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Mini Review

Clinical implications of molecular studies for the diagnosis of thyroid cancer

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The evaluation of patients with nodular thyroid disease is focused on two main aspects, namely the exclusion of thyroid malignancy and the exclusion of hyperthyroidism in regions with borderline iodine supply. However, the identification and preoperative typing of both the substantially less frequently occurring thyroid carcinomas (2-5% of all nodules) and the frequently occurring benign thyroid nodules (30% in the general population) is challenging. Currently, the typing of thyroid carcinomas is based on the morphologic criteria outlined by the WHO classification (Table 1).[1] Due to difficulties involved in clearly differentiating between carcinoma and benign nodular lesions, the categorization of certain thyroid tumours as tumours with low malignant potential has been proposed.[2] However, the molecular pathologic characterization of thyroid nodules seems to offer the opportunity of a more comprehensive definition of thyroid tumour subtypes.

Key words: Thyroid cancer, Follicular thyroid adenoma, Follicular thyroid carcinoma, Molecular studies

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Thyroid ultrasound is used to evaluate the size and structure of the thyroid and its nodules. It is also helpful to guide Fine Needle Biopsy (FNB) and to monitor changes in the size of thyroid nodules. However, the value of sonography to discriminate malignant from benign thyroid nodules is limited. Although ultrasound-based features like hypoechoic structure, presence of calcifications, irregular margins, absence of a halo, solid composition, and intranodular vascularity have been associated with an increased risk of thyroid cancer, particularly in the US American literature, the results differ considerably between studies; no single feature or combination of features offers both the sensitivity and high positive predictive value required to unanimously accept this method for the diagnosis of thyroid cancer.[3,4]

FNB, currently the most sensitive and specific tool for the preoperative identification of thyroid malignancy, also has substantial limitations. While approximately 75% of FNBs reveal benign lesions, and 5% malignancy, up to 20% of FNBs belong to the group of follicular proliferations [Follicular Adenoma (FA), Follicular Thyroid Carcinoma (FTC), or the Follicular Variant of Papillary Thyroid Carcinomas (FV-PTC)], which can be distinguished only histologically, thus necessitating thyroid surgery.[5] As a consequence, the percentage of malignancies among the resected thyroids with follicular proliferations is rather low (the highest reported thyroid carcinoma frequency with vigorous FNB selection of all operated nodules was 34%[6]) and hence the majority (up to 80% according

to Löwhagen et al) of the respective patients end up being operated on for a benign nodule.[7]

Whereas both the histologic diagnosis of non-minimally invasive FTC and the majority of FA show an acceptable inter- and intraobserver variability, the differential diagnosis of minimally invasive FTC and hypercellular (atypical) FA may be extremely difficult. Even the most objective histologic criteria for the diagnosis of minimally invasive FTC, e.g. vascular and/or capsular invasion, show high inter- and intraobserver variations.[8-10] Lang et al[11] demonstrated as much as 20 years ago that the examination of an adequate number of tissue blocks is required to reliably differentiate FTC from FA. These results clearly indicate that the diagnosis of FTC depends on both the experience and training status of the pathologist as well the pathologist's efforts to sufficiently investigate thyroid nodules. Hence, the limitations in the diagnosis of minimally invasive FTCs questions the validity of published results concerning the incidence, prognosis, treatment, and outcome of FTCs,[10] since the rate of distant metastasis and death due to follicular carcinomas is highly influenced by the degree of (angio-)invasion.[12,13] Thus, for a precise diagnosis of encapsulated follicular lesions, the use of molecular markers for improving the discrimination between minimally invasive follicular carcinoma and (hypercellular/atypical) follicular adenoma are most desirable.[8]

Although a growing number of immunohistochemical markers for the discrimination between benign and malignant thyroid tumours have been proposed over the last few years,[14] no one marker or marker combination is able to identify tumour aggressiveness.

One obvious reason for the difficulty in precisely discriminating FTC from FA is the limited knowledge about the molecular aetiology of Cold Thyroid Nodules (CTNs) or scintigraphically isocaptant nodules which are histologically defined as follicular adenomas or adenomatoid nodules, which comprise approximately 85% of all thyroid tumours. In contrast to CTNs and the scintigraphically isocaptant nodules, the molecular aetiology of autonomously functioning thyroid nodules is characterized by constitutively activating mutations of the TSH receptor in about 60% of the nodules.[15-17] In FTCs, both activating *RAS* mutations (in about 40-53% of FTCs[18]) and a fusion oncogene between *PAX8* and *PPARγ* (in about 25-63% of FTCs[19]) have been detected; both molecular alterations have been demonstrated, although less frequently, in FA, which hampers their use as markers of malignancy. The vast majority of PTC are characterized by mutations or re-arrangements along the MAPK signalling cascade [depending on the PTC subtype (Table 1), *RET*/*PTC* re-arrangements are found in 13-43%, and *BRAF* mutations in 29-69% of PTC[19]]. In the light of these findings the diagnostic potential of *RET*/*PTC* re-arrangements and *BRAF* mutations has been investigated in FNA samples in several studies.[20-27] The *BRAF* mutation was detected in 38-83% of the investigated FNA samples that were PTC on histopathology,[20,22,26] indicating that the screening for *BRAF* mutations in

Table 1. Tumours of the thyroid according to DeLellis et al. WHO Classification of Tumours 2004[1]

- Papillary carcinoma
  - Histopathological variants:
    - Follicular variant
    - Macrofollicular variant
    - Oncocytic variant
    - Clear cell variant
    - Diffuse sclerosing variant
    - Solid variant
    - Cribriform carcinoma
    - Papillary carcinoma with focal insular component
    - Papillary carcinoma with squamous cell or mucoepidermoid carcinoma
    - Papillary carcinoma with spindle and giant cell carcinoma
    - Combined papillary and medullary carcinoma
    - Papillary microcarcinomas
- Follicular carcinoma
  - Minimally invasive
  - Widely invasive
    - Cytological variants:
      - Oncocytic variant
      - Clear cell variant
- Poorly differentiated carcinoma
- Undifferentiated (anaplastic) carcinoma
- Medullary thyroid carcinoma
- Follicular adenoma

FNB samples may be a useful adjunct technique for the evaluation of thyroid nodules with indeterminate and suspicious cytological findings, taking into consideration that *BRAF* mutations demonstrate a high geographical variability. Thus, compared to most European countries with relatively high incidence of follicular lesions, the diagnostic impact of demonstrating *BRAF* mutations is considerably higher in countries with a high iodine uptake, e.g. Korea.[20,28,29] With respect to the histological subtype, *BRAF* mutations seem to be more common in the conventional variant of PTC than in the FV-PTC.[22,26]

Although the uncovering of the molecular aetiology of about 60% of follicular and papillary thyroid carcinomas did provide new perspectives for the classification and diagnosis of thyroid tumours, it became rapidly apparent that the incidence of detected somatic mutations in FTCs and PTCs varies in different studies[19] and that various methods of mutation screening (e.g. direct sequencing, denaturing gradient gel electrophoresis, LightCycler PCR, PCR-RFLP) are characterized by different sensitivities.[20,25,30,31] In fact, the use of very sensitive methods led to the detection of *RET/PTC* rearrangements, thought to be specific for papillary thyroid carcinoma, in Hashimoto's disease[32] and *PAX8/PPARγ* rearrangements, thought to be specific for thyroid follicular carcinomas, in some thyroid adenomas.[33-35] However, genetic events are certainly already taking place before malignancy is detectable by histology. Therefore, without knowing the precise molecular basis of thyroid carcinomas it is difficult to diagnose thyroid carcinoma by molecular markers.

Apart from standardization of tissue sample processing, one way out of the discrepancy between histologic and molecular tumour classification, and the methodological limitations of mutation screening, might be the diagnostic application of gene expression signatures. In the thyroid field this can be accomplished with the help of microarrays (for review see[36]). However, despite the fact that microarray studies revealed very distinct changes in the expression of certain genes, none of the genes, identified by array studies as differentially regulated, was proven to be an ideal single marker of PTC.[37-46] For example, *DPP4* (dipeptidyl-peptidase 4) shown to be the most up-regulated gene in PTC,[47,48] did not clearly differentiate between PTC and benign tissue.[49] Also, neither oncofibronectin, galectin 3, nor other proposed markers worked properly in a single gene context.[50-55] Therefore, a more promising approach to discriminate between benign and malignant tumours seems to be the generation and application of multigene classifiers. While a discriminating gene set comprising hundreds of genes is not applicable for diagnostic purposes, a powerful set (10 to 20) of discriminating genes generated by sophisticated bioinformatics approaches appears feasible for use in Real Time (RT)-PCR approaches. Interestingly, the 20 gene classifier discriminating PTCs from benign tissues, proposed by Jarzab et al,[48] does not contain many known genes found in other approaches like Fibronectin 1 (*FN1)* or *TIMP*, whereas some other genes previously known for their up-regulation in PTC (e.g. *DPP4, SERPINA1, LGALS3, MET*) and some new genes previously not described in PTC (e.g. *EVA1, LPR4* and *RXRG*) were included. While only some genes show a stable and very distinct difference in expression between PTCs and benign tissues, other genes included give slightly different results. These genes are overexpressed only in a subset of PTC and, in the case of lack of overexpression, the information is completed by the expression of other genes. Interestingly, this gene classifier did not only reliably classify different data sets of papillary carcinomas but also data sets of hot and cold thyroid nodules.[48,56] In other studies, malignant and benign samples could be differentiated by sets of only six genes.[55,57-59] A further possibility to improve the diagnosis in FNB with cytological findings suspicious for thyroid cancer might be the combination of measuring differentially expressed genes and detecting cancer specific mutations (e.g. *BRAF*, *RET/PTC*). A very promising study, based on the combined analysis of galectin-3 and the $BRAF^{V600E}$ mutation, has recently been published.[60] Other studies using a three or four gene profiling of thyroid nodules have accurately distinguished between FTC and FA.[61,62] Although most of these studies are very promising, almost all of them are based on the investigation of thyroid tissue samples. Therefore, the proposed markers need to be studied in FNB samples.

In conclusion, the currently established determinants of tumour diagnosis, classification, and prognosis

based on tumour histology, immunohistochemistry, and tumour staging will be complemented in the future by molecular approaches such as the detection of genetic alterations and the identification of differentially expressed genes. The application of both histologic and molecular markers will refine the definition of thyroid tumours and will help to better characterize tumours with histologically uncertain biological behaviour. Based on the further understanding of the molecular evolution of thyroid tumours, these approaches must go beyond simple correlations of histologic classifications and molecular markers. They will most likely lead to a better prediction of the risk for invasion, metastasis, and progression. Moreover, molecular markers will provide a more solid basis for an optimized and individualized treatment of thyroid cancer and ultimately will improve survival of patients.

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