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Mini-Review

Clinical usefulness of immunocytochemical and molecular markers in fine needle aspiration cytology

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ABSTRACT

Thyroid cancer is present in about 5% of thyroid nodules. Generally it is a well differentiated cancer originating from follicular epithelium. Undifferentiated thyroid carcinomas and medullary thyroid carcinomas arising from C cells are less frequent. The fine needle aspiration cytology (FNAC) allows the diagnosis of nature of thyroid nodules in the majority of cases, but it has some limitations particularly in presence of follicular lesions. Several immunocytochemical and molecular markers have been proposed to ameliorate diagnostic accuracy of this procedures, but only few of them could be employed in routine clinical practice. It is necessary find not only new markers, but also new methods which could be used in routine clinical practice.

KEYWORDS: thyroid cancer, immunocytochemical markers, molecular markers, FNAC

INTRODUCTION

Differentiated thyroid carcinoma is the most frequent endocrine neoplasm. About 7% of adults present palpable thyroid nodules during their lifetime [1]. Ultrasound imaging shows nodules in more than 50% of 65 year old people [2]. Thyroid nodules are benign in most of the cases; 5-15% are malignant lesions [3]. For this reason pre-surgical diagnostic tests are needed. Actually fine needle aspiration cytology (FNAC) is the first choice and the most used procedure. It allows the differential diagnosis of malignant and benign lesions in most of the cases, even though 10 to 40% of all specimens are diagnosed as indeterminate for malignancy, and are often submitted to diagnostic emithyroidectomy [4]. This is due to the fact that cytological features of follicular thyroid lesions are not different enough to distinguish malignant from benign nodules.

The indeterminate cytology has been recently classified into:

1. indeterminate follicular lesion
2. follicular neoplasm or oncocytic neoplasm (Hürtle cells neoplasm)
3. suspicious for malignancy
   with a probability of malignancy about 5%-20%, 20-30% and 50-75%, respectively [5].

The effect of this new classification in the clinical management of patients affected with thyroid nodules has been defined. Actually thyroid nodules are malignant in 8-56% at pathology [6]. For this reason surgery is often unnecessary due to increased morbidity and higher costs [7]. Furthermore, patients with indeterminate cytology are usually submitted to emithyroidectomy followed by controlateral lobectomy, if the nodule is malignant at first pathology. Whereas, 1 to 3% of benign nodule at cytology are false negative and the late diagnosis could increase the risk of progression of the disease before a definitive diagnosis [8].

Hence, further methods are needed to increase the sensitivity and the specificity of cytology with consequent improvement of clinical management of these patients.

Thyroid cancer has generally a favorable prognosis with a 90% survival rate after five years from diagnosis [9]. Prognosis is strictly conditioned to

the time of diagnosis, the initial treatment, age and histotype. For this reason a precocious diagnosis is important to determine further evolution of the disease. Biological markers of thyroid malignancy are necessary to ameliorate the accuracy of the diagnosis and prognosis.

A large number of molecular and immunocytochemical markers have been investigated to overcome the difficulties to distinguish benign from malignant thyroid lesions, but the clinical implications have been demonstrated only for some of them. Beyond their diagnostic value, these biomarkers should be able to offer prognostic criteria and may also play a role in detecting persistent or recurrent disease as well as in choosing the therapeutic strategies.

Diagnostic value of immunocytochemical markers

Immunocytochemistry is a method that is able to identify specific proteins on FNA samples. The expression of actually known biomarkers is extremely variable in specificity and sensitiveness and, for this reason they are not often used in cytology. Immunocytochemical markers have been particularly used in the diagnosis of papillary thyroid carcinomas (*PTCs*), whereas there are no verified biomarkers to facilitate the diagnosis of follicular thyroid carcinomas (FTCs).

The biomarkers that seem to show higher sensitivity and specifity are Hector Battifora mesothelial cell-1 (HBME-1), high molecular weight cytokeratine19 (CK19) and galectin-3 [10].

HBME-1 is a monoclonal antibody generated against the microvillus surface of mesothelial cells of mesothelioma. HBME-1 has been reported to be present in most cases of *PTCs* and negative in benign lesions. So it is specific for *PTCs*, but it has a low sensitivity, particularly in presence of oncocytic cells and a negative result does not preclude the diagnosis of carcinoma [11].

CK-19 is a high-molecular-weight cytocheratin showing a strong sensitivity but a low specificity for PTC. The presence of CK19 in borderline lesions supports the diagnosis of carcinoma, but *CK19* was also found in peritumoral tissue and in benign nodule, thus limiting the usefulness of this marker [12].

Galectin-3, a beta galactoside binding lectin, is strongly expressed in *PTCs*. Its expression was also observed in a number of FTC, and it has been considered to be of some value in differentiating between benign and malignant follicular nodules. Therefore immunocytochemistry with this marker was proposed as a support procedure to conventional cytology in diagnostic work-up of thyroid lesions [13]. However, it has a low specificity because of its reactivity also in benign nodules and thyroiditis. Furthermore, some studies have reported false negative results in specific lesions, such as Hurtle cells proliferations and minimally invasive follicular carcinomas [14]. Based on the literature, none of the above markers appears to be reliable in identifying all malignant thyroid lesions in a highly specific and sensitive manner and a “magic marker” has not been actually found [15]. The combination of two or three markers may represent a more accurate immune cytochemical approach in the differentiation of malignant tumors from their benign counterparts especially in the controversial categories [16]. Table 1 modified.

Diagnostic value of molecular markers

Molecular markers of thyroid are identified from genetic mutations arising in malignant thyroid cells and recognizable by the molecular biology techniques. Several molecular alterations (mutations and/or gene rearrangements) have been described in thyroid malignancies and it has been demonstrated that different genes and signaling pathways are involved in the development of *PTC* and *FTC*. The expression of each molecular marker can be studied on frozen specimens and cells using *PCR* techniques. The more common genetic alterations found in PTC are radio-induced RET/PTCs rearrangements and BRAF and RET genes’ mutations [17]. RAS mutations and the fusion gene *PAX8/PPARγ* have been frequently encountered in *FTC* [Table 2]. *BRAF* is a serine-threonine kinase involved in the mitogen-activated protein-kinase pathways. BRAF mutations represent the most common genetic alterations in *PTC* and it seems to be tumor specific, because it has never been reported in other histotypes [18]. *BRAF* point mutations at 600 (*BRAF600E*) and less frequently *BRAF* 599

Table 1. Differentiation between benign and malignant lesions using single marker or markers associations in thyroid cytology.

Single Marker	SN (%)	SP (%)	PV+ (%)	PV- (%)	AC (%)
GAL3	92	94	96	89	93
HBME-1	80	97	97	76	86
CK19	76	90	92	71	81
Combination of two markers					
GAL3 + HBME-1	97	90	94	96	94
GAL3 + CK19	99	84	90	98	93
HBME-1 + CK19	92	88	92	88	90
Combination of three markers					
GAL3 + HBME-1 + CK19	100	82	79	100	92
Sequence of markers					
GAL3 + HBME-1	97	96	-	-	-
GAL3 + CK19	99	89	-	-	-

SN: sensitivity; SP: specificity; PV+: positive predictive value; PV-: negative predictive value; AC: accuracy.

Table 2. Prevalence of molecular markers in differentiated thyroid carcinomas.

Histotype	Prevalence (%)
Papillary carcinoma	
BRAF	45
RET/PTC	20
RAS	10
Follicular carcinoma	
RAS	45
PAX8-PPARγ	35

and 601, resulting in constitutive activation of kinase pathways, were detected in 26-69% of sporadic *PTCs* of adults. Recently a *BRAF* rearrangement by paracentric inversion of chromosome 7q followed by fusion between *AKAP9* and *BRAF* genes has been recognized in a subset of radio-induced *PTCs* [19].

BRAF mutations were strongly associated to classic variant of *PTCs*, displaying the typical nuclear feature and the papillary architecture. They were observed also in tall cell or columnar cell variants.

In different studies, mutations of *BRAF* were associated with older age of patients, more advanced stage of disease at presentation and higher frequency of recurrence and/or metastases.

Moreover, a significant incidence of *BRAF* mutations was found in undifferentiated thyroid cancer, suggesting that *BRAF* 600 mutations are involved in tumor progression.

However, the relationship between *BRAF* mutations and more aggressive tumor behavior has not been confirmed in their studies and *BRAF* mutations have been observed also in microcarcinomas with a good prognosis [20]. These data confirm the key role of *BRAF* mutations in the developing of papillary carcinomas [21]. Methods for rapid analysis of these mutations have been developed [21].

The second more frequent genetic alteration in *PTCs* is *RET/PTC* rearrangement. Ret is a proto-oncogene, located on chromosome 10q11.2, encoding for a transmembrane tyrosine-kinase receptor. The rearrangements *RET/PTC* lead to a constitutive activation of the tyrosine kinase receptor *RET*, that activates some signaling cascades, thus promoting cell growth and transformation.

These rearrangements are more frequent in pediatric patients and in radio-induced *PTCS*. In fact, the *RET/PTC* rearrangement incidence is about 15-20%, while it has been found in 87% of *PTCs* post-Chernobyl. *RET/PTCs* rearrangements are restricted to *PTC* including both conventional

PTC and oncocytic and diffuse sclerosing variants, thus representing a marker for this type of thyroid tumor. For this reason the identification of *RET/PTCs* rearrangement on thyroid specimens has been proposed as a diagnostic auxiliary tool in cytological examination [22]. However its specificity has been a matter of discussion because of the identification of such alteration also in thyroiditis, in non neoplastic follicular cells, in oncocytic tumors and in other benign lesions and this expression variability should be taken into account for the molecular diagnosis of thyroid lesions.

Point mutations of RAS genes have also been identified in *PTCs.* They are not only present in thyroid *PTCs* but also in *FTCs* and in anaplastic thyroid carcinomas (*ATCs)*. These mutations seem to be more typical of follicular tumors than *PTCs.* The incidence of *RAS* mutations is variable in these different histotypes ranging from 0-50% in PTCs, 14-62% in FTCs and 0-60% in ATCs. Among PTCs, the follicular variant has higher prevalence of RAS mutations and less prevalence of *BRAF* mutations and *RET/PTCs,* compared to conventional *PT*Cs and other variants [23]. Moreover, RAS mutations have also been reported in follicular adenomas, with a frequency ranging from 0-85%. Nevertheless, a higher rate of *RAS* mutations has been observed in malignant lesions rather than in benign nodules. These observations suggest that *RAS* mutations could be related to chromosomal and genomic instability, thus predisposing follicular cells to the accumulation of additional molecular abnormalities.

However *RAS* mutations have no diagnostic significance in distinguishing the follicular adenoma from *FTC*.

PAX8/PPARγ fusion gene may represent another potentially molecular marker of follicular thyroid cancer. The *PAX-8-PPARγ* fusion protein includes the *DNA* binding domain of *PAX8* and the *PPARγ* nuclear receptor domain. These fusion genes frequently are observed in *FTC* and seem to be involved in progression of follicular adenomas (*AF*) to follicular carcinomas. In fact, only 10% of *AF* expresses this gene. *ATC* also expresses infrequently this rearrangement [24]. According to these results, the expression of *PAX8/PPARγ* could be considered as a marker of well differentiated follicular thyroid cancer and its absence as a marker of progression to less differentiated and more aggressive carcinomas.

Although the diagnosis of follicular carcinomas is usually difficult, the use of this marker is limited in the clinical practice because few clinical trials have been carried out to confirm the effectiveness of this biomarker [25].

CONCLUSIONS

We have reported the present knowledge about thyroid tumor markers which could ameliorate the accuracy of FNAC. Only a few markers could be used in routine clinical practice. Thus there is a strong need for new cytological markers which could distinguish benign follicular adenomas from thyroid malignancy. Hence it is necessary to find not only one or more markers but mainly new methods of identifications which could be employed in routine clinical practice.

CONFLICT OF INTEREST STATEMENT

All the authors declare that no conflicting interests exist.

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