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Combined Papillary and Mucoepidermoid Carcinoma of the Thyroid Gland: a Possible Collision Tumor Diagnosed on Fine-Needle Cytology. Report of a Case with Immunocytochemical and Molecular Correlations

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Abstract Fine-needle cytology (FNC) is frequently used to diagnose thyroid nodules discovered by palpation or imaging studies. Molecular tests on FNC material may increase its diagnostic accuracy. We report a case of a classic papillary thyroid carcinoma combined with a mucoepidermoid carcinoma correctly identified on FNC. The papillary component had a classic immunophenotype (CK19+, TTF1+), while the mucoepidermoid one was only focally CK19+. Point mutations (*BRAF* and *RAS*) and rearrangements (*RET*/PTC) of the papillary component have been also investigated on FNC samples, with resulting concurrent rearrangements of *RET*/PTC1 and *RET*/PTC3, but no point mutations. The histogenesis of combined papillary and mucoepidermoid carcinoma of the thyroid still remains partly unsettled, and further genomic studies are needed to shed some more light on this peculiar neoplasm.

Keywords Fine-needle cytology · Papillary thyroid carcinoma · *RET*/PTC rearrangement · *BRAF* and *RAS* point mutations

Introduction

Thyroid cancer is the most common endocrine malignancy. Fine-needle cytology (FNC) currently represents a reliable, accurate, and cost-effective diagnostic tool for the evaluation of thyroid nodules and for the management of nodular disease [1]. Testing FNC samples for a panel of molecular markers significantly increases the diagnostic accuracy of traditional cytology [2, 3]. We report a case of a 34-year-old woman who was diagnosed with a concurrent papillary thyroid carcinoma (PTC) and a mucoepidermoid carcinoma (MEC) based on FNC, whose diagnosis was supported by histology as well as immunocytochemistry. A panel of somatic mutations and rearrangements was also tested on FNC material in order to investigate on the molecular aspect of the PTC component of such tumor.

The current World Health Organization (WHO) classifies these neoplasms into two different categories: PTC belongs to the group of well-differentiated carcinomas of follicular cell origin, whereas MEC has been encompassed within the group of epithelial tumors of different or uncertain cell origin [4]. This may change in the future as new molecular studies will emerge.

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Table 1 Sequences of primers of GAPDH, RET/PTC1, and RET/PTC3 gene rearrangements

Gene	Sequence 5′—>3′
RET/PTC1 forward	ATTGTCATCTCGCCGTTC
RET/PTC1 reverse	CTTTCAGCATCTTCACGG
RET/PTC3 forward	TGGAGAAGAGAGGCTGTATC
RET/PTC3 reverse	CGTTGCCTTGACCACTTTTC
GAPDH forward	CCCTTCATTGACCTCAACTACATG
GAPDH reverse	TGGGATTTCCATTGATGACAAGC

Case Presentation

A 34-year-old woman was referred to our institute because of a firm and fixed nodule in the isthmic region of the thyroid. The patient underwent an ultrasound-guided FNC using a 23G needle without suction. The slides were stained with Diff Quik™ and Papanicolaou. On-site microscopic evaluation of the Diff Quik™-stained slides disclosed a conventional PTC combined with a MEC. Two more FNC samples were collected, and the needle hubs were immediately washed in a 10-μL β-mercaptoethanol solution for subsequent genetic tests.

Immunocytochemistry was carried out for thyroid transcription factor-1 (TTF1) and cytokeratin 19 (CK19). Two months later, the patient underwent total thyroidectomy and immunohistochemical analyses were performed for TTF1; human thyroglobulin (hTg), and carcinoembryonic antigen (CEA). Dedicated FNC samples were tested for gene mutations (*BRAF* and *RAS*) and rearrangements (*RET*/PTC). Of RNA, 0.5 μg was reverse transcribed into cDNA and the applicability of the obtained RNA samples was verified analyzing the housekeeping gene GAPDH.

Table 2 Sequences of primers of B-RAF, H-RAS, K-RAS, and N-RAS genes point mutations

Gene	Sequence 5′—>3′
B-RAF ex15 forward	CTCATCCTAACACATTTCAAGCC
B-RAF ex15 reverse	CTATAGTTGAGACCTTCAATGACTTTC
H-RAS ex1 forward	TCCTTGGCAGGTGGGGC
H-RAS ex2 reverse	CTCACGGGGTTCACCTGTACTG
K-RAS ex 1 forward	GGTACTGGTGGAGTATTTGATAGTG
K-RAS ex 1 reverse	GGTCCTGCACCAGTAATATGC
K-RAS ex 2 forward	TCCCTTCTCAGGATTCCTACAGG
K-RAS ex 2 reverse	CCCACCTATAATGGTGAATATC
N-RAS ex 1 forward	AGAACCAAATGGAAGGTCAC
N-RAS ex 1 reverse	GTGAGAGACAGGATCAGGTC
N-RAS ex 2 forward	TGAGGGACAAACCAGATAGGC
N-RAS ex 2 reverse	CTGTAGAGGTTAATATCCGCAAATG

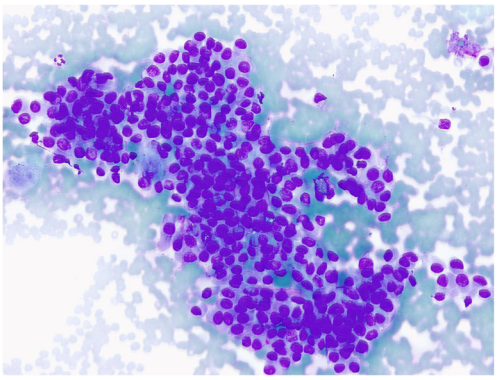

Fig. 1 FNC sample. Papillary carcinoma (PTC) component. A branching sheet of thyrocytes with evident nuclear grooves and micronucleoli can be observed in a necrotic background (DQ, ×400, original magnification)

The primers employed for GAPDH, *RET*/PTC1, and *RET*/PTC3 gene rearrangements are reported in Table 1.

Table 2 shows the primers for *BRAF* gene exon 15 and for point mutations in codons 12/13 and codon 61 (hot spots) of the H-RAS, K-RAS, and N-RAS genes.

PCR amplifications were performed and gel-controlled in the same way in both rearrangements and point mutations. All PCR products were finally analyzed by capillary electrophoresis.

Cytologic Findings

The cytological examination revealed a peculiar epithelial malignancy displaying a mucinous background with necrosis, histiocytes, and lymphoid cells in different maturation stages. The tumor had both aspects of classic PTC and MEC.

The papillary component showed branching sheets and papillaroid clusters of cuboid cells with the typical nuclear features of PTC (ovoid nuclei with finely granular heterochromatin, occasional micronucleoli, nuclear grooves, and intranuclear cytoplasmic inclusions) (Fig. 1). MEC component was made up by strips, sheets, and loose groups of

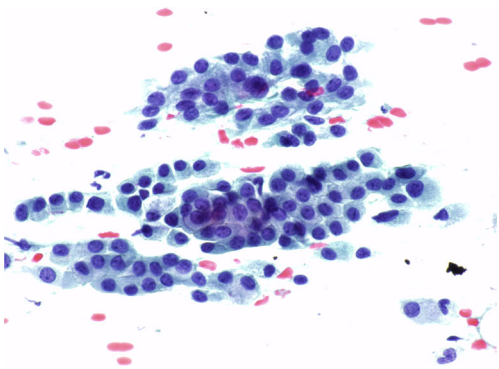

Fig. 2 FNC sample. Mucoepidermoid (MEC) component. Some monolayered sheets of neoplastic cells of cubical shape can be seen. Notice the basal position of nuclei, with well-defined, finely vacuolated cytoplasms often aligned to define a luminal surface (*left* and *right* side of the picture). Papanicolaou, ×400, original magnification

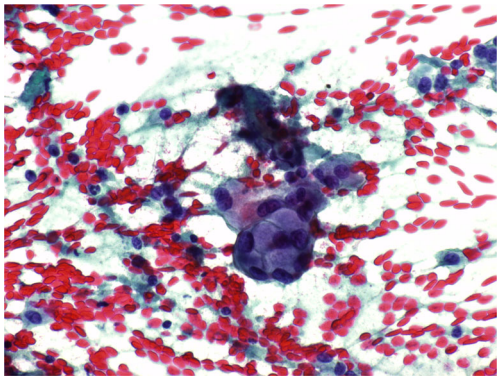

Fig. 3 FNC sample. MEC component. A small nest of neoplastic cells with goblet cell formation can be seen. Papanicolaou, ×600, original magnification

cubic-shaped cells with finely vacuolated cytoplasms in which the nuclei had basal position. These latter had vesicular chromatin structure with one or two small nucleoli; the cytoplasms tended to a triangular or polygonal shape, with their apical portions forming a continuous luminal border (Fig. 2). Sometimes, small nests of mucinous cells were found to have goblet cell differentiation (Fig. 3). Next to well-differentiated mucinous cells, several groups of neoplastic cells with squamoid differentiation (Fig. 4) or clusters of so-called intermediate cells (Fig. 5) could be observed.

Immunocytochemical staining showed a marked and diffuse nuclear positivity for TTF1 in both papillary and mucoepidermoid components (Fig. 6) and a membranous positivity for CK19 in the portion morphologically identifiable as classic PTC, while it was only focally expressed in the MEC component (Fig. 7).

Histologic Findings

A peculiar composite malignancy was found, which consisted of a minor component of classic PTC with psammoma bodies and small papillae and a predominant moderately/well-differentiated MEC component, in a context of a severe chronic thyroiditis (Fig. 8). The low-grade MEC focally infiltrated the peri-thyroid fat tissue.

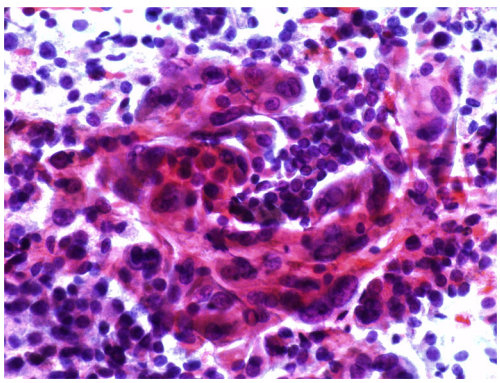

Fig. 4 FNC sample. MEC component. A loose cluster of large neoplastic cells (image center) with evident nuclear atypias and heavily orangiophilic (keratinized) cytoplasms can be seen admixed to smaller mucinous neoplastic cells. Papanicolaou, ×400, original magnification

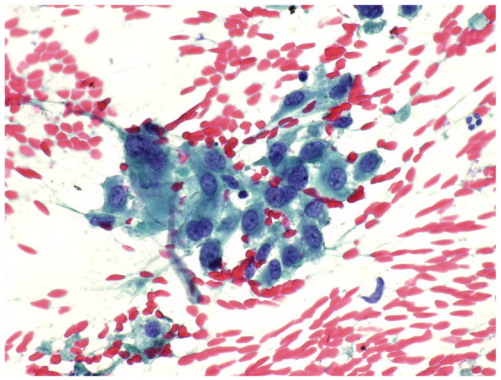

Fig. 5 FNC sample. MEC component. A loose sheet of medium-sized neoplastic epithelial cells show intermediate features between mucinous and squamous differentiation (so-called intermediate cells). Notice the presence of larger, ovoid nuclei with small nucleoli and occasionally folded nuclear membrane, with moderate to ample, well-defined glassy cytoplasms. Papanicolaou, ×600, original magnification

Immunohistochemistry revealed positivity for TTF1 in both papillary and mucoepidermoid areas, CEA was negative in the papillary areas but positive in the mucoepidermoid ones, hTg stained only the papillary part, and Alcian-Pas staining documented vast lakes of mucin-positive neoplastic cells in the context of the neoplasm (Fig. 9).

Molecular Findings

The sequence analysis showed neither mutations in *BRAF* gene nor point mutations in codons 12, 13, and 61 of H-RAS, K-RAS, and N-RAS genes, but it revealed the simultaneous presence of *RET*/PTC1 and *RET*/PTC3 rearrangements (Fig. 10).

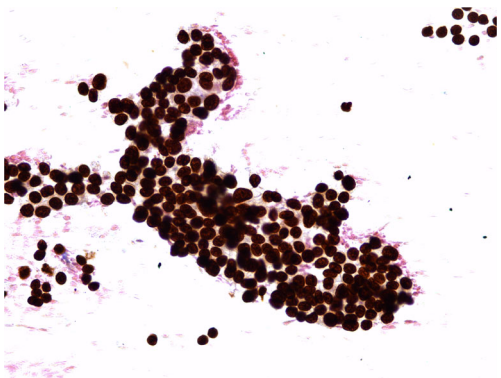

Fig. 6 FNC sample. PTC component. A branching group of thyrocytes shows diffuse nuclear positivity for TFF-1. Immunoperoxidase, ×600, original magnification

Fig. 7 FNC sample. MEC component. A linear sheet of neoplastic mucinous-type cells shows membranous positivity for CK 19. Notice that the apical part of the cytoplasm does not stain for CK 19. Immunoperoxidase, ×400, original magnification

Discussion

FNC represents the gold standard for the differential diagnosis of thyroid nodules [5]. Not only molecular testing of FNC samples for gene mutations and rearrangements helps to improve the diagnostic accuracy of this technique, but it also allows a better stratification of the high-risk patients for clinical management [6].

A number of combined PTC and MEC carcinomas have already been described in the past [7–13]; nevertheless, the increasing number of these entities has not been enough to grant a definite position for these lesions in the WHO classification. Basically, two sets of hypotheses have been put forward: one is in favor of the origin of these tumors from ultimobranchial bodies and/or solid cell nests [14, 15] and the other one is more in favor of metaplastic changes in well-differentiated thyroid carcinoma [16–18]. In our case, the papillary component was clearly of follicular cell origin, both immunocytochemically (TTF1 and CK19 positive) and under

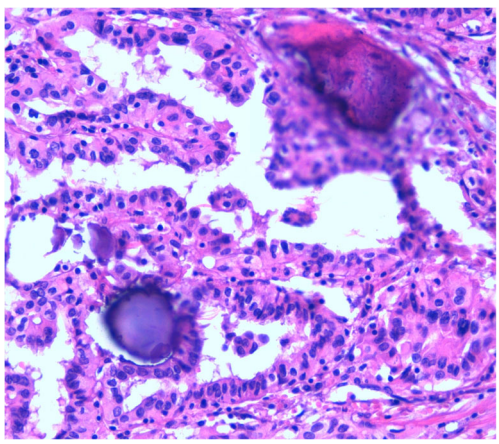

Fig. 8 Surgical sample. A composite neoplasm is shown, made up of a classical papillary carcinoma component with a psammomatous calcification (*right half* of the image) and of a classical low-grade mucoepidermoid carcinoma (lower and left part of the image). H and E, ×600, original magnification

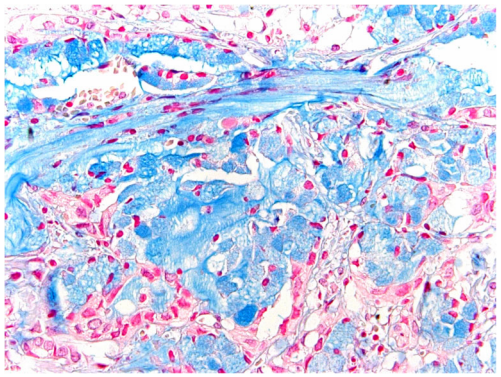

Fig. 9 Surgical sample. Alcian-Pas staining shows wide mucin pools within the neoplasm (×600, original magnification)

its molecular biologic profile. PTC is associated with constitutive activation of the *RET-RAS-RAF-MAPK* pathway, which transduces potent mitogenic and cell survival signals [19, 20]. Pathway activation is usually caused by point mutations in the *BRAF* or *RAS* family genes or *RET*/PTC gene rearrangements. These molecular alterations are mutually exclusive since activation of any single protooncogene confers uncontrolled functioning of downstream effectors [21]. The mutual exclusion also indicates that each of these mutations is independently sufficient to initiate thyroid tumorigenesis [22–24]. Our results showed neither *BRAF* nor *RAS* point mutations of the specific genes but a simultaneous presence of *RET*/PTC1 and *RET*/PTC3 rearrangements.

Double rearrangements have been already reported in previous studies [25, 26]. This simultaneous expression suggests that the additional variant of *RET*/PTC can arise as a progression within a single tumor or that papillary thyroid carcinoma can be of oligoclonal origin [27–29]. Although the latter hypothesis is more likely, further studies will be necessary not

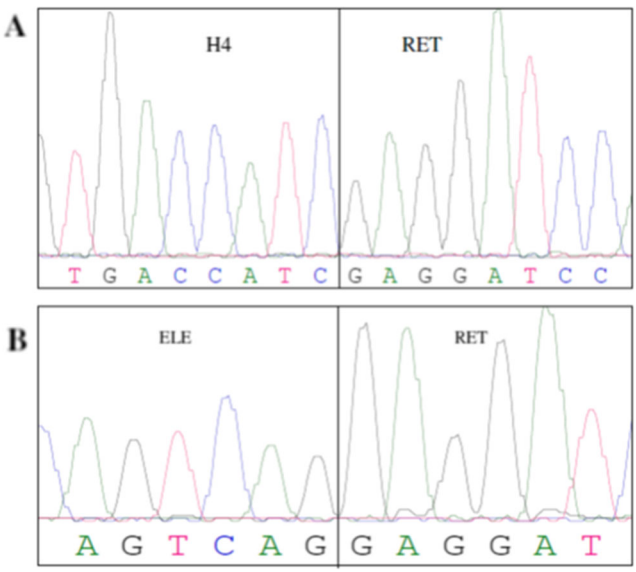

Fig. 10 The sequences show *RET*/PTC1 (**a**) and *RET*/PTC3 (**b**) rearrangements

only to determine the origin and significance of the simultaneous presence of two or more *RET*/PTC rearrangements but also to clarify the genesis and the subsequent classification of combined tumors.

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Conflict of Interest The authors declare no conflict of interest regarding the production of this article. The authors have no personal financial or institutional interest in any of the drugs, materials, or devices described in this article.

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