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Mutations of the BRAF Gene in Papillary Thyroid Carcinoma in a Korean Population

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The B-type Raf kinase (BRAF) protein is a serine/threonine kinase that has an important role in cellular proliferation, differentiation, and programmed cell death. The BRAF gene has been recently found to be mutated in human carcinomas, predominantly in malignant melanoma. The aim of this study was to investigate the frequency of the BRAF mutation in papillary thyroid carcinoma (PTC) of Koreans through direct DNA sequencing of the polymerase chain reaction (PCR)-amplified exon 15 with clinicopathological features. Seventy paraffin-embedded conventional papillary carcinomas in the thyroid gland were evaluated. The BRAF missense mutation at V599E was found in 58 of 70 PTCs (83%). The frequency of our series was much higher than the frequencies of other PTC series (36 - 69%). The frequency of nodal metastasis was also significantly higher in the BRAF mutation group (p= 0.048). These results suggest that the BRAF mutation is involved in the carcinogenesis in most conventional PTCs, especially those occurring in Koreans, and this is a potentially valuable marker for the evaluation of prognosis of patients with PTC. These findings support the specific inhibitors of BRAF being promising targets for the disease outcome.

Key Words: BRAF protein, mutation, papillary carcinoma, thyroid gland

INTRODUCTION

Thyroid carcinomas represent the most common form of malignant endocrine neoplasm. Tumors derived from thyroid epithelial cells are histologically classified as papillary carcinoma, follicular carcinoma, and aggressive anaplastic carcinoma.[1] Medullary thyroid carcinoma is derived from calcitonin-secreting parafollicular C cells. In particular, papillary carcinoma is the most common histologic class of thyroid carcinoma.

Genetic alterations are involved in thyroid carcinogenesis. Recently, activating mutation in the BRAF has been detected at a high frequency in papillary thyroid carcinoma (PTC).[2-5] Raf kinase is a component of the Ras-Raf-MEK-ERK-MAP kinase pathway involved in cellular functions, such as cellular proliferation, differentiation, and programmed cell death.[6] There are three mammalian RAF genes: A-RAF, B-RAF, and C-RAF, each encoding for cytoplasmic serine/threonine kinases.[7,8] Among the RAF genes, BRAF is the strongest activator of this signal pathway, which is located on chromosome.[7,9,10] There are two mutation hot spots in exon 11 and 15, respectively. A thymine to adenine transversion at nucleotide 1796 in exon 15 is the most commonly reported mutation, and this produces the conversion of valine 599 to glutamic acid (V599E).[11] This BRAF mutation can be found in a mutually exclusive relationship with RAS mutations while elevating kinase activity.[11-13] In the present study, we analyzed 70 cases of PTC for the BRAF mutation and detected 58 cases with the BRAF missense mutation.

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MATERIALS AND METHODS

Tissue specimens

The samples were selected from 70 paraffin

embedded thyroidectomy specimens filed at the Department of Pathology, Eulji University Hospital (Daejeon, Korea) and Chungnam National University Hospital (Daejeon, Korea) between 2000 and 2003. Each case was subjected to histological evaluation in order to confirm the diagnosis of conventional PTC, which excluded other variants while relevant clinical and pathological information was retrospectively abstracted from the patient records.

DNA isolation and sequencing

Using hematoxylin and eosin (H & E) stained sections as a guide, PTC areas with papillary or mixed follicular-papillary patterns of growth were marked. Each marked area was prepared from four to five 10 μm-thick sections by a microtome and was transferred into an Eppendorf tube. Microdissected specimens from the paraffin-embedded blocks were subjected to treatment with xylene in order to remove the paraffin. All samples were subjected to digestion with 1.0% sodium dodecyl sulfate and 0.5 mg/mL proteinase K at 55℃ for 16 hours. DNA was isolated from the digested tissue through phenol-chloroform extraction and was precipitated with ethanol in the presence of sodium acetate. The BRAF exon 15 was amplified by the polymerase chain reaction (PCR) using the following primers: forward, 5′-TCATAATGCTTGCTCTGATAGGA-3′, reverse, 5′-GGCCAAAAATTTAATCAGTGGA-3′. PCRs were performed using the following amplification profile: initial denaturation at 95℃ for 5 minutes, followed by 40 cycles for denaturation at 94℃ for 30 seconds, annealing at 58℃ for 30 seconds, and elongation at 72℃ for 30 seconds. After the last cycle, a final extension at 70℃ for 10 minutes was performed. The amplified products were purified by the PCR Purification Kit (Qiagen GmbH, Hilden, Germany). Then, the samples were analyzed by an ABI PRISM 310 automatic sequencer (PE-Applied Biosystems, Foster City, CA, USA).

Data analysis

The results were expressed as percentages and raw numbers. Statistical analysis was performed using the Chi-Square test and the ANOVA test. Any two values were considered to be significantly different when $p < 0.05$.

RESULTS

Seventy PTC were studied for the BRAF mutation, and analyzed the correlation between the BRAF mutation and clinical-pathological features. Using the direct sequencing of DNA, mutations were found in 58 (83%) PTCs. All mutations were involved in a T→A transversion at nucleotide 1796, and all mutations were heterozygous in the nucleotide sequence (Fig. 1). There was a significant correlation between the BRAF mutation and

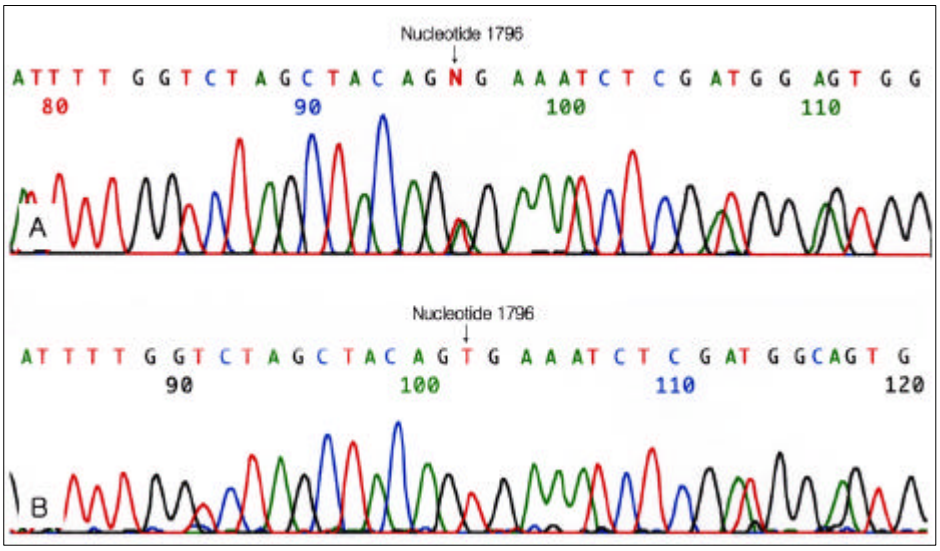

Fig. 1. Representative sequence chromatographs from BRAF exon 15 showing heterozygous mutation (A) and wild-type (B) in papillary thyroid carcinomas.

Table 1. Correlation between the BRAF Mutation and Clinicopathologic Features in Papillary Thyroid Carcinomas

	BRAF-positive N=58 (83%)	BRAF-negative N=12 (17%)	*p*-value
Age, average ± SD (yr)	47.3 ± 14.46	39.9 ± 11.41	0.102
Female : Male ratio	48 : 10	11 : 1	0.675
Tumor size, average ± SD (cm)	2.4 ± 1.14	2.4 ± 0.74	0.948
Thyroid capsular invasion	28 (48.3%)	7 (58.3%)	0.795
Lymph node metastasis	39 (67.2%)	4 (33.3%)	0.048

the nodal metastasis (p=0.048; Chi-Square test), whereas no significant correlation was found in tumor size (p=0.948; ANOVA test), age (p=0.102; ANOVA test), gender (p=0.675; Chi-Square test), and thyroid capsular invasion (p=0.795; Chi-Square test) (Table 1).

DISCUSSION

The BRAF T1796A mutation has been reported in various human carcinomas, and it has especially been reported in more than 65% of malignant melanomas.[11] This mutation has also been studied in several PTC series, all with a high prevalence.[2,4,5] In two recent studies, the BRAF mutation was found in 28 of 53 (53%)[14] and four of five (80%) conventional PTCs.[15] Also in this study, conventional PTC was studied, and a higher frequency of this specific mutation (83%) was detected in our samples. The higher frequency of this mutation that was found in the present study was compatible with the results of previous studies,[2-5,14,15] which confirmed that the mutation is a common genetic event in PTC in Koreans. This result would be of particular importance for the BRAF mutation that is inherently or geographically associated with PTC.

A variety of abnormal genetic alterations have been identified to be involved in thyroid carcinogenesis. RET/PTC and TRK rearrangement have been found in PTC and is known as the earliest identified oncogenic alterations of PTC.[16,17] Activating point mutations of the RAS genes have been frequently identified in follicular thyroid carcinomas.[18] RET mutation leads to familial medullary thyroid carcinoma and some of the sporadic medullary thyroid carcinomas.[19] Mutations of the tumor suppressor gene, p53, seem to be an important mechanism for the dedifferentiation process of thyroid carcinoma.[20] The BRAF T1796A mutation in thyroid tumors was restricted to PTC in initial reports because no mutation was found in other thyroid tumors, including follicular carcinoma, medullary carcinoma and benign adenomas, and in benign hyperplasia. Recently, some authors have reported that the BRAF mutation also occurs in poorly differentiated or anaplastic thyroid carcinoma.[21-23] In a series of 45 anaplastic or poorly differentiated carcinomas, the BRAF mutation is found in five (11%).[23] A papillary carcinoma component existed in all five BRAF mutations. This is consistent with the unique identification of the BRAF mutation in PTC, and offers that the progression from PTC to poorly differentiated and anaplastic carcinoma may have been associated with the BRAF mutation.

In this study, we evaluated the frequency of the BRAF mutation in conventional PTC of Koreans and detected a higher frequency than the frequencies that had been reported in other series.[2-5,14,15] Of the 70 cases of PTC, 58 (83%) had heterozygous mutations in the BRAF gene at codon 599 (V599E). There was also a significant correlation between the BRAF mutation and the nodal metastasis. Our result indicates that the BRAF mutation plays an important role in the carcinogenesis of PTC, which is characterized as advanced carcinomas with nodal metastasis. These findings suggest that the BRAF mutation provide a genetic, diagnostic, and prognostic marker, and a target for exploring cancer therapeutics to treat PTC, but our series is too restricted within a narrow histologic limit and

conventional type of PTC.

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