Clinical, histological and genetic study of malignant melanoma in Koreans

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<TABLE OF CONTENTS>

LIST OF FIGURES

LIST OF TABLES

ABSTRACT

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Background: Melanomas at different sites with different levels of sun exposure are found to have genetic alterations such as *BRAF*, *NRAS*, *KIT*, and *GNAQ/11* mutations. We designed this study to analyze the mutation status of primary tumors in Korean melanoma patients and identify the correlation between the mutations status and clinicopathological features of melanoma.

Methods: Patients diagnosed with malignant melanoma from 2005 to 2012, 188 in number, at Yonsei University Health System, were enrolled for this study.

Results: The most common type was acral type (n=89, 47.3%) followed by non-chronic sun damage induced type (Non-CSD type) (n=32, 17%) and mucosal type (n=31, 16.5%). The overall incidence of somatic mutation was 17.6% in the *BRAF* gene, 12.6% in *NRAS*, and *KIT* amplification was 28.6%. *GNAQ/11* mutation in the uveal type was 66.6%. Of the non-CSD type, 41.9% had the *BRAF* mutation while 35.8% of the acral type had *KIT* amplification. *BRAF* ($P < 0.01$) mutation was associated with advanced stage at diagnosis and was correlated to poor prognosis of the patients compared to wild-type patients.

Conclusion: In conclusion, the mutation status between Korean

melanomas and Caucasian melanomas are similar, but the proportion of the subtypes is distinguishable. In this study, *BRAF* mutation status was identified as an independent prognostic factor in Korean melanoma patients.

Key words : Korean; malignant melanoma; *BRAF; NRAS; C-kit; GNAQ/11*

Clinical, histological and genetic study of malignant melanoma in Koreans

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I. INTRODUCTION

The rising incidence of melanoma and the difficulty to treat the disease has led to many researches in melanoma. Conventionally, cutaneous melanoma has been distinguished by four main types based on their morphology and histology; superficial spreading melanoma (SSM), lentigo maligna melanoma (LMM), nodular melanoma (NM), and acral lentiginous melanoma (ALM) by the World Health Organization (WHO) classification.[1] But recently, genetic alterations such as *BRAF* and *KIT* mutations have been identified in melanomas according to different levels of sun exposure, and a new set of classification has been established.[2]

In 2005, a new classification of malignant melanoma was introduced by Curtin et al. according to the ultraviolet (UV) radiation exposure.[2] Melanomas at different sites with different levels of sun exposure were found to have genetic alterations such as *BRAF*, *NRAS*, *KIT*, and *GNAQ/11* mutations.[3]

Based on the anatomic location of the tumor and the degree of ultraviolet (UV) exposure, melanoma is classified into four subtypes; (1) melanomas that occur on skin without chronic sun-induced damage (non-CSD); (2) melanomas on skin with chronic sun-induced damage (CSD); (3) mucosal melanomas, and (4) acral melanomas.[2]

This new classification by genetic alterations offers targeted therapy, such

as BRAF inhibitor, vemurafenib (PLX4032) or tyrosine kinase inhibitor, imatinib (formerly known as STI571) and this connection between mutation status and therapeutic option makes this new classification valuable.[4]

The purpose of this study was to analyze the mutation status of primary melanoma in Korean patients and to investigate the prevalence of *BRAF*, *NRAS*, *GNAQ/11* mutations and *KIT* amplifications among primary melanomas.

II. MATERIALS AND METHODS

Patients

This study involved tissue samples from 188 melanoma patients, diagnosed during January 2005 to January 2012, at Yonsei University College of Medicine, Severance Hospital in Seoul, Korea. (Fig. 1) Clinical data including age, sex, TNM (tumor-node-metastases) stage, tumor thickness (Breslow), ulceration, and survival (follow-up persisted until the missing of follow-up or the death of patients) were collected. This study protocol was approved by the Institutional Review Board of Yonsei University, Severance Hospital and was conducted according to the Declaration of Helsinki Principles.

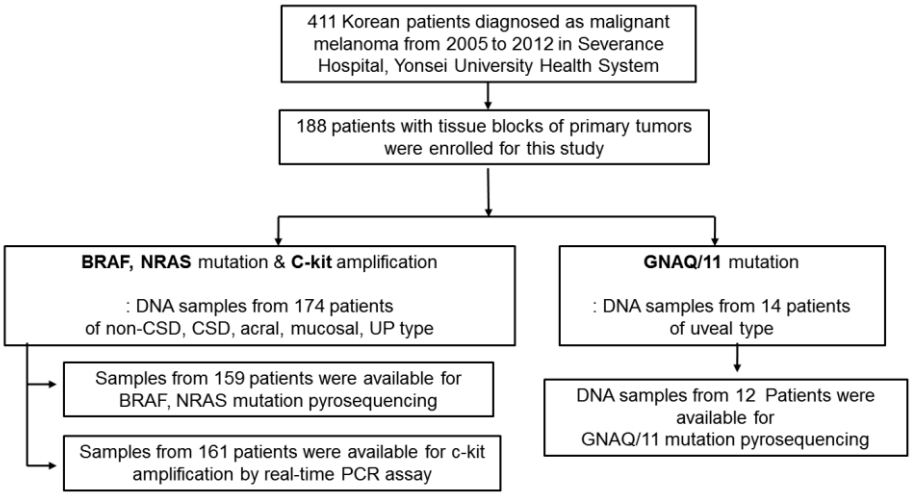


Figure 1. Study overview

DNA preparation and mutation analysis

Formalin-fixed, paraffin-embedded tissue blocks were retrieved from the pathological archives. All pathological specimens were reviewed by two individual pathologists and all patients were confirmed as malignant melanoma. Tumor-rich areas (>80%) were extracted from five paraffin sections of 10 μm thickness containing a representative portion of each tumor block, using the QIAamp DNA FFPE Tissue Kit (Qiagen, Hilden, Germany). To detect hotspot muations, we amplified exons 15 (codon 600) of the *BRAF* gene, exons 1, 2 (codon 12, 13, 61) of *NRAS* gene, and exons 4, 5 (codon 183, 209) of *GNAQ/11* gene by PCR. The primer sequences are listed in table 1.

We performed pyrosequencing using a PyroMark Q24 (Qiagen, Germantown, MD) at room temperature with PyroMark Gold Q24 Reagents (Qiagen Inc.). Sequencing analysis was performed using PyroMark Q24 software (Version 1.0.10; Qiagen Inc.)

Table 1. Primers used in this study

Gene	Exon	Sequence
BRAF	15	F: 5’-biotin-GCTTGCTCTGATAGGAAAATGA-3’
		R: 5’-GACAACTGTTCAAACTGATGGG-3’
		S: 5’-CCACTCCATCGAGATTT-3
NRAS	1	F: 5'-GGTGTGAAATGACTGAGTACAAACTGG-3'
		R: 5'-biotin-CATATTCATCTACAAAGTGGTTCTGGA-3'
		S: 5’-CAAACTGGTGGTGGTTGGAG-3'
	2	F: 5'-GATTCTTACAGAAAACAAGTGGTTATAGAT-3'
		R: 5'-biotin-GCAAATACACAGAGGAAGCCTTCG-3'
		S: 5’-GACATACTGGATACAGCTGG-3'
GNAQ	4	F: 5'-GCCTACGCAACAAGATGTGCT-3'
		R: 5’-biotin-GGTATTCGATGATCCCTGTGGT-3’
		S: 5'-AACAAGATGTGCTTAGAGTT-3'
	5	F: 5’-CAGAATGGTCGATGTAGGG-3’
		R: 5’-biotin-GACATTTTCAAAGCAGTGTATCCA-3’
		S: 5’-AATGGTCGATGTAGGG-3’
GNA11	4	F: 5’-ATCGCCACCTTGGGCTACC-3’
		R: 5’-biotin-CTCGATGATGCCGGTGGT-3’
		S: 5’-GACGTGCTGCGGGTC-3’
	5	F: 5’-biotin-CTGGCGCTGTGTCCTTTCA-3’
		R: 5’-ACTTCCTCCGCTCCGACC-3’
		S: 5’-TCCTCCGCTCCGACC-3’
KIT	17	F: 5’- AAAGATTTGTGATTTTGGTCTAGC-3’
		R: 5’- GAAACTAAAAATCCTTTGCA-3’
GAPDH	2	F: 5’- CACTAGGCGCTCACTGTTCT-3’
		R: 5’- GCGAACTCACCCGTTG-3’

Abbreviations: F, forward primer; R, reverse primer; S, sequencing primer.

Real-time PCR assay for *KIT* copy number

KIT copy number was assessed by quantitative real-time PCR using GAPDH as a control gene. The *KIT* exon 17 primers and glyceraldehydes-3-phophate dehydrogenase (GAPDH) primers are listed in table 1. PCR reactions were done by QuantiTect SYBR Green PCR Kit (Qiagen Inc.), with a 20 μl total volume and 100ng genomic DNA by Rotor-gene 2000 Real-Time Cycler (Corbett Research, Australia). The PCR condition were 1 cycle of 95^{o}C for 15 minutes, followed by 40 cycles of 95^{o}C for 20 seconds, 50^{o}C for 30 seconds, and 72^{o}C for 45 seconds.

Relative copy numbers were calculated by the ΔΔCt method, where Ct is the threshold cycle for amplification.[5,6] For each sample, ΔCt for *KIT* versus GAPDH was calculated as ΔCt = Ct(*KIT*) − Ct(GAPDH).[5,6] The ΔCt value for each experimental test sample was calibrated to a reference pool of human genomic DNA (Promega, Madison, WI), using the formula ΔΔCt = ΔCt(test sample) - ΔCt(reference pool). Relative DNA copy number was calculated using the formula $2^{-\Delta\Delta Ct}$.[5,6]

Statistical analysis

All the statistical analyses were performed using SPSS 18.0 software (SPSS, Chicago, IL) and MedCalc Version 12.7.4. Categorical data are described using frequencies and percentages. Continuous data such as age are described using means ± standard deviations or median (range) for normally distributed data. χ^2 test or Fisher's exact test was used to differentiate the rates of different groups, and differences in measurement data of 2 groups were evaluated by unpaired *t*-test or Mann–Whitney test. Survival curves were established using the Kaplan–Meier method and compared by the log rank test. All statistical analyses were two-sided, and significance was assigned at $p < 0.05$.

III. RESULTS

Patients and tumor tissue samples

Among the 411 patients diagnosed with malignant melanoma from 2005 to 2012 in Yonsei University, 188 patients with adequate tissue blocks for the primary tumor were enrolled for this study. The most common type was acral type (n=89, 47.3%) followed by non-chronic sun damage induced type (Non-CSD type) (n=32, 17%) and mucosal type (n=31, 16.5%). Eighteen patients (9.6%) were chronic sun damage induced type (CSD type) and 14 patients (7.5%) were uveal type, while 4 patients (2.1%) were those who had the tumors of unknown primary origin (UP) (Table 2). The unknown primary origin type (UP) was diagnosed in patients who had initial presentation of melanoma in the lymph nodes, or subcutaneous tissue with no known primary site.

The median age of melanoma patients in this study was 60 years old. The median age of the acral type was 62 years old ranging from 18 to 89, ranking the oldest median age among the subtypes, while the tumors of unknown primary origin had the youngest median age of 47. Non-CSD type had the median age of 52 ranging from 25 to 81, showing tendency of early onset. Male to female ratio of the total included patients was 1: 1.06 (Table 2).

Of the total patients, 62.8% (n=118) were localized melanoma (stages I and II) and 27.2% (n=70) were advanced melanoma (stages III and IV). The CSD and uveal type tend to have more localized tumors than the other types (77.8% and 64.2%) while mucosal type was more likely to have the advanced tumors (54.8%). (Table 2).

Table 2. Clinical characteristics of primary melanoma patients

Clinicopathological factor	Acral	Mucosal	CSD	Non-CSD	Uveal	UP	Total
Patient No. (%)	89 (47.3)	31 (16.5)	18 (9.6)	32 (17)	14 (7.5)	4 (2.1)	188 (100)
Age (year)							
Median (Range)	62 (18-89)	62 (35-82)	60 (39-83)	52 (25-81)	56 (29-75)	47 (37-65)	60 (18-89)
Gender							
Male	39	16	12	17	6	1	91
Female	50	15	6	15	8	3	97
Stages (%)							
I	30 (33.7)	5 (16.1)	5 (27.8)	13 (40.6)	1 (7.1)	0 (0)	54 (28.7)
II	33 (37.0)	9 (29.0)	9 (50.0)	5 (15.6)	8 (57.1)	0 (0)	64 (34.0)
III	18 (20.2)	9 (29.0)	2 (11.1)	8 (25.0)	2 (14.3)	2 (50.0)	41 (21.8)
IV	8 (9.0)	8 (25.8)	2 (11.1)	6 (18.7)	3 (21.4)	2 (50.0)	29 (15.4)

Mutation types and frequencies of *BRAF* and *NRAS* mutation in melanoma

The overall incidence of somatic mutations within the *BRAF* gene was 17.6%, *NRAS* 12.6%, and *KIT* amplification was 28.6%. (Table 3) *GNAQ/11* mutation in the uveal type was 66.6%. Non-CSD type showed the *BRAF* mutation in 41.9% of patients, while acral type had *KIT* amplification in 35.8% of patients. The *BRAF V600E* mutation was the most common genetic alteration in this study, detected in 27 samples among the 28 samples of primary melanoma positive for *BRAF* somatic mutation. *BRAF V600K* was detected in one sample. For the patients containing *NRAS* mutations, 60% (12 patients) of them demonstrated mutations in codon 61, with Q61K as the most frequent mutation in *NRAS* (9 patients) and Q61R mutation in 3 patients. 8 patients (40%) demonstrated *NRAS* mutation in codon 12, 5 patients showing G13R mutation and 3 patients showing G12R mutation. *BRAF* and *NRAS* mutations were mutually exclusive in our study with no patients with simultaneous mutation of the two genes.

Table 3. Mutation status of primary melanoma patients

Subtype	BRAF mutation V600	NRAS mutation Q61 & G12-13	c-KIT amplification	GNAQ/11 mutation
Non-CSD	13/31 (41.9%)	2/31 (6.45%)	3/32 (9.3%)	-
CSD	4/18 (22.2%)	1/18 (5.5%)	4/18 (22.2%)	-
Acral	9/82 (10.9%)	13/82 (15.8%)	29/81 (35.8%)	-
Mucosal	2/26 (7.7%)	3/26 (11.5%)	8/28 (28.6%)	-
Uveal	-	-	-	8/12 (66.6%)
Unknown Primary	0/2 (0%)	1/2 (50%)	2/2 (100%)	-
Total	28/159 (17.6%)	20/159 (12.6%)	46/161 (28.6%)	8/12 (66.6%)

Correlation of *BRAF*, *NRAS* mutations and *KIT* amplification to the clinicopathological features of melanoma

In this study, gender, and subtypes were not significantly different between the patients with genetic mutations or those without mutations. (Table 4) But Korean melanoma patients with *BRAF* mutation tend to be younger (median age: 54 years old) than wild type patients (median age: 62 years old). ($p < 0.01$) Tumor stage was also significantly associated with *BRAF* mutation status in our study. ($p < 0.01$) Advanced tumor with stages III and IV (67.8%) were significantly noticed in patients with *BRAF* mutation while 68.8% of the patients without *BRAF* mutation had localized disease with stages I and II. No such tendency was seen in patients with *NRAS* mutation or *KIT* amplification.

Table 4. Correlation of *BRAF, NRAS, c-KIT* status to clinocopathological features of melanoma

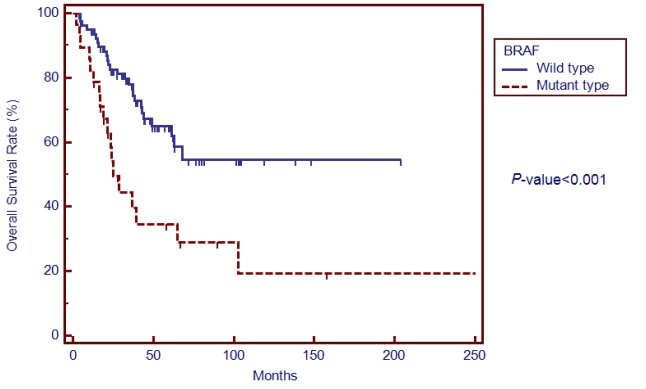
Clinicopathologic features	*BRAF* genotype			*NRAS* genotype			*c-KIT* genotype		
	Mutation (n=28)	Wild type (n=80)	P-value	Mutation (n=20)	Wild type (n=80)	P-value	Amplification (n=46)	Normal (n=78)	P-value
Age, Years									
Median (Range)	54 (30-89)	60 (30-87)	<0.01	56 (25-85)	62 (30-87)	0.36	58 (35-81)	62 (30-87)	0.39
Gender, N (%)									
Male	15 (53.6)	35 (43.7)	0.37	9 (45.0)	35 (43.7)	0.92	28 (60.9)	35 (44.9)	0.09
Female	13 (46.4)	45 (56.3)		11 (55.0)	45 (56.3)		18 (39.1)	43 (55.1)	
Stage, N (%)									
I	4 (14.3)	31 (38.8)		5 (25.0)	31 (38.8)		12 (26.1)	29 (37.2)	
II	5 (17.9)	24 (30.0)	<0.01	9 (45.0)	24 (30.0)	0.12	20 (43.5)	25 (32.1)	0.33
III	10 (35.7)	19 (23.8)		2 (10.0)	19 (23.8)		8 (17.4)	18 (23.1)	
IV	9 (32.1)	6 (7.5)		4 (20.0)	6 (7.5)		6 (13.0)	6 (7.7)	
Subtype, N (%)									
Non-CSD	13 (46.3)	15 (18.8)		2 (10.0)	15 (18.8)		3 (6.5)	15 (19.2)	
CSD	4 (14.3)	10 (12.5)	0.05	1 (5.0)	10 (12.5)	0.49	4 (8.7)	10 (12.8)	0.07
Acral	9 (32.1)	40 (50.0)		13 (65.0)	40 (50.0)		29 (63.0)	37 (47.4)	
Mucosal	2 (7.1)	14 (17.5)		3 (15.0)	14 (17.5)		8 (17.4)	16 (20.5)	
UP	0 (0.0)	1 (1.3)		1 (5.0)	1 (1.3)		2 (4.3)	0 (0.0)	

Prognostic significance of *BRAF*, *NRAS* mutations and *KIT* amplification for overall survival of melanoma

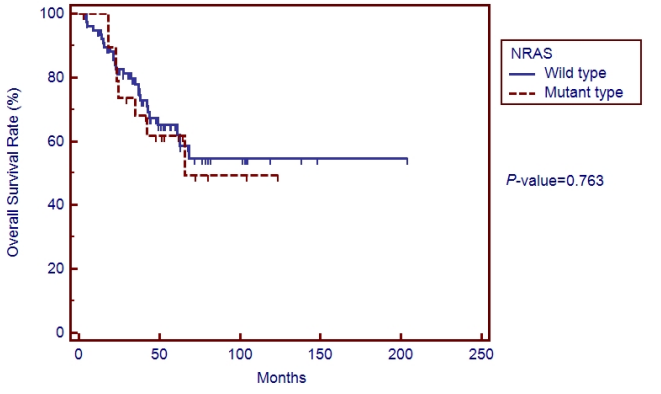
The survival data were collected for patients (n=188) who were diagnosed as primary melanoma or melanoma of unknown primary from the first time of diagnosis as melanoma to January 2012. The median follow-up period was 37 months. We found that the median survival time for patients with *BRAF* mutations (22 months) was significantly shorter than that for patients with wild-type tumors (39 months; p <0.001). (Fig. 2) However, *NRAS* mutation or *KIT* amplification did not show significant effect on survival of melanoma patients.

To exclude the effect of the advanced staging in the patients with *BRAF* mutation, and to see the mutational effect solely on the overall survival rate, Cox multivariate analysis was performed. By the Cox multivariate analysis, *BRAF* mutation (p=0.029) was found to be an independent prognostic factor with the hazard ratios of 2.258 (95% confidence interval (CI): 1.08, 4.69). Among the subtypes, mucosal type (p=0.001) was shown to be a poor prognostic factor as well as increased stage (p=0.000), while female gender (p=0.006) was seen to be a favorable prognostic factor. (Table 5)

(a) Overall survival in relation to *BRAF* mutation



(b) Overall survival in relation to *NRAS* mutation



(c) Overall survival in relation to *KIT* amplification

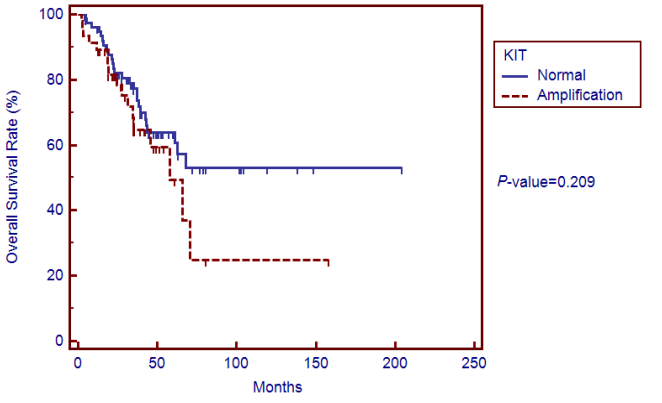


Figure 2. Overall survival of melanoma patients in relation to *BRAF*, *NRAS* mutations and *KIT* amplification.

Table 5. Cox proportional hazard ratios for clinicopathological features and mutation status

	p-value	HR	95% CI
Type			
Non-CSD	.006	1.000	reference
Acral	.177	1.730	0.78-3.83
Mucosal	.001	4.691	1.89-11.64
CSD	.108	2.301	0.83-6.36
UP	.707	1.378	0.25-7.34
Gender			
M		1.000	reference
F	.006	.479	0.28-0.81
Age	.221	1.013	0.99-1.03
Stage			
1 & 2		1.000	reference
3 & 4	.000	4.901	2.80-8.56
Mutation			
WT	.127	1.000	reference
BRAF	.029	2.258	1.08-4.69
NRAS	.488	1.344	0.58-3.09
KIT	.148	1.649	0.83-3.25

Abbreviations: HR, hazard ratio; CI, confidence interval; WT, wild type

IV. DISCUSSION

Incidence of melanoma has increased significantly worldwide over the last several decades.[7,8] Since the classic consensus for the diagnosis of melanoma is based on histopathologic evaluation of biopsy specimens[9], melanoma has been classified according to the morphological and architectural features of the tumor; superficial spreading melanoma (SSM), lentigo maligna melanoma (LMM), nodular melanoma (NM), and acral lentiginous melanoma (ALM).[1] However, this classification based on morphological and architectural features does not have any independent prognostic significance nor cannot be used as factor influencing the treatment plan.[10]

Among the risk factors of melanoma, the most important factor is known to be the periodic and intense sun exposure history during the childhood and adolescent period.[11] Considering the fact that UV light exposure is an inseparable factor for melanoma development[12], Curtin *et al*. reported in 2005 that there are distinct sets of genetic alternations in melanoma with different susceptibility to UV exposure[2]; (1) Melanomas that occur on skin without chronic sun-induced damage (non-CSD); (2) melanomas on skin with chronic sun-induced damage (CSD); lastly, melanomas that arise without obvious exposure to light, which are (3) mucosal melanomas, and (4) acral melanomas.[2]

According to the studies starting from Curtin's study to until now, the *BRAF* and *NRAS* mutations are detected in higher percentages in non-CSD type and CSD type compared to the acral type and mucosal type.[3] Approximately about 45% of non-CSD type and 5 to 30% of of CSD type have *BRAF* mutation, while 10 to 15% of acral type and 5% of mucosal type have *BRAF* mutation.[13] When it goes to *NRAS* mutation, non-CSD type have 15 to 20% and CSD type have 10 to 15% while acral type have 10 to 15% and mucosal type have 5 to 10% of *NRAS* mutation. On the other hand, *KIT* aberrations are found to be more prevalent in acral and mucosal types.[14] In both acral and mucosal type, 15 to 20% have been found to have *KIT* aberrations while less than 1% is found in

non-CSD type and 2 to 17% is found in CSD type.[13] Also in 2010, Van Raamsdonk *et al*, reported that approximately 80% of uveal melanoma has *GNAQ/11* mutations, which is usually not found in mucosal or cutaneous melanomas[15]. But it is important that most of these data are from Caucasian melanoma patients and thus it is questionable if this prevalence of genetic mutations will also fit to Asian patients.

Although there are several Chinese data, it has only reviewed *BRAF, NRAS* mutations and *KIT* aberrations, but not the whole set of known molecular alterations in melanoma, including *GNAQ/11* mutations.[4,16] Likewise, several recently reported data from Korea also deals with only *BRAF* mutation and *KIT* aberrations[17], or has limitations due to its small number of enrolled patients.[18] In this paper, we overviewed the whole set of revealed genetic alterations including *GNAQ/11* mutations, and included 188 patients with primary tumor tissues which were able to obtain abundant amount of DNA.

In this study, the overall incidence of somatic mutations within the *BRAF* gene was 17.6%, *NRAS* 12.6%, and *KIT* amplification was 28.6%. *GNAQ/11* mutation in the uveal type was 66.6% (Table 3). Non-CSD type showed the *BRAF* mutation in 41.9% of patients, while acral type had *KIT* amplification in 35.8% of patients. *BRAF* mutation had definitely smaller portion in the acral and mucosal melanoma (10.9% and 7.7%). Although the percentage of *NRAS* mutation in the non-acral cutaneous type (which refer to non-CSD type and CSD type) was quite small (6.45% in non-CSD type and 5.5% in CSD type), considering the small number of total population who had *NRAS* mutation (20 patients), this study result seems to be consistent with the Caucasian data published in 2012 by Woodman et al.[13] Even though there was no significant difference in the genetic aberration status with Caucasian data, there was a distinguishable difference in the proportion of melanoma subtypes.

In the Caucasian data of 1112 cases by Greaves *et al.*[19], non-acral cutaneous type (non-CSD type and CSD type) constituted 69.6%, while acral

type constituted 10%. In our study, 47.3% constituted of acral type and 16.5% were mucosal type, while the proportion of non-CSD type and CSD type were only 26.6% (Table 2). In the Chinese data of 502 cases representing the Asian population, the most prevalent type was the acral type (38.4%) and mucosal type (33.3%)[4] which showed consistent finding with our data.

Due to its aggressive progression of melanoma, there has been continuous effort to find out the prognostic factors of melanoma.[20] Until now, the single most important prognostic factor for the survival in localized melanoma has been the tumor thickness, measured from the top of the granular layer to the greatest depth of tumor invasion.[21] In this study, we tried to find any association with genetic alterations and clinicopathological features such as age, gender, subtype, and tumor stage based on tumor thickness and metastasis. We could not find any association between *NRAS* mutation and *KIT* amplification with other clinicopathological features. But the patients with *BRAF* mutation tend to have more advanced stage at the time of diagnosis, compared to the patients with wild type melanomas. To see the sole effect of *BRAF* mutation in the overall survival, Cox multivariate analysis was performed, and it was found that the *BRAF* mutation can also act as an independent prognostic factor, especially in the early stage melanomas (Stage 1 and 2). Also, patients with *BRAF* mutation seemed to be about 8 years younger compared to the patients with wild type. It is consistent with the papers reported by Bauer et al.[22] and Ellerhorst et al.[23] in 2011. This finding also supports the fact that *BRAF* works as an oncogene.

V. CONCLUSION

In conclusion, we identified the clinical, histological characteristics and genetic alterations of Korean melanoma patients. Acral melanoma was the most common subtype in Koreans and mucosal melanoma also showed high proportion of the subtype compared to Caucasians. However, there was no

difference in the genetic aberration status among the subtypes compared to Caucasian data. Also, *BRAF* mutation was independently linked to younger age and poor prognosis compared to the wild type patients in our study, proving the evidence as an oncogene.

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ABSTRACT(IN KOREAN)

한국인 악성 흑색종 환자에 대한 임상적, 조직학적 및 유전학적 분석

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배경 : 악성 흑색종은 자외선 조사 정도에 따라 *BRAF, NRAS, KIT,* 그리고 *GNAQ/11* 변이와 같은 유전적 변이가 밝혀진 바 있다. 본 연구에서는 한국인의 악성 흑색종에서 유전자 변이의 종류와 빈도를 파악하고 이에 따른 임상적, 조직학적 특징을 분석하고자 하였다.

방법 : 2005년부터 2012년까지 연세의료원을 방문하여 악성 흑색종으로 진단된 환자들 중, 원발성 병변의 조직을 구할 수 있는 환자들을 대상으로 분석을 시행하였다.

결과 : 한국인의 악성흑색종 중 가장 빈도가 높은 아형은 말단형이었으며 (n=89, 47.3%), 자외선 조사를 만성적으로 받지 않은 피부에 생기는 아형이 그 뒤를 이었다 (n=32, 17%). 전체 흑색종 중 *BRAF* 의 변이는 17.6%, *NRAS* 의 변이는 12.6%, *KIT* 의 증폭은 28.6% 에서 발견되었으며, *GNAQ/11* 의 변이는 안구에 생기는 아형 중 66.6% 에서 발견되었다. 자외선 조사를 만성적으로 받지 않은 피부에 생기는 아형은 41.9%에서 *BRAF* 변이가 발견되었으며, 말단형의 경우 35.8%에서 *KIT* 의 증폭이 관찰되었다. *BRAF* 변이가 있었던 환자들의 경우, 변이가 없는 환자들과 비교하였을 때 더 진행된 병기에서 흑색종이

진단되었으며, 예후가 더 좋지 않았다.
결론 : 결과적으로 한국인의 악성 흑색종 환자에서 관찰되는 유전자 변이의 정도는 서양 환자들의 유전자 변이의 정도와 유사한 양상을 띄는 것으로 밝혀졌으나, 아형의 분포에는 분명한 차이가 있다. 또한, *BRAF* 유전자의 변이는 악성 흑색종의 환자에서 중요한 예후인자로 사용 될 수 있다.

핵심되는 말 : 한국인, 악성 흑색종, *BRAF; NRAS; C-kit; GNAQ/11*