



Accuracy and Reproducibility of Histologic Features Predictive of *BRAF* V600E Mutation in Papillary Thyroid Carcinoma



Renu K Virk, MD¹, Alexander Finkelstein, MD³, Avinash Prasad, MD⁴, Pei Hui, MD¹, David Chhieng, MD¹, Constantine G Theoharis, MD¹, Joanna Gibson, MD¹, Sanziana A Roman, MD², and Manju L Prasad, MD¹

Departments of ¹Pathology and ²Surgery, Yale School of Medicine, New Haven, CT, Department of ³Pathology, New York University, New York, NY, and Department of ⁴Neurology, Hartford Hospital, Hartford, CT

ABSTRACT

Background: We proposed recently that papillary thyroid carcinomas (PTCs) with *BRAF* V600E mutation are morphologically distinct. Here we investigate the accuracy and interobserver reproducibility of a defined set of histological criteria in predicting *BRAF* V600E mutation.

Design: We created a training set of 5 PTCs with and 5 without *BRAF* V600E mutation. The former group included classic, tall cell or subcapsular sclerosing variants, and showed well-developed nuclear features of PTC, tall or polygonal cells with moderate to abundant eosinophilic cytoplasm (plump pink cells), stromal fibrosis/sclerosis/desmoplasia, infiltrative tumor borders and psammoma bodies. The latter group, in general, included follicular variants with subtle nuclear features of PTC, and lacked most or all of the above mentioned histologic features of mutated PTCs. After self-learning on the training set, two pathologists predicted the presence or absence of *BRAF* V600E mutation in 30 PTCs (test set) using the morphologic criteria learnt from the training set. The predictions were evaluated against *BRAF* V600E mutational analysis by single strand conformation polymorphism on tumor DNA.

Results: Table 1 shows the sensitivity, specificity, accuracy, and positive and negative predictive values of the histologic criteria for predicting *BRAF* V600E mutation by each pathologists. There was "excellent" (kappa 0.795) agreement between the two pathologists for predicting *BRAF* V600E mutation (concordance 27/30; 90%).

Predictive Value of Histologic Features for <i>BRAF</i> V600E Mutation		
	Pathologist 1	Pathologist 2
Sensitivity	15/15 (100%)	14/15 (93%)
Specificity	12/15 (80%)	12/15 (80%)
Accuracy	27/30 (90%)	26/30 (87%)
PPV	15/18 (83%)	14/17 (82%)
NPV	12/15 (80%)	12/16 (75%)

Conclusion: Histology can help predict *BRAF* V600E mutation in papillary thyroid carcinomas with accuracy and good interobserver agreement.

BACKGROUND

BRAF V600E mutation has emerged as a marker of aggressive disease in PTC. Recently, we published that PTC with *BRAF* V600E mutation have distinctive morphology when compared with mutation negative PTC and were more likely to show infiltrative tumor borders, tumor-associated desmoplasia/fibrosis/sclerosis, well-developed nuclear features of PTC, plump-pink cells with moderate to abundant cytoplasm, and psammoma bodies (1). Classic, tall cell and subcapsular sclerosing variants of PTC were more frequently associated with *BRAF* V600E mutation whereas follicular variant is more likely to be negative for the mutation. We investigate the accuracy and interobserver reproducibility of these histological features in predicting *BRAF* V600E mutation.

METHODS

Material for the study comprised of a self-training set and a test set of tumors. The training set had 5 PTCs with and 5 without *BRAF* V600E mutation, with a list of six characteristic histologic features based on our previous experience (1). **Table 1** shows the selected histologic features and their definitions. The test set included 15 PTCs each of *BRAF* V600E mutation positive and negative respectively. Two endocrine pathologists were given the list of histologic features with their definition and the training set (n=10) with known *BRAF* V600E mutation status. After self-training, they were asked to predict the *BRAF* V600E mutation status on the test set (n=30).

Table 1

PTC histologic variants:

a. Classic variant (CV) (Figure 1): Classic papillary architecture with true papillae; may be cystic. If predominantly follicular: focal true papillae should be present.

b. Follicular variant (FV) (Figure 2): Exclusive follicular pattern of growth without any true papillae.

c. Tall cell variant (TCV) (Figure 3): >50% of tumor cells with moderate to abundant eosinophilic cytoplasm and at least twice as tall as wide; well-developed nuclear features with frequent pseudonucleoli; may have trabecular architecture with back to back glands with slit-like spaces.

d. Subcapsular Sclerosing Variant (SSV) (Figure 5): Peripheral subcapsular/capsular location; $\geq 20\%$ of the tumor circumference involving the thyroid capsule, central stellate fibrosis \pm ; infiltrating borders; classic nuclei of PTC, a few papillae \pm .

e. Other: Any tumor with features other than above.

Nuclear features:

a. Well-developed: All 6 nuclear features (enlargement, overlapping, grooves, irregular nuclear membrane, clear/powdery chromatin, and pseudoinclusions) present.

b. Subtle: Pseudoinclusions are absent. Rest of the nuclear features may be focal, subtle or less well developed.

Capsule: Absent or present. If present, intact, interrupted or discontinuous.

Presence of desmoplasia/fibrosis/sclerosis (Figure 4): **Desmoplasia** - proliferating fibroblasts in myxoid stroma; **Fibrosis** - Fibroblastic proliferation; **Sclerosis** - paucicellular, eosinophilic, dense bundles of collagen.

Plump eosinophilic cells: Polygonal tumor cells with moderate to abundant eosinophilic cytoplasm, well developed nuclear features of PTC; however, height of tumor cells is less than twice of width (do not meet the "tall" cell criteria); may be focal, usually present at the periphery in the tumor.

Psammoma body: Concentric lamellated calcifications.

RESULTS

Tables 2 and 3 summarize the results for accuracy and reproducibility between two pathologists.

There was excellent (kappa 0.795) agreement between the two pathologists for predicting *BRAF* V600E mutation (concordance 27/30; 90%).

Table 2. Sensitivity, Specificity, Accuracy, Positive (PPV) and Negative Predictive Value (NPV) for Each Pathologist

	Pathologist 1	Pathologist 2
Sensitivity	15/15 (100%)	14/15 (93%)
Specificity	12/15 (80%)	12/15 (80%)
Accuracy	27/30 (90%)	26/30 (87%)
PPV	15/18 (83%)	14/17 (82%)
NPV	12/15 (80%)	12/16 (75%)

Table 3. Interobserver Agreement Between Two Pathologists in Predicting Presence or Absence of *BRAF* V600E Mutation

Correct <i>BRAF</i> prediction	Overall (includes all mutated and non mutated PTCs)	<i>BRAF</i> V600E positive (n=15)	<i>BRAF</i> V600E negative (n=15)
Both Pathologists	25/30 (83%)	14/15 (93%)	11/15 (73%)
At least one pathologist	28/30 (93%)	15/15 (100%)	13/15 (87%)
None of the pathologists	2/30 (7%)	0	2/15 (13%)

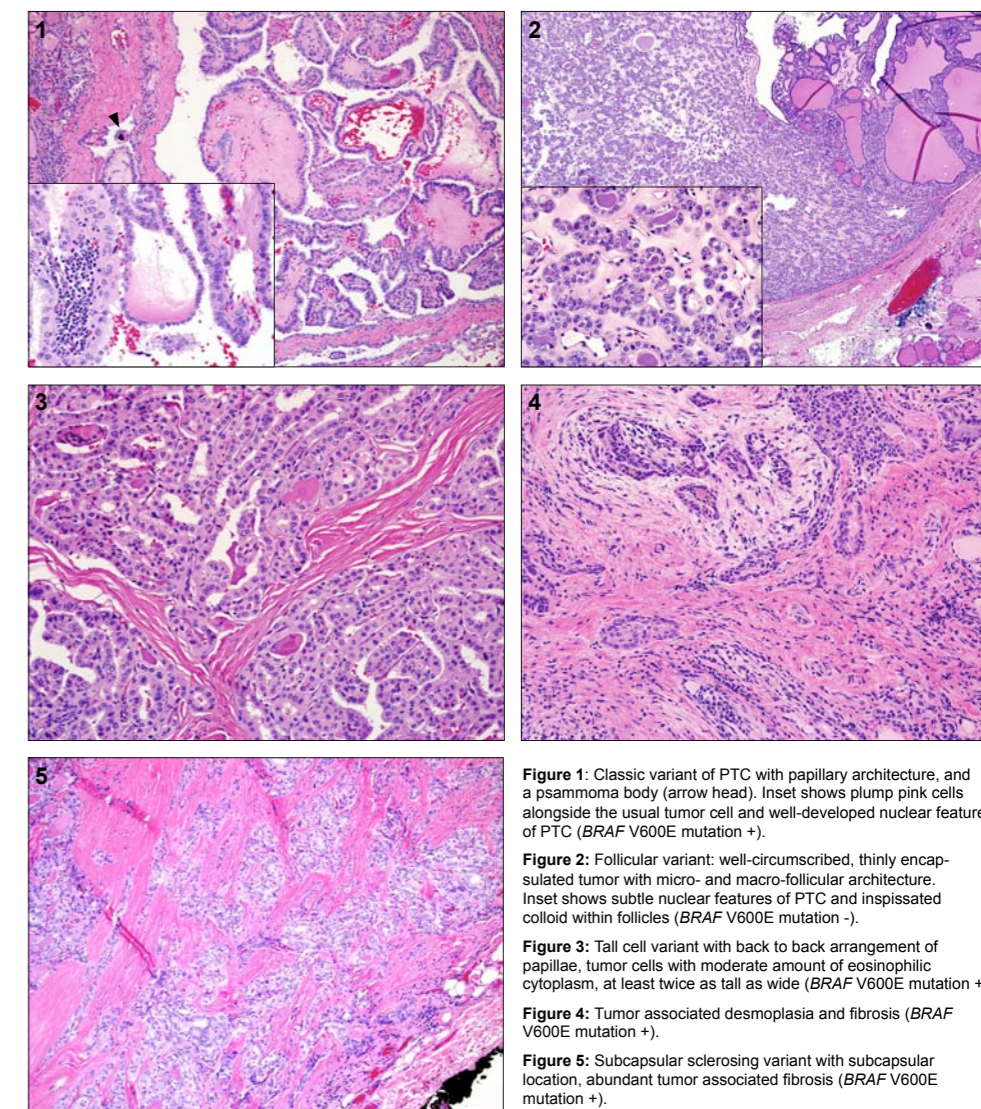


Figure 1: Classic variant of PTC with papillary architecture, and a psammoma body (arrow head). Inset shows plump pink cells alongside the usual tumor cell and well-developed nuclear features of PTC (*BRAF* V600E mutation +).

Figure 2: Follicular variant: well-circumscribed, thinly encapsulated tumor with micro- and macro-follicular architecture. Inset shows subtle nuclear features of PTC and inspissated colloid within follicles (*BRAF* V600E mutation -).

Figure 3: Tall cell variant with back to back arrangement of papillae, tumor cells with moderate amount of eosinophilic cytoplasm, at least twice as tall as wide (*BRAF* V600E mutation +).

Figure 4: Tumor associated desmoplasia and fibrosis (*BRAF* V600E mutation +).

Figure 5: Subcapsular sclerosing variant with subcapsular location, abundant tumor associated fibrosis (*BRAF* V600E mutation +).

CONCLUSIONS

- Histological features can help predict *BRAF* V600E mutation in PTC with accuracy and good interobserver agreement.
- Recognizing the key morphologic features in routine surgical pathology practice may help triage tumors for the more labor intensive and expensive *BRAF* V600E mutational analysis.

REFERENCE

1. Finkelstein A, Levy GH, Hui P, Prasad A, Virk R, Chhieng DC, Carling T, Roman SA, Sosa JA, Udelsman R, Theoharis CG, Prasad ML. Papillary thyroid carcinomas with and without *BRAF* V600E mutations are morphologically distinct. *Histopathology*. 2012 Feb 15 (Epub ahead of print).