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Original contribution

BRAF genetic heterogeneity in papillary thyroid carcinoma and its metastasis☆,☆☆

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Summary Intratumoral heterogeneity is widely recognized as an important determinant of a cancer's initial response and its subsequent resistance to targeted therapy. *BRAF* V600E mutation, common in papillary thyroid carcinoma (PTC), is helpful in fine needle aspiration diagnosis of thyroid nodules and is being evaluated for targeted therapies. This study was designed to assess the presence of *BRAF* mutation heterogeneity within primary PTCs and between paired primary and metastatic lesions. Genetic heterogeneity was evaluated in 47 PTCs (38 differentiated papillary thyroid carcinomas and 9 poorly differentiated PTCs with anaplastic areas). The differentiated papillary thyroid carcinomas included 16 cases with regional lymph node metastases at thyroidectomy and 9 cases with recurrent metastases to regional lymph nodes more than 5 years post thyroidectomy. Genetic heterogeneity of *BRAF* was studied by comparing the mutation status in different samples of tumor as follows: (*a*) 2 separate areas (each >1.5 cm in diameter) within the primary tumor, (*b*) a more than 1.5 cm area of primary carcinoma and a second 5 mm area simulating a fine needle aspiration sample from a different portion of the primary tumor, (*c*) primary carcinoma and its lymph node metastasis at thyroidectomy, (*d*) primary carcinoma and the recurrent metastasis, and (*e*) differentiated and anaplastic areas in the primary carcinoma. *BRAF* mutation status was concordant in 95.2% of the 62 paired samples. Discordant *BRAF* status was detected in only 4.8% of the pairs studied and most frequently involved cases with recurrent metastasis thus suggesting a need for additional testing of these lesions before instituting therapy.

1. Introduction

The *BRAF* V600E mutation (*BRAF* mutation) has been reported in 18% to 87% of thyroid cancers [1,2] including in up to 69% of papillary thyroid carcinomas (PTC) and in 20% to 40% anaplastic thyroid carcinomas (ATC) [3,4]. Detection of the *BRAF* mutation has proven helpful in the management of thyroid nodules that are indeterminate by fine needle aspiration (FNA) [5]. Currently, *BRAF* mutation is being evaluated as a potential stratification tool for extent of thyroid surgery and as a potential therapeutic target in PTC [6] (See Table).

The *BRAF* V600E mutation, located on chromosome 7, involves a thymine-to-adenine substitution at position 1799 in the kinase domain of exon 15 and results in a valine-to-glutamate replacement at position 600. This mutation is thought to play an important role in the pathogenesis of PTC through activation of the mitogen-activated protein kinase pathway [6], which affects the transcriptional regulation and

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Table *BRAF* V600E mutation status in papillary thyroid carcinoma and its metastasis

	Pairs analyzed	Concordant *BRAF* (status no. of pairs)	Discordant *BRAF* (status no. of pairs)	Discordant pairs (%)
Differentiated PTC				
Separate areas within primary tumor				
<1.5 cm area *vs* >1.5 cm area	13	12 (DET, 6; NDE, 6)	1	7.70%
≤0.5 cm area *vs* >1.5 cm area	15	15 (DET, 10; NDE, 5)	0	0%
Primary tumor *vs* metastasis				
At thyroidectomy	16	16 (DET, 11; NDE, 5)	0	0%
>5 y postthyroidectomy	9	7 (DET, 7)	2 (DET in primary and NDE in metastasis)	22.20%
Poorly differentiated/anaplastic PTC				
Papillary *vs* anaplastic areas within primary tumor	9	9 (DET, 7; NDE, 2)	0	0.00%

Abbreviations: PTC, papillary thyroid carcinoma; DET, mutation detected; NDE, no mutation detected.

expression of various genes that are involved in cell proliferation, survival, and cancer progression [7,8]. The *BRAF* mutation is also thought to inhibit the sodium-iodide symporter, which facilitates iodine uptake [9] and has been associated with PTCs that are refractory to radioiodine therapy [10]. Chakravarty et al [11] have shown that kinase inhibitors that target mitogen-activated protein kinase or BRAF can restore iodine avidity to PTCs that contain mutant BRAF, and Brose et al [12] have presented data indicating that sorafenib is beneficial to these patients in clinical trials. Vemurafenib has also shown some efficacy in *BRAF*-mutated ATC [13].

Intratumoral heterogeneity is thought to play an important role in determining a cancer's initial response as well as subsequent resistance to targeted therapy. Initial studies have reported a low incidence of *BRAF* intratumoral heterogeneity in PTCs [14,15], whereas in a recent quantitative analysis of subclonal and individual tumor cell populations for mutant *BRAF* alleles, Guerra et al [16] reported PTCs as containing a heterogeneous population of mutant and wild-type *BRAF* alleles. These techniques are not generally available in clinical laboratories, and the data generated are difficult to translate into patient management. *BRAF* testing for clinical application is performed by real-time polymerase chain reaction (PCR) and uses routinely processed FFPE material to determine the "global" *BRAF* mutation status of the tumor sample. The "global" mutation status, reported as mutation detected (DET) or not detected (NDE), is incorporated into personalized/targeted medical care. Because *BRAF* mutation status within and/or across tumor samples from a patient with PTC can impact patient management, this study was designed to assess "global" *BRAF* mutation heterogeneity within primary PTCs and between paired primary and metastatic lesions.

2. Materials and methods

With Cedars-Sinai Medical Center Institutional Review Board approval, 47 thyroidectomy specimens (38 with differentiated papillary thyroid carcinoma [DPTC] and 9 with poorly differentiated PTC with anaplastic component [PTC-ATC]) were identified in our pathology database. The DPTC (18 conventional PTC, 17 follicular variant PTC [FVPTC], 3 mixed conventional/FVPTC) included cases with regional lymph node metastases at thyroidectomy and cases with recurrent metastases more than 5 years post thyroidectomy. Slides were reviewed, and diagnoses were confirmed (Figs. 1-3). The availability of sufficient primary and metastatic tumor for mutation analysis was the only criterion for case inclusion in the study. Demographics, tumor size, and lymph node status were recorded.

Genetic heterogeneity of *BRAF* in the DPTCs was studied by comparing the mutation status in different samples of tumor as follows:

(a) Two different more than 1.5 cm areas each containing primary carcinoma, that is, 2 whole sections comprising primary carcinoma from 2 different blocks;
(b) A more than 1.5 cm area of primary carcinoma and a second 5 mm area simulating an FNA sample from a different portion of the primary tumor, that is, 1 whole section and a 0.5 cm area from a different block;

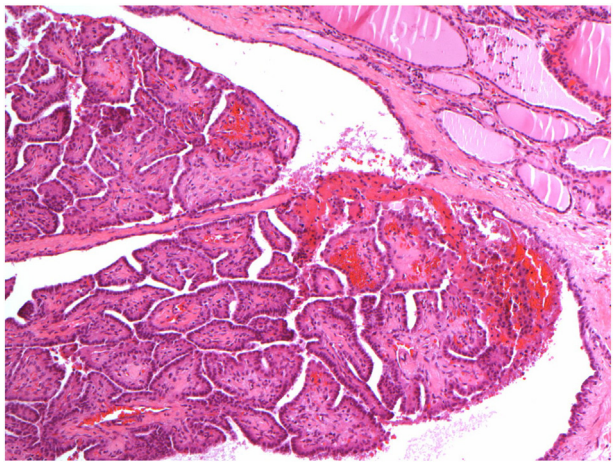

Fig. 1 Conventional papillary thyroid carcinoma.