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Cytologic, histologic and molecular findings of papillary thyroid carcinoma variants, one institution's experience

Nadja K Falk*[1], Swarnamala Ratnayaka[1], Andrew B. Sholl[2], Krzysztof Moroz[1], Tatyana Kalinicheva[3]

[1] *Department of Pathology and Laboratory Medicine, Tulane University School of Medicine, New Orleans, LA, United States*
[2] *Ochsner Department of Pathology, New Orleans, LA, United States*
[3] *Thibodaux Regional Medical Center, Thibodaux, LA, United States*

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ABSTRACT

Papillary thyroid carcinoma (PTC) has two major types, classic (PTCC) and follicular variant (FVPTC), which correlate with molecular findings and have varying clinical implications. We assessed the cytologic findings and subsequent surgical pathology findings with the molecular mutations in these two groups, including microcarcinomas. Fourty-four patients with PTC resections over a one-year period were retrospectively examined in conjunction with previous cytologic diagnoses. BRAF, NRAS and TERT promoter mutations for the resected specimens were analyzed. Correlation with previous cytology in regard to molecular mutations and tumor size (microcarcinoma) were made. Significantly more BRAF V600E mutations were seen with PTCC, whereas significantly more NRAS mutations were seen with FVPTC. TERT mutations were only seen with PTCC. Molecular studies for thyroid carcninomas are becoming increasingly more common and influence treatment and patient prognosis. BRAF and or TERT mutations are associated with a worse prognosis. NRAS mutations associated with FVPTC and may lead to milder cytologic changes compared to the BRAF- and TERT-driven PTCC.

Key Words: Papillary thyroid carcinoma, follicular variant, BRAF, NRAS, TERT

1. INTRODUCTION

Thyroid carcinoma (TC) is the most common endocrine malignancy.[1,2] More than 80% of thyroid carcinomas are PTC, and its incidence is steadily increasing at a rate of 3% per year.[3] The age-standardized incidence rate of thyroid cancer per year in developed countries is 6.62 for men and 20.8 per women per 100,000. PTC accounts for 1.5% of all cancers in the United States (US). Approximately 5% of US individuals fifty years or older have palpable thyroid nodules, however only 5%-15% of thyroid nodules are malignant; and the lifetime risk of TC diagnosis in the US is 1.2%.[4–6] TC occurs 2-3 times more often in women and is the fifth most common malignancy diagnosed in women. Since the 1970s, ultrasound-guided fine needle aspirations (US-FNA) have been performed for the initial diagnosis of thyroid nodules and for management triage.[7–9]

Many PTC subtypes exist, including PTCC, FVPTC, solid/trabecular, tall cell, columnar cell, diffuse sclerosing, hobnail, and cribiriform-morular.[10] Most PTC cases are PTCC or FVPTC histologic sub-types, 55%-65% and 23%-41% respectively. Histologic features of PTCC include nuclear grooves, intranuclear inclusions, and papillary architecture. FVPTC was originally described by Crile and Hazard

*Correspondence: Nadja Falk; Email: nfalk@salud.unm.edu; Address: Department of Pathology, University of New Mexico, Albuquerque, NM 87131-0001, United States.

in 1953 and subsequently categorized by Lindsay et al. and Chem and Rosai.[10–12] FVPTC has a predominantly follicular pattern while retaining the PTC nuclear features and may only have focal papillary architecture.[13,14] US-FNA has a low sensitivity for detecting FVPTC; 45%-77% of FVPTC are classified as atypia of undetermined significance or follicular lesion of undetermined significance and only 7%-48% are diagnosed as malignant.[14–17] Papillary microcarcinoma (PTC-M) is defined by the World Health Organization as a papillary thyroid cancer measuring 10mm or less in diameter. PTC-M is usually indolent, with mortality less than 1% with a locoregional recurrence of approximately 5%.[18]

PTC genetic changes commonly activate the MAPK pathway and are dominated by driving somatic mutations in BRAF and RAS oncogenes. In addition to PTC, somatic mutations BRAF genes have been found in multiple other cancers, including non-Hodgkin lymphoma, colorectal cancer, malignant melanoma, and non-small cell lung carcinoma. BRAF is a serine-threonine kinase that is an activator downstream of the RAS oncogene in the pathway.[19] BRAF V600E mutation makes up the majority of BRAF mutations in PTC, in which a point mutation at codon 600 leads to a valine substitution to glutamic acid.[4] This mutation increases BRAF kinase activity through increasing ERK1/2 phosphorylation and leads to increased cell proliferation, differentiation, survival, tumorigenesis and promotion of epithelial-mesenchymal transition. The average BRAF V600E mutation frequency is 48% in PTCC and 10% in FVPTC.[2,12,20,21] BRAF V600E mutation is > 99% specific for the diagnosis of TC.[22]

NRAS mutations are seen in approximately 40% of FVPTC and only rarely in PTCC.[3,4,7,23] NRAS is a member of the RAS family of GTPase and plays an important role as a mediator of cellular growth signals generated by upstream receptor tyrosine kinases. In addition to PTC, activating mutations of NRAS are found in various types of cancers, including 15%-25% of melanomas, 3%-5% of colorectal cancers, and 5%-10% of hepatocellular carcinomas. The most common NRAS mutations in PTC involve codon 61 (Q61R or Q61K).[20] These point mutations decrease intrinsic GTPase activity, which increases activation of the MAPK and PI3/AKT signaling pathways [24]. RAS mutations are seen in 15%-20% of PTC, 40%-50% of follicular carcinoma, and 20%-40% of follicular adenomas, and 26%-45% of FVPTC.[15,25,26]

In 2013, TERT gene promoter mutations were detected in thyroid carcinoma.[8] TERT is an enzyme involved in telomere elongation. TERT overexpression is caused by a mutation in its promoter region which promotes tumor cell survival. The TERT promoter mutations cluster mainly in two hotspots, c.1-124C >T(C228T) and c.1-146C >T(C250T), which are transition mutations corresponding to nucleotides -124 and -146 upstream of the initiation codon ATG, respectively. TERT mutations have been described in TC, central nervous system tumors, bladder cancer, hepatocellular carcinoma, and melanoma (71% of patients). TERT gene promoter region mutations are reported in 7%-22% of PTC and follicular carcinomas and 29%-95% of dedifferentiated thyroid cancers. Of these, TERT promoter mutations are seen in about 30% of tall cell variant PTC and in approximately 40% of poorly differentiated and anaplastic thyroid carcinomas, which are clinically more aggressive.[7] C228T TERT promoter mutation has been associated BRAF V600E mutation in aggressive thyroid cancer.[5,7,27–29] TERT mutations are uncommon in PTC-M.[17,30,31]

2. METHODS

Forty-four patients, thirty-six females and eight males, with PTC resections over a one-year period were retrospectively studied (see Table 1). The resections, some of which included multifocal carcinoma, included sixty-two PTC foci, of which thirty-three were PTCC and twenty-nine were FVPTC. 55% of these PTC foci had been previously aspirated and evaluated cytologically. The resected PTCC and FVPTC foci were analyzed for BRAF, NRAS and TERT mutations. A two-sample t-test between percent was used to determine significance of molecular alterations between the PTCC and FVPTC groups.

Table 1. Demographic information

Age	26-78 years old (mean = 50 years)
Gender distribution	Females: 36 (82%) Males: 8 (18%)
Number of resected PTC foci evaluated	62 PTC foci, including: PTCC: 33/62 (53%) FVPTC: 29/62 (47%)
Fine needle aspiration cytology	34 (55%) foci

Note. PTC - Papillary thyroid carcinoma; PTCC - Papillary thyroid carcinoma, classical type; FVPTC – Papillary thyroid carcinoma, follicular variant

BRAF and NRAS real time polymerase chain reaction (RT-PCR) testing was performed in the Tulane University Molecular Pathology Laboratory. A designated pathologist identified the presence of at least 20% tumor cells in hematoxylin and eosin-stained slides prepared from formalin-fixed, paraffin-embedded tissue sections. DNA was isolated using Qiagen EZ1 tissue kit (Qiagen, CA). Mutated BRAF or NRAS gene was detected using a validated BRAF or NRAS mutation kits (EntroGen, CA) and RT-PCR methodologies (Light Cycler 480, Roche Applied Science, IN) with an analyti-

cal sensitivity of 1%-5% mutant in a background of wild-type genomic DNA. The following BRAF mutations were tested: V600E (Val600Glu), V600K (Val600Lys), V600D (Val600Asp), V600R (Val600Arg), and V600M (Val600Met) found in codon 600. Somatic mutations located in codons 12, 13, and 61 of the NRAS gene were tested.

TERT Promoter Mutation by next generation sequencing (NGS) (c.1-124C>T (C2228T) and c.1-146C>T (C250T)) analysis was performed at University of Pittsburgh Medical Center, where a molecular pathologist determined lesional cell adequacy for microdissection on hematoxylin and eosin-stained slides. Torrent suite Software v5.2.2 was used for data analysis. The analytical sensitivity for TERT variant detection at >3%-5% mutant allele frequency was >99.9%. The analytical specificity was >99% and the minimal required sequencing depth was 500x.

3. Results

Significantly more BRAF V600E mutations were seen in PTCC foci versus FVPTC foci, 85% versus 18% respectively. Conversely, more NRAS Q61R or Q61K mutations were seen in FVPTC foci versus PTCC foci, 29% versus 4% respectively. TERT C228T mutation was seen in 33% of PTCC foci and not found in FVPTC foci (see Table 2).

Fourteen PTCC foci, of which six were histologically diagnosed as microcarcinoma upon resection, had had previous ultrasound-guided fine needle aspirations and cytologic diagnoses. Ten FVPTC foci, of which three were histologically diagnosed as microcarcinoma upon resection, had had previous ultrasound-guided fine needle aspirations and cytologic diagnoses. The macrocarcinomas (more than 1.0 cm in size) were more often categorized in higher Bethesda categories, including suspicious for follicular neoplasm or suspicious for malignancy, whereas the microcarcinomas were more often categorized in lower Bethesda categories, including benign or atypical. Upon resection, the similar trend of BRAF mutation being present in more PTCC versus FVPTC and NRAS mutations being present in more FVPTC versus PTCC was seen. TERT mutation was seen only in PTCC (see Table 3).

Table 2. Frequency of BRAF, NRAS and TERT mutations identified in resected specimens of PTC, classical type and follicular variant

Mutation	PTCC	FVPTC	Two-sample *t*-test between percent
BRAF V600E c.1799T>A	22/26 (85%)	4/22 (18%)	$p = .00005$*
NRAS Q61R or Q61K	1/26 (4%)	7/24 (29%)	$p = .0199$*
TERT C228T	3/9 (33%)	0/9 (0%)	$p = .0776$

*p-value is less than estimated critical alpha level ($p \leq .05$); PTCC - Papillary thyroid carcinoma, classical type; FVPTC - Papillary thyroid carcinoma, follicular variant.

Table 3. Preceding cytologic aspiration diagnoses for resected foci of PTC, classical type and follicular variant, with correlation to BRAF V600E, NRAS (Q61R or Q61K) and TERT C228T mutations.

PTC types (Total # of foci)	Bethesda II* N (%)	Bethesda III† N (%)	Bethesda IV^ N (%)	Bethesda V & VI‡ N (%)
PTCC (14)	2 (14%)	1 (7%)	0 (0%)	11 (79%)
PTCC-M (6)	2 (33.3%)	2 (33.3%)	0 (0%)	2 (33.3%)
BRAF mutation (16)	3 (19%)	3 (19%)	0 (0%)	10 (62%)
BRAF wild-type (4)	1 (25%)	0 (0%)	0 (0%)	3 (75%)
NRAS mutation (0)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
NRAS wild-type (20)	4 (20%)	3 (15%)	0 (0%)	13 (65%)
TERT mutation (1)	0 (0%)	0 (0%)	0 (0%)	1 (100%)
TERT wild-type (5)	1 (20%)	1 (20%)	0 (0%)	3 (60%)
FVPTC (10)	2 (20%)	4 (40%)	0 (0%)	4 (40%)
FVPTC-M (3)	1 (33.3%)	1 (33.3%)	1 (33.3%)	0 (0%)
BRAF mutation (4)	1 (25%)	1 (25%)	1(25%)	1 (25%)
BRAF wild-type (9)	2 (22%)	4 (44%)	0 (0%)	3 (33%)
NRAS mutation (6)	1 (17%)	3 (50%)	0 (0%)	2 (33%)
NRAS wild-type (7)	2 (28.6%)	2 (28.6%)	1 (14.3%)	2 (28.6%)
TERT mutation (0)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
TERT wild-type (5)	1 (20%)	2 (40%)	0 (0%)	2 (40%)

Note. PTCC - Papillary thyroid carcinoma, classical type; PTCC-M - Papillary thyroid microcarcinoma, classical type; FVPTC - Papillary thyroid carcinoma, follicular variant; FVPTC-M - Papillary thyroid microcarcinoma, follicular variant; N – Number; *Benign; †Atypia of undetermined significance / Follicular lesion of undetermined significance; ^Suspicious for follicular neoplasm or follicular neoplasm; ‡Suspicious for malignancy or malignant

4. DISCUSSION

PTC has a good prognosis with up to 95% survival after ten years. PTCC is generally more aggressive with more metastatic potential than FVPTC. FVPTC patients tend to have a lower prevalence of extrathyroidal extension and lymph node metastasis. PTC treatment usually includes surgery followed by radioactive iodine. Up to 20% of patients, however, relapse after initial treatment, and 5%-10% will metastasize. Poor prognostic features include older age, male sex, large size, and extrathyroidal extension.[32] 5%-10% of the patients with relapsed PTC do not respond to conventional therapies. Therefore, early identification of high-risk patients is paramount.[6, 10, 12, 33]

BRAF mutations are associated with a poor prognosis and tumor aggressiveness (extrathyroidal extension, lymph node metastasis, radioiodine treatment failure, and recurrence) and may be associated with the tumor being more often bilateral.[3, 34] Without other coexisting genetic alterations, RAS mutated PTC lacks an aggressive behavior.[35] TERT promoter mutations correlate with aggressive clinicopathological features and poor outcomes.[36] Concomitant BRAF and TERT mutated tumors have particularly high-risk clinicopathological characteristics and increased recurrence rates. BRAF V600E-activated MAP kinase pathway upregulates ETS transcription factors, which in turn increase the expression of TERT by attaching to the binding site in the TERT promoter created by the C228T or C250T mutation. Patients with BRAF and TERT mutations recur in approximately 70% of cases, whereas patients with only one of these mutations recur in approximately 9% of cases.[7, 27, 30, 38–44]

The MAPK pathway is the predominant therapy target in advanced thyroid carcinomas.[4] Molecular markers may influence targeted therapy for advanced disease, and systemic therapy may be considered in the following circumstances: progressive, locally recurrent, advanced, metastatic, or surgically unresectable disease or tumors not amenable to radioactive iodine. Lenvatinib, sorafenib, sunitinib, axitinib, everolimus, vandetinib, cabozantinib, and pazopanib are examples of kinase inhibitors. Bleeding, hypertension, stroke and liver toxicity are possible side effects, and most are alleviated by discontinuing the systemic therapy.[1]

5. CONCLUSION

TC molecular mutation studies are commonly performed, not only on surgically resected specimens but also on cytologic samples. Diagnosing FVPTC is difficult cytologically; however, the presence of NRAS mutations and lack of BRAF and or TERT mutations may help clinicians to favor this diagnosis. The sheer size of microcarcinomas may hinder accurate cytologic diagnoses due to possible sampling error. Overall, BRAF and TERT individually portend a worse prognosis in PTC; however, concomitant BRAF and TERT mutations may indicate an even worse prognosis for the patient.

CONFLICTS OF INTEREST DISCLOSURE

The author declares no conflict of interest

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