Molecular analysis of multifocal papillary thyroid carcinoma

Xiaoqi Lin, Sydney D Finkelstein¹, Bing Zhu and Jan F Silverman²

Department of Pathology, Feinberg School of Medicine, Northwestern Memorial Hospital, Northwestern University, 251 East Huron Street, Feinberg Building 7-209C, Chicago, Illinois 60611, USA
¹RedPath Integrated Pathology, Inc., Pittsburgh, Pennsylvania, USA
²Department of Pathology, Allegheny General Hospital, Pittsburgh, Pennsylvania, USA

(Correspondence should be addressed to X Lin; Email: xlin@northwestern.edu)

Abstract

Papillary thyroid carcinoma (PTC) frequently presents as a multifocal process. To study the importance of separating independent primary (IP) from intrathyroid metastatic (ITM) PTC, we examined 19 molecular markers on 42 separate tumors from 18 multifocal PTC cases. In 12 of 18 (66.7%) cases, including 6 of 12 (50%) papillary microcarcinoma cases, the same or similar profile of loss of heterozygosity (LOH) and v-raf murine sarcoma viral oncogene homolog B1 (BRAF) mutation was demonstrated, indicating that they were from the same primary and represented ITM. Different profiles of LOHs and BRAF mutation were detected in separate tumors of 6 of 18 cases, indicating that they represented IP. Patients with ITM, including papillary microcarcinoma, had significantly increased lymph node metastasis. The frequencies of LOHs of 17q21, 17p13, 10q23, and 22q13 were higher in tumors with lymph node metastasis, suggesting that these LOHs may be important in increased lymph node metastasis. LOH of 9p21 was found at the highest frequency in PTC (53.8%), followed by 1p36 (46.2%), 10q23 (34.6%), and 22q13 (34.6%). Papillary microcarcinoma had acquired similar genomic mutations as conventional PTC, but higher frequencies of mutations of BRAF, 1p36, 18q, and 22q13 were found in the larger PTC, suggesting that they might play a role in the aggressiveness of PTC. Different profiles of mutations were observed in conventional, follicular variants, and diffuse sclerosing variant of PTC, which might influence the different morphological appearances and clinical courses. In conclusion, molecular analysis can separate multifocal IP PTC from ITM PTC, and may be more important than tumor size in predicting lymph node metastasis, aggressiveness, and prognosis of PTC.

Journal of Molecular Endocrinology (2008) 41, 195–203

Introduction

Papillary thyroid carcinoma (PTC) is the most common malignancy (Harach et al. 1985, Sakorafas et al. 2005). It is considered to be a relatively indolent malignancy in which distant metastasis and death from disease are uncommon (Mazzaferri 1981). PTC, including papillary microcarcinoma (≤1 cm), frequently presents as a multifocal process (Noguchi et al. 1970, Attie et al. 1971). Multifocality in papillary microcarcinoma is reported in 20–47% of cases and up to 40% of these patients can present with lymph node metastases (David et al. 1992, Rodriguez et al. 1997, Baudin et al. 1998, Arem et al. 1999). It was reported that multifocal PTC was more likely to have nodal and pulmonary metastases, as well as persistent disease (Mazzaferri et al. 1977). X-chromosome inactivation in female cases and a narrow panel of loss of heterozygosity (LOH) were used to identify the clonality of multifocal PTC and 50–87% of the cases were found to arise from the same clone (Shattuck et al. 2005, McCarthy et al. 2006). The importance to separate multifocal independent primary (IP) PTC from PTC with intrathyroid metastasis is unclear. It is also not certain which genomic changes are involved in the increased lymph node metastasis, and if PTC with lymph node metastasis increases mortality and morbidity.

Many genomic mutations have been reported in PTC, including two point mutations (V600E and K601E) of BRAF (7q34) (Cohen et al. 2003, Kimura et al. 2003, Soares et al. 2003, Frattini et al. 2004, Trovisco et al. 2005, Fugazzola et al. 2006, Kebebew et al. 2007, Nakayama et al. 2007, Ugolini et al. 2007), Ret (10q11.2) rearranged with PTC gene (Sugg et al. 1998, Salvatore et al. 2004), LOHs of 1q, 4p, 7q, 9p, 9q, and 16q (Kitamura et al. 2000), 1q23.3 (Cowden syndrome) (Alsanea & Clark 2001, Kameyama et al. 2001, Alvarez-Nunez et al. 2006), claudin-10 (Aldred et al. 2004), mutation of adenomatous polyposis coli (APC) gene at 5q21-22 (familial adenomatous polyposis coli) (Lee et al. 2004), insulin-like growth factor-binding protein 6 (Aldred et al. 2004), and cbp/p300-interacting transactivator, with Glu/Asp rich carboxy-terminal domain, 1
(CITED1) protein expression (Aldred et al. 2004, Prasad et al. 2004). Microarray analysis has also been utilized to identify the possible genomic mutations related to the thyroid carcinoma (Yano et al. 2004, Finley et al. 2005). Therefore, it appears that many genomic mutations may be involved in the oncogenesis and pathobiology of PTC via a variety of different mechanisms.

In this study, we investigated whether a broader panel of molecular markers could aid in separating multifocal IP PTC from PTC with intrathyroid metastasis, and the possible genomic mutations associated with local lymph node metastasis, oncogenesis, morphological appearances, and prognosis of PTC.

Materials and methods

Case selection

The institutional review board of Allegheny General Hospital, Pittsburgh, PA, USA approved the study, including ethical approval in accordance to the revised Declaration of Helsinki. Eighteen thyroidectomy cases demonstrating multiple PTC foci were retrieved from 2004 to 2006. A total of 42 separate tumors were examined. The average tumor size was 0.86 ± 0.67 cm, with a range of 0.05–3 cm. The 42 separate tumors included 19 conventional PTC (PTC-C), 16 follicular variant of PTC (PTC-FV), and 7 diffuse sclerosing variant of PTC (PTC-DSV). The average age of the patients was 51.3 ± 13.3. The female to male ratio is 5:1.

Molecular study

For each tumor, a paraffin block that included PTC was selected for molecular analysis. Slides for each tumor were sectioned from the block (one hematoxylin and eosin (H&E) section and six unstained deparaffinized sections followed by another H&E section). The H&E slides were examined, and tumor targets were selected and marked for guiding microdissection of unstained slides under direct visualization using a stereoscopic microscope and a beveled surgical blade. One to seven targets were chosen within each lesion.

DNA was isolated with Qiagen DNA isolation kit (Qiagen Inc). Allelic imbalance for a broad panel of polymorphic microsatellite repeat markers (PMRM) in proximity to known tumor suppressor genes and BRAF point mutation were quantitated using automated PCR with fluorescent-labeled synthesized primers followed by capillary electrophoresis. Fluorescent-labeled oligonucleotide primers were employed for quantitative determination of allelic imbalance based on the peak height ratio of polymorphic microsatellite alleles. The fluorescence signal of each amplified PCR products was picked up by GeneScan (ABI3100, Applied Biosystems, Foster City, CA, USA) and was shown as a peak. The percentage of LOH was calculated by the difference of the two allelic peaks by dividing the high peak and then timing 100%. The percentage of BRAF mutations was calculated by dividing the peak of the mutation base by the sum of the mutation base and normal base peaks and then timing 100%.

Normal microdissected tissue samples were first evaluated to determine whether the patient’s DNA was informative at each specific marker locus. When a particular microsatellite marker in a normal tissue sample manifested only a single peak, the patient was designated as non-informative for that marker. For informative subjects with respect to a specific marker, alleles were assessed as being in balance when the ratio of the individual allele peaks fell within 2 s.d.s of the average calculated from a large series of non-neoplastic microdissected tissue samples for subjects with the same pairing of specific polymorphic alleles. PCRs and subsequent analysis were repeated and confirmed in all cases to avoid misinterpreting allelic dropout as LOH. Thus, it was possible to assign a status as being either non-informative, in allelic balance (no LOH) or positive for imbalance (LOH) with the latter further characterized as being in relative deficiency of the shorter (red) or longer (blue) allele. The temporal sequence of mutation acquisition can be determined by the percentage of mutated cells at a given site. This in turn was based upon a simple model of clonal expansion with forward progression in malignant phenotype of tumor cells. Mutations acquired earlier in time were more likely to present across a wide distance of tumor encompassing all microdissection targets for a given tumor deposit. At the same time, mutations acquired earlier in time were more likely to be clonally expanded and thus present in a higher percentage of cells than mutations acquired later.

A panel of 18 specific PMRM targeting 11 chromosomal loci near some known tumor suppressor genes, including 1p34-36 (CAM and MYCLI), 3p24-26 (VHL, OGGI, retinoic acid receptor-β, and topoisomerase 2-β), 3p12-14 (FHT), 7p31 (c-met), 9p21 (p16 and p14arf), 10q23 (PTEN), 17p13 (p53), 17q21 (NME1, BRCA1), 18q21 (DCC and SMAD4), 21q22 (TFII, PSEN2), and 22q13 (NF2), was evaluated. The short tandem repeats were selected for their high rates of polymorphism (ideally >75%) in the general population. BRAF point mutations at position 599, 600, and 601 were also detected.

For each microdissected focus, the fractional mutation rate (FMR) was calculated by dividing the number of markers with LOH by the total number of informative markers. For each marker, allelic imbalance rate in each diagnostic category was calculated by dividing the number of cases with LOH in any
microdissected focus by the total number of cases informative for that marker.

The tumors were classified as de novo multifocal or metastasis based on three levels of concordance: 1) 50% or more of the same markers were mutated, as metastatic tumor should share most of the genomic mutations with the primary tumor. We found that both primary tumor and metastatic tumor could acquire additional genomic mutations after metastasis occurs. 2) Same alleles were affected. Metastatic tumor must share a mutation on the same allele of the marker as the primary tumor. The mutation on the opposite allele of the marker will be classified as a new genomic mutation. 3) Temporal sequence of the mutations. The higher percentage of a genomic mutation detected in our study indicates the earlier acquisition of the genomic mutation. The sequence of percentage of the mutations indicates the sequence of acquisition of the genomic mutations. Metastatic tumors should share the same sequence of acquisition of genomic mutations as the primary tumor. The percentages of additional mutations after metastasis should be lower than those shared by the metastatic and primary tumors.

Statistical analysis

Student’s t-test, \(\chi^2\)-test, and Fisher’s exact test were employed in the study.

Results

Separation of IP PTC from intrathyroid metastatic (ITM) PTC

Forty-two tumor nodules from 18 multifocal PTC cases were examined for the \(BRAF\) point mutation and LOH of 18 PMRM targeting 11 chromosomal loci near some known tumor suppressor genes (Tables 1 and 2). Based on our three levels of concordance, 12 of 18 cases (66.7%) showed the same or similar profile of LOHs and/or \(BRAF\) mutation, indicating that they were from the same primary tumors and therefore represent intrathyroid metastasis. One case had three tumors, two of which shared the same profile of genetic mutations that were different from the third neoplastic focus, indicating that two tumors were ITM tumors and the third was an IP. Of 18 cases, 6 (33.3%) showed different profile of LOHs and/or \(BRAF\) mutation, indicating that they were from different primaries. We also found that intrathyroid metastasis can involve the same lobe or opposite lobe, and both primary and metastatic tumors can obtain extra genomic mutations after metastasis occurred. Therefore, we believe that the broad panel of molecular markers can separate ITM PTC from an IP PTC.

Of our 18 cases, 12 were papillary microcarcinoma. Of 12 multifocal papillary microcarcinoma cases, 6 showed the same or similar profile of LOHs and/or \(BRAF\) mutation, indicating that they were from the same primary and therefore represented intrathyroid metastasis. Therefore, papillary microcarcinoma can present as multifocal tumors with half the cases having intrathyroid metastasis.

PTC with intrathyroid metastasis have increased local lymph node metastasis

Of our 18 surgical specimens, 10 contained local lymph nodes. Six cases had intrathyroid metastasis and four cases were independent primaries. Five of six (83.3%) PTC with intrathyroid metastasis showed positive lymph node metastasis. By contrast, none of 4 (0.0%) multifocal IP PTC cases had positive local lymph node (Fisher’s exact test, \(P<0.05\)). This suggested that PTC with intrathyroid metastasis had increased local lymph node metastasis.

Seven of the ten cases with lymph node resection were papillary microcarcinomas. Four of these were papillary microcarcinoma with intrathyroid metastasis and three cases were multifocal IP papillary microcarcinoma. Three of four (75%) cases with intrathyroid metastasis showed positive lymph node metastasis. By contrast, none of the 3 (0.0%) cases of multifocal IP papillary microcarcinoma had negative lymph nodes (\(P<0.01\)). The total incidence of lymph node metastasis for papillary microcarcinoma was 42.9% (3 of 7). This suggested that papillary microcarcinoma with intrathyroid metastasis also increased local lymph node metastasis.

We compared the genomic mutations of primary tumor of PTC with intrathyroid metastasis (12 tumors) with those of multifocal IP PTC tumors (Table 2). Three LOHs, 17q21, 17p13, and 10q23, were found in a significantly higher frequency in the primary tumor of PTC with intrathyroid metastasis than in PTC without intrathyroid metastasis, indicating that LOHs of 17q21, 17p13, and 10q23 may play an important role in the increased incidence of lymph node metastasis of multifocal PTC.

For the primary tumors, LOH of chromosome 9p21 was found at the highest frequency in PTC (53.8%), followed by 1p36 (46.2%), 10q23 (34.6%), and 22q13 (34.6%) (Table 2).

Correlation between the tumor size and genomic mutations in PTC

Classifying and managing papillary microcarcinoma are controversial. In this study, we compared the genomic mutation accumulation of papillary microcarcinoma with PTC > 1 cm. The mean number of genomic mutations of papillary microcarcinoma was 2.9 ± 1.5 with a range of 1–5, and PTC > 1 cm had 3.6 ± 0.98 mutations with a range of 2–5 (\(P=0.23\), no statistic
### Table 1  
BRAF mutation and loss of heterozygosity (LOH) in papillary thyroid carcinoma (PTC) nodules

<table>
<thead>
<tr>
<th>Case</th>
<th>1p34-36</th>
<th>3p24-26</th>
<th>3p12-14</th>
<th>7p31</th>
<th>9p21</th>
<th>10q23</th>
<th>17p13</th>
<th>17q12</th>
<th>18q21</th>
<th>21q22</th>
<th>22q13</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>D1S407</td>
<td>D3S193</td>
<td>D3S2303</td>
<td>D3S1</td>
<td>D3S2</td>
<td>D7S2</td>
<td>D9S251</td>
<td>D9S254</td>
<td>D10S1173</td>
<td>D17S1289</td>
<td>D17S1161</td>
</tr>
<tr>
<td>B-RAF</td>
<td>D1S306</td>
<td>D3S193</td>
<td>D3S2303</td>
<td>D3S1</td>
<td>D3S2</td>
<td>D7S2</td>
<td>D9S251</td>
<td>D9S254</td>
<td>D10S1173</td>
<td>D17S1289</td>
<td>D17S1161</td>
</tr>
<tr>
<td></td>
<td>NO MUT</td>
<td>NO MUT</td>
<td>NO MUT</td>
<td>NO MUT</td>
<td>NO MUT</td>
<td>NO MUT</td>
<td>NO MUT</td>
<td>NO MUT</td>
<td>NO MUT</td>
<td>NO MUT</td>
<td>NO MUT</td>
</tr>
</tbody>
</table>

The percentage of LOH was calculated by dividing the difference of the two allelic peaks by the high peak and then timing 100%. The percentage of BRAF mutation was calculated by dividing the peak of mutation base by the sum of mutation base and normal base peaks and then timing 100%. NI, non-informative; NO LOH, no loss of heterozygosity detected.
We compared the frequency of genomic mutations in the three variants of PTC (Table 5). The results showed that none of the molecular markers could separate PTC-C from PTC-FV. All the genomic mutations detected in the study were present in both PTC-C and PTC-FV. The only difference was the \( \text{BRAF} \) point mutation, which was much higher in PTC-FV than PTC-C. Separate papillary carcinomas with a different profile of LOHs could show the same morphological appearance in the same patient (Fig. 1).

Interestingly, the profile of genomic mutations detected in PTC-DSV was different from those in PTC-C and PTC-FV. None of genomic mutations of \( \text{BRAF} \), 1p36, 3p12, 7p31, and 10q23 were detected in the seven PTC-DSVs, but a higher frequency of LOHs of 3p24, 9p21, 17q21, 21q22, and 22q13 were noted in the PTC-DSV. Generally, PTC-DSV had a worse prognosis. Our data suggested that these of mutations (possibly working together) may play an important role in the different morphologic appearance and worse prognosis of PTC-DSV.

**Discussion**

Although many molecular analyses have been reported on PTC, there are very few studies addressing multifocal PTC and the importance in separating PTC with intrathyroid metastasis from multifocal IP PTC. In this study, we applied a broad panel of molecular markers to address these issues.

In this study, we found that 66.7% of multifocal PTC cases are intrathyroidal metastases. This finding differs slightly from those reported by Drs Shattuck and McCarthy (66.7 vs 50% and 87% respectively; Shattuck et al. 2005, McCarthy et al. 2006). These previous studies used either one (X-chromosome inactivation) or four molecular markers (LOHs of 3p25, 9p21, 17q21, and 18p11, and X-chromosome inactivation). X-chromosome inactivation technique is applicable only to female patients. By contrast, 19 molecular markers were utilized in our study and our techniques are applicable to both male and female patients. In addition and most importantly, detection of X-chromosome inactivation alone or combined

---

**Table 2** Genomic mutations in papillary thyroid carcinoma (PTC)

<table>
<thead>
<tr>
<th>Genome</th>
<th>ITM (12)</th>
<th>IP (14)</th>
<th>( P ) value*</th>
<th>Total (26)</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \text{BRAF} )</td>
<td>4 (33.3%)</td>
<td>2 (14.3%)</td>
<td>6 (23.1%)</td>
<td></td>
</tr>
<tr>
<td>1p36</td>
<td>7 (58.3%)</td>
<td>5 (35.7%)</td>
<td>12 (46.2%)</td>
<td></td>
</tr>
<tr>
<td>3p24</td>
<td>2 (16.7%)</td>
<td>4 (28.6%)</td>
<td>6 (23.1%)</td>
<td></td>
</tr>
<tr>
<td>3p12</td>
<td>0 (0%)</td>
<td>3 (21.4%)</td>
<td>3 (11.6%)</td>
<td></td>
</tr>
<tr>
<td>7p31</td>
<td>0 (0%)</td>
<td>2 (14.3%)</td>
<td>2 (7.1%)</td>
<td></td>
</tr>
<tr>
<td>9p21</td>
<td>6 (50.0%)</td>
<td>8 (57.1%)</td>
<td>14 (53.8%)</td>
<td></td>
</tr>
<tr>
<td>10q23</td>
<td>6 (50.0%)</td>
<td>3 (21.4%)</td>
<td>9 (34.6%)</td>
<td></td>
</tr>
<tr>
<td>17p13</td>
<td>4 (33.3%)</td>
<td>1 (7.1%)</td>
<td>5 (19.2%)</td>
<td></td>
</tr>
<tr>
<td>17q21</td>
<td>3 (25.0%)</td>
<td>1 (7.1%)</td>
<td>4 (15.4%)</td>
<td></td>
</tr>
<tr>
<td>18q21</td>
<td>1 (8.3%)</td>
<td>3 (21.4%)</td>
<td>4 (15.4%)</td>
<td></td>
</tr>
<tr>
<td>21q22</td>
<td>3 (25.0%)</td>
<td>3 (21.4%)</td>
<td>6 (23.1%)</td>
<td></td>
</tr>
<tr>
<td>22q13</td>
<td>6 (50.0%)</td>
<td>3 (21.4%)</td>
<td>9 (34.6%)</td>
<td></td>
</tr>
</tbody>
</table>

ITM, primary tumor node of intrathyroid metastasis; IP, primary tumor node of independent primary. Twelve and 14 primary tumor nodules were found in the cases with intrathyroid metastasis and multifocal independent primary tumor respectively. Total primary tumor nodules are 26 (12 + 14).

* \( P \) value was calculated as comparing genomic mutations of primary tumors of intrathyroid metastatic PTC with those of independent primary PTC tumors (\( \chi^2 \)-test).

**Table 3** Correlation between tumor size and genomic mutations

<table>
<thead>
<tr>
<th>Tumor size</th>
<th>No.</th>
<th>Size (cm) (Mean ± S.D.)</th>
<th>FMR(^a) range</th>
<th>FMR (Mean ± S.D.)</th>
<th>( P ) value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \leq 1 ) cm</td>
<td>19</td>
<td>0.50 ± 0.28</td>
<td>0.067–0.357</td>
<td>0.203 ± 0.106</td>
<td>0.251</td>
</tr>
<tr>
<td>&gt; 1 cm</td>
<td>7</td>
<td>1.67 ± 0.66</td>
<td>0.143–0.313</td>
<td>0.245 ± 0.066</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) \( P \) value was calculated as comparison of tumors that were \( \leq 1 \) cm with those that were > 1 cm. There was no statistical significance between two groups (Student’s t-test).

\(^a\)Fractional mutation rate (FMR) is defined as the total number of mutations divided by total number of informative markers.

---

**Table 4** Genomic mutation profile in the conventional, follicular variant, and PTC-DSV

This study consisted of 42 papillary carcinomas composed of 19 PTC-C, 16 PTC-FV, and 7 PTC-DSV.
with a narrow panel of LOHs cannot exclude the possibility that two foci of PTC acquire those mutations by accident. Therefore, the advantages of our method are to more accurately identify clonality in both male and female patients.

The published data on the prognostic significance of lymph node metastasis in PTC remains controversial. Some studies have shown that the presence of cervical lymph node metastases or mediastinal and bilateral cervical lymph node metastases was associated with greater mortality (Sellers et al. 1992, Mazzaferri & Jhiang 1994, Lin et al. 1999) and increased recurrence rates (Mazzaferri et al. 1977, McConahey et al. 1986, Simpson et al. 1987, DeGroot et al. 1990, Akslen & Myking 1992, Mizukami et al. 1992, Lin et al. 1999). However, other studies showed that the presence of lymph node metastases did not significantly impact on cancer mortality (Tubiana et al. 1985, McConahey et al. 1986, Simpson et al. 1987, DeGroot et al. 1990), and even had a lower mortality rate than those without nodal metastases (Cady et al. 1976). Nevertheless, the prognostic importance of lymph node metastasis is emphasized by some investigators (Nemec et al. 1986) and is used in some staging systems (DeGroot et al. 1990). In this study, we found that PTC with intrathyroid metastasis had significantly increased the lymph node metastases, while none of the multifocal de novo independent PTC cases demonstrated lymph node metastasis. We believed that the importance of lymph node metastasis in previous reports is possibly obscured by including a mixture of multifocal metastatic and de novo primary PTC cases. Therefore, we believe that it is important to separate PTC with intrathyroid metastasis from multifocal IP PTC in order to better predict the potential lymph node metastasis and prognosis. We also believed that the molecular study as described in this study is the best method to address this issue to date, since histology and immunohistochemistry examination cannot help in separating ITM PTCs from multifocal IP PTCs.

In this study, we also found that LOHs of chromosomes 1, 7q21, 17p13, 10q23, and 22q13, were more frequently present in PTC with intrathyroid metastasis than multifocal IP PTC, suggesting that the genomic mutations at these foci might play an important role in the increased incidence of lymph node metastasis and worse prognosis of PTC. It is still unknown which genes at these chromosomal foci are involved.

The clinical significance of papillary microcarcinoma is another major controversial issue in PTC. Papillary microcarcinoma can be multifocal, have lymph node metastases, and increase cervical lymph node recurrence especially when multifocal papillary microcarcinomas are present at the time of diagnoses (David et al. 1992, Rodriguez et al. 1997, Baudin et al. 1998, Arem et al. 1999, Chow et al. 2003). However, some studies have indicated that these incidentally found tumors are so clinically insignificant that they should not be treated

<table>
<thead>
<tr>
<th>Genome</th>
<th>PTC-C (19)</th>
<th>PTC-FV (16)</th>
<th>PTC-DSV (7)</th>
<th>P1/P2 value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>BRAF</td>
<td>2 (10-5%)</td>
<td>9 (56-3%)</td>
<td>0 (0-0%)</td>
<td>6.8×10⁻¹¹/6.8×10⁻⁶</td>
</tr>
<tr>
<td>1p36</td>
<td>11 (57-9%)</td>
<td>11 (68-8%)</td>
<td>3 (42-9%)</td>
<td>3×10⁻⁹/7.4×10⁻⁵</td>
</tr>
<tr>
<td>3p24</td>
<td>2 (10-5%)</td>
<td>3 (18-8%)</td>
<td>0 (0-0%)</td>
<td>4.6×10⁻⁹/1.3×10⁻¹⁰</td>
</tr>
<tr>
<td>3p12</td>
<td>2 (10-5%)</td>
<td>2 (12-5%)</td>
<td>5 (71-4%)</td>
<td>8.0×10⁻⁹/0.0003</td>
</tr>
<tr>
<td>7p31</td>
<td>1 (5-3%)</td>
<td>3 (18-8%)</td>
<td>2 (28-6%)</td>
<td>1.4×10⁻⁶/0.0005</td>
</tr>
<tr>
<td>9p21</td>
<td>9 (47-4%)</td>
<td>9 (56-3%)</td>
<td>5 (71-4%)</td>
<td>3×10⁻⁹/7.4×10⁻⁵</td>
</tr>
<tr>
<td>9q22</td>
<td>2 (10-5%)</td>
<td>5 (31-3%)</td>
<td>2 (28-6%)</td>
<td>8.0×10⁻⁹/0.0003</td>
</tr>
<tr>
<td>1p36</td>
<td>6 (31-6%)</td>
<td>6 (37-5%)</td>
<td>3 (42-9%)</td>
<td>1.4×10⁻⁶/0.0005</td>
</tr>
</tbody>
</table>

PTC-C, papillary thyroid carcinoma, conventional type; PTC-FV, PTC, follicular variant; PTC-DSV, PTC, diffuse sclerosing variant. *P value was calculated by comparison of PTC-DSV with PTC-C and P2 value by comparison of PTC-DSV with PTC-FV (χ²-test).
(Mazzaferrl 1981, Sugino et al. 1998) or should not be treated aggressively (Rodriguez et al. 1997, Sakorafas et al. 2005). Some authors suggest that such tumors no longer be termed ‘carcinoma’ but rather be diagnosed as ‘papillary microtumor’ (Rosai et al. 2003). Currently, there are no established criteria for the management of papillary microcarcinoma, although surgery is the usual mode of treatment (Rodriguez et al. 1997, Sakorafas et al. 2005). In this study, we found that the incidence of lymph node metastasis for multifocal papillary microcarcinoma was 42.9%, which is the similar to previous studies (David et al. 1992, Rodriguez et al. 1997, Baudin et al. 1998, Arem et al. 1999). We also found that 75% of papillary microcarcinomas with intrathyroid metastasis were positive for lymph node metastasis, and no patients with multifocal IP papillary microcarcinomas had metastatic disease. We believed that the previous studies (David et al. 1992, Rodriguez et al. 1997, Baudin et al. 1998, Arem et al. 1999) did not separate papillary microcarcinoma with intrathyroid metastasis from those with multifocal independent primaries, resulting in a lower percentage of positive lymph nodes than ours. Therefore, we believe that it is important to separate papillary microcarcinoma with intrathyroid metastasis from multifocal IP tumors.

In this study, we found that papillary microcarcinoma acquired all the genomic mutations as PTC > 1 cm and there was no significant correlation between tumor sizes (papillary microcarcinoma versus PTC > 1 cm) and the accumulation of LOHs for tumor suppression genes and BRAF point mutations, which is similar to previous studies (Sugg et al. 1998, Hunt et al. 2003, Sedliarou et al. 2004, Kim et al. 2005, Trovisco et al. 2005). But, we did find that the frequency of BRAF point mutation and the three LOHs at 1p36 (CMM and MYCL), 18q21 (DCC and SMAD4), and 22q13 (NF2) were significantly higher in the primary tumor of PTC > 1 cm than papillary microcarcinoma, indicating that these genomic changes may play an important role in the aggressiveness and larger tumor size of the PTC. A few previous studies also showed that BRAF mutations might be important in tumor size, extrathyroid extension, lymph node metastasis, distant metastasis, higher TNM stage, and recurrence (Strate et al. 1984, Trovisco et al. 2005, Fugazzola et al. 2006, Kebebew et al. 2007, Nakayama et al. 2007, Rodolico et al. 2007). Therefore, correlating the clinical significance with detection of genomic mutations, especially in papillary microcarcinoma, is worthy of further study for better predicting aggressiveness and prognosis of PTC.

PTC-SC has worse prognosis than PTC-C and PTC-FV. It was reported that gene expression differences exist in PTC-C and PTC-FV (Chevillard et al. 2004). In this study, we found that none of the detected molecular markers could separate PTC-C from PTC-FV, except for the high frequency of BRAF point mutation in the PTC-FV in comparison with PTC-C (56.3% vs 10.5%). However, we did find that the PTC-SC had different genomic mutations from PTC-C and PTC-FV. The higher frequency of LOHs of 9p21 (p16 and p14arf), 17q21 (NME1), 21q22 (TFF1, PSEN2), and 3p24 (VHL, retinoic acid receptor-β, and topoisomerase 2-β), and no LOHs of 1p36, 3p24, 7p31, and 10q23 were noted in the PTC-DSV. This panel of genomic mutations might reflect the morphological appearance and worse prognosis of PTC-SC.

Gene profiling may help further the understanding of thyroid tumorigenesis and significantly enhance the evaluation of thyroid nodules in the future. In this study, we found that LOH of 9p21 (p16 and p14arf) was found in the highest frequency in PTC (53.8%), followed by LOHs of 1p36 (46.2%) (CMM and MYCL), 10q23 (34.6%) (PTEN), and 22q13 (34.6%) (NF2). High frequency of LOH of 9p (36%) was found in another study with a narrow panel of LOHs (Kitamura et al. 2000). In that study, LOH at 4p (21%), 7q (20%), 9q (31%), and 16q (29%) were also identified. Therefore, the present study and the previous study suggest that these genomic mutations might be important in the tumorigenesis of PTC.

Finally, we believe that molecular analysis of multifocal PTC, including papillary microcarcinoma, can separate IP from intrathyroid metastasis PTCs which may be important for predicting the lymph node metastasis, aggressiveness, and prognosis of PTC.

Declaration of interest

There is no conflict of interest that would prejudice the impartiality of the article.
Funding

This work was supported by the Department of Pathology, Allegheny General Hospital, Pittsburgh, PA, USA.

References


Aldred MA, Huang Y, Liyanarachchi S, Pellegrata NS, Gimm O, Jhiang S, Davuluri RV, de la Chapelle A & Eng C 2004 Papillary and follicular thyroid carcinomas show distinctly different microarray expression profiles and can be distinguished by a minimum of five genes. *Journal of Clinical Oncology* 22 3531–5539.


Katamura Y, Shimizu K, Tanaka S, Ito K & Emi M 2000 Association of allelic loss on 1q, 4p, 7q, 9p, 9q, and 10q with postoperative death in papillary thyroid carcinoma. *Clinical Cancer Research* 6 1819–1825.


Lin JD, Liu MJ, Chao TC, Weng HF & Ho YS 1999 Prognostic variables of papillary and follicular thyroid carcinoma patients with lymph node metastases and without distant metastases. *Endocrine-Related Cancer* 6 109–115.


Mazzaferri EL & Jhiang SM 1994 Long-term impact of initial surgical and medical therapy on papillary and follicular thyroid cancer. *American Journal of Medicine* 97 418–428.


Received in final form 23 June 2008
Accepted 15 July 2008
Made available online as an Accepted Preprint 15 July 2008