High Prevalence of BRAF Gene Mutation in Papillary Thyroid Carcinomas and Thyroid Tumor Cell Lines

Xiulong Xu,2 Roderick M. Quiros, Paolo Gattuso, Kenneth B. Ain, and Richard A. Prinz

INTRODUCTION

Thyroid cancer is the most common endocrine neoplasm in the United States. Tumors derived from thyroid epithelial cells display diverse neoplastic phenotypes, including benign follicular adenomas, well-differentiated papillary and follicular carcinomas, and aggressive anaplastic carcinomas (1). Both genetic and epigenetic alterations are involved in the initiation and progression of thyroid carcinomas. Mutation of the thyroid hormone receptor gene (2) or proto-oncogenes (3–5) and GSP (3–5) are frequently involved in PTC. The RAS-RAF-MEK-ERK-MAP kinase pathway mediates the cellular response to extracellular signals that regulate cell proliferation, differentiation, and apoptosis. Mutation of the RET proto-oncogene occurs in various thyroid neoplasms such as papillary thyroid carcinomas (PTCs), follicular thyroid adenomas and carcinomas. A second genetic alteration frequently involved in PTC is RET/PTC rearrangements. Recent studies have shown that BRAF, which is a downstream signaling molecule of RET and RAS, is frequently mutated in melanomas. This study tests whether BRAF is also mutated in thyroid tumors and cell lines. We analyzed BRAF gene mutation at codon 599 in thyroid tumors using mutant-allele-specific PCR and in 10 thyroid tumor cell lines by DNA sequencing of the PCR-amplified exon 15. We found that BRAF was mutated in 8 of 10 thyroid tumor cell lines, including 2 of 2 papillary carcinoma cell lines, 4 of 5 anaplastic carcinoma cell lines, 1 of 2 follicular carcinoma cell lines, and 1 follicular adenoma cell line. BRAF mutation at codon 599 was detected in 21 of 56 PTC (38%) but not in 18 follicular adenomas and 6 goiters. BRAF mutation occurred in PTC at a significantly higher frequency in male patients than in female patients. To test whether BRAF mutation may cooperate with RET/PTC rearrangements in the oncogenesis of PTC, we tested whether BRAF-mutated PTCs were also positive for RET/PTC rearrangements. Immunohistochemical staining was conducted to evaluate RET/PTC rearrangements by using two different anti-RET antibodies. Surprisingly, we found that a large number of BRAF-mutated PTCs (8 of 21) also expressed RET, indicating that the RET proto-oncogene is rearranged in these BRAF-mutated PTCs. These observations suggest that mutated BRAF gene may cooperate with RET/PTC to induce the oncogenesis of PTC.

MATERIALS AND METHODS

Tumor Specimens and Patient Information. Paraffin-embedded tumor blocks from thyroidectomy specimens of patients with thyroid neoplasms were retrieved for analysis of BRAF gene mutation upon approval by the Institutional Review Board of Rush Presbyterian St. Luke’s Medical Center. A total of 80 specimens, all with adequate clinical and pathological information, was studied. These included 56 papillary carcinomas, 18 follicular adenomas, and 6 benign nodular goiters. The presence of metastases was determined by reviewing the patients’ medical records, pathology reports, and subsequent clinical courses. Patients were staged using the tumor-node-metastasis system and classified according to the presence of extrathyroidal extension, cervical nodes, and distant metastases. Nontumor tissue blocks (lymph nodes, parathyroid, and thyroid) from 20 patients with BRAF-mutated PTC were sectioned and used for genomic DNA extraction followed by PCR analysis of BRAF mutation.

Cell Lines. Ten thyroid tumor cell lines were used in this study (Table 1). One follicular adenoma (KAK-1), 1 papillary carcinoma (KAT-10), and 3 anaplastic (KAT-4, KAT-18, and SW1736) carcinoma cell lines were originally established in Dr. Ain’s laboratory and have been described previously (33). The other 5 thyroid tumor cell lines, including 1 papillary (NPA87), 2 follicular (WRO82 and MRO87), and 2 anaplastic carcinomas (ARO81 and DRO90), were kindly provided by Dr. Guy J. F. Juillard at University of California at Los Angeles. All thyroid tumor cell lines were grown in complete RPMI 1640 containing 10% fetal bovine serum.

Received 1/6/03; accepted 5/22/03.

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2 This work was supported, in part, by a grant from Thyroid Research Advisory Council, NIH P20 Grant C06 RR15166, and the Department of General Surgery at Chicago, IL 60612.

3 The abbreviations used are: PTC, papillary thyroid carcinoma; FTA, follicular thyroid adenoma; IHC, immunohistochemistry; MASA, mutant-allele-specific amplification; mAb, monoclonal antibody.
BRAF Gene and Exons 1 and 2 of the NRAS Gene. DNA concentration was quantitated with a spectrophotometer. Genomic DNA extraction from thyroid tumor cell lines and blood followed by proteinase K digestion at 56°C overnight and DNA extraction with phenol. Genomic DNA was precipitated with ethanol in the presence of glycogen. Tumor areas were marked and matched with a dewaxed but unstained set of slides. Tumor areas were examined by a pathologist with expertise in thyroid disease. Tumor areas were matched with a dewaxed but unstained set of slides. Tumor areas were marked and matched with a dewaxed but unstained set of slides. Tumor areas were dissected from unstained slides and transferred into an Eppendorf tube followed by proteinase K digestion at 56°C overnight and DNA extraction with phenol. Genomic DNA was precipitated with ethanol in the presence of glycogen. Genomic DNA extraction from thyroid tumor cell lines and blood of one patient was conducted by using Qiagen genomic DNA extraction kit following the manufacturer’s instructions. DNA concentration was quantitated by A260 absorbance in a spectrophotometer. Exon 15 of the BRAF gene was amplified with two primers in the introns flanking it. The sequence of the forward primer is 5'-GATTTTGGTCCTAGCTACAGT-3' and a reverse primer (5'-GGGCCTCACCTCTATGGTG-3') was designed with a reverse primer (5'-GGGCCTCACCTCTATGGTG-3'). The PCR reaction was set with an initial denaturation of 2 min at 94°C and subsequent denaturation for 30 s at 94°C, annealing for 45 s at 55°C, and extension for 45 s at 72°C. Thirty-five cycles were used to apply the PCR product with the expected size of 215 bp. Exon 1 of the NRAS gene was similarly amplified with a forward primer (5'-GACTGATGACCAACTGTTGGG-3') and a reverse primer (5'-GGGGCTCACCTCTATGGTG-3'); exon 2 of the NRAS gene was amplified with a forward primer (5'-GGTGAAACCTGTTGGTGG-3') and a reverse primer (5'-ATACACAGAGAAGCCTGG-3'). BRAF and NRAS mutations were detected by direct sequencing of the PCR products at the CRC-DNA sequencing facility of the University of Chicago.

**RESULTS**

BRAF Gene Mutation in Thyroid Tumor Cell Lines. We first analyzed BRAF gene mutation in 10 thyroid tumor cell lines by sequencing the PCR-amplified exon 15 of the BRAF gene. As shown in Fig. 1 and Table 1, 2 anaplastic thyroid carcinoma cell lines, AR081 and DRO90, harbored a homozygous BRAF mutation at the nucleotide position 1796 where an adenosine was replaced by a thymidine, leading to the substitution of valine at 599 with glutamic acid. In the other 6 thyroid tumor cell lines (Table 1; 1 follicular adenoma, 2 anaplastic, 2 papillary, and 1 follicular carcinoma cell lines), only one allele of the BRAF gene was mutated at this site (Fig. 1 and Table 1). No mutation at T1796 was detected in an anaplastic cancer cell line (KAT18) and a follicular carcinoma cell line (WRO82; Fig. 1 and Table 1). No other point mutation within exon 15 was detected. Overall, our results show that 8 of 10 thyroid tumor cell lines originally established in two laboratories (Dr. Guy J. F. Juillard, University of California at Los Angeles; Dr. Kenneth B. Ain, University of Kentucky Medical Center) contained BRAF gene mutation at the T1796 nucleotide position. This suggests that BRAF may be mutated at high frequency in thyroid neoplasms.

Somatic BRAF Mutation in Thyroid Neoplasms. We sought to analyze BRAF mutation in benign follicular adenomas and papillary thyroid carcinomas using PCR-based MASA to detect the hot-spot BRAF mutation at the T1796 site. A PCR primer with two mismatches at the 3'-end was used to amplify mutant BRAF, whereas a control primer derived from wild-type BRAF gene that amplifies both wild-type and T1796 mutant BRAF was used as a control. We first tested the feasibility of this method to detect BRAF mutation at T1796 site in well-characterized thyroid tumor cell lines. As shown in Fig. 1B, the BRAF exon 15 was amplified by using the mismatched primer in 4 BRAF-mutant thyroid tumor cell lines (DRO90 and AR081 with homozygous mutation; NPA87 and MRO87 with heterozygous mutation) but not in 2 wild-type cell lines (WRO82 and KAT18). A PCR reaction that amplifies BRAF exon 15 was included as a positive control. BRAF exon 15 was amplified in all samples. These results suggest that screening of BRAF mutation using this MASA-PCR is specific, and this approach can be used to rapidly screen BRAF mutation at codon 599 in a large number of tumor samples.

We next analyzed BRAF mutation in microdissected thyroid tumor tissues by using MASA. As shown in Fig. 2A, BRAF mutation at

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Table 1  **BRAF gene mutation in a panel of thyroid tumor cell lines**

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Tumor type</th>
<th>Passages</th>
<th>Metastasis</th>
<th>BRAF mutation</th>
</tr>
</thead>
<tbody>
<tr>
<td>KAK-1</td>
<td>FTA</td>
<td>50</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>KAT-ATC</td>
<td>ATC</td>
<td>16</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>KAT-18</td>
<td>ATC</td>
<td>35</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>SW1736</td>
<td>ATC</td>
<td>&gt;9</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>ARO81</td>
<td>ATC</td>
<td>65</td>
<td>Yes</td>
<td>Yes*</td>
</tr>
<tr>
<td>DRO90</td>
<td>ATC</td>
<td>35</td>
<td>Yes</td>
<td>Yes*</td>
</tr>
<tr>
<td>MRO87</td>
<td>FTC</td>
<td>46</td>
<td>Unknown</td>
<td>Unknown</td>
</tr>
<tr>
<td>WRO82</td>
<td>FTC</td>
<td>67</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>KAT-10</td>
<td>FTC</td>
<td>36</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>NPA87</td>
<td>FTC</td>
<td>44</td>
<td>Unknown</td>
<td>Unknown</td>
</tr>
</tbody>
</table>

*ATC, anaplastic thyroid carcinoma; FTC, follicular thyroid carcinoma; NPA87, a poorly differentiated PTC cell line.

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*Homozygous/hemizygous BRAF mutation.*
T1796 was detected in 21 of 56 papillary carcinomas but not detected in 18 benign follicular adenomas and 6 nodular goiters. All negative DNA samples were reanalyzed for \( \text{BRAF} \) mutation by PCR reaction with 40 cycles, no \( \text{BRAF} \) mutation was found. Nine of 21 positive samples were randomly selected for DNA sequencing to confirm \( \text{BRAF} \) mutation. All 9 samples were positive with \( \text{BRAF} \) mutation. Shown in Fig. 2B are three representative DNA sequences of \( \text{BRAF} \)-mutated samples, two of which had almost equal peak areas of A and T nucleotides, indicating a high homogeneity of tumor cells in these two microdissected sections. A third sample had a smaller A peak than T peak, reflecting heterogeneity of tumor cells and nontumor cells in the microdissected section. Nevertheless, these sequencing data additionally suggest that \( \text{BRAF} \) mutation detected by MASA is reliable and specific.

To test whether \( \text{BRAF} \) mutation in these PTC is somatically acquired, MASA-PCR was carried out to analyze \( \text{BRAF} \) mutation using the genomic DNA from normal tissues such as lymph nodes, parathyroid, or thyroid, which were separately embedded in paraffin tissue blocks. There was no \( \text{BRAF} \) mutation detected in all 20 normal tissues derived from patients with \( \text{BRAF} \)-mutated PTC (data not shown). One patient with \( \text{BRAF} \)-mutated PTC also developed melanoma at the time of thyroidectomy and thus was highly suspicious of a germ-line \( \text{BRAF} \) mutation. However, direct sequencing of PCR-amplified exon 15 of the \( \text{BRAF} \) gene derived from normal tissue confirmed that there was no germ-line \( \text{BRAF} \) mutation. In another patient with \( \text{BRAF} \)-mutated PTC, genomic DNA from peripheral blood was used in MASA-PCR to analyze for \( \text{BRAF} \) mutation. Again, no germ-line \( \text{BRAF} \) mutation was present in this patient. Thus, we concluded that \( \text{BRAF} \) mutation in all 21 PTC cases was somatic.

**Higher Frequency of \( \text{BRAF} \) Mutation in Male Patients than in Female Patients with PTC.** We analyzed whether \( \text{BRAF} \) mutation correlated with patient age, gender, and tumor stage. Interestingly, we found that \( \text{BRAF} \) mutation occurred in 9 of 14 male PTC patients and in 12 of 42 female patients (Table 2). Statistical analysis revealed that \( \text{BRAF} \) mutation occurred at a significantly higher frequency in male patients than in female patients (\( P < 0.05 \)). In addition, we found that \( \text{BRAF} \) mutation occurred in 15 of 33 PTC patients older than 40 years old and in only 6 of 23 PTC patients younger than 40 years old. \( \text{BRAF} \) mutation in patients with PTC tended to occur at a higher frequency in older patients than in younger patients, but this was not a significant difference (\( P > 0.05 \)). We did not find that \( \text{BRAF} \) mutation was associated with tumor volume (Table 2). \( \text{BRAF} \) was mutated at a higher rate in PTC with invasive and metastatic potential (Table 2) than in the noninvasive tumors, but again, this was not statistically significant (\( P > 0.05 \)).

**Overlapping of \( \text{BRAF} \) Mutation with other Genetic Alterations in PTC.** \( \text{RET/PTC} \) rearrangements frequently occur in papillary thyroid carcinomas. To test whether \( \text{BRAF} \) mutation concurs with \( \text{RET/PTC} \) rearrangements, we conducted IHC to determine the status of \( \text{RET/PTC} \) rearrangement. IHC analysis using an anti-RET rabbit serum revealed that \( \text{RET/PTC} \) expression was abundantly present in the cytoplasm of the tumor cells in a \( \text{BRAF} \)-mutated specimen (Fig. 3A, inset) and a PTC with wild-type \( \text{BRAF} \) but not in the neighboring normal thyroid follicular cells (Fig. 3A). Similar results were obtained when an anti-RET mAb was used (Fig. 3, C and D). Normal mouse IgG and normal rabbit serum included as negative controls did not show any positive signal (data not shown). All samples analyzed for \( \text{RET/PTC} \) expression using anti-RET rabbit serum were additionally tested for \( \text{RET/PTC} \) expression by IHC with an anti-RET mAb. We found that \( \text{RET/PTC} \) expression was consistent in 85% of the specimens. The samples graded as having \( \text{RET/PTC} \) expression with either method were considered as \( \text{RET/PTC} \) positive.

We next analyzed whether \( \text{RET/PTC} \) rearrangements were overlapping with \( \text{BRAF} \) mutation in PTC. As shown in Table 3, we found that 21 of 56 PTCs (38%) were \( \text{RET/PTC} \) positive. Among them, 13 of 35 PTCs (37%) with wild-type \( \text{BRAF} \) were \( \text{RET/PTC} \) positive. Unexpectedly, we found that 8 of 21 \( \text{BRAF} \)-mutated samples (38%) were also \( \text{RET/PTC} \) positive, suggesting that \( \text{BRAF} \) mutation and \( \text{RET/PTC} \) rearrangements are overlapping in a large number of PTC.

All three \( \text{RAS} \) oncogenes (\( \text{NRAS} \), \( \text{KRAS} \), and \( \text{HRAS} \)) were equally mutated in thyroid neoplasms. In this study, we selectively analyzed \( \text{NRAS} \) mutation in \( \text{BRAF} \)-mutated PTC to determine whether \( \text{BRAF} \) and \( \text{NRAS} \) mutations were overlapping. Exons 1 and 2 of the \( \text{NRAS} \) gene were amplified individually, and the PCR products were directly sequenced with a forward primer to amplify exon 15 of the \( \text{BRAF} \) gene. Three sequences representing the wild-type \( \text{BRAF} \) gene (WRO81), homozygous/hemizygous \( \text{BRAF} \) mutation (ARO81), and heterozygous \( \text{BRAF} \) mutation (SPA87) are shown. B, MASA analysis of \( \text{BRAF} \) gene mutation in thyroid tumor cell lines. Genomic DNA samples of 6 representative thyroid tumor cell lines were used as templates in PCR reactions to specifically amplify mutant \( \text{BRAF} \) gene by using a mutant-specific primer. Exon 15 was amplified as a positive control by using two intron primers flanking it. The PCR conditions were 94°C, 2 min (94°C 30°, 52°C 45°, 72°C 45°) × 33 cycles; 72°C, 8 min. PCR products were analyzed in a 3% agarose gel and stained with ethidium bromide.
sequenced to monitor NRAS mutation at codons 12 and 13 in exon 1 and at codon 61 in exon 2. As shown in Table 3, we did not find any NRAS mutations in all 21 BRAF-mutated specimens, indicating that there is no overlapping in NRAS and BRAF mutations in these PTC.

Because KRAS and HRAS mutations were not analyzed in this study, we could not exclude the possibility that KRAS and HRAS mutation might be overlapping with BRAF mutation. However, a recent study by Kimura et al. (39) demonstrated that 28 BRAF-mutated PTCs did not contain any form of mutated RAS genes.

DISCUSSION

Recent studies have demonstrated that BRAF mutation at codon 599 comprises ~80–90% of all mutations detected in several types of tumors, in particular in melanomas and colon cancers (31, 40). Several other sites in the kinase domain of BRAF in exons 11 and 15 can also be mutated but with much lower frequency (31, 40). Our present study using MASA demonstrated that BRAF mutation at the hot-spot codon 599 occurred in 21 of 56 papillary carcinomas (38%). While our manuscript was being revised, Kimura et al. (39) reported that BRAF mutation occurs in 28 of 78 PTCs (35.8%) and that all mutations are located at codon 599. Thus, these observations collectively suggest that the rate of BRAF mutation in PTC is the second highest to that in melanomas (>60%; Refs. 31, 41, 42) and is much higher than other cancers such as colorectal adenocarcinomas (5–10%; Refs. 31, 40, 43) and lung cancers (1.8%; Ref. 44).

Although our present study revealed that BRAF was mutated in 38% of PTC at codon 599, we did not find BRAF mutation at this site...
in any of 18 follicular adenomas studied. Statistical analysis revealed that BRAF was differentially mutated in these two different types of thyroid neoplasms (Table 1; \( P = 0.006 \)). Consistent with this observation, Kimura et al. (39) reported that no BRAF mutation was detected in 14 FTAs, as well as in 10 follicular thyroid carcinomas. These observations are in sharp contrast to previous studies showing that the upstream activators of BRAF, the RAS genes (HRAS, KRAS, and NRAS), are mutated in both benign FTAs and papillary thyroid carcinomas (10, 45, 46). It is not clear why BRAF is only mutated in papillary thyroid carcinomas but not in FTAs, whereas RAS is mutated in both benign and malignant thyroid neoplasms. Nevertheless, a higher BRAF mutation rate in carcinomas than in adenomas was also observed in another type of tumor: Rajagopalan et al. (40) reported that BRAF is mutated in 30 of 276 clinical colon cancers (11%) but is mutated in only 2 of 54 colon adenomas (3.8%); Similarly, Yuen et al. (41) reported that BRAF is mutated in 11 of 215 colorectal adenocarcinomas (5.1%) and in 3 of 108 (2.8%) sporadic adenomas.

The RET proto-oncogene is rearranged in ~40% of spontaneous PTC when analyzed by IHC staining and/or reverse transcription-PCR followed by Southern hybridization (36–38) and with a much higher rate in radiation-associated PTC (56–84%) as well as in pediatric PTCs (70%; Ref. 47). A few prior studies demonstrated that combined RET/PTC rearrangements or TSHR and RAS mutation can occur in papillary thyroid carcinomas (18, 46, 48–50). For example, Bounacer et al. (48) reported that combined RAS mutation and RET/PTC rearrangements are detected in 2 of 28 radiation-associated thyroid tumors. In another study, Sugg et al. (50) analyzed genetic alterations in 20 PTCs and found one of them harboring both NRAS mutation and a RET/PTC3 rearrangement. In this study, we tested whether BRAF mutation would occur together with RET/PTC rearrangements in PTC. To our surprise, IHC analysis revealed that a large number (8 of 21) of BRAF-mutated PTC-expressed RET, indicating that the RET proto-oncogene is rearranged in these tumors. This observation is totally unexpected not only because it contradicts recent studies showing that BRAF mutation at codon 599 does not overlap with RET/PTC rearrangements nor with RAS mutation (39) in PTC, as well as in other malignancies such as melanomas and colorectal adenocarcinomas (31, 40, 43) but also because two combined genetic alterations in the same signaling pathway appear to be redundant and therefore to be unnecessary. Nevertheless, based on our observation that 38% of the 21 BRAF-mutated PTCs also harbored RET/PTC rearrangements, we speculate that RET/PTC may cooperate with mutated BRAF to induce clinically overt PTC (24). In fact, both mutant BRAF gene and RET/PTC have a relatively low oncogenic potential. For example, the oncogenic potential of mutant BRAF gene is ~50-fold lower than V12 HRAS when tested in NIH3T3 cells (31), whereas RET/PTC may cooperate with RAS oncogenes to fully transform PC13 cells (24). It is possible that BRAF may cooperate with RET/PTC to fully transform thyroid epithelial cells, leading to the progression of occult

Table 2. BRAF gene mutation in thyroid neoplasms

<table>
<thead>
<tr>
<th>Tumor type</th>
<th>No.</th>
<th>Positive (%)</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Follicular adenoma</td>
<td>18</td>
<td>0 (0%)</td>
<td></td>
</tr>
<tr>
<td>Papillary carcinoma</td>
<td>56</td>
<td>21 (38%)</td>
<td>0.006</td>
</tr>
</tbody>
</table>

Tumor stage (PTC)

<table>
<thead>
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<th>Age (PTC)</th>
<th>No.</th>
<th>Positive (%)</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;40 years</td>
<td>23</td>
<td>6 (26%)</td>
<td></td>
</tr>
<tr>
<td>&gt;40 years</td>
<td>33</td>
<td>15 (46%)</td>
<td>0.233</td>
</tr>
</tbody>
</table>

Gender (PTC)

<table>
<thead>
<tr>
<th>Gender</th>
<th>No.</th>
<th>Positive (%)</th>
<th>( P )</th>
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<tbody>
<tr>
<td>Male</td>
<td>14</td>
<td>9 (64%)</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>42</td>
<td>12 (27%)</td>
<td>0.038</td>
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</tbody>
</table>

Node metastasis (PTC)

<table>
<thead>
<tr>
<th>Node metastasis (PTC)</th>
<th>No.</th>
<th>Positive (%)</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>13</td>
<td>7 (54%)</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>43</td>
<td>14 (32%)</td>
<td>0.288</td>
</tr>
</tbody>
</table>

Table 3. Overlapping of BRAF mutation with other genetic alterations in PTC

<table>
<thead>
<tr>
<th>BRAF mutation</th>
<th>No. of tumors</th>
<th>RET/PTC positive</th>
<th>NRAS mutation*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>21</td>
<td>8 (38%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>No</td>
<td>35</td>
<td>13 (37%)</td>
<td>ND*</td>
</tr>
<tr>
<td>Total</td>
<td>56</td>
<td>21 (38%)</td>
<td>ND*</td>
</tr>
</tbody>
</table>

*Exons 1 and 2 of the NRAS gene were PCR amplified individually and sequenced to detect NRAS mutation at codons 12, 13, and 61.

Although it is clear that several genetic alterations cooperate and may cooperate to induce clinically overt PTC, it is nonetheless possible that other genetic alterations cooperate and may cooperate to fully transform PC13 cells (24). It is possible that BRAF may cooperate with RET/PTC to fully transform thyroid epithelial cells, leading to the progression of occult
BRAF GENE MUTATION IN THYROID NEOPLASMS

In summary, our present study demonstrates that BRAF was mutated at a high frequency in papillary thyroid carcinomas but not in follicular adenomas and that a large number of BRAF-mutated PTC also harbored RET/PTC rearrangements. These observations provide a molecular basis for the oncogenesis of PTC and suggest that PTC and FTC may be developed through the mutation of different oncogenes within the same signaling pathway. It will be interesting to find out whether quantitative and/or qualitative differences in the RAS/BRAF-signaling pathway determine the type of thyroid neoplasm.

ACKNOWLEDGMENTS

We are grateful to Dr. Guy J. Fu Jilliard (University of California at Los Angeles) for kindly providing thyroid tumor cell lines.

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