Papillary Thyroid Carcinomas with Cervical Lymph Node Metastases Can Be Stratified into Clinically Relevant Prognostic Categories Using Oncogenic *BRAF*, the Number of Nodal Metastases, and Extra-Nodal Extension

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Background: Papillary thyroid carcinoma (PTC) patients presenting with cervical lymph nodes (LN) metastases (M) have a variable outcome. The objective of this study is to assess the value of meticulous histopathologic examination and genotyping in stratifying these patients into clinically relevant prognostic subgroups.

Methods: This was a retrospective clinical and histopathological review of PTC patients with lymph node metastases at presentation identified between 1980 and 2002 in a single institution. Primary tumors from patients who later recurred were matched to a group of patients who did not recur and subjected to mass spectrometry genotyping encompassing the most significant oncogenes in thyroid carcinomas.

Results: There were 246 patients who satisfied the inclusion criteria. The median follow-up was 10.8 years. The presence of >3 metastatic nodes was an independent predictor of decreased recurrence free survival ($p = 0.03$). In patients <45 years, none of 45 with 1–2 metastatic LN recurred, including 26 patients followed for a median of 13 years without radioactive iodine (RAI) therapy. *BRAF* mutations were found in 28 (78%) of 36 genotyped tumors. Combined positivity for *BRAF* and extra-nodal extension was much stronger in predicting disease specific survival (DSS) ($p = 0.004$) than the single analysis of *BRAF* ($p = 0.12$) or extra-nodal extension ($p = 0.02$).

Conclusions: (i) The number of metastatic LN is an independent predictor of recurrence in all age groups and identifies a subset of young patients with excellent prognosis who may not benefit from RAI therapy. (ii) Combined positivity for *BRAF* and extra-nodal extension has additive prognostic value in predicting DSS. (iii) Classification systems that assign the same magnitude of risk for recurrence or death to all patients with N1 disease should be revisited.

Introduction

THE PRESENCE OF any cervical lymph node metastasis (LNM), whether microscopic or single, upgrades a patient with papillary thyroid carcinoma (PTC) from a low to an intermediate risk category according to the American Thyroid Association (ATA) guidelines (1). Using the tumor node metastasis American Joint Committee on Cancer staging system (AJCC), an older individual (>45 years) with papillary microcarcinoma will move from AJCC stage I (T1N0Mx) to stage III (T1N1aMx) if a metastatic LN is identified in the central neck and to stage IVa (T1N1bMx) if the LN is identified in the lateral neck (2). This upstaging often leads to more aggressive therapy (3,4), as the risk of persistent/recurrent disease is

significantly higher in ATA intermediate risk (21%) than ATA low risk patients (3%) (5), and the risk of death is higher in stage III and IV patients (12.2% and 59.3% respectively) than in Stage I patients (0.2%) (6). However, the most recently updated guidelines from the ATA acknowledge the need to stratify patients with LNM (N1disease) into high and low risk groups, as additional therapy such as radioactive iodine (RAI) remnant ablation may be indicated in one but not the other (1). Several studies have shown that patients with N1 disease have variable outcomes. Indeed, the presence of palpable LNM (7), a higher number of metastatic nodes, large metastatic nodes, and extranodal extension have been shown to correlate with poorer outcome (8–10). Although the histopathologic and clinical analysis were detailed in some of these

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studies, none have accounted for the size of the metastatic foci within the lymph nodes (LN), the subtyping of the primary tumor, and of the metastatic foci into histologic variants of PTC. In addition, the prognostic value of the molecular alterations in these tumors has not been assessed. The aims of this study were to characterize PTC patients with LNM at the histopathologic and molecular levels, and to identify predictors of outcome in N1 disease.

Materials and Methods

Patient population and inclusion criteria

The institutional database was searched for all cases with a diagnosis of thyroid carcinoma treated at Memorial Sloan-Kettering Cancer Center (MSKCC) between January 1980 and December 2002. The slides from the cases included in the study were examined by two head and neck pathologists with special interest in thyroid neoplasia (R.A.G. and M.R.). The pathologists were blinded to the clinical outcome of all patients studied. The thyroid carcinomas were classified according to the last World Health Organization classification of endocrine tumors, except for PTC tall cell variant and poorly differentiated thyroid carcinomas (11). The latter tumor was defined as a carcinoma displaying high mitotic activity (≥ 5 mitosis/10 high power fields, $400\times$) and/or tumor necrosis, and showing follicular cell differentiation at the morphologic or immunohistochemical level (12). Tall cell variant was characterized as a papillary carcinoma composed of $>50\%$ of tall cells. The latter cell type was defined as having a height at least twice its width with an oncocytic cytoplasm.

Patients were included in the study if they had (i) PTC as their primary tumor with (ii) LNM at presentation. The study was approved by the Institutional Review Board of MSKCC.

Clinical parameters

The patient's electronic medical records were reviewed for the age at diagnosis, type of surgery, and adjuvant treatment including RAI therapy. The disease status at recurrence or follow-up was based on a combination of clinical and imaging assessments, including history, physical examination, RAI dosimetry, cross sectional imaging, and/or positron emission tomography scanning. Since many cases from the 1980s did not have adequate biochemical data, biochemical recurrence was not assessed. The type of cervical LN dissection was recorded as Central; Lateral; Central and Lateral; Other. The latter category included mostly peri-thyroidal LNM removed with the thyroid specimen, or patients with mediastinal LNM or LN biopsies. The date of initial surgery, first recurrence, and last date of follow-up were documented. The status at last follow-up was recorded as follows: no evidence of disease; alive with disease; dead of other causes; and dead of disease.

Histopathologic analysis

The largest dimension of the tumor was recorded as dictated during gross examination. The mitotic rate of the tumor was determined by counting 10 contiguous high power fields ($400\times$) corresponding to $2.4\,mm^2$. Tumor necrosis was defined by a "comedo-like" appearance composed of degenerating cytoplasm and punctate, karyorrhectic nuclear debris. The presence of vascular invasion was noted only when such foci were present within or beyond the capsule in accordance

with criteria outlined by the Armed Forces Institute of Pathology fascicle (13). Areas of vascular invasion that were closely adjacent to one another were counted as separate foci. Angioinvasion was categorized as present or absent. The capsule status was recorded as complete, partial, or absent. A tumor was considered infiltrative if it lacked a complete capsule and invaded in between non-neoplastic follicles in an irregular fashion in the form of invasive islands. Extra-thyroid extension (ETE) was defined as tumor cells invading beyond the thyroid capsule into peri-thyroid soft tissue or organs. ETE was subdivided into (i) None (ii) Focal: Presence of 1–2 microscopic foci of ETE measuring $\leq 1\,mm$ each. (iii) Extensive: Presence of >2 microscopic foci of ETE ($\leq 1\,mm$ in size each) or any foci $>1\,mm$ in size. The latter category includes patients with gross ETE into muscle and adjacent viscera. The status of the resection margins was reported as positive (tumor present at the surgical margin) or negative (no tumor at surgical margin). The number of LN examined microscopically as well as the number of nodes with metastatic carcinoma was recorded. For the survival analysis, the number of metastatic nodes was categorized using two alternative cutoffs: that is, <3 and >3, or <5 and >5 metastatic nodes. The greatest diameter of the largest metastatic node was measured as well as the largest size of the largest metastatic tumor focus. For the survival analysis, the size of the largest metastatic node and of the largest metastatic focus were categorized as $<1\,cm$ and $>1\,cm$. The most predominant histologic type of tumor in the LN was recorded. Extra-nodal extension was defined as invasion of peri-nodal tissue (Fig. 1) and recorded as present or absent.

Genotyping

Four sections of $10\,\mu m$ from each formalin-fixed paraffin-embedded tissue block were subjected to DNA extraction using the PUREGene Genomic DNA purification kit (Gentra, Inc., Minneapolis, MN). Mutation detection was performed as previously described (14). We used mass spectrometry Sequenom–based genotyping assay (Sequenom Mass array; Sequenom, San Diego CA), which consists of 116 assays, to interrogate mutations in 16 genes, including the most common thyroid oncogenes such as *BRAF*, *NRAS*, *HRAS*, *KRAS*, *PIK3CA*, and *AKT1*. Since the mass spectrometry genotyping assays for codons 12 and 13 of *HRAS* were not informative, we designed primers for this region and sequenced all the tumors that were wild type for *BRAF* or *RAS* mutations by mass spectrometry genotyping (14).

Screening for RET/PTC rearrangements

RET rearrangements were analyzed for tumors that were wild type for *RAS* and *BRAF*. We used tumor cDNA as template for quantitative PCR to analyze for unbalanced expression of exons 10–11 relative to 12–13 of *RET*, which flank the rearrangement site in intron 11. Samples with 12–13 $>$ 10–11 expression were screened for specific *RET* recombination events using primers bracketing the fusion points of *RET/PTC1*, and *RET/PTC3*, respectively, as previously described (14,15).

Statistical analysis of survival outcomes

Recurrence free survival (RFS) was defined as the time interval between the date of initial surgery and date of 1st
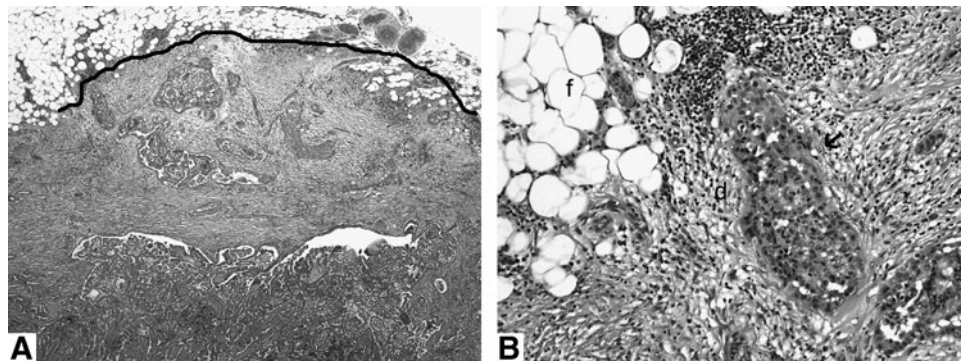
FIG. 1. **(A)** Low-power view of a metastatic lymph node displaying an irregular contour (thick black line) due to extra-nodal extension in a 77-year-old man with multiple nodal metastases (>3) who recurred ~3 years after diagnosis. **(B)** High-power view of the same lymph node. The extra-nodal tumor nest (arrow) has elicited a desmoplastic response (d) in the peri-nodal adipose tissue (f).

recurrence. Disease specific survival (DSS) was calculated from the date of initial surgery to last follow-up. DSS was defined as the presence or absence of death of disease at last follow-up. Two-tailed Fisher exact test was used to assess the relation between categorical variables. Survival outcomes were calculated using the Kaplan–Meier method and compared using the log-rank. A probability (p) value ≤ 0.05 was considered significant. Univariate analysis was carried out using the log rank test and multivariate analysis using a Cox proportional hazard model. Statistical analysis was carried out using commercially available software (SPSS for Windows, version 17.0 [SPSS Inc, Chicago, IL] and JMP version 4.0 [SAS Institute Inc, Cary, NC]).

Results

Clinico-pathologic data and survival analysis on the whole study population

Out of 829 carcinomas of follicular cell origin in the database, 246 (30%) had PTC in their primary tumor with LNM at presentation and were, therefore, included in the study. Table 1 shows the clinico-pathologic features of the cases, including their histotype. Of note, 214 (87%) patients had no capsule or were partially encapsulated, and were infiltrative tumors. The number of LN removed and examined microscopically ranged from 1 to 119 with a median of 24 LN resected per patient at the initial surgery. Table 2 displays the prognostic value of the various histopathologic and clinical parameters in the whole patient cohort. At 10 years of follow-up, the DSS of the whole patient population was 98%, and the overall (any recurrence) RFS was 86%. The neck and distant RFS at 10 years were 91% and 94%, respectively. In regard to overall RFS, older age, extensive ETE and number of positives nodes (>3) (Fig. 2A) were independent predictors of outcome in multivariate analysis (Table 3). Older age, positive margins, and extra-nodal extension predicted for death of disease in univariate analysis. It was not possible to perform multivariate analysis for DSS since very few patients died.

Survival analysis in young patients

In the 168 patients under the age of 45, there was no death of disease with a median follow-up of 12 years. Eighteen patients recurred (15 in the neck and 3 at distant sites), with a 10 year overall RFS of 91%. The 10 year neck and distant RFS

were 93% and 98%, respectively. By univariate analysis, the presence of multicentricity and number of metastatic nodes (>3) correlated with decreased overall RFS ($p = 0.02$). In multivariate analysis, the number of positive nodes (>3) was the only independent predictor of overall RFS (Fig. 2B) (Table 3). In regard to neck RFS, only the number of positive nodes (>3) predicted worse outcome ($p = 0.04$). This factor was, however, not significant in multivariate analysis. Large tumor size was the only factor that correlated with decreased distant RFS ($p = 0.04$). In patients <45 years, none of 45 cases with 1–2 metastatic nodes recurred including 26 patients followed for a median of 13 years without RAI therapy (Table 4).

Survival analysis in older patients

There were 5 deaths of disease in the 78 patients >45 years of age, with a 10 year DSS of 95% and a median follow-up of 9.8 years. Sixteen patients recurred (9 neck and 9 distant recurrences) with a 10-year overall RFS of 76%. The 10 year neck and distant RFS was each 86%. In regard to neck RFS, the number of positive nodes (>5) was the only independent predictor of outcome ($p = 0.02$) (Fig. 2C) (Table 3). On multivariate analysis, the presence of positive margins was the only independent factor for decreased distant RFS (Table 3). By univariate analysis, the presence of positive margins ($p < 0.0001$), a combined central and lateral neck dissection ($p = 0.02$) and the presence of extra-nodal extension ($p = 0.008$) correlated with decreased DSS.

Survival analysis in patients without RAI therapy

In the 106 patients who did not receive RAI therapy, there was no death of disease with a median follow-up of 15.4 years. Fifteen patients recurred (12 in the neck and 3 at distant sites) with a 10 year overall RFS of 89%. The 10 year neck and distant RFS were 92 and 97%, respectively. By univariate analysis, several variables correlated with outcome, but none was an independent predictor in multivariate analysis (Table 5).

Survival analysis in patients treated with RAI

In the 140 patients treated with RAI, there were 4 deaths of disease with a 10 years DSS of 97% and a median follow-up of 9.6 years. Nineteen patients recurred (12 neck and 9 distant recurrences) with a 10 year overall RFS of 84%. The 10 year neck and distant RFS was 89% and 93%,

Table 1. Clinico-Pathologic Features of All 246 Papillary Carcinomas with Lymph Node Metastasis

Characteristic	Number of patients (%)	Characteristic	Number of patients (%)
Age, years		Predominant histologic type	
Median	36	of tumor in lymph node[g]	
<45	168 (68%)	Classic PTC	179 (75%)
>45	78 (32%)	FVPTC	11 (4%)
Gender		Tall cell	45 (19%)
Female	155 (63%)	Poorly differentiated	3 (1%)
Male	91 (37%)	Psammoma bodies	2 (1%)
Tumor size (cm)[a]		Most aggressive PTC subtype	
Median	1.9	in lymph node[g]	
<1.5	100 (42%)	Classic PTC	179 (76%)
>1.5	139 (58%)	FVPTC	11 (5%)
PTC subtypes		Tall cell	45 (19%)
Classic PTC (87% infiltrative)	136 (55.3%)	Extra-nodal extension[h]	
FVPTC (16 infiltrative, 7 encapsulated)	23 (9.3%)	No	161 (68%)
Tall cell PTC	44 (18%)	Yes	75 (32%)
Microcarcinoma	35 (14.2%)	Multicentricity[i]	
Other[b]	8 (3.2%)	No	136 (57%)
Tumor capsule		Yes	102 (43%)
Completely encapsulated	28 (11%)	Thyroid surgery	
Not/partially encapsulated	218 (89%)	Less than TT	69 (28%)
Vascular invasion[c]		TT	177 (72%)
Absent	222 (91%)	RAI ablation	
Present	22 (9%)	Yes	140 (57%)
Extra-thyroid extension[d]		None	106 (43%)
None	87 (36%)	Neck dissection	
Focal	41 (17%)	Central	70 (28%)
Extensive	115 (47%)	Lateral	63 (26%)
Margins[e]		Central and Lateral	86 (35%)
Negative	198 (81%)	Other	27 (11%)
Positive	46 (19%)	Recurrence[j]	
Number of metastatic nodes		Present	34 (14%)
Median	6	Absent	202 (86%)
≤3	95 (39%)	Status at last FU[j]	
>3	151 (61%)	DOD	5 (2%)
Size of largest metastatic node (cm)[f]		AWD	16 (7%)
Median (range)	1.3 (0.1–4.5)	NED	215 (91%)
≤1	103 (44%)	Follow-up	
>1	133 (56%)	Median (range)	10.8 (0.1–28.8)
Size of largest metastatic focus in lymph node (cm)[f]			
Median (range)	1.1 (0.1–4.5)		
≤1	113 (48%)		
>1	123 (52%)		

[a]Tumor size could not be accurately assessed in seven cases.
[b]Other includes diffuse sclerosing variant and solid variant PTC.
[c]In two cases, angioinvasion could not be accurately assessed.
[d]Extra-thyroid extension could not be evaluated in three patients.
[e]Margin status was not assessable in two cases.
[f]In 10 cases, the size of the largest metastatic node and the largest metastatic foci in lymph node (LN) could not be accurately assessed.
[g]In six cases, the PTC subtype could not be evaluated in the LN because of inadequate LN material (two patients had only psammoma bodies in LN, two cases displayed poorly differentiated thyroid carcinoma [PDTC], and one case was considered as PTC progressing toward PDTC in the LN).
[h]In 10 cases, extra-nodal extension was equivocal or could not be assessed because of inadequate material.
[i]Multicentricity defined as >2 foci of carcinoma could not be accurately evaluated in eight cases.
[j]Ten patients were lost for follow-up (FU).
RAI, radioactive iodine; PTC, papillary thyroid carcinoma; FVPTC, follicular variant of papillary thyroid carcinoma; NED, no evidence of disease; AWD, alive with disease; DOD, death of disease; TT, total thyroidectomy.

respectively. By multivariate analysis, only age was an independent predictor of overall RFS with a risk ratio (RR) [95% confidence interval (CI)] of 3.2 [1.3–8.2]; $p = 0.01$. In regard to neck RFS, none of the variables studied were significant in univariate analysis. Both age and extra-nodal extension were independent factors in predicting distant

RFS in multivariate analysis with RR [95% CI] of 5.9 [1.2–28.6]; $p = 0.03$ and 5.2 [1–26.1]; $p = 0.04$, respectively. By univariate analysis, older age and positive margins correlated with decreased DSS ($p = 0.008$ and 0.02 respectively), while there was a trend toward worse DSS with extra-nodal extension ($p = 0.07$).

TABLE 2. PROGNOSTIC FACTORS OF SURVIVAL BY UNIVARIATE ANALYSIS IN PAPILLARY
THYROID CARCINOMA WITH NODAL METASTASIS

Characteristic	Overall RFS		Neck RFS		Distant RFS		DSS	
	10 years RFS (%)	p	10 years RFS (%)	p	10 years RFS (%)	p	10 years DSS (%)	p
Age, years		0.008		0.23		0.0008		0.0002
<45	91		93		98		100	
>45	76		86		86		95	
Gender		0.66		0.9		0.68		0.93
Female	85		89		95		100	
Male	87		92		94		97	
Tumor size (cm)		0.2		0.35		0.49		0.89
≤1.5	89		92		95		99	
>1.5	84		90		93		98	
PTC subtypes		0.06		0.17		0.39		0.08
FVPTC	100		100		100		100	
Microcarcinoma	93		96		97		100	
Classic PTC	86		90		95		99	
Tall cell PTC	79		87		89		94	
DFS and solid variant	75		88		88		100	
Vascular invasion		0.52		0.81		0.32		0.46
Absent	87		91		95		98	
Present	79		89		90		100	
Extra-thyroid extension		0.007		0.1		0.37		0.32
None	94		96		96		98	
Focal	95		97		97		100	
Extensive	77		85		92		98	
Margins		0.59		0.25		0.03		0.0001
Negative	87		90		96		99	
Positive	82		95		87		94	
Number of metastatic nodes		0.009		0.02		0.8		0.68
≤3	93		96		94		98	
>3	82		88		94		98	
Size of largest metastatic node (cm)		0.05		0.28		025		0.48
≤1	90		93		96		100	
>1	83		90		93		98	
Size of largest metastatic focus in lymph node (cm)		0.1		0.54		0.14		0.38
≤1	89		91		97		100	
>1	84		91		92		98	
Most predominant PTC subtype in lymph node[a]		0.16		0.3		0.66		0.62
FVPTC	100		100		100		100	
Classic PTC	87		92		95		99	
Tall cell	78		85		93		96	
Most aggressive PTC subtype in lymph node[a]		0.05		0.09		0.64		0.19
FVPTC	100		100		100		100	
Classic PTC	87		92		95		100	
Tall cell	80		87		93		95	
Extra-nodal extension		0.009		0.19		0.02		0.004
No	92		94		97		100	
Yes	75		86		92		97	
Multicentricity		0.12		0.06		0.56		0.32
No	88		94		95		98	
Yes	82		86		93		98	
Thyroid surgery		0.6		0.64		0.24		0.23
TT	84		90		92		97	
Sub TT	100		100		100		100	
Lobectomy	91		93		98		100	

TABLE 2. (CONTINUED)

Characteristic	Overall RFS 10 years RFS (%)	p	Neck RFS 10 years RFS (%)	p	Distant RFS 10 years RFS (%)	p	DSS 10 years DSS (%)	p
Neck dissection		0.1		0.44		0.002		0.01
Central	87		88		98		100	
Lateral	93		93		100		100	
Central and lateral	78		90		86		95	
Other	93		93		93		100	
RAI therapy		0.65		0.96		0.19		0.12
Yes	89		92		97		100	
None	84		89		93		97	

[a]Only classic PTC, tall cell PTC, and follicular variant PTC were included in the survival analysis.
RFS, recurrence free survival; DSS, disease specific survival; DFS, diffuse sclerosing variant PTC; Sub TT, subtotal thyroidectomy.

Effect of RAI therapy on survival

There was no statistical difference within each clinico-pathologic category (e.g., within cases with <3 metastatic nodes) between patients who were treated with RAI and those who were not. It is noteworthy that RAI treated patients as a group harbored more aggressive clinico-pathologic features than those who did not receive RAI therapy. Indeed, patients treated with RAI were significantly older ($p < 0.001$), had larger primary tumor ($p = 0.02$), harbored
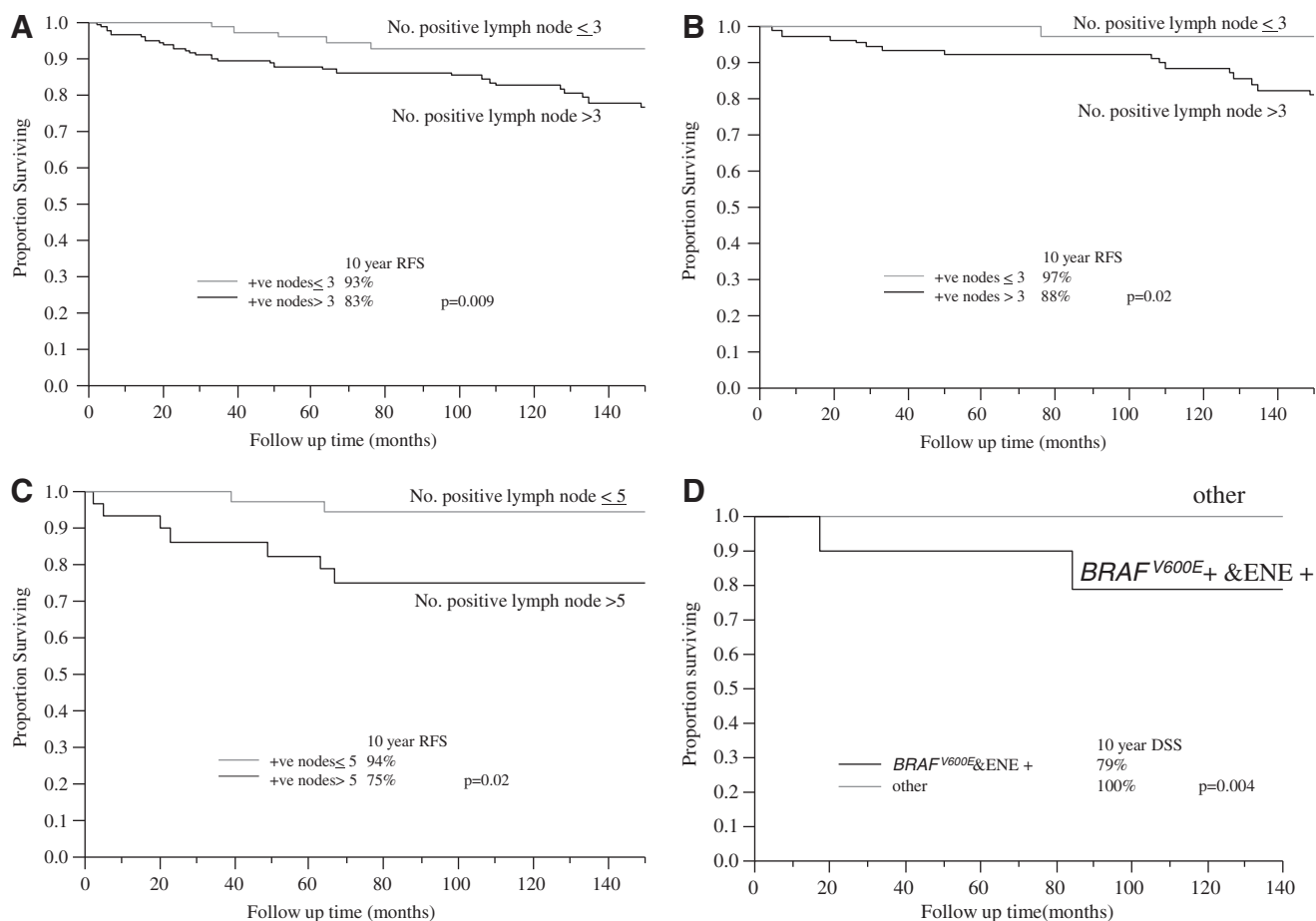

FIG. 2. (A) Overall recurrence free survival (RFS) stratified by number of positive nodes in the whole patient cohort. Patients with >3 metastatic nodes have a significantly lower RFS than those with <3 metastatic nodes. (B) Overall RFS stratified by number of positive nodes in patients <45 years old. Patients with >3 metastatic nodes have a significantly lower RFS than those with <3 metastatic nodes. (C) Neck RFS stratified by number of positive nodes in patients >45 years old. Patients with >5 metastatic nodes have a significantly lower RFS than those with ≤5 metastatic nodes. (D) Disease specific survival (DSS) stratified by combined $BRAF^{V600E}$ mutational and extra-nodal extension (ENE) status in 36 genotyped patients (15 with recurrence and 25 without) matched for various clinico-histopathologic parameters. Patients with extra-nodal extension who are $BRAF^{V600E}$ positive have a lower DSS than the remaining individuals. +ve, positive nodes.

Table 3. Independent Prognostic Factors
for Recurrence in Multivariate
Analysis According to Age

Whole patient population	RR	[95% CI]	p
Overall RFS			
Age	2.5	[1.2–5.1]	0.01
Extra-thyroid extension	2.6	[1–6.5]	0.05
No of metastatic nodes (>3)	3.2	[1.1–8.8]	0.03
Neck RFS			
No of metastatic nodes (>3)	4.3	[1.2–15.2]	0.03
Distant RFS			
Age	7.6	[2.0–28.8]	0.003
Patients <45 years old			
Overall RFS			
No of metastatic nodes (>3)	8.2	[0.9–72.2]	0.05
Neck RFS			
None found			
Distant RFS			
NA			
Patients ≥45 years old			
Overall RFS			
None found			
Neck RFS			
No of metastatic nodes (>5)	9.6	[1.1–83.3]	0.04
Distant RFS			
Margins	4.7	[1–21.6]	0.04

RR, risk ratio; CI, confidence interval; NA, not applicable (multivariate was not possible in view of the low number of events).

Table 4. Outcome of 26 Patients <45 Years
Old with 1–2 Metastatic Nodes
and No Radioactive Iodine Therapy

Recurrence	0/26
Age (median)	33.5 years
Primary tumor size (median)	1.8 cm
Positive margins	2/26 (8%)
Gross ETE[a]	3/25 (12%)
Follow-up (median)	12.99 years

[a]Gross ETE was recorded on the basis of the operating room report.
ETE, Extra-thyroid extension.

more aggressive PTC subtype in the primary ($p = 0.004$), were more multicentric ($p = 0.02$), displayed a higher rate of positive margins ($p = 0.002$), and extra-nodal extension ($p = 0.0001$) than their RAI naïve counterparts.

Genotyping of primary tumors

Of the 34 patients who recurred, 15 had available paraffin embedded tissue for genomic analysis. These 15 recurring patients were matched with 21 patients who did not relapse for the following: age, gender, primary tumor size, frequency of tall cell variant in primary, presence and extent of ETE, number of metastatic nodes, and presence of extra-nodal extension (Table 6). We could not match patients for RAI therapy. $BRAF^{V600E}$ mutations were found in 28 (78%) of 36 primary tumors. *RET-PTC* fusion was found in 3 patients, who all had nonencapsulated classical PTC. These three patients had *RET/PTC* recombination events not detected by the specific reverse transcriptase PCR reactions for *RET/PTC 1*

Table 5. Prognostic Factors of Recurrence by Univariate Analysis in Papillary Thyroid Carcinoma
with Nodal Metastasis Not Treated with Radioactive Iodine Therapy

	Overall RFS		Neck RFS		Distant RFS	
Characteristic	10 years RFS (%)	p	10 years RFS (%)	p	10 years RFS (%)	p
Age, years		0.06		0.44		0.03
<45	93		95		99	
≥45	71		83		88	
PTC subtypes		0.003		0.49		0.009
FVPTC	100		100		100	
Microcarcinoma	95		95		100	
Classic PTC	91		93		98	
Tall cell PTC	76		89		88	
DFS and solid variant PTC	33		67		67	
Extra-thyroid extension		0.06		0.08		0.59
None	97		100		97	
Focal	95		95		100	
Extensive	79		84		95	
Number of metastatic nodes		0.05		0.04		0.93
≤3	94		97		97	
>3	86		90		96	
Most aggressive PTC subtype in lymph node[a]		0.05		0.005		0.66
FVPTC	100		100		100	
Classic PTC	90		95		96	
Tall cell	81		81		100	
Extra-nodal extension		0.05		0.09		0.52
No	93		96		97	
Yes	69		76		93	

[a]Only classic PTC, tall cell PTC, and follicular variant PTC were included in the survival analysis.

TABLE 6. CLINICO-PATHOLOGIC FEATURES OF 15 RECURRING AND 21 NONRECURRING MATCHED PAPILLARY THYROID CANCER CASES WITH NODAL METASTASIS SUBJECTED TO GENOTYPING

	Recurring (n = 15)	*Not recurring* (n = 21)	p-*value*[a]
Median age (years)	52	51	
Female:male ratio	11/15 (73%)	13/21 (61%)	0.7
Median size of primary (cm)	1.8	1.8	
Tall cell frequency in primary tumor	6/15 (40%)	8/21 (38%)	1
ETE present	12/15 (80%)	15/21 (71%)	0.7
ETE extensive	10/15 (66%)	12/21 (57%)	0.7
Median number of metastatic nodes	6	6	
>3 metastatic nodes	11/15 (73%)	13/21 (61%)	0.7
Extra-nodal extension present	7/14 (50%)	5/20 (25%)	0.16
RAI therapy	7/15 (47%)	21/21 (100%)	0.0002

[a]Fisher's exact test.

and *3*. Five patients were wild type for all the genetic aberrations tested. The clinico-pathologic features of the cases according to $BRAF^{V600E}$ status are displayed in Table 7. Of note, *BRAF* positivity correlated with the presence of tall cell variant of papillary carcinoma ($p = 0.01$). There was no significant correlation between *BRAF* positivity and RFS. In regard to DSS, there was a trend toward a decrease in DSS in *BRAF* positive compared with *BRAF* negative patients by univariate analysis ($p = 0.12$). Within this group of genotyped tumors, extra-nodal extension correlated with decreased DSS ($p = 0.02$). The combined presence of *BRAF* and extra-nodal extension provided additive prognostic value. Positivity for both $BRAF^{V600E}$ and extra-nodal extension imparted a lower DSS (79% at 10 years) than negativity for both or either variable (100% at 10 years) ($p = 0.004$) (Fig. 2D).

Discussion

The vast majority of PTCs in this study (87%) were infiltrative tumors lacking complete encapsulation. This high frequency is expected in view of the fact that infiltrative variants of PTC, such as tall cell and classical PTC, are known to have a high nodal metastatic rate (16), and constituted almost 75% of our study cohort. Infiltrative follicular variant of papillary thyroid carcinoma (FVPTC) compose a minor fraction of all FVPTC (~18%), and yet they were overrepresented in this patient population, confirming our previous findings that infiltrative FVPTC correlates with a high rate of metastatic nodes, whereas encapsulated FVPTC rarely metastasize to LN (17). Most of the genotyped tumors in the current series harbored *BRAF* mutations. This is easily explained by the fact that classical, tall cell, and infiltrative follicular variant tumors, which have a high *BRAF* mutation rate (16,17), accounted for 97% of our genotyped cases. As shown in previous publications (16), we found a very strong correlation between $BRAF^{V600E}$ positivity and the identification of tall cell variant PTC ($p = 0.01$). The latter subtype of PTC is known to be almost always infiltrative. While *BRAF* mutation was very common, none of the genotyped cases displayed *RAS* mutations. The strong correlation between the presence of *BRAF* mutation, infiltrative variants of PTC, and nodal metastases confirms that $BRAF^{V600E}$ is a marker of invasiveness in thyroid carcinomas, whereas *RAS* mutation correlates with tumor encapsulation (17). Although the exact mechanism for tumor infiltration in *BRAF* mutated carcinoma is not

TABLE 7. CLINICO-PATHOLOGIC FEATURES ACCORDING TO $BRAF^{V600E}$ STATUS IN 36 PATIENTS WHOSE PRIMARY TUMOR WAS GENOTYPED

	BRAF positive	*BRAF negative*	p[a]
Age			
<45	7 (25%)	5 (62.5%)	0.086
>45	21 (75%)	3 (37.5%)	
Gender			
Male	11 (39%)	1 (12.5%)	0.2
Female	17 (61%)	7 (87.5%)	
Primary tumor size			
≤1.5 cm	12 (43%)	4 (50%)	1
>1.5 cm	16 (57%)	4 (50%)	
PTC subtypes			
Tall cell (*n* = 14)	14 (50%)	0	0.01[b]
Classical (*n* = 19)	13 (46%)	6 (75%)	
Infiltrative FVPTC (*n* = 2)	0	2 (25%)	
Microcarcinoma, infiltrative (*n* = 1)	1 (4%)	0	
No. of positive nodes			
≤3	10 (36%)	1 (12.5%)	0.388
>3	18 (64%)	7 (87.5%)	
Extra-nodal extension (*n* = 34)[c]			
Yes	10 (37%)	2 (40%)	1
No	17 (63%)	5 (60%)	
Size of largest positive node (*n* = 35)[d]			
≤1 cm	14 (51%)	1 (14%)	0.1
>1 cm	13 (49%)	7 (86%)	
Size of largest metastatic focus (*n* = 35)[d]			
≤1 cm	15 (56%)	2 (25%)	0.2
>1 cm	12 (44%)	6 (75%)	
Vascular invasion			
Present	0	1 (12.5%)	0.22
Absent	28 (100%)	7 (87.5%)	
ETE			
Extensive	18 (64%)	4 (50%)	0.6
Focal	2 (7%)	3 (37.5%)	
None	8 (29%)	1 (12.5%)	

[a]Fisher's exact test.
[b]Tall cell vs. nontall cell.
[c]In 2 cases, extra nodal extension could not be evaluated.
[d]In one individual, the size of the metastatic node and largest metastatic focus could not be assessed.

known, $BRAF^{V600E}$ seems to promote tumor invasiveness via an increase in metalloproteinases, secreted enzymes that cause extracellular matrix degradation favoring tumor invasion (18,19).

The data just mentioned demonstrate that PTC patients with LNM can be stratified into prognostically relevant categories using traditional clinico-pathologic features. Indeed, in the current study, a simple count of the number of metastatic nodes was an independent and powerful predictor of RFS in patients with PTC. Leboulleux *et al.* have found that the presence of >3 metastatic nodes correlates with higher recurrence in PTC, but this finding was restricted to those nodes with extra-nodal extension (8). In the papers by Sugitani *et al.* and Ito *et al.*, the presence of five or more metastatic nodes was an independent predictor of recurrence but only in young patients (9,20). Our results confirm the importance of the number of metastatic nodes in predicting recurrence, and extend the findings of previous publications. Indeed, we found that the number of metastatic nodes, irrespective of whether they also had extra-nodal extension, is an independent variable for recurrence not only in young patients but also in older individuals. Although the presence of a large size metastatic node correlated with worse RFS in our series, the size of the largest metastatic tumor focus was unable to predict outcome in patients with PTC. This lack of correlation could be due to the difficulty in assessing metastatic tumor burden in a LN using a unidimensional measurement of the largest tumor focus. The latter may not be an accurate representation of metastatic tumor volume. While extra-nodal extension was a predictor of recurrence and more importantly death of disease, one should acknowledge that in practice the microscopic analysis of extra-nodal extension is subject to significant inter-observer variability due to lack of stringent criteria for its recognition.

Perhaps, one of the most important and interesting finding in this study is the lack of recurrence in any of the 45 young patients with 1–2 metastatic nodes including 26 patients followed for a median of 13 years without RAI therapy. While these results provide reassuring data that the simple presence of LNM in young patients does not convey a high risk of recurrence, these findings, however, cannot be used to study the effect of RAI therapy on survival, as RAI-treated patients as a group harbored much more aggressive clinico-pathologic features than those who did not receive RAI therapy.

While many papers have analyzed the prognostic value of *BRAF* and its relationship with nodal metastasis (21–25), none have assessed its value in stratifying patients with N1 disease. In that regard, we found a trend toward worse DSS in those patients with $BRAF^{V600E}$ mutations. The prognostic value of $BRAF^{V600E}$ was markedly increased when combined with the presence of extra-nodal extension. Indeed, the correlation between positivity for both parameters and death of disease ($p = 0.004$) is much stronger than the one predicted by the single analysis of *BRAF* ($p = 0.12$) or extra-nodal extension ($p = 0.02$). This could be due to the fact that tumor initiation by oncogenic *BRAF* is a required but insufficient event for progression of *BRAF* positive PTC. Indeed, Knauf *et al.* have shown in a preclinical model that progression of *BRAF*-induced thyroid cancer to a more aggressive phenotype is associated with epithelial-mesenchymal transition requiring concomitant MAP kinase and TGFβ signaling transition (26). Similarly, an oncogenic event responsible or associated with

extra-nodal extension may have increased the metastatic potential of the *BRAF* positive tumors in this study leading to higher death rate. Alternatively, the presence of extra-nodal extension may be a marker of tumor cell dissemination. Patients with $BRAF^{V600E}$ tumor without extra-nodal extension may have locoregional confined disease that is cured by surgery, while those with *BRAF* positive tumor with extra-nodal extension have already disseminated and are difficult to treat in view of the propensity of *BRAF* tumor cells to be resistant to RAI therapy (27). Since almost all the genotyped cases in this study were infiltrative PTC and the vast majority were $BRAF^{V600E}$ positive, this survival data suggest that *BRAF* mutation when combined with extra-nodal extension has prognostic value for DSS even within infiltrative PTC.

In conclusion, simple histologic parameters such as the number of metastatic nodes are able to stratify patients with N1 disease and may have important clinical implications. It may help identify a subset of patients at low risk (similar to the one with N0 or Nx disease) that may not require RAI administration sparing numerous young patients the side effects and costs of such therapy. Although the data are conflicting as to whether adjuvant therapy improves outcome in N1 disease (1), the results of this study will help restrict the use of adjuvant RAI therapy to those patients with N1 disease at highest risk of recurrence. The presence of $BRAF^{V600E}$ in the primary tumor when combined with extra-nodal extension seems to have some additive prognostic value in predicting death. The findings just mentioned should prompt re-evaluation of classification systems that assign the same magnitude of risk of recurrence or death to all patients with N1 disease.

Disclosure Statement

The authors declare that no competing financial interests exist.

References

1. Cooper DS, Doherty GM, Haugen BR, Kloos RT, Lee SL, Mandel SJ, Mazzaferri EL, Mclver B, Pacini F, Schlumberger M, Sherman SI, Stewart DL, Tuttle RM 2009 Revised American Thyroid Association management guidelines for patients with thyroid nodules and differentiated thyroid cancer. Thyroid **19:**1167–1214.
2. Edge SB, Byrd DR, Compton CC, Fritz AG, Greene FL, Trotti A (eds) 2010 AJCC Cancer Staging Manual, 7th edition. Springer, New York, NY, pp 67–74.
3. Hughes DT, White ML, Miller BS, Gauger PG, Burney RE, Doherty GM 2010 Influence of prophylactic central lymph node dissection on postoperative thyroglobulin levels and radioiodine treatment in papillary thyroid cancer. Surgery **148:**1100–1106; discussion 1006–1007.
4. Bonnet S, Hartl D, Leboulleux S, Baudin E, Lumbroso JD, Al Ghuzlan A, Chami L, Schlumberger M, Travagli JP 2009 Prophylactic lymph node dissection for papillary thyroid cancer less than 2 cm: implications for radioiodine treatment. J Clin Endocrinol Metab **94:**1162–1167.
5. Tuttle RM, Tala H, Shah J, Leboeuf R, Ghossein R, Gonen M, Brokhin M, Omry G, Fagin JA, Shaha A 2010 Estimating risk of recurrence in differentiated thyroid cancer after total thyroidectomy and radioactive iodine remnant ablation: using response to therapy variables to modify the initial risk

estimates predicted by the new American thyroid association staging system. Thyroid 20:1341–1349.

6. Ries LAG, Young JL, Keel GE, Eisner MP, Lin YD, Horner M-J (eds) 2007 SEER Survival Monograph: Cancer Survival Among Adults: U.S. SEER Program, 1988–2001, Patient and Tumor Characteristics. National Cancer Institute, SEER Program, NIH Pub. No. 07–6215, Bethesda, MD.

7. Bardet S, Malville E, Rame JP, Babin E, Samama G, De Raucourt D, Michels JJ, Reznik Y, Henry-Amar M 2008 Macroscopic lymph-node involvement and neck dissection predict lymph-node recurrence in papillary thyroid carcinoma. Eur J Endocrinol 158:551–560.

8. Leboulleux S, Rubino C, Baudin E, Caillou B, Hartl DM, Bidart JM, Travagli JP, Schlumberger M 2005 Prognostic factors for persistent or recurrent disease of papillary thyroid carcinoma with neck lymph node metastases and/or tumor extension beyond the thyroid capsule at initial diagnosis. J Clin Endocrinol Metab 90:5723–5729.

9. Sugitani I, Kasai N, Fujimoto Y, Yanagisawa A 2004 A novel classification system for patients with PTC: addition of the new variables of large (3 cm or greater) nodal metastases and reclassification during the follow-up period. Surgery 135:139–148.

10. Cranshaw IM, Carnaille B 2008 Micrometastases in thyroid cancer. An important finding? Surg Oncol 17:253–258.

11. DeLellis RA, Lloyd RV, Heitz PU, Eng C (eds) 2004 World Health Organization Classification of Tumours. Pathology & Genetics Tumours of the Endocrine Organs. IARC Press, Lyon.

12. Hiltzik D, Carlson DL, Tuttle RM, Chuai S, Ishill N, Shaha A, Shah JP, Singh B, Ghossein RA 2006 Poorly differentiated thyroid carcinomas defined on the basis of mitosis and necrosis: a clinicopathologic study of 58 patients. Cancer 106:1286–1295.

13. Rosai J, Carcangiu ML, Delellis RA 1992 Tumors of the thyroid gland In: Rosai J, Sobin LH (eds) Atlas of Tumor Pathology, vol. 5. Armed Forces Institute of Pathology, New York, pp 161–182.

14. Ricarte-Filho JCM, Ryder M, Chitale DA, Rivera M, Heguy A, Ladanyi M, Janakiraman M, Solit D, Knauf JA, Tuttle RM, Ghossein RA, Fagin JA 2009 Mutational profile of advanced primary and metastatic radioactive iodine-refractory thyroid cancers reveals distinct pathogenetic roles for BRAF, PIK3-CA and AKT1. Cancer Res 69:4885–4893.

15. Imkamp F, von Wasielewski R, Musholt TJ, Musholt PB 2007 Rearrangement analysis in archival thyroid tissues: punching microdissection and artificial RET/PTC 1–12 transcripts. J Surg Res 143:350–363.

16. Adeniran AJ, Zhu Z, Gandhi M, Steward DL, Fidler JP, Giordano TJ, Biddinger PW, Nikiforov YE 2006 Correlation between genetic alterations and microscopic features. Clinical manifestations, and prognostic characteristics of thyroid papillary carcinomas. Am J Surg Pathol 30:216–222.

17. Rivera M, Ricarte-Filho J, Knauf J, Shaha A, Tuttle RM, Fagin JA, Ghossein RA 2010 Molecular genotyping of papillary thyroid carcinoma follicular variant according to its histologic subtypes (encapsulated vs infiltrative) reveals distinct BRAF and RAS mutation patterns. Mod Pathol 23:1191–1200.

18. Mesa C Jr., Mirza M, Mitsutake N, Sartor M, Medvedovic M, Tomlinson C, Knauf JA, Weber GF, Fagin JA 2006 Conditional activation of RET/PTC3 and BRAF^{V600E} in thyroid cells is associated with gene expression profiles that predict a preferential role of BRAF in extracellular matrix remodeling. Cancer Res 66:6521–6529.

19. Frasca F, Nucera C, Pellegriti G, Gangemi P, Attard M, Stella M, Loda M, Vella V, Giordano C, Trimarchi F, Mazzon E, Belfiore A, Vigneri R 2008 BRAF^{V600E} mutation and the biology of papillary thyroid cancer. Endocr Relat Cancer 15:191–205.

20. Ito Y, Fukushima M, Tomoda C, Inoue H, Kihara M, Higashiyama T, Uruno T, Takamura Y, Miya A, Kobayashi K, Matsuzuka F, Miyauchi A 2009 Prognosis of patients with papillary thyroidcarcinoma having clinically apparent metastasis to the lateral compartment. Endocr J 56:759–766.

21. Kim TY, Kim WB, Rhee YS, Song JY, Kim JM, Gong G, Lee S, Kim SY, Kim SC, Hong SJ, Shong YK 2006 The BRAF mutation is useful for prediction of clinicalrecurrence in low-risk patients with conventional papillary thyroid carcinoma. Clin Endocrinol 65:364–368.

22. Jung CK, Kang YG, Bae JS, Lim DJ, Choi YJ, Lee KY 2010 Unique patterns of tumor growth related with the risk of lymph node metastasis in papillary thyroid carcinoma. Mod Pathol 23:1201–1208.

23. Kebebew E, Weng J, Bauer J, Ranvier G, Clark OH, Duh QY, Shibru D, Bastian B, Griffin A 2007 The Prevalence and Prognostic Value of BRAF Mutation in Thyroid Cancer. Ann Surg 246:466–471.

24. Frasca F, Nucera C, Pellegriti G, Gangemi P, Attard M, Stella M, Loda M, Vella V, Giordano C, Trimarchi F, Mazzon E, Belfiore A, Vigneri R 2008 BRAF^{V600E} mutation and the biology of papillary thyroid cancer. Endocr-Relat Cancer 15:191–205.

25. Sykorova V, Dvorakova S, Ryska A, Vcelak J, Vaclavikova E, Laco J, Kodetova D, Kodet R, Cibula A, Duskova J, Hlobilkova A, Astl J, Vesely D, Betka J, Hoch J, Smutny S, Cap J, Vlcek P, Novak Z, Bendlova B 2010 BRAFV600E mutation in the pathogenesis of a large series of papillary thyroid carcinoma in Czech Republic. J Endocrinol Invest 33:318–324.

26. Knauf JA, Sartor MA, Medvedovic M, Lundsmith E, Ryder M, Salzano M, Nikiforov YE, Giordano TJ, Ghossein RA, Fagin JA 2011 Progression of BRAF-induced thyroid cancer is associated with epithelial-mesenchymal transition requiring concomitant MAP kinase and TGFβ signaling. Oncogene 30:3154–3162.

27. Xing M 2010 Prognostic utility of BRAF mutation in papillary thyroid cancer. Mol Cell Endocrinol 321:86–93.

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