[Frontiers in Bioscience 6, 1-14, January 1, 2014]

Molecular diagnosis of carcinomas of the thyroid gland

Dario de Biase[1], Michela Visani[2], Annalisa Pession[2], Giovanni Tallini[1]

[1]Anatomic Pathology, Bellaria Hospital, University of Bologna School of Medicine, Bologna, Italy, [2] Department of Pharmacy and Biotechnology (FABIT), University of Bologna, Bologna, Italy

TABLE OF CONTENTS

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

Table 1. Molecular alterations in thyroid carcinomas

Histotype	*BRAF* (%)	*RAS* (%)	*PAX8/PPARg* (%)	*RET/PTC* (%)	*RET* (%)	Others alterations (%)
PTC conventional (65% of thyroid carcinomas)	60	<5	0	20[1]	0	*NTRK* rearrangements (<5)
PTC follicular variant (20% of thyroid carcinomas)	10	35	10	0	0	0
FTC (5-10 of thyroid carcinomas)	0	40	30	0	0	*PIK3CA*(<10)[3] *PTEN* (<10)
PDC (<5 of thyroid carcinomas)	10[2]	30	0	<5[2]	0	*CTNNB1* (15) *p53* (30) *PIK3CA* (<10)[3] *AKT1* (5)[3]
ATC (<5 of thyroid carcinomas)	20[2]	40	0	<5[2]	0	*p53* (70) *CTNNB1*(50) *PIK3CA* (15)[3] *AKT1* (5)[3] *PTEN* (15)
MTC (5 of thyroid carcinomas)	<5	10[4]	0	0	40[5]	

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. [1]*RET/PTC* rearrangement incidence is higher (up to ~80%) in patients with history of radiation exposure. [2]The proportion of *BRAF* mutated cases is higher in cases were a PTC component can be demonstrated. [3]The proportion of cases with molecular alterations reported in the literature depends on the method used for detection. [4]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; [5]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 type 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 Hürthle 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/g089guidanceontherepportingofthyroidcytologyfinal.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 - Atypia 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

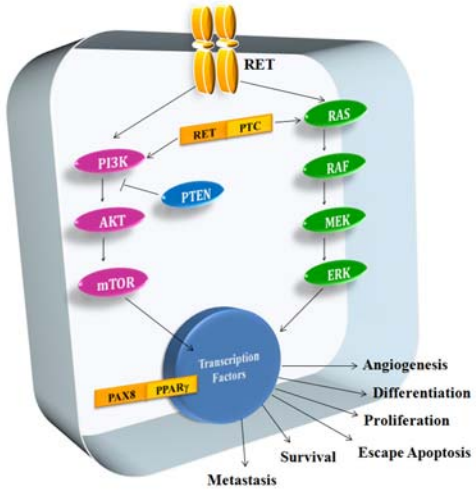

Figure 1. Molecular signalling in thyroid cells.

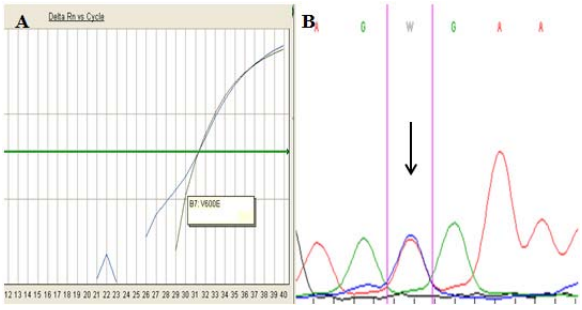

Figure 2. Methods for molecular analysis of *BRAF*. A) Allele specific PCR of a $BRAF^{V600E}$ mutated sample. B) Sanger sequencing profile of a sample with $BRAF^{V600E}$.

specific mutations varies in the different tumor types, most genes are mutated in tumors that originate from follicular cells (PTC, FTC, PDC, ATC) as well as in those of parafollicular cells (MTC) (Table 1). Alterations of the RET tyrosine kinase are particularly important for thyroid tumorigenesis. Germline point mutation of *RET* define the hereditary form of medullary carcinoma (Multiple Endocrine Neoplasia-MEN syndromes 2a and 2b, Familial Medullary Thyroid carcinoma-FMTC), while *RET* point mutations are present in ~40% of sporadic MTC. *RET* is rearranged in PTC *(RET/PTC)* and, together with *PAX8/PPARg* present in a subset of FTC, is the main chromosomal alteration identified in thyroid carcinomas. These genetic changes (point mutations and gene rearrangements) cause activation of the mitogen activated protein kinase (MAPK) and of the PI3K/Akt/mTOR pathways (Figure 1).

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 $BRAF^{V600E}$. $BRAF^{V600E}$ is a Valine to Glutammate 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. $BRAF^{K601E}$) 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 $BRAF^{V600E}$ (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).

$BRAF^{V600E}$ 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 $BRAF^{V600E}$ (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, $BRAF^{V600E}$ mutation specific techniques are frequently used. Commercial kits (e.g. BRAF RGQ kit by Qiagen or Cobas 4800 by Roche

Table 2. Commonly used detection methods for the more frequent alterations observed in thyroid tumors

Gene	Main Type of Alterations in thyroid tumors	Clinical significance	Commonly used detection methods
BRAF	Point mutations	Diagnosis (FNA) Prognosis ?	Sequencing Mutation specific methods IHC
RAS (N61, H61, K12-13)	Point mutations	Diagnosis (FNA) Prognosis ?	Sequencing Mutation specific methods[1]
RET	Point mutations	Diagnosis of hereditary MTC Prognosis ?	Sequencing
RET/PTC	Rearrangements	Diagnosis (FNA)	qRT-PCR FISH
PAX8/PPARg	Rearrangements	Diagnosis (FNA)	qRT-PCR FISH

N61 and H61 means codon 61 of *N-RAS* and *H-RAS* genes espectively; K12-13 means codons 12-13 of *K-RAS* gene. [1]Mutation specific methods should be not focused only on *K-RAS* codons 12-13

diagnostic), homemade tests (e.g. allele specific LNA qPCR – ASLNAqPCR (37) (Figure 1A) - or dual priming 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 pyrosequencing, 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 has been successfully used 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 tumors 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 *N-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 transmenbrane 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 the 80% of *RET* mutations are on codon 634 (especially C634R, TGC-CGC → Cys-Arg, or C634T, TGC-TAC → 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

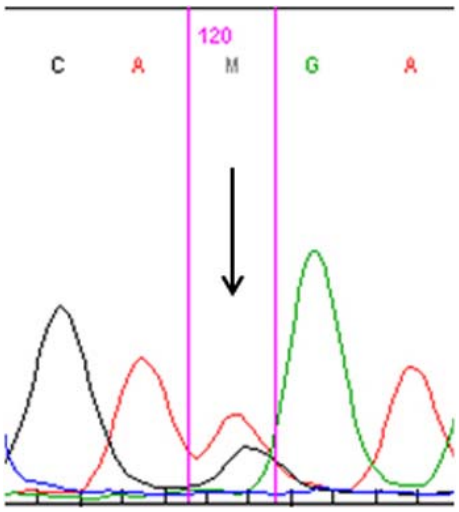

Figure 3. Sanger sequencing profile of a *N-RAS* - Q61H mutated sample.

most common, several other nucleotide substitutions are known (as C630R or A883F (61)).

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 exons 5, 8, 10, 11, 13, 14, 15 and 16 are analyzed. Exons 10 e 11 should be tested first because they are more frequently mutated. If no mutations are identified exons 13, 14, 15 e 16 are tested next, and if no mutations are present exons 5 and 8 are tested last.

The main method to identify RET mutations is Sanger sequencing because it allows to detect all mutations, even those that are less common. Other methods can be used including high resolution melting analysis (63), heteroduplex analysis (64), oligonucleotide microarray (65), lightcycler PCR assay (66), nonradioactive single-strand conformation polymorphism (67). For the detection of germline alterations in the peripheral blood Sanger sequencing allows to obtain very reliable results. The use of next generation sequencing platforms may prove very useful because it allows to investigate all exons with high sensitivity. Methods with high analytical sensitivity may be important to identify somatic alterations in tumor cases with low neoplastic cell enrichment. RET M918T and RET C634R testing of FNA samples may be considered if a medullary carcinoma is suspected preoperatively.

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, cytogenetically 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/PTC1 and *RET/PTC3* represent more than 90% of all rearrangements, with *RET/PTC1* being detected in approximately two thirds and *RET/PTC3* in approximately one third of *RET/PTC* positive cases (72). *RET/PTC2* (*RET* fusion with *PRKAR1A*) (73) represents less than 5% of all rearrangements, while the remaining variants are extremely rare and usually found only in radiation-associated tumors (57,72).

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).

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).

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 (pre-neoplastic?) thyroid nodules (80) or in the follicular cells of Hasimoto's thyroiditis (81).

3.4.1. Detection methods

Even if an antibodies against the tyrosine kinase domain of RET can be used to detect by immunohistochemistry the aberrant expression of RET in follicular cells, that become immunoreactive only if the rearrangement has occurred (72) (Table 2). However, cytoplasmatic immunoreactivity for RET with commercially available antibodies is often weak and highly variable (82). No rearrangement specific antibodies are currently available. The most reliable method to detect *RET/PTC* rearrangement is real-time RT-PCR that has an analytical sensitivity of ~1% (Figure 4A). Fluorescence *in situ* hybridization with specific hybridization probes can also be used (Figure 4B) (23, 83), provided that appropriate cut offs are set (78). Using FISH de Vries and colleagues found *RET/PTC* rearrangement in 6 out of 17 (35.3%) follicular Hürthle cell carcinomas analyzed (84).

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

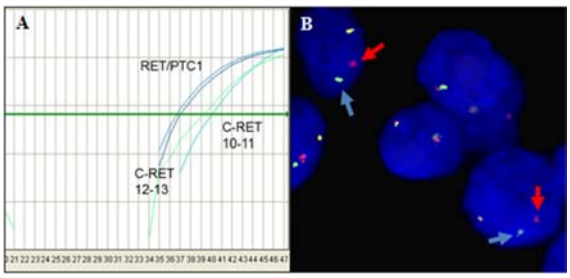

Figure 4. Detection methods for *RET/PTC* rearrangement. A) real time RT-PCR of a thyroid carcinoma with *RET/PTC1*; B) *In situ* hybridization with RET break-apart bacterial artificial chromosome (BAC) probes; the red signal is proximal (centromeric), the green signal distal (telomeric) to RET (Courtesy of Dr. R. Vanni).

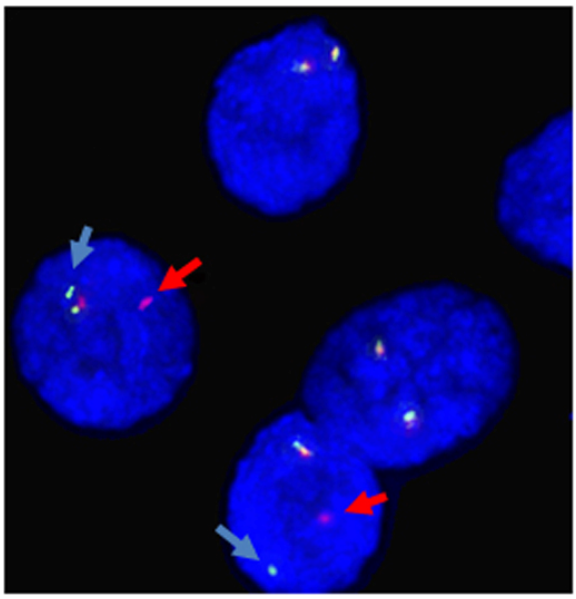

Figure 5. FISH detection of *PAX8/PPARg* rearrangement. *In situ* hybridization with *PPARg* break-apart bacterial artificial chromosome (BAC) probes; the red signal is proximal (centromeric), the green signal distal (telomeric) to *PPARg* (Courtesy of Dr. R. Vanni).

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 *Gsα*. 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).

4. AKNOWLEDGMENTS

This work was supported in part by an Italian Government MURST grant (Grant no. 20093XZC57_003) to GT.

5. REFERENCES

1. R. A. DeLellis, R. V. Lloyd, P. U. Heitz and C. Eng: Pathology and Genetics of Tumours of Endocrine Organs. Lyon: IARC press, (2004)

2. G. Tallini and C. Gallo: Fine-needle aspiration and intraoperative consultation in thyroid pathology: when and how? *Int J Surg Pathol*, 19(2), 141-4 (2011)

3. Y. E. Nikiforov, N. P. Ohori, S. P. Hodak, S. E. Carty, S. O. LeBeau, R. L. Ferris, L. Yip, R. R. Seethala, M. E. Tublin, M. T. Stang, C. Coyne, J. T. Johnson, A. F. Stewart and M. N. Nikiforova: Impact of mutational testing on the diagnosis and management of patients with cytologically indeterminate thyroid nodules: a prospective analysis of 1056 FNA samples. *J Clin Endocrinol Metab*, 96(11), 3390-7 (2011)

4. N. P. Ohori, R. Singhal, M. N. Nikiforova, L. Yip, K. E. Schoedel, C. Coyne, K. L. McCoy, S. O. Lebeau, S. P.

Hodak, S. E. Carty and Y. E. Nikiforov: BRAF mutation detection in indeterminate thyroid cytology specimens: Underlying cytologic, molecular, and pathologic characteristics of papillary thyroid carcinoma. *Cancer Cytopathol* (2012)

5. Y. E. Nikiforov, D. L. Steward, T. M. Robinson-Smith, B. R. Haugen, J. P. Klopper, Z. Zhu, J. A. Fagin, M. Falciglia, K. Weber and M. N. Nikiforova: Molecular testing for mutations in improving the fine-needle aspiration diagnosis of thyroid nodules. *J Clin Endocrinol Metab*, 94(6), 2092-8 (2009)

6. T. Kondo, S. Ezzat and S. L. Asa: Pathogenetic mechanisms in thyroid follicular-cell neoplasia. *Nat Rev Cancer*, 6(4), 292-306 (2006)

7. Y. E. Nikiforov and M. N. Nikiforova: Molecular genetics and diagnosis of thyroid cancer. *Nat Rev Endocrinol*, 7(10), 569-80 (2011)

8. M. Xing: BRAF mutation in papillary thyroid cancer: pathogenic role, molecular bases, and clinical implications. *Endocr Rev*, 28(7), 742-62 (2007)

9. J. A. Knauf, M. A. Sartor, M. Medvedovic, E. Lundsmith, M. Ryder, M. Salzano, Y. E. Nikiforov, T. J. Giordano, R. A. Ghossein and J. A. Fagin: Progression of BRAF-induced thyroid cancer is associated with epithelial-mesenchymal transition requiring concomitant MAP kinase and TGFbeta signaling. *Oncogene*, 30(28), 3153-62 (2011)

10. C. Nucera, A. Porrello, Z. A. Antonello, M. Mekel, M. A. Nehs, T. J. Giordano, D. Gerald, L. E. Benjamin, C. Priolo, E. Puxeddu, S. Finn, B. Jarzab, R. A. Hodin, A. Pontecorvi, V. Nose, J. Lawler and S. Parangi: B-Raf(V600E) and thrombospondin-1 promote thyroid cancer progression. *Proc Natl Acad Sci U S A*, 107(23), 10649-54 (2010)

11. A. M. Costa, A. Herrero, M. F. Fresno, J. Heymann, J. A. Alvarez, J. Cameselle-Teijeiro and G. Garcia-Rostan: BRAF mutation associated with other genetic events identifies a subset of aggressive papillary thyroid carcinoma. *Clin Endocrinol (Oxf)*, 68(4), 618-34 (2008)

12. V. Vasko, S. Hu, G. Wu, J. C. Xing, A. Larin, V. Savchenko, B. Trink and M. Xing: High prevalence and possible de novo formation of BRAF mutation in metastasized papillary thyroid cancer in lymph nodes. *J Clin Endocrinol Metab*, 90(9), 5265-9 (2005)

13. M. Xing: BRAF mutation in thyroid cancer. *Endocr Relat Cancer*, 12(2), 245-62 (2005)

14. Y. Cohen, M. Xing, E. Mambo, Z. Guo, G. Wu, B. Trink, U. Beller, W. H. Westra, P. W. Ladenson and D. Sidransky: BRAF mutation in papillary thyroid carcinoma. *J Natl Cancer Inst*, 95(8), 625-7 (2003)

15. E. T. Kimura, M. N. Nikiforova, Z. Zhu, J. A. Knauf, Y. E. Nikiforov and J. A. Fagin: High prevalence of BRAF mutations in thyroid cancer: genetic evidence for constitutive activation of the RET/PTC-RAS-BRAF signaling pathway in papillary thyroid carcinoma. *Cancer Res*, 63(7), 1454-7 (2003)

16. T. Fukushima, S. Suzuki, M. Mashiko, T. Ohtake, Y. Endo, Y. Takebayashi, K. Sekikawa, K. Hagiwara and S. Takenoshita: BRAF mutations in papillary carcinomas of the thyroid. *Oncogene*, 22(41), 6455-7 (2003)

17. V. Trovisco, P. Soares, A. Preto, I. V. de Castro, J. Lima, P. Castro, V. Maximo, T. Botelho, S. Moreira, A. M. Meireles, J. Magalhaes, A. Abrosimov, J. Cameselle-Teijeiro and M. Sobrinho-Simoes: Type and prevalence of BRAF mutations are closely associated with papillary thyroid carcinoma histotype and patients' age but not with tumour aggressiveness. *Virchows Arch*, 446(6), 589-95 (2005)

18. V. De Falco, R. Giannini, A. Tamburrino, C. Ugolini, C. Lupi, E. Puxeddu, M. Santoro and F. Basolo: Functional characterization of the novel T599I-VKSRdel BRAF mutation in a follicular variant papillary thyroid carcinoma. *J Clin Endocrinol Metab*, 93(11), 4398-402 (2008)

19. A. J. Adeniran, Z. Zhu, M. Gandhi, D. L. Steward, J. P. Fidler, T. J. Giordano, P. W. Biddinger and Y. E. Nikiforov: Correlation between genetic alterations and microscopic features, clinical manifestations, and prognostic characteristics of thyroid papillary carcinomas. *Am J Surg Pathol*, 30(2), 216-22 (2006)

20. K. W. Chung, S. K. Yang, G. K. Lee, E. Y. Kim, S. Kwon, S. H. Lee, J. Park do, H. S. Lee, B. Y. Cho, E. S. Lee and S. W. Kim: Detection of BRAFV600E mutation on fine needle aspiration specimens of thyroid nodule refines cyto-pathology diagnosis, especially in BRAF600E mutation-prevalent area. *Clin Endocrinol (Oxf)*, 65(5), 660-6 (2006)

21. M. N. Nikiforova and Y. E. Nikiforov: Molecular diagnostics and predictors in thyroid cancer. *Thyroid*, 19(12), 1351-61 (2009)

22. A. L. Melck, L. Yip and S. E. Carty: The utility of BRAF testing in the management of papillary thyroid cancer. *Oncologist*, 15(12), 1285-93 (2010)

23. P. Caria and R. Vanni: Cytogenetic and molecular events in adenoma and well-differentiated thyroid follicular-cell neoplasia. *Cancer Genet Cytogenet*, 203(1), 21-9 (2010)

24. M. N. Nikiforova, R. A. Lynch, P. W. Biddinger, E. K. Alexander, G. W. Dorn, 2nd, G. Tallini, T. G. Kroll and Y. E. Nikiforov: RAS point mutations and PAX8-PPAR gamma rearrangement in thyroid tumors: evidence for distinct molecular pathways in thyroid follicular carcinoma. *J Clin Endocrinol Metab*, 88(5), 2318-26 (2003)

25. C. Lupi, R. Giannini, C. Ugolini, A. Proietti, P. Berti, M. Minuto, G. Materazzi, R. Elisei, M. Santoro, P. Miccoli

and F. Basolo: Association of BRAF V600E mutation with poor clinicopathological outcomes in 500 consecutive cases of papillary thyroid carcinoma. *J Clin Endocrinol Metab*, 92(11), 4085-90 (2007)

26. M. Xing, W. H. Westra, R. P. Tufano, Y. Cohen, E. Rosenbaum, K. J. Rhoden, K. A. Carson, V. Vasko, A. Larin, G. Tallini, S. Tolaney, E. H. Holt, P. Hui, C. B. Umbricht, S. Basaria, M. Ewertz, A. P. Tufaro, J. A. Califano, M. D. Ringel, M. A. Zeiger, D. Sidransky and P. W. Ladenson: BRAF mutation predicts a poorer clinical prognosis for papillary thyroid cancer. *J Clin Endocrinol Metab*, 90(12), 6373-9 (2005)

27. R. Elisei, C. Ugolini, D. Viola, C. Lupi, A. Biagini, R. Giannini, C. Romei, P. Miccoli, A. Pinchera and F. Basolo: BRAF(V600E) mutation and outcome of patients with papillary thyroid carcinoma: a 15-year median follow-up study. *J Clin Endocrinol Metab*, 93(10), 3943-9 (2008)

28. F. Basolo, L. Torregrossa, R. Giannini, M. Miccoli, C. Lupi, E. Sensi, P. Berti, R. Elisei, P. Vitti, A. Baggiani and P. Miccoli: Correlation between the BRAF V600E mutation and tumor invasiveness in papillary thyroid carcinomas smaller than 20 millimeters: analysis of 1060 cases. *J Clin Endocrinol Metab*, 95(9), 4197-205 (2010)

29. Y. Ito, H. Yoshida, R. Maruo, S. Morita, T. Takano, M. Hirokawa, T. Yabuta, M. Fukushima, H. Inoue, C. Tomoda, M. Kihara, T. Uruno, T. Higashiyama, Y. Takamura, A. Miya, K. Kobayashi, F. Matsuzuka and A. Miyauchi: BRAF mutation in papillary thyroid carcinoma in a Japanese population: its lack of correlation with high-risk clinicopathological features and disease-free survival of patients. *Endocr J*, 56(1), 89-97 (2009)

30. L. Fugazzola, E. Puxeddu, N. Avenia, C. Romei, V. Cirello, A. Cavaliere, P. Faviana, D. Mannavola, S. Moretti, S. Rossi, M. Sculli, V. Bottici, P. Beck-Peccoz, F. Pacini, A. Pinchera, F. Santeusanio and R. Elisei: Correlation between B-RAFV600E mutation and clinico-pathologic parameters in papillary thyroid carcinoma: data from a multicentric Italian study and review of the literature. *Endocr Relat Cancer*, 13(2), 455-64 (2006)

31. V. Sancisi, D. Nicoli, M. Ragazzi, S. Piana and A. Ciarrocchi: BRAFV600E mutation does not mean distant metastasis in thyroid papillary carcinomas. *J Clin Endocrinol Metab*, 97(9), E1745-9 (2012)

32. L. R. Rowe, B. G. Bentz and J. S. Bentz: Detection of BRAF V600E activating mutation in papillary thyroid carcinoma using PCR with allele-specific fluorescent probe melting curve analysis. *J Clin Pathol*, 60(11), 1211-5 (2007)

33. G. Salvatore, R. Giannini, P. Faviana, A. Caleo, I. Migliaccio, J. A. Fagin, Y. E. Nikiforov, G. Troncone, L. Palombini, F. Basolo and M. Santoro: Analysis of BRAF point mutation and RET/PTC rearrangement refines the fine-needle aspiration diagnosis of papillary thyroid carcinoma. *J Clin Endocrinol Metab*, 89(10), 5175-80 (2004)

34. Y. S. Jo, S. Huang, Y. J. Kim, I. S. Lee, S. S. Kim, J. R. Kim, T. Oh, Y. Moon, S. An, H. K. Ro, J. M. Kim and M. Shong: Diagnostic value of pyrosequencing for the BRAF V600E mutation in ultrasound-guided fine-needle aspiration biopsy samples of thyroid incidentalomas. *Clin Endocrinol (Oxf)*, 70(1), 139-44 (2009)

35. M. R. James, T. Dumeni, M. S. Stark, D. L. Duffy, G. W. Montgomery, N. G. Martin and N. K. Hayward: Rapid screening of 4000 individuals for germ-line variations in the BRAF gene. *Clin Chem*, 52(9), 1675-8 (2006)

36. I. J. Kim, H. C. Kang, S. G. Jang, S. A. Ahn, H. J. Yoon and J. G. Park: Development and applications of a BRAF oligonucleotide microarray. *J Mol Diagn*, 9(1), 55-63 (2007)

37. L. Morandi, D. de Biase, M. Visani, V. Cesari, G. De Maglio, S. Pizzolitto, A. Pession and G. Tallini: Allele specific locked nucleic acid quantitative PCR (ASLNAqPCR): an accurate and cost-effective assay to diagnose and quantify KRAS and BRAF mutation. *PLoS One*, 7(4), e36084 (2012)

38. J. Y. Kwak, E. K. Kim, J. K. Kim, J. H. Han, S. W. Hong, T. S. Park and J. R. Choi: Dual priming oligonucleotide-based multiplex PCR analysis for detection of BRAFV600E mutation in FNAB samples of thyroid nodules in BRAFV600E mutation-prevalent area. *Head Neck*, 32(4), 490-8 (2010)

39. A. Marchetti, L. Felicioni, S. Malatesta, M. Grazia Sciarrotta, L. Guetti, A. Chella, P. Viola, C. Pullara, F. Mucilli and F. Buttitta: Clinical features and outcome of patients with non-small-cell lung cancer harboring BRAF mutations. *J Clin Oncol*, 29(26), 3574-9 (2011)

40. T. E. Kim, E. S. Jung, C. K. Jung, J. S. Bae, S. N. Kim, G. S. Kim, H. N. Lee, C. S. Kang and Y. J. Choi: DHPLC is a highly sensitive and rapid screening method to detect BRAF(V600E) mutation in papillary thyroid carcinoma. *Exp Mol Pathol*, 94(1), 203-209 (2012)

41. A. Guerra, M. R. Sapio, V. Marotta, E. Campanile, S. Rossi, I. Forno, L. Fugazzola, A. Budillon, T. Moccia, G. Fenzi and M. Vitale: The primary occurrence of BRAF(V600E) is a rare clonal event in papillary thyroid carcinoma. *J Clin Endocrinol Metab*, 97(2), 517-24 (2012)

42. O. Koperek, C. Kornauth, D. Capper, A. S. Berghoff, R. Asari, B. Niederle, A. von Deimling, P. Birner and M. Preusser: Immunohistochemical detection of the BRAF V600E-mutated protein in papillary thyroid carcinoma. *Am J Surg Pathol*, 36(6), 844-50 (2012)

43. V. Vasko, M. Ferrand, J. Di Cristofaro, P. Carayon, J. F. Henry and C. de Micco: Specific pattern of RAS oncogene mutations in follicular thyroid tumors. *J Clin Endocrinol Metab*, 88(6), 2745-52 (2003)

44. P. Castro, A. P. Rebocho, R. J. Soares, J. Magalhaes, L. Roque, V. Trovisco, I. Vieira de Castro, M. Cardoso-de-

Oliveira, E. Fonseca, P. Soares and M. Sobrinho-Simoes: PAX8-PPARgamma rearrangement is frequently detected in the follicular variant of papillary thyroid carcinoma. *J Clin Endocrinol Metab*, 91(1), 213-20 (2006)

45. M. B. Myers, K. L. McKim and B. L. Parsons: A subset of papillary thyroid carcinomas contain KRAS mutant subpopulations at levels above normal thyroid. *Mol Carcinog* (2012)

46. G. Garcia-Rostan, H. Zhao, R. L. Camp, M. Pollan, A. Herrero, J. Pardo, R. Wu, M. L. Carcangiu, J. Costa and G. Tallini: ras mutations are associated with aggressive tumor phenotypes and poor prognosis in thyroid cancer. *J Clin Oncol*, 21(17), 3226-35 (2003)

47. F. Basolo, F. Pisaturo, L. E. Pollina, G. Fontanini, R. Elisei, E. Molinaro, P. Iacconi, P. Miccoli and F. Pacini: N-ras mutation in poorly differentiated thyroid carcinomas: correlation with bone metastases and inverse correlation to thyroglobulin expression. *Thyroid*, 10(1), 19-23 (2000)

48. H. Karga, J. K. Lee, A. L. Vickery, Jr., A. Thor, R. D. Gaz and J. L. Jameson: Ras oncogene mutations in benign and malignant thyroid neoplasms. *J Clin Endocrinol Metab*, 73(4), 832-6 (1991)

49. M. Volante, I. Rapa, M. Gandhi, G. Bussolati, D. Giachino, M. Papotti and Y. E. Nikiforov: RAS mutations are the predominant molecular alteration in poorly differentiated thyroid carcinomas and bear prognostic impact. *J Clin Endocrinol Metab*, 94(12), 4735-41 (2009)

50. A. Boichard, L. Croux, A. Al Ghuzlan, S. Broutin, C. Dupuy, S. Leboulleux, M. Schlumberger, J. M. Bidart and L. Lacroix: Somatic RAS mutations occur in a large proportion of sporadic RET-negative medullary thyroid carcinomas and extend to a previously unidentified exon. *J Clin Endocrinol Metab*, 97(10), E2031-5 (2012)

51. R. Ciampi, C. Mian, L. Fugazzola, B. Cosci, C. Romei, S. Barollo, V. Cirello, V. Bottici, G. Marconcini, P. M. Rosa, M. G. Borrello, F. Basolo, C. Ugolini, G. Materazzi, A. Pinchera and R. Elisei: Evidence of a low prevalence of RAS mutations in a large medullary thyroid cancer series. *Thyroid*, 23(1), 50-7 (2013)

52. M. M. Moura, B. M. Cavaco, A. E. Pinto and V. Leite: High prevalence of RAS mutations in RET-negative sporadic medullary thyroid carcinomas. *J Clin Endocrinol Metab*, 96(5), E863-8 (2011)

53. N. Agrawal, Y. Jiao, M. Sausen, R. Leary, C. Bettegowda, N. J. Roberts, S. Bhan, A. S. Ho, Z. Khan, J. Bishop, W. H. Westra, L. D. Wood, R. H. Hruban, R. P. Tufano, B. Robinson, H. Dralle, S. P. Toledo, R. A. Toledo, L. G. Morris, R. A. Ghossein, J. A. Fagin, T. A. Chan, V. E. Velculescu, B. Vogelstein, K. W. Kinzler, N. Papadopoulos, B. D. Nelkin and D. W. Ball: Exomic Sequencing of Medullary Thyroid Cancer Reveals Dominant and Mutually Exclusive Oncogenic Mutations in RET and RAS. *J Clin Endocrinol Metab*, 98(2), E364-9 (2013)

54. W. Moses, J. Weng, I. Sansano, M. Peng, E. Khanafshar, B. M. Ljung, Q. Y. Duh, O. H. Clark and E. Kebebew: Molecular testing for somatic mutations improves the accuracy of thyroid fine-needle aspiration biopsy. *World J Surg*, 34(11), 2589-94 (2010)

55. Y. E. Nikiforov, M. N. Nikiforova, D. R. Gnepp and J. A. Fagin: Prevalence of mutations of ras and p53 in benign and malignant thyroid tumors from children exposed to radiation after the Chernobyl nuclear accident. *Oncogene*, 13(4), 687-93 (1996)

56. K. Y. Na, R. M. Kim, E. M. Song, J. H. Lee, J. Lee and E. Y. Soh: Allelic loss of susceptibility loci and the occurrence of BRAF and RAS mutations in patients with familial non-medullary thyroid cancer. *J Surg Oncol*, 105(1), 10-4 (2012)

57. M. D. Castellone and M. Santoro: Dysregulated RET signaling in thyroid cancer. *Endocrinol Metab Clin North Am*, 37(2), 363-74, viii (2008)

58. R. Elisei, B. Cosci, C. Romei, V. Bottici, G. Renzini, E. Molinaro, L. Agate, A. Vivaldi, P. Faviana, F. Basolo, P. Miccoli, P. Berti, F. Pacini and A. Pinchera: Prognostic significance of somatic RET oncogene mutations in sporadic medullary thyroid cancer: a 10-year follow-up study. *J Clin Endocrinol Metab*, 93(3), 682-7 (2008)

59. M. K. Punales, H. Graf, J. L. Gross and A. L. Maia: RET codon 634 mutations in multiple endocrine neoplasia type 2: variable clinical features and clinical outcome. *J Clin Endocrinol Metab*, 88(6), 2644-9 (2003)

60. T. Schilling, J. Burck, H. P. Sinn, A. Clemens, H. F. Otto, W. Hoppner, C. Herfarth, R. Ziegler, M. Schwab and F. Raue: Prognostic value of codon 918 (ATG-->ACG) RET proto-oncogene mutations in sporadic medullary thyroid carcinoma. *Int J Cancer*, 95(1), 62-6 (2001)

61. S. A. Forbes, N. Bindal, S. Bamford, C. Cole, C. Y. Kok, D. Beare, M. Jia, R. Shepherd, K. Leung, A. Menzies, J. W. Teague, P. J. Campbell, M. R. Stratton and P. A. Futreal: COSMIC: mining complete cancer genomes in the Catalogue of Somatic Mutations in Cancer. *Nucleic Acids Res*, 39(Database issue), D945-50 (2011)

62. R. T. Kloos, C. Eng, D. B. Evans, G. L. Francis, R. F. Gagel, H. Gharib, J. F. Moley, F. Pacini, M. D. Ringel, M. Schlumberger and S. A. Wells, Jr.: Medullary thyroid cancer: management guidelines of the American Thyroid Association. *Thyroid*, 19(6), 565-612 (2009)

63. R. L. Margraf, R. Mao, W. E. Highsmith, L. M. Holtegaard and C. T. Wittwer: Mutation scanning of the RET protooncogene using high-resolution melting analysis. *Clin Chem*, 52(1), 138-41 (2006)

64. M. Kambouris, C. E. Jackson and G. L. Feldman: Diagnosis of multiple endocrine neoplasia [MEN] 2A, 2B and familial medullary thyroid cancer [FMTC] by multiplex PCR and heteroduplex analyses of RET proto-oncogene mutations. *Hum Mutat*, 8(1), 64-70 (1996)

65. I. J. Kim, H. C. Kang, J. H. Park, J. L. Ku, J. S. Lee, H. J. Kwon, K. A. Yoon, S. C. Heo, H. Y. Yang, B. Y. Cho, S. Y. Kim, S. K. Oh, Y. K. Youn, D. J. Park, M. S. Lee, K. W. Lee and J. G. Park: RET oligonucleotide microarray for the detection of RET mutations in multiple endocrine neoplasia type 2 syndromes. *Clin Cancer Res*, 8(2), 457-63 (2002)

66. M. de la Fuente, C. Quinteiro, F. Dominguez and L. Loidi: LightCycler PCR assay for genotyping codon 634 mutations in the RET protooncogene. *Clin Chem*, 47(6), 1131-2 (2001)

67. P. Komminoth, E. Kunz, O. Hiort, S. Schroder, X. Matias-Guiu, G. Christiansen, J. Roth and P. U. Heitz: Detection of RET proto-oncogene point mutations in paraffin-embedded pheochromocytoma specimens by nonradioactive single-strand conformation polymorphism analysis and direct sequencing. *Am J Pathol*, 145(4), 922-9 (1994)

68. M. Grieco, M. Santoro, M. T. Berlingieri, R. M. Melillo, R. Donghi, I. Bongarzone, M. A. Pierotti, G. Della Porta, A. Fusco and G. Vecchio: PTC is a novel rearranged form of the ret proto-oncogene and is frequently detected *in vivo* in human thyroid papillary carcinomas. *Cell*, 60(4), 557-63 (1990)

69. A. Fusco, M. Grieco, M. Santoro, M. T. Berlingieri, S. Pilotti, M. A. Pierotti, G. Della Porta and G. Vecchio: A new oncogene in human thyroid papillary carcinomas and their lymph-nodal metastases. *Nature*, 328(6126), 170-2 (1987)

70. M. A. Pierotti, M. Santoro, R. B. Jenkins, G. Sozzi, I. Bongarzone, M. Grieco, N. Monzini, M. Miozzo, M. A. Herrmann, A. Fusco and *et al.*: Characterization of an inversion on the long arm of chromosome 10 juxtaposing D10S170 and RET and creating the oncogenic sequence RET/PTC. *Proc Natl Acad Sci U S A*, 89(5), 1616-20 (1992)

71. M. Santoro, N. A. Dathan, M. T. Berlingieri, I. Bongarzone, C. Paulin, M. Grieco, M. A. Pierotti, G. Vecchio and A. Fusco: Molecular characterization of RET/PTC3; a novel rearranged version of the RETproto-oncogene in a human thyroid papillary carcinoma. *Oncogene*, 9(2), 509-16 (1994)

72. G. Tallini and S. L. Asa: RET oncogene activation in papillary thyroid carcinoma. *Adv Anat Pathol*, 8(6), 345-54 (2001)

73. I. Bongarzone, N. Monzini, M. G. Borrello, C. Carcano, G. Ferraresi, E. Arighi, P. Mondellini, G. Della Porta and M. A. Pierotti: Molecular characterization of a thyroid tumor-specific transforming sequence formed by the fusion of ret tyrosine kinase and the regulatory subunit RI alpha of cyclic AMP-dependent protein kinase A. *Mol Cell Biol*, 13(1), 358-66 (1993)

74. H. M. Rabes, E. P. Demidchik, J. D. Sidorow, E. Lengfelder, C. Beimfohr, D. Hoelzel and S. Klugbauer: Pattern of radiation-induced RET and NTRK1 rearrangements in 191 post-chernobyl papillary thyroid carcinomas: biological, phenotypic, and clinical implications. *Clin Cancer Res*, 6(3), 1093-103 (2000)

75. C. L. Fenton, Y. Lukes, D. Nicholson, C. A. Dinauer, G. L. Francis and R. M. Tuttle: The ret/PTC mutations are common in sporadic papillary thyroid carcinoma of children and young adults. *J Clin Endocrinol Metab*, 85(3), 1170-5 (2000)

76. M. Santoro, F. Carlomagno, I. D. Hay, M. A. Herrmann, M. Grieco, R. Melillo, M. A. Pierotti, I. Bongarzone, G. Della Porta, N. Berger and *et al.*: Ret oncogene activation in human thyroid neoplasms is restricted to the papillary cancer subtype. *J Clin Invest*, 89(5), 1517-22 (1992)

77. K. J. Rhoden, C. Johnson, G. Brandao, J. G. Howe, B. R. Smith and G. Tallini: Real-time quantitative RT-PCR identifies distinct c-RET, RET/PTC1 and RET/PTC3 expression patterns in papillary thyroid carcinoma. *Lab Invest*, 84(12), 1557-70 (2004)

78. Z. Zhu, R. Ciampi, M. N. Nikiforova, M. Gandhi and Y. E. Nikiforov: Prevalence of RET/PTC rearrangements in thyroid papillary carcinomas: effects of the detection methods and genetic heterogeneity. *J Clin Endocrinol Metab*, 91(9), 3603-10 (2006)

79. G. Tallini and G. Brandao: Assessment of RET/PTC oncogene activation in thyroid nodules utilizing laser microdissection followed by nested RT-PCR. *Methods Mol Biol*, 293, 103-11 (2005)

80. A. Fusco, G. Chiappetta, P. Hui, G. Garcia-Rostan, L. Golden, B. K. Kinder, D. A. Dillon, A. Giuliano, A. M. Cirafici, M. Santoro, J. Rosai and G. Tallini: Assessment of RET/PTC oncogene activation and clonality in thyroid nodules with incomplete morphological evidence of papillary carcinoma: a search for the early precursors of papillary cancer. *Am J Pathol*, 160(6), 2157-67 (2002)

81. K. J. Rhoden, K. Unger, G. Salvatore, Y. Yilmaz, V. Vovk, G. Chiappetta, M. B. Qumsiyeh, J. L. Rothstein, A. Fusco, M. Santoro, H. Zitzelsberger and G. Tallini: RET/papillary thyroid cancer rearrangement in nonneoplastic thyrocytes: follicular cells of Hashimoto's thyroiditis share low-level recombination events with a subset of papillary carcinoma. *J Clin Endocrinol Metab*, 91(6), 2414-23 (2006)

82. V. Marotta, A. Guerra, M. R. Sapio and M. Vitale: RET/PTC rearrangement in benign and malignant thyroid diseases: a clinical standpoint. *Eur J Endocrinol*, 165(4), 499-507 (2011)

83. C. Ferraz, C. Rehfeld, A. Krogdahl, E. M. Precht Jensen, E. Bosenberg, F. Narz, L. Hegedus, R. Paschke and M. Eszlinger: Detection of PAX8/PPARG and RET/PTC rearrangements is feasible in routine air-dried fine needle aspiration smears. *Thyroid*, 22(10), 1025-30 (2012)

84. M. M. de Vries, R. Celestino, P. Castro, C. Eloy, V. Maximo, J. E. van der Wal, J. T. Plukker, T. P. Links, R. M. Hofstra, M. Sobrinho-Simoes and P. Soares: RET/PTC rearrangement is prevalent in follicular Hurthle cell carcinomas. *Histopathology*, 61(5), 833-43 (2012)

85. T. G. Kroll, P. Sarraf, L. Pecciarini, C. J. Chen, E. Mueller, B. M. Spiegelman and J. A. Fletcher: PAX8-PPARgamma1 fusion oncogene in human thyroid carcinoma [corrected]. *Science*, 289(5483), 1357-60 (2000)

86. M. N. Nikiforova, P. W. Biddinger, C. M. Caudill, T. G. Kroll and Y. E. Nikiforov: PAX8-PPARgamma rearrangement in thyroid tumors: RT-PCR and immunohistochemical analyses. *Am J Surg Pathol*, 26(8), 1016-23 (2002)

87. W. O. Lui, L. Zeng, V. Rehrmann, S. Deshpande, M. Tretiakova, E. L. Kaplan, I. Leibiger, B. Leibiger, U. Enberg, A. Hoog, C. Larsson and T. G. Kroll: CREB3L2-PPARgamma fusion mutation identifies a thyroid signaling pathway regulated by intramembrane proteolysis. *Cancer Res*, 68(17), 7156-64 (2008)

88. M. Sahin, B. L. Allard, M. Yates, J. G. Powell, X. L. Wang, I. D. Hay, Y. Zhao, J. R. Goellner, T. J. Sebo, S. K. Grebe, N. L. Eberhardt and B. McIver: PPARgamma staining as a surrogate for PAX8/PPARgamma fusion oncogene expression in follicular neoplasms: clinicopathological correlation and histopathological diagnostic value. *J Clin Endocrinol Metab*, 90(1), 463-8 (2005)

89. T. Dwight, S. R. Thoppe, T. Foukakis, W. O. Lui, G. Wallin, A. Hoog, T. Frisk, C. Larsson and J. Zedenius: Involvement of the PAX8/peroxisome proliferator-activated receptor gamma rearrangement in follicular thyroid tumors. *J Clin Endocrinol Metab*, 88(9), 4440-5 (2003)

90. R. J. Koenig: Detection of the PAX8-PPARgamma fusion protein in thyroid tumors. *Clin Chem*, 56(3), 331-3 (2010)

91. L. Lacroix, C. Mian, T. Barrier, M. Talbot, B. Caillou, M. Schlumberger and J. M. Bidart: PAX8 and peroxisome proliferator-activated receptor gamma 1 gene expression status in benign and malignant thyroid tissues. *Eur J Endocrinol*, 151(3), 367-74 (2004)

92. A. Algeciras-Schimnich, D. Milosevic, B. McIver, H. Flynn, H. V. Reddi, N. L. Eberhardt and S. K. Grebe: Evaluation of the PAX8/PPARG translocation in follicular thyroid cancer with a 4-color reverse-transcription PCR assay and automated high-resolution fragment analysis. *Clin Chem*, 56(3), 391-8 (2010)

93. P. Radice, G. Sozzi, M. Miozzo, V. De Benedetti, T. Cariani, I. Bongarzone, N. K. Spurr, M. A. Pierotti and G. Della Porta: The human tropomyosin gene involved in the generation of the TRK oncogene maps to chromosome 1q31. *Oncogene*, 6(11), 2145-8 (1991)

94. A. Greco, C. Mariani, C. Miranda, A. Lupas, S. Pagliardini, M. Pomati and M. A. Pierotti: The DNA rearrangement that generates the TRK-T3 oncogene involves a novel gene on chromosome 3 whose product has a potential coiled-coil domain. *Mol Cell Biol*, 15(11), 6118-27 (1995)

95. A. Greco, M. A. Pierotti, I. Bongarzone, S. Pagliardini, C. Lanzi and G. Della Porta: TRK-T1 is a novel oncogene formed by the fusion of TPR and TRK genes in human papillary thyroid carcinomas. *Oncogene*, 7(2), 237-42 (1992)

96. A. Greco, C. Miranda, S. Pagliardini, L. Fusetti, I. Bongarzone and M. A. Pierotti: Chromosome 1 rearrangements involving the genes TPR and NTRK1 produce structurally different thyroid-specific TRK oncogenes. *Genes Chromosomes Cancer*, 19(2), 112-23 (1997)

97. H. M. Rabes: Gene rearrangements in radiation-induced thyroid carcinogenesis. *Med Pediatr Oncol*, 36(5), 574-82 (2001)

98. J. P. Russell, D. J. Powell, M. Cunnane, A. Greco, G. Portella, M. Santoro, A. Fusco and J. L. Rothstein: The TRK-T1 fusion protein induces neoplastic transformation of thyroid epithelium. *Oncogene*, 19(50), 5729-35 (2000)

99. J. C. Ricarte-Filho, M. Ryder, D. A. Chitale, M. Rivera, A. Heguy, M. Ladanyi, M. Janakiraman, D. Solit, J. A. Knauf, R. M. Tuttle, R. A. Ghossein and J. A. Fagin: Mutational profile of advanced primary and metastatic radioactive iodine-refractory thyroid cancers reveals distinct pathogenetic roles for BRAF, PIK3CA, and AKT1. *Cancer Res*, 69(11), 4885-93 (2009)

100. J. E. Paes and M. D. Ringel: Dysregulation of the phosphatidylinositol 3-kinase pathway in thyroid neoplasia. *Endocrinol Metab Clin North Am*, 37(2), 375-87, viii-ix (2008)

101. L. Santarpia, A. K. El-Naggar, G. J. Cote, J. N. Myers and S. I. Sherman: Phosphatidylinositol 3-kinase/akt and ras/raf-mitogen-activated protein kinase pathway mutations in anaplastic thyroid cancer. *J Clin Endocrinol Metab*, 93(1), 278-84 (2008)

102. G. Garcia-Rostan, A. M. Costa, I. Pereira-Castro, G. Salvatore, R. Hernandez, M. J. Hermsem, A. Herrero, A. Fusco, J. Cameselle-Teijeiro and M. Santoro: Mutation of the PIK3CA gene in anaplastic thyroid cancer. *Cancer Res*, 65(22), 10199-207 (2005)

103. J. Parma, L. Duprez, J. Van Sande, P. Cochaux, C. Gervy, J. Mockel, J. Dumont and G. Vassart: Somatic

mutations in the thyrotropin receptor gene cause hyperfunctioning thyroid adenomas. *Nature*, 365(6447), 649-51 (1993)

104. K. Krohn, D. Fuhrer, Y. Bayer, M. Eszlinger, V. Brauer, S. Neumann and R. Paschke: Molecular pathogenesis of euthyroid and toxic multinodular goiter. *Endocr Rev*, 26(4), 504-24 (2005)

105. J. Lyons, C. A. Landis, G. Harsh, L. Vallar, K. Grunewald, H. Feichtinger, Q. Y. Duh, O. H. Clark, E. Kawasaki, H. R. Bourne and *et al.*: Two G protein oncogenes in human endocrine tumors. *Science*, 249(4969), 655-9 (1990)

106. D. Spambalg, N. Sharifi, R. Elisei, J. L. Gross, G. Medeiros-Neto and J. A. Fagin: Structural studies of the thyrotropin receptor and Gs alpha in human thyroid cancers: low prevalence of mutations predicts infrequent involvement in malignant transformation. *J Clin Endocrinol Metab*, 81(11), 3898-901 (1996)

107. G. Garcia-Rostan, R. L. Camp, A. Herrero, M. L. Carcangiu, D. L. Rimm and G. Tallini: Beta-catenin dysregulation in thyroid neoplasms: down-regulation, aberrant nuclear expression, and CTNNB1 exon 3 mutations are markers for aggressive tumor phenotypes and poor prognosis. *Am J Pathol*, 158(3), 987-96 (2001)

108. G. Garcia-Rostan, G. Tallini, A. Herrero, T. G. D'Aquila, M. L. Carcangiu and D. L. Rimm: Frequent mutation and nuclear localization of beta-catenin in anaplastic thyroid carcinoma. *Cancer Res*, 59(8), 1811-5 (1999)

109. R. Donghi, A. Longoni, S. Pilotti, P. Michieli, G. Della Porta and M. A. Pierotti: Gene p53 mutations are restricted to poorly differentiated and undifferentiated carcinomas of the thyroid gland. *J Clin Invest*, 91(4), 1753-60 (1993)

110. Y. Dobashi, H. Sugimura, A. Sakamoto, M. Mernyei, M. Mori, T. Oyama and R. Machinami: Stepwise participation of p53 gene mutation during dedifferentiation of human thyroid carcinomas. *Diagn Mol Pathol*, 3(1), 9-14 (1994)

111. D. P. Bartel: MicroRNAs: genomics, biogenesis, mechanism, and function. *Cell*, 116(2), 281-97 (2004)

112. H. He, K. Jazdzewski, W. Li, S. Liyanarachchi, R. Nagy, S. Volinia, G. A. Calin, C. G. Liu, K. Franssila, S. Suster, R. T. Kloos, C. M. Croce and A. de la Chapelle: The role of microRNA genes in papillary thyroid carcinoma. *Proc Natl Acad Sci U S A*, 102(52), 19075-80 (2005)

113. M. N. Nikiforova, G. C. Tseng, D. Steward, D. Diorio and Y. E. Nikiforov: MicroRNA expression profiling of thyroid tumors: biological significance and diagnostic utility. *J Clin Endocrinol Metab*, 93(5), 1600-8 (2008) doi:10.1210/jc.2007-2696

114. P. Pallante, R. Visone, M. Ferracin, A. Ferraro, M. T. Berlingieri, G. Troncone, G. Chiappetta, C. G. Liu, M. Santoro, M. Negrini, C. M. Croce and A. Fusco: MicroRNA deregulation in human thyroid papillary carcinomas. *Endocr Relat Cancer*, 13(2), 497-508 (2006)

115. M. T. Tetzlaff, A. Liu, X. Xu, S. R. Master, D. A. Baldwin, J. W. Tobias, V. A. Livolsi and Z. W. Baloch: Differential expression of miRNAs in papillary thyroid carcinoma compared to multinodular goiter using formalin fixed paraffin embedded tissues. *Endocr Pathol*, 18(3), 163-73 (2007)

116. L. Yip, L. Kelly, Y. Shuai, M. J. Armstrong, Y. E. Nikiforov, S. E. Carty and M. N. Nikiforova: MicroRNA signature distinguishes the degree of aggressiveness of papillary thyroid carcinoma. *Ann Surg Oncol*, 18(7), 2035-41 (2011)

117. C. K. Chou, R. F. Chen, F. F. Chou, H. W. Chang, Y. J. Chen, Y. F. Lee, K. D. Yang, J. T. Cheng, C. C. Huang and R. T. Liu: miR-146b is highly expressed in adult papillary thyroid carcinomas with high risk features including extrathyroidal invasion and the BRAF(V600E) mutation. *Thyroid*, 20(5), 489-94 (2010)

118. S. Y. Sheu, F. Grabellus, S. Schwertheim, K. Worm, M. Broecker-Preuss and K. W. Schmid: Differential miRNA expression profiles in variants of papillary thyroid carcinoma and encapsulated follicular thyroid tumours. *Br J Cancer*, 102(2), 376-82 (2010)

119. S. Schwertheim, S. Y. Sheu, K. Worm, F. Grabellus and K. W. Schmid: Analysis of deregulated miRNAs is helpful to distinguish poorly differentiated thyroid carcinoma from papillary thyroid carcinoma. *Horm Metab Res*, 41(6), 475-81 (2009)

120. Y. T. Chen, N. Kitabayashi, X. K. Zhou, T. J. Fahey, 3rd and T. Scognamiglio: MicroRNA analysis as a potential diagnostic tool for papillary thyroid carcinoma. *Mod Pathol*, 21(9), 1139-46 (2008)

121. H. Mazeh, I. Mizrahi, D. Halle, N. Ilyayev, A. Stojadinovic, B. Trink, S. Mitrani-Rosenbaum, M. Roistacher, I. Ariel, A. Eid, H. R. Freund and A. Nissan: Development of a microRNA-based molecular assay for the detection of papillary thyroid carcinoma in aspiration biopsy samples. *Thyroid*, 21(2), 111-8 (2011)

122. V. Leone, D. D'Angelo, I. Rubio, P. M. de Freitas, A. Federico, M. Colamaio, P. Pallante, G. Medeiros-Neto and A. Fusco: MiR-1 is a tumor suppressor in thyroid carcinogenesis targeting CCND2, CXCR4, and SDF-1alpha. *J Clin Endocrinol Metab*, 96(9), E1388-98 (2011)

123. R. Visone, P. Pallante, A. Vecchione, R. Cirombella, M. Ferracin, A. Ferraro, S. Volinia, S. Coluzzi, V. Leone, E. Borbone, C. G. Liu, F. Petrocca, G. Troncone, G. A. Calin, A. Scarpa, C. Colato, G. Tallini, M. Santoro, C. M. Croce and A. Fusco: Specific microRNAs are downregulated in human thyroid anaplastic carcinomas. *Oncogene*, 26(54), 7590-5 (2007)

124. J. Braun, C. Hoang-Vu, H. Dralle and S. Huttelmaier: Downregulation of microRNAs directs the EMT and invasive potential of anaplastic thyroid carcinomas. *Oncogene*, 29(29), 4237-44 (2010)

125. F. Weber, R. E. Teresi, C. E. Broelsch, A. Frilling and C. Eng: A limited set of human MicroRNA is deregulated in follicular thyroid carcinoma. *J Clin Endocrinol Metab*, 91(9), 3584-91 (2006)

126. M. Dettmer, A. Vogetseder, M. B. Durso, H. Moch, P. Komminoth, A. Perren, Y. E. Nikiforov and M. N. Nikiforova: MicroRNA expression array identifies novel diagnostic markers for conventional and oncocytic follicular thyroid carcinomas. *J Clin Endocrinol Metab*, 98(1), E1-7 (2013)

127. M. S. Dettmer, A. Perren, H. Moch, P. Komminoth, Y. E. Nikiforov and M. N. Nikiforova: Comprehensive microRNA expression profiling identifies novel markers in follicular variant of papillary thyroid carcinoma. *Thyroid* (2013)

128. C. Mian, G. Pennelli, M. Fassan, M. Balistreri, S. Barollo, E. Cavedon, F. Galuppini, M. Pizzi, F. Vianello, M. R. Pelizzo, M. E. Girelli, M. Rugge and G. Opocher: MicroRNA profiles in familial and sporadic medullary thyroid carcinoma: preliminary relationships with RET status and outcome. *Thyroid*, 22(9), 890-6 (2012)

129. Y. Huang, M. Prasad, W. J. Lemon, H. Hampel, F. A. Wright, K. Kornacker, V. LiVolsi, W. Frankel, R. T. Kloos, C. Eng, N. S. Pellegata and A. de la Chapelle: Gene expression in papillary thyroid carcinoma reveals highly consistent profiles. *Proc Natl Acad Sci U S A*, 98(26), 15044-9 (2001)

130. M. A. Aldred, Y. Huang, S. Liyanarachchi, N. S. Pellegata, O. Gimm, S. Jhiang, R. V. Davuluri, A. de la Chapelle and C. Eng: Papillary and follicular thyroid carcinomas show distinctly different microarray expression profiles and can be distinguished by a minimum of five genes. *J Clin Oncol*, 22(17), 3531-9 (2004)

131. S. Chevillard, N. Ugolin, P. Vielh, K. Ory, C. Levalois, D. Elliott, G. L. Clayman and A. K. El-Naggar: Gene expression profiling of differentiated thyroid neoplasms: diagnostic and clinical implications. *Clin Cancer Res*, 10(19), 6586-97 (2004)

132. T. J. Giordano, R. Kuick, D. G. Thomas, D. E. Misek, M. Vinco, D. Sanders, Z. Zhu, R. Ciampi, M. Roh, K. Shedden, P. Gauger, G. Doherty, N. W. Thompson, S. Hanash, R. J. Koenig and Y. E. Nikiforov: Molecular classification of papillary thyroid carcinoma: distinct BRAF, RAS, and RET/PTC mutation-specific gene expression profiles discovered by DNA microarray analysis. *Oncogene*, 24(44), 6646-56 (2005)

133. O. L. Griffith, A. Melck, S. J. Jones and S. M. Wiseman: Meta-analysis and meta-review of thyroid cancer gene expression profiling studies identifies important diagnostic biomarkers. *J Clin Oncol*, 24(31), 5043-51 (2006)

134. F. Weber, L. Shen, M. A. Aldred, C. D. Morrison, A. Frilling, M. Saji, F. Schuppert, C. E. Broelsch, M. D. Ringel and C. Eng: Genetic classification of benign and malignant thyroid follicular neoplasia based on a three-gene combination. *J Clin Endocrinol Metab*, 90(5), 2512-21 (2005)

135. B. S. Stolf, M. M. Santos, D. F. Simao, J. P. Diaz, E. B. Cristo, R. Hirata, Jr., M. P. Curado, E. J. Neves, L. P. Kowalski and A. F. Carvalho: Class distinction between follicular adenomas and follicular carcinomas of the thyroid gland on the basis of their signature expression. *Cancer*, 106(9), 1891-900 (2006)

136. G. Chiappetta, A. Ferraro, E. Vuttariello, M. Monaco, F. Galdiero, V. De Simone, D. Califano, P. Pallante, G. Botti, L. Pezzullo, G. M. Pierantoni, M. Santoro and A. Fusco: HMGA2 mRNA expression correlates with the malignant phenotype in human thyroid neoplasias. *Eur J Cancer*, 44(7), 1015-21 (2008)

137. N. B. Prasad, H. Somervell, R. P. Tufano, A. P. Dackiw, M. R. Marohn, J. A. Califano, Y. Wang, W. H. Westra, D. P. Clark, C. B. Umbricht, S. K. Libutti and M. A. Zeiger: Identification of genes differentially expressed in benign versus malignant thyroid tumors. *Clin Cancer Res*, 14(11), 3327-37 (2008)

138. J. L. Jameson: Minimizing unnecessary surgery for thyroid nodules. *N Engl J Med*, 367(8), 765-7 (2012)

139. E. K. Alexander, G. C. Kennedy, Z. W. Baloch, E. S. Cibas, D. Chudova, J. Diggans, L. Friedman, R. T. Kloos, V. A. LiVolsi, S. J. Mandel, S. S. Raab, J. Rosai, D. L. Steward, P. S. Walsh, J. I. Wilde, M. A. Zeiger, R. B. Lanman and B. R. Haugen: Preoperative diagnosis of benign thyroid nodules with indeterminate cytology. *N Engl J Med*, 367(8), 705-15 (2012)

Key Words: Review, Thyroid carcinomas, Molecular diagnostic, BRAF, Molecular techniques, Review

Send correspondence to: Giovanni Tallini, University of Bologna School of Medicine, Anatomic Pathology, Bellaria Hospital, via Altura, 3 40139 Bologna, Italy, Tel: +39 051 622.57.57, Fax: +39 051 622.57.59, E-mail: giovanni.tallini@unibo.it

.