Molecular diagnosis of carcinomas of the thyroid gland

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1. ABSTRACT

Our understanding of the molecular pathology of thyroid cancer has progressed significantly. It is now apparent that thyroid tumors show a very good correlation between genotype and phenotype, a correlation that is much stronger than that observed in tumors of many other organs. Activation of classic oncogenes (BRAF, RAS, RET) activate MAPK signalling. Other pathways like the PI3K/PTEN/AKT cascade are also active in many thyroid tumors. The analysis of molecular profiles is generating data that can be applied to improve patient management. The common occurrence of thyroid nodules in the general population and the widespread use of fine needle aspiration for the preoperative diagnosis of thyroid nodules creates an unprecedented opportunity to apply what we have learnt from the molecular alterations of thyroid cancer to the clinical arena.

2. INTRODUCTION

The large majority of thyroid tumours derives from follicular cells. Carcinomas of follicular cell derivation are classified as papillary carcinoma (PTC), follicular carcinoma, poorly differentiated carcinoma and anaplastic (undifferentiated) carcinoma (Table 1) (1). Papillary carcinoma is the most common type of thyroid cancer (~85% of thyroid carcinomas). It is characterized by papillary growth and diagnosed based on a specific set of cytologic alterations of neoplastic cell nuclei (nuclear pseudoinclusions, grooves, chromatin clearing, irregularities of the nuclear membrane contour). In addition to the classic form of papillary carcinoma numerous morphologic subtypes of papillary carcinoma are recognized. These include the tall cell variant and the follicular variant, as well as uncommon subtypes, such as the diffuse sclerosing, solid, papillary oncocytic, columnar cell and cribriform-morular variants. The term papillary
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Table 1. Molecular alterations in thyroid carcinomas

<table>
<thead>
<tr>
<th>Histotype</th>
<th>BRAF (%)</th>
<th>RAS (%)</th>
<th>PAX8/PPARγ (%)</th>
<th>RET/PTC (%)</th>
<th>RET (%)</th>
<th>Others alterations (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PTC conventional (65% of thyroid carcinomas)</td>
<td>60</td>
<td>&lt;5</td>
<td>0</td>
<td>20</td>
<td>0</td>
<td>AXTR rearrangements (&lt;5)</td>
</tr>
<tr>
<td>PTC follicular variant (20% of thyroid carcinomas)</td>
<td>10</td>
<td>35</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>RET/PTC (&lt;10)</td>
</tr>
<tr>
<td>FTC (5-10% of thyroid carcinomas)</td>
<td>0</td>
<td>40</td>
<td>30</td>
<td>0</td>
<td>0</td>
<td>PIK3CA (&lt;10)</td>
</tr>
<tr>
<td>PDC (&lt;5% of thyroid carcinomas)</td>
<td>10</td>
<td>30</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>CTNNB1 (15)</td>
</tr>
<tr>
<td>ATC (&lt;5% of thyroid carcinomas)</td>
<td>20</td>
<td>40</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>PIK3CA (&lt;10) AKT1 (5)</td>
</tr>
<tr>
<td>MTC (5% of thyroid carcinomas)</td>
<td>&lt;5</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>P53 (10)</td>
</tr>
</tbody>
</table>

PTC, papillary thyroid carcinoma; FTC, follicular thyroid carcinoma; PDC, poorly differentiated carcinoma; ATC, anaplastic thyroid carcinoma; MTC, medullary thyroid carcinoma. The percentage figures are estimated from the literature and/or based on the personal experience of the authors. RET/PTC rearrangement incidence is higher (up to ~80%) in patients with history of radiation exposure. The proportion of BRAF mutated cases is higher in cases were a PTC component can be demonstrated. The proportion of cases with molecular alterations reported in the literature depends on the method used for detection. The percentage figure refers to sporadic MTC, but other series have found a higher proportion of RAS mutated sporadic MTC; percentage figures are higher if only RET-negative sporadic MTC are considered because RET and RAS mutations are mutually exclusive in MTC; RAS mutations are very rare in hereditary MTC; the percentage figure refers to sporadic MTC; germline RET mutations are present in >95% of hereditary MTC.

Microcarcinoma (mPTC) is used to designate small tumors that measure less than one centimetre. Follicular carcinoma (FTC), defined by the identification of invasion of blood vessels or of the tumor capsule, is the second most common histologic subtype of thyroid cancer (~5-10% of thyroid carcinomas). Two types are recognized: minimally invasive and widely invasive follicular carcinoma. The most important and common morphologic subtype is the oncocytic follicular carcinoma (also known as Hurthle cell carcinoma). Poorly differentiated carcinoma (PDC, <5% of thyroid carcinomas) and anaplastic (undifferentiated) carcinoma (ATC, <5% of thyroid carcinomas) are high grade tumors characterized by loss of differentiation that can derive from well differentiated tumours or arise de novo. Medullary thyroid carcinoma (MTC) originates not from the parafollicular cells (Calcitonin producing C-cells) and represents ~5% of thyroid carcinomas.

Thyroid nodules are very common in the general population and tend to increase with age. They can be identified by palpation in ~5% of the general population and in an even higher proportion of people using ultrasound scans. The large majority of these nodules are benign, but a small proportion are malignant. Fine needle aspiration (FNA) of thyroid nodules has become the most effective and widely utilized tool for the preoperative evaluation of thyroid lesions. Its main role is in the triage of patients that require surgery and has succeeded in greatly reducing unnecessary “diagnostic” thyroid resections, performed for the main purpose of defining which nodule is benign and which is malignant after histologic examination (2).

Among thyroid nodules that undergo successful FNA, ~60-70% are cytologically benign, whereas ~5-10% are usually diagnosed as malignant. The remaining 20-30% exhibit features which are not diagnostic of either benignancy or malignancy. Various diagnostic terminologies, including “atypical”, “indeterminate”, and “suspicious for malignancy” are used to describe them (2). Significant efforts have been made to standardise the reporting of thyroid FNA diagnoses. The Bethesda system for reporting thyroid cytopathology is currently used in the United States and has been well received in Europe. The Royal College of Pathologists has modified its traditional Thy1-5 reporting scheme to accommodate the suggestions of the Bethesda system (http://www.rcpath.org/resources/pdf/g089guidanceonthereportingofthyroidcytologyfinal.pdf, accessed October 29, 2010). The following categories are recognized in both system: Thy1/Bethesda system category I - Non-Diagnostic or Unsatisfactory (ND/UNS); Thy2/Bethesda system category II - Benign; Thy3a/Bethesda system category III - Atyopia of Undetermined Significance or Follicular Lesion of Undetermined Significance (AUS/FLUS); Thy3f/Bethesda system category IV - Follicular Neoplasm or Suspicious for a Follicular Neoplasm (FN/SFN); Thy4/Bethesda system category V - Suspicious for Malignancy; Thy5/Bethesda system category VI – Malignant (2). This process of standardization allows to precisely identify the diagnostic categories that can benefit the most from the application of molecular analysis to FNA samples. These are the Thy3f/Bethesda system category IV FN/SFN, the Thy4/Bethesda system category V - Suspicious for Malignancy, and - in particular - the Thy3a/Bethesda system category III - AUS/FLUS (3-5).

A variety of genetic alterations occur in thyroid carcinoma, including chromosomal rearrangements and point mutations at hot spots of specific genes (6, 7).

The most frequent nucleotide substitutions involve BRAF, RAS (H-, N- and K-RAS) and the RET genes. Although the relative proportion of cases with
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3. MOLECULAR ALTERATIONS IN THYROID TUMORS

3.1. BRAF

BRAF is a gene codifying for a serine/threonine protein kinase that plays a role in MAP kinase signaling pathway. BRAF activation due to nucleotides substitutions plays a key role in the development of papillary thyroid carcinomas (PTC) (8-10). Several studies suggest that BRAF mutations may also occur as a later alteration, acquired during the progression of thyroid cancer (11, 12). More than 65 BRAF mutations are known, but the most frequent, up to 98% of BRAF mutations in a wide range of human cancers, is BRAFV600E. BRAFV600E is a Valine to Glutamate trasversion at aminoacid 600 of the gene (nucleotide 1799, from Thymine to Alanine, c.1799 T>A) (13). This mutation was characterized for the first time in thyroid carcinomas in 2003 (14-16). Other mutations (e.g. BRAFV601E) have been found, but only in a small percentage of cases (up to 7%) of PTC that usually belong to the follicular variant of PTC (17, 18). BRAF mutations are strongly associated with papillary phenotype, in fact ~50% of PTC have the BRAFV600E (13). There is a good phenotype-genotype relationship between PTC variants and BRAF mutations. BRAF has been found mutated in 60% of classic PTC (PTC-CL), 80% of tall cell variant PTC (PTC-TC) and in 10% of follicular variant PTC (PTC-FV) (19).

A number of published papers reported that BRAF mutational analysis improves the sensitivity of the fine needle aspirate (FNA) evaluation of thyroid nodules, especially in those cases diagnosed cytologically as atypia of undetermined significance (Thy-3a) or classified as inadequate (Thy-1) (3, 4, 20-23).

BRAFV600E mutation has been associated with aggressive tumour characteristics, including advanced tumour stage, extrathyroidal extension or poor outcome (24-28). However, it should be noted that not all studies support this association. In a series of more than 630 patients with PTC, Ito et al. observed that BRAFV600E does not reflect aggressive behaviour nor does it show any relationship with tumor size or stage (29). Fugazzola et al. confirmed the strong association of BRAFV600E with papillary growth pattern, but found no correlation with poor tumor differentiation or adverse prognosis (30). In a recent study Sancisi and colleagues did not observe in a series of 47 well-differentiated PTC with distant metastases significant differences in the BRAF V600E distribution in relation to sex, stages, presence of regional or vascular invasion, or histotype in comparison with not metastatic PTC (31).

3.1.1. Detection methods

BRAF mutational analysis can be performed using many different methods, that range from standard (Sanger) sequencing to mutation specific PCR. Melting curve analysis and single strand conformation polymorphism (SSCP) assay are both suitable and effective to detect the BRAFV600E (32, 33) (Figure 2, Table 2). Pyrosequencing allows to detect BRAF mutations with greater sensitivity than Sanger sequencing (34). Other methods include matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) (35) or oligonucleotide microarray (36), but they are time-consuming and/or require the use of sophisticated platforms not always affordable by pathology laboratories. Considering the high prevalence of the V600E substitution in thyroid carcinomas, BRAFV600E mutation specific techniques are frequently used. Commercial kits (e.g. BRAF RGQ kit by Qiagen or Cobas 4800 by Roche...
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<table>
<thead>
<tr>
<th>Gene</th>
<th>Main Type of Alterations in thyroid tumors</th>
<th>Clinical significance</th>
<th>Commonly used detection methods</th>
</tr>
</thead>
<tbody>
<tr>
<td>BRAF</td>
<td>Point mutations</td>
<td>Diagnosis (FNA)</td>
<td>Sequencing</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Prognosis ?</td>
<td>Mutation specific methods</td>
</tr>
<tr>
<td>RAS (N61, H61, K12-13)</td>
<td>Point mutations</td>
<td>Diagnosis (FNA)</td>
<td>Sequencing</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Prognosis ?</td>
<td>Mutation specific methods</td>
</tr>
<tr>
<td>RET</td>
<td>Point mutations</td>
<td>Diagnosis of hereditary MTC</td>
<td>Sequencing</td>
</tr>
<tr>
<td>RET/PTC</td>
<td>Rearrangements</td>
<td>Diagnosis (FNA)</td>
<td>qRT-PCR</td>
</tr>
<tr>
<td>PAX8/PPARg</td>
<td>Rearrangements</td>
<td>Diagnosis (FNA)</td>
<td>FISH</td>
</tr>
</tbody>
</table>

N61 and H61 mean codon 61 of N-RAS and H-RAS genes respectively; K12-13 means codons 12-13 of K-RAS gene.

1Mutation specific methods should be not focused only on K-RAS codons 12-13

Recently a novel mutation specific antibody for BRAF V600E-mutated protein in papillary thyroid carcinomas has been successfully used for the detection of the BRAF V600E, as well as strip oligonucleotide-based multiplex PCR - DPO-PCR (38)) are successfully used to identify BRAF V600E, as well as strip hybridization assays (39) or denaturing high-performance liquid chromatography (40). Restriction fragment length analysis is not generally recommended, due to its low sensitivity and specificity (20). Highly sensitive methods, such as ASLNAqPCR or pyro sequencing, should be used when the percentage of tumor cells in the specimen is low or when tumor cell enrichment by microdissection is not feasible, as in the case of FNA specimens (37, 41).

Recently a novel mutation specific antibody for the detection of the BRAF V600E-mutated protein in papillary thyroid carcinomas (42).

3.2. RAS

The RAS genes family is composed by K-RAS, H-RAS and N-RAS, that codify for G-proteins which play a fundamental role in transduction of intracellular signals from the cell membrane. Constitutive activation of these three genes has been observed in all thyroid tumors originating from follicular cells. RAS mutations are observed in thyroid lesions with a variable frequency. They are not specific for malignancy since they are also present in benign hyperplastic nodules (43). They are found in follicular adenomas (~35%, range 20-40%), follicular carcinomas (~40%, range 30-50%), and in the follicular variant of papillary carcinomas (~35%, range 25-45%) (6, 7).

They are uncommon in conventional papillary carcinomas (44), but minor KRAS mutant subpopulations have been reported (45). RAS substitutions are frequently detected in anaplastic and poorly differentiated thyroid carcinomas. The mutations reported more often in thyroid tumours are at codon 61 of N-RAS and in H-RAS, even if other mutations have been found in other codons of all three genes (24) (43). A meta-analysis by Vasko et al. has shown that most of the mutations in follicular carcinomas (and adenomas) are at codon 61 of N-RAS, that codon 61 N-RAS mutations are more frequent in follicular carcinomas than in adenomas, that codon 61 H-RAS mutations are more frequent in follicular carcinomas than in benign nodules, and that codon 12 and 13 K-RAS mutations are more frequent in malignant tumors (follicular and papillary) than in benign nodules (43).

The presence of RAS mutations in thyroid carcinomas has been found to define tumours with a more aggressive behaviour and a less favourable prognosis (46-49). Mutations of H- and K-RAS, but not usually of N-RAS, have been observed in sporadic medullary thyroid carcinomas (50-53).

Analysis of RAS mutational status of thyroid FNA has been shown to improve the diagnostic accuracy of malignant nodules (54).

3.2.1. Detection methods

As for BRAF analysis, RAS mutations can be detected using Sanger sequencing, melting curve analysis, SSCP, pyrosequencing or mutation specific techniques (24, 51, 55, 56) (Figure 3, Table 2). It should be considered that, seen the high prevalence of H- and K-RAS codon 61 substitutions in thyroid tumours, mutational assays designed for the molecular analysis of colorectal carcinoma that detect only mutations of K-RAS exon 2 are not adequate. Assays investigating mutations in codon 61 of N-RAS and at least H-RAS codon 61 are necessary for molecular analysis. The use of next generation sequencing platforms may prove very useful because it allows to investigate the whole sequence of N- and H-RAS exon 3, as well as that of K-RAS exon 2 with high sensitivity.

3.3. RET

RET (REarranged during Transfection) is a proto-oncogene that encodes a transmembrane tyrosine-kinase receptor. The gene maps on chromosome 10 (cytoband q11.2) and its activation stimulates several signalling pathways, including the MAP kinase (MAPK) cascade.

RET is not normally expressed in the follicular cells of the thyroid gland. Germline point mutation of RET are identified in about 98% of hereditary MTC and in approximately 40% (range 30-70%) of the sporadic cases as somatic mutations (57). While in MEN type 2A about 80% of RET mutations are on codon 634 (especially C634R and Cys-Arg, or C634T, TGC-CAC Cys-Tyr), in MEN2B and in sporadic MTC the most frequent alteration is the M918T substitution (ATG-ACG Met-Thr) (58, 59). In the sporadic medullary thyroid carcinomas the M918T RET mutation has been associated with poor prognosis and persistence of the disease (58, 60). Although the C634R and the M918T mutations are the
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thyroidectomy is performed (62). Usually the germline mutation is found in one of them – a prophylactic mutations (Table 2). Obviously, RET testing has important implications for the relatives of the affected patient, and if a mutation is detected, the blood of all patients with MTC should be tested since in a small proportion (~7%) of patients with apparently sporadic medullary carcinoma there are germ-line mutations (57,72).

3.3.1. Detection methods

According to current guidelines (62), RET should be tested in the blood of all patients with MTC, since in a significant proportion (~7%) of patients with apparently sporadic medullary carcinoma there are germ-line mutations (Table 2). Obviously, RET testing has important implications for the relatives of the affected patient, and if a germline mutation is found in one of them – a prophylactic thyroidectomy is performed (62). Usually the RET gene is mutated in approximately two thirds and RET/PTC in approximately one third of RET/PTC positive cases (72).

RET/PTC rearrangements have been identified in up to up to ~80% of PTC from radiation exposed patients (74). They are also common in children and young adults (75).

In PTC the rearrangement can be present as a subclonal event in a minority of the neoplastic cells (77, 78). If very sensitive methods are used (78, 79) the rearrangement can be found in non-neoplastic (preneoplastic?) thyroid nodules (80) or in the follicular cells of Hashimoto’s thyroiditis (81).

3.4. RET/PTC

Point mutations are not the only alteration involving RET. The gene can also be altered because of chromosomal rearrangements leading to the RET/PTC fusion gene (PTC from Papillary Thyroid Carcinoma) (68, 69). More than 17 different RET/PTC products are currently known (57). Those that are more common are RET fusion with CCD6 (coiled-coil domain containing 6, cytogetenically detected as inv10(q11.2q21.2) – called RET/PTC1 (70), and RET fusion with NCO4 (Nuclear coactivator 4, consequence of paracentric inversion on chromosome 10q, not detectable by cytogenetic analysis) – called RET/PTC3 (71).

RET/PTC rearrangements have been identified in up to up to ~80% of PTC from radiation exposed patients (74). They are also common in children and young adults (75).

RET/PTC is a marker for papillary thyroid carcinoma (76), but it is detected with variable frequency in different studies that have used detection methods of different analytical sensitivity (72, 77).

Methods with high analytical sensitivity should not be used for molecular diagnosis, because they may detect the rearrangement in small neoplastic cell clones or identify low level rearrangement in non-neoplastic samples (77, 78).

3.5. PAX8/PPARg

PAX8/PPARg rearrangement was described for the first time in FTC by Kroll et al. in the year 2000 (85). The fusion gene is the results of a translocation between chromosome 3 and chromosome 2 – t(2;3)(q13;p25). The...
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Figure 4. Detection methods for RET/PTC rearrangement. A) real time RT-PCR of a thyroid carcinoma with RET/PTC1; B) In situ hybridization with RET break-apart bacterial artificial chromosome (BAC) probes; the red signal is proximal (centromeric), the green signal distal (telomeric) to RET (Courtesy of Dr. R. Vanni).

Figure 5. FISH detection of PAX8/PPARg rearrangement. In situ hybridization with PPARg break-apart bacterial artificial chromosome (BAC) probes; the red signal is proximal (centromeric), the green signal distal (telomeric) to PPARg (Courtesy of Dr. R. Vanni).

earrangement combines exons 1-6 of PPARg (Peroxisomal Proliferator-Activated Receptor g) with exons 7-9 of PAX8 (Paired Box 8).

PPARg is expressed at high level in adipocytes where it regulates cell function and differentiation. PAX8 encodes a transcription factor essential for the maintenance of the differentiated phenotype of thyroid follicular cells.

The rearrangement is found in ~30% (range 20-50%) of FTC (7, 85) (Nikiforova, Lynch et al. 2003), but also in the follicular variant of PTC- (~10% of cases) (7, 44) and in a few follicular adenomas (<10%) (Nikiforova, Lynch et al. 2003) (7, 86). Another translocation involving PPARg is the t(3;7)(p25;q34), in which there is in frame fusion of PPARg with exon 1 and 2 of CREB3L2 (cAMP responsive element binding protein 3-like 2). This rearrangement has been reported only in a small subset of FTC (87).

3.5.1. Detection methods
Several techniques are used to detect PAX8/PPARg fusion gene: immunohistochemistry, fluorescence in situ hybridization (FISH) and reverse-transcription PCR (RT-PCR) (85, 86, 88, 89) (Table 2). Immunohistochemistry identifies the upregulation of the PPARg protein that happens only if the rearrangement has occurred (Koenig 2010). However, it can be difficult to score and may give variable results (86, 90, 91). FISH (Figure 5) is considered the most useful method (23, 85), but it can be technically challenging and labor intensive. Real time RT-PCR is also useful and has a good correlation with the FISH results (83, 86). Other methods have been proposed, such as a 4-color reverse-transcription PCR assay (92).

3.6. Other molecular alterations
NTRK1. NTRK1 gene maps on chromosome 1q21-22 and encodes for a tyrosine kinase receptor. It is not expressed in thyroid follicular cells, but the rearrangements with different genes (TPM3, TPR, TFG) (93-96) result in constitutive activation of the protein. NTRK1 rearrangements (also known as TRK rearrangements) are a feature of a subset of papillary thyroid carcinomas. They share with RET/PTC similar oncogenetic mechanisms, but are much less common than RET/PTC (97, 98).

AKT. AKT, also known as protein kinase B (PKB), is a key component of the PI3K/PTEN/AKT signaling cascade. Mutations in AKT gene leading to constitutive activation of AKT have been detected in aggressive, high grade forms of thyroid cancer that do not respond to radioactive iodine treatment. AKT are heterogeneously present in neoplastic cells and have been associated with tumor progression (99).

PIK3CA. PIK3CA belongs to a family of transducer enzymes (PI3Ks) and is a key component of the PI3K/PTEN/AKT pathway. Mutations in these genes are concentrated on exon 9 and exon 20. These substitutions have been reported in follicular carcinomas (<10%), anaplastic thyroid cancers (~15%, range 5-25%) and rarely in papillary carcinomas (99, 100). PIK3CA amplification has been detected up to 40% of anaplastic carcinomas and in ~25% of follicular carcinomas (100-102).

PTEN. PTEN (phosphatase and tensin homolog) is a protein phosphatase that negatively regulates AKT and a key regulator of PI3K/Pten/AKT signalling. Loss of function germline PTEN mutations cause Cowden syndrome that is characterized by the development of follicular carcinoma in 10% of the patients, as well as by the occurrence of benign adenomas and hyperplastic adenomatous nodules. Inactivating mutations of PTEN have been detected in ~15% (range 5-20%) of anaplastic carcinomas, in up to 10% of follicular carcinomas and rarely in papillary carcinomas (100). Chromosome deletions and epigenetic modifications appear to be a more
common cause of PTEN loss of function than point mutations (100).

TSHR and Gsa. TSH (thyroid-stimulating hormone) binding to the TSH receptor leads to the production of cAMP mediated by Gsα. Mutations of TSHR, described for the first time in 1993 (103), occur especially in exon 10 (104). Gsα mutations, described for the first time in 1990 (105), cause constitutive activation of the Gsα protein. TSHR and Gsα mutations are typically present in hyperfunctioning nodules that are “hot” by thyroid scintigraphy (104). They are rare in thyroid carcinomas (106).

CTNNB1. The CTNNB1 gene encodes the β-catenin protein that is a cell adhesion molecule, that also acts as an effector of Wnt signalling. CTNNB1 mutations are common in anaplastic carcinomas where they have been identified in up to 65% of cases, and also occur in a subset of poorly differentiated tumours. CTNNB1 mutations are present in some sporadic cases of the cribriform morular variant of PTC, a rare histologic subtype of PTC that typically occurs in patients with familial adenomatous polyposis (FAP) (107, 108).

TP53. Tumour Protein 53 (TP53) is encoded by the p53 gene, that maps at chromosome 17p13.1. About 50% of human cancers are mutated for TP53 and exons 5 to 8 are usually analyzed for mutation detection. Mutations in p53 gene are found almost exclusively in high grade carcinomas that are poorly differentiated or anaplastic (109, 110).

3.7. MicroRNAs

MicroRNAs, or miRNAs, are small molecules (19-23 nucleotides) that regulates gene expression (111). They can be analyzed on formalin fixed samples and in fine needle aspiration specimens and their use as diagnostic or prognostic markers is being evaluated with encouraging results (7).

In PTC several studies have reported the up-regulation of miR-221 and miR-222 (112-121), miR-181b (114, 115, 118, 119), miR-146b (113, 115-118, 120, 121), miR-31 (113, 115, 116, 121), and miR-213 (114, 115). Less data are available about miRNAs that are down-regulated in PTC. Significant down-regulation of miR-1, identified as a tumor suppressor gene (122), and of miR-345, found down-regulated by two independent study (112, 115) has been reported.

miRNAs usually found up-regulated in PTC are not deregulated in anaplastic thyroid carcinomas (ATC) (123), even if Schwertheim et al. found miR-221 and miR-222 up-regulated also in this type of tumor (119). In ATC the miRNAs with higher overexpression appear to be miR-302, miR-205 and miR-137 (123, 124), while the most down-regulated miRNAs appear to be miR-30d, miR-125b, miR-26a, miR-30a, miR-138 and miR-125a (123). Distinct miRNA profiles have been shown for FTC, oncocytic FTC and the follicular variant of PTC(113, 125-127).

In medullary thyroid carcinomas (MTC), a study by Mian et al. found 9 miRNAs up-regulated (miR-9*, miR-21, mir-127, mir-183, mir-154, miR-224, miR-323, miR-370, miR-375), with no differences between sporadic and hereditary MTC (128).

3.8. Expression (cDNA) profiling

The analysis of cDNA expression profiles shows that PTC has distinct features that are different from FTC and other thyroid tumors (129-131). The expression signature has been correlated to papillary carcinoma subtypes, including the follicular and tall cell variants, and to specific oncogenic alterations like RET/PTC, BRAF and RAS mutations (132).

Studies that have analyzed the expression profile of follicular neoplasms, usually FTC, and have also shown distinct features (130, 133). Signatures to reliably distinguish between follicular adenoma and FTC have been proposed, but they need validation (134, 135). Among the proposed markers is HGMA2, that appears to be overexpressed in benign compared with malignant thyroid nodules (136, 137).

The analysis of expression profiles of thyroid cancer is being successfully applied to the molecular diagnosis of FNA samples. Molecular profiles of thyroid nodules that are associated with a benign histologic diagnosis have been identified, and data used to generate a molecular test with a high negative predictive value to be utilized for the preoperative diagnosis of thyroid nodules. Analysis of the expression profile of 167 proprietary genes (Veracyte) has been validated as a useful tool help decide which nodules with indeterminate cytologic diagnoses should be surgically removed and which can be safely followed because they have a very low risk of being malignant (138, 139).

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