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Original article

Clinicopathologic correlations of the *BRAF*^{V600E} mutation, BRAF V600E immunohistochemistry, and *BRAF* RNA *in situ* hybridization in papillary thyroid carcinoma

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ABSTRACT

Background: The *BRAF*^{V600E} mutation is the most common genetic alteration in papillary thyroid carcinoma (PTC). The aim of this study is to analyze the clinicopathologic correlations of the *BRAF*^{V600E} mutation, BRAF V600E immunohistochemistry (IHC) and *BRAF* RNA *in situ* hybridization (ISH) in PTC.
Methods: This study included 467 patients with PTC who underwent surgical resection. We studied the *BRAF*^{V600E} mutation using real-time PCR and BRAF V600E and *BRAF* RNA ISH using tissue microarray (TMA).
Results: The frequencies of a positive *BRAF*^{V600E} mutation by real-time PCR, positive BRAF V600E IHC, and high *BRAF* RNA ISH were 84%, 86%, and 70%, respectively, in PTC. Conventional PTC had higher positive rates in all three tests than other histologic types. The *BRAF*^{V600E} mutation, BRAF V600E IHC, low ΔCt, and high *BRAF* RNA ISH were significantly associated with lymph node metastasis. The *BRAF*^{V600E} mutation was significantly associated with positive immunostaining for BRAF V600E mutant protein ($P < 0.001$) overall, with high *BRAF* RNA ISH only in the follicular variant ($P = 0.035$). No significant correlation was noted between BRAF V600E IHC and *BRAF* RNA ISH. The sensitivity of BRAF V600E IHC for the *BRAF*^{V600E} mutation was 95%, and the specificity was 61% overall, 96% and 54% in the conventional type, and 85% and 70% in the follicular variant.
Conclusions: Our results showed that positive BRAF V600E IHC significantly correlated with the *BRAF*^{V600E} mutation. This suggests its clinical utility as a screening tool for the *BRAF*^{V600E} mutation. In addition, a high *BRAF* RNA ISH score could be a candidate marker of aggressive behavior in *BRAF*^{V600E} mutation-positive cases of PTC.

Introduction

Papillary thyroid carcinoma (PTC) is the most common subtype of thyroid cancer, accounting for about 80% of all thyroid malignancies [2]. The *BRAF*^{V600E} mutation is the most common type of *BRAF* mutation, and has been detected in 30–83% of PTCs [14]. The *BRAF* oncogene encodes the human gene for B-type Raf kinase. Over 30 mutations of *BRAF* associated with human cancers have been identified [7], the majority of which are located within the kinase domain. In an analysis of 22 *BRAF* mutants, 18 had elevated kinase activity and signaled to ERK *in vivo*. Three other mutants had reduced kinase activity toward MEK *in vitro* but, by activating *CRAF in vivo*, signaled to ERK in cells [26].

The T1799A point mutation in exon 15 of *BRAF* (thymidine-to-adenine transversion) results in a valine-to-glutamate substitution at position 600 (V600E) and activates the RAS/RAF/MAPK signaling pathway by disrupting hydrophobic interactions between residues in both the activation loop and the ATP binding site [26]. This pathway is hyperactivated in about 30% of cancers including malignant melanoma, papillary thyroid carcinoma, pilocytic astrocytoma, adenocarcinoma of the lung, ovarian neoplasms, hairy cell

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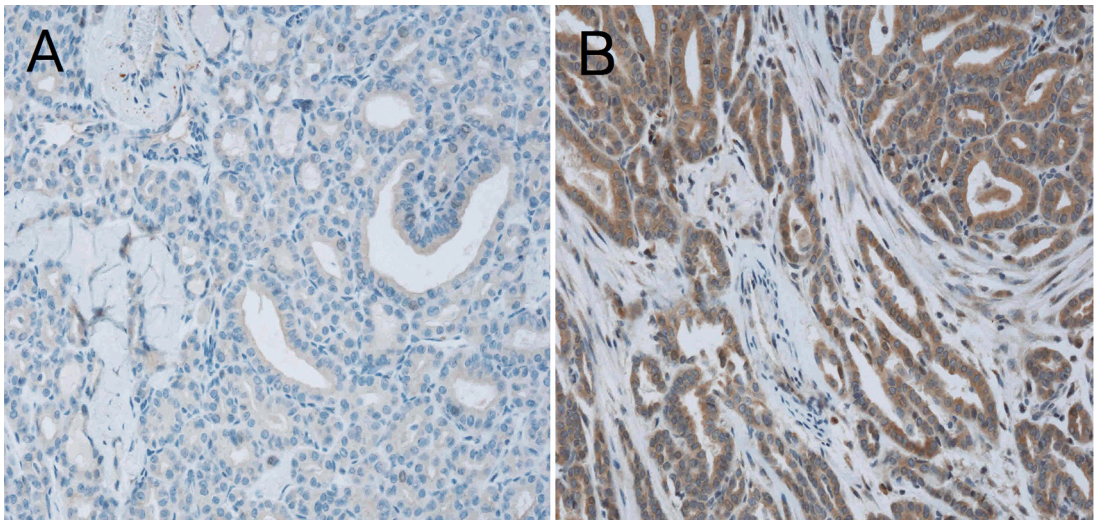

Fig. 1. BRAF V600E immunohistochemistry shows cytoplasmic localization of BRAF V600E protein in PTC. (A) Negative staining; (B) positive staining.

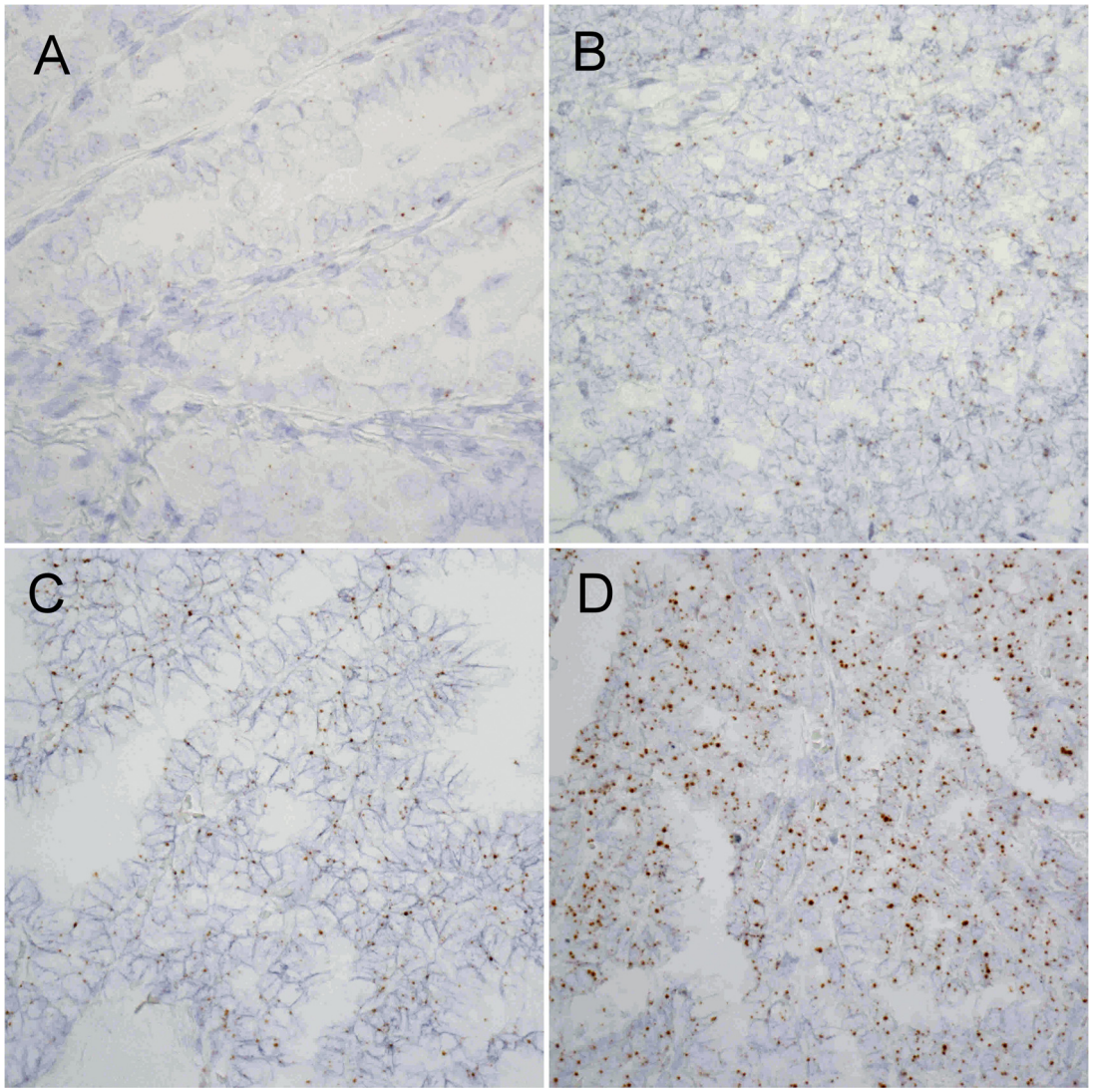

Fig. 2. BRAF mRNA expression level evaluated by RNA ISH in PTC. (A) Score 0; (B) score 1; (C) score 2; (D) score 4; (original magnification 400×).