BRAF Mutation in Papillary Thyroid Carcinoma

*Yoram Cohen, Mingzhao Xing,
Elizabeth Mambo, Zhongmin Guo,
Guogun Wu, Barry Trink, Uziel
Beller, William H. Westra, Paul W.
Ladenson, David Sidransky*

The BRAF gene has been found to be activated by mutation in human cancers, predominantly in malignant melanoma. We tested 476 primary tumors, including 214 lung, 126 head and neck, 54 thyroid, 27 bladder, 38 cervical, and 17 prostate cancers, for the BRAF T1796A mutation by polymerase chain reaction (PCR)–restriction enzyme analysis of BRAF exon 15. In 24 (69%) of the 35 papillary thyroid carcinomas examined, we found a missense thymine (T)→adenine (A) transversion at nucleotide 1796 in the BRAF gene (T1796A). The T1796A mutation was detected in four lung cancers and in six head and neck cancers but not in bladder, cervical, or prostate cancers. Our data suggest that activating BRAF mutations may be an important event in the development of papillary thyroid cancer. [J Natl Cancer Inst 2003;95:625–7]

The RAF proteins are highly conserved serine/threonine protein kinases that have an important role in cell proliferation, differentiation, and programmed cell death *(1)*. The RAF proteins activate mitogen-activated protein kinase kinase (MEK), which in turn activates the mitogen-activated protein kinase (MAPK) pathway *(2)*. Inappropriate and/or continuous activation of this pathway provides a potent promitogenic force resulting in abnormal proliferation and differentiation in many human cancers *(3)*. Davies et al. *(4)* reported that BRAF is frequently mutated in a variety of human tumors, especially in malignant melanoma and colon carcinoma. The most common reported mutation was a missense thymine (T)→adenine (A) transversion at nucleotide 1796 (T1796A; amino acid change in the BRAF protein = Val^{599}→Glu^{599}) observed in 80% of the malignant melanoma tumors. Functional analysis revealed that this transversion was the only detected mutation that caused constitutive activation of BRAF kinase activity, independent of RAS activation, by converting BRAF into a dominant transforming protein *(4)*. In this study, we investigated the frequency of BRAF T1796A mutation and further elucidated the importance of this mutation in various primary human tumors.

We screened 476 primary tumors, including 214 lung, 126 head and neck, 54 thyroid, 27 bladder, 38 cervical, and 17 prostate cancers for the BRAF T1796A mutation by polymerase chain reaction (PCR)–restriction enzyme analysis. The samples were obtained from patients treated at The Johns Hopkins Medical

Affiliations of authors: Y. Cohen, E. Mambo, Z. Guo, G. Wu, B. Trink, W. H. Westra, D. Sidransky (Division of Head and Neck Cancer Research, Department of Otolaryngology–Head and Neck Surgery), M. Xing, P. W. Ladenson (Division of Endocrinology and Metabolism), The Johns Hopkins University School of Medicine, Baltimore, MD; U. Beller, Department of Obstetrics and Gynecology, Shaare Zedek Medical Center, Ben-Gurion University of the Negev, Jerusalem, Israel.

Correspondence to: David Sidransky, M.D., Division of Head and Neck Cancer Research, Department of Otolaryngology–Head and Neck Surgery, The Johns Hopkins University School of Medicine, 720 Rutland Ave., Ross Bldg. 818, Baltimore, MD 21205-2196 (e-mail: dsidrans@jhmi.edu).

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Institutions (Baltimore, MD) and were collected in our tissue bank. Written informed consent was obtained from each patient in accordance with the institutional review board at The Johns Hopkins Medical Institutions. PCR amplification of exon 15 followed by digestion of the exon 15 products by the restriction endonuclease *Tsp*RI identified the BRAF T1796A mutation. *Tsp*RI digestion of the PCR fragment yielded three major bands at 125 base pairs (bp), 87 bp, and 12 bp in the wild-type allele. The T1796A mutation abolished the restriction site, resulting in a prominent 212-bp band from the mutant allele and residual bands from the normal allele (Fig. 1, A). Reamplification of BRAF exon 15 followed by direct manual sequencing of five samples validated the results of the *Tsp*RI assay (Fig. 1, B). As positive controls for the BRAF T1796A mutation, we used melanoma cell lines HTB71, HTB72, and A2058; for negative controls, we used cell lines ME180 (cervical cancer) and HCT116 (colorectal carcinoma).

The BRAF T1796A mutation was identified in 24 (69%) of 35 papillary thyroid carcinomas (Table 1), six (4.8%) of 126 head and neck cancers, and four (1.9%) of 214 lung cancers. Moreover, we analyzed nine common thyroid cell lines (KAK1, KAT5, KAT7, KAT10, DRO, ARO, MRO 87–1, WRO–821, and C643) and found the same BRAF mutation in six (67%) of the nine lines. We also completely sequenced exons 11

Table 1. BRAF mutations in primary human tumors and thyroid cell lines

Tumor type	No. of samples screened	No. of T1796A mutations (%)
Thyroid		
Papillary cancer	35	24 (69)
Follicular cancer	13	0 (0)
Hürthle cancer	3	0 (0)
Medullary cancer	3	0 (0)
Benign tumors	20	0 (0)
Cell lines*	9	6 (67)
Others		
Lung cancer†	214	4 (1.9)
Head and neck cancer‡	126	6 (4.8)
Cervical cancer§	38	0 (0)
Prostate cancer	17	0 (0)
Bladder cancer	27	0 (0)
Total	505	40 (8)

*Including KAK1, KAT5, KAT7, KAT10, DRO, ARO, MRO 87-1, WRO-821, and C643 cell lines.

†Four of 116 lung adenocarcinomas.

‡Six of 77 head and neck squamous cell carcinomas.

§Including 22 squamous cell carcinomas and 16 adenocarcinomas of the uterine cervix.

and 15 in all T1796A-negative papillary thyroid cancers and in 10 T1796A-positive tumors but did not identify additional BRAF mutations. We did not identify any mutations in bladder, cervical, and prostate primary tumors, and no mutation was identified in biopsy samples from 20 patients with benign thyroid conditions (nodular goiter, follicular adenoma, atypical follicular adenoma, and adenomatous hyperplasia), 13 patients with follicular thyroid carcinoma, three patients with medullary thyroid carcinoma, and three patients with Hürthle cell carcinoma.

Papillary and follicular thyroid carcinomas originate from thyroid follicular epithelial cells. To date, oncogenic mutations in RAS and RET/PTC rearrangements have been observed in follicular thyroid carcinoma and papillary thyroid carcinomas, respectively *(5,6)*. RAS mutations are common in follicular thyroid cancers, reaching 50% in some studies, but are less common (5%–20%) in papillary thyroid tumors *(5)*. Our observation of a high frequency of BRAF-activating mutations in papillary thyroid carcinoma suggests that BRAF activation and, in turn, activation of the RAF/MEK/MAPK signaling pathway, is a common biologic mechanism in the development of human papillary thyroid carcinoma. This observation is also consistent with the reported inverse association between the presence of BRAF and RAS mutations in other cancer types *(4,7,8)*. The relationship between BRAF T1796A mutation and RET/PTC rearrangements remains to be explored.

The importance of the RAS pathway in thyroid cancers is further suggested by the common presence of RET mutations in medullary thyroid tumors and their transforming effect through activation of the RAS/RAF/MEK pathway *(9)*. Moreover, activation of the RAS/RAF/MEK/MAPK pathway is known to induce genomic instability in thyroid PCCL-3 cells *(10)*, and inhibition of the MAPK pathway has led to decreased cellular proliferation of human thyroid cancer cell lines *(11)*. Thus, activation at various points in the RAS/RAF/MEK/MAPK pathway is a key event in the most common type of malignant thyroid tumor. The high frequency of BRAF mutations in melanoma and papillary thyroid carcinoma suggests that inhibition of BRAF activity by the newly developed RAF kinase inhibitors *(12)* may offer a new strategy in the treatment of these tumors. Our results have identified the BRAF T1796A mutation and likely activation of the RAF/MEK/MAPK signaling pathway as a major mechanism in the development of primary papillary thyroid carcinoma.

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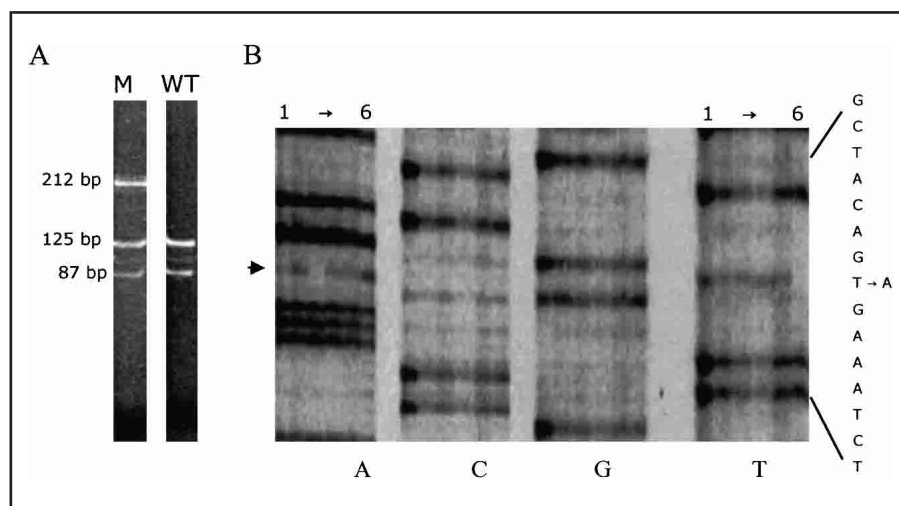
Fig. 1. *Tsp*RI restriction enzyme analysis (**A**) and exon 15 sequence analysis (**B**) of BRAF. **A)** Restriction pattern of the T1796A mutation. **Lane M** = mutant T299; **lane WT** = wild-type T486. **B)** Manual DNA sequence gel of exon 15 from papillary thyroid samples harboring the T1796A mutation (**arrowhead**). **Lane 1** = T569; **lane 2** = T203; **lane 3** = a thyroid adenomatous hyperplasia (T530) negative for the T1796A mutation; **lane 4** = T228; **lane 5** = T171; and **lane 6** = melanoma cell line HTB72 that carries a homozygous T1796A mutation. The sequence is to the right.

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