Clinical effect of MUC1 and its relevance to BRAF V600E mutation in papillary thyroid carcinoma: a case–control study

Quan Li*
Wen-Xu Jin*
Yi-Xiang Jin
Zhou-Ci Zheng
Xiao-Fen Zhou
Qing-Xuan Wang
Dan-Rong Ye
Yi-Han Sun
Xiao-Hua Zhang
Ou-Chen Wang
En-Dong Chen
Ye-Feng Cai

Department of Breast and Thyroid Surgery, The First Affiliated Hospital of Wenzhou Medical University, Wenzhou, Zhejiang, China

*These authors contributed equally to this work

Aim: To investigate the clinical effects of MUC1 on papillary thyroid cancer (PTC) and explore the relationship between MUC1 expression and BRAF mutation.

Methods: The data of 69 patients subjected to fine-needle aspiration biopsy in our hospital and 486 patient data downloaded from The Cancer Genome Atlas (TCGA) database were used. Univariate and multivariate analyses were performed.

Results: The results on the 486 patients recorded in the TCGA indicated that high MUC1 expression was independently related to BRAF mutation, lymph node metastasis (LNM), and unifocal type. In the 69 fine-needle aspiration biopsy patients with PTC, high MUC1 expression was significantly related to LNM and extrathyroid extension (ETE). The result of Pearson’s correlation coefficient showed that BRAF mutation and MUC1 expression were moderately correlated. Moreover, in the subgroup with low MUC1 expression, the patients with BRAF mutation had higher ETE frequency and LNM than those without BRAF mutation. In the subgroup with BRAF mutation, patients with high MUC1 expression exhibited higher ETE frequency than those with low MUC1 expression, and high MUC1 expression occurred in older patients. In the subgroup with BRAF wild-type mutation, patients with high MUC1 expression had a higher incidence of ETE and LNM than those with low expression.

Conclusion: We demonstrated that the MUC1 is an important oncogene in PTC and may have great significance on therapeutic cancer vaccine development.

Keywords: MUC1, BRAF mutation, papillary thyroid cancer, prognosis, lymph node metastasis, extrathyroid extension

Introduction

Thyroid cancer is the fifth most common cancer that occurs in women. In the USA, 56,870 new cases were estimated in 2017.1 In these cases, papillary thyroid cancer (PTC), which has a rapidly increasing incidence worldwide, is the most common histologic type.2 PTC exhibits a broad range of clinical behaviors, and most types of PTC are relatively indolent and highly curable. Meanwhile, the aggressive types, particularly tall cell variants, distant metastases, and those that exhibit extrathyroidal extension (ETE), continue to have high incidence rates,3–5 prompting the need to improve preoperative evaluation for patients with aggressive PTC. Traditional pathologic diagnosis was recently discovered, which has increased the potential benefits when performed in tandem with molecular diagnosis.6 Generally, genetic markers have a high specificity for malignancy identification and can be used in operation procedure and for overtreatment prevention.7
MUC1 encodes a membrane-bound protein that is a member of the mucin family. Mucins are O-glycosylated proteins that form protective mucus barriers on epithelial surfaces. This protein is expressed on the apical surface of epithelial cells that line the mucosal surfaces of various tissues, including lung, breast, stomach, and pancreatic tissues. Overexpression, aberrant intracellular localization, and changes in the glycosylation of this protein have been associated with many carcinomas, such as esophageal squamous cell carcinoma, gastric carcinoma, colorectal carcinoma, breast carcinoma, pancreatic cancer, and PTC. Several studies reported that MUC1 is an independent marker of PTC with aggressive behavior; however, MUC1 as a marker of worse prognosis remains controversial.

BRAF mutation, which is the most common genetic alteration in thyroid cancer, occurs in about 45% of sporadic PTCs and is a major cause of aberrant activation of the mitogen-activated protein kinase (MAPK) pathway in human cancers. The T1799A BRAF mutation causes a V600E amino acid change in the BRAF protein and occurs uniquely in PTC and in some PTC-derived anaplastic thyroid cancers. This mutation is associated with aggressive clinicopathologic characteristics, such as extrathyroidal invasion, lymph node metastasis (LNM), and advanced tumor stages, which are close to tumor progression and recurrence.

However, studies that focused on the effect of MUC1 on PTC and its relation with BRAF mutation are few. Thus, in this study, we investigated the clinical effect of MUC1 on PTC and explored the relationship between MUC1 expression and BRAF mutation. Furthermore, we explored whether high MUC1 expression in fine-needle aspiration biopsy (FNAB) can predict the aggressive characteristics of PTC.

Patients and methods

Patients and clinicopathologic parameters

The study included 69 thyroid cancer patients who underwent total thyroidectomy or lobectomy with lymph node dissection at the First Affiliated Hospital of Wenzhou Medical University from January 2015 to December 2015. All samples were confirmed as PTC by postoperative histopathologic examination. Records of patients who underwent total thyroidectomy or lobectomy with lymph node dissection at LNM, ETE, T stage, N stage, M stage, and histologic type were obtained from electronic medical records.

The Cancer Genome Atlas database

A total of 486 patients with clinical and DNA mutation data were downloaded from The Cancer Genome Atlas (TCGA) database (http://cancergenome.nih.gov/) for analysis. The TCGA database was obtained from primary PTC tissue. Also, MUC1 expression was normalized against normal thyroid tissue. We used the data to confirm the relationship between MUC1 and clinicopathologic features of PTC. Normalized mRNA expression counts were obtained via the TCGA portal and expressed as RNA-Seq by transcripts per kilobase million values. All patient information was anonymous and deidentified in this database.

FNAB specimens

FNAB was performed preoperatively on the primary thyroid tumor in each of the 69 patients. Ultrasound was performed with Acuson Sequoia and 128XP sonographic scanners (Siemens Medical Solutions, Mountain View, CA, USA) equipped with 8–13 MHz linear probes. Four to five ultrasonic guidance fine-needle aspirations with a 25-gauge needle were made to collect material for cytological and molecular analyses. Each sample was mixed with TRIzol in an Eppendorf tube, snap-frozen in liquid nitrogen immediately, and then stored at −80°C before RNA extraction.

RNA isolation and reverse transcription quantitative polymerase chain reaction (PCR)

Total RNA was isolated from the FNAB samples using TRIzol Reagent (Thermo Fisher Scientific, Waltham, MA, USA), and reverse transcription (TOYOBO, Osaka, Japan) was performed according to the manufacturer’s instructions. Each sample was mixed with reverse transcription PCR, and real-time PCR analysis was performed in triplicate on the ABI prism 7500 sequence detection system (Thermo Fisher Scientific) using the THUNDERBIRD SYBR qPCR Mix (TOYOBO), according to manufacturer’s instructions. GAPDH mRNA level was used for normalization. Primer sequences were as follows: MUC1 5’-TGCGCCGCTAGTACG-3’ and 5’-TGGGTTACTCGCTCATAGGAT-3’; GAPDH 5’-GGTCCAGTCACGGATTTTG-3’ and 5’-ATGAGCCCAGCCTTCTCCAT-3’.

Statistical analysis

Categorical variables were expressed as percentage and were compared with chi-square test or Fisher’s exact test, as appropriate. The normally distributed continuous data were expressed as mean ± SD, and the non-normal distribution was expressed as median ± quartile. For the continuous data, independent t and Wilcoxon–Mann–Whitney tests were used for normal and non-normal distribution, respectively. Logistic
regression analysis was also performed to estimate the odds ratios (ORs) of certain parameters. Variables with \( P < 0.05 \) in the univariate analysis were progressed to a multivariate analysis using forward stepwise selection. All \( P \) values were two sided, and \( P \) values <0.05 were considered statistically significant. Statistical analysis was performed with SPSS software version 22.0 (IBM Corporation, Armonk, NY, USA).

**Ethics approval and informed consent**

The research protocol used in this study was approved by the Ethics Committee of the First Affiliated Hospital of Wenzhou Medical University. All of the patients provided written informed consent.

**Results**

**Relationship between \( MUC1 \) expression and clinical features**

To analyze whether \( MUC1 \) played a role in PTC development, we investigated the relationship between \( MUC1 \) and clinical features of 486 PTC patients in the TCGA. A total of 279 patients were positive for \( BRAF \) V600E mutation (57.4%). Meanwhile, high \( MUC1 \) expression was related to \( BRAF \) mutation \( (P < 0.001) \), ETE \( (P < 0.001) \), advanced T stage \( (P < 0.001) \), unifocal type \( (P = 0.013) \), advanced pathologic stage \( (P < 0.001) \), and LNM \( (P < 0.001) \); Table 1). Multivariate analysis results showed that high \( MUC1 \) expression was significant in the presence of \( BRAF \) mutation (odds ratio \( [OR]=9.837, 95\% \) confidence interval \([CI]\) 6.065–15.955, \( P < 0.001) \), LNM \( (OR=1.836, 95\% \) CI 1.147–2.939, \( P < 0.01) \), and the unifocal type \( (OR=0.457, 95\% \) CI 0.288–0.726, \( P < 0.001) \); Table 2).

To explore whether high \( MUC1 \) expression in FNAB predicts aggressive characteristics in PTC, we investigated the relationship between \( MUC1 \) and clinical features in the FNABs of 69 PTC patients. The patients were divided into low and high \( MUC1 \) expression groups according to the result of reverse transcription quantitative PCR and on the basis of the median value of \( MUC1 \) \((2^{(\Delta \Delta CT(N/T))}, \text{value}=2.12)\). According to the univariate analysis results, LNM \( (P = 0.002) \) and ETE \( (P = 0.029) \) were significantly related to high \( MUC1 \) expression (Table 3). Meanwhile, multivariate analysis results revealed that LNM \( (OR=3.596, 95\% \) CI 1.029–12.564, \( P = 0.045) \) and ETE \( (OR=3.528, 95\% \) CI 1.044–11.921, \( P = 0.042) \) were independently associated with high \( MUC1 \) expression (Table 4).

**Relationship between \( MUC1 \) and \( BRAF \) mutation in PTC**

In the previous analysis, \( BRAF \) mutation was considered an independent risk factor for high \( MUC1 \) expression \( (OR=8.129, 95\% \) CI 4.979–13.271, \( P < 0.001) \), ETE \( (OR=2.396, 95\% \) CI 1.063–5.399, \( P = 0.035) \), and LNM \( (OR=1.705, 95\% \) CI 1.046–2.778, \( P = 0.032) \); Table 5). The

### Table 1 Relationship between \( MUC1 \) expression and clinical features in 486 PTC patients from TCGA database

| Characteristics | \( MUC1 \) High expression | \( MUC1 \) Low expression | \( P \)-value |
|-----------------|--------------------------|-------------------------|-------------|
| Size (cm)       | 2.79±1.81                | 2.64±1.66               | 0.356       |
| Age (years)     | 47.69±16.21              | 46.85±15.34             | 0.558       |
| \( BRAF \) mutation | Yes, n (%) 203 (83.5) | Yes, n (%) 76 (31.3) | <0.001     |
|                 | No, n (%) 40 (16.5)      | No, n (%) 167 (68.7)    | <0.001     |
| Extrathyroid extension | Yes, n (%) 103 (43.6) | Yes, n (%) 44 (18.1) | <0.001     |
|                 | No, n (%) 133 (56.3)     | No, n (%) 188 (81.9)    | <0.001     |
| Gender          | Male, n (%) 74 (30.5)    | Male, n (%) 58 (23.9)   | 0.126      |
|                 | Female, n (%) 169 (69.5) | Female, n (%) 185 (76.1) |          |
| Focus           | Unifocal, n (%) 143 (59.8) | Unifocal, n (%) 114 (48.1) | 0.013     |
|                 | Multifocal, n (%) 96 (40.2) | Multifocal, n (%) 123 (51.9) |          |
| T stage         | T1–T2, n (%) 124 (23.2)  | T1–T2, n (%) 176 (25.4) | <0.001     |
|                 | T3–T4, n (%) 117 (21.1)  | T3–T4, n (%) 67 (25.9)  | <0.001     |
| N stage         | N0, n (%) 88 (39.1)      | N0, n (%) 134 (63.2)    | 0.738      |
|                 | N1, n (%) 137 (60.9)     | N1, n (%) 78 (36.8)     |            |
| M stage         | M0, n (%) 153 (66.8)     | M0, n (%) 122 (97.6)    | <0.001     |
|                 | M1, n (%) 5 (2.2)        | M1, n (%) 3 (2.4)       |            |
| Pathologic stage| I–II, n (%) 143 (53.1)   | I–II, n (%) 184 (60.7)  | <0.001     |
|                 | III–IV, n (%) 100 (37.7) | III–IV, n (%) 58 (17.4) |            |
| Lymph node metastasis | Yes, n (%) 137 (56.4) | Yes, n (%) 78 (32.1) | <0.001     |
|                 | No, n (%) 106 (43.6)     | No, n (%) 165 (67.9)    |            |
| Hashimoto’s thyroiditis | Yes, n (%) 31 (14.8) | Yes, n (%) 40 (18.2) | 0.340      |
|                 | No, n (%) 179 (85.2)     | No, n (%) 180 (81.8)    |            |

**Abbreviations:** \( MUC1 \), mucin 1; PTC, papillary thyroid cancer; TCGA, The Cancer Genome Atlas.

### Table 2 Multivariate analysis for \( MUC1 \) expression and clinical features in 486 PTC patients from TCGA database

| Characteristics | OR     | 95\% CI | \( P \)-value |
|-----------------|--------|---------|-------------|
| \( BRAF \) mutation | 9.837  | 6.065–15.955 | 0.000       |
| Extrathyroid extension | 1.821  | 0.0910–3.644 | 0.090       |
| Histologic type | 1.207  | 0.846–1.722 | 0.300       |
| Lymph node metastasis | 1.836  | 1.147–2.939 | 0.011       |
| T stage         | 1.134  | 0.785–1.637 | 0.503       |
| Pathologic stage | 1.008  | 0.798–1.273 | 0.949       |
| Unifocal        | 0.457  | 0.288–0.726 | 0.001       |

**Abbreviations:** CI, confidence interval; \( MUC1 \), mucin 1; OR, odds ratio; PTC, papillary thyroid cancer; TCGA, The Cancer Genome Atlas.
Table 3 | Relationship between MUC1 expression and clinical features in the FNAB specimen of 69 PTC patients

| Characteristics | Low expression, n=34 | High expression, n=35 | P-value |
|-----------------|----------------------|-----------------------|---------|
| Size (cm)       | 0.85 (0.41)          | 0.94 (0.45)           | 0.396   |
| Age (years)     | 45.2 (10.0)          | 45.5 (11.6)           | 0.898   |
| ≤45, n (%)      | 18 (52.9)            | 18 (51.4)             | 0.9     |
| >45, n (%)      | 16 (47.1)            | 17 (48.6)             |         |
| Gender          |                      |                       | 0.722   |
| Female, n (%)   | 26 (76.5)            | 28 (80.0)             |         |
| Male, n (%)     | 8 (23.5)             | 7 (20.0)              |         |
| Lymph node metastasis |                |                       | 0.002   |
| Yes, n (%)      | 7 (20.6)             | 20 (57.1)             |         |
| No, n (%)       | 27 (79.4)            | 15 (42.9)             |         |
| Focus           |                      |                       | 0.096   |
| Unilocular, n (%) | 25 (73.5)          | 19 (54.3)             |         |
| Multilocular, n (%) | 9 (26.5)           | 16 (45.7)             |         |
| Extrathyroid extension |            |                       | 0.029   |
| Yes, n (%)      | 10 (29.4)            | 20 (57.1)             |         |
| No, n (%)       | 24 (70.6)            | 15 (42.9)             |         |
| Hashimoto’s thyroiditis |           |                       | 0.722   |
| Yes, n (%)      | 8 (23.5)             | 7 (20)                |         |
| No, n (%)       | 26 (76.5)            | 28 (80)               |         |

Abbreviations: FNAB, fine-needle aspiration biopsy; MUC1, mucin 1; PTC, papillary thyroid cancer.

Table 4 | Multivariate analysis for MUC1 expression and clinical features in the FNAB specimen of 69 PTC patients

| Characteristics | OR 95% CI | P-value |
|-----------------|-----------|---------|
| Size            | 1.002     | 0.885–1.134 | 0.978   |
| Age             | 1.003     | 0.948–1.062 | 0.918   |
| Gender          | 0.815     | 0.202–3.285 | 0.774   |
| Lymph node metastasis | 3.596 | 1.029–12.564 | 0.045   |
| Unilocular      | 3.355     | 0.931–12.084 | 0.064   |
| Extrathyroid extension | 3.528 | 1.044–11.921 | 0.042   |
| Hashimoto’s thyroiditis | 0.860 | 0.188–3.932 | 0.846   |

Abbreviations: CI, confidence interval; FNAB, fine-needle aspiration biopsy; MUC1, mucin 1; OR, odds ratio; PTC, papillary thyroid cancer.

Table 5 | Multivariate analysis for the BRAF mutation and clinical features in 486 PTC patients from TCGA database

| Characteristic   | OR 95% CI | P-value |
|-----------------|-----------|---------|
| MUC1 high expression | 8.129 | 4.979–13.271 | 0.000   |
| Extrathyroid extension | 2.396 | 1.063–5.399 | 0.035   |
| Lymph node metastasis | 1.705 | 1.046–2.778 | 0.032   |
| T stage          | 0.475     | 0.214–1.050 | 0.066   |
| Pathologic stage | 1.165     | 0.648–2.095 | 0.611   |
| Unilocular       | 1.404     | 0.875–2.255 | 0.161   |

Abbreviations: CI, confidence interval; MUC1, mucin 1; OR, odds ratio; PTC, papillary thyroid cancer; TCGA, The Cancer Genome Atlas.

correlation between BRAF mutation and MUC1 expression was analyzed by using the Pearson’s correlation coefficient. The result showed that BRAF mutation was moderately correlated with MUC1 expression (R=0.528, P<0.001; Table 6).

Table 6 | Pearson’s correlation coefficient between MUC1 expression and BRAF mutation

| Characteristic      | R value | P-value | Evaluation          |
|---------------------|---------|---------|---------------------|
| Pearson’s correlation coefficient | 0.528   | <0.001  | Moderate correlation |

Abbreviation: MUC1, mucin 1.

Table 7 | Effect of high MUC1 expression and BRAF mutation on LNM and ETE in 486 PTC patients from TCGA database

| Characteristics | OR 95% CI | P-value |
|-----------------|-----------|---------|
| LNM             | High MUC1 expression | 2.486 | 1.672–3.698 | <0.001 |
|                 | BRAF mutation | 2.552 | 1.696–3.839 | <0.001 |
| ETE             | High MUC1 expression | 3.140 | 2.030–4.856 | <0.001 |
|                 | BRAF mutation | 2.547 | 1.623–3.998 | <0.001 |

Abbreviations: CI, confidence interval; ETE, extrathyroid extension; LNM, lymph node metastasis; MUC1, mucin 1; OR, odds ratio; PTC, papillary thyroid cancer; TCGA, The Cancer Genome Atlas.

LNM and ETE are unfavorable factors for PTC, and thus lead to poor prognosis. The result of logistic univariate analysis for LNM showed that BRAF mutation (OR=2.552, 95% CI 1.696–3.839, P<0.001) and high MUC1 expression (OR=2.486, 95% CI 1.672–3.698, P<0.001) had equal effects. By contrast, the logistic univariate analysis results for ETE showed that high MUC1 expression (OR=3.140, 95% CI 2.030–4.856, P<0.001) had a higher effect than BRAF mutation (OR=2.547, 95% CI 1.623–3.998, P<0.001; Table 7).

Influence of different states of MUC1 expression and BRAF mutation in PTC

To investigate the influence of MUC1 expression in PTC patients with or without BRAF mutation, we divided the patients into the following subgroups on the basis of MUC1 expression and BRAF status: MUC1 (+) BRAF (+) (high MUC1 expression and BRAF mutation), MUC1 (+) BRAF (−) (high MUC1 expression and BRAF wild type), MUC1 (−) BRAF (+) (low MUC1 expression and BRAF mutation), and MUC1 (−) BRAF (−) (low MUC1 expression and BRAF wild type). We then compared the four groups with one another (Table 8).

MUC1 (−) BRAF (−) versus MUC1 (−) BRAF (+)

The MUC1 (−) BRAF (+) group had a higher ETE frequency (27.6% versus 14.7%, P=0.021) and LNM (54.9% versus 27.6%, P<0.001) than MUC1 (−) BRAF (−) group. These results showed that the BRAF mutation is an aggressive factor of PTC in patients without high MUC1 expression.
Table 8 Correlation between MUC1 expression and BRAF status in 486 PTC patients from TCGA database

| Variables                  | MUC1 (+) | MUC1 (+) | MUC1 (+) | MUC1 (+) | P-value | P-value | P-value | P-value |
|----------------------------|----------|----------|----------|----------|---------|---------|---------|---------|
|                           | BRAF (+) | BRAF (+) | BRAF (+) | BRAF (+) |         |         |         |         |
| Size (cm)                  | n=203    | n=40     | n=76     | n=167    | 0.817   | 0.337   | 0.062   | 0.521   |
| Age (years)                | 2.9±1.7  | 3.0±1.6  | 2.6±1.6  | 2.8±1.6  | 0.013   | 0.046   | 0.055   |         |
| Extrathyroid extension     |          |          |          |          | 0.021   | 0.009   | 0.002   |         |
| Yes, n (%)                 | 89 (44.9)| 14 (36.8)| 21 (27.6)| 23 (14.7)|         |         |         |         |
| No, n (%)                  | 109 (55.1)| 24 (63.2)| 55 (72.4)| 133 (85.3)| 0.188   | 0.748   | 0.363   | 0.075   |
| Gender                     |          |          |          |          | 0.002   | 0.001   | 0.001   |         |
| Male, n (%)                | 58 (28.6)| 16 (40)  | 17 (22.4)| 41 (24.6)|         |         |         |         |
| Female, n (%)              | 145 (71.4)| 24 (60)  | 59 (77.6)| 126 (75.4)|         |         |         |         |
| Lymph node metastasis      |          |          |          |          | 0.1      | 0.396   | 0.001   |         |
| Yes, n (%)                 | 113 (60.8)| 24 (61.5)| 39 (54.9)| 39 (27.6)|         |         |         |         |
| No, n (%)                  | 73 (39.2)| 15 (38.5)| 32 (45.1)| 102 (72.3)|         |         |         |         |

Notes: MUC1 (+) = high expression of MUC1, MUC1 (-) = low expression of MUC1, BRAF (+) = BRAF mutation, BRAF (-) = BRAF wild type. P value represents MUC1 (+) BRAF (+) versus MUC1 (+) BRAF (-). P value represents MUC1 (-) BRAF (+) versus MUC1 (-) BRAF (-). P value represents MUC1 (+) BRAF (+) versus MUC1 (-) BRAF (+). P value represents MUC1 (+) BRAF (+) versus MUC1 (-) BRAF (-).

Abbreviations: MUC1, mucin 1; PTC, papillary thyroid cancer; TCGA, The Cancer Genome Atlas.

MUC1 (+) BRAF (+) versus MUC1 (-) BRAF (+)

The MUC1 (+) BRAF (+) group exhibited a higher risk of ETE (44.9% versus 27.6%, P=0.009) and old age of patient (49±16 versus 45±13 years, P=0.046). High MUC1 expression can lead to ETE in patients with BRAF mutation. No significant difference was observed between the LNM values of the MUC1 (+) BRAF (+) and MUC1 (-) BRAF (+) groups. This result is due to the role of BRAF mutation, which may be approximately equal to high MUC1 expression in the LNM aspect of PTC.

MUC1 (+) BRAF (-) versus MUC1 (-) BRAF (-)

The MUC1 (+) BRAF (-) group had a higher incidence of ETE (36.8% versus 14.7%, P=0.002) and LNM (61.5% versus 27.6%, P<0.001) than that of the MUC1 (-) BRAF (-) group. This result showed that high MUC1 expression exhibited an aggressive effect on PTC with BRAF wild-type mutation.

MUC1 (+) BRAF (+) versus MUC1 (+) BRAF (-)

The MUC1 (+) BRAF (+) group was diagnosed older than MUC1 (+) BRAF (-) group (49±16 versus 42±17 years, P=0.021) and MUC1 (-) BRAF (+) group (49±16 versus 45±13 years, P=0.046). This result indicated that MUC1 and BRAF synergistically act in PTC, and high MUC1 expression and BRAF mutation tend to occur in old patients.

Discussion

Thyroid cancer is the most common endocrine malignancy and is becoming the fastest growing type of cancer in recent years.1-20 Thus, developing molecular and genetic markers that enhance the detection rate of potential aggressive cancers in thyroid nodules is necessary; these markers may enable surgeons and endocrinologists to formulate a comprehensive operative plan, including thyroidectomy, possible lymphadenectomy, and postoperative radioactive iodine administration.4

The overexpression and membrane delocalization of MUC1 is associated with poor prognosis and decreased survival rate in breast, colon, kidney, prostate, and gastrointestinal cancers.21 Weiss et al22 reported that MUC1 expressed in FNAB is higher in PTC than in benign samples. Some studies reported that MUC1 expression fails to predict LNM in papillary thyroid microcarcinomas or demonstrates poor prognosis in PTC.15,16 However, one research reported that MUC1 overexpression is associated with the aggressive behavior of PTC and is a prognostic marker and potential therapeutic target in PTC.13 Another study reported that MUC1 expression is correlated with BRAF mutation and LNM, which is the most important risk factor of relapse.23 In our study, the results of the univariate and multivariate analyses on 69 patients with FNAB PTC indicated that high MUC1 expression is correlated with LNM and ETE, and thus are in line with the previous study.23 Also, similar results are validated by the TCGA database. Therefore, high MUC1 expression may be an aggressive factor in PTC. Furthermore, the correlation analysis results showed that high MUC1 expression and BRAF mutation are moderately related. BRAF mutation is an important genetic event in PTC and leads to serious clinicopathologic characteristics and poor prognosis. Thus, MUC1 may be another important genetic event in PTC and
has a more important progressive effect on PTC than on
\textit{BRAF} mutation.

Many studies found that \textit{BRAF} mutation is associated
with some progressive clinicopathologic features, including
LNM and ETE.\textsuperscript{19,24} In our study, the results showed
that \textit{BRAF} mutation and high \textit{MUC1} expression have an
equal effect on LNM. The MAPK pathway is continuously
activated in some tumor cells and closely related to tumor
development and progression. \textit{BRAF} mutation and \textit{MUC1}
are both involved in this pathway. For instance, ERK1/2
activation in the mammary glands of \textit{MUC1} transgenic
mice sharply increases in contrast to that in \textit{MUC1} null
and wild-type animals, although RAS mutation and MEK
inhibitors can prevent this effect.\textsuperscript{25} Furthermore, \textit{MUC1}
activates the ERK→C/EBPβ→ALDH1A1 pathway, which
upregulates ALDH activity associated with stemness in
breast cancer cells.\textsuperscript{26} In one study, the researchers used the
GST pull-down assay in vitro and co-immunoprecipitation
and found that \textit{MUC1} binds to JNK1, which is an important
member of the MAPK superfamily, and activates it.\textsuperscript{27} Koga
et al\textsuperscript{28} found that the TNFR1→MEK1/2→ERK1→Sp1
pathway mediates TNF-α-induced \textit{MUC1} promoter activity.
Therefore, \textit{MUC1} may be an important molecule in the
MAPK signaling pathway and may have the same effects as
\textit{BRAF} mutation in PTC. Moreover, we found that \textit{MUC1}
is moderately correlated with \textit{BRAF} mutation. On analyzing
the four subgroups in the 486 PTC patients, the \textit{MUC1} and
\textit{BRAF} mutation were found to have nearly equal OR values
for LNM in PTC, which could explain that they may have
similar effects when they participate in the same MAPK
pathway.

Meanwhile, our study found that high \textit{MUC1} expression
reflects a high incidence of ETE, regardless of the \textit{BRAF}
status. In the \textit{BRAF} mutation group, the patients with high
\textit{MUC1} expression levels exhibited a higher frequency
of ETE than those patients with low \textit{MUC1} expression
levels. A similar pattern was observed in the \textit{BRAF} wild-type
group. Interestingly, when \textit{MUC1} expression was low, \textit{BRAF}
status was significantly associated with ETE. The incidence of ETE was higher in the \textit{BRAF} mutation
group than in the \textit{BRAF} wild-type group. At high \textit{MUC1}
expression levels, the risk of ETE in the \textit{BRAF} mutation
group was not significantly different from that of the \textit{BRAF}
wild-type group. Logistic regression analysis revealed that
\textit{MUC1} had higher risk in ETE than \textit{BRAF} mutation in PTC.
These results indicated that \textit{MUC1} plays a more important
role in ETE than the \textit{BRAF} status, and is an important
oncogene in PTC.

\textbf{Limitations}

Although the combination between the two molecular mark-
ers enabled the prediction of the aggressive characteristics
of PTC, the current study has several limitations. First, the
FNAB sample size was small and the study was carried out
in a single center. A large sample size and a multicenter study
are needed for the validation of the current study. Second,
no long-term follow-up information is available, and thus,
the relationship between the two molecular markers and the
prognosis of PTC cannot be directly concluded. Therefore,
a longer study period is necessary.

\textbf{Conclusion}

Our analysis results on \textit{MUC1} expression in PTC revealed
several interesting results. First, by performing univariate
and multivariate analyses on 69 patients with FNAB PTC
and 486 patients in TCGA PTC, we found that high \textit{MUC1}
expression is associated with \textit{BRAF} mutation, LNM, and
ETE, which are considered poor prognostic factors. Second,
we showed that \textit{MUC1} expression and \textit{BRAF} mutation are
moderately correlated. In LNM, high \textit{MUC1} expression
and \textit{BRAF} mutation have similar effects on LNM. In ETE,
high \textit{MUC1} expression has a higher risk on ETE than \textit{BRAF}
muutation. Third, subgroups with both high \textit{MUC1} expres-
sion and \textit{BRAF} mutation had higher risk to ETE than those
with either \textit{MUC1} expression or \textit{BRAF} mutation alone,
whereas no synergistic effect in the LNM was observed. Finally, the two molecular markers may have a synergistic action on age.

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**Disclosure**

The authors report no conflicts of interest in this work.

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