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ORIGINAL RESEARCH

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

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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]

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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.[8–13] Several studies reported that *MUC1* is an independent marker of PTC with aggressive behavior; however, *MUC1* as a marker of worse prognosis remains controversial.[13–16]

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.[17] 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.[18] 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.[19]

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′-TGCCGCCGAAAGAACTACG-3′ and 5′-TGGGGTACTCGCTCATAGGAT-3′; GAPDH 5′-GGTCGGAGTCAACGGATTTG-3′ and 5′-ATGAGCCCCAGCCTTCTCCAT-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* ($2^{-\Delta\Delta CT\ (T/N)}$, 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).

Table 1 Relationship between *MUC1* expression and clinical features in 486 PTC patients from TCGA database

Characteristics	*MUC1*		***P*-value**
	High expression	**Low expression**	
	n=243	**n=243**	
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			<0.001
Yes, n (%)	203 (83.5)	76 (31.3)	
No, n (%)	40 (16.5)	167 (68.7)	
Extrathyroid extension			<0.001
Yes, n (%)	103 (43.6)	44 (18.1)	
No, n (%)	133 (56.3)	188 (81.9)	
Gender			0.126
Male, n (%)	74 (30.5)	58 (23.9)	
Female, n (%)	169 (69.5)	185 (76.1)	
Focus			0.013
Unifocal, n (%)	143 (59.8)	114 (48.1)	
Multifocal, n (%)	96 (40.2)	123 (51.9)	
T stage			<0.001
T1–T2, n (%)	124 (23.2)	176 (34.2)	
T3–T4, n (%)	117 (41.1)	67 (25.9)	
N stage			<0.001
N0, n (%)	88 (39.1)	134 (63.2)	
N1, n (%)	137 (60.9)	78 (36.8)	
M stage			0.738
M0, n (%)	153 (96.8)	122 (97.6)	
M1, n (%)	5 (3.2)	3 (2.4)	
Pathologic stage			<0.001
I–II, n (%)	143 (53.1)	184 (60.7)	
III–IV, n (%)	100 (26.7)	58 (17.4)	
Lymph node metastasis			<0.001
Yes, n (%)	137 (56.4)	78 (32.1)	
No, n (%)	106 (43.6)	165 (67.9)	
Hashimoto's thyroiditis			0.340
Yes, n (%)	31 (14.8)	40 (18.2)	
No, n (%)	179 (85.2)	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	0785–1.637	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.

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 3 Relationship between *MUC1* expression and clinical features in the FNAB specimen of 69 PTC patients

Characteristics	*MUC1*		*P*-value
	Low expression, n=34	**High expression, n=35**	
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
Unifocal, n (%)	25 (73.5)	19 (54.3)	
Multifocal, 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
Unifocal	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
Unifocal	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 on 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* (+) *BRAF* (+) n=203	*MUC1* (+) *BRAF* (–) n=40	*MUC1* (–) *BRAF* (+) n=76	*MUC1* (–) *BRAF* (–) n=167	*P*-value[a]	*P*-value[b]	*P*-value[c]	*P*-value[d]
Size (cm)	2.9±1.7	3.0±1.6	2.6±1.6	2.8±1.6	0.817	0.337	0.062	0.521
Age (years)	48.8±16.0	42.3±16.6	44.7±12.8	47.8±16.3	0.021	0.136	0.046	0.055
Extrathyroid extension					0.378	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)				
Gender					0.188	0.748	0.363	0.075
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					1	<0.001	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. [a]*P* value represents *MUC1* (+) *BRAF* (+) versus *MUC1* (+) *BRAF* (–). [b]*P* value represents *MUC1* (–) *BRAF* (+) versus *MUC1* (–) *BRAF* (–). [c]*P* value represents *MUC1* (+) *BRAF* (+) versus *MUC1* (–) *BRAF* (+). [d]*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 al[22] 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 *BRAF* mutation.

Many studies found that *BRAF* mutation is associated with some progressive clinicopathologic features, including LNM and ETE.[19,24] In our study, the results showed that *BRAF* mutation and high *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. *BRAF* mutation and *MUC1* are both involved in this pathway. For instance, ERK1/2 activation in the mammary glands of *MUC1* transgenic mice sharply increases in contrast to that in *MUC1* null and wild-type animals, although RAS mutation and MEK inhibitors can prevent this effect.[25] Furthermore, *MUC1* activates the ERK→C/EBPβ→ALDH1A1 pathway, which upregulates ALDH activity associated with stemness in breast cancer cells.[26] In one study, the researchers used the GST pull-down assay in vitro and co-immunoprecipitation and found that *MUC1* binds to JNK1, which is an important member of the MAPK superfamily, and activates it.[27] Koga et al[28] found that the TNFR1→MEK1/2→ERK1→Sp1 pathway mediates TNF-α-induced *MUC1* promoter activity. Therefore, *MUC1* may be an important molecule in the MAPK signaling pathway and may have the same effects as *BRAF* mutation in PTC. Moreover, we found that *MUC1* is moderately correlated with *BRAF* mutation. On analyzing the four subgroups in the 486 PTC patients, the *MUC1* and *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 *MUC1* expression reflects a high incidence of ETE, regardless of the *BRAF* status. In the *BRAF* mutation group, the patients with high *MUC1* expression levels exhibited a higher frequency of ETE than those patients with low *MUC1* expression levels. A similar pattern was observed in the *BRAF* wild-type group. Interestingly, when *MUC1* expression was low, *BRAF* status was significantly associated with ETE. The incidence of ETE was higher in the *BRAF* mutation group than in the *BRAF* wild-type group. At high *MUC1* expression levels, the risk of ETE in the *BRAF* mutation group was not significantly different from that of the *BRAF* wild-type group. Logistic regression analysis revealed that *MUC1* had higher risk in ETE than *BRAF* mutation in PTC. These results indicated that *MUC1* plays a more important role in ETE than the *BRAF* status, and is an important oncogene in PTC.

FNAB is recommended preoperatively for the evaluation of thyroid nodules. Our results showed that *MUC1* and *BRAF* can be used for the prediction of aggressive clinicopathologic features. Therefore, preoperative detection of the two molecular markers by FNAB can be utilized in practice and helps surgeons formulate individualized operative plans.

Therapeutic cancer vaccines are tumor antigen-like molecules that stimulate humoral and/or cell-mediated immunity and recognize and selectively kill cancer cells. Theratope (Biomira, Inc., Edmonton, AB, Canada), a therapeutic cancer vaccine, employs a synthetic antigen that mimics *MUC1* antigens and is conjugated to the high-molecular-weight protein carrier keyhole limpet hemocyanin.[29] It has promising safety and immunogenicity profiles in breast cancer patients, as demonstrated in Phases I, II, and III clinical trials.[30–37] Furthermore, adding theratope to endocrine therapy may improve the clinical outcomes and result in few adverse effects in women with metastatic breast cancer.[29] In our study, we demonstrated that *MUC1* is highly expressed and an important oncogene in PTC, and thus has great significance on therapeutic cancer vaccine development.

Limitations

Although the combination between the two molecular markers 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.

Conclusion

Our analysis results on *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 *MUC1* expression is associated with *BRAF* mutation, LNM, and ETE, which are considered poor prognostic factors. Second, we showed that *MUC1* expression and *BRAF* mutation are moderately correlated. In LNM, high *MUC1* expression and *BRAF* mutation have similar effects on LNM. In ETE, high *MUC1* expression has a higher risk on ETE than *BRAF* mutation. Third, subgroups with both high *MUC1* expression and *BRAF* mutation had higher risk to ETE than those with either *MUC1* expression or *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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