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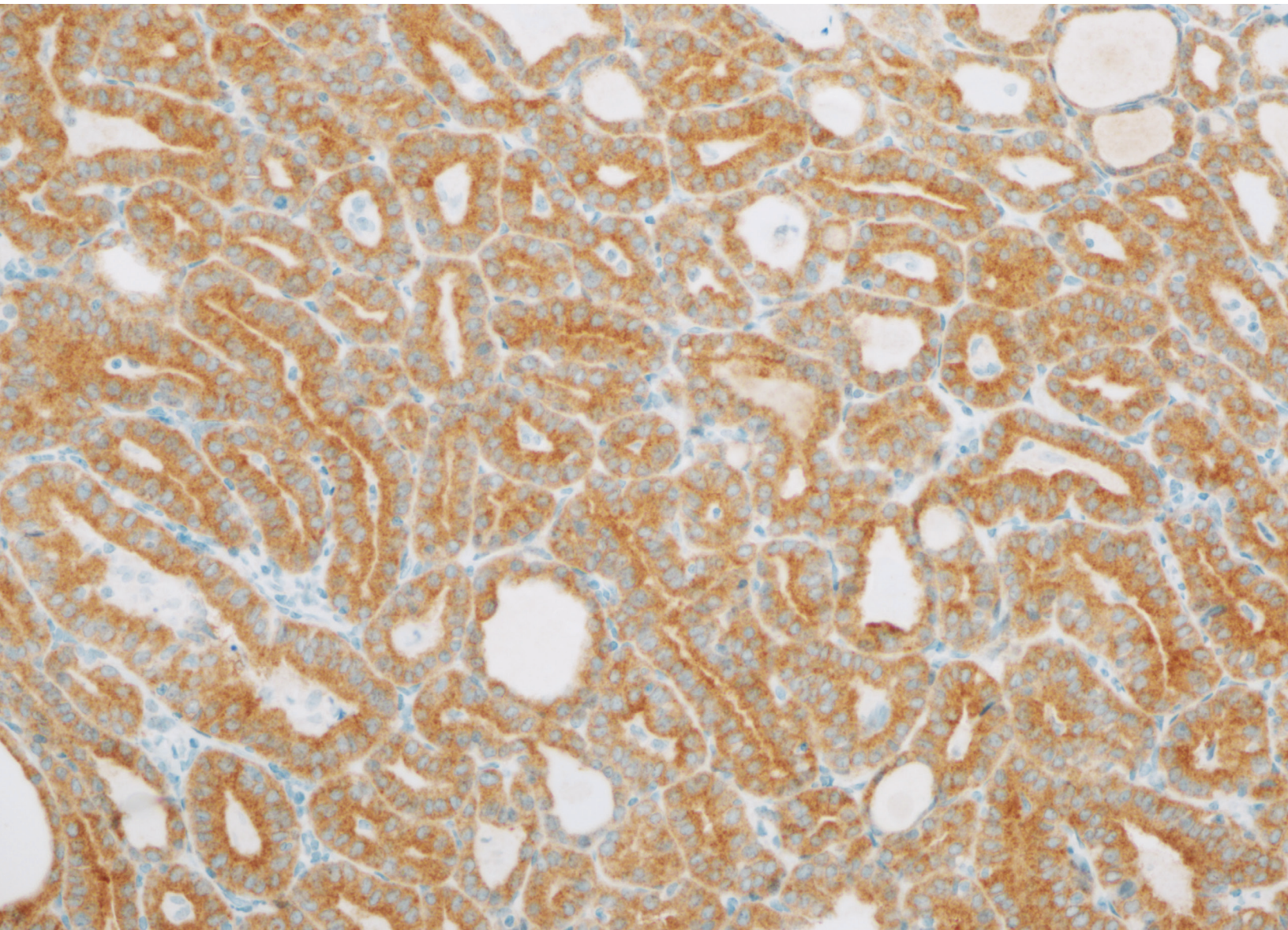

Evaluation of BRAF (V600E) Mutation by Immunohistochemical Staining with anti-BRAF V600E (VE1) Antibody: A Comparison with Sanger Sequencing

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I. Background

BRAF gene located on chromosome 7q34 encodes a cytoplasmic serine-threonine kinase. This kinase acts upstream of the mitogen-activated protein kinase (MAPK) signaling pathway[1]. The oncogenic mutations in the kinase region of BRAF gene result in the production of a protein kinase that is no longer sensitive to the normal inhibitory signals. This mutated BRAF protein constitutively activates the MAPK signaling pathway, which results in increased cell proliferation, apoptosis resistance and tumor progression.[1,2] BRAF mutations are usually found in tumors that are wild-type for KRAS and NRAS, KIT, and other driver mutations.

The majority of the BRAF V600 mutations are V600E, other mutations such as V600K, V600M, V600R, V600D and V600G are less common.[3] This most frequent BRAF mutation is caused by transversion T→A at nucleotide 1799 (T1799A) and results in a substitution of valine (V) to glutamic acid (E) at the position 600 of the amino acid sequence.[1,2] BRAF V600E mutation was detected in approximately 7% of all solid tumors, including 45% of papillary thyroid carcinomas, 40–60% melanomas, 5–15% of colorectal adenocarcinomas, 35% serous ovarian carcinomas, 1–3% of lung cancers, and other cancers.[4] Furthermore, BRAF V600E mutation was found in 100% of hairy cell leukemia.[5]

Currently, the gold standard for the detection of BRAF mutation is direct sequencing of the tumor DNA. However, the cost of molecular analysis makes this screening impractical for some laboratories. BRAF V600E mutation evaluation by Immunohistochemistry (IHC), however, could be added as a diagnostic aid in the identification of BRAF V600E mutation in most laboratories with minimal effort.

The primary goal of this study was to compare a newly developed immunohistochemical method using anti-BRAF V600E (VE1) antibody and the Sanger sequencing of BRAF gene. The anti-BRAF V600E (VE1 clone) is a mutation specific mouse monoclonal antibody that was raised against a synthetic peptide representing the BRAF V600E mutated amino acid sequence from amino acids 596 to 606 (GLATEKSRWSG).[5,6] The samples from patients with colon cancer and thyroid papillary carcinoma were used in these studies.

II. Methods

Tumor specimens

A total of 275 formalin-fixed paraffin embedded (FFPE) tissues were investigated in the present study and included 220 colon cancer cases and 55 thyroid papillary carcinoma cases. The tissue samples were obtained from the tissue bank at Ventana Medical Systems, Inc. (Tucson, USA) and from the University of Melbourne (Australia).

BRAF Sequencing

Genomic DNA was extracted from 20 µm thick sections from 275 FFPE samples (220 colon cancer cases, 55 thyroid papillary carcinoma cases). Mutation analysis across a BRAF V600 target region was performed using PCR amplification followed by Sanger sequencing or SNaPshot™ assay. Primers were designed to amplify region of the exon 15 of the BRAF gene coding sequences at mutation site and a few nucleotides in the intron on both ends. Both forward and reverse strands were sequenced on an Applied Biosystem's 3730xl DNA Analyzer or ABI3130xl Genetic Analyzer and analyzed using Sequence Scanner v1.0 software (Applied Biosystems) or GeneMapper® v4 software (Applied Biosystems).

BRAF Immunohistochemistry

The immunohistochemical method using anti-BRAF V600E (VE1) antibody was developed at Ventana Medical Systems, Inc. Sections (5µm) were cut from the FFPE blocks. The testing was performed on a Benchmark XT IHC/ISH staining instrument with Cell Conditioning 1 for 64 min, pre-peroxidase inhibition and primary antibody incubation for 16 minutes. Final concentration of the Anti-BRAF V600E (VE1) Mouse Monoclonal Primary Antibody was ~12µg/ml. OptiView DAB IHC Detection Kit was used to detect BRAF V600E protein expression. To counterstain tissues the slides were incubated with Hematoxylin II and Bluing Reagent for 4 minutes. To measure the level of non-specific background signal, each tissue was also stained with a mouse monoclonal antibody (MOPC-211) [Negative Control (Monoclonal), Ventana Medical Systems, Inc.]. This antibody is not directed against any known epitope present in human tissue. The slides were scored on the scale 0–3. The strong cytoplasmic staining was scored as a 3, a medium cytoplasmic staining as 2, a weak cytoplasmic staining intensity as 1, the absence of staining was scored as 0. The scores 1–3 represent positive staining, while the score less than 1 is considered a negative staining.

III. Results

All 275 cases of colon cancer and thyroid papillary carcinoma were evaluated for BRAF V600E mutations by immunohistochemistry with anti-BRAF V600E (VE1) mouse monoclonal antibody and by sequencing (220 colon cancer cases, 55 papillary thyroid carcinoma cases). Our data demonstrated concordance between IHC and DNA sequencing in 266 out of total 275 cases (96.7%).

The 98.2% of cases (162/165) that were negative for BRAF V600E mutation by IHC were confirmed to be BRAF V600E negative by sequencing (Figure 1). Three cases out of 165 total cases were positive for the BRAF V600E mutation by sequencing, but negative by IHC. All of these discordant cases were colon cancer tissues. Of the 110 cases that were IHC positive for BRAF V600E, 104 cases were confirmed by sequencing (94.5%) and 6 cases were discordant. Of these 6 discordant cases, two cases that were negative by sequencing were weakly positive by IHC (score 1, two colon cancer cases), three cases showed moderate BRAF V600E (VE1) IHC staining (score 2, two thyroid carcinomas and one colon cancer) and one case contained a different mutation at codon 600 (V600K, colon cancer). The summary of the data are shown in Table 1. Figure 2 shows the thyroid cancer and colon cancer cases positive/negative for BRAF V600E mutation by Sanger sequencing stained with anti-BRAF V600E (VE1) antibody, mouse negative control, and H&E. Overall, there was high concordance between IHC and DNA sequencing: negative predictive value was 98.2%, positive predictive value was 94.5%, sensitivity was 97.2%, and specificity was 96.4%. The overall percentage agreement across all cases was 96.7% (266/275 cases).

IV. Conclusion

The data from this study demonstrated a high concordance between Sanger sequencing and IHC using anti-BRAF V600E (VE1) antibody for detection of the BRAF V600E mutation in colorectal cancer and thyroid cancer FFPE tissues.

Table 1. Summary of the data

IHC and sequencing data for the colon cancer cases

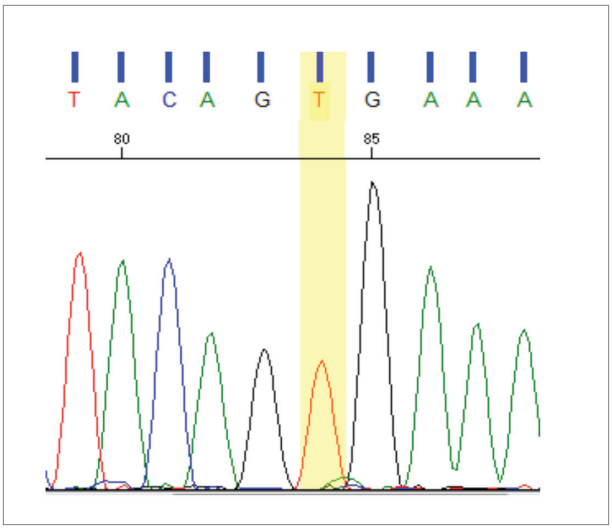
BRAF V600E Results (N=220)	BRAF V600E Sequence positive	BRAF V600E Sequence negative
IHC positive	65	4
IHC negative	3	148

IHC and sequencing data for the papillary thyroid carcinoma cases

BRAF V600E Results (N=55)	BRAF V600E Sequence positive	BRAF V600E Sequence negative
IHC positive	39	2
IHC negative	0	14

IHC and sequencing data for all cases

BRAF V600E Results (N=275)	BRAF V600E Sequence positive	BRAF V600E Sequence negative
IHC positive	104	6
IHC negative	3	162



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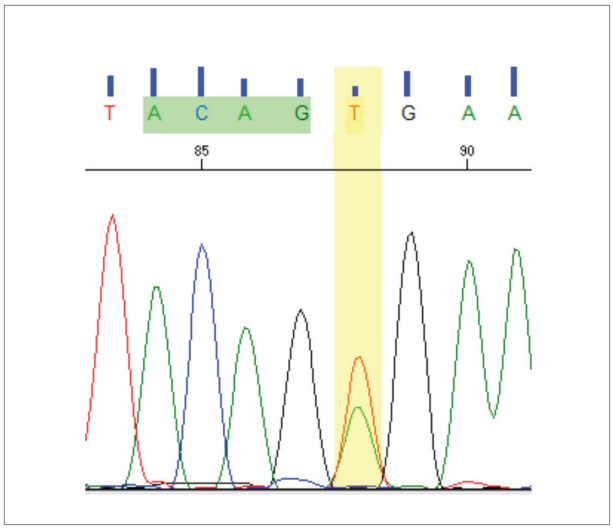

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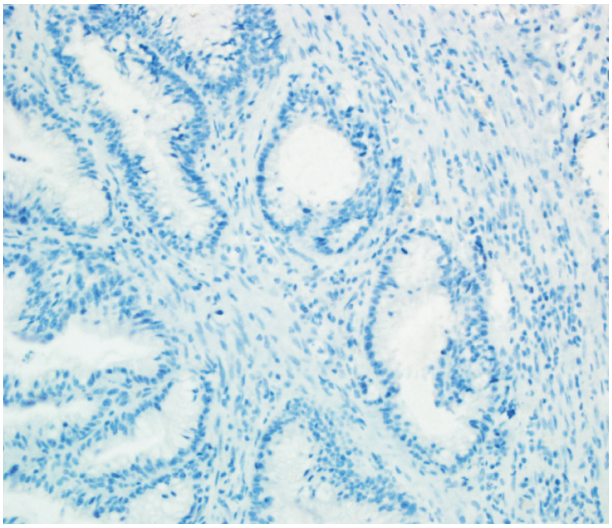

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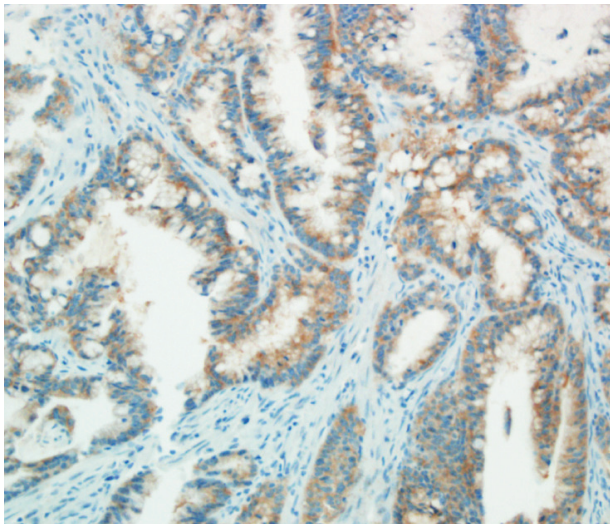

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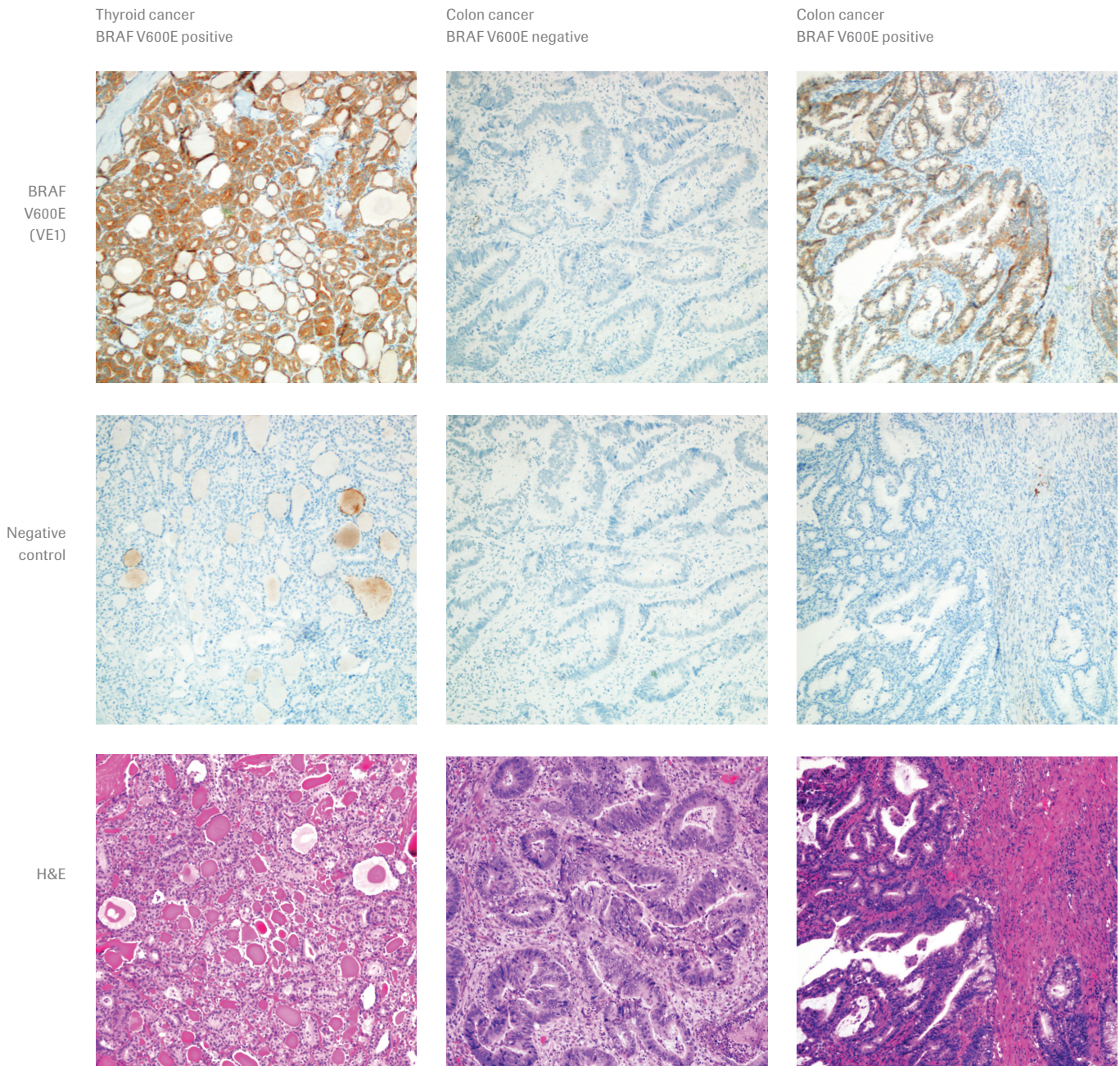
VI. Figure 1

Mutation analysis of BRAF gene in colon cancer tissues. An example of sequencing electropherogram with BRAF codon wild type (A) and V600E heterozygous (B). The corresponding images of tissues stained with anti-BRAF V600E (VE1) mouse monoclonal antibody are included (40x). Brown color indicates a positive staining with anti-BRAF V600E (VE1) antibody.



VII. Figure 2.

The images of colon cancer and thyroid cancer tissues stained with anti-BRAF V600E (VE1) mouse monoclonal antibody, negative mouse monoclonal control and H&E (10X).

V. References

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