Immunohistochemistry of thyroid gland carcinomas: clinical utility and diagnostic pitfalls

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Abstract

There are several settings in which immunohistochemistry could be employed in thyroid pathology. Ancillary immunohistochemistry can aid in differentiating tumour types and has also been used to support a diagnosis of malignancy in equivocal follicular lesions. Theoretically, immunostains could serve as prognostic markers as well. Here, we focus on the role of immunohistochemistry as a diagnostic tool mainly in less well-differentiated carcinomas, to confirm cell lineage. In this setting, it is important to perform a panel of immunostains and interpret them in the context of the tumour morphology and the clinical picture, since staining patterns can overlap with other non-thyroid tumour types. We also address the controversial use of immunostains to separate malignant from benign follicular lesions. Lastly, the potential for immunohistochemistry as prognostic markers will be considered. This review emphasizes the application of immunohistochemistry to thyroid histopathology rather than cytology.

Keywords *BRAF V600E*; calcitonin; cytokeratin; immunohistochemistry; monoclonal carcinoembryonic antigen (mCEA); napsin A; PAX8; solid cell nests; thyroglobulin; thyroid carcinoma; thyroid transcription factor-1 (TTF-1)

Introduction

Most thyroid carcinomas originate from follicular epithelial cells, the predominant cell type in the thyroid gland. The vast majority is well-differentiated, either papillary thyroid carcinoma (PTC) or follicular thyroid carcinoma (FTC), which resemble normal thyroid follicles and often contain colloid. These can usually be diagnosed histologically without the aid of ancillary immunohistochemistry. However, poorly differentiated thyroid carcinomas (PDTCs) and anaplastic (undifferentiated) thyroid carcinomas (ATCs), which together account for less than 5% of thyroid malignancies, have less histologic evidence of follicular differentiation. As such, they may require immunohistochemistry for definitive diagnosis, especially when arising *de novo* without an associated well-differentiated component. Medullary thyroid carcinoma (MTC), which accounts for less than 5% of thyroid malignancies, is the only tumour type derived from parafollicular c cells. However, it can show a variety of histologic patterns that may mimic follicular cell-derived thyroid carcinomas. Lineage specific markers for parafollicular c cells, which are neuroendocrine, are quite useful to identify such cases.

Thyroid carcinomas may show overlapping immunophenotypes with carcinomas from other sites. Therefore, it is important to consider metastases to the thyroid gland or secondary involvement by direct extension from a non-thyroid malignancy in the neck or airway, when interrogating a carcinoma in the thyroid with immunohistochemistry. One should perform a panel of immunostains rather than relying on a single marker and correlate with clinical and radiographic findings in difficult cases. The same is true when presented with a lymph node or distant metastasis for which the differential diagnosis includes thyroid carcinoma.

Immunohistochemistry has not just been employed to confirm cell of origin/tumour type in thyroid carcinomas but has also been used to aid in the diagnosis of malignancy itself. Well-differentiated PTC can be quite challenging to distinguish from benign thyroid disease histologically when the nuclear features are not well developed. However, as of yet, there is no single definitive "cancer stain" to diagnose PTC. While a variety of markers may be more commonly over or under expressed in thyroid cancer, none is entirely sensitive or specific.

Here, we review the utility of immunohistochemistry in the diagnosis and differential diagnosis of thyroid carcinomas. We will focus on the use of lineage restricted markers to aid in determining cell/site of origin in histologically challenging cases. In addition, we will briefly discuss the literature regarding immunohistochemistry to support a diagnosis of malignancy in lesions with borderline nuclear features of PTC. Lastly, the possibility of prognostic markers will be explored.

Thyroid and follicular cell lineage markers

Immunohistochemical markers of the thyroid, including follicular cell lineage, are most useful to confirm the diagnosis of PDTC or ATC, which may show less histologic clues of thyroid follicular cell origin. In addition, immunostains may be helpful in small biopsy material of unusual variants of PTC, such as the hobnail variant, which shows similar micropapillary architecture seen in micropapillary adenocarcinomas from other organ systems, such as the lung, bladder or breast (Figure 1).

PDTC is characterized by solid, insular or trabecular growth with sparse to absent thyroid follicles and no nuclear features of PTC. Mitotic activity or necrosis is usually present. By definition, histologic resemblance to normal thyroid follicles is often lacking. Differential diagnostic considerations for PDTC include MTC (discussed below) as well as metastatic disease to the thyroid gland. Metastases to the thyroid gland represent less than 1% of all thyroid malignancies.[1,2] Common primary sites include the kidney, head and neck (squamous cell carcinomas), lung and breast.[1,2] Renal cell carcinoma, in particular, may have a nested pattern similar to PDTC. In addition, abundant clear cytoplasm, typical of renal cell carcinomas, may also be seen in PDTC or FTC (Figure 2). Although rare, parathyroid carcinoma may enter the differential diagnosis as well, given the close proximity of the parathyroid glands to the thyroid and similar monotonous population of tumour cells with solid, nested or trabecular growth. Mucin production is very unusual in thyroid carcinomas and should raise the possibility of metastasis.

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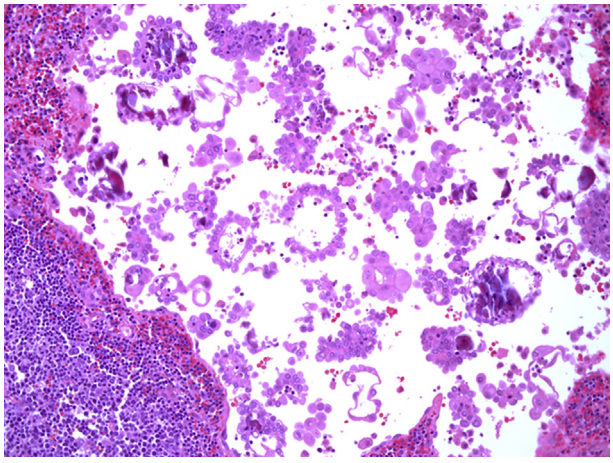

Figure 1 Hobnail variant of PTC in a lymph node. This variant shows small papilla that lack fibrovascular cores and may resemble micropapillary adenocarcinomas from other organ systems on small biopsy.

ATC is an undifferentiated malignancy composed of sheets of spindled, epithelioid, giant or squamoid cells. The differential diagnosis usually includes other undifferentiated or sarcomatoid carcinomas, true sarcomas or poorly differentiated squamous cell carcinoma. Diagnosis may require clinicopathologic correlation. Patients with ATC typically present with a rapidly growing thyroid mass and without another primary site suspected on imaging.

Specific thyroid and follicular cell lineage markers are described below, with emphasis on their utility in sorting out entities in the differential diagnosis of PDTC and ATC.

Thyroglobulin

Thyroglobulin is the precursor molecule of thyroid hormones triiodothyronine (T3) and thyroxine (T4) that is synthesized in thyroid follicular cells and stored in the colloid. As a result, it is considered a specific marker of follicular cell derived carcinomas. In a previous survey of the literature, thyroglobulin expression was not found in a variety of other tissues and tumour types including lung, stomach, pancreas, ovary, kidney, salivary gland, colon, prostate, breast and parathyroid.[3] However, thyroglobulin may stain entrapped follicular cells within MTC or other neoplasms involving the thyroid gland and also diffuse through tissues to produce artifactual staining that can be mistaken for positivity. Anecdotally, we have encountered parathyroid adenomas with patchy positivity for thyroglobulin (Figure 3).

Thyroglobulin stains both cytoplasm and extracellular colloid. It has been reported to be positive in 100% of PTCs, >75% of FTCs and 50–90% of PDTCs.[3] The staining pattern may be focal or patchy in poorly differentiated thyroid carcinomas, whereas thyroglobulin is almost always negative in ATC. ATCs are simply too undifferentiated and, although derived from follicular cells, they no longer synthesize thyroglobulin. As a result, thyroglobulin is a useful marker for PDTC when it is positive and artifactual staining has been excluded, but it may be negative in a significant subset. It is not a useful marker of ATC.

Thyroid transcription factor-1

Thyroid transcription factor-1 (TTF-1) is a lineage restricted nuclear transcription factor that is important in the development of the lung, thyroid, ventral forebrain and pituitary gland.[4] It shows a nuclear pattern of staining and is normally expressed in the lung and both thyroid follicular and parafollicular c-cells. In thyroid neoplasms, TTF-1 is expressed in 90% or more of well-differentiated PTC and FTCs as well as in PDTCs and MTCs; expression is significantly lower in oncocytic (Hurthle cell) carcinomas.[5] Less than 20% of ATCs retain expression of TTF-1.[5] TTF-1 is of course expressed in the majority (approximately 70–80%) of lung adenocarcinomas. However, it also is positive in a subset of lung squamous cell carcinomas as well as a small minority of various non-pulmonary adenocarcinomas including from the genitourinary and gastrointestinal tracts and breast.[5] TTF-1 is also notorious for positive immunoreactivity in small cell carcinomas from various sites, not just the lung. Non-pulmonary well-differentiated neuroendocrine tumours are less frequently TTF-1 positive. Additionally, awareness of TTF-1 antibody clone is important as the SPT24 clone is known to be less specific, albeit more sensitive for lung adenocarcinomas, than the 8G7G3/1 clone.

TTF-1 is a useful marker of PDTC but cannot be relied upon alone, especially if lung adenocarcinoma is in the differential diagnosis. Since only a minority of ATCs express TTF-1, its utility is limited in this setting, but helpful when positive. It is important to note that a small subset of PDTCs, ATCs and PTCs, including the hobnail pattern, also express napsin A, which is often used as a marker of lung adenocarcinomas.[6] Therefore, the combination of TTF-1 and napsin A positivity does not necessarily indicate a lung primary as this staining pattern can also be seen in thyroid carcinomas (Figure 4). TTF-1, of course, does not help differentiate PDTC from MTC, since both are positive, nor does it help differentiate MTC from neuroendocrine tumours of the lung or small cell carcinoma from many organ systems.

PAX8

PAX8 is a more recently characterized transcription factor that is a member of the paired-box family of genes and is important for the development of the thyroid gland, kidney and Mullerian tract.[7,8] Like TTF-1, PAX8 shows a nuclear pattern of staining. Greater than 90% of FTCs and PDTCs, and up to 100% of PTCs express PAX8.[8–10] Importantly, PAX8 is much more frequently positive in ATC (approximately 2/3 of cases) than TTF-1.[11] PAX8 is, therefore, a very useful marker for ATC, which often lacks expression of other thyroid specific markers (such as thyroglobulin and TTF-1). PAX8 expression is variable in MTC.[8–10]

Among other carcinomas, PAX8 is frequently expressed in renal cell carcinomas and carcinomas of the gynaecologic tract and thymus, but may also be expressed in a minority of carcinomas from other sites including the pancreas, bladder and squamous cell carcinoma of the lung.[8,10] PAX8 expression may be seen in neuroendocrine tumours from a variety of organ systems, including the parathyroid (but notably only rarely in the lung). However, immunoreactivity may depend on the particular antibody used.[12] All neuroendocrine tumours, including 5 MTCs, were negative for PAX8 in one study when monoclonal antibodies specific for the less conserved C-terminal portion of PAX8 were used.[12] PAX8 expression has also been reported in non-epithelial malignancies, including rhabdomyosarcoma.[10]

PAX8 is useful to confirm the diagnosis of PDTCs and ATCs, given the frequent expression in both tumour types. Including

