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IMMUNOHISTOCHEMICAL AND MOLECULAR MARKERS IN THE DIAGNOSIS OF WELL-DIFFERENTIATED FOLLICULAR-DERIVED THYROID TUMORS
-ABSTRACT-

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Introduction

Thyroid cancer accounts for approximately 1% of all malignancies in developed countries. Although rare, studies have reported increasing incidence of thyroid cancer in USA, Canada, as well as Europe in the last 40 years. This growing incidence is almost entirely attributed to papillary thyroid carcinoma (PTC), the most common thyroid malignancy, whereas the rates of follicular (FC), medullary and anaplastic thyroid carcinoma did not change significantly.

The diagnosis of well-differentiated follicular-derived thyroid tumors rests primarily on morphological examination. However, the pathologist is often facing difficult situations in which the distinction between a benign or malignant lesion is extremely difficult, all these with important consequences for the management and follow-up of the patients.

Many authors have explored immunohistochemical and molecular markers as a complement to the morphological criteria for the diagnosis of these tumors. However, controversies exist over the best antibody or panel of antibodies, over the best molecular markers, and, to present date, a clear consensus regarding the place of ancillary techniques in the diagnosis of the difficult cases does not exist.

My thesis is structured into three distinct parts.

1. Incidence trends of well-differentiated follicular-derived thyroid tumors in Mureş country: a 20 year retrospective study (1990-2009)

In the first part of my thesis, **the aim** was to describe recent trends in the incidence rates of well-differentiated follicular-derived thyroid tumors (PTC, FC and thyroid tumors of uncertain malignant potential) in Mureş County in the last 20 years (1990-2009), in an attempt to better explain the evolution and major changes in the epidemiology of these tumors. Our study demonstrated increasing trends in the incidence of PTC in the last 20 years, with a statistically significant increase of the follicular variant of PTC (FVPTC)'s incidence, as compared to classic PTC (CPTC)'s incidence. These results can be explained by a better understanding and description of the morphological criteria of the FVPTC. An increased incidence of papillary microcarcinomas was also noticed, translating major changes in sampling techniques, with an increase in the detection of small tumors.

2. Immunohistochemical markers in the diagnosis of well-differentiated follicular-derived thyroid tumors: the promising role of combined immunostaining using HBME-1 and CD56. A tissue microarray analysis.

In the second part of my thesis, **the aim** was to evaluate the diagnostic value of five antibodies and their possible combinations (HBME-1, Galectin-3, CK19, CD56, p63) in a very large series of unequivocal PTC (including FVPTC) and FC cases. The results obtained from this first series of patients were then applied to assess the expression and the possible diagnostic role of these markers in 18 cases of thyroid tumors of uncertain malignant potential.

This work was performed at the Department of Pathology, Centre Hospitalier Lyon Sud (HCL, Lyon, France) and it is the result of a multidisciplinary collaboration between the Rhone-Alpin Thyroid Cancer Registry and its affiliated institutions from Lyon, France (Department of Pathology, Surgery, Endocrinology and Clinical Research, Hospices Civils de Lyon) and the Department of Histology, University of Medicine and Pharmacy, Targu-Mures, Romania.

Material and methods: We performed an immunohistochemical analysis on a tissue micro-array of 204 PTCs (98 CPTCs, 90 FVPTCs, 16 other variants), 2 FC and 18 thyroid tumors of uncertain malignant potential.

Results: HBME-1 was the most sensitive marker, staining 95.9% of CPTCs and 81.1% of FVPTCs. CD56, a marker whose expression is reduced or absent in thyroid carcinoma, revealed a negative, "malignant" profile in 93.9% of CPTCs and 73.3% of FVPTCs. Galectin-3, CK19 and p63 were positive in 64.7%, 45.6% and 6.9% of PTCs, respectively. The immunopanel consisting of HBME-1, CD56 and/or CK19 reached the highest sensitivity (95.6%). The co-expression of 2 or more proteins was observed in 88.2% of PTCs, with HBME-1 and CD56 being the most frequent positive association (79.4%). Thyroid tumors of uncertain malignant revealed more heterogeneous immunohistochemical profiles, with 7/18 and 10/18 cases staining positively for HBME-1 and CD56, respectively.

Conclusion: We report a new panel of antibodies consisting of HBME-1, CK19 and CD56, that was found to be highly sensitive for both CPTC and FVPTC. To the best of our knowledge, this is the first time that this three-marker panel has been reported in the literature and used in a very large series of PTCs. In our opinion, these results could be of particular interest as a complement to the morphological criteria in the diagnosis of difficult FVPTC cases. Thyroid tumors of uncertain malignant potential demonstrated very heterogeneous immunohistochemical profiles, suggesting that immunohistochemistry is still to be regarded more as a supporting factor, while morphological criteria should always prime in the diagnostic decision for these tumors.

3. Detection of *BRAF* V600E mutation in thyroid Fine Needle Aspiration specimens by High Resolution Melting (HRM) analysis

The third part of my thesis was focused on molecular markers in the diagnosis of well-differentiated follicular-derived thyroid tumors. **The aim** was to test the feasibility of a new, innovative PCR (polymerase chain reaction) method, High Resolution Melting (HRM) analysis for detection of *BRAF* V600E mutation in FNA (fine-needle aspiration) specimens from patients with PTC.

Material and methods: We analyzed fresh thyroid aspirates and smears from eight cases of PTC (3 CPTCs, 3 FVPTCs, 1 tall cell and 1 oncocytic variant of PTC). DNA extraction was

performed using a MasterPure purification kit. The isolated DNA quantity was assessed using a NanoDrop spectrophotometer and the DNA quality was tested by PCR amplification of beta-globine gene and by native DNA electrophoresis. HRM was performed on a LightCycler 480 (Roche). We amplified the 15th exon of BRAF gene, using selected primers to flank the *BRAF* V600E mutation point.

Results: For all types of cytological specimens, the quantity of isolated DNA was adequate and allowed amplification. Similarly, the DNA quality control did not show signs of DNA degradation and the DNA was amplifiable for β-globine gene. Four cases revealed the *BRAF* V600E mutation (2 CPTCs, 1 oncocytic PTC, 1 tall cell PTC). None of the three cases of FVPTC had this mutation.

Conclusion: Our results are original due to the novelty of the technique used for the detection of *BRAF* V600E mutation (the HRM analysis) in FNA specimens, but also for clarifying several aspects related to the diagnostic and prognostic role of *BRAF* V600E mutation in papillary thyroid carcinoma.

HRM analysis represents a feasible and reproducible molecular technique, offering new perspectives for detecting *BRAF* mutation in various FNA specimens. In our study *BRAF* V600E mutation revealed a strong association with specific histological variants of PTC: highly specific for CPTC, tall cell and oncocytic PTC, but negative in all cases of FVPTC.

Keywords: thyroid cancer, papillary; immunohistochemistry; HBME-1 antigen; CD56 antigen; thyroid fine needle aspiration biopsy; *BRAF* V600E.