Gene Mutation Characteristics and Clinical Prognosis of Head and Neck Mucosal Melanoma

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Abstract

Background: As a highly malignant tumor type, the mucosal type is the second most common pathological subtype in China. In this study, we analyzed the results and clinical data for C-KIT, NRAS, PDGFRA and BRAF genotypes in patients, to explore the characteristics of gene mutation in head and neck mucosal melanoma and the correlation between the four common gene mutation types and prognosis.

Methods: C-KIT (exons 9, 11, 13, 17 and 18), NRAS (exons 1 and 2), PDGFRA (exons 12, 14 and 18) and BRAF (exons 11 and 15) were analyzed by PCR amplification and Sanger sequencing in patients. The Chi-squared test, Log rank test and Cox regression model were used to analyze the risk factors.

Results: In total, 96 patients were included in the study. 14 (14.58%) patients had the C-KIT mutation, 6 (6.25%) had the BRAF mutation, 23 (23.96%) had the PDGFRA mutation, and 12 (12.50%) had the NRAS mutation. The NRAS mutation (P = 0.037, 95%CI: 1.050–4.572) was an independent factor affecting postoperative distant metastasis. The mutation types of different primary tumor sites were different. The BRAF mutation was more common in the nasopharynx and other sites (P = 0.008), whereas the NRAS mutation was more common in the nasal cavity/sinus (P = 0.043).

Conclusion: The specificity of this genotype may have some guiding significance for treatment of patients with head and neck mucosal melanoma, but this study did not find any correlation with survival time, and this needs further analysis with a larger dataset.

1. Background

Mucosal melanoma is a highly aggressive subtype of melanoma, and Asians have the highest number of cases of mucosal melanoma worldwide: 70% of Asian patients with melanoma have acral or mucosal types [1]. The incidence of malignant melanoma in China is increasing gradually. The incidence rate of new patients is more than 20000 per year [2]. Patients with mucosal-type melanoma account for 22.6% [1], while patients with primary melanoma in the head and neck account for 23–55% [3–5]. Compared to other subtypes of melanoma, the prognosis of patients with head and neck mucosal melanoma is significantly worse, and the 5-year survival rate is only 4–25%, accompanied by high recurrence and a high rate of distant metastasis [6–8]. BRAF mutation activity is very common in the skin (> 50%), while the mutation rate in the mucous membrane is only about 10% [9, 10]. Other mutations in the mitogen-activated protein kinase (MAPK) pathway (NRAS, C-KIT and PDGFRA) were also different in patients with mucosal-type compared with skin-type melanoma. However, the expression of these four genes in relation to the MAPK pathway in head and neck mucosal melanoma is still unclear. Therefore, this paper analyzes the expression and clinical characteristics of BRAF, NRAS, C-KIT and PDGFRA in patients with head and neck mucosal melanoma, so as to clarify the genotype characteristics of head and neck mucosal melanoma.

2. Material And Methods

2.1 General information

From June 2004 to October 2018, 96 patients with head and neck mucosal malignant melanoma were diagnosed by pathology in our hospital. PCR amplification and Sanger sequencing were used to analyze the mutations of C-KIT (exons 9, 11, 13, 17 and 18), NRAS (exons 1 and 2), PDGFRA (exons 12, 14 and 18), and BRAF (exons 11 and 15). This study aimed to collect and summarize the past medical history, diagnosis and treatment, relevant auxiliary examination, pathological condition and follow-up information, and the results of gene testing in the 96 patients. The study was approved by the hospital medical ethics committee. All patients gave their informed consent verbally and signed consent forms.

2.2 Methods

2.2.1 DNA preparation and mutation screening

Genomic DNA was extracted from formalin fixed, paraffin embedded (FFPE) sections using a QIAamp DNA FFPE Tissue kit (Qiagen, Hilden, Germany). 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 separate preparations of genomic DNA (the primer sequences are listed in Supplementary Table S1). After PCR, PCR products were purified using QIAquick (Qiagen), followed by Sanger sequencing (Tianyihuiyuan Company, Beijing, China). The specific DNA preparation and mutation detection methods were consistent with previous reports [11].

2.2.2 Statistical methods

Microsoft Excel 2017 (Microsoft Corp., Redmond, WA, USA) was used to establish the database, and SPSS version 22.0 statistical software (IBM Corp., Armonk, NY, USA) was used to conduct single factor Chi-squared tests on various risk factors. The Chi-squared test was used to compare frequency data between groups. The Log rank test was used to compare the curves between groups. A Cox regression model was used to analyze the risk factors of statistically significant variables obtained by univariate analysis. $P < 0.05$ was taken to indicate a statistically significant difference between groups.

3. Results

3.1 Detection of gene mutations

Among the 96 patients, 82 had wild-type C-KIT (85.42%, 82/96), 14 had mutant-type (14.58%, 14/96), 90 had wild-type BRAF (93.75%, 90/96), 6 had mutant-type (6.25%, 6/96), 73 had wild-type PDGFRA (76.04%, 73/96), 23 had mutant-type (23.96%, 3/96), 84 had wild-type NRAS (87.50%, 84/96), and 12 had mutant-type (12.50%, 12/96).

3.2 General information on patients

As of December 2019, the follow-up time ranged from 14 to 186 months, with a median of 27.5 months. There were 54 males and 42 females. Age of onset was 29–84 years, and median age was 60 years. The primary site of the tumor was as follows: 74 patients with nasal cavity/paranasal sinus, 22 patients with nasopharynx and other locations (inner canthus, nasolacrimal duct, tongue, vocal cord, epiglottis). According to TNM staging, there were 51 patients with T3N0M0, 29 patients with T4aN0M0, 4 patients with T4aN1M0, 10 patients with T4bN0M0, and 2 patients with T4bN1M0. The median survival time was 20 months. Among the 96 patients, 32 (33.33%, 32/96) had a local recurrence, and 44 (45.83%, 44/96) had distant metastasis. The metastatic sites were lung (34.09%, 15/44), abdomen (4.54%, 2/44), bone (2.27%, 1/44), multiple organs (31.82%, 14/44), and 7 patients (7.29%, 7/96) had overall lymph node metastasis.

3.3 Analysis of the differences among patients with different genotypes

The factors that may be related to the patient's genotype were analyzed between groups, including age, sex, primary tumor location, tumor stage, pigment classification, postoperative distant metastasis, location of postoperative distant metastasis, lymph node metastasis, postoperative local recurrence, and overall recurrence (Table 1).

Table 1
Analysis of phenotype related factors for C-KIT, BRAF, PDGFRA and NRAS in all 96 patients

	Total	C-KIT			BRAF			PDGFRA			NRAS			□□□□		
		Wild	Mut	P	Wild	Mut	P	Wild	Mut	P	Wild	Mut	P	NoMut	Any Mut	P
Sex				0.512			0.750			0.651			0.276			0.8
Male	54	37	9		51	3		42	12		49	5		28	26	
Female	42	45	5		39	3		31	11		35	7		21	21	
Age		58.65	58.14	0.885	59.77	40.67	0.000	58.81	57.83	0.734	58.04	62.33	0.247	59.92	57.17	0.3
Pigment typing				0.106			0.843			0.126			0.771			0.1
Amelanotic	19	14	5		18	1		17	2		17	2		9	10	
Pigment	77	68	9		72	5		56	21		67	10		40	37	
TNM Stage				0.857			0.520			0.920			0.763			0.3
T3N0M0	51	43	8		48	3		39	12		46	5		28	23	
T4aN0M0	29	25	4		27	2		21	8		24	5		12	17	
T4aN1M0	4	4	0		3	1		3	1		3	1		1	3	
T4bN0M0	10	9	2		10	0		8	2		9	1		6	4	
T4bN1M0	2	2	0		2	0		2	0		2	0		2	0	
Total lymph node metastasis				0.256			0.362			0.766			0.882			0.0
Yes	7	7	0		6	1		5	2		6	1		3	4	
No	89	75	14		84	5		68	21		78	11		46	43	
Local recurrence after operation				0.413			0.371			0.866			1.000			0.1
Yes	32	26	6		29	3		24	8		28	4		13	19	
No	64	56	8		61	3		49	15		56	8		36	28	
Postoperative distant metastasis				0.809			0.526			0.097			0.030			0.0
Yes	44	38	6		42	2		30	14		35	9		18	26	
No	52	44	8		48	4		43	9		49	3		31	21	
Location of distant metastasis after operation				0.752			0.751			0.457			0.575			0.5
Lung	15	12	3		14	1		11	4		13	2		6	9	
Abdomen	12	10	2		11	1		9	3		12	0		7	5	
Bone	1	1	0		1	0		0	1		1	0		0	1	
Multiple organs	14	13	1		14	0		10	4		12	2		8	6	
Overall recurrence				0.455			0.828			0.422			0.339			0.4
Yes	60	50	10		56	4		44	16		54	6		29	31	
No	36	32	4		34	2		29	7		30	6		20	16	
Primary location				0.886			0.008			0.678			0.043			0.9
Nasal cavity/sinus	74	63	11		72	2		57	17		62	12		38	36	

	Total	C-KIT			BRAF			PDGFRA			NRAS			□□□□		
		Wild	Mut	P	Wild	Mut	P	Wild	Mut	P	Wild	Mut	P	NoMut	Any Mut	P
Nasopharynx and other locations	22	19	3		18	4		16	6		22	0		11	11	
Survival				0.283			0.082			0.724			0.487			0.9
Yes	47	42	5		42	5		35	12		40	7		24	23	
No	49	40	9		48	1		38	11		44	5		25	24	

(1) The mean age of patients with mutant-type BRAF was significantly lower than that of patients with wild-type BRAF (40.67 vs 59.77 years), but the number of BRAF mutation cases was low (6/90; 6.25%), which may lead to bias.

(2) There were statistically significant differences in mutations in different types of primary lesion site. BRAF mutations were more common in lesion sites outside the nasal cavity and paranasal sinuses (4 vs 2; P = 0.008); on the other hand, NRAS mutations were more common in lesion sites in the nasal cavity/paranasal sinuses. The difference was statistically significant (12 vs 0; P = 0.043);

(3) The presence of postoperative distant metastasis was statistically significantly different between the wild-type and mutant NRAS gene (P = 0.030). There were no significant differences in overall survival, recurrence, postoperative distant metastasis, and lymph node metastasis between patients with wild-type and other genotype.

The possible factors related to postoperative distant metastasis in the 96 patients were analyzed. TNM staging in patients with distant metastasis was significantly different from the staging in patients with no distant metastasis (p = 0.006). There were no significant differences for the other factors (Table 2).

Table 2
Analysis of factors related to postoperative distant metastasis in the 96 patients in this study

	With distant metastasis	Without distant metastasis	X^2/T	p
Sex			0.011	0.918
Male	25	29		
Female	19	23		
Age	59.00	58.07	0.378	0.706
Pigment typing			0.133	0.716
Amelanotic	8	11		
Pigment	36	41		
TNM Stage			14.292	0.006
T3N0M0	17	34		
T4aN0M0	19	10		
T4aN1M0	0	4		
T4bN0M0	6	4		
T4bN1M0	2	0		
Operation			0.019	0.890
Endoscope	26	30		
Open	18	22		
Postoperative chemotherapy			0.000	0.986
Yes	17	20		
No	27	32		
Postoperative radiotherapy			0.357	0.550
Yes	23	24		
No	21	28		
Postoperative immunotherapy			1.392	0.238
Yes	13	10		
No	31	42		

Cox regression analysis was then performed on TNM stage and NRAS phenotype to further verify the independent factors related to postoperative distant metastasis (Table 3). The NRAS phenotype (P = 0.037, 95%CI: 1.050–4.572) and TNM stage (P = 0.000, 95%CI: 1.192–1.787) were independent factors influencing postoperative distant metastasis. The risk curves for wild-type and mutant NRAS are shown in Fig. 1.

Table 3
Cox regression analysis on TNM stage and NRAS phenotype in the 96 patients in this study

	B	P	Exp (B)	95%CI
NRAS mutation	0.784	0.037	2.191	1.050–4.572
TNM stage	0.378	0.000	1.459	1.192–1.787

3.4 Group analysis of different primary tumor locations

The analysis mentioned above showed that there may be differences in genotype in different lesion locations, so the patients with different primary lesion locations were grouped and analyzed: (1) group with lesions in the nasal cavity/paranasal sinus/nasal cavity and sinus; (2) group with lesions in the nasopharynx and other locations.

3.4.1 Group with lesions in the nasal cavity/sinus/nasal cavity

In total, 74 patients (40 male and 34 female) whose primary lesions were located in the nasal cavity and paranasal sinuses were included in this group. Age of onset was 37–84 years, and median age was 60 years. According to TNM staging, there were 36 patients with T3N0M0, 27 patients with T4aN0M0, 1 patient with T4aN1M0, and 10 patients with T4bN0M0. According to the specific locations of the lesions, they were divided into three groups: simple nasal cavity (40

cases), single sinus/nasal cavity + single sinus (21 cases), multiple sinuses/nasal cavity + multiple sinuses (13 cases). In total, 37 cases died, 37 cases survived, and the median survival time was 19 months. Local recurrence occurred in 25 cases (33.78%, 25/74), distant metastasis in 36 cases (48.65%, 36/74), and overall lymph node metastasis in 1 case (1.35%, 1/74). The possible differences between groups were analyzed (Table 4).

Table 4
Analysis of genotype related factors for C-KIT, BRAF, PDGFRA and NRAS in the 74 patients with lesions in the nasal cavity/sinuses/nasal cavity and paranasal sinuses

	Total	C-KIT			BRAF			PDGFRA			NRAS		
		Wild	Mut	P	Wild	Mut	P	Wild	Mut	P	Wild	Mut	P
Sex				0.489			0.907			0.510			0.347
Male	40	33	7		39	1		32	8		35	5	
Female	34	30	4		33	1		25	9		27	7	
Age		59.62	58.73	0.810	59.79	48.50	0.163	59.21	60.41	0.702	58.94	62.33	0.342
Pigment typing				0.531			0.289			0.320			0.734
Amelanotic	15	12	3		14	1		13	2		13	2	
Pigment	59	51	8		58	1		44	15		49	10	
TNM Stage				0.855			0.311			0.920			0.110
T3N0M0	36	30	6		36	0		28	8		31	5	
T4aN0M0	27	24	3		25	2		20	7		22	5	
T4aN1M0	1	1	0		1	0		1	0		0	1	
T4bN0M0	10	8	2		10	0		8	2		9	1	
Primary location				0.996			0.703			0.206			0.759
Nasal cavity	40	34	6		39	1		34	6		34	6	
Single sinus/nasal cavity + single sinus	21	18	3		20	1		14	7		18	3	
Multiple sinuses/nasal cavity + multiple sinuses	13	11	2		13	0		9	4		10	3	
Total lymph node metastasis				0.674			0.867			0.582			0.162
Yes	1	1	0		1	0		1	0		0	1	
No	73	62	11		71	2		56	17		62	11	
Local recurrence after operation				0.375			0.306			0.664			0.971
Yes	25	20	5		25	0		20	5		21	4	
No	49	43	6		47	2		37	12		41	8	
Postoperative distant metastasis				0.672			0.163			0.131			0.046
Yes	36	30	6		36	0		25	11		27	9	
No	38	33	5		36	2		32	6		35	3	
Survival				0.744			0.152			0.782			0.528
Yes	37	32	5		35	2		29	8		30	7	
No	37	31	6		37	0		28	9		32	5	

(1) There was no statistically significant differences in general factors such as age, sex, disease stage, pigmentation classification, and primary disease location among groups.

(2) There was a statistically significant difference in the occurrence of postoperative distant metastasis between the wild-type and mutant NRAS gene (9/36; 25%; P = 0.046). There were no significant differences in overall survival, recurrence, and lymph node metastasis between patients with wild-type and mutant NRAS.

The possible factors related to postoperative distant metastasis in the 74 patients with lesions in the nasal cavity and paranasal sinuses were analyzed. TNM staging in patients with distant metastasis was significantly different from the staging in patients with no distant metastasis (0.039). There were no significant differences for the other factors (Table 5).

Table 5
Analysis of factors related to postoperative distant metastasis in the 74 patients with lesions in the nasal cavity and paranasal sinuses

	With distant metastasis	Without distant metastasis	X^2/T	p
Sex			0.064	0.801
Male	20	20		
Female	16	18		
Age	59.00	58.07	0.378	0.706
Pigment typing			0.165	0.684
Amelanotic	8	7		
Pigment	28	31		
TNM Stage			8.352	0.039
T3N0M0	12	24		
T4aN0M0	18	9		
T4aN1M0	0	1		
T4bN0M0	6	4		
Operation			0.004	0.948
Endoscope	23	24		
Open	13	14		
Postoperative radiotherapy			0.845	0.358
Yes	19	16		
No	17	22		
Postoperative chemotherapy			0.645	0.422
Yes	11	15		
No	25	23		
Postoperative immunotherapy			0.499	0.480
Yes	8	6		
No	28	32		

Cox regression analysis was performed on TNM stage and NRAS phenotype to further verify the independent factors related to postoperative distant metastasis (Table 6). The NRAS phenotype (P = 0.046, 95%CI: 1.014–4.618) and TNM stage (P = 0.003, 95%CI: 1.130–1.809) were independent factors influencing postoperative distant metastasis in patients with nasal cavity/paranasal sinuses. The risk curves for wild-type and mutant NRAS are shown in Fig. 2.

Table 6
Cox regression analysis on TNM stage and NRAS phenotype in the 74 patients with lesions in the nasal cavity and paranasal sinuses

	B	P	Exp(B)	95%CI
NRAS mutation	0.772	0.046	2.164	1.014–4.618
TNM stage	0.358	0.003	1.430	1.130–1.809

3.4.2 Group with lesions in the nasopharynx and other locations

In total, 22 patients (14 male and 8 female) whose primary lesions were in the nasopharynx or other locations (inner canthus, nasolacrimal duct, tongue, vocal cord, epiglottis) were included in this group. Age of onset was 29–82 years, and median age was 57.5 years. According to TNM staging, there were 15 patients with T3N0M0, 2 patients with T4aN0M0, 3 patients with T4aN1M0, and 2 patients with T4bN1M0. The median survival time was 24 months. There were local lesion recurrences in 7 patients (31.82%, 7/22), distant metastasis in 8 patients (36.36%, 8/22), and overall lymph node metastasis in 6 patients (27.27%, 6/22). The possible differences between groups were analyzed (Table 7).

Table 7
Analysis of phenotype related factors for C-KIT, BRAF, PDGFRA and NRAS in the 22 patients with lesions in the nasopharynx or other locations

	Total	C-KIT			BRAF			PDGFRA			NRAS		
		Wild	Mut	P	Wild	Mut	P	Wild	Mut	P	Wild	Mut	P
Sex				0.907			0.531			0.856			-
Male	14	12	2		12	2		10	4		14	0	
Female	8	7	1		6	2		6	2		8	0	
Age		55.42	56.00	0.949	50.67	36.75	0.001	57.38	50.50	0.316	55.50	-	-
Pigment typing				0.028			0.264			0.197			-
Amelanotic	4	2	2		4	0		4	0		4	0	
Pigment	16	15	1		12	4		11	5		16	0	
TNM Stage				0.386			0.709			0.722			-
T3N0M0	15	13	2		12	3		11	4		15	0	
T4aN0M0	2	1	1		2	0		1	1		2	0	
T4aN1M0	3	3	0		2	1		2	1		3	0	
T4bN1M0	2	2	0		2	0		2	0		2	0	
Postoperative lymph node metastasis				0.459			0.380			0.099			-
Yes	3	3	0		3	0		1	2		3	0	
No	19	16	3		15	4		15	4		19	0	
Total lymph node metastasis				0.254			0.910			0.696			-
Yes	6	6	0		5	1		4	2		6	0	
No	16	13	3		13	3		12	4		16	0	
Local recurrence after operation				0.952			0.040			0.262			-
Yes	7	6	1		4	3		4	3		7	0	
No	15	13	2		14	1		12	3		15	0	
Postoperative distant metastasis				0.159			0.531			0.416			-
Yes	8	8	0		6	2		5	3		8	0	
No	14	11	3		12	2		11	3		14	0	
Survival				0.089			0.190			0.221			-
Yes	10	10	0		7	3		6	4		10	0	
No	12	9	3		11	1		10	2		12	0	

(1) The mean age of patients with mutant-type BRAF was significantly lower than that of patients with wild-type BRAF (36.75 vs 50.67 years); however, the number of BRAF mutation cases was low (18.18%, 4/22, P = 0.001), which may lead to bias.

(2) According to the pigment classification of the lesions, it can be seen that there was a significant difference between patients with wild-type and mutant-type C-KIT. The non-pigmented type was more likely to be present in patients with C-KIT mutations (50%, 2/4, P = 0.028), but this result is also limited by the number of cases, so there may be some bias.

(3) There was a statistically significant difference in postoperative local recurrence between patients with wild-type and mutant-type BRAF. Postoperative local recurrences were more likely to be present in patients with BRAF mutations (42.86%, 3/7, p = 0.040), but again, this result is limited by the low number of cases, so there may be some bias.

Therefore, we analyzed the factors related to postoperative local recurrence in the group of patients with lesions in the nasopharynx or other locations, and clarified the influence of mutations on local recurrence in these patients (Table 8). There were no significant differences in the factors in terms of local recurrence of lesions in the nasopharynx or other locations in this group of patients. Therefore, the results shown in Table 8 exclude the other factors that may affect local recurrence and confirm the previous conclusion drawn from Table 7 that there was a statistically significant difference in postoperative local recurrence between wild-type and mutant-type BRAF in the 22 patients with lesions in the nasopharynx or other locations. The risk curves for wild-type and mutant BRAF are shown in Fig. 3.

Table 8
Analysis of factors related to local recurrence in the 22 patients with lesions in the nasopharynx or other locations

	With Local recurrence	Without Local recurrence	X^2/T	p
Sex			1.916	0.166
Male	3	11		
Female	4	4		
Age	50.86	57.67	1.068	0.298
Pigment typing			0.220	0.639
Amelanotic	1	3		
Pigment	6	10		
TNM Stage			5.406	0.144
T3N0M0	4	11		
T4aN0M0	2	0		
T4aN1M0	1	2		
T4bN1M0	0	2		
Operation			1.119	0.290
Endoscope	4	5		
Open	3	10		
Postoperative radiotherapy			0.210	0.647
Yes	4	7		
No	3	8		
Postoperative chemotherapy			0.566	0.452
Yes	3	9		
No	4	6		
Postoperative immunotherapy			1.119	0.290
Yes	4	5		
No	3	10		

4. Discussion

Mucosal melanoma accounts for about 23% of the total incidence of melanoma in the Chinese population [1], and the head and neck mucosa is one of the main locations for melanoma. The location of the lesion is specific and there are no tumor-specific signs. It is accompanied by a high degree of malignancy and invasiveness, but the reason for this is unclear. At present, there are only a few studies on head and neck mucosal melanoma, most of which are limited by the number of cases reported. Most studies are presented in the form of case reports or small-scale retrospective analyses, so it is difficult to formulate evidence-based guidelines for clinical management. Three studies reported 5-year overall survival rates and these ranged from a maximum of 55.6–79.3% to a minimum of 4–25% [6, 8, 12]. Therefore, more reliable data are needed to analyze the biological characteristics of head and neck mucosal melanoma. In addition, at the present time, there has not been a breakthrough in clinical treatment which could improve the prognosis for patients with head and neck mucosal melanoma. The application of targeted drugs in patients with cutaneous melanoma has shown that it is possible to clarify the genotype characteristics of patients with head and neck mucosal melanoma to guide clinical treatment.

Previous studies have shown that mitogen activated protein kinase (MAPK) and phosphatidylinositol 3 kinase (PI3K/Akt) are the most important signal transduction pathways in melanoma [13, 14]. An increasing number of studies have shown that these pathways are involved in the occurrence and treatment resistance of melanoma. Among the pathways, the expression of many genes is different in different subtypes of melanoma and even in patients with mucosal melanoma in different locations. Skin melanoma can be divided into four genomic subtypes: BRAF, RAS (n/h/k), NF1 and Triple-WT. However, this classification of gene mutation subtypes is not suitable for mucosal melanoma and has an impact on its treatment. According to the study published in *Cell* in 2015 [15], we compared the genotype differences of patients with different subtypes of melanoma. Among them, the total mutation rates of MAPK, PI3K, RTKs, and cell cycle pathway related genes in skin melanoma patients were 92%, 56%, 49%, and 40%, respectively, while the corresponding mutation rates of acral and mucosal melanoma were only 60%, 23%, 40%, and 16%. There were significant differences in the overall genetic characteristics, but that study was also limited by the small number of patients.

At present, the largest study in Asia on 2793 patients with melanoma [11] showed that the somatic mutation rates of BRAF, NRAS, C-KIT, and PDGFRA genes were 23.7%, 10.4%, 8.0%, and 1.4%, respectively. That study included 755 (27%) patients with mucosal type melanoma. In addition, the earlier reported analysis of 706 patients with mucosal melanoma showed that the mutation rates of C-KIT and BRAF were 10% and 12%, respectively [16]. Compared with the data for simple head and neck mucosal lesions in this study, genotype differences of mucosal subtypes are present in different locations. The mutation rates of the above genes were 6.25%, 12.50%, 14.58%, and 23.96%, respectively, i.e. BRAF < NRAS < C-KIT < PDGFRA. Other researchers [17] have studied 65 cases of head and neck mucosal melanoma, showing that the BRAF mutation rate was 3.1%, the NRAS mutation rate was 6.2%, the NF1 mutation rate was 7.8%, and the C-KIT mutation rate was 23.1%. In the analysis of our data, PDGFRA was the most common mutation at 23.96%, while in melanoma, the overall frequency of PDGFRA mutations was lower (4.6%). Compared with the prevalence of PDGFRA mutations in melanoma in skin with chronic sun damage (0%), the prevalences in limbs (6.8%), mucosa (3.6%) and skin without chronic sun injury (1.8%) were significantly higher [18].

According to the previous literature [19, 20], the BRAF mutation rate was about 40–60% in melanoma, and was mainly present in superficial diffusion-type of skin melanin. The NRAS mutation rate was 15–30% in patients with non-chronic sunlight damage type melanin, which was more common than in the nodular type and chronic sunlight damage type of skin melanin. The C-KIT mutation rate was 1–2%, which is more common in patients with mucosal and acral melanoma, while the C-KIT mutation rate was 1–2%. C-KIT was the most frequently mutated gene in mucosal melanoma (10–30%), which was significantly higher than in cutaneous melanoma (4%). PDGFRA was also found in acral and mucosal melanoma.

In this study, data from 96 patients with different BRAF phenotypes were analyzed and BRAF mutations were found to be more common in young patients, which is consistent with previous studies on cutaneous melanoma. In addition, the primary location of head and neck was further divided into two groups: nasal cavity/paranasal sinus, and nasopharynx/other locations (inner canthus, nasolacrimal duct, tongue, vocal cord, epiglottis). BRAF mutations were more common in the nasopharynx/other locations than in the nasal cavity and paranasal sinuses, while NRAS mutations were more common in the nasal cavity/paranasal sinuses. A clinical analysis of 25 patients with melanoma in the nasal cavity and paranasal sinuses showed that BRAF (32%) was the most common mutation type, followed by RAS (12%) and C-KIT (12%) [21]. There were fewer patients in that study compared with ours, and this has probably resulted in the difference in conclusions. For the influence of genotype on the prognosis of patients with head and neck mucosal melanoma, our results showed that the NRAS phenotype (P = 0.037, 95% CI: 1.050–4.572) may be an independent factor affecting the prognosis of patients with head and neck mucosal melanoma. Some studies have shown that the occurrence of the C-KIT mutation may be an independent factor affecting the prognosis of patients [11], but this was not seen in our study.

Having found that there may be differences in the genotypes of patients with head and neck mucosal melanoma in different primary locations, we further analyzed the clinical characteristics of the two groups of patients. No correlation was found between genotypes and general clinical characteristics in the 74 patients with nasal cavity/paranasal sinus lesions, but the NRAS phenotype was confirmed to be an independent factor affecting postoperative distant metastasis (P = 0.046, 95% CI: 1.014–4.618). The analysis of data from the 22 patients with lesions in the nasopharynx or other locations (inner canthus, nasolacrimal duct, tongue, vocal cord, epiglottis) confirmed that the mean age of patients with mutant-type BRAF was significantly lower than that of patients with wild-type BRAF (36.75 vs 50.67 years). When considering different pathological pigmentation types in that same group of patients, the non-pigmented type was more likely to occur in patients with the C-KIT mutation (50%, 2/4). Previous studies have also noted this, and the prognosis of patients with non-pigmented type was found to be poorer in previous studies in our center. Therefore, this conclusion also provides a predictor for the prognosis of patients, but it still needs to be supported by studies with larger datasets. In addition, in patients with lesions in the nasopharynx or other locations, postoperative local recurrences were more likely to appear in patients with BRAF mutations, but this conclusion is also limited by the number of patients (4/22). Therefore, the specific impact of different genotypes on the prognosis of patients needs further clinical analysis.

In addition, other pathways involved in tumorigenesis and development include DNA damage repair, MAPK, growth factors and their receptors, cell cycle, immune response and Wnt/notch. In recent years, studies on related pathways have also been carried out. For example, amplifications of TERT, CDK4, MDM2 and agap2 were found in tissue samples from patients with head and neck mucosal melanoma, with values of 65% (52/80), 78.75% (63/80), 50% (40/80) and 48.75% (39/80), respectively, and the possibility of CDK4 as a target therapy was verified by the mucosal melanoma patient-derived xenograft (PDX) trial [17].

For the current targeted treatment of melanoma patients, drug therapy for patients with the BRAF-V600 mutation, using drugs such as vemurafenib and dabrafenib, have been approved by the FDA and recommended in different guidelines, but they are mainly used in patients with cutaneous melanoma. Although BRAF inhibitors alone show good therapeutic effect in the treatment of BRAF mutant melanoma, patients can easily develop drug resistance by upregulating RTK or NRAS [22, 23]. Therefore, the combination of BRAF and MEK inhibitors can block the growth pathway of melanoma in many ways, and improve the emergence of drug resistance.

In a phase III clinical trial involving 402 patients with stage IIIC or IV NRAS mutant melanoma, the survival rate and effective rate with binimetinib treatment were higher than those with dacarbazine [24]. However, in general, treatments for NRAS mutant melanoma are still relatively rare.

Drugs targeted in C-KIT mutations include imatinib, nilotinib and sunitinib. A retrospective study of 78 patients with melanoma and having the C-KIT gene mutation showed that the mean progression-free survival (m PFS) and mean overall survival (mOS) of imatinib-treated patients were 13.1 months and 4.2 months, respectively, and the disease control rate was 60.3%. The treatment also showed considerable disease control. Nilotinib has also shown the prospect of treating C-KIT mutations in phase 2 clinical trials [25, 26]. However, because of the low incidence rate, there is little clinical test data on the use of different drugs.

Other drugs that can be considered for patients with melanoma, such as PARP inhibitors, can be used in tumors with mutation of the DNA damage repair pathway. For melanoma with cell cycle and PI3K mTOR related pathway mutation, CDK4/6 inhibitor and PI3K mTOR pathway inhibitor can play an effective role [27, 28]. Gene mutations in immune response related pathways, such as Jak1/2, have been shown to be associated with secondary drug resistance in immunotherapy [29], and a combination of targeted therapy may have a better therapeutic effect. Moreover, with the progression of research on mucosal

melanoma in each center, different new targets have also been suggested. Combined with the support from larger datasets, this may bring new treatment options for patients with mucosal melanoma.

5. Conclusions

The genetic characteristics of head and neck mucosal melanoma are different from other subtypes of melanoma, and also necessitate different treatment options. At present, all targeted therapies are at the stage of clinical exploration. It would be helpful to further clarify the characteristics of head and neck mucosal melanoma by carrying out clinical programs. However, the more common mutations such as C-KIT and PDGFRA in patients with mucosal melanoma still need to be studied further. In depth mechanism research is required to develop a precise treatment plan. The implementation of gene detection in patients with head and neck mucosal melanoma is very important, and will provide guidance for the development of comprehensive follow-up treatments.

Abbreviations

MAPK
Mitogen-activated protein kinase
PI3K
Phosphatidylinositol 3 kinase
PCR
Polymerase Chain Reaction

Declarations

Ethics approval and consent to participate：Ethics Committee of Beijing Tongren Hospital Affiliated to Capital Medical University（TRECKY2018-059)

Consent for publication：We have the special institutional consent form to patients and can provide the copy at any stage.

Availability of data and materials: All data generated or analysed during this study are included in this published article and also available from the corresponding author on reasonable request.

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Figures

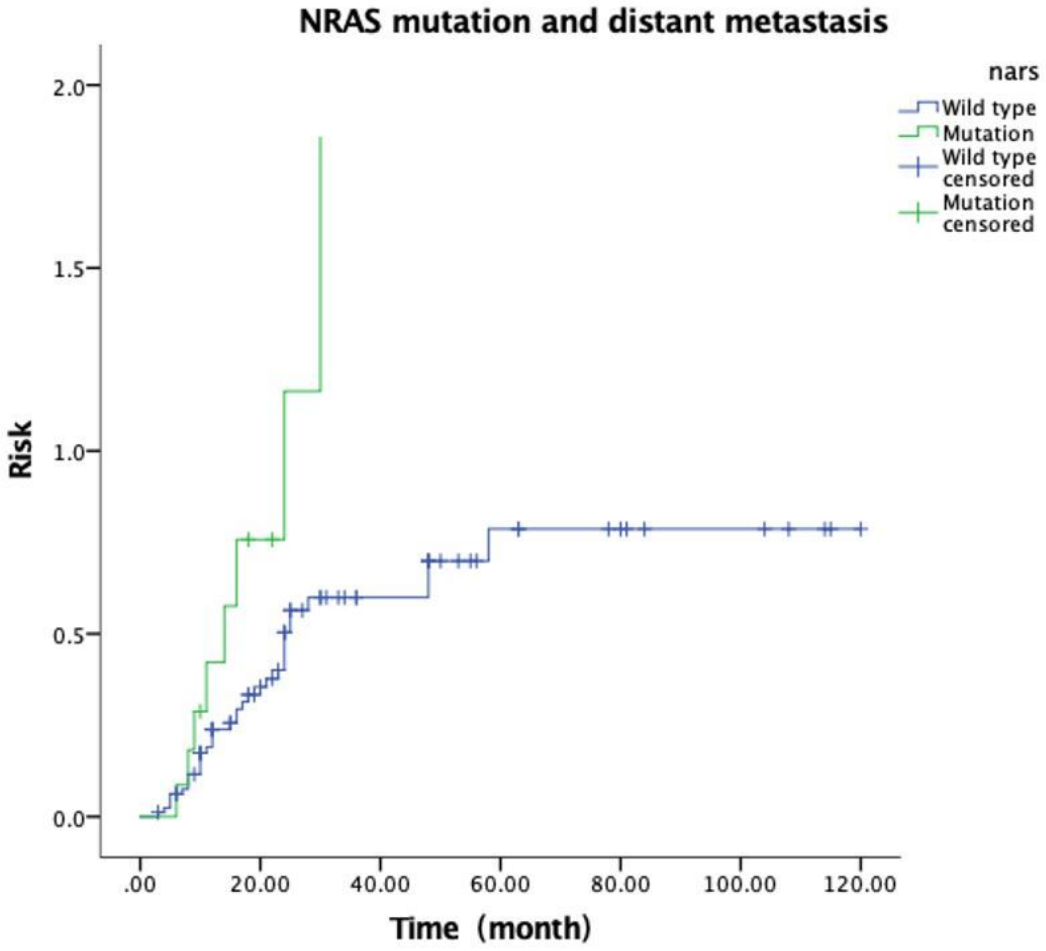

Figure 1

Correlation between distant metastasis and NRAS phenotype in the 96 patients in this study.

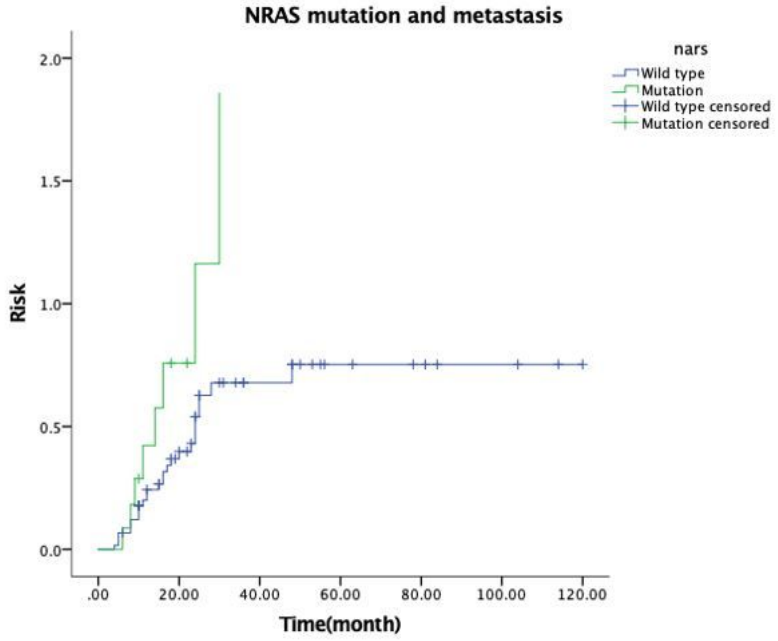

Figure 2

Correlation between distant metastasis and NRAS phenotype in the 74 patients with lesions in the nasal cavity and paranasal sinuses

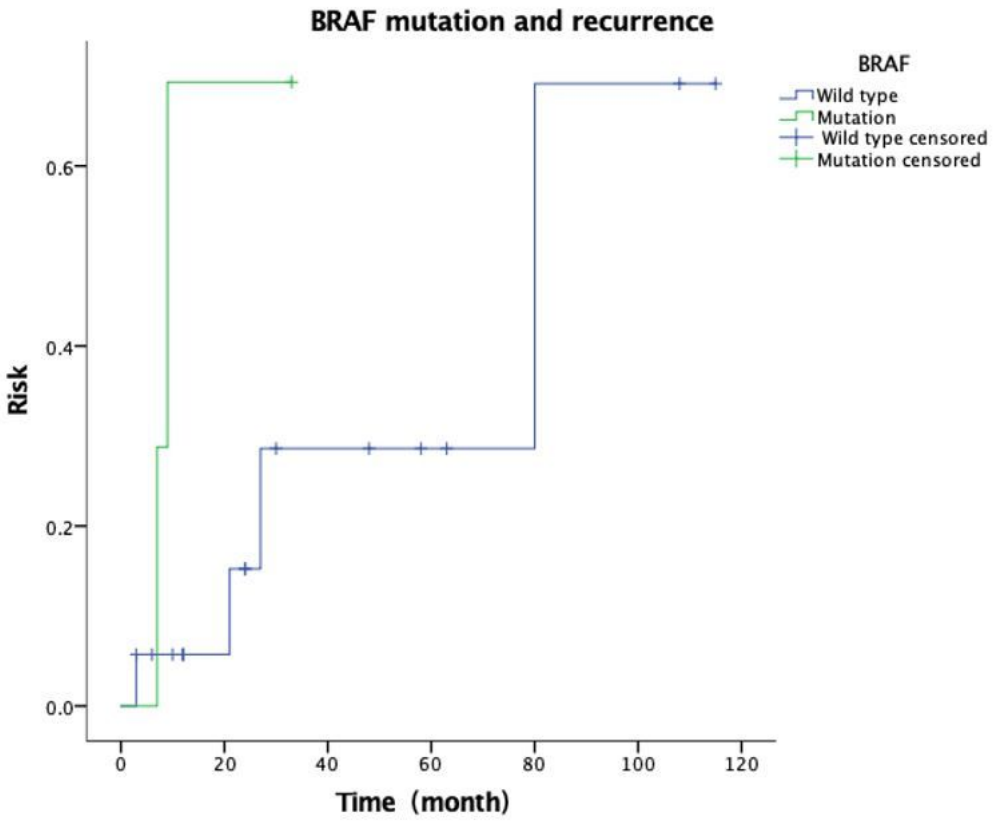

Figure 3

Correlation between local recurrence and BRAF phenotype in the 22 patients with lesions in the nasopharyngeal or other locations.

Supplementary Files

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- SupplementaryData.docx