cMET-N375S germline mutation is associated with poor prognosis of melanoma in Chinese patients

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Background: cMET has been known to play an essential role in malignant melanoma tumor progression. Recent studies have shown that a germline missense variant in exon 2 of the *cMET*, N375S (rs33917957 A>G) occurred at a much higher frequency in East Asian patients with lung cancer. However, the status of this mutation in melanoma is unclear. In this study, we examined the mutation frequency of *cMET-N375S* in 181 melanoma samples and analyzed its clinicopathological significance.

Methods: Tissue samples (n=181) were analyzed for *cMET-N375S*, *BRAF*, *NRAS*, and *CKIT* mutation in genomic DNA by polymerase chain reaction amplification and Sanger sequencing. The levels of p-cMET protein were determined by immunohistochemistry. Clinical data of patients were also collected.

Results: We identified a highly frequent variant (N375S) of *cMET* in 13.26% (24/181) of the patients with melanoma. This mutation was relatively more frequent in acral (22.8%) and mucosal (12.7%) melanomas (the two most common melanoma subtypes in Asian, but not in Caucasians) than in non-chronic sun induced damage (non-CSD; 5.9%) and CSD (8.3%) melanomas. Among the 24 cases with *cMET-N375S* mutations, six cases were found to harbor the *BRAF* mutation, four cases harbored *NRAS* mutations, and two cases harbored *CKIT* mutations. And there was no correlation between this mutation and p-cMET levels. Kaplan-Meier analysis showed significant differences in overall survival (OS) and disease-free survival (DFS) between the N375S mutation group and non-N375S mutation group (P=0.039; P=0.030). The Cox proportional hazards model revealed that *cMET-N375S* mutation was an independent adverse prognostic factor for OS [P=0.003, hazard ratio (HR) =3.577].

Conclusions: Our findings suggested that the *cMET-N375S* mutation was an adverse prognostic factor for melanoma in a Chinese cohort.

Keywords: *cMET-N375S*; mutation; Chinese melanoma; prognosis

Introduction

Melanoma is a highly malignant tumor with rapid development and poor prognosis (1). In recent years, almost 200,000 new cases of melanoma were diagnosed globally, and an estimated 50,000 deaths occurred due to the disease (2). Despite the extensively study results from western countries, knowledge of melanoma in Asian patients is scarce. Different from the rates in Caucasians, acral and mucosal melanomas are the common types in most Asian patients with melanoma. In an analysis of 522 Asian patients diagnosed with malignant melanoma, these two subtypes are almost accounted for 65% of all patients (3). However, acral and mucosal melanomas showed a markedly different genomic landscape from cutaneous melanoma, with a far lower mutation burden dominated by large-scale structural variants (4). Thus, while the individualized targeted therapy has achieved success in recent years, a lot of Chinese patients with melanoma still do not have validated targets for targeted therapy.

cMET, a receptor tyrosine kinase (RTK) for hepatocyte growth factor (HGF), has been shown to play a key role in malignant melanoma tumor progression and is significantly related to patient survival (5-7). HGF is a mitogen of human melanocytes and contributes to the acquisition of the invasive phenotype in melanoma (5). In many cancer cells, cMET signaling can be constitutively activated through HGF-induced paracrine and autocrine signaling and through the presence of *cMET* mutations or protein overexpression (6,8-10). Several studies have shown that cMET can induce the activation of downstream signal transduction pathways, including the phosphatidylinositol 3-kinase/Akt and mitogen-activated protein kinase pathways, which are key targets for melanoma (11,12). Due to this activation mechanism, immediate resistance to BRAF (13-15) and NRAS (16) inhibitors can occur in melanoma cells. Thus, cMET is a potential new therapeutic target for melanoma treatment (17,18).

cMET consists of a heterodimer with 50-kDa α chain and 140-kDa β chain (19). As shown in *Figure 1A*, the canonical human cMET family includes the Sema (semaphorins), PSI (plexins, semaphorins, integrins), four IPT repeat (immunoglobulins, plexins, transcription factors), transmembrane (TM), juxtamembrane (JM) and tyrosine kinase (TK) domains (20). Among them, extracellular N-terminal 500 residues fold into a Sema domain, which is necessary for HGF ligand binding and receptor dimerization. A number of nonsynonymous
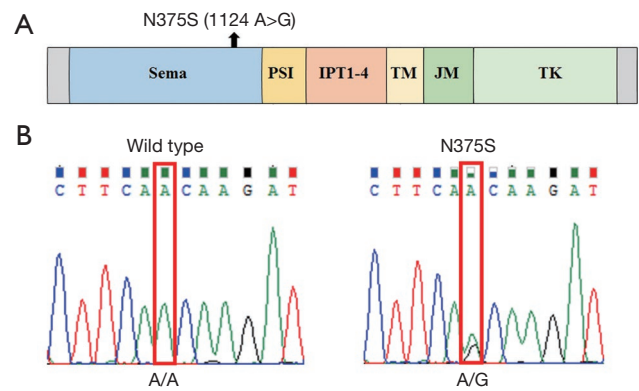

Figure 1 Sequence analysis revealed the *cMET-N375S* polymorphism in melanoma. (A) Boxes represent functional domains: the Sema (semaphorins), PSI (plexins, semaphorins, integrins), four IPT repeat (immunoglobulin, plexins, transcription factors), transmembrane (TM), juxtamembrane (JM), and tyrosine kinase (TK) domains; (B) representative sequencing results of the *cMET-N375S* polymorphism are shown.

mutations have been shown to be localized to this ligand binding domain in many cancers (10,21).

In a previous study of lung cancer, the N375S germline mutation was found to occur at a higher frequency in East Asians than Caucasians (13% *vs*. 1%) (21). It has been reported that this germline missense variation may decrease susceptibility to gastric cancer in a relatively large Chinese population (22). But the status of this mutation in melanoma is unknown. Therefore, in this study, we aim to examine the frequency of *cMET-N375S* and correlate it with phospho-cMET levels and other frequently mutated genes, such as BRAF and NRAS. We also evaluated correlations of *cMET-N375S* mutation with clinicopathological parameters and prognosis in patients with melanoma. This study may provide potential therapeutic targets for melanoma patients with *cMET-N375S* mutation.

Methods

Patients and tumor tissue samples

Paraffin-embedded tissue specimens from 181 patients with melanoma were obtained from Peking University Cancer Hospital & Institute. These samples were analyzed by hematoxylin and eosin (H&E) staining and by immunohistochemistry to confirm the diagnosis of melanoma. Clinical data, including age, sex, stage,

thickness, ulceration, and survival (follow-up through October 2015), were collected. For patients during stage II/III, surgical resection and high-dose interferon adjuvant treatment are the main therapeutic regimens. For stage IV patients, the main types of therapies are dacarbazine-based chemotherapy regimens. No more than 5% of patients were treated with BRAF inhibitors or immunotherapies. The study was approved by the Medical Ethics Committee of the Peking University Cancer Hospital & Institute.

DNA extraction and mutation analysis

We extracted genomic DNA from formalin-fixed, paraffin-embedded (FFPE) sections using a QIAamp DNA FFPE Tissue Kit (Qiagen, Valencia, CA, USA) according to the manufacturer's instructions. We amplified exon 2 of the *cMET* gene, exons 11 and 15 of the *BRAF* gene, exons 1 and 2 of the *NRAS* gene and exons 9, 11, 13, 15 and 17 of the *CKIT* gene by polymerase chain reaction (PCR) in three separate preparations of genomic DNA. The primer sequences are listed in *Table S1*. We purified PCR products with QIAquick (Qiagen), and directly sequenced them using Big Dye Terminator sequencing chemistry on an ABI3130 automated sequencer (Applied Biosystems, Foster City, CA, USA). All aberrations were verified by repeated bidirectional sequencing on the ABI sequencer. In the case of single nucleotide polymorphisms, blood samples were obtained, and genomic DNA was extracted from peripheral lymphocytes using standard methods.

Immunohistochemistry

Immunohistochemical (IHC) analyses were performed using antibodies against p-Met (Cell Signaling Technology, Beverly, MA, USA), followed by a standard avidin-biotin detection protocol using 3-amino-9 ethylcarbazole (AEC). The staining score for each sample, based on the intensity and density of the staining, was graded as 0, 1, 2, and 3 (0 as negative and 3 as strongest; or 0 as negative and 1, 2, and 3 as positive, respectively) by three pathologists independently, without knowledge of the *cMET* mutation status of these patients.

Statistical methods for clinical correlation

SPSS 16.0 software was used for all statistical analyses. Categorical data, such as sex, were described using frequencies and percentages. Continuous data, such as age and thickness, were described using means ± standard deviations for normally distributed data. We used unpaired t-tests to evaluate differences in measurement data of two groups and Pearson χ^2 tests, Fisher's exact tests, or Kruskal-Wallis tests to analyze the correlation between clinicopathological parameters and different groups. Overall survival (OS) and disease-free survival (DFS) curves were calculated according to the Kaplan-Meier method, and comparisons were performed using log-rank tests. All statistical analyses were two sided, and differences with P values of less than 0.05 were considered statistically significant.

Results

cMET mutations in melanoma subtypes

Of the 181 melanoma samples analyzed, 26 (14.4%) were found to contain nonsynonymous *cMET* mutations. With only two exceptions (C788T and C463A mutations), all mutations were N375S [24 (13.26%), *Figure 1B*]. The frequencies of *cMET-N375S* mutations in acral and mucosal melanoma, melanomas of the skin without chronic sun induced damage (non-CSD), melanomas of the skin with chronic sun induced damage (CSD) melanoma, and unknown primary subtypes were 22.8%, 12.7%, 5.9%, 8.3%, and 4.3%, respectively (*Figure 2A*). The N375S mutation was found at a higher frequency in acral and mucosal melanomas (20/112, 17.9%) than in other types of melanomas (4/69, 5.8%; P=0.036). Consistent with previous conclusions (21), this N375S mutation could be found in peripheral blood lymphocyte DNA, indicating that it was a germline polymorphism.

Correlation of the cMET-N375S mutation with clinicopathological parameters of melanoma

In our cohort, we analyzed the correlations of *cMET-N375S* mutations with the characteristics of patients with melanoma, including age, sex, ulceration, thickness, and clinical stage (*Table 1*). There were no significant differences between patients with melanoma with or without *cMET-N375S* mutation in terms of age, sex, and clinical stage.

Ulceration and the Breslow thickness of melanomas are important prognostic indicators. Since most patients' data regarding mitotic rate, vascular invasion and degree of tumor-infiltrating lymphocytes were not available, we did not analyze these clinical features. Among the 181 samples
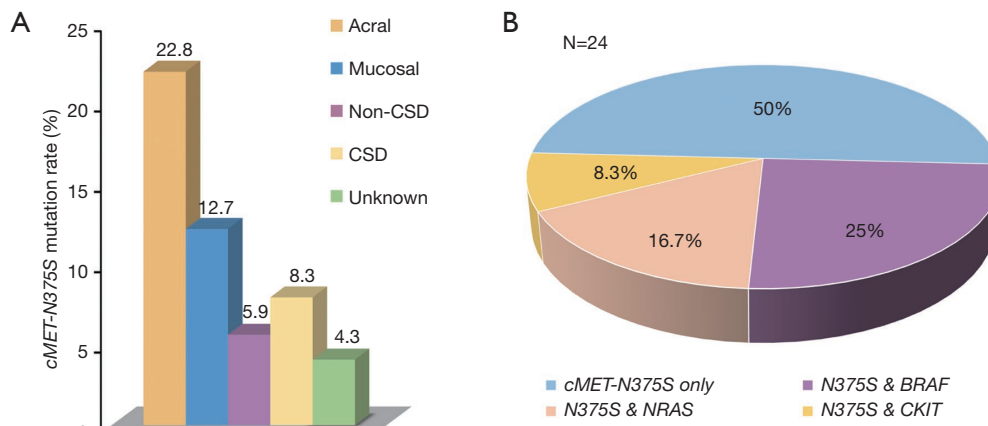
Figure 2 *cMET-N375S* mutations in melanoma. (A) Frequency distribution of *cMET-N375S* mutations in melanoma subtypes; (B) the pie chart shows tumors with mutations in *cMET-N375S* alone, *cMET-N375S* and *BRAF*, *cMET-N375S* and *NRAS*, and *cMET-N375S* and *CKIT* as a percentage of the total number of *cMET* mutation carriers.

Table 1 Correlation of *cMET-N375S* and p-cMET with clinical characteristics of melanoma

Characteristics	*cMET-N375S* mutation			p-cMET levels		
	Mutation (n=24)	WT (n=157)	P[a]	High (n=69)	Low (n=112)	P[a]
Age	55.4±18.7	58.3±14.6	0.650	58±13.2	58.5±14	0.811
Sex (female, %)	11 (45.8)	69 (43.9)	0.863	35 (50.7)	45 (40.2)	0.088
Thickness (mm)	4.4±3.9	4.1±2.8	0.198	5.3±2.3	4.7±3.3	0.192
Ulceration, n (%)	8 (33.3)	53 (33.8)	0.971	23 (33.3)	37 (33.0)	0.716
Stage, n (%)			0.244			0.007
I	0 (0)	2 (1.3)		1 (1.4)	1 (0.9)	
II	8 (33.3)	25 (15.9)		5 (7.2)	28 (25.0)	
III	4 (16.7)	32 (20.4)		12 (17.4)	24 (21.4)	
IV	12 (50.0)	98 (62.4)		51 (73.9)	59 (52.7)	
Subtypes, n (%)			0.188			0.157
Acral	13 (54.2)	44 (28.0)		23 (33.3)	34 (30.4)	
Mucosal	7 (29.2)	48 (30.6)		20 (29.0)	35 (31.2)	
Non-CSD	2 (8.3)	32 (20.4)		19 (27.5)	15 (13.4)	
CSD	1 (4.2)	11 (7.0)		3 (4.3)	9 (8.0)	
Unknown	1 (4.2)	22 (14.0)		4 (6.0)	19 (17.0)	
Other mutations, n (%)			0.310			0.252
BRAF	6 (25.0)	21 (13.4)		13 (18.8)	14 (12.5)	
NRAS	4 (16.7)	15 (9.6)		8 (11.6)	11 (9.8)	
CKIT	2 (8.3)	20 (12.7)		10 (14.5)	12 (10.7)	

[a], the P value of 181 patients for age, thickness was analyzed by *t*-test, Pearson χ^2 test for sex, ulceration, Fisher's exact tests for and subtypes, and Kruskal-Wallis test for stage analysis.
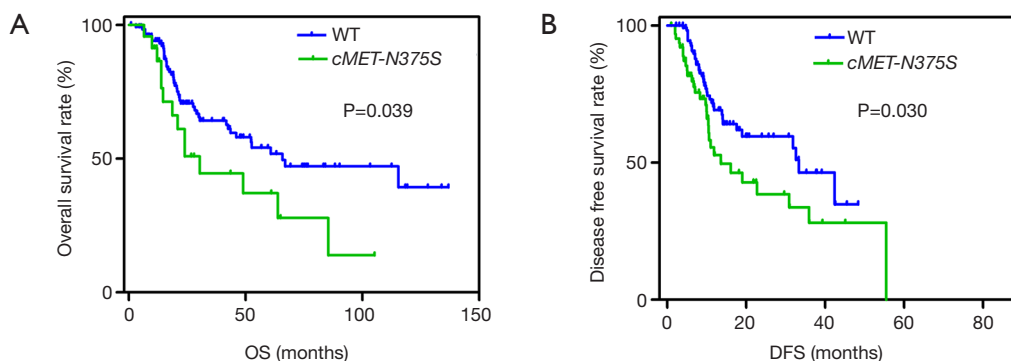
Figure 3 Effects of cMET-N375S mutation on the prognosis of patients with melanoma. Kaplan-Meier estimates illustrating the OS (A) and DFS (B) of patients based on *cMET-N375S* mutations. P values were calculated using the log rank test. OS, overall survival; DFS, disease-free survival.

with data available, the average thickness of samples with *cMET-N375S* mutations was 4.4±3.9 mm, whereas that of samples without this mutation was 4.1±2.8 mm (P=0.198). Additionally, the ulceration rate was not significantly different between patients with and without *cMET-N375S* mutations (P=0.971).

Next, to elucidate the relationships between the type and frequency of *cMET* mutations with other common mutations in melanoma, we also examined mutations in well-established target genes (e.g., *BRAF*, *CKIT*, and *NRAS*) in the 181 melanoma samples (*Table 1*). *BRAF*, *NRAS* and *CKIT* mutations were found in 27 (14.9%), 19 (10.5%), 22 (12.1%) of our cohort, respectively (*Table S2*). Among the 24 cases with *cMET-N375S* mutations, six cases were found to harbor the *BRAF-V600E* mutation, four cases harbored *NRAS* mutations (one case of G12C, one case of L53L, one case of Q61R, and one case of Q61H), and two cases harbored *CKIT* mutations (one case of L589L, one case of L862L) (*Figure 2B*). Further analysis of the frequencies of *BRAF* and *NRAS* mutations in patients of our cohort showed that the mutation frequencies of *BRAF* and *NRAS* did differ significantly between patients with or without *cMET-N375S* mutations (P=0.039).

Effects of cMET-N375S mutation on the prognosis of melanoma

We then analyzed the prognostic significance of the *cMET-N375S* mutation for OS (*Figure 3A*). The OS rates were determined for 181 patients. The results showed that the median follow-up period was 24 (range, 4–231) months, and the median survival time for patients with *cMET-*

N375S [30.5 months; 95% confidence interval (CI), 6.6–105.2 months] was significantly shorter than that for patients without the nonsynonymous *cMET-N375S* mutation (65.9 months; 95% CI, 3.9–136.9 months; P=0.039). Therefore, the *cMET-N375S* mutation may be an important prognostic factor for patients with melanoma, and melanoma patients with this mutation may show higher risk of death. In multivariate Cox regression assays (*Table 2*), the N375S mutation was validated as an independent prognostic factor for OS [P=0.003, hazard ratio (HR) =3.557]. Also, *cMET-N375S* was associated with DFS (P=0.030, *Figure 3B*). The DFS rates were determined for 176 patients. The median survival time for patients with *cMET-N375S* (13.6 months) was significantly shorter than that for patients without the nonsynonymous *cMET-N375S* mutation (33.3 months).

p-cMET levels in melanoma subtypes

p-cMET levels were divided into high (IHC score =2 or 3) and low (IHC score =0 or 1) groups (*Figure 4*). Patient and clinic characteristics based on p-cMET levels are summarized in *Table 1*. There were statistically significant differences in stage (P=0.007) between patients with high or low levels of p-cMET.

Among the 181 samples, the overall rate of detection of p-cMET staining was 64.1% (116/181). Positive p-cMET staining was observed in 63.2% (36/57) of acral, 63.6% (35/55) of mucosal, 73.5% (25/34) of non-CSD, 50.0% (6/12) of CSD, and 60.9% (14/23) of unknown primary site melanomas. We found that p-cMET levels were not significantly different between these subtypes (P=0.316; *Table 3*).

Table 2 Cox regression analysis of *cMet-N375S* mutation and clinicopathologic factors with overall survival

Factors	Group	Univariate analysis			Multivariate analysis		
		HR	95% CI	P value	HR	95% CI	P value
Sex (n=181)	Female *vs.* male	1.574	0.964–2.571	0.070	0.975	–	0.855
Age (n=181)	≥60 *vs.* <60 years	0.896	0.553–1.449	0.653	0.091	–	0.763
Thickness (n=94)	≥2 *vs.*<2 mm	0.816	0.894–1.115	0.073	0.526	–	0.468
Ulceration (n=81)	Ulceration *vs.* non-ulceration	1.479	0.627–3.488	0.371	0.330	–	0.566
TNM stage (n=181)	III and IV *vs.* I and II	2.286	1.269–4.117	0.006	2.947	0.973–8.925	0.056
cMet-N375S mutation (n=181)	Mut *vs.* WT	2.158	1.039–4.481	0.039	3.557	1.551–8.156	0.003
BRAF mutation (n=181)	Mut *vs.* WT	3.609	1.245–5.546	0.018	1.359	–	0.244
NRAS mutation (n=181)	Mut *vs.* WT	1.206	0.546–2.666	0.643	1.466	–	0.226
CKIT mutation (n=181)	Mut *vs.* WT	1.136	0.561–2.300	0.723	0.572	–	0.449

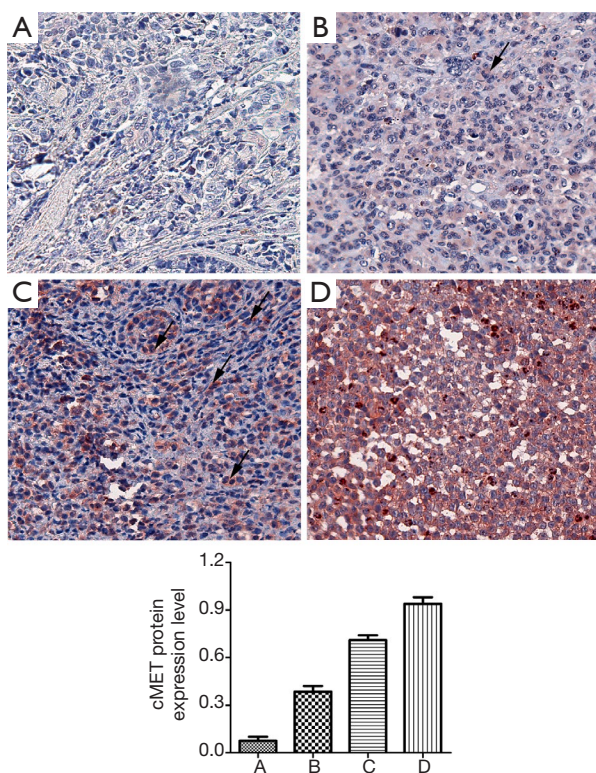CI, confidence interval; Mut, mutation; WT, wild type.

Figure 4 Immunohistochemistry of p-cMET levels in melanomas. p-cMET staining was mainly detected in the cytomembrane and cytoplasm of melanoma cells. The staining score for each sample, based on the intensity and density of the staining, was graded as 0 (A), 1 (B), 2 (C), and 3 (D) (0 as negative and 3 as the strongest). Arrows point to the examples of the positive staining cells. Scale bars, 500 µm; magnification, ×50.

Next, we analyzed the correlation between *cMET-N375S* mutations and cMET expression levels. Among the 24 cases with *cMET-N375S* mutations, the IHC detection rate for p-cMET was 75.0% (18/24), which was not significantly higher (P=0.232) than that in cases without N375S mutation (62.4%, 98/157; *Table 4*).

Discussion

In a previous study of lung cancer, the N375S germline mutation was found to occur at a higher frequency in East Asians than Caucasians (13% *vs.* 1%) (21). Here, we found that the frequency of the *cMET-N375S* germline mutation was 13.26% (24/181) in patients with melanoma in our cohort.

Different from Caucasians, acral and mucosal melanomas are the common types of melanoma diagnosed in Asian patients. The mechanism, clinical features and treatment principle of mucosal and acral melanomas are different from those of cutaneous melanoma. The principal mutation mechanisms driving these two subtypes of melanoma were not attributable to ultraviolet radiation and imply novel carcinogenic exposures (4). Moreover, we found that the incidence of the *cMET-N375S* mutation was much higher in patients with acral and mucosal melanomas than in patients with other subtypes. According to our findings, it is suggested that larger sample size and further research is necessary to clarify potential links between *cMET-N375S* mutation and these two special subtypes of melanoma in China.

Table 3 Phospho-cMET levels in melanoma subtypes

Subtype	IHC scores[a]				NES	NPS[b]	%
	0	1	2	3			
Acral	21	13	19	4	57	36	63.2
Mucosal	20	15	15	5	55	35	63.6
Non-CSD	9	6	16	3	34	25	73.5
CSD	6	3	2	1	12	6	50
Unknown	9	10	2	2	23	14	60.9
Total	65	47	54	15	181	116	64.1
P[c]	0.316	–	–	–	–	–	–

[a], the signal intensity of immunohistochemistry results were determined by three individual pathologists and scored as 0, 1, 2, and 3, with a score of 0 as a negative and a score 3 as the strongest; [b], samples with signal intensity of scores 1, 2, or 3 were regarded as p-cMET positive; [c], the P value was analyzed by Pearson χ^2. NES, number of examined samples; NPS; number of positive samples.

Table 4 Correlations of *cMET-N375S* with p-cMET levels

Subtype	*cMET-N375S* mutation	
	Mutation	WT
IHC scores		
0	6	59
1	8	42
2	7	44
3	3	12
Positive rate (%)	18/24 (75.0)	98/157(62.4)
P[a]	0.232	–

[a], the P value was analyzed by Pearson χ^2.

Resistance to vemurafenib (a first-in-class small-molecule BRAF inhibitor) has become a major issue in patients with melanoma. cMET activation in melanoma cell lines shows primary resistance to vemurafenib, and BRAF/cMET combination therapy results in reversal of vemurafenib resistance (13,14). In addition, the Sema domain of cMET harbors the ligand binding site; the effects of mutations in this domain on the function of the protein should be assessed. And, replacement of asparagine 375 with serine can disrupt HGF ligand binding by altering the molecular structure. Thus, *cMET-N375S* mutation leads to the emergence of Met inhibitor resistance (23). Furthermore, we found that the *cMET-N375S* mutation often co-existed with *BRAF* mutations. Accordingly, we suggest that alterations in BRAF and elevated p-cMet may be strong

indications for combination therapy, although careful attention is required to exclude *cMET-N375S* mutation.

Next, we intended to find out the relationship between this mutation and the prognosis of Chinese melanoma patients. *cMET-N375S* was found to decrease gastric cancer susceptibility (22) and was not shown to be associated with lung cancer prognosis (23). However, our data showed that OS and DFS differed significantly between patients with and without the *cMET-N375S* mutation. This may due to differences in cancer type. Thus, additional large-scale studies are needed to clarify the effects of the *cMET-N375S* mutation on cancer prognosis, especially in acral and mucosal melanoma.

We also evaluated the p-cMET expression levels in 181 patients. Several studies have shown correlation between cMET expression and progression or aggressive behavior of many cancers (9,10,24). Moreover, a previous study showed that cMET phosphorylation was twice as high in patients with melanoma than in patients with nevus. Though we find out that *cMET-N375S* mutation is associated with poor prognosis of Chinese melanoma patients, there was no correlation between this mutation and p-cMET levels (P=0.232). The accurate molecular mechanisms remain unclear.

Conclusions

Our study demonstrated that the *cMET-N375S* germline mutation was an independent adverse prognostic factor for melanoma. Although we did not perform functional experiments *in vivo* or *in vitro*, our study provided clinical

data to support further investigations on cMET as a potential therapeutic target in Chinese patients with melanoma.

Footnote

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at http://dx.doi.org/10.21037/tcr.2018.02.13). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study protocol was approved by the Medical Ethics Committee of the Peking University Cancer Hospital & Institute. All procedures performed in studies involving human participants were in accordance with the ethical standards of Peking University Cancer Hospital & Institute and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Written informed consent was obtained from all patients.

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Table S1 Primers sequences

Gene	Exon	Primer Set 1	Primer Set 2
cMET	2	F:5'-ATAAACCTCTCATAATGAAGGCCC-3'	–
		R:5'-CCTATTAAAGCAGTGCTCATGATT-3'	–
BRAF	11	F:5'-CAGGTAACCATTTATTTGTTCTCT-3'	F:5'-AACCATTTATTTGTTCTCTCTCCA-3'
		R:5'-TGGAACAAAACAAAGGAAGC-3'	R:5'-GAACAAAACAAAGGAAGCCACT-3'
	15	F:5'-TTATTGACTCTAAGAGGAAAGATGAAG-3'	F:5'-TTATTGACTCTAAGAGGAAAGATGAAG-3'
		R:5'-TGATTTTTGTGAATACTGGGAAC-3'	R:5'-GGCCAAAAATTTAATCAGTGGA-3'
NRAS	1	F:5'- AGGGTTTTCATTTCCATTGATTAT-3'	F:5'-GGGTTTTCATTTCCATTGATTATA-3'
		R:5'-TTTTACTTTCTCTCCTCTTATTCCTT-3'	R:5'-TTTACTTTCTCTCCTCTTATTCCTTT-3'
	2	F:5'-CCAGATAGGCAGAAATGGGC-3'	F:5'-CAATAGCATTGCATTCCCTG-3'
		R:5'-ATCTTCCCTAGTGTGGTAAC-3'	R:5'-TTCAGAACACAAAGATCATC-3'
CKIT	9	F:5'-AACTCAGTGTTGGTGGGGGT-3'	F:5'-AAAGTATGCCACATCCCAAG-3'
		R:5'-TTGTTCTAATTCTGTTTGGGTG-3'	R:5'-ACAGAGCCTAAACATCCCCT-3'
	11	F:5'-TATTGTGATGATGATTCTGACCTAC-3'	F:5'-GTGCTCTAATGACTGAGACAAT-3'
		R:5'-TTTTTCCTACGATGTTCTCTATG-3'	R:5'-GGAACAAAACAAAGGAAGC-3'
	13	F:5'-TTTCGGGAAGGTTGTTGAGG-3'	F:5'-ACTGTCGCTGTAAAGATGCTCA-3'
		R:5'-ATAACTAGGGTATGTCCTGGGC-3'	R:5'-ACAACAGTCTGGGTAAAAAAAT-3'
	15	F:5'-TACTTTTGATTTTTATTTTTGG-3'	F:5'-AGTTAGTTTTCACTCTTTACAA-3'
		R:5'-ACCTACATTTGTTACACTTGAG-3'	R:5'-TAAAATGTGTGATATCCCTAGA-3'
	17	F:5'-ATGTATTTCAGAGGTGATTGGG-3'	F:5'-TTCAGCAACAGCAGCATCTATA-3'
		R:5'-GTGTTCAGGGCTGAGCATCC-3'	R:5'-GCAGGACACCAATGAAACTT-3'

F, forward primer; R, reverse primer.

Table S2 BRAF, NRAS and CKIT mutations identified in 181 melanomas

Gene	Exon	Amino acid change	No.	Subtype [n]
BRAF	15	V600E	25	AM [4], MM [3], non-CSD [3], CSD [13], unknown [2]
	15	V600K	2	MM [1], non-CSD [1]
NRAS	1	G13D	7	MM [5], CSD [2]
	2	Q61R	6	AM [5], MM [1]
	2	Q61L	1	Unknown
	2	Q61H	2	AM
	2	L53L	2	MM
CKIT	9	V489I	1	AM
	9	F483I	2	AM
	9	D496N	1	Non-CSD
	11	V560D	1	MM
	11	V559A	1	MM
	11	N587N	1	AM
	11	L589L	1	AM
	11	L576P	1	AM
	11	Y578H	1	CSD
	13	K642E	1	AM
	13	G648G	1	Non-CSD
	17	Y823S	1	MM
	17	N823S	1	MM
	17	D820Y	2	AM [1], MM [1]
	18	L862L	6	AM [2], MM [2], non-CSD [1], CSD [1]

AM, acral melanoma; MM, mucosal melanoma; non-CSD, melanomas of the skin without chronic sun induced damage; unknown, melanoma of unknown primary lesion.