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MAPK Pathway and *TERT* Promoter Gene Mutation Pattern and Its Prognostic Value in Melanoma Patients: A Retrospective Study of 2,793 Cases

Xue Bai, Yan Kong, Zhihong Chi, Xinan Sheng, Chuanliang Cui, Xuan Wang, Lili Mao, Bixia Tang, Siming Li, Bin Lian, Xieqiao Yan, Li Zhou, Jie Dai, Jun Guo, and Lu Si

Abstract

Purpose: Ethnic differences are conspicuous in melanoma. This study is to obtain a comprehensive view of a genomic landscape and a better understanding of the correlations of gene mutation status with clinicopathologic characteristics and disease prognosis in the Asian population.

Experimental Design: A total of 2,793 melanoma patient samples were retrospectively collected and analyzed for mutations in C-KIT, BRAF, NRAS, and PDGFRA coding regions and telomerase reverse transcriptase (TERT) promoter region by Sanger sequencing. Mutations were correlated to clinicopathologic features and overall survival.

Results: The incidences of somatic mutations within the BRAF, NRAS, C-KIT, TERT-228, TERT-250, and PDGFRA genes were 23.7%, 10.4%, 8.0%, 5.9%, 5.5%, and 1.4%, respectively. Hotspot mutations accounted for 95.8% and 87.2% of BRAF and NRAS mutations, respectively; meanwhile, C-KIT and PDGFRA mutations showed more heterogeneity. BRAF, C-KIT, and NRAS mutations were mutually exclusive. BRAF, C-KIT, NRAS, and numbers of gene mutations of the MAPK pathway were all independent negative prognostic factors ($P = 0.007$, other $P < 0.001$, respectively). In acral melanoma, BRAF, C-KIT, and NRAS mutations were all independent prognostic factors of worse overall survival (all $P < 0.001$), whereas in mucosal melanoma, only C-KIT was ($P = 0.006$). Although correlated with BRAF mutations ($P = 0.001$ and $P < 0.001$ for C228T and C250T, respectively), TERT promoter gene mutations were not correlated with overall survival ($P = 0.406$ and 0.256, respectively).

Conclusions: The MAPK pathway and TERT promoter gene mutations are differentially represented in the Asian population. Mutations in BRAF, C-KIT, and NRAS have prognostic values that vary by melanoma subtypes. Clinical treatment targeting these critical pathways should be aimed directly at these poor-prognosis subpopulations for maximum potential impact. *Clin Cancer Res; 23(20); 6120–7. ©2017 AACR.*

Introduction

Melanoma is the most deadly skin cancer which is anticipated to cause approximately 10,000 deaths in the United States and thousands more worldwide (1). In China, there was estimated to be about 8,000 new cases and 3,200 deaths in 2015 (2). The epidemiology differs greatly between Caucasian and Asian. Instead of cutaneous melanoma as the major subtype in Caucasian, 70% of Asian patients are diagnosed with acral and mucosal melanoma (3), compared with 5% in Caucasian (4, 5). The difference in subtypes might indicate disparities in genetic profiles between Caucasian and Asian melanoma patients and could direct to different therapeutic treatments. For instance, BRAF mutation is one of the most frequently mutated genes in Caucasian (40%–60%; refs. 6, 7), whereas only about 25% of Asian patients were reported harboring BRAF mutation (8). Racial disparities have been observed in other gene mutations in the MAPK pathway, namely NRAS (8–10), C-KIT (11), and platelet-derived growth factor α receptor (PDGFRA; ref. 12). And gene mutation types of these above-mentioned pivotal genes in the MAPK pathway and their correlations with each other remain unclear. Besides the MAPK pathway, telomerase reverse transcriptase (TERT) is another important gene in melanoma. TERT promoter mutations have been reported in up to 50% of cutaneous melanoma (13, 14), but only 0% to 7% of acral melanoma (15–17), indicating potential racial discrepancy also. Besides, in Caucasian populations, TERT promoter mutations have been reported to be correlated with BRAF and NRAS mutations (16); however, it remains unclear whether there is correlation in Asian counterparts.

To delineate the mutation profiles in Asian melanoma patients, to better understand their correlations with melanoma subtypes and disease prognosis, and to direct future targeted therapies at those poor prognosis subpopulations for maximum potential impact, we retrospectively collected 2,793 primary melanoma samples and evaluated four most frequently mutated genes in the MAPK pathway (BRAF, C-KIT, NRAS, and PDGFRA) and two most frequently mutated genes in TERT promoter (228 and 250). The associations between mutation status and clinicopathologic

The Key Laboratory of Carcinogenesis and Translational Research (Ministry of Education/Beijing), Department of Renal Cancer and Melanoma, Peking University Cancer Hospital and Institute, Beijing, China.

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X. Bai and Y. Kong contributed equally to this article.

Corresponding Authors: Lu Si, Peking University Cancer Hospital and Institute, 52# Fucheng Road, Haidian District, Beijing 100142, China. Phone: 86-10-8819-6951; E-mail: silu15_silu@126.com; and Jun Guo, guoj307@126.com

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Translational Relevance

Gene mutations in the MAPK pathway and telomerase reverse transcriptase (TERT) promoter region are key events in melanoma tumorigenesis and of significant prognostic values in Caucasian populations. Targeted therapy inhibiting those mutated oncogenes has improved outcomes for distinct subpopulations. However, huge disparities in genetic profiles exist among different ethnicities. Therefore, there is a need to delineate the gene mutational profiles and their prognostic values in the Asian population to help guide the development of targeted therapy for poor-prognosis subpopulations for maximal potential impact. Here, we retrospectively analyzed 2,793 Asian melanoma samples and found significantly different gene mutation patterns and their prognostic roles: C-KIT and NRAS mutations were of greater prognostic value, whereas TERT promoter gene mutations were not prognostic factors. We propose that future development of targeted therapy for Asian melanoma patients would be most impactful if we focused on C-KIT, NRAS, and BRAF mutations, which robustly predicted poorer overall survival.

features, as well as the correlations among different gene mutations to each other were investigated. Our study demonstrated subtype-specific mutation profiles and varied prognostic value of each mutation in different patient subgroups. These findings could help clinicians choose more precise treatments in their future practice, with a focus on mutations associated with adverse clinical outcome.

Materials and Methods

Patient samples

Formalin-fixed paraffin-embedded (FFPE) tissues from 2,793 Chinese patients were retrieved from the files of the Department of Melanoma at Peking University Cancer Hospital. All the samples were collected between July 2011 and December 2015. Clinical data, including age, gender, tumor–node–metastases stage (TNM), Breslow thickness, ulceration status, and survival (follow-up persisted until the death of patients), were collected. Last follow-up was carried out in September 2016; median follow-up time was 29.0 months (range, 2.0–300.0 months).

Samples were analyzed by hematoxylin and eosin (H&E) staining and by immunohistochemistry for melanoma markers (S-100, HMB-45, or MART-1) to confirm the diagnosis of melanoma and their subtypes. Sun-induced damage was defined microscopically by the presence of marked solar elastosis on H&E-stained sections, and was determined by at least two individual pathologists as previously described. This study was approved by the medical ethics committee of the Peking University Cancer Hospital & Institute and was conducted according to the Declaration of Helsinki Principles.

DNA preparation and mutation screening

Genomic DNA was extracted from FFPE sections using a QIAamp DNA FFPE Tissue kit (Qiagen). To detect hotspot mutations, we amplified exons 11 and 15 of BRAF gene, exons 9, 11, 13, 17, and 18 of C-KIT gene, exons 1 and 2 of NRAS gene, and exons 12, 14, and 18 of PDGFRA gene by PCR in at least two separated preparations of genomic DNA and promoter of TERT gene by PCR in one preparation of genomic DNA. The primer sequences were listed in Supplementary Table S1, and PCR conditions had been described previously (8, 11, 12). Examples of hotspot gene mutations of BRAF, C-KIT, NRAS, PDGFRA, and TERT promoter region were shown in Supplementary Figs. S1–S5. After PCR, PCR products were purified using QIAquick (Qiagen), followed by Sanger sequencing (Tianyihuiyuan Company). All mutations were confirmed by bidirectional sequencing on an ABI 313 automated sequencer.

DNAs from the peripheral blood mononuclear cells from all the patients harboring BRAF/C-KIT/NRAS/PDGFRA mutations were extracted, and corresponding mutation status was examined to exclude the possibility that the detected mutations were due to polymorphisms.

Statistical analysis

Variance analysis and Pearson's χ^2 test were performed to investigate the correlation between gene mutations and clinicopathologic data of the patients. Survival analysis was performed by the method of Kaplan–Meier survival curve and compared by the log-rank test. Multivariate survival analysis was carried out via the Cox regression test. All statistical tests were two-sided, and $P < 0.05$ was judged as of significance. All statistical analyses were performed using SPSS version 20.0.

Results

Basic clinicopathologic characteristics and gene mutation rates

Similar to our previously published study (3), we found that in this melanoma cohort, the most prevalent melanoma subtypes were acral (42.8%) and mucosal (27.0%; Table 1). The chronic sun-damaged (CSD) melanoma was relatively rare (8.9%). Non-CSD (NCSD) melanoma, the most common subtype in Caucasians, accounted for only 13.3% of all melanomas in this cohort. In addition, melanomas of unknown primary (UP; e.g., melanomas found in lymph nodes, liver, lung, brain, etc. upon hospitalization) accounted for 8.0% of all melanomas. Almost half of the patients in this cohort were diagnosed with Breslow's depth measurement over 4 mm, indicating an overall poor prognosis in this population.

In this cohort, gene mutation rates of BRAF, NRAS, C-KIT, TERT C228T, TERT C250T, and PDGFRA, were 23.7% (641/2,706), 10.4% (242/2,325), 8.0% (223/2,793), 5.9% (32/545), 5.5% (30/545), and 1.4% (40/2,325), respectively. Mutation rates of BRAF, C-KIT, and NRAS were similar as reported before in Asian population (8, 11). As for PDGFRA, which was reported be around 4.6% (16/351) previously (12), in this cohort, the mutation rate was only 1.4% (40/2,793). This phenomenon might be due to the profound impact of the absolute number of PDGFRA-mutant patients in a certain cohort caused by its rarity; it may also be because of the prevalence of synonymous mutations in this cohort. It has been demonstrated that all mutations mentioned above were somatic.

MAPK pathway gene mutations

Correlation between MAPK pathway gene mutations and clinicopathologic features of melanoma patients. The distribution of MAPK pathway gene mutations among patients with different clinicopathologic features was analyzed, and significant distribution disparities were observed regarding BRAF, C-KIT, and NRAS

Table 1. Clinicopathologic characteristics of patients ($N = 2,793$)

Characteristics	N (%)
Sex	
Male	1,362 (48.8)
Female	1,431 (51.2)
Age/year	
≥60	860 (30.8)
<60	1,933 (69.2)
Subtype	
Acral	1196 (42.8)
Mucosal	755 (27.0)
CSD	247 (8.9)
NCSD	372 (13.3)
UP	223 (8.0)
Breslow thickness (mm)	
≤1.00	234 (8.4)
1.01–2.00	312 (11.2)
2.01–4.00	771 (27.6)
>4.00	1,341 (48.0)
NA	135 (4.8)
Ulceration	
Yes	1,519 (54.4)
No	1,142 (40.9)
NA	132 (4.7)
Stage	
I	173 (6.2)
II	1,192 (42.7)
III	668 (23.9)
IV	760 (27.2)
Gene mutation	
BRAF ($n = 2,706$)	641 (23.7)
C-KIT ($n = 2,793$)	223 (8.0)
NRAS ($n = 2,325$)	242 (10.4)
PDGFR ($n = 2,325$)	40 (1.4)
TERT228 ($n = 545$)	32 (5.9)
TERT250 ($n = 545$)	30 (5.5)

Abbreviation: NA, not applicable.

gene mutations, the three most commonly mutated genes in the MAPK pathway.

BRAF mutation rate was higher in patients of younger age (<60 years; $P < 0.001$), female ($P = 0.038$), CSD/NCSD/UP subtypes ($P < 0.001$), and advanced stage (stage III/IV; $P < 0.001$). C-KIT mutation was more commonly observed in elderly patients (≥60 years; $P = 0.002$) and acral and mucosal melanoma subtypes ($P = 0.018$). NRAS mutation was more frequently seen in elderly patients (≥60 years; $P = 0.017$), female ($P = 0.010$), and patients in advanced stage ($P = 0.011$).

The summary of associations between clinicopathologic features and MAPK pathway gene mutations is shown in Supplementary Table S2.

Gene mutation types of BRAF, C-KIT, NRAS, and PDGFRA. We further analyzed the gene mutation types of BRAF, C-KIT, NRAS, and PDGFRA. As for 641 patients who harbored BRAF mutations, 614 (95.8%) targeted the well-known V600 amino acid residue, namely V600E ($n = 591$, 92.2%), V600K ($n = 22$, 3.4%), and V600R ($n = 1$, 0.2%). The second most common mutated residue was D594 ($n = 9$, 1.4%), followed by G596 ($n = 3$, 0.5%). As for 223 patients with C-KIT mutations, 49 (22.0%) targeted L576 amino acid residue, followed by K642 ($n = 22$, 9.9%), F483, and V559 (both $n = 9$, 4.0%). Overall, 242 patients had NRAS mutations, of those, 211 (87.2%) had hotspot mutations, which included Q61 ($n = 170$, 70.2%), G12 ($n = 30$, 12.4%), and G13 ($n = 11$, 4.6%). For 40 patients carrying PDGFRA mutations, 2 shared C664 mutation, 2 M642 mutation, and the rest all harboring different mutations, indicating huge heterogeneity among this subgroup of patients. Details are presented in Table 2.

Gene mutation patterns of BRAF, C-KIT, and NRAS in melanoma patients. Mutations of BRAF, C-KIT, and NRAS were negatively correlated ($P < 0.001$ for BRAF-C-KIT and BRAF-NRAS, $P = 0.001$ for C-KIT-NRAS; Fig. 1; details seen in Supplementary Table S3). We further analyzed the subgroup of patients harboring dual mutations simultaneously and found that hotspot mutations of BRAF and C-KIT were mutually exclusive, whereas hotspot mutations of BRAF and NRAS were not, universally (13 patients harbored both BRAF V600E and NRAS Q61R/K or G12D mutations). PDGFRA mutation was not correlated with BRAF, C-KIT, or NRAS mutations ($P = 0.976$, 0.089, and 0.132, respectively), implying that PDGFRA mutation would be an independent event. MAPK pathway gene mutation pattern is presented in Fig. 1.

Gene mutation types of BRAF, C-KIT, and NRAS and their correlation with clinicopathologic features. By correlating different gene mutation types of BRAF, C-KIT, and NRAS with clinicopathologic features, we found that BRAF V600E mutation rate was higher in young (<60 years; $P = 0.004$) and female patients ($P = 0.008$), and lower in mucosal subtype ($P < 0.001$). On the contrary, V600K mutation was more frequently seen in elders (≥60 years; $P = 0.004$), male ($P = 0.008$), and CSD/NCSD/UP subtypes ($P < 0.001$). BRAF nonhotspot mutations were more commonly observed in mucosal melanoma ($P < 0.001$). Details are presented in Supplementary Table S4.

No correlation between C-KIT mutation subtypes and clinicopathologic features was observed (Supplementary Table S5).

As for NRAS mutations, mutations targeting Q61 amino acid residue were more commonly seen in acral and NCSD subtypes, G12 in mucosal subtype, and nonhotspot mutations in CSD and UP subtypes ($P = 0.001$; Supplementary Table S6).

Correlation between MAPK pathway gene mutations and prognosis of melanoma patients. We sought to reveal the effect of individual gene mutation in the MAPK pathway on melanoma patient survival regardless of disease stage, subtype, and other clinicopathologic features, and demonstrated that C-KIT and NRAS mutations were correlated with worse prognosis (both $P < 0.001$; Fig. 2B and C), whereas BRAF and PDGFRA mutations

Table 2. MAPK pathway gene mutation pattern: subtype distribution

BRAF ($N = 641$)		C-KIT ($N = 223$)		NRAS ($N = 242$)		PDGFRA ($N = 40$)	
Subtypes	n (%)	Subtypes	n (%)	Subtypes	n (%)	Subtypes	n (%)
V600	614 (95.8)	L576	49 (22.0)	Q61	170 (70.2)	C664	2 (5.0)
D594	9 (1.4)	K642	22 (9.9)	G12	30 (12.4)	M642	2 (5.0)
G596	3 (0.5)	F483	9 (4.0)	G13	11 (4.6)	Others	36 (90.0)
Others	15 (2.3)	V559	9 (4.0)	Others	31 (12.8)		
		Others	134 (60.1)				

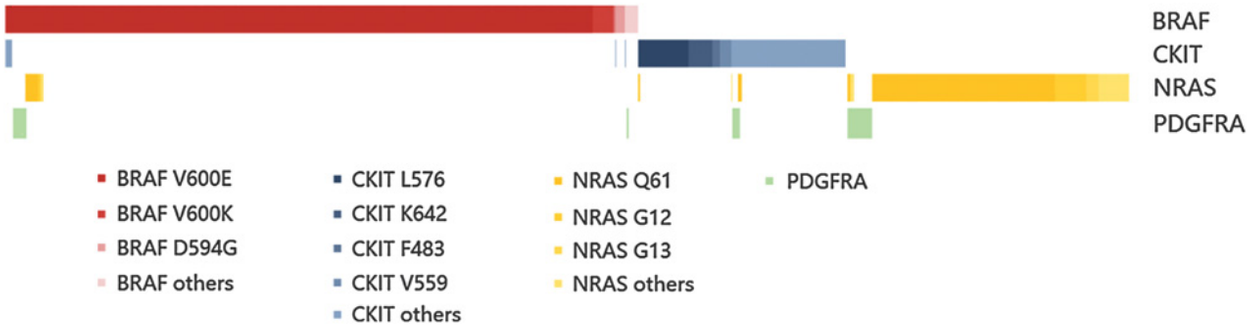

Figure 1.

MAPK pathway gene mutation pattern of melanoma ($n = 933$). Patients harboring at least one gene mutation(s) of BRAF, C-KIT, NRAS, and PDGFRA are presented. BRAF, C-KIT, and NRAS gene mutations were negatively correlated ($P < 0.001$ for BRAF-C-KIT and BRAF-NRAS, $P = 0.001$ for C-KIT-NRAS). Hotspot mutations of BRAF, NRAS, and C-KIT were mutually exclusive, with few exceptions for BRAF and NRAS. PDGFRA mutation was not correlated with BRAF, C-KIT, or NRAS mutations ($P = 0.976$, 0.089, and 0.132, respectively).

had the tendency to predict shorter overall survival (OS), yet not statistically significant ($P = 0.064$ and 0.061, respectively). OS data and more details are presented in Supplementary Table S7.

In multivariate analysis, BRAF, C-KIT, and NRAS mutations were all negative independent prognostic factors of OS ($P = 0.007$, <0.001, and <0.001, respectively). Details are presented in Supplementary Table S8. Compared with BRAF by 1.26 times, C-KIT and NRAS had greater impact on prognosis, the risk ratio increased by 1.57 and 1.58 times, respectively. However, PDGFRA was not an independent prognostic factor of OS ($P = 0.160$). Details are presented in Supplementary Table S8.

To better understand the role of mutations in prognosis, we further looked into the MAPK pathway mutation profiles in acral and mucosal melanomas, the most common subtypes in Asian population. In both uni- and multivariate analyses, BRAF, C-KIT, and NRAS were negative prognostic factors in acral melanoma, and the influence of MAPK pathway gene mutations on prognosis ranked as NRAS > BRAF > C-KIT (multivariate analysis, HR = 2.508, 2.182, and 1.864, respectively; all $P < 0.001$), whereas only C-KIT predicted poorer OS in mucosal melanoma (multivariate analysis, HR = 1.799, $P = 0.006$). Details are presented in Supplementary Tables S9–S11.

Correlation between MAPK pathway gene mutation types and prognosis. We then correlated the types of gene mutations of BRAF, C-KIT, and NRAS with prognosis. For patients harboring at least one gene mutation(s) of BRAF, C-KIT, or NRAS ($n = 933$), different types of gene mutations were not correlated with OS. Details are listed in Supplementary Table S12.

Correlation between MAPK pathway gene mutation numbers and prognosis. To further explore the prognostic value of MAPK pathway gene mutations (including BRAF, C-KIT, NRAS, and PDGFRA), we analyzed the number of gene mutations and its correlation with OS. Both uni- and multivariate analyses showed that the number of MAPK pathway gene mutations each individual patient harboring was correlated negatively with OS (both $P < 0.001$; Fig. 3). Median OS of patients harboring 0, 1, 2, or 3 gene mutations simultaneously were 52.0, 41.0, 31.0, and 16.0 months, respectively. Details are presented in Supplementary Table S13.

Taken into consideration the heterogeneity of patients harboring dual or triple MAPK pathway mutations ($n = 54$), we further looked into the prognostic value of different types of comutations in this cohort and found no statistically significant correlation. Details are presented in Supplementary Table S14.

MAPK pathway gene mutations. In total, 545 samples in this cohort were tested for the hotspot mutations of TERT promoter gene, namely C228T and C250T. As mentioned above, the overall mutation rates were 5.9% (32/545) and 5.5% (30/545), respectively.

Correlation between TERT promoter gene mutations and clinicopathologic features. Regarding the distribution of TERT promoter C228T and C250T mutations among patients with different clinicopathologic characteristics, it is notice-worthy that mutation rate of C228T was significantly higher in subtypes of CSD/NCSD/UP ($P < 0.001$), and C250T had borderline significance to show subtype distribution imbalance—more commonly seen also in CSD/NCSD/UP ($P = 0.050$). This phenomenon was consistent with the overall low mutation rates of TERT promoter genes in Asian melanomas which are dominated by acral and mucosal subtypes. As infrequently observed in acral and mucosal subtypes, TERT promoter gene mutations were anticipated to be less important in Asian populations. The summary of the associations between clinicopathologic features and TERT promoter gene mutations is shown in Supplementary Table S15.

Correlation between MAPK pathway and TERT promoter gene mutations. TERT C228T and C250T mutations were more commonly observed among BRAF-mutant patients ($P = 0.001$ and $P < 0.001$, respectively; Supplementary Table S16), with the majority of them seen in patients bearing V600E mutation (Fig. 4). This would probably be because of the prevalence of V600E in all BRAF mutations and not the distribution imbalance of TERT promoter gene mutation among different types of BRAF-mutant patients ($P = 0.421$; details are shown in Supplementary Table S17). It is interesting that TERT C228T was correlated with PDGFRA mutation ($P = 0.007$), yet this would be interpreted with caution, considering that only 7 patients were PDGFRA mutation positive in this whole cohort. TERT C228T and C250T mutations were not

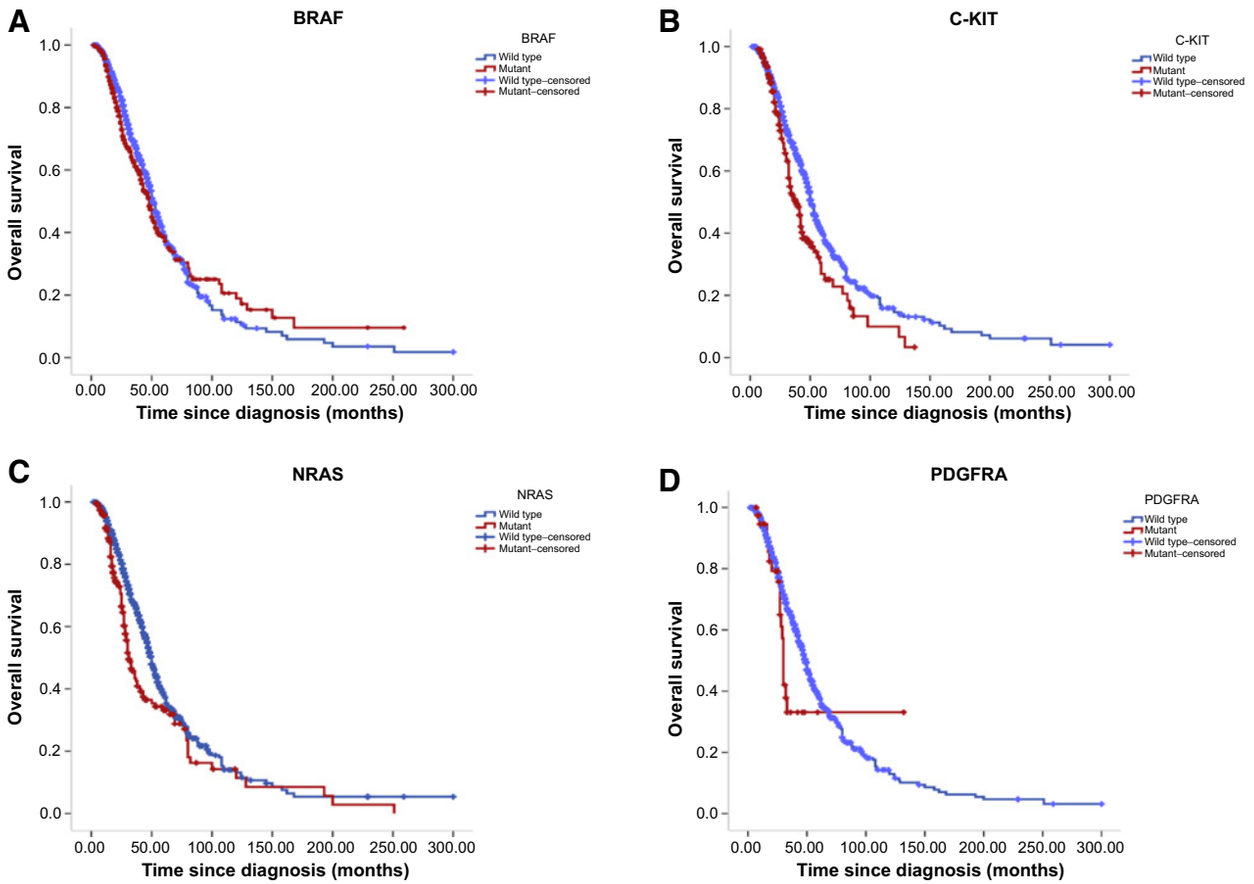

Figure 2.

Correlations of MAPK pathway gene mutations and prognosis. Presence of C-KIT and NRAS mutations was associated with poorer prognosis compared with wild-type patients [both $P < 0.001$; shown in (**B**) and (**C**), respectively]. BRAF and PDGFRA mutations had the tendency toward shorter OS, yet not statistically significant [$P = 0.064$ and 0.061, respectively; (**A**) and (**D**)].

correlated with C-KIT or NRAS mutations ($P = 0.595$ and 0.451 for TERT C228T, 0.645 and 0.694 for TERT C250T, respectively; Supplementary Table S16).

Prognostic value of TERT promoter genes. Both TERT promoter gene mutations (C228T and C250T) were not correlated with OS ($P = 0.406$ and $P = 0.256$, respectively; details are shown in Supplementary Table S18). Taken into consideration their relatively low mutation rates and subtype distribution imbalance (more commonly seen in CSD/NCSD/UP), TERT promoter gene mutations were less likely to be pivotal events in Asian population.

Discussion

Whole-genome sequencing provided comprehensive and nonbiased mutation profiles in Caucasian melanoma patients, dominated by cutaneous melanoma (CSD and NCSD; ref. 18). Mutations on MAPK signaling pathway and TERT promoter have been shown to be frequently seen in cutaneous melanoma (13–16, 19–23). In addition to being targeted therapy indicators, mutation profile also provides a clinical parameter on disease status and prognosis (8, 11).

Previous studies have shown that subtypes of melanoma in Asian population are distinct from that in Caucasian, prevalent with acral and mucosal melanomas, and with a distinct gene mutation pattern (3, 8, 11). In this study, we focused on mutations on MAPK pathway gene hotspots (namely BRAF, C-KIT, NRAS, and PDGFRA) and most frequently mutated genes on TERT promoter (C228T and C250T), with an aim to correlate mutations with clinicopathologic characteristics, particular subtypes, and to reveal their prognostic values.

Similar as reported previously (8, 11, 12), the incidence of MAPK pathway–related genes, namely, BRAF, NRAS, C-KIT, and PDGFRA, was 23.7%, 10.4%, 8.0%, and 1.4%, respectively, indicating that the MAPK pathway was activated in about half of patients, and BRAF/MEK inhibitors which are in clinical trials in China would benefit patients profoundly. As for PDGFRA, which was reported to be around 4.6% (16/351) previously (12), in this cohort, the mutation rate was only 1.4% (40/2,793), which might be due to the paramount impact of the absolute number of PDGFRA-mutant patients in a certain cohort caused by its rarity.

When correlating MAPK pathway gene mutations with clinicopathologic features, imbalance was observed regarding age, gender, stages, and subtype distribution of BRAF and NRAS gene

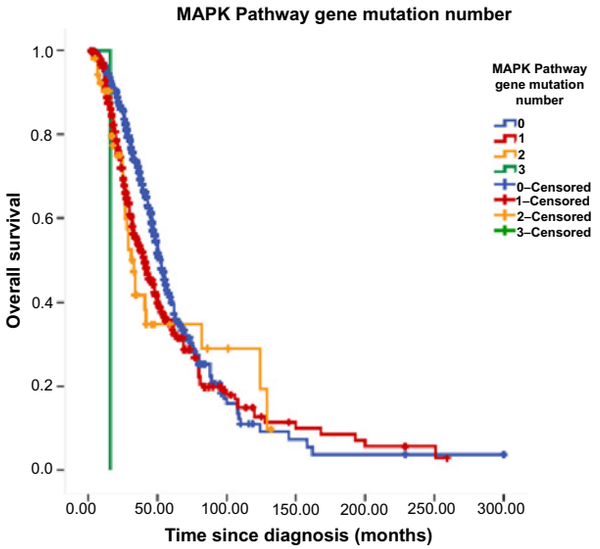

Figure 3.
MAPK pathway gene mutation numbers and OS ($n = 2265$). The number of MAPK pathway gene mutations each individual patient harboring was correlated negatively with OS (both $P < 0.001$ in uni- and multivariate analyses). Median OS of patients harboring 0, 1, 2, or 3 gene mutations simultaneously were 52.0, 41.0, 31.0, and 16.0 months, respectively.

mutations. Consistent with previous meta-analysis (24), BRAF mutations were more frequently seen in younger, female patients, patients of advanced stage, and CSD/NCSD/UP subtypes in this cohort; and NRAS more in elders, male, and patients of advanced stage. Although there is canonical hypothesis that BRAF and NRAS mutations occur early during melanoma initiation and are maintained during melanoma progression (25, 26), our finding suggested that BRAF and NRAS mutations correlate more with tumor progression rather than initiation, and is supported by an alternative view proposed by Dong and colleagues (27). Evidences in line with our finding include that (1) BRAF and NRAS mutation rates increased from melanoma *in situ*, radial growth phase to vertical growth phase (28); (2) patients with BRAF and NRAS mutations had higher tendency to develop regional lymph node (29) and distant metastasis (30). Underlying mechanism might be that heterogeneity is a general characteristic of melanoma (31), and selection pressure helps BRAF and NRAS mutations prevail as melanoma progresses. In our study, BRAF mutation dominated in CSD, NCSD, and UP subtypes of melanoma, whereas the more common subtypes in China (acral and mucosal melanomas, 70% of patients) demonstrated a different pattern of MAPK pathway gene mutations, with lower BRAF mutation rate and higher frequency of C-KIT mutations (8, 32), indicating distinct melanoma progression trajectory of different pathologic subtypes.

This is so far the study of the largest sample size in Asian population which focused on the gene mutation types of key molecules in the MAPK pathway and delineated their characteristics. Similar as reported previously in Caucasian population (18), BRAF mutations were dominated by V600 hotspot mutations (95.8%), and NRAS by Q61, G12, and G13 (87.2%). Meanwhile, C-KIT and PDGFRA mutations demonstrated comparatively more heterogeneity, hotspot mutations (including mutations targeting L576, K642, F483, and V559) accounted for only around 40% of C-KIT mutations, and no hotspot mutations were identified regarding PDGFRA. Interestingly, it has been demonstrated that BRAF, C-KIT, and NRAS mutations are mutually negatively correlated, indicating their roles as driver rather than passenger genes in individual patients.

Regarding clinicopathologic features of different gene mutation types, for BRAF, V600E was correlated with younger, female patients and V600K with elderly, male patients, which agrees with previous report that cumulative sun-induced damage correlated with V600K but not V600E mutation (6). For BRAF and NRAS hotspot mutations, distinct distribution patterns regarding melanoma subtypes were also observed, namely V600E dominated in NCSD and non-V600E comparatively more common in mucosal melanoma; Q61 prevailed in acral/NCSD and non-Q61 in mucosal/UP subtypes, indicating distinct molecular mechanisms of different pathologic subtypes, which probably worth further investigation.

Regarding the prognostic value of MAPK pathway key molecule mutations, in multivariate analysis, C-KIT, NRAS, and BRAF mutations were all negative independent prognostic factors, consistent with our previous reports (8, 11), and the impact on prognosis ranked as C-KIT=NRAS>BRAF, indicating that C-KIT and NRAS mutations play more important roles in Asian population, agreeing with the pivotal prognostic roles of NRAS in acral and C-KIT in mucosal melanomas, as will be mentioned later. Interestingly, it is demonstrated for the first time that the mutational burden (numbers) of MAPK pathway genes correlated negatively with OS. Subgroup analysis was carried out for the first time regarding the two most common subtypes (acral and mucosal) and yielded positive results. In acral melanoma, influence of MAPK pathway gene mutations on prognosis ranked as

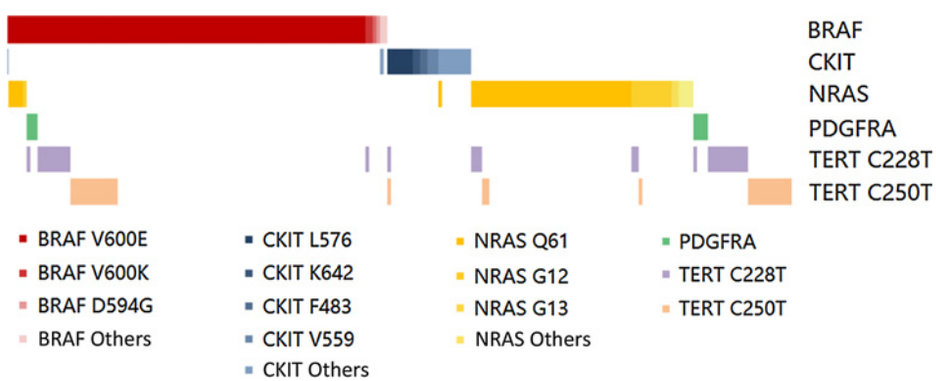

Figure 4.
MAPK pathway and TERT promoter gene mutation pattern of melanoma ($n = 216$). Patients harboring at least one gene mutation of BRAF, C-KIT, NRAS, PDGFRA, TERT C228T, and TERT C250T are presented. TERT C228T and C250T mutations were correlated with BRAF mutation: higher TERT promoter gene mutation rates were seen in BRAF-mutant patients ($P = 0.001$ and $P < 0.001$ for C228T and C250T, respectively).

NRAS>BRAF>C-KIT; in mucosal counterpart, only C-KIT mutation was an independent prognostic factor.

This is so far the study of largest sample size to analyze TERT promoter mutation in Asian melanoma patients. In our study, TERT promoter mutation rates were 5.9% (32/545) and 5.5% (30/545) for C228T and C250T, respectively. This result was conspicuously lower compared with those reported in Caucasian cutaneous melanoma (22%–85%; refs. 13–16, 20–23). Besides, imbalance has been observed regarding their subtype distribution, and the mutation rates were much lower in acral and mucosal subtypes comparing with cutaneous melanoma, similar to as reported before (15–17, 33, 34). This is probably due to ethic genetic background differences between Caucasian and Asian. Besides, in contrast with their counterparts in Caucasian (15, 16, 22, 35), TERT promoter mutations in Asian were correlated with neither clinicopathologic characteristics other than pathologic subtypes nor OS. All these data suggest distinct genetic patterns among different ethnicities. It is suggested that in Asian population, based on their rarity, irrelevance with most clinicopathologic characteristics, and prognosis, TERT promoter mutations could hardly be counted as driver events. Limitation of this study is that the number of patients harboring TERT promoter gene mutations was relatively low and permitted no stratified analysis; thus, this result could only be considered as preliminary.

It is worth noting that BRAF mutation was positively correlated with both TERT promoter mutations, consistent with previous reports (13, 16, 35). This finding added evidence to the hypothesis that multistep processes are required in melanoma oncogenesis. It is suggested that for melanoma development, BRAF mutation alone, which promotes tumor cell mitosis, is not adequate; additional TERT activation, which immortalizes tumor cells, is essential (36). However, considering the rarity of TERT promoter mutation in our cohort, there is anticipated to be some other unknown mechanism lying underneath. And future studies are required to clarify this question.

A limitation of this study is that in our test we did not include NF1 gene, which encodes neurofibromin 1, activating RAS GTPase leading to suppression of RAS signaling (37). Recently, The Cancer Genome Atlas has defined NF1-mutant subtype as one of the four main subsets of cutaneous melanoma (18), which is correlated strongly with UV mutation signature (38). As non–UV-radiation-correlated subtypes (namely acral and mucosal melanomas) prevail in Asian populations, whether or not NF1 mutation accounts for such an important role in Asian as in Caucasian population remains to be further clarified. It is interesting that we identified a subgroup of patients carrying PDGFRA or TERT promoter gene mutations yet without other tested driver genes in this cohort, and it remains unclear whether or not they simultaneously harbor NF1 mutation, which demonstrated relative high prevalence in this particular patient subset, as previously reported in Caucasian populations (10, 39, 40). Besides, as demonstrated previously that protein neurofibromin 1 both regulates and is regulated by c-Kit (41, 42), and NF1 has high tendency to be comutated with C-KIT in mucosal melanoma (43), it warrants further investigation of NF1 gene to get a more comprehensive view of gene mutation landscape in Asian populations.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: X. Bai, J. Guo, L. Si, Y. Kong
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): X. Bai, Z. Chi, X. Sheng, C. Cui, L. Mao, B. Tang, S. Li, X. Yan, L. Zhou, J. Dai, J. Guo, L. Si, Y. Kong, X. Wang, B. Lian
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): X. Bai, L. Si, Y. Kong
Writing, review, and/or revision of the manuscript: X. Bai, L. Si, Y. Kong
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): X. Bai, L. Si
Study supervision: L. Si

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