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Research Article

Current and future markers for the diagnosis of thyroid cancer

Descamps Géraldine[1], Saussez Sven[1,2*] and Journe Fabrice[1,3]

[1]*Laboratory of Human Anatomy and Experimental Oncology, University of Mons, Belgium*
[2]*Department of Oto-Rhino-Laryngology, Université Libre de Bruxelles (ULB), CHU Saint-Pierre, 1000 Brussels, Belgium*
[3]*Laboratory of Oncology and Experimental Surgery, Institut Jules Bordet, ULB, Belgium*

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ABSTRACT

Today, immunohistochemical markers are routinely used alone or in association to examine thyroid lesions but without sufficient sensitivity and specificity regarding to cancer diagnosis. Additional markers are currently identified among genetic alterations or miRNA panels carrying significant diagnostic values. Combining immunostaining data, mutation status, gene rearrangement and miRNA expression should help to define an integrative signature for the accurate diagnosis of thyroid carcinomas.

Introduction

Whereas thyroid nodules are frequent pathologies of thyroid gland, thyroid cancers are quite rare (<10%) [1-2]. Thyroid FNA and subsequent cytopathological evaluation are the most common preoperative techniques used for the diagnosis of malignant thyroid tumors. However, this invasive procedure showed inconclusive results in 10-20% of cases and many benign lesions with suspicious of cancer are thus abusively referred to surgery [3]. In particular, the diagnosis of follicular lesions is always challenging to clinicians since cytological features overlap between follicular adenoma and carcinoma.

In this context, increasing number of studies had evaluated immunohistochemical markers, such galectin-3, CK19, HBME-1, TPO; DNA alterations, mainly including BRAF and RAS point mutations, RET/PTC and PAX8/PPARg rearrangements; miRNA signatures and circulating tumor cells for diagnosis of thyroid cancers [4-6, 8-12]. While some markers are promising for differential diagnosis, none of them is individually conclusive because of limitations due to significant prevalence in benign thyroid nodules to a notable extent.

Immunohistochemical markers

Among recent evaluations of new IHC markers, galectin-1 was reported to display a higher specificity (97%) than galectin-3 and CK19 which showed higher sensitivity (97% and 98%, respectively), showing complementary diagnosis values [13]. In addition, Ki67 was also found as a suitable single marker for distinguishing carcinomas from benign thyroid diseases [14]. Combinations of markers were evaluated to improve differential diagnosis, especially regarding the classification of thyroid follicular lesions [15-17]. The association of positivity for galectin-3, CK19 and HBME-1 proved to be the most relevant combination (97% specificity, 95% sensitivity) in the distinction between PTC and FA [18]. Additional new marker combinations are currently opening novel opportunities as diagnosis tools. Combined positive emerin (including nuclear level changes) and negative CD56 (lost in cancer) showed 72% sensitivity and 100% specificity and could be additional helpful markers in overlapping cases with high diagnostic validity (high specificity and PPV) [19]. Similarly, the differential expression of TROP-2 and SLP-2 has been evaluated by quantitative PCR and IHC in PTC and FA and results showed that marker expressions were significantly increased in carcinomas. Moreover, when TROP-2

Correspondence to: Sven Saussez, MD, PhD, Department of Human Anatomy and Experimental Oncology, Faculty of Medicine and Pharmacy, University of Mons, Pentagone 2A, Avenue du Champ de Mars, 6, B-7000 Mons, Belgium; Email: sven.saussez@umons.ac.be

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were combined to CD56, the sensitivity and NPV increased to 100% and had a better diagnostic accuracy, further supporting the interest for CD56 [20]. Again, NRG1 was also recently reported to be highly expressed in PTC compared with adjacent normal tissues [21].

Genetic alterations

In parallel to immunoprofiling, significant progress has been made in developing molecular markers for clinical use in FNA materials, including gene mutation panels and gene expression signatures [22]. For examples, TERT promoter and TP53 mutations are more frequent in less differentiated carcinomas, potentially helping in the thyroid cancer classification [23-25]. Clinical tests include 3 approaches to investigate the molecular profile of FNA: (1) the seven genes panel, (2) the Afirma classifier, and (3) the NGS assays [26]. Indeed, a test detecting alterations in seven main genes responsible of 70% of all thyroid cancers was designed, but despite a good sensitivity, the NPV was too low for clinical use [27-28]. In order to assess the risk of cancer in ITN, the Afirma test was introduced to distinguish "benign" from "suspicious" nodules, based on the analysis of a 142 gene expression profile [29].

Finally, the NGS detect various types of genetic alterations in a small amount of cells with high sensitivity [30-32]. In this context, the Thyroseq test identifies several point mutations, hotspot mutations in 14 genes, 42 types of gene fusions and expression levels of 16 genes. Thyroseq is reported to have a NPV of 96-97% and a PPV of 77-83% [33, 34]. Recently, Taye et al. also evaluated the Thyroseq v2 performance in 156 ITN and conclude that it is likely to be an appropriate "rule out" test (NPV >95%) but they warn with the positive results and advice to refine the result based on the mutation and histological type. Indeed, the PPV is dependant of cancer prevalence in patient populations [2]. Their observations agree with those of two other studies that have highlighted an NPV of 91-94% and a PPV of 42-66% [35, 36]. Lastly, a bioinformatic analysis has identified seven-hub genes, including FN1, SERPINA1, ECM1, MMRN1, PROS1, CFD and TIMP1, which may helpful for the development of gene panel for thyroid nodule diagnosis [37].

Micro RNA panels

In addition to gene expression signature, miRNA profiling was further developed as an important main for the diagnosis of thyroid carcinomas [38]. The mechanisms of miRNA implication in cancer development are linked to the downregulation of tumor suppressor genes or the upregulation of oncogenes. As for previous markers, a set of multiple miRNAs seems to be more sensitive (87%) than a single miRNA (71%) although there is a discrepancy in terms of set of miRNA proposed among studies [39, 40]. Nevertheless, a set of 15 miRNAs emerge as the more powerful diagnostic panel for indeterminate lesions [26]. Of course, future prospective and retrospective researches are recommended on a larger cohort of indeterminate lesions to validate the diagnostic value of this panel. A meta-analysis reported a significant difference in miRNA-221/222 expression in thyroid cancer compared to normal thyroid or benign thyroid lesions, further supporting miRNAs as promising molecular markers to improve diagnosis of thyroid cancer [41]. In this context, two additional diagnostic tests using miRNAs were introduced to predict malignancy in thyroid nodules. The first one, ThyGenX/ThyraMIR, combines a 7-gene mutation panel and a group of 10 miRNA markers. The second one, Rosetta GX Reveal test, is based on the detection of 24 miRNA markers. In addition, a study aiming to compare the performance of both tests on 10 FNA by correlating the results to histopathology data showed a 100% NPV while RosettaGX disclosed a 75% PPV in comparison to 60% for ThyGenX/ThyraMIR [42]. Finally, a review of the 4 commercially available molecular tests concludes that they offer unique approaches to improve the risk stratification of thyroid nodules [43]. Interestingly, the study of Mazeh et al. reinforces the interest of these technologies combining NGS and miRNA since they were able to classify ITN with the greatest accuracy in comparison with the molecular tests currently marketed [44].

Conclusions

Nowadays, novel marker panels/signatures are always under validation step to define additional criteria for the distinction between thyroid lesions, especially regarding the classification of thyroid follicular lesions. Based on meta-analyses, new combinations of markers including immunohistochemical protein detection, genetic alteration evaluations (mutation and rearrangement) and miRNA levels (up/down regulation) should be assessed and validated in a large series of tissues to propose an integrative signature with the highest sensitivity and specificity to improve the diagnosis and the management of thyroid cancer diseases.

Abbreviations

FNA:	Fine-needle aspiration
IHC:	Immunohistochemical
HBME-1:	Hector battifora mesothelial-1
TPO:	Thyroid peroxidase
CK19:	Cytokeratin 19
PTC:	Papillary thyroid carcinoma
FA:	Follicular adenoma
TROP-2:	Trophoblast cell surface antigen-2
SLP-2:	Stomatin-like protein-2
PCR:	Polymerase chain reaction
NRG1:	Neuregulin 1
miRNA:	micro RNA
TERT:	Telomerase reverse transcriptase
TP53:	protein 53
NGS:	Next generation sequencing
NPV:	Negative predictive value
PPV:	Positive predictive value
ITN:	Indeterminate thyroid nodule
FN1:	Fibronectin
SERPINA1:	Serpin peptidase inhibitor clade A member 1
ECM1:	Extracellular matrix protein 1
MMRN1:	Multimerin 1
PROS1:	Protein S
CFD:	Complement factor D
TIMP1:	Tissue inhibitors of metalloproteinases

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