Mps1 is associated with the BRAF^{V600E} mutation but does not rely on the classic RAS/RAF/MEK/ERK signaling pathway in thyroid carcinoma

YIKE LI[1*], YANYAN ZHANG[1*], SHUAISHUAI XIAO[2], PENGZHOU KONG[3,4], CAIXIA CHENG[5], RUYI SHI[3,4], FANG WANG[3,4], LING ZHANG[3,4], JUAN WANG[3,4], ZHIWU JIA[3,4], SHUAI WU[1], YUN LIU[1], JIANSHENG GUO[1], XIAOLONG CHENG[3,4], YONGPING CUI[3,4] and JING LIU[1]

[1]Department of General Surgery, First Hospital of Shanxi Medical University, Taiyuan, Shanxi 030001; [2]Department of General Surgery, Affiliated Tumor Hospital of Shanxi Medical University, Taiyuan, Shanxi 030013; [3]Translational Medicine Research Center, Shanxi Medical University; [4]Key Laboratory of Cellular Physiology, Ministry of Education, Shanxi Medical University; [5]Department of Pathology, First Hospital of Shanxi Medical University, Taiyuan, Shanxi 030001, P.R. China

Abstract. In previous studies, the B-Raf proto-oncogene, serine/threonine kinase (BRAF)^{V600E} mutation has been identified in multiple malignant tumors. BRAF^{V600E} has been revealed to contribute to tumorigenesis by the activation of phospho-mitogen-activated protein kinases (MAPKs) and their downstream Monopolar spindle 1 (Mps1), leading to chromosome euploidy and tumor development. In the present study, the presence of phospho-MAPK and Mps1 in 161 thyroid carcinoma cases with complete clinical parameters was analyzed by immunohistochemistry, and the BRAF mutation was detected by polymerase chain reaction-direct sequencing. It was revealed that BRAF^{V600E} was present in ~34% of thyroid cancer cases and was associated with age, clinical tumor stage and lymph node stage. However, the association of BRAF^{V600E} with overall survival was not statistically significant. The expression of Mps1 was significantly increased in tumor tissues with BRAF^{V600E}, however, this did not affect the expression of phospho-MAPK in thyroid carcinomas. Collectively, the results of the present study suggested that BRAF^{V600E} may regulate the expression of Mps1 in MAP kinase independent ways in thyroid carcinoma. Therefore, Mps1 expression is associated with BRAF^{V600E} while the upstream signaling of phospho-MAPK has no relevance. The specific mechanisms of BRAF^{V600E} and the unknown pathway associated with Mps1 exhibit potential for further study, and provide a theoretical basis for the molecular treatment of thyroid carcinoma.

Introduction

Thyroid carcinoma is one of the major common malignant tumors of the endocrine system. Its incidence has grown to become the fifth most common cancer in females globally (1,2). In total, >50% of thyroid cancers in women and >45% of those in men were diagnosed at age 50+ (3). The increased incidence rates of thyroid cancer may be accounted for by changes in environmental risk factors, including obesity and molecular pathways (4-6). Thyroid carcinoma is classed into four main types, including papillary, follicular, medullary and anaplastic carcinomas (7). Among these four types of thyroid carcinoma, papillary thyroid carcinoma (PTC) is the predominant type, accounting for ~70% of all thyroid malignancies (8). Traditional clinical treatments for thyroid carcinoma include surgery, radioactive iodine and endocrine suppression therapy. PTC generally has an indolent course and favorable prognosis (9). However, there remain 20-30% of patients where PTC recrudesces and became resistant to radioactive iodine treatment, particularly in patients with advanced thyroid carcinoma, yielding poor prognosis and shorter survival (10,11). Therefore, there is an urgent requirement to elucidate the molecular mechanism underlying thyroid carcinoma progression and to improve the survival rate of patients with thyroid carcinoma.

Previously, there has been extensive research into the molecular alterations in thyroid carcinomas, with particular focus on the investigation of several oncogenic pathways

Correspondence to: Professor Jing Liu, Department of General Surgery, First Hospital of Shanxi Medical University, 56 Xinjian Nan Road, Taiyuan, Shanxi 030001, P.R. China
E-mail: liujing5585@163.com

Professor Yongping Cui, Translational Medicine Research Center, Shanxi Medical University, 56 Xinjian Nan Road, Taiyuan, Shanxi 030001, P.R. China
E-mail: cuiyp@sxmu.edu.cn

*Contributed equally

that contribute to various cancer types (12,13). Numerous molecular markers associated with the prognosis of thyroid carcinomas have been identified, including RAS, phosphatidylinositol-4,5-bisphosphate 3-kinase, phosphatase and tensin homolog, tumour protein p53, anaplastic lymphoma kinase and B-Raf proto-oncogene serine/threonine kinase (BRAF). The most common gene alteration identified in thyroid carcinoma is BRAF mutation, which i§s associated with ~50% of these tumors (14,15). BRAF mutations are relatively specific for PTC and have not been identified in other types of thyroid carcinomas. The specific mutation that occurs in thyroid carcinomas is a threonine-to alanine nucleotide transversion at position 1799 in exon 15, which results in a valine-to-glutamate substitution at codon 600, named BRAF[V600E], which has been revealed to be a potential prognostic factor in thyroid carcinoma (16,17).

BRAF belongs to the RAF protein kinase family, and is a serine/threonine kinase which serves an important function in the mitogen activated protein kinase (MAPK) signaling pathway (18). The mutation in this gene may activate the RAF/mitogen-activated protein kinase/extracellular regulated kinase (MEK)/extracellular signal regulated kinase (ERK) signaling pathway, promoting cell proliferation and inhibiting apoptosis. BRAF has been revealed to be mutated in a variety of malignant tumors. For example, BRAF mutations have been detected in ~70% of skin cancer (19), ~50% of thyroid carcinoma (20), ~20% of colorectal cancer (21), 14-30% of ovarian cancer (22), ~15% of hepatocellular carcinoma (23) and ~5% of lung cancer and breast cancer (24,25). Notably, BRAF mutation has also been identified as a lethal factor. Multiple studies have demonstrated that tumors with a BRAF mutation are resistant to traditional treatment, leading to poor prognosis (26,27). Therefore, the development of a novel therapeutic strategy for cancer with BRAF mutation is urgently required.

Previous studies have indicated that BRAF[V600E] mutation status may predict recurrence and prognosis in patients with thyroid carcinomas (15,28,29). BRAF[V600E] contributes to tumorigenesis by the continual activation of phospho-MAPK and the downstream Monopolar spindle 1 (Mps1) in thyroid carcinomas, leading to chromosome euploidy which promotes tumor development (30). Although there has been extensive research into the high frequency of BRAF mutations in thyroid carcinomas, the exact molecular mechanisms of BRAF mutation are yet to be fully understood. The present study will focus on observations of the BRAF[V600E] mutation in thyroid carcinomas, the associations between the BRAF[V600E] mutation, phospho-MAPK, Mps1 and clinical parameters of thyroid carcinomas and its prognostic significance.

Materials and methods

Ethics statement and clinical specimens. Cancer tissue samples were obtained from 161 patients with thyroid carcinomas who underwent surgical resection at the First Hospital of Shanxi Medical University, (Shanxi, China) and Shanxi Provincial People's Hospital, (Shanxi, China) between January 2009 and June 2015 (Table I). The present study was approved by the Ethics Committee of the First Hospital of Shanxi Medical University and written informed consent was obtained from the patients. All patients were enrolled at the time of surgery and did not receive treatment prior to surgery, including radiotherapy or chemotherapy. Surgically removed tissue was fixed in 10% buffered formalin for 24 h at room temperature, and embedded in paraffin. Hematoxylin and eosin-stained tumor tissues (stained for 5 min and 30 sec at room temperature, respectively) were reviewed by two pathologists. The tumor area was marked and then manually cut into 2 tissue volumes (10-μm thick) to collect tumor cells for DNA extraction. DNA was extracted using the FFPE DNA kit (Omega Bio-Tek, Inc., Norcross, GA, USA) following the manufacturer's protocol. The extracted DNA was quantified using a spectrophotometer (Thermo Fisher Scientific, Inc., Waltham, MA, USA).

BRAF[V600E] mutation analysis. To screen for the BRAF[V600E] mutation, DNA from tumor tissue was analyzed by polymerase chain reaction (PCR)-direct sequencing. Exon 15 of the BRAF gene, which contains the T1796A mutation, was amplified using the following specific primers: Forward, 5'-CTCTTC ATAATGCTTGCTCTGATAGG-3'; and reverse, 5'-CTCTTC ATAATGCTTGCTCTGATAGG-3'. The cycling conditions for PCR were as follows: Initial denaturation (98°C, 3 min) followed by 30 cycles of denaturation 98°C for 30 sec, annealing 58°C for 30 sec and then an extension 72°C for 30 sec. All PCR products were visualized using electrophoresis on a 2% agarose gel. Sanger sequencing was performed by the Beijing Genomics Institute (Shenzhen, China).

Immunohistochemistry (IHC). Phospho-MAPK or Mps1 protein levels in thyroid carcinomas were determined by IHC with phospho-MAPK antibody (cat. no. 4370, Cell Signaling Technology, Inc., Danvers, MA, USA) or Mps1 antibody (cat. no. ab1118, Abcam, Cambridge, UK). IHC was performed as follows: In brief, sections were incubated with primary antibodies against phospho-MAPK (1:100) or Mps1 (1:50) overnight at 4°C, followed by detection using the PV8000 kit (Beijing Zhongshan Jinqiao Biotechnology Co., Ltd., Beijing, China) and DAB detection kit (Fuzhou Maixin Biotech Co., Ltd., Fuzhou, China), according to the manufacturer's protocol. Slides were counterstained with hematoxylin for 50 sec at room temperature. All images were captured using an Aprio automatic biopsy scanner at x100 magnification. The percentage and intensity of positive staining of phospho-MAPK and Mps1 were analyzed using Aperio Cytoplasma 2.0 software (Leica Microsystems, Inc., Buffalo Grove, IL, USA). Statistical analyses were performed using GraphPad Prism v.6.0 software package (GraphPad Software, Inc., La Jolla, CA, USA). Using a scoring system from (-) to (+++) respectively, nucleus Phospho-MAPK expression was measured as negative for 0-25 scores (-), weak for 25-50 scores (+), median for 50-75 scores (++) or strong for >130 scores (+++). Cytoplasm Mps1 expression was measured as negative for 0-50 scores (-), weak for 50-90 scores (+), median for 90-130 scores (++) or strong for >130 scores (+++).

Statistical analysis. 60 cases were selected for analysis, including 30 BRAF[V600E]-positive cases, which were randomly matched with 30 BRAF[V600E]-negative cases. The expression of phospho-MAPK and Mps1 levels between the paired samples were compared using χ^2 tests or Fisher's exact tests. This was also

Table I. Clinicopathological features of 161 patients with thyroid carcinomas.

Clinicopathological feature	No. (161)
Sex	
Male	34
Female	127
Age, years	
>35	139
≤35	22
Smoking status	
Yes	9
No	152
Drinking status	
Yes	6
No	155
Familial history	
Yes	2
No	159
Tumor grade	
Papillocarcinoma	157
Non-papillocarcinoma	4
Pathological T stage	
T1-T2	133
T3-T4	28
Pathological N stage	
N0	64
N1	97
Clinical M	
M0	161
M1	0
Pathological stage	
I-II	106
III-IV	55

T, tumor; N, lymph node; M, metastasis.

used to assess the association between BRAF mutation status and clinical parameters. Cox regression was used to measure the association between $BRAF^{V600E}$ mutation and the clinical pathological information, linear regression was used to analyze the association between phospho-MAPK and Mps1 expression and survival analysis was performed using Kaplan-Meier analysis and the Log-rank test. Univariate and multivariate survival analyses were performed using a Cox proportional hazards regression model. P<0.05 was considered to indicate a statistically significant difference. All statistical tests were performed using IBM SPSS 19.0 (IBM Corp., Armonk, NY, USA).

Results

$BRAF^{V600E}$ mutation and its association with clinical parameters in thyroid carcinomas. Single-base substitutions were detected by Sanger sequencing, which were presented in BRAF exon 15: T1796A, leading to a substitution of valine to glutamic acid at position 600 (V600E) in thyroid cancer (Fig. 1A). In 161 cases of thyroid carcinomas, 55 cases (34%) of $BRAF^{V600E}$ mutation were identified. A statistical analysis of BRAF mutations and clinical parameters revealed that BRAF mutations were significantly associated with age, tumor (T) stage and lymph node (N) stage, were more prevalent in younger patients (≤35 years), pathological T1-T2 stage patients and pathological N0 stage patients (χ^2 test or Fisher's exact test, P<0.05, Table II; Cox regression analysis, P<0.05, Fig. 1C). However, there was no significant association between BRAF mutation status and other clinical parameters, including sex, smoking status, drinking status, familial history, tumor grade, clinical metastasis (M) and pathological stage (χ^2 test or Fisher's exact test, P>0.05, Table II; Cox regression analysis, P>0.05, Fig. 1C). Although patients with thyroid carcinomas with $BRAF^{V600E}$ mutations had a higher risk of mortality, the association of $BRAF^{V600E}$ mutations with overall survival (OS) was not statistically significant [Log-rank (MantelCox), P=0.9132, Fig. 1B]. Furthermore, the Cox regression model revealed that age, T stage and N stage were associated with $BRAF^{V600E}$ mutation in thyroid cancer (Fig. 1C).

Cox regression analysis was used to assess the impact of BRAF mutations and clinical parameters on OS. Notably, the results revealed that the association of BRAF mutations with OS was not statistically significant (Cox regression univariate analyses, P=0.913, Fig. 2A; Cox regression multivariate analyses, P=0.954; Fig. 2B). Additionally, the association of clinical parameters with OS was not statistically significant (Cox regression univariate analyses, P>0.05; Fig. 2A; Cox regression multivariate analyses, P>0.05; Fig. 2B). This data indicates that there were no significantly associated prognostic factors in thyroid carcinoma.

IHC and evaluation of phospho-MAPK and Mps1 in thyroid carcinomas. Aside from a number of the papillary thyroid micro carcinoma cases, which were excluded due to the tumor tissue being too thin to obtain, 30 $BRAF^{V600E}$ cases and 30 $BRAF^{WT}$ cases matched in age, sex, pathological type and tumor size were selected to undergo IHC.

The results of IHC demonstrated that phospho-MAPK was expressed in the nucleus of normal tissues. The expression of phospho-MAPK was negative and had no difference between $BRAF^{V600E}$ and $BRAF^{WT}$ thyroid carcinomas tissues (Fig. 3A; χ^2 test or Fisher's exact test, P>0.05, Table III). However, unexpectedly, phospho-MAPK levels were significantly decreased in tumor tissues compared with those in matched normal thyroid tissues, particularly in the tissues with $BRAF^{V600E}$ mutations (P<0.05; Fig. 3A). In the $BRAF^{V600E}$ samples, only 3.33% (1/30) demonstrated positive stained nuclei phospho-MAPK in tumor tissues, while 50% (15/30) demonstrated strongly stained nuclei in normal thyroid tissues (Fig. 3B and C). However, in the $BRAF^{WT}$ cases, only 3.33% (1/30) had nuclei that stained positive for phospho-MAPK in the tumor tissues, while 36.67% (11/30) demonstrated strongly positive nuclei staining in normal thyroid tissues (Fig. 3B and C).

Further evaluations were made concerning the expression of Mps1 in cytoplasm. The expression of Mps1 was significantly increased in tumor tissues compared with that
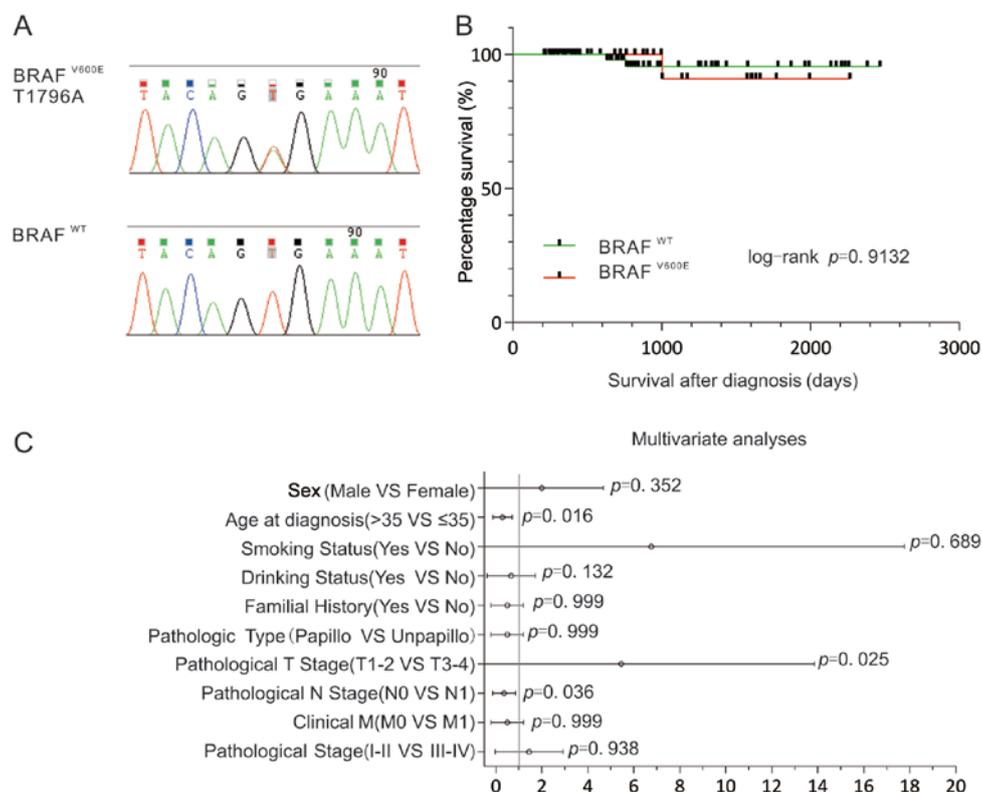
Figure 1. BRAF[V600E] mutation and its association with clinical parameters in thyroid carcinomas. (A) Sanger sequencing peak figure of BRAF gene, The BRAF[V600E] mutation shows additional peaks compared with the wild-type, (B) Kaplan-Meier survival curves for patients with BRAF[V600E] mutations or wild-type in the cohort, (C) Cox regression analyses were used to assess the association of BRAF[V600E] mutation with clinical parameters in thyroid carcinomas. BRAF, B-Raf proto-oncogene serine/threonine kinase.

in matched normal tissues (P<0.05; Fig. 3A), and it was significantly increased in BRAF[V600E] mutation-positive tissues compared with that in BRAF[WT] tissues (Fig. 3A; χ^2 test or Fisher's exact test, P<0.05, Table III). Furthermore, 71.67% (43/60) of the samples demonstrated cytoplasmic stained Mps1 in the tumor tissues, while 13.3% (8/60) stained positive in normal tissues (Fig. 3B). When the BRAF status was analyzed, 96.67% (29/30) of the samples were cytoplasmic-positive for Mps1 in BRAF[V600E] samples, while 60% (18/30) were cytoplasmic-positive in BRAF[WT] samples (Fig. 3B and 3C).

Association between phospho-MAPK or Msp1 expression and clinical parameters. Analysis of phospho-MAPK expression and clinical parameters revealed that the expression of phospho-MAPK in thyroid carcinomas had no significant association with clinical parameters, including sex, age, smoking status, drinking status, familial history, tumor grade, pathological N stage, clinical M and pathological stage (P>0.05, Table II). The expression of Mps1 had no significant association with clinical parameters, including sex, age, smoking status, drinking status, familial history, tumor grade, pathological T stage, pathological N stage, clinical M and pathological stage (P>0.05, Table II).

Associations between phospho-MAPK or Msp1 expression and BRAF mutation. The associations between phospho-MAPK or Msp1 expression and BRAF mutation were analyzed. No association was identified between the expression of phospho-MAPK and BRAF mutation (P>0.05, Table III).

However, there was significant association between the expression of Mps1 and BRAF mutation (P<0.05, Table III). The expression of phospho-MAPK and Mps1 were not significantly associated (Linear regression correlation coefficient $R^2=0.032$, P>0.05).

Discussion

The majority of patients with thyroid carcinoma are cured routinely and have a relatively good prognosis (31). Generally, patients with thyroid carcinomas are treated with thyroidectomy, and then radioiodine to remove residual tumor tissue and metastasis (12,32). However, in certain patients, thyroid cancer is diagnosed as a poorly differentiated carcinoma or anaplastic thyroid carcinoma rather than well-differentiated PTC. These patients also have significantly reduced survival (32). Therefore, it is important to identify novel therapeutic approaches for these types of thyroid carcinomas.

Thyroid carcinomas are associated with RAF-MEK-MAPK signaling (33). It has been revealed that BRAF[V600E] causes a 500-fold increase in activation of BRAF, and activates MEK-MAPK signaling constantly to regulate the expression of a variety of malignant tumor-associated genes, resulting in cell proliferation and differentiation (34-36). Thus, it serves an important function in the occurrence and progression of cancer.

BRAF[V600E] mutation appears to be the most frequent ontogenetic event in thyroid carcinomas. Due to its high frequency and specificity for thyroid carcinomas, the BRAF[V600E] mutation serves a unique and fundamental

Table II. Associations between BRAF[V600E] mutation/phospho-MAPK/Mps1 and clinicopathological parameters in thyroid carcinomas.

Clinicopathological feature	B-RAFV600E mutation (n=161)			Phospho-MAPK in tumor (n=60)			MpS1 in tumor (n=60)		
	+	-	P-value	+	-	P-value	+	-	P-value
No. (%)	54 (34%)	107 (67%)		2 (3%)	58 (97%)		47 (78%)	13 (22%)	
Sex			0.514			>0.999			>0.999
Male	13	21		1	29		10	3	
Female	41	86		1	29		37	10	
Age, years			0.025			0.472			>0.999
>35	42	97		2	28		38	11	
≤35	12	10		0	30		9	2	
Smoking status			0.727			0.472			>0.999
Yes	4	5		0	30		2	1	
No	50	102		2	28		45	12	
Drinking status			0.190			0.472			>0.999
Yes	4	2		0	30		2	0	
No	50	105		2	28		45	13	
Familial history			0.211			0.472			
Yes	2	0		0	30		0	0	
No	52	107		2	28		47	13	
Tumor grade			0.367			0.472			
Papillocarcinoma	54	103		2	28		47	13	
Non-papillocarcinoma	0	4		0	30		0	0	
Pathological T stage			0.018			0.472			0.629
T1-T2	50	83		2	28		41	10	
T3-T4	4	24		0	30		6	3	
Pathological N stage			0.004			0.472			0.898
N0	30	34		0	30		28	8	
N1	24	73		2	28		19	5	
Clinical M						0.472			
M0	54	107		2	28		47	13	
M1	0	0		0	30		0	0	
Pathological stage			0.585			0.472			>0.999
I-II	34	72		0	30		33	9	
III-IV	20	35		2	28		14	4	

BRAF, B-Raf proto-oncogene serine/threonine kinase; phospho-MAPK, phospho-mitogen-activated protein kinases; T, tumor; N, lymph node; M, metastasis.

function in thyroid carcinomas (37). Therefore, it is of great importance to investigate the function and molecular mechanism of BRAF[V600E] in thyroid cancer, in order to identify novel treatment strategies, and to improve the survival rate. In the present study, 55 cases (34%) with BRAF[V600E] mutation in 161 cases of thyroid carcinomas were identified. Multiple studies have reported that the frequency of BRAF[V600E] mutation is 30-50% in thyroid carcinomas, and is associated with lymph node metastasis, extra thyroidal extension, tumor size, recurrence and drug tolerance (38-41). Xing (42) conducted a multicenter retrospective study, and after a mean follow-up of

33 months, they identified that the BRAF[V600E] mutation significantly increased cancer-associated mortality. However, other research from Japan and South Korea yielded different results, demonstrating that B-RAF[V600E] was not associated with poor prognosis in thyroid cancer (43,44). The results of the present study revealed that BRAF mutations were significantly more prevalent in younger patients (≤35 years), pathological T1-T2 stage patients, and pathological N0 stage patients. The association of BRAF[V600E] mutations with OS was not statistically significant. This may be due to the good prognosis of thyroid carcinomas, short follow-up time, difference of analysis

Table III. Associations between phospho-MAPK and Msp1 expression, and BRAF mutation status.

PTC	Phospho-MAPK			MpS1		
	+ (%)	- (%)	P-value	+ (%)	- (%)	P-value
BRAF[V600E]	1 (3)	29 (97)		29 (97)	1 (3)	
BRAF[WT]	1 (3)	29 (97)	-	18 (60)	12 (40)	0.002

PTC, papillary thyroid carcinoma; BRAF, B-Raf proto-oncogene serine/threonine kinase; phospho-MAPK, phospho-mitogen-activated protein kinases.
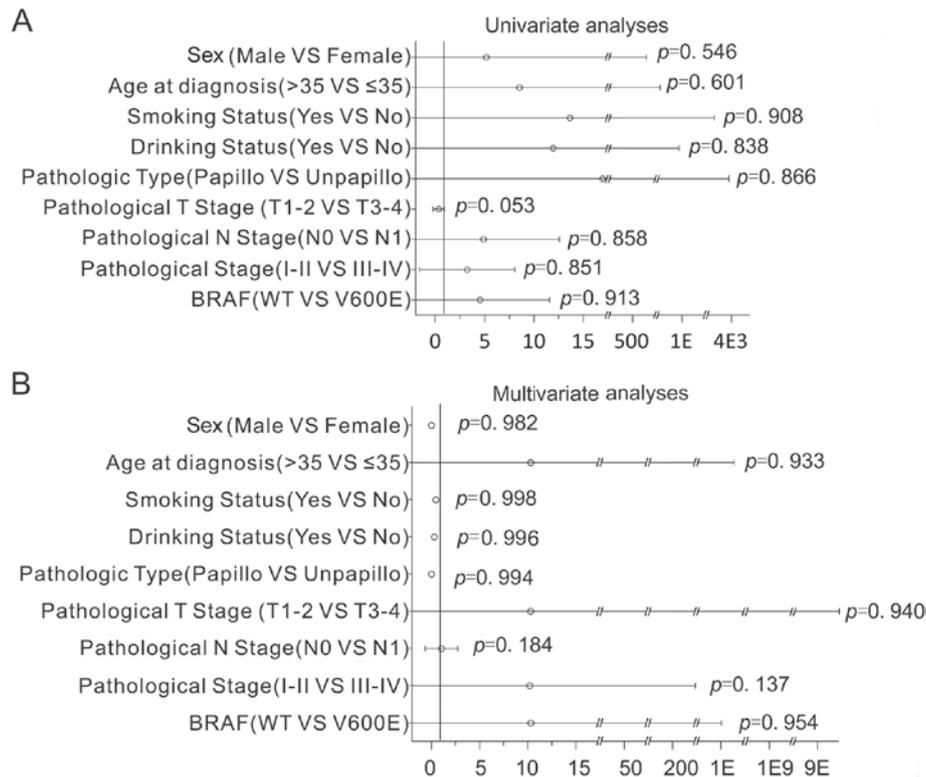

Figure 2. Cox regression analyses according to BRAF mutation status. Cox regression analysis was used to adjust for traditional prognostic factors, (A) Cox univariate regression analyses were used to examine the associations between clinical pathological parameters and prognosis, (B) Cox multivariate regression analyses were used to examine the associations between clinical pathological parameters and prognosis. BRAF, B-Raf proto-oncogene serine/threonine kinase.

method and the genetic difference between those of eastern Asian and Caucasian descent. The specific mechanisms of BRAF[V600E] still require further study.

The development of specific kinase inhibitors targeting BRAF, and the BRAF[V600E] allele in particular, has been achieved. One of these is inhibitor PLX4032. It is a highly selective inhibitor of BRAF kinase and has an anti-proliferative effect on the A375 melanoma cell line, which is BRAF[V600E]-positive. However, no differences in apoptosis and cell cycle in the thyroid carcinoma cell line NPA and ARO were observed, which also carries the BRAF[V600E] mutation. These results may be due to the opposite direction of regulation of p21cip1/waf1 between melanoma and thyroid cancer cells (45). Previous preclinical studies indicated that combination with BAY43-9006 or other agents to increase its efficacy was thought to be a novel strategy for effective clinical therapy (46).

Previous studies have identified Mps1 to be a downstream target of B-RAF[V600E], and demonstrated that is significantly associated with phospho-MAPK in melanoma (30). Based on the high mutation rate of BRAF[V600E] in thyroid carcinoma, the present study detected the expression of phospho-MAPK and Mps1 in thyroid carcinoma by IHC. Compared with normal thyroid tissues, phospho-MAPK was significantly decreased in patients with thyroid carcinomas with the BRAF[V600E] mutation, which was consistent with previous studies (47,48). Furthermore, the expression of phospho-MAPK was not associated with tumor size or clinical stage. The expression of Mps1 in patients with the BRAF[V600E] mutation was significantly higher than that in patients with BRAF[WT], it was significantly higher in tumor tissues than in paired normal tissue, and was not associated with clinical factors. The present study revealed that the expression of phospho-MAPK and Mps1 were not associated in patients with
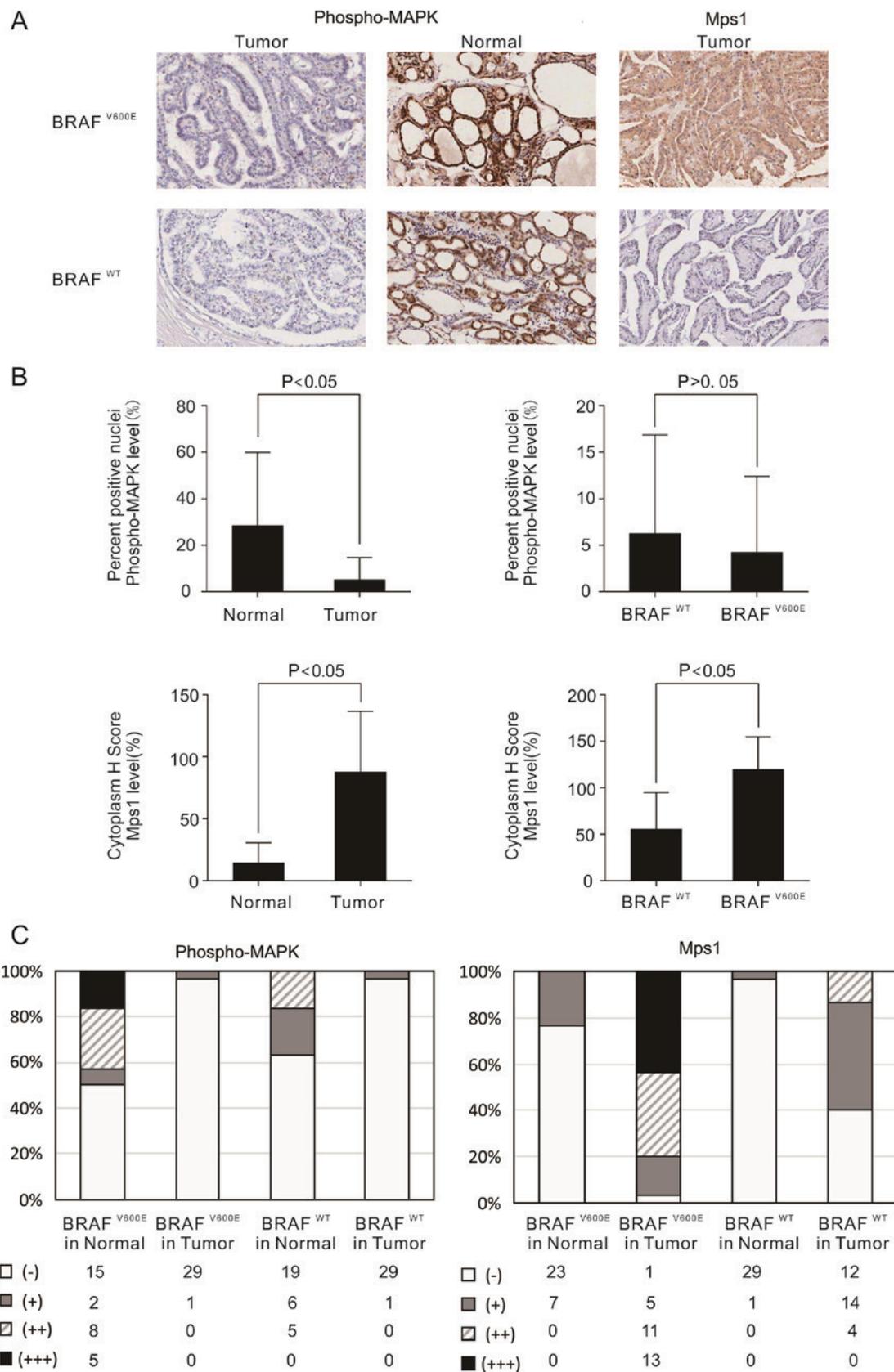
Figure 3. Immunohistochemical staining and evaluation of phospho-MAPK and Mps1 in thyroid carcinomas. (A) Representative immunohistochemistry images revealed phospho-MAPK or Mps1 staining in thyroid carcinomas and corresponding normal tissues with BRAF[WT] or BRAF[V600E] mutations, at x100 magnification. In the BRAF[WT] and BRAF[V600E] mutation of thyroid carcinomas, the expression of phospho-MAPK was negative (first row), while in the normal tissues with BRAF[WT] and BRAF[V600E] mutation, the expression of phospho-MAPK was strongly positive in the nuclei (second row). In the thyroid carcinomas with BRAF[WT], the expression of Mps1 was negative (third row left), while in the thyroid carcinomas with BRAF[V600E] mutation, the expression of Mps1 was strongly positive in the cytoplasm (third row right). (B) The bar graph revealed there was no significant difference in expression of Phospho-MAPK. (C) Cumulative bar chart revealing phospho-MAPK (left panel) and Mps1 (right panel) immunoreactivity in thyroid carcinomas. BRAF, B-Raf proto-oncogene serine/threonine kinase; MAPK, mitogen-associated protein kinase.

BRAF^{V600E}. The classical theory of BRAF^{V600E}, which is that it serves an important function in thyroid carcinomas through the continuous activation of the RAF-MEK-ERK signaling pathway, has been demonstrated in numerous studies *in vivo* and *in vitro* (49,50). However, the results of these studies contradict one another. Potential reasons include the high tumor metabolic rate of thyroid carcinomas. This suggests that the tumor specimens lost their blood supply following operation, and thus the phosphorylation process would be forced to stop due to the lack of ATP, which may lead to the low-level of phospho-MAPK (47). Therefore, the level of phospho-MAPK observed in specimens may not represent the actual level of phospho-MAPK *in vivo*. In addition, MAPK pathway signaling may be important in a context-dependent manner (30). As ERK phosphorylation was not associated with the presence of activating BRAF mutations, the way in which activated BRAF contributes to oncogenesis may be more complex than previously studied. This has important implications for therapeutic approaches targeting the MAP kinase pathway.

In conclusion, the results of the present study suggested that Mps1 expression is associated with BRAF^{V600E} mutation while its upstream signal phospho-MAPK has no relevance. However, as a downstream gene of BRAF, the expression of Mps1 is affected not only by BRAF^{V600E} but also by BRAF^{WT}. These results revealed that BRAF^{V600E} may regulate the expression of Mps1 in MAP kinase independent ways in thyroid carcinoma. In addition, these results demonstrated that the expression of Mps1 was not directly associated with prognosis of thyroid cancer, which may be due to the overall positive prognosis of thyroid cancer and limited number of samples. In future studies, sample size should be expanded to further research and explore the unknown pathways associated with Mps1, in order to provide a theoretical basis for molecular treatment of thyroid cancer.

Acknowledgements

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