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ORIGINAL ARTICLE

Prognostic and Clinicopathologic Associations of *BRAF* Mutation in Primary Acral Lentiginous Melanoma in Korean Patients: A Preliminary Study

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Background: In the majority of melanomas, the RAS/RAF/MEK/ERK signaling pathway is constitutively activated, due to oncogenic mutations in the *BRAF* and *NRAS* genes. The *BRAF* mutation has been mainly described in Caucasian melanomas. However, there is a lack of study evaluating the status, and the clinical significance, of *BRAF* mutation in the Asian population. **Objective:** This study was aimed to determine the frequency of *BRAF* mutation, and to evaluate the correlation of *BRAF* status with clinicopathologic features and outcomes, in Korean primary acral lentiginous melanoma (ALM) patients. **Methods:** ALM samples (n=36) were analyzed for the *BRAF V600E* mutation, by dual-priming oligonucleotide (DPO) based real-time polymerase chain reaction. The clinicopathologic features and prognosis of the patients were analyzed with *BRAF* mutation status. **Results:** The incidence of *BRAF V600E* mutation was 19.4% (7/36). The *BRAF V600E* mutations were not associated with clinicopathologic features, except for the age factor. All of the *BRAF*-mutant patients survived without recurrence or metastasis, and have a better clinical outcome than *BRAF* wild-type patients. **Conclusion:** In Korean primary ALM, a low frequency of *BRAF* mutation was shown; and *BRAF* mutation presented with a favorable prognosis. These results indicate that other distinctive genetic mechanisms may have more important roles in the development and progression of disease. Further multicenter study with large sample size is firmly needed, to confirm the results of our preliminary study. **(Ann Dermatol 26(2) 195～202, 2014)**

-Keywords-
Acral lentiginous melanoma, *BRAF* mutation, Korean, Prognosis

INTRODUCTION

Cutaneous melanoma is a potentially fatal neoplasm, with a complex and heterogeneous etiology. Several genes and signaling cascades, including the RAS/RAF/MEK/ERK signaling pathway, have been implicated in the pathogenesis of cutaneous melanoma. Somatic oncogenic mutations of *BRAF* have been identified most commonly in primary human melanomas, where mutational frequencies have been reported to be as high as 70%[1,2]. All the mutations were found within the kinase domain of *BRAF*, with a single substitution (T to A) of glutamate for valine at codon 600 (V600E) being responsible for 90% of the observed mutation[1]. This mutated kinase promotes constitutive ERK signaling, stimulating proliferation and survival, and providing essential tumor growth.

In the Asian population, the incidence of cutaneous melanoma is much lower than in the Caucasian population. Furthermore, the clinical and histological types of melanoma vary among different ethnicities, such that Caucasian patients are often afflicted with superficial spreading melanoma and nodular melanoma, whereas Asian patients present with acral lentiginous melanoma (ALM). Recent studies have revealed that the frequency of *BRAF* mutation in ALM was lower than in other types of melanoma[3,4], and

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the effects of mutation on clinicopathologic features and clinical outcome remain uncertain, with previous studies reporting conflicting results[2,5-9]. However these observations are mostly conducted in Caucasian populations, and there have been few studies about the status and clinical significance of *BRAF* mutation in Asian patients[10-14].
Furthermore, there has been only one report about the effects of *BRAF* mutations on the clinical features and outcome of melanoma in Korean patients[13]. Thus, the purpose of this study was to determine the frequency of *BRAF* mutation, and to evaluate the clinical significance of *BRAF* mutation in Korean primary ALM patients.

MATERIALS AND METHODS

Patients

We retrospectively analyzed the clinical records of 36 patients who had been treated for pathologically proven melanoma that occurred in acral site at Dong-A University Medical Center (Busan, Korea), between July 1997 and October 2008. Clinical data, including age, sex, American Joint Committee on Cancer (AJCC) pathologic stage[15], thickness (Breslow), ulceration, recurrence or metastasis of disease after initial diagnosis, and survival (follow-up persisted until September 2011, or until the missing of follow-up, or death of patients), were collected. This study was approved by the institutional review board of Dong-A University Medical Center (IRB 12-032), and written informed consent was obtained from all patients.

Tumor tissue samples and nucleic acid isolation

1) Tumor tissue samples preparation

Thirty-six tumor specimens were obtained as surgical biopsies. For each case, formalin fixed, paraffin wax embedded sections (10 μm thick) were cut, using a sterile microtome blade, with two sections used for each sample. Each method was run three times, to ensure reproducibility.

2) Nucleic acid isolation

The commercial QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) was used for DNA extraction, according to the manufacturer's protocol. The tissues were dewaxed with two washes of xylene, followed by the addition of 1 ml of 100% ethanol, to remove residual xylene. After dewaxing, tissues were digested with 200 μl of ALT buffer, containing proteinase K at 200 μg/ml, overnight, at 56°C. After digestion, 200 μl of AL buffer was added, and incubated at 70°C for 10 minutes, followed by mixing with 200 μl of 100% ethanol. The solution was transferred into a spin column, centrifuged for one minute, and washed with AW1 and AW2 buffers. DNA was eluted with 200 μl of AE buffer preheated to 70°C, and further incubated at 70°C for five minutes, before collection by centrifugation. The buffers and the proteinase were provided in the extraction kit.

Detection of the *BRAF V600E* mutation

1) Dual-priming oligonucleotide based real-time polymerase chain reaction

The *BRAF V600E* mutation was detected using Anyplex™ *BRAF V600E* Real time detection system (Seegene Inc., Seoul, Korea). The reaction mixture was prepared as follows: for 1 reaction, the mixture contained 2 μl of 10X *BRAF* Oligo Mix (OM) containing amplification and detection reagents, 3 μl of 8-methoxypsoralen (8-Mop) solution to prevent carryover contamination, 10 μl of 2X Anyplex polymerase chain reaction (PCR) Master Mix (Seegene Inc.) containing DNA polymerase, and buffer with deoxynucleoside triphosphates. The reaction mixture tube was agitated, by inverting it 5 times, or by quick vortexing. 15 μl of the reaction mixture was dispensed into 0.2-ml PCR tubes. 15 μl of each sample's nucleic acid was added to the reaction mixture tube, in order to reach a total reaction volume of 20 μl. Real-time PCR was performed on a CFX96™ real-time PCR System (Bio-Rad, Hercules, CA, USA), under the following conditions: 15 min at 95°C, followed by 15 cycles of 15 seconds at 95°C, and 30 seconds at 60°C, and then 35 cycles of 30 seconds at 95°C, and 32 seconds at 60°C.

2) Interpretation

For real-time PCR, the cycle threshold (Ct) is the cycle at which a significant increase in fluorescence occurs. A sample and internal control that had a Ct value below 40 and 40, respectively, were considered positive. Each run contained a positive control, and negative control.

Statistical analysis

Data were summarized using descriptive statistics: frequency and percentage for categorical variables, and mean and standard deviation for continuous variables. Differences in patients' demographic and clinical characteristics were compared across subgroups, with Fisher's exact test for categorical variables, and t-test for continuous variables. Survival was estimated, using Kaplan-Meier curves. The associations of *BRAF* mutation and other clinicopathological factors with survival were also analyzed, using the Cox proportional hazards regression model. The 95% confidence intervals for hazard ratios

were calculated, and reported, for the multivariate statistical model. Overall survival (OS) was defined as the time from date of first diagnosis, to death. Disease free survival (DFS) was defined as the time from date of first diagnosis, to local recurrence or metastasis, or death. Survival curves were compared between groups, using the log-rank test. All *p*-values less than 0.05 were considered statically significant. All statistical analyses were carried out using PASW Statistics 18.0 version (IBM Co., Armonk, NY, USA) and MedCalc 11.6.1 version (MedCalc Software, Mariakerke, Belgium) statistical software.

Table 1. Patients' baseline and clinical characteristics

Variable	Overall	*BRAF* mutation		*p*-value
		Mutation	Wild	
All patients	36 (100)	7 (19.0)	29 (81.0)	
Sex				
Male	15 (41.7)	4 (57.1)	11 (38.0)	0.42
Female	21 (58.3)	3 (42.9)	18 (62.0)	
Age (yr)	58.2±14.9	45.6±14.7	61.2±13.5	0.01
	60 (16~85)	49 (16~60)	62 (33~85)	
Site				
Finger	12 (33.3)	3 (42.8)	9 (31.0)	0.86
Heel	6 (16.7)	1 (14.4)	5 (17.3)	
Palm	1 (2.8)	0 (0.0)	1 (3.4)	
Sole	12 (33.3)	3 (42.8)	9 (31.0)	
Toe	5 (13.9)	0 (0.0)	5 (17.3)	
Thickness (mm)	4.0±3.8	4.1±3.2	4.0±3.9	0.99
	3.25 (0.2~21.0)	2.5 (1.2~9.0)	3.5 (0.2~21.0)	
Breslow thickness (mm)				
0.01~1.00	3 (8.3)	0 (0.0)	3 (10.4)	0.70
1.01~2.00	8 (22.2)	3 (42.8)	5 (17.2)	
2.01~4.00	14 (38.9)	2 (28.6)	12 (41.4)	
>4.00	11 (30.6)	2 (28.6)	9 (31.0)	
Ulceration				
Yes	21 (58.3)	4 (57.1)	17 (58.6)	1.00
No	15 (41.7)	3 (42.9)	12 (41.4)	
AJCC pathologic stage*				
IB	9 (25.0)	1 (14.4)	8 (27.7)	0.69
IIA	8 (22.2)	2 (28.4)	6 (20.7)	
IIB	11 (30.6)	2 (28.4)	9 (31.0)	
IIC	3 (8.3)	0 (0.0)	3 (10.3)	
IIIA	1 (2.8)	1 (14.4)	0 (0.0)	
IIIB	3 (8.3)	1 (14.4)	2 (6.9)	
IV	1 (2.8)	0 (0.0)	1 (3.4)	
Local recurrence				
Yes	5 (13.9)	0 (0.0)	5 (17.2)	0.56
No	31 (86.1)	7 (100.0)	24 (82.8)	
Metastasis				
Yes	8 (22.2)	0 (0.0)	8 (27.6)	0.31
No	28 (77.8)	7 (100.0)	21 (72.4)	
Status				
Dead	7 (19.4)	0 (0.0)	7 (24.0)	0.26
Loss to follow-up	13 (36.2)	2 (28.6)	11 (38.0)	
Alive	16 (44.4)	5 (71.4)	11 (38.0)	

Values are presented as number (%), mean±standard deviation, or median (range). *American Joint Committee on Cancer (AJCC) staging system for cutaneous melanoma.

RESULTS

Clinical characteristics of patients, and the frequency of *BRAF V600E* mutations

A total of 36 patients with primary ALM were included in this study (15 men and 21 women). The mean age at diagnosis of all patients was 58.2 years (range: 16～85 years); and all tumors were located in the acral area (hand: 36.1% and foot: 63.9%) (Table 1). Primary ALM lesions from a total of 36 patients were screened for *BRAF V600E* mutations, using dual-priming oligonucleotide (DPO)-based real-time PCR. *BRAF V600E* mutations were detected in 7 of the 36 patients (19.4%). The characteristics of the *BRAF* mutant patients are shown in Table 2.

Correlation of *BRAF* mutation to the clinicopathologic features of acral lentiginous melanoma

Only the mean age was significantly different between patients with *BRAF* mutations, and those without *BRAF* mutations; while other clinicopathologic features, including sex ($p=0.42$), site ($p=0.86$), tumor thickness (mean tumor thickness: $p=0.99$ and Breslow thickness: $p=0.70$), presence of ulceration ($p=1.00$), and AJCC pathologic stage ($p=0.69$), were not. The mean age (45.6 ± 14.7 years) of patients bearing *BRAF* mutations was younger, than that of patients without *BRAF* mutations (61.2 ± 13.5 years; $p=0.01$). The mean tumor thickness (4.1 ± 3.2 mm) of patients with *BRAF* mutations was thicker, than that of patients without *BRAF* mutations (4.0 ± 3.9 mm; $p=0.99$); while the proportion of Breslow thickness that was more than 2.01 mm in the non-*BRAF* mutant group (72.4%), was higher than that in the *BRAF* mutant group (57.2%; $p=0.70$) (Table 1).

Prognostic significance of *BRAF* mutations for the survival of primary acral lentiginous melanoma

The recurrence, metastasis and survival data were collected for patients who were diagnosed as primary ALM, from the first time of diagnosis as melanoma, to September 2011.

Thirteen (36.2%) of the 36 patients were lost to follow-up. The median follow-up period was 65 months (range: 1～146 months). Overall, local recurrence occurred in 5 patients (13.9%), and 8 patients (22.2%) developed distant metastasis. Seven (19.4%) of the 36 patients are known to have died (Table 1). Among the 7 patients with *BRAF* mutation, all of the 5 patients who were able to be followed-up were alive, without recurrence or metastasis (Table 1, 2). We found that the patients with *BRAF* mutation tended to have a better OS ($p=0.071$), and had a better DFS ($p=0.045$), than patients with wild-type tumors (Fig. 1). The Cox multivariate analysis of the prognostic factors (*BRAF* mutation, sex, age, Breslow thickness, pathological T stage, pathological TNM stage, and ulceration) for OS and DFS are shown in Table 3, 4, respectively. In this study, we could not check whether the *BRAF* mutation is a prognostic factor, or not, for OS and DFS in the Cox's multivariate analysis, because the events (local recurrence, metastasis, and death) did not occur in *BRAF* mutant patients. But, OS of the ALM patients was associated with several factors in Cox multivariate analyses (Table 3), and age and Breslow thickness of the tumor significantly correlated with OS. Similarly, DFS was also associated with several prognostic

Table 2. Clinical characteristics in patients with *BRAF* mutation

Case	Sex/age (yr)	Primary site	Thickness (mm)	Ulceration	AJCC pathologic stage*	Recurrence	Metastasis	Live/death (mo)
1	Female/49	Left sole	9.0	No	T4aN0M0 (Stage IIB)	No	No	Follow-up loss (58)
2	Male/44	Left thumb nail bed	4.0	No	T3aN0M0 (Stage IIA)	No	No	Live (145)
3	Male/49	Right heel	8.0	Yes	T4bN2aM0 (Stage IIIB)	No	No	Follow-up loss (8)
4	Female/60	Right thumb nail	1.2	Yes	T2bN0M0 (Stage IIA)	No	No	Live (139)
5	Female/42	Left sole	2.0	No	T2aN0M0 (Stage IB)	No	No	Live (115)
6	Male/16	Left finger	1.7	No	T2aN1aM0 (Stage IIIA)	No	No	Live (95)
7	Male/59	Left sole	2.5	Yes	T3bN0M0 (Stage IIB)	No	No	Live (36)

*American Joint Committee on Cancer (AJCC) staging system for cutaneous melanoma.

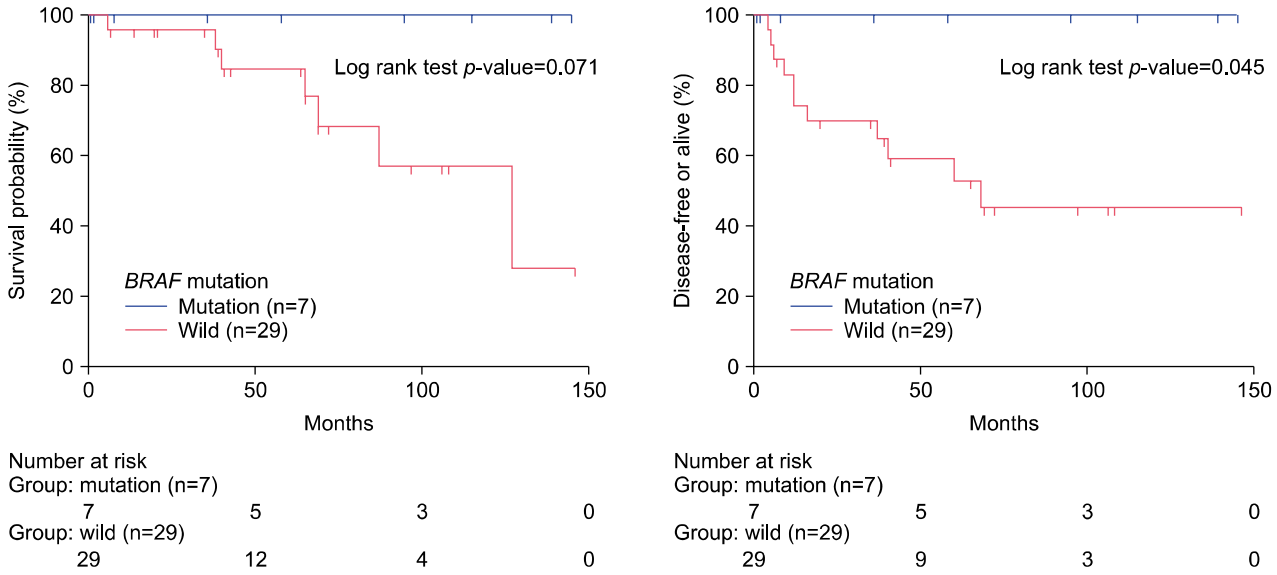

Fig. 1. Kaplan-Meier curve for overall survival and disease free survival across subgroup, based on *BRAF* mutation.

Table 3. Multivariate analysis for overall survival

Variable	Multivariate		
	Hazard ratio	95% confidence interval	*p*-value
BRAF (mutation vs. wild)	-	-	-
Sex (male vs. female)	1.33	(0.29～6.02)	0.72
Age (≥60 years vs. <60 years)	1.91	(0.96～8.61)	0.02
Thickness (mm)	1.23	(1.02～1.49)	0.03
Pathologic T stage (T3, T4 vs. Tis, T1, T2)	2.01	(0.38～10.6)	0.41
Pathologic TMN stage*			
II vs. I	0.63	(0.10～3.94)	0.62
III vs. I	1.39	(0.18～10.8)	0.75
VI vs. I	0.00	(0.00～)	1.00
Ulceration (yes vs. no)	1.31	(0.29～6.01)	0.73

-: cannot be checked. *American Joint Committee on Cancer (AJCC) staging system for cutaneous melanoma.

Table 4. Multivariate analysis for disease-free survival

Variable	Multivariate		
	Hazard ratio	95% confidence interval	*p*-value
BRAF (mutation vs. wild)	-	-	-
Sex (male vs. female)	1.06	(0.32～3.47)	0.93
Age (≥60 years vs. <60 years)	2.35	(0.98～9.11)	0.04
Thickness (mm)	2.10	(1.00～2.38)	0.02
Pathologic T stage (T3, T4 vs. Tis, T1, T2)	1.68	(0.44～6.38)	0.45
Pathologic TMN stage*			
II vs. I	0.55	(0.12～2.46)	0.43
III vs. I	2.33	(0.39～14.7)	0.37
VI vs. I	2.39	(0.24～23.1)	0.35
Ulceration (yes vs. no)	1.07	(0.32～3.51)	0.91

-: cannot be checked. *American Joint Committee on Cancer (AJCC) staging system for cutaneous melanoma.

factors in Cox multivariate analyses (Table 4), with age and Breslow thickness showing a significant correlation with DFS.

DISCUSSION

In the last decade, significant progress has been made in understanding of the genetic alterations in melanocytic tumors. The most exciting finding is the discovery of oncogenic *BRAF* and *NRAS* mutations in melanoma. A number of recent studies have shown that the RAS/RAF/MEK/ERK signaling pathway plays a crucial role in melanoma development, with ERK being constitutively activated in up to 90% of melanoma[16]. In melanoma, ERK activation is most commonly due to mutations of *NRAS*, and especially, *BRAF* genes[1].

The RAF family of serine/threonine protein kinase are components of a kinase signaling cascade that links extracellular signals to downstream cellular effectors. In mammals, there are three highly conserved *RAR* genes: *ARAF*, *BRAF*, and *CRAF*. The *BRAF* gene encodes a serine/threonine kinase involved in signaling from RAS to ERK signaling pathway. *BRAF* signaling regulates a variety of cellular processes, including growth, differentiation and apoptosis[17]. The most common *BRAF* mutation, which accounts for more than 90% of cases of cancer involving this gene, is glutamic acid for valine substitution, at position 600 (V600E)[1]. *BRAF V600E* has elevated kinase activity, when compared with wild type *BRAF*;[1] and it induces constitutive ERK signaling, through hyperactivation of the RAS/RAF/MEK/ERK pathway, stimulating proliferation, survival and transformation. *BRAF* is mutated in up to 70% of primary human melanoma[1,2], and surprisingly, a high frequency of *BRAF* mutation has also been reported in common benign nevi[18]. However, *BRAF V600E* induces senescence in benign nevi, through transcriptional upregulation of cell cycle inhibitor $p16^{INK4a}$ [19,20]. Thus, *BRAF* plays an important role in cancer induction, maintenance and progression, given that it is mutated early in the initiation process. However, oncogenic *BRAF* by itself is not sufficient for cancer, and must cooperate with other processes, to induce the fully cancerous state.

Recent studies by Boris Bastian's group have revealed that there exist site-specific genetic alterations in melanoma[21-23]. They classified melanomas into four groups: melanoma on skin with chronic sun-damage (CSD melanoma), melanoma on skin without chronic sun-damage (non-CSD melanoma), melanoma on palms, soles and nail bed (acral melanoma), and melanoma on mucous membrane (mucosal melanoma). Non-CSD melanoma roughly corresponds to superficial spreading melanoma, CSD melanoma to lentigo maligna melanoma, and acral melanoma to ALM. They found that non-CSD melanomas were characterized by the high frequency of *BRAF* mutations (reaching up to 75%); and ALM (23%), mucosal (11%) and CSD melanoma (11%) showed a low frequency of *BRAF* mutation[21-23]. It was concluded that *BRAF* mutations are associated with acral melanomas originating in areas of repetitive acute sun exposure; but not in areas with chronic ultraviolet radiation (UVR) exposure, or those are protected from UVR altogether. Other studies have also revealed that the frequency of *BRAF* mutation in ALM was lower, than in other types of melanoma[3,4]. Most of these studies are conducted in the Caucasian population, but there have been only few reports about *BRAF* mutation in Asian patients[10-14]. In the Asian population, the most common type of melanoma is ALM; and to our knowledge, there is only one report about the status and the clinical significance of *BRAF* mutation in melanoma of the Korean population[13]. So, we determined the frequency of *BRAF V600E* mutation in ALM by DPO based real-time PCR, and the incidence of *BRAF* mutation was found to be 19.4%. According to recent studies conducted in Asian populations, the frequencies of *BRAF* mutation in ALM were 15.5% to 15.7%[10,11]. These results were similar to the previous Western studies. Notwithstanding the methodological difference between sequencing and PCR for detecting *BRAF* mutation, the result of our study is sufficient to explain the low frequency of the *BRAF* mutation seen in ALM Korean patients. Benlloch et al.[24] compared the frequency of the *BRAF* mutation between the PCR and sequencing. There was no significant difference in the frequency of the *BRAF* mutation between the two groups. Because ALMs have a low frequency of *BRAF* mutations, it can be suggested that the activation of other distinctive genetic mechanisms serves an independent oncogenic function in ALMs lacking *BRAF* or *NRAS* mutations. In addition, these findings support the notion that divergent molecular pathways exist during melanoma development, which would explain the heterogeneous nature of this malignancy, which has been observed clinically.

Previously, several studies have been carried out to examine whether mutations in *BRAF* confer different pathological features and clinical behavior. The effects of its mutation on clinicopathologic features and clinical outcome remain uncertain, with previous studies reporting conflicting results[2,5-9]. Some reports have shown that the *BRAF* mutation is associated with thinner tumor thickness, and lower rate of proliferation[5,9]; and these observations indicate that *BRAF* mutation may be associated with a more differentiated form of melanoma, with a slower cell

proliferation rate. However, other studies were not able to find any association[2,6]. In this study, despite the statistical insignificance between the two groups, the proportion of Breslow thickness of more than 2.01 mm was lower in *BRAF* mutant patients, compared with wild type. Previous studies reveal that *BRAF* mutations were found to be inversely correlated with patients' age[2,8,9,23,25]. Although the close relationships of patient age, anatomic site, and sun-induced damage have made it difficult to segregate their individual associations with *BRAF* mutations, Bauer et al.[25] have recently confirmed that patient age is independently associated with *BRAF* mutation frequency. We can also observe that *BRAF* mutant patients were younger, than *BRAF* wild-type patients. However, there was no significant association between *BRAF* mutations, and other clinicopathologic features.

In the majority of studies, *BRAF* mutations in primary melanoma have no apparent impact on DFS and OS[2,5,6,8]. Furthermore, a recent study by Jin et al.[13] examined *BRAF* and KIT mutations in 202 Korean patients, and found no prognostic impact on *BRAF* mutation status, by multivariate analysis. In our study, we could not check whether or not the *BRAF* mutation is a prognostic factor for OS and DFS in the Cox's multivariate analysis, because the events (local recurrence, metastasis, and death) did not occur in *BRAF* mutant patients, and the number of patients in our study was too small for analysis. But, in the Kaplan-Meier curve, the *BRAF* mutant patients had a better clinical outcome, than *BRAF* wild patients. Our study showed that the *BRAF* mutant patients were younger, than *BRAF* wild patients. Moreover, in our study, younger patients had a better clinical outcome. We thought that this resulted from the differences in the melanoma sites and histological subtypes of the study population, between previous studies, and our study.

In addition, our data showed that *BRAF* wild type patients tend to have thicker tumor, compared with *BRAF* mutant patients, albeit without statistical significance. A large number of previous studies reported that *NRAS* mutations were associated with thicker tumor and higher mitotic rate, when compared to *BRAF* mutation[2,5]. Also, Devitt et al.[5] identified that the presence of *NRAS* mutations is an adverse prognostic factor, leading to shorter melanoma specific survival. In general, *BRAF* and *NRAS* mutations are mutually exclusive; thus, the effect of these mutations on clinical outcomes may be different. Furthermore, previous studies demonstrated that the *NRAS* mutations were associated with thicker tumor, older age, and poor clinical outcome, compared to *BRAF* mutation[2,5].

In conclusion, we assumed in our study that the *BRAF* wild patients might have *NRAS* mutation, which suggests that additional study on the *NRAS* mutation in ALM could be necessary, to confirm the effects of RAS/RAF/MEK/ERK signaling pathway on clinical outcome. Though a large scale analysis study on the survival rates associated with *BRAF* and *NRAS* mutation was conducted in Asian patients, the study included all types of melanoma, in contrast to this study, which included only ALM[10]. In addition, because the incidence of melanoma is far lower in the Asian, than in the Western population, and there are few systemic studies on the ALM and its survival rates associated with these mutations, further multicenter studies with larger sample size are needed, to confirm the result of this preliminary study in Korea.

Because the ALM has a low frequency of *BRAF* mutations, the potential therapeutic targets may be different from other types of melanoma. However, there are suggestions that ALM is also likely to be a target of *BRAF* kinase inhibitors, for the reason that in the majority of ALM, the RAS/RAF/MEK/ERK pathway is constitutively activated[26]. The stronger relationship between the RAS/RAF/MEK/ERK pathway and the ALM would be confirmed through additional *NRAS* mutation study in Korean patients, so that the *BRAF* kinase (e.g. Sorafenib) and selective RAF inhibitor (e.g. PLX4032, GSK2118436) could be reasonable treatments.

We acknowledge a number of limitations to our study. This study was a single-center study, with a limited number of patients. In addition, the incidence of melanoma is far lower in the Korean, than in the Western population; and there is a lack of systemic studies on ALM in Korea. For these reasons, we could not absolutely demonstrate the results of our study. Therefore, a multicenter study with a large sample size should be performed, to confirm the results of our preliminary study in Korea.

So in conclusion, a low frequency of *BRAF* mutation was shown, and *BRAF* mutation presented with a favorable prognosis in Korean primary ALM. These results indicate that other distinctive genetic mechanisms may have more important roles, in the development and progression of disease. So, further study is warranted into the molecular characterization of ALM, including the examination of *CCND1*, *PTEN* and *KIT*; as aberrations of these genes are likely to interact with *BRAF* and *NRAS*, to further drive clinical outcome.

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