BRAF, C-KIT, and NRAS mutations correlated with different clinicopathological features: an analysis of 691 melanoma patients from a single center

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Abstract

Aims

To analyze the prevalence and relevance of pathogenetic mutations in BRAF, C-KIT, and NRAS in Chinese melanoma patients to provide some potential reference for the targeted treatment in China.

Methods

We described the frequency and distribution of BRAF, NRAS, and C-KIT mutation in 691 melanoma patients, and analyze the statistical significance of different gene mutation with clinicopathological features.

Results

BRAF mutation was found in 166 patients (24.0%), and V600E was the prominent genetic alteration (87.3%). Statistical analysis showed that younger patients had a higher BRAF mutation rate than the older. Furthermore, the frequency of BRAF mutation in patients with extremity location, ALM type, thinner thickness and no ulceration was more likely to be lower. The rate of NRAS mutation was 12.6% (38/302), mainly involved codon 61 in exon 3 and codon 12 in exon 2. C-KIT mutation was detected in 65 patients (9.4%), and the most common sites of mutations was L576 in exon 11 (44.6%). Patients with NRAS or C-KIT mutation had higher Clark level and more likely to be located in extremity than those without mutation. The concordance of BRAF, NRAS, and C-KIT mutations between paired primary and metastatic lesions was 89.6% (60/67), and visceral metastases showed a highest similar distribution of gene mutations versus primary melanomas compared with lymph nodes and cutaneous metastases.

Conclusions

In this large cohort on Chinese population, the frequency of BRAF and NRAS mutation was relatively lower than that in Caucasian, but the clinicopathological features of BRAF, C-KIT and NRAS mutation were similar. Paired primary and metastatic lesions showed high concordance of gene mutations.

Introduction

Melanoma is an extremely aggressive tumor with a high tumor mutation burden (TMB). Based on the pattern of the most prevalent significantly mutated genes, melanoma was classified into four genomic types including mutant BRAF, mutant RAS, mutant NF1, and Triple-WT (wild-type), and KIT mutations is revealed enrichment in the wild-type¹². With the successfully approved of BRAF and MEK dual-targeted inhibitors in China, more melanoma patients have the opportunity to receive accurate and effective targeted therapy. However, there is a remarkable discrepancy of disease characteristics between different

ethnic groups in melanoma³. Compared with western Caucasian (34.4 per 100,000 men and 20.9 per 100,000 women), the incidence of melanoma is relatively low (about 0.5 per 100,000), and the predominant histological types as well as corresponding genetic alterations are also obviously discrepant in Asians⁴⁵. Therefore, there is of great clinical value to further explore the prevalence of mutation characteristics in Chinese melanoma patients. Our study aimed to retrospectively analyze the prevalence and relevance of pathogenetic mutations in BRAF, C-KIT, and NRAS in a single-institution series of 691 melanoma patients to provide some potential references for the targeted treatment in China.

Materials And Methods

Patients and samples

We included 691 patients who were diagnosed with melanoma from 2013 to 2019 in Fudan University Shanghai Cancer Center. All patients in our study had pathological confirmed diagnoses. Clinical information (sex, age of diagnosis, primary/metastatic/unknown melanoma, tumor location) and histological features (histological subtype of melanoma, Breslow thickness, Clark level, ulceration and status of lymph nodes at diagnosis) were all completely obtained. Mutation analyses of BRAF (exon 15) and C-KIT (exon 9, 11, 13 and 17) were performed in all 691 patients, and 302 patients were assessed the mutation in NRAS exon 2, 3 and 4. All of the procedures were conducted conforming to the guidelines approved by Institutional Ethics Committee at Fudan University Shanghai Cancer Center.

DNA extraction

Genomic DNA was extracted from 4–5 µm unstained serial formalin-fixed paraffin-embedded sections. The standard xylene-phenol protocol was used to dissolve paraffin, and the tissue specimens were digested with proteinase K. Genomic DNA was extracted using a QIAamp DNA extraction kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. DNA concentration and quality were determined on a NanoDrop spectrophotometer (ND-1000, Thermo Fisher Scientific, Wilmington, DE, USA).

Direct sequencing of BRAF, C-KIT, and NRAS

PCR amplification and direct sequencing of exon 15 of BRAF gene, exons 9, 11, 13, and 17 of C-KIT gene, exons 2, 3, and 4 of NRAS gene were performed. The primer sequences of BRAF, C-KIT, and NRAS were listed in Supplementary Table S1. The PCR conditions were described as previously: 94°C for 10 minutes, then 38 cycles for denaturing at 94°C for 45 seconds, annealing at 60°C for 45 seconds, extension at 72°C for 45 seconds, and final extension at 72°C for 7 minutes. PCR products were purified using a QIAquick gel extraction kit (Qiagen, Germany) and were used to prepare sequencing reactions. Sequencing was performed with the Big Dye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, Foster City, CA, USA) and the following PCR conditions: 94°C for 1 minute, 24 cycles of denaturing at 94°C for 10 seconds, annealing at 50°C for 5 seconds, extension at 60°C for 1 minute, and final extension at 72°C for 5 minutes. Sequenced PCR products were purified and all mutations were

confirmed by bidirectional sequencing on an ABI 3500 Genetic Analyzer (Applied Biosystems, Foster City, USA).

Statistical analysis

Chi-square or Fisher's exact test was used to compare the gene mutation frequency in different clinicopathological features. All statistical analyses were performed with SPSS 22.0 software (SPS, Inc) and a P-value of < 0.05 (two-sided) was considered statistically significant.

Results

Clinicopathological characteristics

A total of 691 Chinese patients were included in our cohort, and there were 480 primary, 87 metastatic, 57 unknown primary (including melanomas only detected in lymph nodes, liver, brain etc), and 67 paired primary and metastatic lesions. The age of patients ranged from 20 to 89 years (median 60 years) and the ratio of male to female was 0.89. In 547 primary patients, there were 361 cutaneous and 186 mucosal melanomas. Among 361 cutaneous melanomas, 18 located in head and neck, 56 in trunk, 51 in limbs, and 236 in extremity. And among 186 mucosal melanomas, 65 located in gastrointestinal tract, 53 in respiratory tract, 63 in urogenital tract, and 5 in conjunctiva. And 109 primary patients (19.7%) involved lymph node metastasis at diagnosis.

In 361 primary cutaneous melanomas, acral-lentiginous melanoma (ALM, $n = 212$) was the most common histological subtype, followed by superficial spreading melanoma (SSM, $n = 91$), nodular melanoma (NM, $n = 50$), lentigo maligna melanoma (LMM, $n = 5$), and others ($n = 3$). The median Breslow thickness of all lesions was 3.8 mm. Ulceration was found in 110 patients (30.5%). The detailed information of clinical and pathological characteristics in cutaneous and mucosal melanoma groups was shown in Table 1.

Table 1

Basic clinical, pathological information and prevalence of mutation in 691 melanoma patients.

Clinicopathological features	All patients	Cutaneous melanoma	Mucosal melanoma
Total	691	361	186
Sex			
Male	326	193	58
Female	365	168	128
Age			
Median	60.0	60	61
< 60	336	169	89
≥ 60	355	192	97
Lymph nodes metastasis at diagnosis of primary tumor (n = 547)			
Yes	109	71	38
No	438	290	148
BRAF mutation			
Total	166	100	11
V600E	145	88	8
V600K	7	5	0
Others	14	7	3
C-KIT mutation			
Total	65	34	23
Exon9	2	1	0
Exon 11 (L576P)	25	16	9
Exon 11 (others)	22	11	4
Exon 13 (K642E)	9	3	6
Exon 13 (others)	3	2	1

There were 155 cutaneous melanoma cases who were analyzed NRAS mutation.

* There were 94 mucosal melanoma cases who were analyzed NRAS mutation.

Clinicopathological features	All patients	Cutaneous melanoma	Mucosal melanoma
Exon 17	4	1	3
NRAS mutation (n = 302)			
Total	38	17 [#]	12 [*]
Exon 2	12	4	4
Exon 3	26	13	8
Exon 4	0	0	0
[#] There were 155 cutaneous melanoma cases who were analyzed NRAS mutation.			
[*] There were 94 mucosal melanoma cases who were analyzed NRAS mutation.			

BRAF, C-KIT, and NRAS mutations in melanoma

BRAF exon 15 mutations were detected in 166 of 691 melanoma patients (24.0%, Table 1). The most common site of mutation was codon 600 (n = 154, 94.8%), and V600E was the prominent genetic alteration (n = 145, 87.3%). C-KIT gene mutations were detected in 65 of 691 patients (9.4%, Table 1), mainly occurred in exon 11 and 13. Furthermore, L576 in exon 11 (n = 29, 44.6%) was the predominant genetic alternation in C-KIT mutation, and the common amino acid changes were L576P in exon 11 (n = 25) and K642E in exon 13 (n = 9). Among 302 patients detected with NRAS mutation, 38 had a mutation in NRAS. The most common site of mutation were codon 61 in exon 3 (63.2%, 24/38) and codon 12 in exon 2 (23.7%, 9/38). Common amino acid changes were Q61R > Q61K > Q61H in codon 61 of exon 3 and G12D > G12R > G12C in codon 12 of exon 2, but there was no mutation in NRAS exon 4. Representative figures of BRAF, C-KIT, and NRAS mutation were shown in Supplementary Figure S1.

Four patients harbored BRAF and C-KIT gene mutations simultaneously (S614P in BRAF plus 570_576 deletion in C-KIT exon11, G593D in BRAF plus 570_576 deletion in C-KIT exon11, V600E in BRAF plus K484R in C-KIT exon 9, and V600E in BRAF plus D820Y in C-KIT exon 17), one patient harbored both Q61R in NRAS exon 3 and T500A in C-KIT exon 9, as well as one patient carried two C-KIT mutations including V560D in exon 11 and N822H in exon 17.

Correlation of BRAF, C-KIT and NRAS mutations with clinicopathological features

In our cohort, the frequency of BRAF mutation in younger patients (< 60) was statistically higher than that in the older (≥ 60) (31.3% vs. 17.2%, $P < 0.001$). There was no sex difference in BRAF mutation rates. BRAF mutation was observed in 41.4% of metastases, a significant increase in mutation frequency compared with that in primary melanoma (20.3%, $P < 0.001$). In terms of different primary tumor location, BRAF mutations were detected in 27.7% (100/361) of cutaneous melanoma but only in 5.9% of mucosal melanoma ($P < 0.001$). Moreover, the location of head and neck, trunk, and limbs had significantly higher

BRAF mutation rate than extremity ($P < 0.001$). And gastrointestinal tract mucosal melanoma more likely had BRAF mutation than respiratory tract, urogenital tract and conjunctival melanoma ($P < 0.001$). When mutations were stratified by histological subtypes, the frequency of BRAF mutation in ALM was lower than that in other subtypes of melanoma ($P < 0.001$). In our series, patients with BRAF mutation had thicker tumors and less ulceration than those without BRAF mutation (Table 2).

Table 2

Correlation of BRAF, C-KIT, and NRAS mutations with clinicopathological features in 361 cutaneous melanoma cases.

Clinicopathological features	BRAF mutation			C-KIT mutation			NRAS mutation		
	Mutant	Wt	P	Mutant	Wt	P	Mutant	Wt	P
Sex									
Male	51	142	.561	19	174	.778	9	72	.952
Female	49	119		15	153		8	66	
Age									
< 60	66	103	.000	7	162	.001	6	63	.417
≥ 60	34	158		27	165		11	75	
Location of cutaneous melanoma									
Extremity	30	206	.000	30	206	.003	16	86	.009
Others	70	55		4	121		1	52	
Lymph nodes metastasis at diagnosis of primary tumor									
Yes	22	49	.490	7	64	.887	2	27	.741
No	78	212		27	263		15	111	
Histological type of cutaneous melanoma									
ALM	25	187	.000	25	187	.218	14	82	.066
Others	75	74		9	140		3	56	
Breslow thickness of cutaneous melanoma, mm									
≤ 1.0	6	34	.047	2	39	.401	0	16	.045
> 1.0	94	227		32	288		17	122	
Clark level of cutaneous melanoma									
I-III	13	47	.253	3	57	.035	0	27	.047
IV-V	87	214		31	170		17	111	
Ulceration of cutaneous melanoma									
Absent	22	88	.030	15	95	.069	6	43	.700
Present	78	173		19	232		11	97	

Table 3
Correlation of BRAF, C-KIT, and NRAS mutations with clinicopathological features in 186 mucosal melanoma cases.

Clinicopathological features	BRAF mutation			C-KIT mutation			NRAS mutation		
	Mutant	Wt	P	Mutant	Wt	P	Mutant	Wt	P
Sex									
Male	6	52	.100	6	52	.573	3	31	.526
Female	5	123		17	111		9	51	
Age									
< 60	5	84	.870	9	80	.371	4	33	.759
≥ 60	6	91		14	83		8	49	
Location of mucosal melanoma									
Gastrointestinal tract	7	58	.000	9	56	.653	0	35	.003
Others	4	117		14	107		12	47	
Lymph nodes metastasis at diagnosis of primary tumor									
Yes	3	35	.971	4	34	.699	1	19	.450
No	8	91		19	129		11	63	

Contrary to the features of BRAF mutation, the frequency of C-KIT mutation in younger patients was lower than that in the older ($P = 0.006$), and extremity had higher C-KIT mutation frequency than other location in cutaneous patients. Similarly, patients with C-KIT mutation had higher Clark level than those without C-KIT mutation. However, no relationship was found between sex, histological subtype, ulceration, lymph node metastasis with C-KIT mutation (Table 2).

NRAS mutation according to age and sex was not significantly different in 302 patients. The frequency of NRAS mutation in gastrointestinal tract was statistically lower than that in other tumor location in mucosal melanoma. Similar to the features of C-KIT mutation, NRAS mutation was more frequently detected in cases featured with extremity location and higher Clark level (Table 2).

Concordance of mutation between paired primary and metastatic lesions

Among 67 paired primary and metastatic lesions, there were 44 lymph nodes, 18 cutaneous and 5 visceral metastases. Mutation inconsistencies of BRAF, C-KIT, and NRAS gene were found in 7 patients (10.4%), including 4 BRAF discordance (9.1%), 1 C-KIT discordance (2.3%), and 2 NRAS discordance (2/39, 5.1%). Stratified by different types of metastasis, visceral metastases (100.0% consistency)

presented highest similar distribution of BRAF/C-KIT/NRAS mutations versus primary melanomas, followed by lymph nodes (90.9% consistency) and cutaneous metastases (83.3% consistency).

Discussion

Until now, most of studies on the landscape of genomic changes in melanoma have focused on western population⁶⁷, so there is still a need to fully delineate the oncogenic difference between Caucasian and Asian population. Based on the large-scale single-institution study of genetic mutations in melanoma, we further disclosed the particular features of BRAF, C-KIT, and NRAS mutations which had significant clinical value for the treatment of Chinese melanoma patients. On the aspect of clinical characteristics, this study and previous studies on eastern population uniformly showed that the tumor in Asian melanoma patients are more likely to be located in extremities, thicker and have higher rate of ulceration, accompanied by poorer prognoses compared to that in western patients^{38–10}.

BRAF gene, as the major mutated gene in melanoma, is described to be mutated in about 50–60% among western melanoma patients and associated with intermittent ultraviolet (UV) exposure¹¹¹². However, because the most common tumor locations are sun-protected extremity and mucosa (70% patients), the frequency of BRAF mutation is only around 20% in Asians⁸¹³¹⁴. Although the relatively limited frequency of BRAF mutation in Chinese population, our data and previous studies unanimously confirmed that the dominated position is codon 600, and more than 90% of the amino acid change is V600E which could active the function of this kinase⁸¹³. Consistent with previous reported studies, BRAF mutation prevailed in younger patients⁴¹⁵, but was rare in extremities and corresponding ALM type confirming the UV-induced feature of BRAF mutation⁴¹⁶. Our study also revealed that thicker thickness and metastatic lesions had markedly higher rates of BRAF mutation than thinner and primary lesions^{17–19}, indicting a sequential increase in BRAF mutation rates during melanoma prognosis which needs more studies to further investigate the role of BRAF mutation in melanomagenesis and progression. Additionally, BRAF mutation was found in 5.9% of mucosal melanoma patients in our cohort, but non-V600E was comparatively more common (3/11, 27.3%) compared to that in cutaneous melanoma (12/100, 12.0%), in accordance with the findings by Bai et al in 2793 Chinese cases¹³. Country to the results form a meta-analysis of mucosal melanoma²⁰, we found gastrointestinal tract melanomas, rather than conjunctival, harbored more BRAF mutation, probably owing to relatively few conjunctival melanomas patients in our serious.

Consistent with reported previously^{1321–23}, the incidence of C-KIT and NRAS in melanoma was about 8.0% and 10.4%, respectively, and C-KIT mutations were dominated by L576 and K642 (38/65, 58.5%), NRAS by Q61, G12, and G13 (36/38, 94.7%). In contrast to the clinicopathological features of BRAF mutation, the frequency of C-KIT and NRAS mutations in older patients were higher than that in the younger. Extremity location and ALM subtype were prone to harbor more C-KIT or NRAS mutation, compared to that in previous reports^{12182124–26}. Furthermore, our study found that NRAS mutation positive patients showed higher Clark level and deeper Breslow thickness as compared to negative

patients, and C-KIT mutation was also correlated with higher Clark level. However, most of studies didn't find the significant correlation between C-KIT or NRAS mutation with level of tumor invasion^{9-10, 16, 18, 27}, and Thomas et al²⁸ found that NRAS mutation was associated with lower ulceration rate which was nonsignificant in our series. The discrepancies on the clinical relevance of C-KIT and NRAS mutations in different studies may be related to distinct ethnic groups and size of sample, and may reveal the peculiar genetic information in Chinese population.

The above different genetic pattern of BRAF, C-KIT and NRAS mutation between Asian and Western population mainly Generally, hotspots of mutations of BRAF, C-KIT, or NRAS gene, as the key molecules in MAPK pathway, were mutually exclusive in melanoma¹. In our series, six patients simultaneously harbored two of BRAF, C-KIT, or NRAS mutations, but at least one of these gene mutations was non-hotspot whose biochemical and clinical value needed to be further investigated. However, in the report by Bai et al¹³, there were 13 patients harbored both BRAF V600E and NRAS Q61R/K or G12D mutations, suggesting that we should be aware of the exceptional genetic alterations so that patients could get appropriate treatment in our routine practice.

Several previous studies have disclosed the heterogeneity of genetic changes between primary and corresponding metastatic lesions¹⁹²⁹⁻³¹. In our cohort, the concordance rates of BRAF, C-KIT, and NRAS mutations between paired primary and metastatic lesions were 94.0%, 97.7%, and 94.9% respectively. A recent study conducted by Cormican et al showed the high concordance of BRAF mutational status in matched primary and metastatic melanoma (52/53, 98%)³⁰, which was higher than the results in our study. However, the study conducted by Manca et al revealed that mutational concordance in pathogenic/pathogenic like mutations between primary and metastatic melanoma was only 76%⁶, even different site from the same lesion could exist diverse genetic alterations³²⁻³⁴. Because the size of paired primary and metastatic samples was relatively limited, the high concordance of gene mutations in paired primaries and metastases in our study needed to be further evaluated in large cohort containing more balanced distribution of different melanoma subtypes. The intra- and inter-tumor heterogeneity of gene mutation indicates that different site and period lesions all should be evaluated the status of mutation, in order to avoid omitting any patients who are possible to benefit from the targeted therapy. For different types of metastasis, we demonstrated that visceral and lymph nodes metastases, compared to cutaneous metastases, presented highly similar mutation status versus primary lesions, which was consistent with the studies by Colombino et al²⁹.

In conclusion, the present study showed that compared to the mutation distribution in Caucasian, Chinese patients harbored relatively lower BRAF mutation and higher frequency of C-KIT and NRAS mutations, but the common genotypes of BRAF, C-KIT, and NRAS mutations were parallel. The diverse clinicopathological characteristics of BRAF, C-KIT, and NRAS mutations delineated the peculiar genomic landscape of Chinese melanoma patients. The discrepancy in mutation status between primary and metastatic lesions indicated the needs to fully comprehend the genetic background of patients who have opportunities to receive targeted treatment. Further studies using comprehensive and accurate detection

methods, including next generation sequencing and whole exome sequencing, are warrant to clarify the pathogenesis and possible therapeutic targets to facilitate the treatment of melanoma in China.

Declarations

Ethics approval and consent to participate: Institutional Ethics Committee at Fudan University Shanghai Cancer Center has approved this study.

Availability of data and materials: All data generated or analysed during this study are included in this published article [and its supplementary information files].

Competing interests: The authors have disclosed no conflicts of interest.

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Authors' contributions: Min Ren edited the manuscript and analyzed the data. Jing Zhang performed the experiments and collected original data. Yunyi Kong and Yong Chen provided part of data. Qianming Bai, Peng Qi, Ling Zhang, and Qian Wang carried out part of experiments. Xiaoli Zhu and Xiaoyan Zhou conceived and designed the study and revised the paper. All authors read and approved the manuscript.

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