

RESEARCH COMMUNICATION

Preoperative BRAF Mutation is Predictive of Occult Contralateral Carcinoma in Patients with Unilateral Papillary Thyroid Microcarcinoma

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Abstract

Background and Objective: The optimal resection extent for clinically unilateral papillary thyroid microcarcinoma (PTMC) remains controversial. The objective was to investigate risk factors associated with occult contralateral carcinoma, and put emphasis on the predictive value of preoperative BRAF mutation. **Materials and Methods:** 100 clinically unilateral PTMC patients all newly diagnosed, previously untreated were analyzed in a prospective cohort study. We assessed the T1799A BRAF mutation status in FNAB specimens obtained from all PTMC patients before undergoing total thyroidectomy (TT) and central lymph node dissection (CLND) for PTMC. Univariate and multivariate analyses were used to reveal the incidence of contralateral occult cancer, difference of risk factors and predictive value, with respect to the following variables: preoperative BRAF mutation status, age, gender, tumor size, multifocality of primary tumor, capsular invasion, presence of Hashimoto thyroiditis and central lymph node metastasis. **Results:** 20 of 100 patients (20%) had occult contralateral lobe carcinoma. On multi-variate analysis, preoperative BRAF mutation ($p = 0.030$, OR = 3.439) and multifocality of the primary tumor ($p = 0.004$, OR = 9.570) were independent predictive factors for occult contralateral PTMC presence. However, there were no significant differences between the presence of occult contralateral carcinomas and age, gender, tumor size, capsular invasion, Hashimoto thyroiditis and central lymph node metastasis. **Conclusions:** Total thyroidectomy, including the contralateral lobe, should be considered for the treatment of unilateral PTMC if preoperative BRAF mutation is positive and/or if the observed lesion presents as a multifocal tumor in the unilateral lobe.

Keywords: Papillary thyroid micro-carcinoma - occult contralateral carcinoma - BRAF mutation - lymph node metastasis

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Introduction

Thyroid cancer is the most common endocrine malignancy, accounting for 1% of all malignant tumors in the USA (Sherman, 2003) and 5.9% in one district of Wenzhou (a coastal city in Southeast China, with a incidence of 21/100,000) (Zheng and Zhang, 2007). The incidence of papillary thyroid carcinoma, which is the most common histologic type of thyroid malignancy, is increasing worldwide (Davies and Welch, 2006). Recently, widespread use of ultrasonography (US) and US-guided fine needle aspiration biopsy (FNAB) has facilitated the diagnosis of papillary thyroid microcarcinoma (PTMC), which is defined by the World Health Organization as a papillary carcinoma measured at 10 mm or less in its maximal diameter (Davies and Welch, 2006). However, the management of PTMC remains controversial. Although some clinicians believe that observation is appropriate,

most prefer surgical resection (Ito et al., 2003; Shindo et al., 2006).

Overall, total or neartotal thyroidectomy is performed when tumor foci is preoperatively detected in a bilateral lobe. However, when PTMC confined to the unilateral lobe, either total thyroidectomy or unilateral lobectomy seems to be applicable (Hay et al., 1987; Noguchi et al., 1996; Baudin et al., 1998), though unilateral lobectomy may present possibility of recurrent or persistent carcinoma in the remnant contralateral lobe. The rate of contralateral PTC discovered in completion thyroidectomy or total thyroidectomy specimens was reported to be from 13 to 56% (Pasiaka et al., 1992; Schonberger et al., 2007; Pitt et al., 2009; Wang et al., 2012). Other studies had revealed the incidence of contralateral PTMC was from 10 to 30% (Chow et al., 2003; Jacquot-Laperriere et al., 2007; Schonberger et al., 2007; Hay et al., 2008; Mercante et al., 2009). Due to the rate of contralateral PTMC does

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not justify routine total thyroidectomy for all patients with preoperative unilateral PTMC, some studies had began to investigate risk factors associated with occult contralateral carcinoma which may identify a subset of PTMC patients who may benefit from more aggressive surgical intervention with a total thyroidectomy. They found that multifocality in uni-lateral lobe could be used to predictive contralateral PTC (Pasięka et al., 1992; Hwang et al., 2010; Koo et al., 2010; Connor et al., 2011). Unfortunately, multifocality in unilateral lobe should be conformed by histology in surgical operation, and prospective predictive factors for occult contralateral carcinoma is unknown. Recent studies reported that poorer outcomes of bilateral PTC patients may be at least partially explained by the high incidence of BRAF V600E mutation (Wang et al., 2012), and bilateral PTC often arise from a single clone with concordant BRAF status (Wang et al., 2010). Based on above, T1799A BRAF mutation status in FNAB specimens may be expected to be independent predictive factor for contralateral carcinoma. The current study is designed to investigate this hypothesis. We investigated the incidence of contralateral carcinoma in clinically unilateral PTMC patients, the presenting risk factors. Our study is the first to specifically investigate the predictive value of BRAF mutation status in prospective FNAB specimens. In doing this, we hope to identify a subset of clinically unilateral PTMC patients who may benefit from more aggressive surgical intervention with a total thyroidectomy before initial surgical operation.

Materials and Methods

Study Cohort and Clinicopathological Features

This research involves a prospective cohort study of 100 clinically unilateral PTMC patients all newly diagnosed, previously untreated presenting to our First Affiliated Hospital of Wenzhou Medical College from November 2010 to November 2011. All patients underwent preoperative ultrasonography and computed tomography with contrast and fine needle aspiration cytology of the primary tumor. Patients were pre-operatively diagnosed with PTMC in a unilateral lobe by pathology. All patient underwent TT and preventive CLND. The specimens after surgery were routinely sectioned every 3 mm and were stained with hematoxylin and eosin for histopathologic examination. All histologic diagnoses were made by 2 pathologists according to the recommendations of the World Health Organization. Patients with other pathologic types of thyroid malignancies, preoperatively bilateral papillary thyroid cancer and traditional PTC (foci > 1cm) were excluded. The study was approval by our institutional review board and patient consent.

Demographic factor (age and gender), Clinicopathological factors included tumor size, multifocality of primary tumor, presence of capsular invasion, presence of Hashimoto thyroiditis and central lymph node metastasis by pathologic finding and preoperative BRAF mutation status were considered into risk analysis of contralateral carcinoma in clinically unilateral PTMC patients. Tumor size in multifocal PTMC patients who have multiple foci in one or each lobe was

according to maximum diameter. Multifocality was defined as having more than 1 tumor focus in the unilateral lobe of the primary tumor.

FNAB Specimens and paraffin-embedded specimens

FNAB was performed on the primary thyroid tumor in each of the 100 patients before the modified neck dissection operation. Briefly, three to four aspirations with a 25-gauge needle were made to collect material for cytological and molecular analyses. Each sample was washed in phosphate buffer solution (PBS) in a plastic tube and centrifuged. After centrifugation, the pellet was resuspended, washed twice in PBS, and stored at -80 °C until use.

Paraffin-embedded specimens obtained from these patients who were found contralateral carcinoma by final pathology were collected and stored until for DNA extraction.

BRAF Mutation Analysis

DNA was extracted from the FNAB samples with a QIAamp DNA Micro Kit (QIAGEN), according to the manufacturer's protocol and QIAamp DNA FFPE Tissue Kit (QIAGEN) was used for paraffinembedded specimens. We amplified the BRAF exon 15 by polymerase chain reaction (PCR) with the following primers designed by Gu et al. (2009): forward, 5'-TCATAATGCTTGCTCTGATAGGA-3', reverse, 5'-GGCCAAAATTAAATCAGTGG-3'. The amplicon size was 215 bp. The PCR conditions were: initial denaturation at 94 °C for 2 min, followed by 35 cycles of denaturation at 94 °C for 15 s, annealing at 60 °C for 30 s, and elongation at 68 °C for 20 s. The specificity and integrity of the PCR were confirmed by visualization of a single band PCR product with the expected molecular weight on a 1.5% agarose gel. The samples were analyzed on an ABI PRISM 3700 DNA Analyzer (Applied Biosystems) to identify the mutation

Statistical Analysis

Chi-square test or Fisher exact test was used in enumeration data, while Logistic regression multivariate analysis was used for further study. Application of two-tailed test P value < 0.05 was considered statistically significant. Statistical analysis was performed using SPSS 18.0.

Results

Characteristics and incidence of contralateral carcinoma in clinically unilateral PTMC patients

The 100 patients consisted of 81 women and 19 men with a median age of 48 years (range, 23-78 years). The median size of the primary thyroid cancer was 0.63 cm (range, 0.1-1.0 cm). Unilateral multifocal cancer lesions were found in 10 patients (10%). Capsular invasion, presence of Hashimoto thyroiditis and Central lymph node metastases were found in 6 (6%), 32 (28%), and 46 (46%) patients, respectively. Pre-operative BRAF mutation positive status was found in 31 patients (31%) (Table 1).

Table 1. Characteristics of 100 Patients

| Characteristics | Negative contralateral carcinoma | Positive contralateral carcinoma | Total |
|---------------------------------|----------------------------------|----------------------------------|------------|
| No. of patients | 80 | 20 | 100 |
| Mean age± SD | 48 ±11 | 50 ±10 | 48 ±11 |
| Gender (M/F) | 15/65 | 4/16 | 19/81 |
| Primary mean tumor size ± SD | 0.62 ±0.25 | 0.70 ±0.29 | 0.63 ±0.26 |
| Multifocality (%) | 3(3.8) | 7(35.0) | 10(10.0) |
| Capsular invasion (%) | 3(3.8) | 3(15.0) | 6(6.0) |
| With Hashimoto thyroiditis(%) | 24(30.0) | 8(40.0) | 32(32.0) |
| Positive central lymph node (%) | 34(42.5) | 12(60.0) | 46(46.0) |
| Preoperative BRAF mutation(%) | 19(23.8) | 12(60.0) | 31(31.0) |

Table 2. Correspondence of BRAF Mutation Status in Paired Preoperative FNAB and Resected Tissue Specimens

| Patient no. | BRAF mutation status | | |
|-------------|----------------------|-----------------------------|-------------------------------|
| | FNAB sample | Ipsilateral resected tissue | contralateral resected tissue |
| 1 | Positive | Positive | Positive |
| 2 | Positive | Positive | Negative* |
| 3 | Positive | Positive | Positive |
| 4 | Negative | Negative | Negative |
| 5 | Positive | Positive | Positive |
| 6 | Negative | Negative | Negative |
| 7 | Negative* | Positive | Negative* |
| 8 | Positive | Positive | Positive |
| 9 | Negative | Negative | Negative |
| 10 | Positive | Positive | Positive |
| 11 | Positive | Positive | Positive |
| 12 | Negative | Negative | Negative |
| 13 | Negative | Negative | Negative |
| 14 | Positive | Positive | Positive |
| 15 | Negative | Negative | Negative |
| 16 | Positive | Positive | Positive |
| 17 | Negative | Negative | Negative |
| 18 | Positive | Positive | Positive |
| 19 | Positive | Positive | Positive |
| 20 | Positive | Positive | Positive |

*Discordant results

Of the 100 patients with clinically unilateral PTMC, 20 (20%) had occult contralateral carcinomas, which were all characterized as papillary microcarcinomas. In 20 patients with occult contralateral carcinoma, the mean size of the primary papillary carcinoma was 0.63 ± 0.26 (range, 0.4-1.0 cm). Of the 20 patients with occult contralateral papillary carcinoma, 7 (35%) had multifocal primary tumors in a unilateral lobe. Capsular invasion, presence of Hashimoto thyroiditis and Central lymph node metastases were found in 3 (15%), 8 (40%), and 12 (60%) patients, respectively. Preoperative BRAF mutation positive status was found in 12 (60%) (Table 1).

Correspondence of BRAF Mutation Status in Paired Preoperative FNAB and Resected Tissue Specimens

Correspondence of BRAF mutation Status in paired

Table 4. Multivariate Logistic Regression for Occult Carcinoma of Contralateral Lobe

| Variables | β | S.E. | Wald | P value | Exp(B) | 95%CI |
|---------------|---------|-------|--------|---------|--------|--------------|
| Multifocality | 2.259 | 0.786 | 8.261 | 0.004* | 9.57 | 2.051-44.644 |
| BRAF Mutation | 1.235 | 0.57 | 4.699 | 0.030* | 3.439 | 1.126-10.504 |
| Contant | -2.219 | 0.4 | 30.811 | | | |

*P <0.05 between the two categories for a given variable

Table 3. Univariate Analysis of Potential Clinicopathologic Factors Associated with Occult Carcinomas of the Contralateral Lobe

| Variables | Number of patients with occult carcinoma of contralateral lobe (%) | P value |
|-------------------------------|--|--------------------|
| Age, year | | 0.75 |
| <45 years | 6/33(18.2) | |
| ≥45 years | 14/67(20.9) | |
| Gender | | 1.000 ^a |
| Male | 4/19(21.1) | |
| Female | 16/81(19.8) | |
| Tumor size | | 0.560 ^a |
| ≥0.5cm | 14/76(18.4) | |
| <0.5cm | 6/24(25.0) | |
| Multifocality | | <0.001* |
| Yes | 7/10(70.0) | |
| No | 13/90(14.4) | |
| Capsular invasion | | 0.092 ^a |
| Yes | 3/6(50.0) | |
| No | 17/94(18.1) | |
| With Hashimoto thyroiditis | | 0.391 |
| Yes | 8/32(25.0) | |
| No | 12/68(17.6) | |
| Central lymph node metastases | | 0.16 |
| Yes | 12/46(26.1) | |
| No | 8/54(14.8) | |
| Preoperative BRAF mutation | | 0.002* |
| Yes | 12/31(38.7) | |
| No | 8/69(11.6) | |

^aFisher exact test was used; Chi-square test was used in others;

*P < 0.05 between the two categories for a given variable

preoperative FNAB and resected Tissue Specimens were compared in 20 patients who were found contralateral carcinoma. There was 95% concordance in the paired FNAB and ipsilateral tumor resection specimens and 90% concordance between contralateral and ipsilateral tumor resection specimens in terms of BRAF status (Table 2).

Association of Contralateral PTMC With Preoperative BRAF Mutation Status and Pathologic Factors

Univariate analysis of potential clinicopathologic factors associated with occult carcinomas of the contralateral lobe based on our patients with clinically

determined unilateral PTMC is shown in Table 3. Occult contralateral papillary carcinoma was significantly more frequent in patients with multifocality of the primary carcinoma in the unilateral lobe ($p < 0.001$) and preoperative BRAF mutation positive status ($p = 0.002$). There were no significant differences between the presence of occult contralateral carcinoma and age, gender, tumor size, capsular invasion, presence of Hashimoto thyroiditis and central lymph node metastasis (Table 3).

Preoperative BRAF Mutation and Multifocality in Unilateral Lobe were Predictors for Occult Contralateral Carcinoma

Multivariate analysis also revealed that multifocality of the primary carcinoma in the unilateral lobe ($p = 0.004$, OR = 9.570) and Preoperative BRAF Mutation ($p = 0.030$, OR = 3.439) were independent predictors for the presence of occult contralateral carcinomas in patients with clinically unilateral PTMC (Table 4).

Discussion

PTMC will likely draw continued attention given its increasing incidence in recent years (Davies and Welch, 2006). Although the American Thyroid Association (ATA) guidelines for patients with thyroid nodules recommend total thyroidectomy for papillary thyroid carcinoma > 1 cm, the management for PTMC remains controversial (Cooper et al., 2006). It is well known that PTMC is usually slow growing and has an excellent prognosis. Therefore, conservative treatment such as unilateral lobectomy has been advocated for patients with these tumors (Hay et al., 2008; Cooper et al., 2009). Noguchi et al. (1996) from an analysis of 867 patients affected by PTMC, concluded that total thyroidectomy is not necessary. ATA also states that lobectomy alone "may be sufficient" for low-risk PTMC patients with disease isolated to the thyroid (Cooper et al., 2009). Unfortunately, some PTMCs may be occult and with bilateral involvement and have an aggressive behavior which can cause local regional recurrences and cervical lymph node metastases (Grant et al., 1988; Baudin et al., 1998; Chow et al., 2003). In some studies, where bilateral PTC was identified following completion thyroidectomy in patients who had already undergone a unilateral resection, factors that would predict the presence of cancer in the remaining lobe were investigated (Pasiaka et al., 1992; Pacini et al., 2001; Kim et al., 2004). Hay et al. (1987) and Baudin et al. (1998) both reported that extent of initial surgery was significant factors for recurrence. Therefore, they asserted total or near-total thyroidectomy. So far, total or near-total thyroidectomy is generally accepted to be performed for preoperatively detected bilateral PTMC. However, the optimal extent of surgical resection in cases with preoperative unilateral PTMC remains a topic of debate.

We termed an 'occult carcinoma' which was often discovered on pathology previously undetected carcinoma in the contralateral lobe when total thyroidectomy was performed in patients with preoperative unilateral PTMC (Pelizzo et al., 2006). Nevertheless, reported rates of contralateral PTC discovered in completion thyroidectomy

or total thyroidectomy specimens ranges from 13 to 56 percent (Pasiaka et al., 1992; Schonberger et al., 2007; Pitt et al., 2009; Wang et al., 2012). For PTMCs, the incidence of contralateral PTMC had been reported from 10% to 30% (Chow et al., 2003; Jacquot-Laperriere et al., 2007; Schonberger et al., 2007; Hay et al., 2008; Mercante et al., 2009). Our study found that 20% of clinically unilateral PTMC patients had occult contralateral carcinoma, which is consistent with these previous reports (Koo et al., 2010; Connor et al., 2011). In our opinion, a 20% occult ratio for a contralateral carcinoma does not justify routine total thyroidectomy for all patients with preoperative unilateral PTMC. So, we further examined for any possible predictive factors especially preoperative BRAF mutation associated with presence of occult contralateral carcinoma to help determine which patients with clinically unilateral PTMC should undergo total thyroidectomy.

In our study, we found that up to 70% of PTMC patients with multifocality of the primary carcinoma in the unilateral lobe had occult contralateral carcinoma, which was significantly different from that (14.4%) in non-multifocal patients. Further multivariate logistic regression confirmed that multifocality was an independent predictor for occult contralateral carcinoma in clinically unilateral PTMC patients ($p = 0.004$, OR = 9.570). Other previous studies also reported similar findings to our study that ipsilateral multifocal disease could be used to predict PTC in the contralateral lobe (Pasiaka et al., 1992; Connor et al., 2011; Koo et al., 2010; Wang et al., 2012). Pitt and colleagues showed that the presence of contralateral PTC appeared to be unrelated to the size of the primary tumor (Pitt et al., 2009), which was confirmed by our study. Pacini et al. reported in a study 182 patients treated with complete thyroidectomy after lobectomy for papillary thyroid carcinoma that the presence of lymph node metastases at the first surgical treatment and time interval between first treatment and completion thyroidectomy correlated with higher frequency rates of bilaterality (Pacini et al., 2001). Other studies on bilateral PTC had reported positive results of T stage and extrathyroidal which showed that bilateral PTCs were interrelated with advanced T stage and high incidence of extrathyroidal (Hwang et al., 2010; Wang et al., 2012). However, we did not obtain the same results in PTMC cases as previous studies on bilateral PTMC (Koo et al., 2010; Connor et al., 2011). The reasons were considered that PTMC, found early and microfoci, may not yet demonstrate full biological behavior and the number of cases was not enough. Other factors such as gender, age and with HT were not found significant difference as other papers reported (Hwang E et al., 2008; Koo et al., 2010; Connor et al., 2011).

Although ipsilateral multifocal disease had been accepted for a predictive factor of occult contralateral carcinoma to identify a subset of clinically unilateral PTMC patients who may benefit from a total thyroidectomy, it should be confirmed by intraoperative histology. It may be not satisfied for preoperative evaluation and preoperative preparation and lead to intraoperative waiting. On the other hand, surgeon could not design optimal incision size preoperatively. Therefore, it is significant to seek for preoperative predictive factors of occult

contralateral carcinoma. Koo et al. (2010) believed that the presence of coexistent benign nodule in the contralateral lobe was an independent predictive factors for occult contralateral PTMC presence. We attempted to seek for new predictors from molecular analysis of preoperative FNAB. Previous studies had shown that BRAFV600E was the most significant genetic alterations in PTCs (Kimura et al., 2003). This mutation had been reported to be associated with one or more conventional high-risk clinicopathological characteristics of PTC, such as lymph node metastasis, multifocality, extrathyroidal invasion, and advanced disease stage (Xing, 2007; Nikiforova et al., 2003). Moreover, BRAF V600E mutation was confirmed to be an important independent prognostic factor which may influence therapy options (Elisei et al., 2008; Oler and Cerutti, 2009). Our recent report had also highlighted that preoperative BRAF mutation was predictive of aggressive clinicopathological characteristics such as lateral lymph node metastasis in PTMCs (Lin et al., 2010). Given that BRAF mutation is associated with high-risk clinicopathological characteristics such as multifocality which had been confirmed as a risk factor of occult contralateral carcinoma, BRAF mutation may be interrelated with occult contralateral carcinoma. Latest report showed that poorer outcomes of bilateral PTC patients may be at least partially explained by the high incidence of BRAF V600E mutation (Wang et al., 2012). Another study provided evidence that bilateral PTCs often arise from a single clone and that intrathyroidal metastasis. They evaluated BRAF gene mutation analysis combined with X-chromosome inactivation in 25 pairs of bilateral PTCs and found that 85.7% cases showed concordant BRAF status in tumors from both thyroid lobes (Wang et al., 2010). Based on above all, we assumed to detect preoperative BRAF mutation status from unilateral PTMC foci to reflect tumor invasiveness and contralateral involvement. Through research, we found that the BRAF mutation status of bilateral PTMC was matched up to 90%, which supported the monoclonal theory of bilateral PTC (Wang et al., 2010). The preoperative BRAF mutation rate as high as 60% in patients who had occult contralateral carcinoma was significantly higher than that (23.8%) in final unilateral PTMC patients. The result agreed with the prior report on synchronous bilateral PTC (Wang et al., 2012). Further multivariate logistic regression analysis confirmed that BRAF mutation assessment from preoperative FNAB specimens could be used as an independent predictor of occult contralateral PTMC ($p = 0.030$, $OR = 3.439$).

In our study, we analyzed for the incidence and predictive factors of occult contralateral carcinomas in patients with clinically unilateral PTMC. If we can predict the occurrence rate of an occult contralateral carcinoma undetected by preoperative evaluation in patients with clinically unilateral PTMC, we can avoid delayed removal and a second operation. Therefore, we found BRAF mutation from FNAB specimens as a preoperative predictor. Although our analysis is limited because of the lack of both long-term follow-up results and prognostic implication of occult contralateral carcinomas, we did highlight that preoperative BRAF mutation might compensate the lack of preoperative sensitivity to reveal

undetected contralateral lobe carcinomas in patients with clinically unilateral PTMC.

In conclusion, multifocality of the primary carcinoma in the unilateral lobe and pre-operative BRAF mutation from FNAB can help predict the presence of an occult contralateral papillary carcinoma. Therefore, we suggest that total thyroidectomy should be considered for the treatment of a unilateral PTMC if such significant risk factors for occult contralateral carcinoma are present.

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