Mps1 is associated with the BRAF^{V600E} mutation and predicts poor outcome in patients with colorectal cancer

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Abstract. Colorectal cancer (CRC) with the V600E mutation of B-Raf proto-oncogene serine/threonine kinase (BRAF^{V600E}) mutation is insensitive to chemotherapy and is indicative of a poor patient prognosis. Although BRAF inhibitors have a marked effect on malignant melanoma harboring the BRAF^{V600E} mutation, they have a limited effect on patients with CRC with the same BRAF mutation. A previous study identified a novel gene, monopolar spindle protein kinase 1 (Mps1), a downstream target of BRAF^{V600E} only, rather than of wild-type BRAF as well, which contributes to tumorigenesis in melanoma. In the present study, the incidence of BRAF^{V600E} in patients with CRC was identified and the correlation of Mps1, phospho-extracellular-signal-regulated kinase (p-ERK) and BRAF^{V600E} was investigated. The results indicated that the mutation rate of BRAF^{V600E} was 5.2% in CRC. Poorly differentiated tumors and mucinous tumors have a significantly higher incidence of BRAF^{V600E} compared with well-differentiated tumors and non-mucinous tumors (P<0.05). Kaplan-Meier survival analysis indicated that the survival rate was markedly lower in patients with BRAF^{V600E} compared with in patients with wild-type BRAF (BRAF^{WT}). The expression of p-ERK and Mps1 in CRC with BRAF^{V600E} was significantly higher compared with in CRC with BRAF^{WT} (P<0.05), and their expression is associated with cancer classification, degree of differentiation and lymph node transfusion (P<0.05). In addition p-ERK expression was positively correlated with Mps1 expression, with a contingency coefficient of 0.679 (P=0.002). In conclusion, the results of the present study indicated that Mps1 was significantly associated with BRAF^{V600E}/p-ERK and may serve a crucial function in the development of CRC. The results of the present study raise the possibility that targeting the oncogenic BRAF and Mps1, particularly when in conjunction, could provide promising therapeutic opportunities for the treatment of CRC.

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Introduction

Colorectal cancer (CRC) is one of the most common malignant tumors and is the third highest cancer for incidence and has the second highest mortality (9.2%) of different types of cancer worldwide (1). The principal treatments used for CRC are surgery, radiation therapy, chemotherapy and molecular targeted therapy. As a novel treatment type, molecular targeted therapy has been used in a variety of tumors including CRC (2,3). The therapeutic strategy to target the selected epidermal growth factor receptor has been developed in clinical trials (4). However, it possesses drug resistance in the treatment of patients with CRC harboring the V600E mutation of B-Raf proto-oncogene serine/threonine kinase (BRAF^{V600E}) mutation (5,6). BRAF is an important oncogene and mutant BRAF has been implicated in the pathogenesis of several types of cancer. The 1796T>A mutation results in an amino acid substitution at position 600 in BRAF, from valine to glutamic acid. This mutation occurs within the activation segment of the kinase domain and leads to the continuous activation of the

MAPK/ERK signaling pathway (7,8). Although oral BRAF inhibitors have remarkable clinical activity in metastatic melanomas with BRAF^{V600E}, resistance to therapy invariably develops in patients with CRC with the same BRAF mutation (9,10). Therefore, there is an urgent requirement to develop a novel and effective treatment for these patients.

Our previous study identified a novel gene monopolar spindle protein kinase 1 (Mps1), which is a downstream target of BRAF^{V600E}, and continuously activated BRAF^{V600E} signaling may be a potential mechanism for the deregulation of Mps1 stability and kinase activity in human malignancies (11,12). Persistent phosphorylation of Mps1 through BRAF^{V600E} signaling is a key event in disrupting the control of centrosome duplication and chromosome stability that may contribute to tumorigenesis (13,14). Notably, phospho (p)-Ser^{281} Mps1 staining was demonstrated to be positively associated with p-mitogen-activated protein kinase (MAPK) [extracellular-signal-regulated kinase ERK)1/2] in human melanoma tissues (13). However, to the best of our knowledge, no previous study has investigated whether a correlation exists between BRAF and Mps1 in CRC.

In the present study, the incidence of BRAF^{V600E} was determined in CRC tissues, and the correlation of Mps1 and BRAF^{V600E} and p-ERK in Chinese patients with CRC was determined. The results raise the possibility that targeting the oncogenic BRAF and Mps1, particularly when used in combination, may potentially provide effective therapeutic opportunities for the treatment of CRC.

Materials and methods

Patients and samples. The present study was approved by the Ethics Committee of Shanxi Medical University (Taiyuan, China), and patients provided written informed consent for their inclusion. A total of 288 (156 male and 132 female) paraffin-embedded tissue sections containing the carcinoma and its adjacent non-neoplastic colorectal tissue were obtained from The First Hospital of Shanxi Medical University and TaiYuan Municipal No. 2 People's Hospital collected between January 2009 and June 2015. Among them, there were 284 adenocarcinoma, 1 glandular squamous cell carcinoma, 1 signet-ring cell carcinoma and 2 neuroendocrine carcinoma tissues. The age of patients at the time of diagnosis with CRC ranged between 25 and 92 years, and the median age was 64 years. On the basis of Tumor-Node-Metastasis classification, there were 169 patients at stage I and II, 119 patients at stage IIIb and IV (15) 110 cases with lymph metastasis, and 178 cases without lymph node (LN) metastasis (Table I). All patients were diagnosed with CRC, with no previous history of other malignant tumor types, and had not received other treatments prior to surgery. In 183 cases [168 cases with wild-type BRAF (BRAF^{WT}) and 15 cases with BRAF^{V600E}], no cases of squamous cell carcinoma were identified. The complete clinical data and follow-up data were included in the survival analysis.

DNA extraction. 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 independently reviewed by two pathologists. DNA was extracted using the FFPE DNA kit (Omega Bio-Tek, Inc., Norcross, GA, USA), according to the manufacturer's protocol. The DNA concentration was determined using a NanoDrop 2000 instrument and the 260/280 nm ratio was calculated to evaluate the quality of DNA. The sample concentration was adjusted to 200-300 ng/μl, and DNA was stored at -80˚C.

Polymerase chain reaction (PCR). Using the DNA template above, and a 252-bp fragment of BRAF exon 15 was obtained using PCR. The primers of BRAF were 5'-CTTGCCACA GGTCTCCCC-3' (forward) and 5'-TCTAGTAACTCAGCA GCATCTCAGG-3' (reverse). The PCR was carried out in 10 μl PCR buffer, 4 μl dNTP, 2 μl DNA, 1.5 μl forward primer, 1.5 μl reverse primer, 1 μl DNA polymerase (PrimeSTAR GXL DNA Polymerase TAKARA Japan) and double-distilled water for a total reaction volume of 50 μl. The amplification procedure was pre-denaturation for 3 min at 98˚C, followed by 30 cycles of 30 sec at 98˚C, 30 sec at 58˚C and 30 sec at 72˚C. The PCR product was examined by 2% agarose electrophoresis and stained by ethidium bromide, then detected under UV. Sanger sequencing was performed by Beijing Liuhe Huada Gene Technology Company (Beijing, China).

Immunohistochemistry (IHC). According to the sequencing results, 15 cases harboring BRAF^{V600E} and the same number of patients harboring BRAF^{WT} were randomly selected for IHC, with rabbit anti-human Mps1 antibody (cat. no. ab135819; Abcam, Cambridge, UK) and rabbit anti-human p-ERK antibody (cat. no. 4376; Cell Signaling Technology, Inc., Danvers, MA, USA) as primary antibodies, and horseradish peroxide (HRP)-labeled goat anti-rabbit immunoglobulin G (IgG; cat. no. A20120A0704; BioTNT, Shanghai, China) as a secondary antibody. A known positive tissue section served as a positive control. A negative control was established using PBS instead of the primary antibody.

The IHC analysis was performed as follows: 4 micron thick sections were moved to anhydrous ethanol for 2 min at room temperature, then placed in 95% ethanol liquid cylinder for 2 min, then moved to 85% ethanol liquid cylinder for 2 min, and finally placed in 75% ethanol liquid cylinder for 2 min, and then incubated in 3% hydrogen peroxide to block endogenous peroxidase for 15 min at room temperature, followed by washing with PBS three times and soaking in distilled water for 5 min. Antigen retrieval was performed with citrate liquid in a microwave at 92-98˚C for 15 min. Sections were blocked with 10% goat serum (1201A Shanghai Biyun day Biotechnology Co., Ltd China) at 37˚C for 30 min and rabbit anti-human Mps1 monoclonal antibody (1:100) or anti-p-ERK antibody (1:400) was added, and incubated at 4˚C overnight. The slides were then washed with PBS three times and incubated with HRP-labeled goat anti-rabbit IgG at 37˚C for 20 min, followed by washing with PBS three times and using 3,3'-diaminobenzidine as a chromogen. Sections were counterstained with hematoxylin for 1 min at room temperature, dehydrated in graded alcohol and sealed with resin sealing agent.

Fully automatic digital pathological scanning apparatus (Leica Microsystems, Inc., Buffalo Grove, IL, USA) were used to obtain high-resolution digital images. A total of five high-power fields of vision (x400 magnification) were randomly selected and analyzed using Image Scope software

Table I. Clinicopathological information of the 288 patients with colorectal cancer in the present study.

Clinicopathological feature	n
Sex	
Male	156
Female	132
Age, years	
>60	195
≤60	93
Smoking status	
Yes	47
No	241
Drinking status	
Yes	259
No	29
Differentiation	
Well	51
Medium and poor	237
Pathological pattern	
Mucinous carcinoma	24
Other	264
T[a] (infiltration depth)	
>T3	79
≤T3	209
Lymph node metastasis	
Positive	110
Negative	178
Clinical stage (15)	
I-II	169
III-IV	119
Location	
Rectum	125
Colon	163

[a]>T3 includes T4a (tumor penetrates to the surface of the visceral peritoneum) and T4b (tumor directly invades or is adherent to other organs or structures). ≤T3 includes T1 (tumor invades submucosa), T2 (tumor invades muscularis propria) and T3 (tumor invades through the muscularis propria into the pericolorectal tissues) according to NCCN Guidelines version 2.2015 Staging Colon Cancer (15). LN, lymph node.

(12.0; Leica Microsystems, Inc.). The average value of the positive rate of five randomly selected fields of view was obtained using standardized cell nuclear analysis parameters. Nuclear p-ERK expression was classified as negative for 0-35 and positive for >35. Mps1 expression in the cytoplasm was classified as negative for 0-70 and positive for >70.

Statistical analysis. Continuous variables are presented as the mean (SD), and categorical variables are presented as count values (percentages). The p-ERK and Mps1 positive expression levels were divided into four groups based on inter-quartile range respectively, which were 35-45.71, 45.71-51.14 51.14-65.54, and ≥65.54 for p-ERK, as well as 70-88.73, 88.73-117.33, 117.33-157.12 and ≥157.12 for Mps1. The differences were analyzed by χ^2 test. χ^2 test or Fisher's exact test was also used to assess the association between BRAF mutation status and clinical parameters. Univariate and multivariate survival analyses were performed using a Cox proportional hazards regression model. The contingency coefficient was used to evaluate the correlation between p-ERK and Mps1 expression. A sensitivity analysis was conducted using Spearman's rank analysis was performed to determine the correlation between p-ERK and Mps1. Kaplan-Meier survival analysis and the log-rank test were used to analyze the association between $BRAF^{V600E}$ and prognosis. Data were analyzed using SPSS software (version 20.0; IBM Corp., Armonk, NY, USA). P<0.05 was considered to indicate a statistically significant difference.

Results

$BRAF^{V600E}$ mutation and its association with clinical parameters in CRC. Sanger DNA sequencing was used to detect the 1796T>A (V600E) mutation, which was the most frequently observed mutation site in BRAF (Fig. 1A and B). In 288 cases of colorectal cancer, 15 cases of BRAF mutation were identified. The rate of $BRAF^{V600E}$ was 5.2% in CRC. A statistical analysis of BRAF mutations and clinical parameters revealed that $BRAF^{V600E}$ was further associated with the age, infiltrating depth, pathological pattern of CRC, were more prevalent in older patients (>60 years), infiltrating depth>T3 stage patients and the mucinous tumors (χ^2 test or Fisher's exact test P<0.05; Table II). However, no association of the BRAF mutation with location, clinical stage, LN metastasis, differentiation and sex were identified (χ^2 test or Fisher's exact test P>0.05; Table II). Despite previous studies attempting to identify specific risk factors, no dietary and lifestyle factors have been clearly associated with the development of BRAF mutated CRC (16-18). The results of the present study suggest that the BRAF mutation was not associated with smoking, alcohol intake (χ^2 test or Fisher's exact test P>0.05; Table II).

$BRAF^{V600E}$ mutation is associated with a poor prognosis of patients with CRC. Kaplan-Meier survival analysis indicated that the survival rate was significantly lower in patients with $BRAF^{V600E}$ mutation compared with those with $BRAF^{WT}$ (Fig. 1C). The median survival time of patients with $BRAF^{V600E}$ and $BRAF^{WT}$ were 300 and 429.5 days respectively.

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 statistically significant (Cox regression multivariate analysis HR=0.32, P=0.051; Fig. 2A; Cox regression univariate analyses, HR=0.36, P=0.03, Fig. 2B). Although the P-value of $BRAF^{V600E}$ in the multivariate analysis was 0.051These results suggested that $BRAF^{V600E}$ may serve an important function in specific pathological CRC, and may function as an independent prognostic factor and a novel oncological therapeutic strategy.

IHC and evaluation of p-ERK and Mps1 in CRC. According to the sequencing results, 15 cases harboring $BRAF^{V600E}$

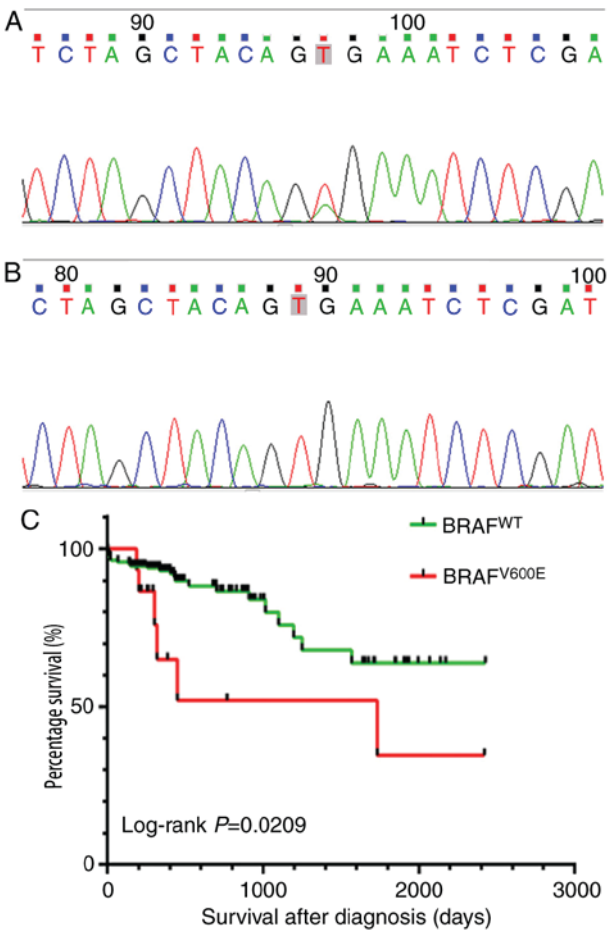

Figure 1. Sanger sequencing peak map of BRAF. (A) Sanger sequencing peak map of BRAF^{V600E}. The BRAF^{V600E} mutation shows additional peaks compared with the wild-type, which is indicated by the grey box. (B) Sanger Sequencing peak map of BRAF^{WT}. The site at position 1796 was not mutated. (C) Kaplan-Meier survival analysis indicated that the survival rate was significantly lower in patients with BRAF^{V600E} mutation compared with those with BRAF. BRAF, B-Raf proto-oncogene serine/threonine kinase; WT, wild-type.

and the same number of patients harboring BRAF^{WT} were randomly selected for IHC with anti-Mps1 and anti-p-ERK antibodies. The positive expression of p-ERK protein was brown and localized in the nucleus (Fig. 3). And the positive rate of p-ERK expression was 93.3% in colorectal cancer tissue with BRAF^{V600E}, while the positive rate of p-ERK expression was 6.7% in paired normal tissues, which the difference was statistically significant in BRAF^{V600E} mutation cases (Fig. 4A, χ^2 test, P<0.05). However, In BRAF^{WT} cases, there was no significant difference in the expression of p-ERK between colorectal cancer and paired normal tissues (Fig. 4A, χ^2 test, P>0.05).

Further evaluations were made concerning the expression of Mps1 in colorectal cancer. the positive expression of Mps1 protein was brown and localized in the cytoplasm (Fig. 3). The positive expression of Mps1 in CRC with BRAF^{V600E} was significantly higher compared with that in paired normal tissues (Fig. 4B, χ^2 test, P<0.05). While there was no significant difference in the expression of Mps1 between colorectal cancer and paired normal tissues in BRAF^{WT} cases. (Fig. 4B, χ^2 test, P>0.05).

Table II. Association between BRAF^{V600E} mutation and clinicopathological parameters in colorectal cancer.

Clinicopathological feature	BRAF^{V600E} mutation + (n=15; 5.2%)	- (n=273; 94.8%)	P-value
Sex			0.739
Male	7	149	
Female	8	124	
Age, years			0.043
>60	14	181	
≤60	1	92	
Smoking status			0.142
Yes	0	47	
No	15	226	
Drinking status			0.378
Yes	0	29	
No	15	244	
Differentiation			0.082
Well	0	51	
Medium and poor	15	222	
Pathological pattern			0.001
Mucinous carcinoma	6	18	
Others	9	255	
T[a] (infiltration depth)			<0.001
>T3	15	64	
≤T3	0	209	
Lymph node metastasis			0.882
Positive	6	104	
Negative	9	169	
Clinical stage (15)			0.871
I-II	8	161	
III-IV	7	112	
Location			0.107
Rectum	3	122	
Colon	12	151	

[a]>T3 includes T4a (tumor penetrates to the surface of the visceral peritoneum) and T4b (tumor directly invades or is adherent to other organs or structures). ≤T3 includes T1 (tumor invades submucosa), T2 (tumor invades muscularis propria) and T3 (tumor invades through the muscularis propria into the pericolorectal tissues) according to NCCN Guidelines version 2.2015 Staging Colon Cancer (15). BRAF, B-Raf proto-oncogene serine/threonine kinase.

Association between p-ERK or Mps1 expression and clinical parameters. Subsequently, the association of expression of p-ERK and Mps1 with clinical pathological features was analyzed (Table III). p-ERK expression was associated with LN metastasis, pathology type and degree of differentiation (χ^2 test, P<0.05; Table III). The expression of p-ERK was significantly higher in poorly differentiated adenocarcinoma and mucinous adenocarcinoma compared with that in highly differentiated

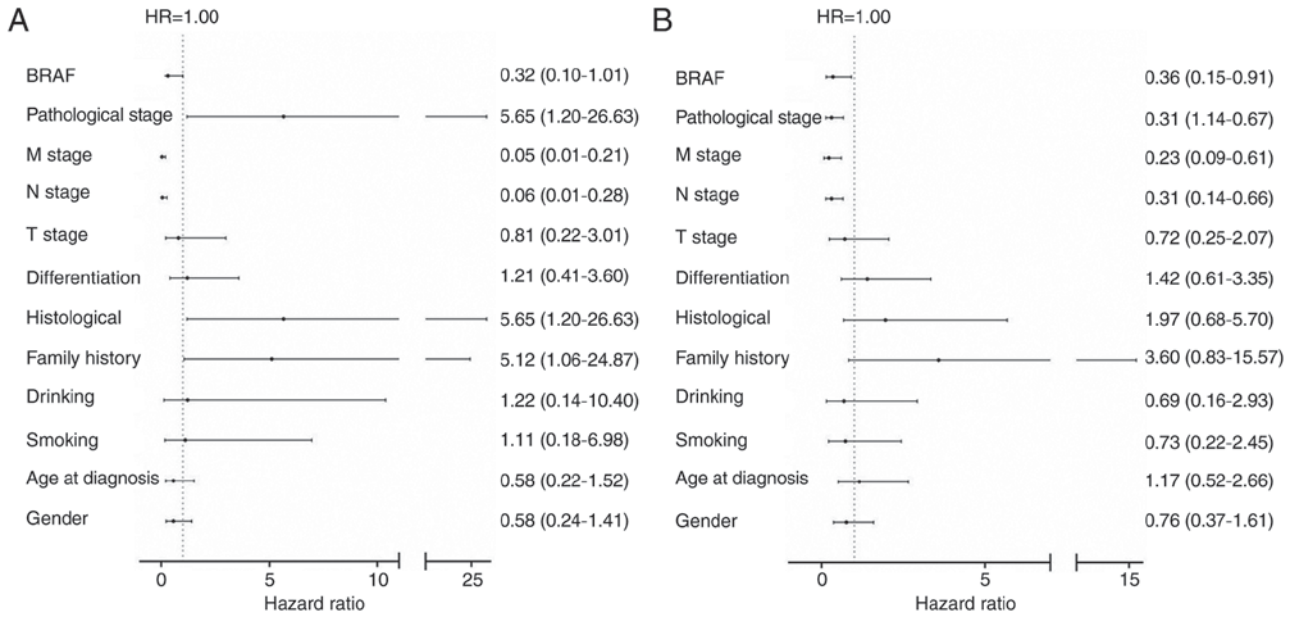

Figure 2. Multivariate and univariate analyses of survival prognosis in CRC. (A) Multivariate analyses of survival prognosis in CRC (B) Univariate analysis of survival prognosis in CRC. BRAF, WT vs. V600E; pathological stage, I-II vs. III-IV; M stage, M0 vs. M1; N stage, N0 vs. N1; T stage, T1-2 vs. T3-4; differentiation, poor vs. medium + well; histological, mucous adenocarcinoma vs. others; family history, yes vs. no; drinking, yes vs. no; smoking, yes vs. no; age at diagnosis, ≥60 years vs. <60 years; sex, male vs. female. CRC, colorectal cancer; BRAF, B-Raf proto-oncogene serine/threonine kinase; WT, wild-type; HR, hazard ratio; T, tumor; N, node; M, metastasis. The bracketed values represent 95% confidence intervals.

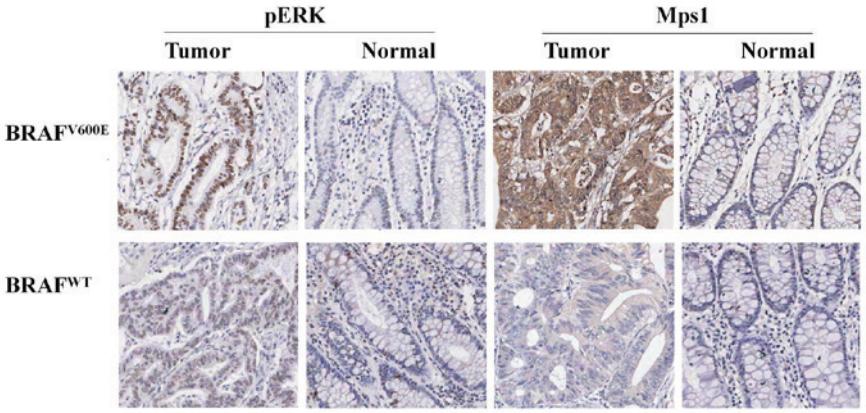

Figure 3. Representative immunohistochemistry images revealed p-ERK or Mps1 staining in colorectal cancer and corresponding normal tissues with $BRAF^{WT}$ or $BRAF^{V600E}$ mutations, at x100 magnifcation. In the $BRAF^{V600E}$ mutation of colorectal cancer, the positive expression of p-ERK and Mps1 protein were brown and localized in the nucleus and the cytoplasm respectively, while in the normal tissues with $BRAF^{V600E}$ mutation, the expression of pERK and Mps1 was negtive. In the colorectal cancer with $BRAF^{WT}$, there was no significant difference in the expression of p-ERK or Mps1 between colorectal cancer and paired normal tissues. p-ERK, phospho-extracellular-signal-regulated kinase; Mps1, monopolar spindle protein kinase 1. (x100 magnification).

adenocarcinoma and non-mucinous adenocarcinoma, as well as in the group with LN metastasis compared with without LN metastasis. (χ^2 test, P<0.05; Table III); however, p-ERK expression was not associated with age, sex, smoking status, drinking status, location or T stage in CRC (χ^2 test, P>0.05; Table III).

Positive Mps1 expression was significantly greater in poorly differentiated carcinoma compared with in well-differentiated adenocarcinoma in CRC (χ^2 test, P<0.05, Table III). There were no significant associations between positive expression of Mps1 and age, sex, smoking status, drinking status, location, pathological type, LN metastasis or T stage in CRC (χ^2 test, P>0.05; Table III).

In addition, the expression of p-ERK and Mps1 between the $BRAF^{V600E}$ and $BRAF^{WT}$ groups were then compared, and the expression of p-ERK and Mps1 in the $BRAF^{V600E}$ group was higher compared with those in the $BRAF^{WT}$ group (Fisher's exact test, P<0.05). Expression of p-ERK was correlated positively with the Mps1 expression, with a contingency coefficient of 0.679 (P=0.002; Table IV). In the sensitivity analysis, it was also identified that p-ERK expression was positively correlated with the expression of Mps1 (Spearman's rank correlation analysis correlation coefficient 0.623; P<0.001; Fig. 4C).

Discussion

The results of the present study demonstrated that the incidence of $BRAF^{V600E}$ was 5.2% in CRC, which was consistent with previously published rates, between 5 and 15% (19-24). The

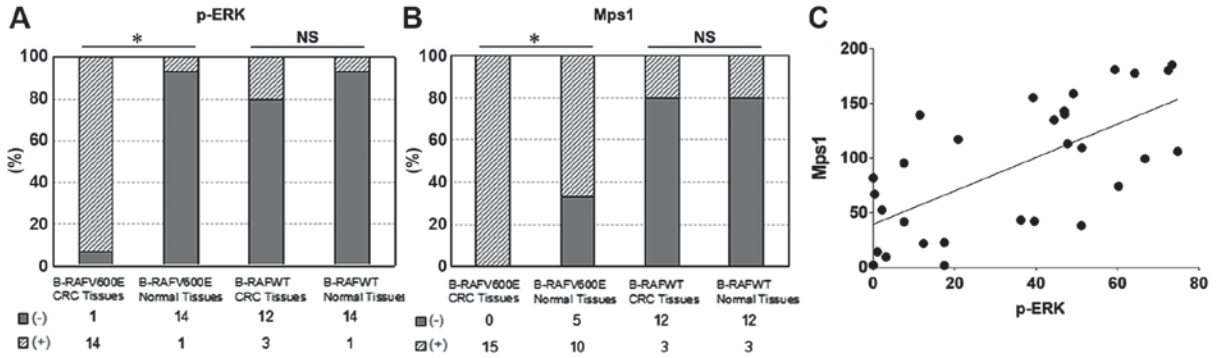

Figure 4. Mps1 is significantly associated with BRAF^{V600E}/p-ERK. (A) The expression of p-ERK between BRAF^{V600E} and BRAF^{WT}. (B) The expression of Mps1 between BRAF^{V600E} and BRAF^{WT}. (C) A sensitivity analysis was conducted using Spearman's rank correlation analysis in order to evaluate the correlation between p-ERK and Mps1. The results demonstrated that p-ERK expression was positively correlated with Mps1 expression (contingency coefficient, 0.623; P<0.001). BRAF, B-Raf proto-oncogene serine/threonine kinase; Mps1, monopolar spindle protein kinase 1; p-ERK, phospho-extracellular-signal-regulated kinase; WT, wild-type *P<0.05. The p-ERK and Mps1 positive expression levels between colorectal cancer and paired normal tissues in BRAF^{V600E} group is statistically significant (P<0.05); NS. The p-ERK and Mps1 positive expression levels between colorectal cancer and paired normal tissues in BRAF^{WT} group is not statistically significant (P>0.05).

difference mentioned above may be due to the complicated genetic background of different ethnicities. In the study by Yoon *et al* (25), BRAF mutation frequency in CRC from Caucasians (13.9%) was twice that of tumors from Asians (5.6%) or individuals of African (6.4%) descent.

Malignant tumors with the BRAF^{V600E} mutation have been demonstrated to be associated with mortality in patients with colorectal cancer (26). Numerous studies have demonstrated that the malignant tumor with BRAF^{V600E} is insensitive to the traditional treatments and patients have a poor prognosis (27-29). With the success of BRAF inhibitors in malignant melanoma, there is concern about the efficacy of BRAF inhibitors in other tumors with BRAF^{V600E} mutations (9,30). However, BRAF inhibitors exhibited severe adverse effects in the treatment of patients with CRC harboring the BRAFV600E mutation (31). A previous clinical study compared the expression of p-ERK between pre- and post-treatment with BRAF inhibitors, but the results showed that the downregulation of p-ERK only occurred in 47% of patients with CRC harboring the BRAF^{V600E} mutation (32). This indicates that the inhibition of the MAPK signaling pathway by this BRAF inhibitor is insufficient, which may be a principal reason for the low response rate of BRAF inhibitors. Thus, identifying novel strategies for the full and sustained inhibition of the MAPK pathway in patients with CRC with the BRAF mutant is of marked clinical importance.

Mps1, a member of the spindle-monitoring complex, is involved in centrosome duplication and spindle checkpoint (33) and cell cycle regulation, and has maximum kinase activity in the M phase of the cell cycle (34). Typically, Mps1 is an unstable protein, which is degraded by the ubiquitin-proteasome pathway when centrosome duplication is completed, and cells enter anaphase (35). It has been reported that either too high or too low Mps1 kinase activity results in aberrations in centrosome duplication (36), lead to aneuploidy formation and result in malignant tumor formation (37). Currently, Mps1 has been reported in the breast, colon and other malignant tumors with high expression, and may facilitate tumor cell evasion from apoptosis, culminating in carcinogenesis (38-40). Our previous study identified that Mps1 is a downstream target of BRAF^{V600E} (12). Persistent phosphorylation of Mps1 through BRAF^{V600E} signaling is a key event in disrupting the control of centrosome duplication and chromosome stability that may contribute to tumorigenesis (13). Thus, Mps1 may serve as a novel therapeutic target for patients with CRC harboring the BRAF^{V600E} mutation.

The effect of Mps1 kinase inhibitors have been investigated in a variety of malignant tumors, with promising results (41,42). The present study initially identified that Mps1 was significantly associated with BRAF^{V600E}/p-ERK in CRC. It was indicated that Mps1, the downstream target of the BRAF^{V600E}/MAPK/ERK kinase/ERK signaling pathway, may serve a significant function in the development of CRC. The use of a BRAF inhibitor combined with an Mps1 inhibitor may provide a novel therapeutic approach for treating patients with CRC harboring the BRAF^{V600E} mutation, who were previously resistant or insensitive to the BRAF inhibitor.

However, there were some limitations to the present study. For example, the data pertaining to the 5-year survival rate are still being collected, and the sample size of BRAF^{V600E} is not large enough, which leads to the lack of representativeness. However, even if the sample size of BRAF^{V600E} is small, the data still conform to the normal distribution, ensuring the accuracy and integrity of the results. We will analyze the 5-year survival data in further study. More samples of BRAF^{V600E} will be selected in statistical analysis in further research.

In conclusion, the present study demonstrated that Mps1 was significantly associated with BRAF^{V600E}/p-ERK and may serve a crucial function in the development of CRC. Targeting the oncogenic BRAF^{V600E} and Mps1, particularly when used in combination, could potentially provide therapeutic opportunities for the treatment of cancer.

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Table III. Association between p-ERK/Mps1 and clinicopathological parameters in colorectal cancer.

	p-ERK in tumor			Mps1 in tumor		
Clinicopathological feature	+ (n=17; 56.7%)	- (n=13; 13.3%)	P-value	+ (n=18; 60%)	- (n=12; 40%)	P-value
Sex						
Male	10	9		9	10	
Female	7	4	0.558	9	2	0.121
Age, years						
>60	15	11		17	9	
≤60	2	2	0.773	1	3	0.274
Smoking status						
Yes	1	1		0	2	
No	16	12	0.844	18	10	0.152
Drinking status						
Yes	1	0		0	1	
No	16	13	1.000	18	11	0.400
Differentiation						
Well	1	6		1	6	
Medium and poor	16	7	0.01	17	6	0.009
Pathological pattern						
Mucinous carcinoma	6	0		6	0	
Others	11	13	0.024	12	12	0.057
T[a] (infiltration depth)						
>T3	4	1		5	0	
≤T3	13	12	0.355	13	12	0.066
Lymph node metastasis						
Positive	8	1		6	3	
Negative	9	12	0.02	12	9	0.626
Clinical stage (15)						
I-II	8	9		10	7	
III-IV	9	4	0.225	8	5	0.88
Location						
Rectum	13	9		14	8	
Colon	4	4	0.698	4	4	0.679

[a]>T3 includes T4a (tumor penetrates to the surface of the visceral peritoneum) and T4b (tumor directly invades or is adherent to other organs or structures). ≤T3 includes T1 (tumor invades submucosa), T2 (tumor invades muscularis propria) and T3 (tumor invades through the muscularis propria into the pericolorectal tissues) according to NCCN Guidelines version 2.2015 Staging Colon Cancer (15). p-ERK, phospho-extracellular-signal-regulated kinase; Mps1, monopolar spindle protein kinase 1.

Table IV. Association between the p-ERK or Mps1 expression and BRAF mutation in colorectal cancer.

	p-ERK			Mps1		
CRC	+	-	P-value	+	-	P-value
$BRAF^{V600E}$	14 (93.3%)	1 (6.7%)		15 (100%)	0 (0%)	
$BRAF^{WT}$	3 (20%)	12 (80%)	<0.001	3 (20%)	12 (80%)	0.002

CRC, colorectal cancer; BRAF, B-Raf proto-oncogene serine/threonine kinase; p-ERK, phospho-extracellular-signal-regulated kinase; Mps1, monopolar spindle protein kinase 1; WT, wild-type.

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Availability of data and materials

The datasets used or analyzed during the present study are available from the corresponding author on reasonable request.

Authors' contributions

JL and JG analyzed and interpreted the data of the study. YZ, JD, RS and YL are responsible for specific experimental work including DNA extraction of colorectal cancer tissues and polymerase chain reaction. CC, BS, YB and HH took charge for collection of case samples and IHC examination. LF, PK and LZ performed the statistical analysis. YZ was also a major contributor in writing the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The present study was approved by the Ethics Committee of Shanxi Medical University and patients provided written informed consent.

Patient consent for publication

There is no disclosure of any personal, identifiable, non-anonymized patient information in the manuscript. The patients in the study provided their consent for the publication of this data and any associated images.

Competing interests

The authors declare that they have no competing interests.

References

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA and Jemal A: Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 68: 394-424, 2018.
2. Brenner H, Kloor M and Pox CP: Colorectal cancer. Lancet 383: 1490-1502, 2014.
3. Stintzing S: Recent advances in understanding colorectal cancer. F1000Res 7: F1000 Faculty Rev-1528, 2018.
4. Tímár J, Hegedüs B and Rásó E: KRAS mutation testing of colorectal cancer for anti-EGFR therapy: Dogmas versus evidence. Curr Cancer Drug Targets 10: 813-823, 2010.
5. Prahallad A, Sun C, Huang S, Di Nicolantonio F, Salazar R, Zecchin D, Beijersbergen RL, Bardelli A and Bernards R: Unresponsiveness of colon cancer to BRAF(V600E) inhibition through feedback activation of EGFR. Nature 483: 100-103, 2012.
6. Königsberg R, Hulla W, Klimpfinger M, Reiner-Concin A, Steininger T, Büchler W, Terkola R and Dittrich C: Clinical and economic aspects of KRAS mutational status as predictor for epidermal growth factor receptor inhibitor therapy in metastatic colorectal cancer patients. Oncology 81: 359-364, 2011.
7. Davies H, Bignell GR, Cox C, Stephens P, Edkins S, Clegg S, Teague J, Woffendin H, Garnett MJ, Bottomley W, *et al*: Mutations of the BRAF gene in human cancer. Nature 417: 949-954, 2002.
8. Dizdar L, Werner TA, Drusenheimer JC, Möhlendick B, Raba K, Boeck I, Anlauf M, Schott M, Göring W, Esposito I, *et al*: BRAFV600E mutation: A promising target in colorectal neuroendocrine carcinoma. Int J Cancer, 2018.
9. Kopetz S, Desai J, Chan E, Hecht JR, O'Dwyer PJ, Maru D, Morris V, Janku F, Dasari A, Chung W, *et al*: Phase II pilot study of vemurafenib in patients with metastatic BRAF-mutated colorectal cancer. J Clin Oncol 33: 4032-4038, 2015.
10. Su F, Viros A, Milagre C, Trunzer K, Bollag G, Spleiss O, Reis-Filho JS, Kong X, Koya RC, Flaherty KT, *et al*: RAS mutations in cutaneous squamous-cell carcinomas in patients treated with BRAF inhibitors. N Engl J Med 366: 207-215, 2012.
11. Cui Y and Guadagno TM: B-Raf(V600E) signaling deregulates the mitotic spindle checkpoint through stabilizing Mps1 levels in melanoma cells. Oncogene 27: 3122-3133, 2008.
12. Cui Y, Borysova MK, Johnson JO and Guadagno TM: Oncogenic B-Raf(V600E) induces spindle abnormalities, supernumerary centrosomes, an aneuploidy in human melanocytic cells. Cancer Res 70: 675-684, 2010.
13. Liu J, Cheng X, Zhang Y, Li S, Cui H, Zhang L, Shi R, Zhao Z, He C, Wang C, *et al*: Phosphorylation of Mps1 by BRAFV600E prevents Mps1 degradation and contributes to chromosome instability in melanoma. Oncogene 32: 713-723, 2012.
14. Zhang L, Shi R, He C, Cheng C, Song B, Cui H, Zhang Y, Zhao Z, Bi Y, Yang X, *et al*: Oncogenic B-Raf(V600E) abrogates the AKT/B-Raf/Mps1 interaction in melanoma cells. Cancer Lett 337: 125-132, 2013.
15. NCCN Guidelines version 2.2015 Staging Colon Cancer. [https://www.nccn.org/professionals/physician_gls/default.aspx#site].
16. Fu X, Huang Y, Fan X, Deng Y, Liu H, Zou H, Wu P, Chen Z, Huang J, Wang J, *et al*: Demographic trends and KRAS/BRAF^{V600E} mutations in colorectal cancer patients of South China: A single-site report. Int J Cancer, 2018 Nov 10. Doi: 10.1002/ijc.31973.
17. Sawada K, Nakamura Y, Yamanaka T, Kuboki Y, Yamaguchi D, Yuki S, Yoshino T, Komatsu Y, Sakamoto N, Okamoto W and Fujii S: Prognostic and predictive value of HER2 amplification in patients with metastatic colorectal cancer. Clin Colorectal Cancer 17: 198-205, 2018.
18. Bläker H, Alwers E, Arnold A, Herpel E, Tagscherer KE, Roth W, Jansen L, Walter V, Kloor M, Chang-Claude J, *et al*: The association between mutations in BRAF and colorectal cancer-specific survival depends on microsatellite status and tumor stage. Clin Gastroenterol Hepatol: S1542-3565(18)30371-9, 2018.
19. Tie J, Gibbs P, Lipton L, Christie M, Jorissen RN, Burgess AW, Croxford M, Jones I, Langland R, Kosmider S, *et al*: Optimizing targeted therapeutic development: Analysis of a colorectal cancer patient population with the BRAF(V600E) mutation. Int J Cancer 128: 2075-2084, 2011.
20. Pinheiro M, Ahlquist T, Danielsen SA, Lind GE, Veiga I, Pinto C, Costa V, Afonso L, Sousa O, Fragoso M, *et al*: Colorectal carcinomas with microsatellite instability display a different pattern of target gene mutations according to large bowel site of origin. BMC Cancer 10: 587, 2010.
21. Shaukat A, Arain M, Thaygarajan B, Bond JH and Sawhney M: Is BRAF mutation associated with interval colorectal cancers? Dig Dis Sci 55: 2352-2356, 2010.
22. Ogino S, Nosho K, Kirkner GJ, Kawasaki T, Meyerhardt JA, Loda M, Giovannucci EL and Fuchs CS: CpG island methylator phenotype, microsatellite instability, BRAF mutation and clinical outcome in colon cancer. Gut 58: 90-96, 2009.
23. Rozek LS, Herron CM, Greenson JK, Moreno V, Capella G, Rennert G and Gruber SB: Smoking, gender, and ethnicity predict somatic BRAF mutations in colorectal cancer. Cancer Epidemiol Biomarkers Prev 19: 838-843, 2010.
24. Fariña-Sarasqueta A, van Lijnschoten G, Moerland E, Creemers GJ, Lemmens VE, Rutten HJ and van den Brule AJ: The BRAF^{V600E} mutation is an independent prognostic factor for survival in stage II and stage III colon cancer patients. Ann Oncol 21: 2396-2402, 2010.

25. Yoon HH, Shi Q, Alberts SR, Goldberg RM, Thibodeau SN, Sargent DJ and Sinicrope FA; Alliance for Clinical Trials in Oncology: Racial differences in BRAF/KRAS mutation rates and survival in stage III colon cancer patients. J Natl Cancer Inst 107: djv186, 2015.
26. Barras D, Missiaglia E, Wirapati P, Sieber OM, Jorissen RN, Love C, Molloy PL, Jones IT, McLaughlin S, Gibbs P, *et al*: BRAFV600E mutant colorectal cancer subtypes based on gene expression. Clin Cancer Res 23: 104-115, 2017.
27. Bokemeyer C, Bondarenko I, Hartmann JT, de Braud F, Schuch G, Zubel A, Celik I, Schlichting M and Koralewski P: Efficacy according to biomarker status of cetuximab plus FOLFOX-4 as first line treatment for metastatic colorectal cancer: The OPUS study. Ann Oncol 22: 1535-1546, 2011.
28. Strub T, Ghiraldini FG, Carcamo S, Li M, Wroblewska A, Singh R, Goldberg MS, Hasson D, Wang Z, Gallagher SJ, *et al*: SIRT6 haploinsufficiency induces $BRAF^{V600E}$ melanoma cell resistance to MAPK inhibitors via IGF signalling. Nat Commun 9: 3440, 2018.
29. Zhang G, Frederick DT, Wu L, Wei Z, Krepler C, Srinivasan S, Chae YC, Xu X, Choi H, Dimwamwa E, *et al*: Targeting mitochondrial biogenesis to overcome drug resistance to MAPK inhibitors. J Clin Invest 126: 1834-1856, 2016.
30. Knauf JA, Luckett KA, Chen KY, Voza F, Socci ND, Ghossein R and Fagin JA: Hgf/Met activation mediates resistance to BRAF inhibition in murine anaplastic thyroid cancers. J Clin Invest 128: 4086-4097, 2018.
31. Korphaisarn K and Kopetz S: BRAF-directed therapy in metastatic colorectal cancer. Cancer J 22: 175-178, 2016.
32. Corcoran RB, Atreya CE, Falchook GS, Kwak EL, Ryan DP, Bendell JC, Hamid O, Messersmith WA, Daud A, Kurzrock R, *et al*: Combined BRAF and MEK inhibition with dabrafenib and trametinib in BRAFV600-mutant colorectal cancer. J Clin Oncol 33: 4023-4031, 2015.
33. Kasbek C, Yang CH and Fisk HA: Mps1 as a link between centrosomes and genomic instability. Environ Mol Mutagen 50: 654-665, 2009.
34. Restuccia A, Yang F, Chen C, Lu L and Dai W: Mps1 is SUMO-modified during the cell cycle. Oncotarget 7: 3158-3170, 2016.
35. Gorbsky GJ: The spindle checkpoint and chromosome segregation in meiosis. FEBS J 282: 2471-2487, 2015.
36. Edelmann MJ, Nicholson B and Kessler BM: Pharmacological targets in the ubiquitin system offer new ways of treating cancer, neurodegenerative disorders and infectious diseases. Expert Rev Mol Med 13: e35, 2011.
37. Hiruma Y, Sacristan C, Pachis ST, Adamopoulos A, Kuijt T, Ubbink M, von Castelmur E, Perrakis A and Kops GJ: CELL DIVISION CYCLE. Competition between MPS1 and microtubules at kinetochores regulates spindle checkpoint signaling. Science 348: 1264-1267, 2015.
38. Daniel J, Coulter J, Woo JH, Wilsbach K and Gabrielson E: High levels of the Mps1 checkpoint protein are protective of aneuploidy in breast cancer cells. Proc Natl Acad Sci USA 108: 5384-5389, 2011.
39. Shankavaram U, Maachani UB, Zhao S, Camphausen K and Tandle A: Molecular profiling of MPS1 gene silencing in U251 glioma cell line. Genom Data 6: 36-39, 2015.
40. Ling Y, Zhang X, Bai Y, Li P, Wei C, Song T, Zheng Z, Guan K, Zhang Y, Zhang B, *et al*: Overexpression of Mps1 in colon cancer cells attenuates the spindle assembly checkpoint and increases aneuploidy. Biochem Biophys Res Commun 450: 1690-1695, 2014.
41. Dominguez-Brauer C, Thu KL, Mason JM, Blaser H, Bray MR and Mak TW: Targeting mitosis in cancer: Emerging strategies. Mol Cell 60: 524-536, 2015.
42. Martinez R, Blasina A, Hallin JF, Hu W, Rymer I, Fan J, Hoffman RL, Murphy S, Marx M, Yanochko G, *et al*: Mitotic checkpoint kinase Mps1 has a role in normal physiology which impacts clinical utility. PLoS One 10: e0138616, 2015.