From nodule to differentiated thyroid carcinoma: Contributions of molecular analysis in 2012

Du nodule au cancer thyroïdien différencié : apports de la biologie moléculaire en 2012

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Abstract

Today there is a better understanding of the events involved in the initiation and progression of thyroid cancer. It is indeed now known that BRAF and RAS mutations and RET/PTC and PAX8/PPARγ rearrangements account for the majority of molecular alterations detected in differentiated thyroid cancers. Abnormal regulation of microRNAs (miRNAs) is also a promising way of research. The diagnostic utility and prognostic value of detecting these molecular events has been analyzed in several recent studies. BRAF mutation analysis improves the performance of fine-needle aspiration diagnosis by increasing specificity in “indeterminate” cytologies and sensitivity in false negatives. Testing for a “panel of mutations” (BRAF, RAS, RET/PTC and PAX8/PPARγ) improves the performance, detecting papillary carcinomas with non-classic histology. The specificity of these analyzes is excellent but their sensitivity is still insufficient. In the future, specific miRNAs expression profiles in thyroid carcinoma and identification of new mutations might provide interesting information. Several studies have found that BRAF mutations are associated with a more aggressive tumor behavior, a higher risk of recurrence and treatment failure. With regard to the other mutations and rearrangements, current data are conflicting and it seems premature to draw practical conclusions applicable in routine practice. Lastly, targeted therapy with tyrosine kinase inhibitors, based on our understanding of the molecular mechanisms of thyroid oncogenesis, has shown promise in metastatic, progressive, and radioactive iodine-refractory differentiated thyroid carcinomas.

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Résumé

Il y a aujourd’hui une meilleure compréhension des événements impliqués dans l’initiation et la progression des cancers thyroïdiens. Ainsi, les mutations BRAF, RAS et les réarrangements RET/PTC et PAX8/PPARγ représentent la majorité des mutations détectées dans les cancers thyroïdiens différenciés. Les anomalies d’expression de microARNs (miARNs) sont également une voie prometteuse. L’intérêt diagnostique et pronostique de la recherche de ces anomalies moléculaires a été analysé dans plusieurs études récentes. La recherche de la mutation BRAF améliore les performances de la cytoponction avec un gain de spécificité, dans les cytologies « indéterminées », et de sensibilité dans les faux négatifs. La recherche d’un « panel de mutation » (BRAF, RAS, RET/PTC et PAX8/PPARγ) est encore plus performante, détectant des carcinomes papillaires d’architecture non classique. La spécificité de ces techniques est excellente, mais leur sensibilité est encore insuffisante. Dans l’avenir, la détermination de profils d’expression de miARNs spécifiques des cancers thyroïdiens et l’identification de nouvelles mutations pourraient être un apport complémentaire intéressant. Dans plusieurs études, la mutation BRAF est associée à un comportement tumoral plus agressif, avec un risque élevé de récidive et de résistance au traitement. Pour les autres mutations ou réarrangements, les données actuelles sont discordantes. Il est encore tôt pour tirer des conséquences pratiques en routine. Enfin, la thérapie ciblée par inhibiteur des tyrosines kinases, basée sur la compréhension des mécanismes moléculaires de l’oncogène thyroidienne, semble être une voie prometteuse dans les cancers thyroïdiens différenciés métastatiques, progressifs et réfractaires au traitement par iode radioactif.

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1. Introduction

The prevalence of thyroid nodules depends on the method of detection, ranging from approximately 4% for nodules detected by palpation (with a diameter greater than 1 cm) [1] to as high as 67% by high resolution ultrasonography [2]. The latter value agrees with the prevalence of 35–65% found at autopsy [3]. Most thyroid nodules are benign, and thyroid cancer accounts for only 5% of cases [4]. However, with an incidence of 140,000 cases per year worldwide, thyroid cancer represents 1% of all cancers [5]. The goal of any physician is therefore to differentiate between a benign nodule and a malignant nodule so that each patient receives appropriate management while avoiding unnecessary surgery.

Currently available methods — mainly ultrasonography and fine-needle aspiration (FNA) — do not provide definitive confirmation that a nodule is malignant.

Several ultrasonographic features are associated with malignancy and their association increases diagnostic performance. Two recent studies developed a stratification of these features into several categories, based on their association, reflecting an increasing probability of malignancy, by analogy with breast nodules [6,7]. Elastography, a newer technique, measures the elasticity index of the nodule, an indicator of stiffness, which is higher in malignant nodules. Two techniques are available to date, compression and Shear Wave. The overall sensitivity and specificity of elastography are good, but the false negative rate remains high: 9.7% in a recent review of 17 studies comprising 1544 nodules of which 402 were malignant [8]. On the whole, ultrasonography and elastography (when available) are essential first-line evaluations, and are especially useful for selecting nodules to biopsy in a multiheteronodular goiter.

Fine-needle aspiration biopsy (FNAB) with cytological analysis is currently the gold standard for the management of thyroid nodules. The new Bethesda classification proposed in 2007 (Table 1, [9]) includes six classes (that some cytopathologists identify by the numbers 1 to 6: 1 = non-diagnostic, 2 = benign to 6 = malignant). Nodules of uncertain cytological significance make up three categories:

- “Atypia of undetermined significance or follicular lesion of undetermined significance”. This is a heterogeneous category which includes suspicious follicular lesions or neoplasms, characterized by minimal cellular atypia, architectural atypia or low cellularity [10]. The clinical significance of this category is unclear, although the risk of malignancy is estimated at 5–15%. A repeat FNAB is recommended which in some cases will establish the diagnosis and thereby leads to appropriate management;
- “Follicular neoplasm or suspicious for a follicular neoplasm”. This category has a higher probability of malignancy (15–30%) and lobectomy is recommended to conclusively confirm or refute malignancy through histological evaluation [9];
- the category “suspicious for malignancy” comprises 60–75% malignant nodules; lobectomy or even total or subtotal thyroidectomy is advised.

The difficulties with cytological evaluation concern mainly under-detection of cancers among the uncertain cytological results and in particular in the first two categories described above (atypia of undetermined significance or follicular lesion of undetermined significance and follicular neoplasm or suspicious for a follicular neoplasm). With current analytical methods, only 8–56% of surgically excised thyroid nodules turn out to be malignant [11–14], meaning that many surgeries, with their attendant risks and costs, could be avoided [15]. Furthermore, most patients with these cytological features undergo partial thyroidectomy and may later require total thyroidectomy if final diagnosis is malignancy.

The second difficulty is that FNAB results may give rise to false negatives which can progress to malignancy. In these cases, diagnosis and treatment of the cancer are delayed, with a non-negligible risk of disease progression in the meantime [16]. The American Thyroid Association (ATA) estimated the false negative rate at 5% in 2009 [17], but higher values closer to 10% have been reported in several studies [18,19].

The existence of “indeterminate” or “uncertain” categories and the significant rate of false negatives highlight the importance of developing new, more effective methods that are also practically feasible and of reasonable cost.

Several immunohistochemical markers (Galectin-3, HBME-1, TPO, fibronectin-1, CITED-1, cytokeratin-19, etc.) have recently been studied with the aim of improving differential diagnosis between benign and malignant nodules. However,

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Table 1

The Bethesda System for reporting thyroid cytopathology.

<table>
<thead>
<tr>
<th>Diagnostic category</th>
<th>Risk of malignancy</th>
<th>Usual management</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-diagnostic or unsatisfactory</td>
<td>–</td>
<td>Repeat FNAb with ultrasound guidance</td>
</tr>
<tr>
<td>Benign</td>
<td>0–3</td>
<td>Clinical follow-up</td>
</tr>
<tr>
<td>Atypia of undetermined significance or follicular lesion of undetermined significance</td>
<td>5–15</td>
<td>Repeat FNAb</td>
</tr>
<tr>
<td>Follicular neoplasm or suspicious for a follicular neoplasm</td>
<td>15–30</td>
<td>Surgical lobectomy</td>
</tr>
<tr>
<td>Suspicious of malignancy</td>
<td>60–75</td>
<td>Near-total thyroidectomy or Surgical lobectomy</td>
</tr>
<tr>
<td>Malignant</td>
<td>97–99</td>
<td>Near-total thyroidectomy</td>
</tr>
</tbody>
</table>

*upon The Bethesda System for Reporting Thyroid Cytopathology [9].

FNAb: Fine-Needle Aspiration.
the sensitivity and specificity of these markers are insufficient to reliably replace a histological diagnosis [20] and the recent SFE consensus considers that there is not adequate proof to allow this method to be used as a marker of malignant or benign disease in undetermined or suspicious FNAB results [4].

Much hope has been placed on molecular biology. Indeed, the molecular mechanisms that deregulate signaling pathways in thyroid tumorigenesis are now more fully understood. Several groups have used these fundamental data for investigations of thyroid nodules [21]. It turns out that detection of molecular markers not only has diagnostic utility (better preoperative selection of patients at high risk of malignancy) but also prognostic value in identifying thyroid carcinomas with a poor prognosis, requiring more aggressive management [22].

The aim of this review is to make an inventory of the contributions of molecular analysis to diagnosis, prognosis and treatment of differentiated thyroid carcinomas. The molecular alterations in the currently known signaling pathways involved in thyroid carcinogenesis will be reviewed first, followed by a description of the additional information that molecular analysis could provide to FNAB in diagnosis and the prognosis value of this method. Lastly, we will discuss the therapeutic interest of this analysis in terms of mutations in certain signaling pathways that could be potential molecular targets for treatment.

2. Molecular basis

Anomalies in the genes coding for tyrosine kinase receptors or proteins located farther downstream in intracellular signaling pathways have been identified in thyroid cancers, resulting in defects in cell regulation mechanisms (Fig. 1). For instance, mutations of the \( \text{BRAF} \) and \( \text{RAS} \) genes and \( \text{RET/PTC} \) and \( \text{PAX8/PPAR}_{\gamma} \) rearrangements account for the majority of molecular alterations detected in papillary and follicular carcinomas and have the largest impact on tumor diagnosis and prognosis (Table 2, [23]). Abnormal microRNAs (miRNAs)

\[ \text{Table 2} \]

<table>
<thead>
<tr>
<th>Tumor type</th>
<th>Prevalence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Papillary carcinoma</td>
<td></td>
</tr>
<tr>
<td>( \text{BRAF} )</td>
<td>45</td>
</tr>
<tr>
<td>( \text{RET/PTC} )</td>
<td>20</td>
</tr>
<tr>
<td>( \text{RAS} )</td>
<td>10</td>
</tr>
<tr>
<td>( \text{TRK} )</td>
<td>&lt; 5</td>
</tr>
<tr>
<td>Follicular carcinoma</td>
<td></td>
</tr>
<tr>
<td>( \text{RAS} )</td>
<td>45</td>
</tr>
<tr>
<td>( \text{PAX8/PPAR}_{\gamma} )</td>
<td>35</td>
</tr>
<tr>
<td>( \text{PIK3CA} )</td>
<td>&lt; 10</td>
</tr>
<tr>
<td>( \text{PTEN} )</td>
<td>&lt; 10</td>
</tr>
<tr>
<td>Poorly differentiated carcinoma</td>
<td></td>
</tr>
<tr>
<td>( \text{RAS} )</td>
<td>35</td>
</tr>
<tr>
<td>( \beta)-\text{Catene} (\text{CTNNB1})</td>
<td>20</td>
</tr>
<tr>
<td>( \text{TP53} )</td>
<td>20</td>
</tr>
<tr>
<td>( \text{BRAF} )</td>
<td>15</td>
</tr>
</tbody>
</table>

Upon [23].
expression also appears to be a promising diagnostic marker of differentiated thyroid carcinoma.

This review will not discuss the molecular alterations involved in other types of thyroid cancers: TP53 and CTNNB1 gene mutations in poorly differentiated or anaplastic thyroid carcinoma [21], RET gene mutations in hereditary and sporadic medullary carcinomas [24].

2.1. BRAF

Activating mutations of the BRAF gene (the B isoform of the serine-threonine kinase RAF) are the most frequent genetic events associated with papillary carcinomas, and are found in about 45% of these tumors [25]. Approximately 95% of these mutations concern nucleotide 1799 and result in a valine-to-glutamate substitution at residue 600 (V600E). This mutation leads to constitutive activation of BRAF kinase and chronic stimulation of the MAP kinase pathway, resulting in increased cell proliferation and survival and therefore orienting the cell towards tumorigenesis [26]. Less frequent mechanisms of BRAF activation have been described in papillary carcinomas, including D601E mutations, small insertions or deletions around codon 600 [27] or AKAP9/BRAF rearrangements, usually found in radiation-induced papillary carcinomas [28].

The BRAF (V600E) mutation, typical of papillary carcinomas with classic histology and tall cell variants, is much less frequent in the follicular variant of papillary carcinoma [29]. It has also been found in anaplastic or poorly differentiated carcinomas arising from papillary carcinomas [30]. This mutation has not been found in other types of differentiated thyroid carcinomas or in benign thyroid nodules [31]. Therefore, it represents a specific marker of papillary carcinoma.

2.2. RAS

Mutations of RAS genes (HRAS, KRAS and NRAS) are not specific of thyroid tumor type and have been detected in follicular carcinomas, papillary carcinomas and follicular adenomas. RAS mutations give rise to a mutant protein that continuously stimulates the MAP kinase pathway as well as other signaling pathways such as P13 K/AKT. RAS mutations have been detected in 10–20% of papillary carcinomas [32], mostly follicular variants. RAS mutations have also been detected in 40–50% of follicular carcinomas and 20–40% of follicular adenomas [33]. A lower incidence has been observed in oncocytic tumors [34]. The presence of RAS mutations in follicular adenomas and carcinomas suggests that RAS activation may be an early step in thyroid tumorigenesis.

2.3. RET/PTC

RET/PTC rearrangements are another genetic event found in papillary thyroid carcinomas [35]. They are formed by the fusion between the 3’ portion of the RET gene and the 5’ portion of various PTC genes (paracentric inversions in the case of PTC1 and 3, the most frequent; interchromosomal translocations for the others) [36]. All these rearrangements enable the RET/PTC chimeric protein to activate the RAS-RAF-MAP kinase cascade and thereby initiate tumorigenesis [37].

RET/PTC rearrangements are present in approximately 20% of papillary carcinomas in adults, with a prevalence that is variable according to geographical factors and sensitivity of the detection methods [38]. They are more common in radiation-induced papillary carcinomas (50–80%) and in carcinomas from children and young adults (40–70%) [39]. Some studies have observed this type of rearrangement in benign thyroid lesions (Hashimoto thyroiditis, adenoma), but clonal RET/PTC rearrangements (involving the majority of tumor cells) are specific for papillary thyroid carcinoma [40].

2.4. PAX8/PPARγ

PAX8/PPARγ rearrangements are due to the translocation (t2;3) (q13,p25) leading to the fusion between the PAX8 gene encoding a thyroid-specific transcription factor and the PPARγ gene encoding the peroxisome proliferator-activated receptor. This leads to strong overexpression of the PPARγ protein, resulting in an activation of its target genes [41]. PAX8/PPARγ rearrangements are detected in 30–40% of follicular carcinomas and with lower prevalence in oncocytic carcinomas. They are also found to a small extent in follicular adenomas (2–10%) and occasionally in the follicular variant of papillary carcinoma [42]. However, follicular adenomas harboring this rearrangement typically have a thick capsule and an immunohistochemical profile characteristic of thyroid carcinoma, suggesting that they may be preinvasive follicular carcinomas or malignant tumors whose invasiveness was not detected by histologic evaluation [43].

2.5. MicroRNAs

MicroRNAs (miRNAs) constitute a class of small endogenous non-coding RNAs that act as negative regulators of gene expression and are involved in many cellular processes. Specific miRNA profiles have been described in several types of cancer including lung and breast. Several miRNAs are upregulated in papillary carcinomas, which may play a role in thyroid tumorigenesis [44]. Interestingly, level of upregulation of some of these miRNAs is correlated with the mutational status of papillary carcinomas; examples include miR-187 and tumors harboring RET/PTC rearrangements; miR-221 and 222 and tumors harboring BRAF and RAS mutations or papillary carcinomas without an identified mutation. MicroRNA upregulation has also been described in follicular carcinomas [45].

So far, only a few specialized laboratories are able to perform such molecular analyzes. Before these methods to be used in “routine” clinical practice, both the extraction techniques and the molecular analysis must be standardized. The goal of these molecular methods is therefore to attain maximum sensitivity and reproducibility so as to be exploitable in clinical practice. Nonetheless, caution is advised to avoid excess sensitivity which could increase the rate of false positives and result in a loss of specificity.
3. Applications of molecular analyses in clinical practice

3.1. Diagnostic utility: Fine-needle aspiration biopsy

Currently available methods in molecular biology are able to detect these molecular events in FNA samples and surgically removed tumor tissues, thereby providing useful information for the diagnosis and management of thyroid nodules (Fig. 2). Some studies have shown that molecular testing in FNAB significantly improves the sensitivity and specificity of cytological diagnosis of thyroid nodules.

3.1.1. BRAF

The majority of studies have focused on the diagnostic utility of the BRAF mutation. Nikiforova et al. analyzed nine prospective and seven retrospective studies on FNAB samples and two studies on surgical specimens conducted between 2004 and 2009 [46]. Among 581 BRAF-positive samples, 580 were papillary carcinomas, with a single false positive (0.2%). It is noteworthy that in these studies, a considerable proportion (15–39%) of BRAF-positive FNA samples had indeterminate or non-diagnostic cytology, thus demonstrating the diagnostic utility of this molecular analysis when cytological features are inconclusive. Moreover, some nodules initially considered benign by cytology harbored BRAF mutation and were ultimately identified as papillary carcinomas at histology [47,48]. This method could therefore allow correcting the false negatives of FNA cytology.

Nam et al. used a different strategy: they classified 244 nodules as suspicious for malignancy or not suspicious, based on ultrasonographic features, and then performed cytological and BRAFV600E mutation analysis. For nodules with indeterminate or non-diagnostic cytology, the BRAF mutation was present in 45% of ultrasonographic suspicious nodules. The authors concluded that adding BRAF molecular testing to cytological analysis could provide important information for ultrasonographic suspicious nodules, especially when cytology is inconclusive [49]. In a large prospective series of 1074 patients with thyroid nodules, this same Korean group found that BRAFV600E mutation analysis significantly improved the sensitivity of FNA cytology, from 67.5% to 89.6%. Note that their technique produced five BRAF false positives (multiplex PCR based on dual-priming oligonucleotides) [50]. Lastly, this group proposed a strategy of “systematic surgery” in patients with “atypical follicular lesion of undetermined significance” cytology with BRAFV600E mutation; 29/30 patients with these findings had a papillary carcinoma. The authors concluded that BRAF mutation analysis in this type of nodule is a useful tool in the therapeutic decision [51].

It should nevertheless be kept in mind that this progress in the understanding of BRAF mutation status is only useful for improving the diagnostic accuracy of papillary carcinoma, and usually with classic histology. It provides no additional evidence for the diagnosis of follicular carcinoma, the subtype for which cytological diagnosis is most difficult. So, it is necessary to identify other molecular markers to improve the performance of FNA cytology in follicular carcinomas.

3.1.2. RET/PTC, RAS and PAX8/PPARγ

Several studies have explored the possibility of testing for RET/PTC, PAX8/PPARγ rearrangements or RAS mutations in FNA samples. It was initially suggested that analysis of RET/PTC (1, 2 and 3) rearrangements could improve the diagnosis of papillary carcinomas, with excellent specificity, particularly for cytologically indeterminate samples [52]. However, this specificity was challenged by the fact that RET/PTC rearrangements have also been found in Hashimoto thyroiditis and thyroid adenomas using ultrasensitive detection methods. Therefore, it is currently recommended to use a less sensitive method (such as real-time PCR) or FISH analysis to better make the difference between a malignant and benign nodule and limit the rate of false positives [40].

RAS oncogene analysis also appears to improve the sensitivity of FNA cytology [53], although the possible presence of this type of mutation in follicular adenomas makes its use potentially less specific. Nonetheless, it is interesting to note that RAS mutation detection permits the identification of tumors with inconclusive cytology, such as follicular variant of papillary carcinomas, and follicular carcinomas.

PAX8/PPARγ rearrangements can also be a diagnostic aid in these difficult cases, although they are found at a much lower frequency [47].

3.1.3. Panel of mutations

Testing for a panel of mutations has proved superior to testing for a single mutation. A recent study used a mutation panel consisting of BRAF, RAS, RET/PTC and PAX8/PPARγ in 470 specimens and found 32 mutations (18 BRAF, eight RAS, five RET/PTC and one PAX8/PPARγ) [47]. The presence of a mutation was a strong indicator of cancer: 97% of mutation-positive nodules were histologically malignant (28 papillary carcinomas and three follicular carcinomas); a mutation was present in a benign nodule in only one case. Furthermore, the sensitivity of FNA cytology was improved by molecular testing, increasing from 44% (cytologically malignant) to 62% (mutation detected) and to 80% when associating both methods. Considering only nodules with indeterminate cytology, the probability of malignancy ranged from approximately 40% with cytology alone, to 100% in the presence of a mutation and 14% if no mutation was found. This molecular diagnosis was particularly useful in cytological analysis of “atypical follicular lesions of undetermined significance” (100% of mutation-positive nodules were malignant, with no false positives). Moreover, these molecular analyses lowered the rate of cytological false negatives from 2.1% to 0.9%.

A more recent study focusing on “follicular lesion or atypia of undetermined significance” also used a mutation panel comprising BRAF, RAS, RET/PTC and PAX8/PPARγ. Among 117 samples with histological correlation, 12 molecular alterations (three BRAF, classic and tall cell variant of papillary carcinoma, seven NRAS, one HRAS and one PAX8/PPARγ corresponding to follicular variant of papillary carcinoma) were found in a total of 20 papillary carcinomas. This molecular analysis therefore showed excellent specificity with no false positive results; however, thyroid carcinoma was present in 7.6% of cases with
indeterminate cytology and no molecular alteration. Repeat FNAB allowed the diagnosis of malignancy in eight of these patients, less than molecular testing. However, in three of these eight patients, no mutation had been found. These two strategies could therefore be complementary [54].

In a recent analysis of the four main studies which tested a panel of somatic mutations (BRAF, RAS, RET/PTC and PAX8/PPARγ) in thyroid FNA samples, Ferraz et al. found a mean sensitivity of 63.7% (38–85.7) and a specificity of 98% (95–100) for indeterminate lesions. A comparison of these findings with the data from four studies which only tested for the BRAF mutation in cytological “indeterminate” samples revealed a marked improvement in sensitivity: 12.9% sensitivity with BRAF alone versus 63.7% with the panel of mutations, but with an increase in false positives: 0.5 false positive rate (mean) with BRAF alone versus 1.25 with the panel. Finally, it is important to keep in mind that despite a negative molecular result, 9/80 indeterminate cytology tumors (11.25%) were ultimately malignant on histological evaluation [55]. These data were confirmed in another study by Nikiforov et al. on more than 1000 FNA with indeterminate cytology. This study found an 11% probability of cancer among the indeterminate cytologies when no mutation was detected in this panel [56]. These studies emphasize the need to identify more molecular markers so as to reduce the number of unnecessary or diagnostic surgeries.

In practice, given the excellent positive predictive value of the presence of these mutations in different studies, it appears logical to propose total thyroidectomy as first-line treatment for patients with indeterminate cytology and a BRAF, RAS, RET/PTC or PAX8/PPARγ mutation. This would avoid repeat surgery to complete thyroidectomy in cancer and the associated morbidities and costs thereof.

In light of the mounting evidence for molecular diagnosis, the American Thyroid Association has validated these methods and included them in its newest version of management guidelines for patients with thyroid nodules or differentiated thyroid cancer. These guidelines recommend testing for these molecular markers as a tool in the diagnosis of nodules with indeterminate cytology [17].

3.1.4. MicroRNAs and other molecular markers

The sensitivity of detecting thyroid cancer can be improved by adding other molecular markers such as miRNAs, some of which are upregulated in differentiated thyroid carcinomas. Pallante et al. reported upregulation of three miRNAs (miR-221, 222 and 181b) in 88% (7/8) of FNA samples of papillary carcinomas [57]. In another study in FNAB, miRNAs expression profiles were significantly different according to the histological tumor type. Thereby the authors validated a panel of seven miRNAs which are significantly upregulated in thyroid cancers as compared to hyperplasic nodules, with different profiles specific of tumor type (miR-187, 221, 222, 146b, 155, 224 and 197) and excellent sensitivity and specificity (88% sensitivity when three or more miRNAs were upregulated with 100% specificity) [44].

Studies have also been carried out to identify expression profiles of genes differentially expressed in benign and malignant nodules (genes that would play an important role in disease development or progression) by transcriptome analysis. A meta-analysis of genes whose expression is frequently found to be altered in thyroid cancer identified 12 genes differentially expressed in thyroid cancer, potential candidates for a panel of markers which could improve the diagnostic sensitivity and specificity of FNA cytology [58]. Several groups have made use of this differential expression to distinguish between healthy and malignant thyroid tissue and apply it to thyroid nodule FNAB samples [59]. In a recent study of more than 240,000 transcripts in 315 thyroid nodules (FNA samples and surgical tissue), Chudova et al. found that some genes were over-represented. This allowed them to develop a molecular classification providing 84% specificity with 96% negative predictive value [60] (Fig. 2).

Molecular testing during surgery on the tumor tissue might also be helpful in diagnosis, although extemporaneous analysis would have to be done in “real time”, which is not yet possible with current methods. Thus, for the time being, molecular testing on the surgically removed tumor would mainly have a prognostic role.

3.2. Prognostic utility

Most well-differentiated thyroid carcinomas, especially small, localized tumors, progress slowly and can be treated conservatively. However, some tumors are highly aggressive, and molecular markers might serve as early indicators of a poor prognosis.

3.2.1. BRAF

The BRAF V600E mutation is generally associated with more aggressive tumor behavior, as shown in many studies. In fact, the majority of studies report a correlation between BRAF mutation and extrathyroidal extension, lymph nodes, distant metastases and a more advanced disease stage at presentation [61–63]. This correlation was not found by other authors, such as Kim et al. [64] and Costa et al. [65]. There is no clear explanation for the variability of these results from one study to another, but possibilities include differences in methodology, consensus, or tumors diagnosed at different stages. In the majority of studies, BRAF mutation tends to be associated with a poorer clinical and pathological state, although this correlation is not always significant [66]. Moreover, BRAF mutations occur frequently in subsets of papillary carcinoma that are known to be more aggressive, such as tall cell variants [25].

The BRAFV600E mutation has also been shown to be an independent predictor of treatment failure and tumor recurrence, even in patients with low stage disease [67], and also an independent risk factor of cancer-related mortality in a long-term follow-up study [68]. The correlation between the presence of the BRAFV600E mutation and poor tumor outcome remains significant in FNA analyses. Thereby, Xing et al. proposed the use of BRAF mutation testing for risk stratification in papillary carcinoma to adapt the type of surgery (especially the extent of surgery) according to the mutation status of the FNA sample [69].

BRAF activation through the BRAFV600E mutation in thyroid cells appears to result in altered function of the iodine
3.2.2. RAS

The predictive role of RAS mutations in tumor aggressiveness is not as clearly defined as that of BRAF because RAS mutations can be found in both follicular adenomas and carcinomas (cf. supra).

Several studies report a significant correlation between RAS mutations and metastatic behavior of follicular carcinomas [74]. RAS mutations are also associated with more aggressive tumor phenotypes in papillary carcinomas, especially with respect to distant metastasis and mortality [75]. Volante et al. found that RAS mutations were the most frequently detected molecular alteration in poorly differentiated and anaplastic carcinomas [71].

In papillary microcarcinomas, which are usually diagnosed incidentally, classically during thyroid surgery performed for reasons unrelated to cancer, the BRAF mutation could have important prognostic implications. Most of these tumors have a low potential for progression and are cured by resection, but some are more aggressive, recur and require further treatment [22]. Several recent studies have found a correlation between BRAF mutation and a high rate of extrathyroidal extension and metastatic lymph nodes in papillary microcarcinomas [72,73], which might mean that the BRAF mutation is associated with a more aggressive tumor in this subtype of cancer.

However, even if the BRAF mutation appears to be a good marker of aggressiveness that can be used in particular for papillary microcarcinomas, not all BRAF-positive papillary carcinomas are aggressive and not all aggressive papillary carcinomas necessarily harbor this mutation.

3.2.3. RET/PTC and PAX8/PPARγ

In contrast to other molecular alterations, presence of RET/PTC rearrangement is associated with low-aggressive papillary carcinomas and, for RET/PTC1 in particular, with a low probability of progression to poorly differentiated or anaplastic tumors and an association with classic papillary carcinomas or microcarcinoma subtypes [78]. Indeed, this rearrangement is only present in a small fraction of poorly differentiated thyroid cancers, suggesting that this oncogene does not confer a high risk of tumor progression [79].

However, Durand et al. found that gene expression profiles in papillary carcinomas negative for BRAF mutation or RET/PTC rearrangement were closer to those of healthy thyroid tissue. The absence of these mutations would therefore indicate a better prognosis [80]. Furthermore, Adeniran et al. found that tumors harboring RET/PTC rearrangements occurred in patients who were younger with a higher rate of lymph node metastasis [29]. Thus, the correlation between this rearrangement and the prognosis of papillary carcinoma requires further study.

Data concerning the prognostic value of rearrangements PAX8/PPARγ are scarce. Tumors harboring this type of rearrangement tend to occur at an early age, are smaller in size and more frequently have vascular invasion [81].

3.2.4. MicroRNA

Some authors have found a correlation between upregulation of some miRNAs (miR221, 222 and 146b), extrathyroidal invasion and high risk characteristics in papillary carcinomas. Significantly higher level of miR-146b has also been found in papillary carcinomas harboring the BRAF mutation. These observations suggest that these miRNAs may be negative prognostic factors in papillary carcinoma [82].

4. Therapeutic perspectives

The use of targeted therapies for the treatment of differentiated thyroid carcinomas requires an understanding of the molecular mechanisms of carcinogenesis. Therefore, candidate molecules should “ideally” inhibit signaling pathways activated by RET/PTC, PAX8/PPARγ rearrangements and RAS and BRAF mutations. Novel treatments specifically targeting these signaling pathways and/or inhibitors of neoangiogenesis are being developed. Several tyrosine kinase inhibitors are under study in thyroid cancer, including imatinib, gefitinib, axitinib, sorafenib, motesanib, vandetanib, sunitinib, XL184, pazopanib, lenvatinib (E7080). The majority of these molecules inhibit several proteins potentially involved in tumor growth and most also inhibit angiogenesis via the vascular endothelial growth factor (VEGF) receptor [83] (Table 3).

For the moment these molecules are under investigation in phase II and III clinical trials, particularly in advanced differentiated thyroid carcinomas that are no longer sensitive to conventional treatment (radioiodine-refractory).

Unfortunately, these new molecules often cause side effects, such as gastrointestinal symptoms, hypertension, skin reactions or rash, fatigue, weight loss, etc., that have to be monitored and that may require dose reduction or even treatment discontinuation.

It is worth presenting some recent data in this rapidly moving field. Sorafenib and sunitinib have shown encouraging preliminary results in progressive differentiated thyroid carcinomas. Cabanillas et al. treated 15 patients with progressive papillary (eight) or follicular (seven) carcinomas with sorafenib (13) or sunitinib (two) and observed a clinical benefit in 80% of the patients (20% partial response, 60% stable disease), with a predominant response in lung metastases and a median progression-free survival of 19 months [84].
principal tyrosine kinase inhibitors studied in clinical trials and their targets.

<table>
<thead>
<tr>
<th>Drug</th>
<th>VEGFR1</th>
<th>VEGFR2</th>
<th>VE6FR3</th>
<th>PDGFR</th>
<th>RET</th>
<th>RET/PTC</th>
<th>BRAF</th>
<th>EGFR</th>
<th>FGFR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Motesanib</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
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<tr>
<td>Sunitinib</td>
<td>+</td>
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<td>+</td>
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<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Vandetanib</td>
<td>–</td>
<td>+</td>
<td>+</td>
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<td>+</td>
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<tr>
<td>Sorafenib</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<td>–</td>
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</tr>
<tr>
<td>XL184</td>
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<td>+</td>
<td>–</td>
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<td>+</td>
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<tr>
<td>Pazopanib</td>
<td>+</td>
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<td>–</td>
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<td>–</td>
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<tr>
<td>Axitinib</td>
<td>+</td>
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<td>–</td>
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</tr>
<tr>
<td>Lenvatinib (E7080)</td>
<td>+</td>
<td>+</td>
<td>+</td>
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</tbody>
</table>

Upon [83].

Sherman et al. reported data from a phase II trial of lenvatinib (E7080) in 58 patients with advanced, progressive, radioiodine-refractory thyroid cancer [85]. A partial response was achieved in 50% of patients with a median progression-free survival of 12.6 months. Interestingly, a correlation was found between the presence of a RAS mutation and the efficacy on tumor response. A phase III study is now under way.

A phase II study with sorafenib found a correlation between longer progression-free survival and presence of the BRAFV600E mutation [86]. These findings suggest for the first time the validity of a specific target in the pharmacological treatment of thyroid cancer and highlight the importance of patient selection in future studies.

Thus, to optimize the use of these drugs and target those which would potentially be most effective, would it not be necessary to perform molecular profiling of each patient’s tumor, identifying the causal mutation and specifically acting on the signaling pathway involved? The question is still under study.

5. Conclusion

The selection of suspicious nodules has greatly improved since the introduction of FNAB (3.1% of carcinomas in operated nodules without FNA versus 34% with fine-needle aspiration) [87].

Nowadays, a better understanding of the molecular mechanisms underlying thyroid oncogenesis, together with the identification of specific mutations and rearrangements and different gene expression profiles and miRNAs profiles in thyroid tumor tissue, provide a solid scientific basis for developing new diagnostic and prognostic tools in a not so faraway future.

So far, the detection of molecular alterations in the signaling pathways involved in thyroid carcinogenesis (BRAF, RAS mutations and RET/PTC, PAX8/PPARγ rearrangements) appears to be of diagnostic utility (Fig. 3), especially in “indeterminate” cytology, even for false negative fine-needle aspirates, with excellent specificity but a still insufficient sensitivity, particularly for follicular carcinomas. It therefore seems worthwhile to pursue research on other molecular alterations or mutations specific of thyroid tumor tissue so as to improve diagnostic sensitivity. miRNA or gene expression profiles, currently under study, could provide additional valuable diagnostic information and would, especially, enhance the sensitivity.

The prognostic value of the method still requires confirmation, the idea being to eventually adapt the treatment strategy to the molecular results, particularly in papillary microcarcinomas.

Nevertheless, for the moment these tests are only available in a few specialized laboratories and their routine use will require standardization of extraction and analysis techniques, so as to achieve optimum sensitivity and specificity. Furthermore, these methods will have to be usable and reliable on thyroid tissue as well as on FNAB samples.

In the future, identification of these molecular markers will permit better characterization of thyroid tumors, helping to guide the treatment decision, particularly the indication for and extent of thyroid surgery, according to the potential for malignancy and the tumor prognosis, and will even permit, if necessary, to select a medical therapy targeting a specific molecular alteration.

Disclosure of interest

The authors declare that they have no conflicts of interest concerning this article.

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