Clinical study

**PAX8 is expressed in anaplastic thyroid carcinoma diagnosed by fine-needle aspiration: a study of three cases with histological correlates**

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**Abstract**

**Objective:** It is difficult to diagnose anaplastic thyroid carcinoma (ATC) in a fine-needle aspiration (FNA) sample because, given the loss of morphological and immunophenotypical follicular thyroid features, its cytology resembles that of other undifferentiated neoplasms. Recent studies have shown that immunostaining for paired box gene 8 (PAX8), a transcription factor expressed in normal thyroid, is effective for diagnosing ATCs on histology. The aim of this study was to evaluate whether PAX8 could be used to identify ATCs on cytology also.

**Design and methods:** We selected three PAX8-immunostained undifferentiated FNA samples previously diagnosed as suspected ATCs, whose cell block had been negative for the expression of TGB and thyroid transcription factor-1. Matched histological samples, available in two cases, were also processed for PAX8 immunohistochemistry.

**Results:** All three FNA samples were PAX8 positive. Two samples that had an epithelioid pattern showed a diffuse, intense nuclear signal. The third sample, which had a spindle-cell pattern, showed less intense and more patchy staining. Matched histology yielded overlapping results.

**Conclusions:** PAX8 immunocytochemistry can help cytopathologists to diagnose ATCs.

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**Introduction**

Anaplastic thyroid carcinoma (ATC) is a highly aggressive disease. Most cases derive from a pre-existing differentiated thyroid carcinoma (DTC) that has lost its morphological and immunophenotypical follicular cell features (1). However, a co-existing DTC can elude detection in some cases (1). Thus, it is difficult to differentiate ATCs from sarcomas, high-grade lymphomas, infiltrating head and neck squamous cell carcinomas, and thyroid metastases even on histology (2).

Fine-needle aspiration (FNA) is important in the management of ATCs for at least two reasons: first, patients often present with an advanced stage of disease, thereby precluding surgery as a first therapeutic choice (1), and second, the diagnosis of ATCs by FNA can help in the triaging of patients for BRAF mutational testing so that targeted therapy can be initiated in case of a positive result (3, 4, 5). However, in most cases, only undifferentiated cells are aspirated. Microscopic identification of these cells as ATC-derived cells is difficult because of their variable appearance, which reflects different histological patterns (6); consequently, ancillary staining is necessary not only in cytology but also in histology.

The progression from a DTC to an ATC is characterised by the gain of several malignancy-associated markers. For example, the p53 (TP53) tumour suppressor gene is expressed by the anaplastic component in ATCs that progress from papillary thyroid carcinomas (PTCs) (7). Similarly, we had reported previously that UBCH10 (UBE2C), a cell-cycle-related protein, is consistently overexpressed at both transcriptional and translational levels in ATCs (8). However, these malignancy-associated markers are not thyroid specific and have little value in routine diagnostics. Unfortunately, de-differentiation results in the loss of expression of the canonical thyroid tissue markers thyroglobulin (TGB) and thyroid transcription factor-1 (TTF1) (2). More recently, paired box gene 8 (PAX8) has been identified as a marker of thyroid linkage. This gene is a transcription factor required for normal thyroid development, and it is mutated in sporadic and familial thyroid hypoplasia and ectopy (9, 10). Its interaction with TTF1 and TTF2 induces the biosynthesis of thyroid hormones (11). From a technical viewpoint, PAX8
yields robust nuclear staining in paraffin-embedded sections. It labels both normal and neoplastic follicular cells, being strongly positive in most thyroid carcinomas, regardless of the histotype and degree of differentiation (2, 12, 13).

Little is known about the usefulness of PAX8 in cytology. In cell blocks (CBs) obtained from thyroid FNA samples, Schmitt et al. (14) demonstrated PAX8 nuclear positivity in > 90% of the PTC samples (31/32 cases) and in all the 20 benign nodules examined. In the only report of PAX8 staining of ATCs available, none of the five CBs studied stained positive, although, as stated by the authors, Bouin’s processing could have negatively affected their results (15). Given the inconclusive data on the usefulness of PAX8 in ATC cytology, in this study, we investigated its expression in ATC FNA samples.

Subjects and methods

Patients and samples

A computerised search was carried out to identify cases of ATC diagnosed by FNA at our Department for which an adequate CB was available. The criterion for CB adequacy was the presence of three or more groups of follicular cells or two or more tissue fragments according to the method of Sanchez & Selvaggi (16). Three cases were found. In each case, smears (both May–Grunwald–Giemsa- and Papanicolaou-stained) were reviewed. CB slides relative to TTF1, TGB, cytokeratin and vimentin staining were also retrieved to verify whether PAX8 MAB reacts in lymphoid B cells, we used a CB relative to a histologically proven neck B-cell lymphoma.

Case 1 was a 73-year-old male who suffered from a long-standing goitre and presented with a rapidly growing neck mass, hoarseness and elevated serum thyroglobulin levels. The lesion was first sampled by FNA and subsequently by large needle biopsy. Case 2 was a 64-year-old man with a 7 cm palpable hard lump in the left thyroid lobe. Shortly after FNA, the thyroid was removed. The histology of cases 1 and 2 was reviewed. Case 3 was a 78-year-old woman with a family history of thyroid cancer. A fast-growing left lobe mass and an enlarged palpable lateral neck lymph node were aspirated. Histological data were not available for this patient.

PAX8 immunocytochemistry

Four-micron sections of formalin-fixed paraffin-embedded CBs were deparaffinised and dehydrated. We used the following primary MABs: PAX8 (clone: MRQ-50), TTF1, TGB, cytokeratin, vimentin, S100 and HMB45 (all obtained from Ventana, Roche). After heat-induced antigen retrieval, the slides were processed by Benchmark Autostainer (Ventana, Roche) using the UltraView Polymer Detection kit. Negative controls were obtained by omitting the primary antibody. The intensity and distribution of PAX8 staining were scored according to the method of Schmitt et al. (14). Briefly, < 5% of positive nuclei was considered negative, 5–10% focal positive, 10–50% intermediate and > 50% diffuse. In terms of staining intensity, samples were defined negative (0), weak (1 +), intermediate (2 +) or strong (3 +).

BRAF mutational status assessment

Three CB sections of 4 μm each were scraped and deparaffinised to assess BRAF mutational status. After DNA extraction, BRAF exon 15 PCR products were directly sequenced in both directions (AB 3130XL,
Applied Biosystems) (17, 18, 19). The matched histological samples were also \textit{BRAF} tested.

\section*{Results}

\subsection*{Review of morphological and immunophenotypic features}

On smear review, the smears of cases 1 and 3 showed highly atypical dissociated cells with an epithelioid morphology. The cytoplasms were dense and ‘glassy’ in case 1 and thin and vacuolated in case 3 (Fig. 1a and b). In the latter, the background was necrotic and rich in neutrophils. The aspirate obtained from the adjacent lymph node showed overlapping features. The smears of case 2 showed a spindle-cell sarcoma-like cytomorphology with scattered pleomorphic large cells (Fig. 1c). Neither TTF1 nor TGB was expressed in any sample, whereas both cytokeratin and vimentin yielded strong staining. S100 and HMB45 stainings, which had been performed in case 1 to rule out metastatic melanoma, were also negative.

On histology, the dense and ‘glassy’ cytoplasms observed in the smears of case 1 reflected the rhabdoid phenotype (20), namely dense hyaline cytoplasmic inclusions displacing the nucleus eccentrically (Fig. 2a). The smears of case 2 showed a spindle-cell sarcoma-like cytomorphology with scattered pleomorphic large cells (Fig. 1c). Neither TTF1 nor TGB was expressed in any sample, whereas both cytokeratin and vimentin yielded strong staining. S100 and HMB45 stainings, which had been performed in case 1 to rule out metastatic melanoma, were also negative.

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\subsection*{PAX8 immunocytochemical expression}

Table 1 summarises the results of PAX8 immunostaining on CBs and matched histology. In the smears of cases 1 and 3, which showed an epithelioid cell pattern, nuclei were strongly and diffusely positive (Fig. 3a and b). Case 2, the smears of which showed a spindle-cell pattern, was also scored positive (Fig. 3c), with 10–50% of the nuclei displaying a moderate signal. Signal intensity and distribution overlapped (Fig. 4a and b) on the matched histology. As shown in Fig. 5, the B-cell lymphoma smear (Fig. 5a), whose CB was available (Fig. 5b), showed CD20 staining (Fig. 5c), whereas it lacked PAX8 reactivity (Fig. 5d).

\begin{table}[h]
\centering
\caption{PAX8 positivity in cell blocks and histological sections. Staining intensity and distribution were scored based on the method of Schmitt et al. (14).}
\begin{tabular}{|c|c|c|}
\hline
\textbf{Cases} & \textbf{Cell block} & \textbf{Histology} \\
\hline
Case 1 & 3+ (>50\%) & 3+ (>50\%) \\
Case 2 & 3+ (>50\%) & - \\
Case 3 & 2+ (5–50\%) & 2+ (5–50\%) \\
\hline
\end{tabular}
\end{table}

\section*{BRAF mutational status}

Cases 1 and 2, but not case 3, harboured the \textit{BRAF} V600E mutation. Cytological and matched histological samples yielded concordant genotyping.

\section*{Discussion}

ATC is highly aggressive and frequently presents with a rapidly enlarging thyroid mass. The extension of the tumour into adjacent tissues, including extrathyroidal soft tissue, trachea, larynx and oesophagus, often prevents surgical excision as the first therapeutic choice. Thus, FNA may be the only source of diagnostic material, and efforts to efficiently diagnose ATCs in cytological samples are justified. Unfortunately, ATCs do not often show evidence of thyroid differentiation at the microscopic or immunohistochemical level (2). Although a co-existing differentiated carcinoma can be occasionally observed on histology by serial sectioning (21), this component is rarely observed in aspirates (6). Since the \textit{BRAF} V600E mutation is found in 25\% of the ATCs (22), a correct ATC diagnosis can lead to \textit{BRAF} gene testing and, if a mutation is found, to a tailored therapeutic regimen (3, 5).

\textit{PAX8} is a transcription factor whose expression is retained in ATCs. Its diagnostic usefulness is well proven in histology (2, 13, 23, 24), but not in CBs. In this study, we retrospectively evaluated the expression of PAX8 in three ATC FNA samples whose CBs were available. There are no reports of PAX8 immunocytochemistry of direct smears or monolayer preparations (12). Therefore, the feasibility of PAX8 immunostaining in cytological preparations other than CBs remains to be established. The CBs that we examined were formalin fixed and in all cases were PAX8 positive. Two cases...
showed an epithelioid morphology, and one case showed spindle-cell sarcomatoid features. The nuclear signal was diffuse and strong in the two cases with epithelioid cells and less intense and patchy in the spindle-cell ATC sample. PAX8 efficiently differentiates ATCs from other poorly differentiated/anaplastic malignancies occurring in the head and neck region (2). Indeed, ATCs with a predominant squamous cell pattern are stained by PAX8, whereas squamous cell head and neck carcinomas are negative (2). Soft tissue neoplasms are rare in the thyroid. Mesenchymal tumours with a spindle-cell morphology (e.g. leiomyosarcoma and solitary fibrous tumour) may also be distinguished from ATCs based on their PAX8-negative staining (2). Conversely, PAX8 is less useful when ATCs spread to the mediastinum. Thymic carcinomas and thymic anaplastic carcinomas also express PAX8 (26). In these cases, additional markers (e.g. CD5 and CD117) can exclude an origin from the thymus. By contrast, spindle epithelial tumours with thymus-like differentiation and carcinomas showing thymus-like differentiation have not yet been tested for PAX8. Medullary carcinomas may display spindle-cell features that can overlap with the features of ATCs. The expression of PAX8 has been studied in C-cell-derived neoplasms with variable results ranging from negativity to focal positivity (13, 23, 24, 27). In doubtful cases, positivity for medullary cancer markers (calcitonin, synaptophysin and chromogranin) is diagnostic (28).

High-grade lymphomas closely mimic ATCs on FNA (29). It is noteworthy that the type of PAX8 antibody used may be important. In fact, the polyclonal antibody cross-reacts with the PAX5 N-terminal region, stains lymphoma cells and leads to a misdiagnosis (12, 30). Conversely, the MAB that we used recognises the more specific C-terminal region, which differs widely among the PAX gene products (12). Thus, negative staining with the PAX8 MAB and lymphoid marker (e.g. LCA, CD20 and CD3) positivity suggest a high-grade lymphoma on undifferentiated thyroid FNA (Fig. 5).

Among the most frequent thyroid metastases, only renal cell carcinomas (RCCs) express PAX8. The morphology and immunophenotype of RCCs usually do not overlap with those of ATCs (31); only the rare sarcomatoid variant may be similar. In this setting, the PAX2 marker stains RCCs, but not ATCs (32).

In conclusion, this study has shown that PAX8 is a useful immunocytochemical tool that can aid cytopathologists in the diagnosis of ATC and hence initiate correct management of this aggressive disease.

**Declaration of interest**

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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