BRAF Mutations in Thyroid Tumors Are Restricted to Papillary Carcinomas and Anaplastic or Poorly Differentiated Carcinomas Arising from Papillary Carcinomas

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Activating point mutations of the *BRAF* gene have been recently reported in papillary thyroid carcinomas. In this study, we analyzed 320 thyroid tumors and six anaplastic carcinoma cell lines and detected *BRAF* mutations in 45 (38%) papillary carcinomas, two (13%) poorly-differentiated carcinomas, three (10%) anaplastic carcinomas, and five (83%) thyroid anaplastic carcinomas, follicular and Hürthle cell, and medullary carcinomas, follicular and Hürthle cell adenomas, or benign hyperplastic nodules. All mutations involved a $T \rightarrow A$ transversion at nucleotide 1796. In papillary carcinomas, *BRAF* mutations were associated with older age, classic papillary carcinoma or tall cell variant histology, extrathyroidal

VER THE LAST 2 decades, significant progress has been achieved in the understanding of genetic events in follicular cell-derived thyroid tumors. RET/PTC and TRK rearrangement have been identified and characterized as specific events in papillary thyroid carcinogenesis (1-3). Another type of chromosomal rearrangement, PAX8- $PPAR\gamma$, has been found predominantly in follicular carcinomas (4, 5). Activating point mutations of the RAS genes occur in follicular carcinomas and adenomas, poorly differentiated and anaplastic carcinomas, and in some papillary carcinomas, predominantly of the follicular variant (6–8). Though PAX8-*PPAR* γ rearrangement and *RAS* mutations are found in up to 80% of all follicular carcinomas, until recently, identifiable genetic alterations were observed in only 30-40% of papillary carcinomas, suggesting that additional genetic defect remained undiscovered.

Recently, a somatic point mutation in the *BRAF* gene has been identified as the most common genetic event in papillary thyroid carcinoma (9). In a series of 78 papillary carcinomas, 36% of tumors harbored a thymine-to-adenine transversion at nucleotide position 1796, which results in a valine-to-glutamate substitution at residue 599 (V599E). In extension, and more frequent presentation at stages III and IV. All *BRAF*-positive poorly differentiated and anaplastic carcinomas contained areas of preexisting papillary carcinoma, and mutation was present in both the well-differentiated and dedifferentiated components. These data indicate that *BRAF* mutations are restricted to papillary carcinomas and poorly differentiated and anaplastic carcinomas arising from papillary carcinomas. They are associated with distinct phenotypical and biological properties of papillary carcinomas and may participate in progression to poorly differentiated and anaplastic carcinol Metab 88: 5399–5404, 2003)

another study, a higher prevalence (69%) of this mutation was observed in a series of 35 papillary carcinomas (10). Nucleotide 1796, the only mutational site in thyroid carcinomas, was the most frequently affected hot spot in other types of human cancers where *BRAF* is mutated, such as malignant melanoma and colorectal carcinoma (11, 12). However, in these neoplasms, additional mutations were revealed in exons 11 and 15 of *BRAF*.

The *BRAF* gene codes a cytoplasmic serine/threonine kinase that is regulated by binding RAS. The V599E mutation is believed to mimic the phosphorylation in the activation segment by insertion of an acidic residue close to a site of regulated phosphorylation at serine 598. No overlap was observed between papillary carcinomas harboring *BRAF*, *RAS*, and *RET*/PTC mutations (9), consistent with the notion that *BRAF* acts along the RET/PTC-RAS-BRAF-MAPK pathway in thyroid cells.

The initial reports indicated that *BRAF* mutations in thyroid tumors are restricted to papillary carcinoma, because no mutation was observed in other types of well-differentiated thyroid cancer, including follicular carcinoma, Hürthle carcinoma and medullary carcinoma, and in benign thyroid tumors (9, 10). However, it remains unclear whether this gene is also mutated in poorly differentiated and anaplastic thyroid carcinomas, the most aggressive types of thyroid neoplasm. In addition, it is important to determine whether

Abbreviations: FMCA, Fluorescence melting curve analysis; SSCP, single-strand conformational polymorphism; V599E, valine-to-gluta-mate substitution at residue 599; WT, wild-type.

BRAF mutation confers papillary carcinomas with distinct phenotypical and biological properties.

In this study, we analyzed a series of 320 thyroid tumors and benign nodules for *BRAF* mutation, report a novel method of screening for the mutation, and provide a detailed comparison of clinical-pathologic features between thyroid tumors according to their *BRAF* status.

Materials and Methods

Tumor samples and cell lines

We analyzed 119 papillary carcinomas, 32 follicular and Hürthle cell carcinomas, 16 poorly-differentiated carcinomas, 31 anaplastic carcinomas, 13 medullary carcinomas, 45 follicular and Hürthle cell adenomas, and 64 benign hyperplastic nodules. The tissues were obtained from the Department of Pathology at the University Hospital in Cincinnati with the help of the University of Cincinnati General Clinical Research Center Tissue Procurement Facility (249 samples) and through the Cooperative Human Tissue Network (34 samples). An additional 37 tumor samples were obtained from the Department of Oncology of the University of Pisa, Italy. In all cases, the protocols of the study were approved by the respective Institutional Review Board committees. In 269 cases, snapfrozen tissue was available; and in 51 cases, paraffin-embedded tissue was available. After the initial review and selection of cases (by Y. E. Nikiforov or F. Basolo), glass slides from 104 papillary carcinomas were re-examined, in a blinded fashion, by two pathologists (P. W. Biddinger and Y. E. Nikiforov) and subclassified as classic papillary carcinoma or as distinct histologic variant (13, 14), based of the following criteria: follicular variant, more than 50% of tumor has follicular architecture and no well-formed papillae found; solid variant, more than 50% of tumor has solid and/or trabecular architecture and no well-formed papillae found; papillary microcarcinoma, tumor measures 1.0 cm or less irrespective of the growth pattern; tall cell variant, more than 50% of tumor cells are twice as tall as they are wide; and diffuse sclerosing variant, diffuse involvement of the gland with multiple tumor aggregates in lymphatic vessels, prominent lymphocytic infiltration, fibrosis, and squamous metaplasia.

In addition, six cell lines were studied, including four anaplastic thyroid carcinoma cell lines, FB1 (15), CAL62 (16), KAT4 (17), and ARO (18), and two cell lines, BHT101 (19) and 8505C (20), established from anaplastic carcinoma containing areas of papillary carcinoma. They were purchased from DSMZ, Braunschweig, Germany.

DNA and RNA isolation

Genomic DNA was isolated using proteinase K digestion, phenolchloroform extraction, and ethanol precipitation as previously described (21). RNA isolation from snap-frozen and paraffin-embedded tissue was performed using Trizol reagent (Invitrogen, Carlsbad, CA) as previously described (22) or using the RNA extraction midi Kit (Qiagen, Valencia, CA). Two to three micrograms of total RNA, extracted from frozen tissue, and 5 μ l of RNA extracted from paraffin-embedded tissue were reverse transcribed in a vol of 20 μ l using random hexamer priming and Superscript II RT (Invitrogen) according to the manufacturer's protocol. All cDNA samples obtained from paraffin-embedded tissue and selective samples from frozen tissue were tested to assess the adequacy of RNA by amplifying a 247-bp control sequence of the 3'-phosphoglycerate kinase (*PGK*) gene as reported elsewhere (23).

Laser capture microdissection

In nine cases, tumor tissue was microdissected to either obtain DNA from a small focus of papillary microcarcinoma or to study separately well-differentiated and poorly differentiated or anaplastic areas within the same tumor nodule. For microdissection, $5-\mu$ m sections from paraffin-embedded tissue were put on glass slides and weakly stained with hematoxylin and eosin. The areas of interest were microdissected using the Laser Capture Method (Arcturus, Mountain View, CA). The tissue samples (500–1,000 cells) were then digested overnight using proteinase K (0.4 μ g/ μ l) in 30 μ l of digestion buffer [10 mM Tris-HCL (pH 8.0), 1

mM EDTA, and 1% Tween-20]. After heat inactivation of the enzyme, 1 μ l of the mixture was used as a DNA template for PCR.

Detection of BRAF mutations

Mutations in the BRAF gene were detected using three different methods.

LightCycler PCR and fluorescence melting curve analysis (FMCA). Taking advantage of the fact that all BRAF mutations identified to date in thyroid carcinomas were restricted to nucleotide 1796, we developed a new method of BRAF detection using real-time PCR and FMCA on the LightCycler instrument (Roche Molecular Biochemicals, Mannheim, Germany). A pair of oligonucleotide primers flanking the mutation site was designed (5'-TCCTTTACTTACACCTCAG-3' and 5'-CATCT-CAGGGCCAAAAAT-3'), together with two fluorescent probes (sensor 5'-AGCTACAGTGAAATCTCGATGGAG-Fluorescein-3' and anchor 5'-LC Red 705-GGTCCCATCAGTTTGAACAGTTGTCTGGA-Phosphate-3'), with the sensor probe spanning the nucleotide position 1796. All primers and probes were purchased from TIB Molbiol (Berlin, Germany). Amplification was performed in a glass capillary using 50 ng of DNÁ in a 20- μ l vol containing 2 μ l of 10× LightCycler DNA Master Hybridization Probes (containing PCR buffer, deoxynucleotide triphosphates, 10 mM MgCl₂, and Taq polymerase) (Roche), 1.6 µl of 25 mM MgCl₂, 40 pmol or each primer, and 2 pmol of each hybridization probe. The reaction mixture was subjected to 45 cycles of rapid PCR consisting of denaturation at 94 C for 1 sec, annealing at 55 C for 20 sec, and extension at 72 C for 10 sec. Postamplification FMCA was performed by gradual heating of samples at a rate of 0.2 C/sec from 45 C to 95 C. Fluorescence melting peaks were built by plotting of the negative derivative of fluorescent signal corresponding to the temperature -dF/dT).

Normal placental DNA was used as a negative control, and DNA from a tumor sample with the known *BRAF* nucleotide 1796 mutation served as a positive control. All PCR products that showed deviation from the wild-type (WT) (placental DNA) melting peak were sequenced after purification through a Microcon PCR kit (Millipore Corp., Billerica, MA) to verify the presence of mutation.

Single-strand conformational polymorphism (SSCP). DNA samples were screened for mutations within exons 11 and 15 of the *BRAF* gene by SSCP as previously described (9). For exon 15 mutations, we used DNA from the melanoma cell line SKMel28 and thyroid NPA cells (*BRAF^{V599E}*); and for exon 11 mutations, we used DNA from the small cell carcinoma cell line NCI-H1755 (*BRAF^{G468A}*) as positive controls. The aberrant SSCP bands were cut out directly from dried gels and sequenced.

Direct sequencing. Thirty-seven tumor samples from Italy and five cell lines were analyzed by direct sequencing of exons 11 and 15 of BRAF at the RNA and DNA levels. For cDNA amplification, the following primers were used for exon 11: 5' GAC GGG ACT CGA GTG ATG AT 3' [BRAF(R) 11F] and 5' CTG CTG AGG TGT AGG TGC TG 3' [BRAF(R) 11R]; for exon 15: 5' GCA CAG GGC ATG GAT TAC TT 3' [BRAF(R) 15F] and 5' GAT GAC TTC TGG TGC CAT CC 3' [BRAF(R) 15R]. For genomic DNA, PCR primers were designed to amplify target exons plus approximately 50-bp flanking intron sequences in both upstream and downstream directions. The following primers were used for exon 11: 5' TCC CTC TCA GGC ATA AGG TAA 3' [BRAF(G) 11F] and 5' CGA ACA GTG AAT ATT TCC TTT GAT 3' [BRAF(G) 11R]; for exon 15: 5' TCA TAA TGC TTG CTC TGA TAG GA 3' [BRAF(G) 15F] and 5' GGC CAA AAA TTT AAT CAG TGG A 3' [BRAF(G) 15R]. Amplification was carried out for 40 cycles (94 C for 30 sec, 60 C for 30 sec, 72 C for 1 min) on a thermal cycler (Perkin-Elmer, Wellesley, MA). All PCR products were visualized by electrophoresis in 2% agarose gel and purified using a PCR purification kit (Qiagen). PCR products were sequenced using an automated sequencer.

Analysis of clinical-pathological features and tumor staging

Surgical pathology reports were reviewed to extract demographic information and pathologic characteristics of the tumors. Glass slides were reviewed to confirm a diagnosis and establish the variant of papillary carcinoma. Tumor staging was based on the most recent American Joint Committee on Cancer recommendations (24).

Statistical analysis

We compared raw numbers of *BRAF*-negative and *BRAF*-positive papillary carcinomas for each category (*i.e.* histological variant, age at presentation, extrathyroidal invasion, and others). Comparison between two groups was performed using the Student's *t* test for continuous data, two-tailed Fisher exact test in cases where the numbers in the cell were less than 5, and standard χ^2 test in all other cases. The difference between two values was considered significant at P < 0.05.

Results

Prevalence of BRAF mutations

Three hundred twenty tumors and benign thyroid nodules were studied for BRAF point mutations (Table 1). Using LightCycler FMCA, SSCP, and direct sequencing of DNA and RNA, mutations were found in 45 (38%) papillary carcinomas, two (13%) poorly differentiated carcinomas, and three (10%) anaplastic thyroid carcinomas (Fig. 1). All mutations were identical and involved a $T \rightarrow A$ transversion at nucleotide 1796. No mutation was detected in a large series of follicular and Hürthle cell carcinomas and adenomas, medullary thyroid carcinomas, and benign hyperplastic nodules. There was no difference between the prevalence of mutation in papillary carcinomas from the United States (38%) and Italy (40%). All mutations were heterozygous, as detected by the presence of a WT peak on the LightCycler melting curve or in the nucleotide sequence (Fig. 1). Among 259 samples studied by both LightCycler FMCA and SSCP, there was a 100% correlation in the detection rate, with no other mutational spots found in exons 11 and 15 by the SSCP analysis.

Six thyroid carcinoma cell lines were also tested. They included four cell lines established from pure anaplastic carcinomas (FB1, CAL62, KAT4, and ARO) and two from tumors containing areas of well-differentiated papillary carcinoma in addition to anaplastic carcinoma (BHT101 and 8505C). Five cell lines revealed *BRAF* 1796 T \rightarrow A mutation, whereas CAL62 showed a WT *BRAF* sequence. Four of these cell lines were heterozygous for the mutation, whereas 8505C cells showed a homozygous *BRAF* mutation.

BRAF mutations in the progression of thyroid carcinomas

Glass slides of anaplastic and poorly differentiated carcinomas were further reviewed to determine the presence of areas of a pre-existing well-differentiated thyroid tumor (Table 2). Among poorly differentiated carcinomas, eight tumors had no well-differentiated component, seven had areas of papillary carcinoma, and one of follicular carcinoma. The two *BRAF* mutations found in this group were both in tumors containing a papillary carcinoma component; and in both cases, it was a tall cell variant. Among 29 anaplastic carcinomas, five tumors were found to contain areas of papillary carcinoma, and four revealed foci of follicular or Hürthle cell carcinoma. Three *BRAF* mutations identified in this group were all in anaplastic carcinomas containing areas of preexisting papillary carcinoma. In two tumors, the papillary carcinoma component was a tall cell variant; and in one, it had a classic papillary histology.

To determine whether *BRAF* mutation represents an early clonal event and was present at all stages of tumor evolution, well-differentiated papillary areas were microdissected and studied separately in one poorly differentiated and one anaplastic carcinoma. In both cases, *BRAF* mutations were identified in the well-differentiated papillary component as well as the poorly differentiated or anaplastic carcinoma area (Fig. 2).

Analysis of microscopic features and genotype-phenotype correlations

In 104 papillary carcinomas (98 from the United States and six from Italy), histologic slides and surgical pathology reports were available for further review. Tumors were subclassified into specific histologic variants based on the criteria described above. This information was analyzed in correlation with the status of BRAF. Tumors with BRAF mutation were typically either classic papillary carcinomas (74%) or tall cell variants (16%) (Fig. 3). In addition, two microcarcinomas with typical papillary architecture were positive for *BRAF* mutation, confirming the presence of this alteration at all stages of papillary carcinoma development. Tumors lacking BRAF mutation were either follicular variants (42%) or classic papillary carcinomas (38%). Differences in prevalence of classic papillary, follicular, and tall cell morphology in the two groups was statistically significant (Table 3). In addition, a multidegree *P* value was obtained to assess global changes in histological categories in tumors positive and negative for *BRAF* mutation (df = 5) and was highly significant (P < 0.0001).

Additional clinical-pathologic features of papillary carcinomas with and without *BRAF* mutation are summarized in Table 3. Tumors with *BRAF* mutation were associated with a significantly older age of patients and presented mostly in the fifth decade of life, whereas patients with tumors lacking the mutation were, on average, 14 yr younger. The frequency of extrathyroidal extension was also significantly higher in the *BRAF*-positive group (42% *vs.* 20%), whereas no signifi-

TABLE 1. Prevalence of BRAF mutations in thyroid tumors and benign nodules

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	LightCycler FMCA SSCP I		Direct sequencing	10(8)
Papillary carcinoma	39/104 (38%) ^a	$32/87 (37\%)^a$	6/15 (40%)	45/119 (38%)
Follicular and Hürthle cell carcinomas	0/32	0/32		0/32
Poorly differentiated carcinoma	1/12 (8%)	1/11 (9%)	1/4 (25%)	2/16 (13%)
Anaplastic carcinoma	1/11 (9%)	1/10 (10%)	2/18 (11%)	3/29 (10%)
Medullary carcinoma	0/13	0/11		0/13
Follicular and Hürthle cell adenomas	0/46	0/43		0/46
Hyperplastic nodule	0/65	0/65		0/65

^a All cases studied by SSCP were also analyzed by LightCycler FMCA.



FIG. 1. Left, LightCycler FMCA detection of BRAF mutation based on a distinct melting temperature (T_m) of duplexes formed between the WT probe and either WT or mutant sequences. Whereas T_m of the WT sequence (GTG) was 63.8 C, the GTG \rightarrow GAG mutation at nucleotide 1796 resulted in a T_m shift to 59.1 C. The mutation in this tumor sample is heterozygous, because both mutant and WT peak were detected. *Right*, Nucleotide sequencing of PCR product from a papillary carcinoma shows a T \rightarrow A transversion at nucleotide 1796, as well as a WT peak.

TABLE 2. BRAF mutations in thyroid poorly differentiated and anaplastic carcinomas

		Prevalence of BRAN mutations
Poorly differentiated carcinomas	With no WD component	0/8
	With PC component	2/7 (29%)
	With FC/HCC component	0/1
Anaplastic carcinomas	With no WD component	0/20
	With PC component	3/5 (60%)
	With FC/HCC component	0/4

WD, Well-differentiated; PC, papillary carcinoma; FC, follicular carcinoma; HCC, Hürthle cell carcinoma.

icant difference was found in tumor size and frequency of cervical lymph node and distant metastasis at presentation. When tumor stage was compared between the groups, the *BRAF*-positive papillary carcinomas seemed to present significantly more frequently at stages III and IV (45% *vs.* 8% in *BRAF*-negative tumors). The opposite was true for stage I disease, which was observed in 82% of *BRAF*-negative tumors, compared with 53% of papillary carcinomas positive for *BRAF* mutation.

Discussion

In this study, we report, for the first time, that *BRAF* mutations, which in thyroid tumors were thought to be restricted to papillary carcinomas, also occur in poorly differentiated and anaplastic carcinomas. This is apparent only in those less-differentiated carcinomas that arise from preexisting papillary carcinomas. These findings are also supported by the results obtained on cell lines established from anaplastic carcinomas, the majority of which were found to harbor the mutation.

The pathogenesis of poorly differentiated and anaplastic thyroid carcinomas and their possible association with preexisting well-differentiated thyroid tumors have been debated for a long time. A number of observations have re-



FIG. 2. *Upper panel*, Poorly differentiated (PD) carcinoma containing a focus of residual well-differentiated (WD) papillary carcinoma. *Lower panel*, Anaplastic carcinoma (AC) containing an area of WD papillary carcinoma. *BRAF* mutations were identified in both WD and PD or AC areas after they were microdissected separately for DNA extraction.

ported that 21–79% of anaplastic carcinomas had coexisting areas or previous history of well-differentiated follicular or papillary cancer (25–28). This has offered histologic evidence for the following progression: well-differentiated thyroid carcinoma \rightarrow poorly differentiated carcinoma \rightarrow anaplastic carcinoma. However, little evidence is available, so far, for the molecular conformation of such progression (29, 30). Here, we demonstrate that progression from papillary thyroid carcinoma to poorly differentiated and anaplastic carcinoma in many tumors may have been favored by constitutive activation of *BRAF*.

As for the papillary carcinomas, *BRAF* mutations were found at all stages of progression, including microcarcinomas. Papillary carcinomas with *BRAF* alteration had strong association with classical papillary architecture and tall cell variant. The association with tall cell variant was particularly striking, because all six papillary carcinomas with this histotype harbored the mutation. Moreover, both poorly differentiated carcinomas and two of the three anaplastic carcinomas that tested positive for *BRAF* had adjacent areas of tall cell papillary carcinoma. These findings support the link between mutated *BRAF* and tall cell morphology, and provide evidence for the association between this genetic event and more aggressive tumor behavior. This is consistent with other reports indicating that tall cell variant may represent an aggressive variant of papillary carcinoma (31–33).

Additional evidence for more aggressive tumor biology comes from the analysis of tumor stage. Indeed, whereas the vast majority of *BRAF*-negative papillary carcinomas presented at stage I, this was true only for the half of tumors with the mutation. On the other hand, 45% of *BRAF*-positive tumors were at an advanced stage of the disease (stage III or IV). For the most part, this was attributable to the fact that



FIG. 3. Typical microscopic appearance of papillary carcinomas harboring *BRAF* mutation. A, Classic papillary carcinoma demonstrating a uniform papillary growth pattern and well-developed papillae with central fibrovascular cores, some of which show prominent hyalinization. B, Tall cell variants of papillary carcinoma. The tumor cells have abundant cytoplasm and are at least twice as tall as they are wide (*inset*).

BRAF-positive patients were older and had higher incidence on extrathyroidal extension, because both of these features are important determinants of tumor stage and, consequently, disease prognosis.

The fact that *BRAF* mutations were found both in microcarcinomas/stage I tumors and in advanced papillary carcinomas and dedifferentiated tumors suggests that, being an early event and probably insufficient alone for the fully aggressive phenotype, this mutation may predispose the tumor cells to acquisition of additional genetic alterations, which, in turn, activate more aggressive pathways and lead to dedifferentiation.

An exceedingly low prevalence of *BRAF* mutations was observed in the follicular variant of papillary carcinoma. This, in addition to our recent finding that *RAS* mutations were restricted to the follicular variants and virtually absent in classic papillary carcinoma (8), suggests that these two major variants of papillary carcinoma develop through a distinct set of molecular abnormalities and may have unique biological properties.

There is now compelling genetic evidence that constitutive activation of effectors along the RET/PTC-RAS-BRAF signaling pathway plays a cardinal role in pathogenesis of papillary thyroid carcinoma. The lack of overlap between *RET*/*PTC*, *RAS*, or *BRAF* mutations in papillary carcinomas (9) indicates that any one of these alterations is sufficient to drive this pathway and, either alone or in cooperation with other events, evokes the phenotype. Despite this, certain phenotypic differences are apparent between papillary thyroid cancers harboring mutations of each of these genes [*i.e. BRAF* is associated with classic papillary carcinoma and tall cell histology and *RAS* with the follicular variant (8)]. This is likely attributable to the fact that each of these oncoproteins may engage a distinct repertoire of downstream effectors and activate them with different intensity.

The high prevalence of *BRAF* mutations in papillary carcinomas and the specificity of this alteration to papillary carcinomas or less differentiated tumors developing from papillary carcinoma make it a potentially important marker for tumor diagnosis and prognosis. Testing for *BRAF* mu-

TABLE	3.	Correlation	between	the status	of BRAF	mutation	and	clinico-	patholog	gic fea	atures of	pap	illary	carcinom	as

	$\begin{array}{l} BRAF \text{-positive} \\ n = 38 \end{array}$	$\begin{array}{l} BRAF\text{-negative} \\ n = 66 \end{array}$	P value
Age, average \pm SD (yr)	49.3 ± 16.2	35.0 ± 17.3	< 0.0001
Female:male ratio	2:1	2:1	1
Tumor size, average \pm SD (cm)	2.9 ± 1.6	2.7 ± 1.8	0.5
Histologic variants of PTC			
Classic papillary	28 (74%)	25 (38%)	0.0004
Tall cell variant	6 (16%)	0	0.002
Follicular variant	2(5%)	28 (42%)	< 0.0001
Microcarcinoma	2(5%)	8 (12%)	0.32
Solid variant	0	4 (6%)	0.29
Diffuse sclerosing	0	1(2%)	1
Extrathyroidal extension	16 (42%)	13 (20%)	0.03
Lymph node metastases	23 (61%)	29 (44%)	0.15
Distant metastases	2(5%)	1(2%)	0.55
Tumor stage			
I	20 (53%)	54 (82%)	0.003
II	1(3%)	7(11%)	0.25
III	10 (26%)	2(3%)	0.0006
IV	7 (18%)	3(4%)	0.03

tations in thyroid tumors is simplified by the fact that virtually all of them are restricted to the nucleotide position 1796. Among several methods that can be used, we developed and validated an assay based on LightCycler PCR and post-PCR melting curve analysis. This relatively simple, quick, and sensitive method can be used not only in tissues harvested at the time of surgery but also for the preoperative diagnosis of thyroid FNA specimens. An early detection of BRAF mutations in papillary, but especially in poorly differentiated and anaplastic carcinomas, may also be of significant importance in planning appropriate therapy. In addition, BRAF may be a valuable target for small molecular kinase inhibitors currently being developed to block BRAF activity. This would be of a particular importance for poorly differentiated and anaplastic carcinomas that represent a major source of mortality from thyroid tumors.

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