiles. *Proc Natl Acad Sci U S A*. 2001;98:15044–15049.

112. Prasad ML, Pellegata NS, Kloos RT, et al. CITED1 protein expression suggests papillary thyroid carcinoma in high throughput tissue microarray-based study. *Thyroid*. 2004;14:169–175.

113. Ryu S, Jimi S, Eura Y, et al. Strong intracellular and negative peripheral expression of fibronectin in tumor cells contribute to invasion and metastasis in papillary thyroid carcinoma. *Cancer Lett*. 1999;146:103–109.

114. Liu W, Asa SL, Ezzat S. 1 $\alpha$ ,25-Dihydroxyvitamin D3 targets PTEN-dependent fibronectin expression to restore thyroid cancer cell adhesiveness. *Mol Endocrinol*. 2005;19:2349–2357.

115. Takano T, Miyauchi A, Matsuzuka F, et al. Expression of oncofetal fibronectin messenger ribonucleic acid in fibroblasts in the thyroid: a possible cause of false positive results in molecular-based diagnosis of thyroid carcinomas. *J Clin Endocrinol Metab*. 2000;85:765–768.

116. Gunther U, Hofmann M, Rudy W, et al. A new variant of glycoprotein CD44 confers metastatic potential to rat carcinoma cells. *Cell*. 1991;65:13–24.

117. Arch R, Wirth K, Hofmann M, et al. Participation in normal immune responses of a metastasis-inducing splice variant of CD44. *Science*. 1992;257:682–685.

118. Seiter S, Arch R, Reber S, et al. Prevention of tumor metastasis formation by anti-variant CD44. *J Exp Med*. 1993;177:443–455.

119. Figge J, del Rosario AD, Gerasimov G, et al. Preferential expression of the cell adhesion molecule CD44 in papillary thyroid carcinoma. *Exp Mol Pathol*. 1994;61:203–211.

120. De MC, Vassko V, Henry JF. The value of thyroid peroxidase immunohistochemistry for preoperative fine-needle aspiration diagnosis of the follicular variant of papillary thyroid cancer. *Surgery*. 1999;126:1200–1204.

121. Smanik PA, Fithian LJ, Jhiang SM. Thyroid peroxidase expression and DNA polymorphisms in thyroid cancer. *Biochem Biophys Res Commun*. 1994;198:948–954.

122. De MC, Zoro P, Garcia S, et al. Thyroid peroxidase immunodetection as a tool to assist diagnosis of thyroid nodules on fine-needle aspiration biopsy. *Eur J Endocrinol*. 1994;131:474–479.

123. Christensen L, Blichert-Toft M, Brandt M, et al. Thyroperoxidase (TPO) immunostaining of the solitary cold thyroid nodule. *Clin Endocrinol (Oxf)*. 2000;53:161–169.

124. Raffaelli M, De MC, Lubrano D, et al. [Immunodetection of thyroid peroxidase in the diagnosis of follicular variants of thyroid papillary cancer]. *Ann Chir*. 2001;126:148–151.

125. Czarnocka B, Pastuszko D, Janota-Bzowski M, et al. Is there loss or qualitative changes in the expression of thyroid peroxidase protein in thyroid epithelial cancer? *Br J Cancer*. 2001;85:875–880.

126. Savin S, Cvejic D, Isic T, et al. Thyroid peroxidase immunohistochemistry in differential diagnosis of thyroid tumors. *Endocr Pathol*. 2006;17:53–60.

127. Savin S, Cvejic D, Isic T, et al. The efficacy of the thyroid peroxidase marker for distinguishing follicular thyroid carcinoma from follicular adenoma. *Exp Oncol*. 2006;28:70–74.

128. Scognamiglio T, Hyjek E, Kao J, et al. Diagnostic usefulness of HBME1, galectin-3, CK19, and CITED1 and evaluation of their expression in encapsulated lesions with questionable features of papillary thyroid carcinoma. *Am J Clin Pathol*. 2006;126:700–708.

129. Morin PJ, Sparks AB, Korinek V, et al. Activation of beta-catenin-Tcf signaling in colon cancer by mutations in beta-catenin or APC. *Science*. 1997;275:1787–1790.

130. Lantsov D, Meirmanov S, Nakashima M, et al. Cyclin D1 overexpression in thyroid papillary microcarcinoma: its association with tumour size and aberrant beta-catenin expression. *Histopathology*. 2005;47:248–256.

131. Khoo ML, Beasley NJ, Ezzat S, et al. Overexpression of cyclin D1 and underexpression of p27 predict lymph node metastases in papillary thyroid carcinoma. *J Clin Endocrinol Metab*. 2002;87:1814–1818.

132. Saiz AD, Olvera M, Rezk S, et al. Immunohistochemical expression of cyclin D1, E2F-1, and Ki-67 in benign and malignant thyroid lesions. *J Pathol*. 2002;198:157–162.

133. Nakashima M, Meirmanov S, Naruke Y, et al. Cyclin D1 overexpression in thyroid tumours from a radio-contaminated area and its correlation with Pin1 and aberrant beta-catenin expression. *J Pathol*. 2004;202:446–455.

134. Khoo ML, Ezzat S, Freeman JL, et al. Cyclin D1 protein expression predicts metastatic behavior in thyroid papillary microcarcinomas but is not associated with gene amplification. *J Clin Endocrinol Metab*. 2002;87:1810–1813.

135. Erickson LA, Jin L, Wollan PC, et al. Expression of p27kip1 and Ki-67 in benign and malignant thyroid tumors. *Mod Pathol*. 1998;11:169–174.

136. Resnick MB, Schacter P, Finkelstein Y, et al. Immunohistochemical analysis of p27kip1 expression in thyroid carcinoma. *Mod Pathol*. 1998;11:735–739.

137. Erickson LA, Yousef OM, Jin L, et al. p27kip1 expression distinguishes papillary hyperplasia in Graves' disease from papillary thyroid carcinoma. *Mod Pathol*. 2000;13:1014–1019.

138. Khoo ML, Freeman JL, Witterick IJ, et al. Underexpression of p27/Kip1 in thyroid papillary microcarcinomas with gross metastatic disease. *Arch Otolaryngol Head Neck Surg*. 2002;128:253–257.

139. Ito Y, Uruno T, Takamura Y, et al. Papillary microcarcinomas of the thyroid with preoperatively detectable lymph node metastasis show significantly higher aggressive characteristics on immunohistochemical examination. *Oncology*. 2005;68:87–96.

140. Donghi R, Longoni A, Pilotti S, et al. Gene p53 mutations are restricted

to poorly differentiated and undifferentiated carcinomas of the thyroid gland. *J Clin Invest*. 1993;91:1753–1760.

141. Dobashi Y, Sugimura H, Sakamoto A, et al. Stepwise participation of p53 gene mutation during dedifferentiation of human thyroid carcinomas. *Diagn Mol Pathol*. 1994;3:9–14.

142. Nishida T, Nakao K, Hamaji M, et al. Overexpression of p53 protein and DNA content are important biologic prognostic factors for thyroid cancer. *Surgery*. 1996;119:568–575.

143. Hosal SA, Apel RL, Freeman JL, et al. Immunohistochemical localization of p53 in human thyroid neoplasms: correlation with biological behavior. *Endocr Pathol*. 1997;8:21–28.

144. Civitareale D, Lonigro R, Sinclair AJ, et al. A thyroid-specific nuclear protein essential for tissue-specific expression of the thyroglobulin promoter. *EMBO J*. 1989;8:2537–2542.

145. Bingle CD. Thyroid transcription factor-1. *Int J Biochem Cell Biol*. 1997;29:1471–1473.

146. Endo T, Kaneshige M, Nakazato M, et al. Thyroid transcription factor-1 activates the promoter activity of rat thyroid Na<sup>+</sup>/I<sup>-</sup> symporter gene. *Mol Endocrinol*. 1997;11:1747–1755.

147. Glasser SW, Burhans MS, Eszterhas SK, et al. Human SP-C gene sequences that confer lung epithelium-specific expression in transgenic mice. *Am J Physiol Lung Cell Mol Physiol*. 2000;278:L933–L945.

148. Bruno MD, Bohinski RJ, Huelsman KM, et al. Lung cell-specific expression of the murine surfactant protein A (SP-A) gene is mediated by interactions between the SP-A promoter and thyroid transcription factor-1. *J Biol Chem*. 1995;270:6531–6536.

149. Kelly SE, Bachurski CJ, Burhans MS, et al. Transcription of the lung-specific surfactant protein C gene is mediated by thyroid transcription factor 1. *J Biol Chem*. 1996;271:6881–6888.

150. Zhang L, Whitsett JA, Stripp BR. Regulation of Clara cell secretory protein gene transcription by thyroid transcription factor-1. *Biochim Biophys Acta*. 1997;1350:359–367.

151. Srodon M, Westra WH. Immunohistochemical staining for thyroid transcription factor-1: a helpful aid in discerning primary site of tumor origin in patients with brain metastases. *Hum Pathol*. 2002;33:642–645.

152. Roh MS, Hong SH. Utility of thyroid transcription factor-1 and cytokeratin 20 in identifying the origin of metastatic carcinomas of cervical lymph nodes. *J Korean Med Sci*. 2002;17:512–517.

153. Cai YC, Banner B, Glickman J, et al. Cytokeratin 7 and 20 and thyroid transcription factor 1 can help distinguish pulmonary from gastrointestinal carcinoid and pancreatic endocrine tumors. *Hum Pathol*. 2001;32:1087–1093.

154. Oliveira AM, Tazelaar HD, Myers JL, et al. Thyroid transcription factor-1 distinguishes metastatic pulmonary from well-differentiated neuroendocrine tumors of other sites. *Am J Surg Pathol*. 2001;25:815–819.

155. Sturm N, Rossi G, Lantuejoul S, et al. Expression of thyroid transcription factor-1 in the spectrum of neuroendocrine cell lung proliferations with special interest in carcinoids. *Hum Pathol*. 2002;33:175–182.

156. Kaufmann O, Dietel M. Expression of thyroid transcription factor-1 in pulmonary and extrapulmonary small cell carcinomas and other neuroendocrine carcinomas of various primary sites. *Histopathology*. 2000;36:415–420.

157. Ros P, Rossi DL, Acebron A, et al. Thyroid-specific gene expression in the multi-step process of thyroid carcinogenesis. *Biochimie*. 1999;81:389–396.

158. Bejarano PA, Nikiforov YE, Swenson ES, et al. Thyroid transcription factor-1, thyroglobulin, cytokeratin 7, and cytokeratin 20 in thyroid neoplasms. *Appl Immunohistochem Mol Morphol*. 2000;8:189–194.

159. Fabbro D, Di LC, Beltrami CA, et al. Expression of thyroid-specific transcription factors TTF-1 and PAX-8 in human thyroid neoplasms. *Cancer Res*. 1994;54:4744–4749.

160. Suzuki K, Mori A, Lavaroni S, et al. Thyroglobulin regulates follicular

function and heterogeneity by suppressing thyroid-specific gene expression. *Biochimie*. 1999;81:329–340.

161. Ratnatnga N, Ramadasa S. Immunohistochemical staining for thyroglobulin in poorly differentiated carcinoma of the thyroid. *Ceylon Med J*. 1993;38:113–116.

162. Harach HR, Franssila KO. Thyroglobulin immunostaining in follicular thyroid carcinoma: relationship to the degree of differentiation and cell type. *Histopathology*. 1988;13:43–54.

163. Ozkara SK, Gurbuz Y, Muezzinoglu B, et al. Encapsulated cystic papillary variant of medullary carcinoma of thyroid gland. *Endocr Pathol*. 2002;13:167–171.

164. Huss LJ, Mendelsohn G. Medullary carcinoma of the thyroid gland: an encapsulated variant resembling the hyalinizing trabecular (paraganglioma-like) adenoma of thyroid. *Mod Pathol*. 1990;3:581–585.

165. Sikri KL, Varndell IM, Hamid QA, et al. Medullary carcinoma of the thyroid: an immunocytochemical and histochemical study of 25 cases using eight separate markers. *Cancer*. 1985;56:2481–2491.

166. Gould VE, Wiedenmann B, Lee I, et al. Synaptophysin expression in neuroendocrine neoplasms as determined by immunocytochemistry. *Am J Pathol*. 1987;126:243–257.

167. Baloch ZW, LiVolsi VA. Neuroendocrine tumors of the thyroid gland. *Am J Clin Pathol*. 2001;115(suppl):S56–S67.

168. Saad MF, Ordonez NG, Guido JJ, et al. The prognostic value of calcitonin immunostaining in medullary carcinoma of the thyroid. *J Clin Endocrinol Metab*. 1984;59:850–856.

169. Zajac JD, Penschow J, Mason T, et al. Identification of calcitonin and calcitonin gene-related peptide messenger ribonucleic acid in medullary thyroid carcinomas by hybridization histochemistry. *J Clin Endocrinol Metab*. 1986;62:1037–1043.

170. Lloyd RV. Use of molecular probes in the study of endocrine diseases. *Hum Pathol*. 1987;18:1199–1211.

171. Talerma A, Lindeman J, Kievit-Tyson PA, et al. Demonstration of calcitonin and carcinoembryonic antigen (CEA) in medullary carcinoma of the thyroid (MCT) by immunoperoxidase technique. *Histopathology*. 1979;3:503–510.

172. Lloyd RV, Sisson JC, Marangos PJ. Calcitonin, carcinoembryonic antigen and neuron-specific enolase in medullary thyroid carcinoma. *Cancer*. 1983;51:2234–2239.

173. Dasovic-Knezevic M, Bormer O, Holm R, et al. Carcinoembryonic antigen in medullary thyroid carcinoma: an immunohistochemical study applying six novel monoclonal antibodies. *Mod Pathol*. 1989;2:610–617.

174. Wilson NW, Pambakian H, Richardson TC, et al. Epithelial markers in thyroid carcinoma: an immunoperoxidase study. *Histopathology*. 1986;10:815–829.

175. Uribe M, Fenoglio-Preiser CM, Grimes M, et al. Medullary carcinoma of the thyroid gland. Clinical, pathological, and immunohistochemical features with review of the literature. *Am J Surg Pathol*. 1985;9:577–594.

176. Yamada Y, Ito S, Matsubara Y, et al. Immunohistochemical demonstration of somatostatin-containing cells in the human, dog and rat thyroids. *Tohoku J Exp Med*. 1977;122:87–92.

177. Sundler F, Alumets J, Hakanson R, et al. Somatostatin-immunoreactive cells in medullary carcinoma of the thyroid. *Am J Pathol*. 1977;88:381–386.

178. Birkenhager JC, Upton GV, Seldenrath HJ, et al. Medullary thyroid carcinoma: ectopic production of peptides with ACTH-like, corticotrophin releasing factor-like and prolactin production-stimulating activities. *Acta Endocrinol (Copenh)*. 1976;83:280–292.

179. Matsubayashi S, Yanaihara C, Ohkubo M, et al. Gastrin-releasing peptide immunoreactivity in medullary thyroid carcinoma. *Cancer*. 1984;53:2472–2477.

180. Sunday ME, Wolfe HJ, Roos BA, et al. Gastrin-releasing peptide gene expression in developing, hyperplastic, and neoplastic human thyroid C-cells. *Endocrinology*. 1988;122:1551–1558.