PTEN promoter hypermethylation is associated with Breslow thickness in acral melanoma on the weight-bearing plantar foot

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Hae Seok Park

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<ABSTRACT>

PTEN promoter hypermethylation is associated with Breslow thickness in acral melanoma on the weight-bearing plantar foot

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Acral melanoma occurs on the acral glabrous skin and histologically shows a radial growth pattern in the epidermis. Although the pathogenesis of acral melanoma is not well understood, mechanical stress is thought to induce acral melanoma. The incidence of mutations and promoter methylation has been reported in tumors from acral melanoma; however, an association between genetic/epigenetic alterations and mechanical stress in acral melanoma remains unclear. In this study, we investigate the relationship between clinical/genetic factors and mechanical stress in acral melanoma.

A retrospective review of 56 patients diagnosed with acral melanoma was performed. We reviewed the clinical characteristics of patients, tumor status, and tumor location. Mutations in *BRAF, NRAS,* and the *TERT* promoter along with *KIT* amplification and *PTEN* promoter methylation were analyzed in tumors. The heel (34/56, 60.7%) was the most common anatomical tumor site. Mutations in *BRAF* (6/52, 11.5%), *NRAS* (6/53, 11.3%), and the *TERT* promotor (4/37, 10.8%) along with *KIT* amplification(4/41, 9.8%) and *PTEN* promoter hypermethylation (13/51, 25.5%) were observed in the tumors. On

the weight bearing area of the plantar foot, *PTEN* promoter hypermethylation was significantly associated with Breslow thickness (p =0.001) and ulceration rate (p=0.022). In the non-weight bearing area. there was no significant Breslow thickness or ulceration rate difference regardless of *PTEN* promoter hypermethylation. (p>0.05) In conclusion, *PTEN* promoter hypermethylation is associated with Breslow thickness and tumor ulceration on the weight-bearing plantar foot in acral melanoma in Korean patients.

Key Words : acral melanoma, breslow thickness, malignant melanoma, mechanical stress, PTEN promoter hypermethylation

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I. INTRODUCTION

Cutaneous melanoma, which is the heterogeneous group of tumor, is subdivided into the four types including chronic sun damage-induced melanoma (CSD), non-chronic sun damage-induced melanoma (non-CSD), uveal melanoma and acral melanoma.[1] Among them, acral melanoma occurs on glabrous skin or the nail apparatus and is distinguished with UV related melanoma according to different genetic alteration patterns.[2,3] Acral melanoma mostly shows the histologic features of acral lentiginous melanoma (>80%).[4] Acral melanoma accounts for 2-8% of melanoma from Caucasian, whereas it accounts for more than 40% of melanoma from Asian.[5] Acral melanoma is the most common type of melanoma in the Korean population.[4]

Melanoma harbors the highest somatic mutational burdens among solid malignancies.[6] Acral melanoma is genetically distinct from other cutaneous melanoma. Molecular genetics research has demonstrated several mutational differences between UV-related melanoma and non-UV related melanoma. Acral melanomas have a low mutation burden, different mutated genes, and a different type of genetic alteration.3 UV related mutation signatures, C>T or CC>TT, are not much detected in acral melanoma.[2] *BRAF* mutations (14.8%) and *NRAS* mutations (13.3%) are less frequent observed in acral melanoma than in other cutaneous melanoma (45~60% and 15~25%).[7] *TERT* promoter mutation is reported to be observed in 33-65% of cutaneous melanoma, while less than 10% in acral melanoma.[8] Acral melanoma shows recurrent genomic copy number alterations including gains of *TERT*(5p15), gains of *KIT*(4q12), gains of *CCND1*(11q13) and gains of *AURKA* gene(20q13).[2] These results suggest that acral melanoma may have different genetic causal pathways compared to UV related melanomas.[9]

Besides the genetic alteration, a recent study reported that the epigenetic alterations such as promoter hypermethylation of *RARB* and *PTEN* are the markers representing the prognosis in cutaneous melanoma.[10] Although the frequency of promoter hypermethylation is lower in tumors from Asian than in tumors from Caucasian, *PTEN* promoter hypermethylation is known to be an independent prognostic factor for survival in Asian melanoma.[11] However, the clinical significance of the promoter methylation and somatic mutation in acral melanoma is unclear.

Besides genetic factors, other factor such as mechanical stress is regarded as a factor for non-UV related melanoma.[12-14] Several reports showed a high incidence of acral melanoma on the weight-bearing portion of the soles,[15-17] and more oral mucosal melanoma occurred on hard palatal mucosa and

maxillary gingiva than on other areas of the oral mucosal surface.[14] Based on the studies of sole pressure while standing and walking,[18,19] heel and forefoot showed high peak pressure. Lesser toes and midfoot are known to be the relatively low-pressure area. Also, recent studies show that more acral melanoma occurs on the heel than any other areas of the plantar surface.[12,13]

Based on these relationships of acral melanoma with pressure, we investigated whether there are genetic or clinical factors associated with pressure. We reviewed the *BRAF, NRAS,* and *TERT* promoter mutations, *KIT* amplification, and *PTEN* promoter hypermethylation in primary tumors on the plantar area from Korean acral melanoma patients, and evaluated their association with tumor status, mechanical stress and clinical characteristics.

II. MATERIALS AND METHODS

1. Patients

This study included 56 acral melanoma patients. A Retrospective review of 56 patients diagnosed with acral melanoma was carried out. Medical records and mutation analysis results were reviewed for acral melanoma patient that presented to the Severance Hospital and Yonsei Cancer Hospital from 2002 to 2012. We selected 56 patients with genetic and epigenetic alteration results, which we intend to analyze. Clinical data including age, sex, body mass index, tumor-node-metastasis (TNM) stage, Breslow thickness, ulceration were collected. Of the genetic and epigenetic alteration results, *BRAF/NRAS* mutation, *TERT* promoter mutation, *KIT* amplification, and *PTEN* promoter hypermethylation was reviewed.

All lesions with clinical pictures were standardized in a composite image

and plotted with location according to pressure. (Fig. 1c) We divided the sole into 4 regions (heel, midfoot, forefoot, and toes) and each region into various areas (center, medial, lateral, lesser toes, hallux) based on the previous studies of pedobarography pressure.[19] The forefoot includes the metatarsal lesion and transverse arch. The midfoot contains cuboid bone, medial and lateral arch. The heel is composed of calcaneus and talus bones. Considering 3 arches (2 longitudinal and 1 transverse) and previous literatures[15-17], weight-bearing areas were defined as the forefoot, heel, and hallux. Midfoot and lesser toes were defined as non-weight bearing area. (Fig. 1, Fig. 2 Type A) Additionally, considering pedobarography pressure[20] and the previous study that Korean acral melanoma tended to be predominant in central heel and inner forefoot,[17] the weight bearing area was also defined differently as heel and inner forefoot (Fig. 2 Type B), central heel and forefoot (Fig. 2 Type C), central foot and inner forefoot (Fig. 2 Type D). The staging was determined according to the American Joint Committee on Cancer (AJCC) 8th guidelines for melanoma.

2. DNA preparation and mutation analysis

DNA preparation and mutation analysis was done as previously described.[21, 22] Formalin-fixed, paraffin-embedded tissue blocks diagnosed as acral melanoma was retrieved. Exons 15 (codon 600) of the *BRAF* gene, exons 1, 2 (codon 12, 13, and 61) of *NRAS* gene were amplified by polymerase chain reaction (PCR) to detect hotspot mutations. Also, PCR amplification of the *TERT* promoter region was performed. The primer sequences are listed in Table 1. Pyrosequencing using a PyroMark Q24 (Qiagen, Germantown, MD) was performed at room temperature with

PyroMark. Gold Q24 Reagents (Qiagen) following the manufacturer's instructions. Sequencing analysis was performed using PyroMark Q24 software ver. 1.0.10 (Qiagen) in the allele quantification analysis mode.

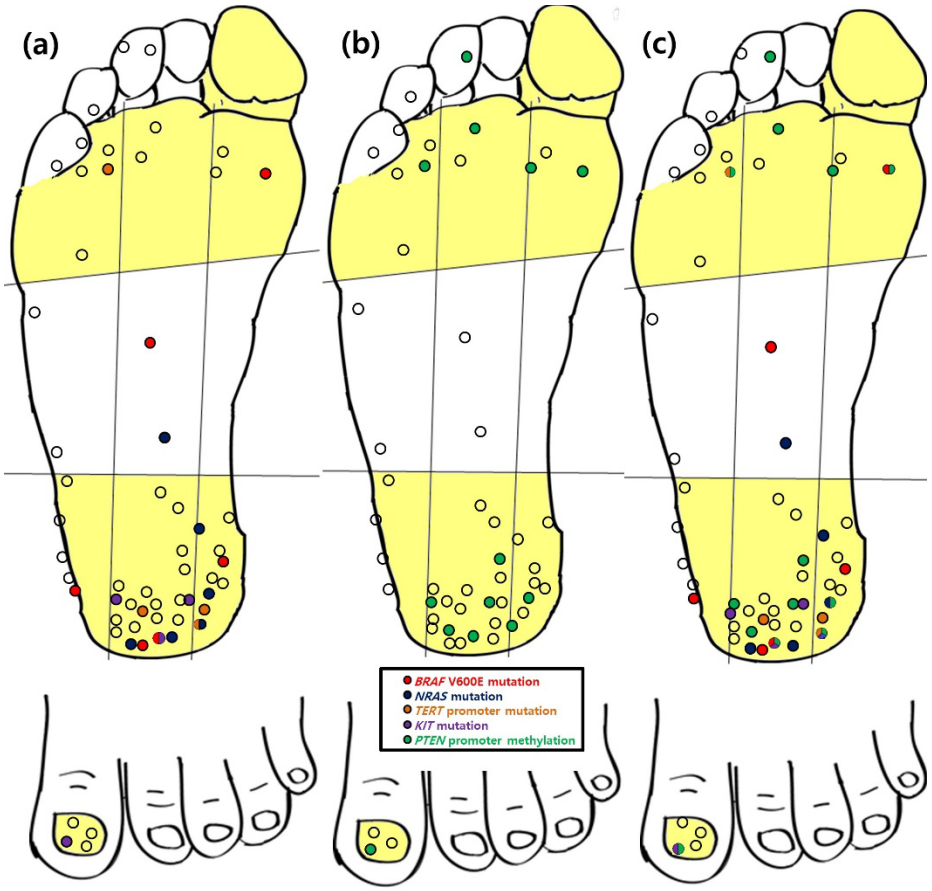

Figure 1. Anatomical mapping of genetic and epigenetic alterations in acral melanoma on the glabrous foot.

Weight-bearing areas are yellow-colored and the center of each lesion is plotted (n=56). Each dot is color-coded according to (A) the types of mutation or (B) PTEN promoter hypermethylation. (C) Both mutations and PTEN promoter hypermethylation are shown in each dot.

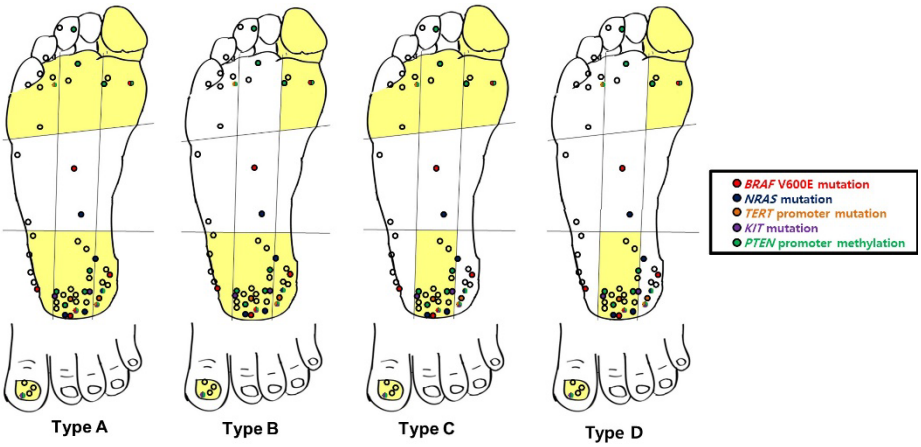

Figure 2. Anatomical mapping of genetic and epigenetic alterations according to weight-bearing patterns in acral melanoma on the glabrous foot.

Four types of weight-bearing patterns are shown in type A to D. Each dot is color-coded according to the types of genetic alteration or/and PTEN promoter hypermethylation.

3. Real-time PCR assay for *KIT* copy number

KIT amplification was analyzed as previously described.[21] *KIT* copy number was assessed by quantitative real-time PCR using glyceraldehydes-3-phosphate dehydrogenase (GAPDH) as a control gene (The *KIT* exon 17 primers and *GAPDH* primers are listed in Table 1). PCR reactions were done by QuantiTect SYBR Green PCR Kit (Qiagen), with a 20 μL total volume and 100 ng genomic DNA by Rotor-gene 2000 Real-Time Cycler (Corbett Research, Mortlake, Australia). Relative copy numbers were calculated by the ΔΔCt method, where Ct is the threshold cycle for amplification. For each sample, ΔCt for *KIT* versus *GAPDH* was calculated as ΔCt=Ct (KIT)–Ct

(GAPDH). 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 $2^{-\Delta\Delta Ct}$.

Table 1. Primers used in referred studies

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:	S: 5′-GACATACTGGATACAGCTGG-3'
TERT promoter		F:	5'-CCCACGTGCGCAGCAGGAC-3'
		R:	5'-Biotin-CTCCCAGTGGATTCGCGGGC-3‘
		S:	5'-AGGGGCTGGGAGGGC
KIT	17	F:	5′-AAAGATTTGTGATTTTGGTCTAGC-3′
		R:	5′-GAAACTAAAAATCCTTTGCA-3′
GAPDH	2	F:	5′-CACTAGGCGCTCACTGTTCT-3′
		R:	5′-GCGAACTCACCCGTTG-3′
PTEN promoter		F:	5'-GGATGTGGGTGTTTGTGTAATTA-3'
		R:	5'-biotin-AATTCCCACTCCCCAATAATAAC-3'
		S:	5'-TTTGTGTAATTAGTTTTTTA-3'

F, forward primer; R, reverse primer; S, sequencing primer

4. *PTEN* promoter methylation analysis

PTEN promoter methylation was analyzed as previously described.[11] Five potential promoter regions, spanning 1,333 base pairs upstream and 1,297 base pairs downstream around the transcription start site of the *PTEN* gene, was analyzed. Thereafter, CpG islands were identified within this core promoter region. For primer design, the DNA sequences were converted in silico to the methylated form of CpG as follows: CG motifs were converted to YG with Y equaling either C/T or G/A, and subsequently, C was converted to T. Using this converted sequence, methylation-specific primers for quantitative sequencing (pyrosequencing) of *PTEN* CpGs were designed using the Biotage Assay Design software (PyroMark Assay Design 2.0) and pyrosequencer PyroMark Q24 version 1.0.10 software (Qiagen, Germantown, MD). The primer sequences are listed in Table 1. 50 ng of bisulfite-treated DNA was used in the PCR reaction with 200 nmol/l forward and reverse primers for pyrosequencing. PCR conditions for *PTEN* were 1 x 95 ˚C for 15 minutes (95 ˚C for 40 seconds, 55 ˚C for 40 seconds, and 72 ˚C for 40 seconds), 50 cycles and 1 x 72 ˚C for 10 minutes using 0.5U of Amplitaq Gold (Applied Biosystem, TX). The percentage methylated fraction (C/T ratio) is automatically calculated. Each site is analyzed as a C/T polymorphism where a 100% C-reading denotes a fully methylated C in the original genomic DNA sample and a 100% T-reading denotes that this C was unmethylated in the gDNA. Intermediate C/T percentages denote partial methylation at the level of the sample. Then the value of methylation was calculated as the peak height methylated/ (peak height methylated + peak height unmethylated) x 100.

5. Statistical analysis

All the statistical analyses were performed using SPSS ver. 23.0 software (SPSS Inc., Chicago, IL). Categorical data are described using frequencies and percentages. Continuous data such as age are described using mean ± standard deviations or median (range) for normally distributed data. Chi-square test or Fisher exact test was used to differentiate the rates of different groups, and differences in measurement data of two groups were evaluated by unpaired t-test or Mann-Whitney test. Hierarchical clustering was performed to identify PTEN "hypermethylated" and "low methylated" samples. All statistical analyses were two-sided, and significance was assigned at $p < 0.05$.

III. RESULTS

1. Baseline clinical characteristics

Fifty-six acral melanoma patients were analysed. (Table 2) There were 28 males and 28 females with a sex ratio of 1:1. The median age was 65 (range 37 to 89 years). Among 56 patients, 52 acral melanoma (92.9%) were on the volar area and 4 patients (7.1%) had nail unit melanoma. Of 4 nail unit melanoma patients, three patients had subungual mass, 1 patient accompanied melanonychia. Ulceration was present in 18 (40.0%) patients, with a mean Breslow thickness of 3.04 mm. There was no significant relationship between high BMI and acral melanoma in the weight-bearing area. ($p>0.05$, Table 2) The rate of mutations was 11.5% for *BRAF* (6/52), 11.3% for *NRAS* (6/53), and 10.8% for *TERT* promoter (4/37). The rate of gene amplification was 9.8% for *KIT* (4/41). *PTEN* promoter hypermethylation (13/51) was also observed in 25.5%. The center of each acral melanoma lesion was plotted on the composite image. Each dot is color-coded according to the types of gene alterations (Fig. 1a) or *PTEN* promoter

hypermethylation. (Fig. 1b) All *BRAF* mutations (n=6) were *BRAF V600E*. In 6 *NRAS* mutation patients, an *NRAS* mutation in codon 61 was found in 2 patients. And G12R in 1 patient, G13R mutation in 3 patients was also detected. *TERT* promoter mutation observed as G > A transition was found at position −124 bp (relative to the ATG start site) in 4 patients. Among 4 patients of subungual melanoma on the great toenail, one patient had both *KIT* amplification and *PTEN* promoter hypermethylation.

Table 2. Baseline clinical characteristics of acral melanoma patients.

	Total	WB	NWB	
Patient - no. (%)	56 (100.0)	47 (83.9)	9 (16.1)	
Median age - year (range)	65 (37-89)	63 (37-89)	67 (37-80)	p=0.337
Female sex - no. (%)	28 (50.0)	24 (51.1)	4 (44.4)	p=0.715
BMI (kg/m2) - no. (n=55)	24.04±3.14	24.36±2.85	22.37±3.90	
BMI ≤23	22	17 (37.0%)	5 (55.5%)	
23 < BMI ≤25	12	11 (23.9%)	1 (11.1%)	p=0.650
BMI >25	21	18 (39.1%)	3 (33.3%)	
Ulceration - no. (n=45)				
Yes	18	17 (47.2%)	1 (11.1%)	p=0.064
No	27	19 (52.8%)	8 (88.8%)	
Thickness - mm (n=51)	3.04±3.00	3.39±3.19	1.37±0.93	p=0.071
Gene alteration - no. (%)				
BRAF (n=52)	6 (11.5)	5	1	
NRAS (n=53)	6 (11.3)	5	1	
TERT promoter (n=37)	4 (10.8)	4	0	
KIT amplification (n=41)	4 (9.8)	4	0	
PTEN promoter hypermethylation (n=51)	13 (25.5)	12	1	

*WB: Weight bearing area
**NWB: Non-weight bearing area

2. Anatomical mapping of acral melanoma

Among 56 patients, 52 acral melanoma (92.9%) were on the volar area. In

the volar area, vertical distribution of lesions was as follows: 34 lesions (60.7%) on the heel, 4 lesions (7.1%) on the midfoot, 9 lesions (16.1%) on the forefoot, and 5 lesions (8.9%) on the toes. The heel (n=34, 60.7%) was the most common site. The parallel distribution of lesions was as follows: 16 lesions (28.6%) on the medial side, 14 lesions (25.0%) on the lateral side, 26 lesions (46.4%) on the central. The anatomic mapping of the site distribution is shown in Fig. 1c.

3. Analysis of the relationship between clinical/genetic factors and pressure

To analyse the relationship with pressure, the foot was divided into pressure prone area and lesser pressure prone area. Weight-bearing areas are yellow-colored on Fig. 1. And we analysed the relationship between clinical/genetic factors and pressure. *BRAF, NRAS, TERT* promoter mutation and *KIT* amplification profiles showed no association with Breslow thickness and ulceration rate in both weight bearing and non-weight bearing lesion. ($p > 0.05$, Table 3) However, *PTEN* promoter hypermethylation showed association with Breslow thickness ($p=0.001$, Table 4) and ulceration ($p=0.022$, Table 4) on the weight-bearing area. In the presence of *PTEN* promoter hypermethylation, Breslow thickness was deep only in the weight-bearing area. In the non-weight bearing area. there was no significant Breslow thickness or ulceration rate difference regardless of *PTEN* promoter hypermethylation. ($p>0.05$, Table 4) In order to confirm the relationship between weight-bearing area and tumor status (Breslow thickness, ulceration rate) in *PTEN* promoter hypermethylation, weight-bearing areas were defined with a various method containing the forefoot, heel, and hallux. (Fig. 2) The relationship was verified by various methods (Type A, B, C, D) using sensitivity analysis, and the results were

similar. (Table 5)

Table 3. Genetic alteration profiles associated with Breslow thickness and ulceration in acral melanoma.

		BRAF		*NRAS*		*KIT* amplification		*TERT* promoter	
		YES	NO	YES	NO	YES	NO	YES	NO
Weight bearing	Breslow thickness – No. (%)								
	≤ 1	1 (20.0)	10 (28.6)	0	11 (29.7)	0	9 (33.3)	1 (33.3)	7 (26.9)
	1.0-2.0	1 (20.0)	10 (28.6)	1 (25.0)	10 (27.0)	2 (50.0)	6 (22.2)	1 (33.3)	2 (7.7)
	2.0-4.0	0	6 (17.1)	2 (50.0)	4 (10.8)	0	2 (7.4)	0	6 (23.1)
	> 4	3 (60.0)	9 (25.7)	1 (25.0)	12(32.4)	2 (50.0)	10 (37.0)	1 (33.3)	11 (42.3)
			p=0.295		*p*=0.395		*p*=0.452		*p*=0.541
	Ulceration - No. (%)								
	Yes	2 (66.6)	14 (43.7)	2 (50.0)	14 (45.1)	3 (75.0)	10 (43.5)	0	12 (54.5)
	No	1 (33.3)	18(56.2)	2 (50.0)	17(54.9)	1 (25.0)	13(56.5)	2 (100.0)	10 (45.5)
			p=0.446		*p*>0.999		*p*=0.325		*p*=0.239
Non weight bearing	Breslow thickness – No. (%)								
	≤ 1	0	4 (50.0)	0	4 (50.0)	0	3 (50.0)	0	0
	1.0-2.0	0	2 (25.0)	1 (100.0)	1 (12.5)	0	2 (33.3)	0	3 (75.0)
	2.0-4.0	1 (100.0)	2 (25.0)	0	3 (37.5)	0	1 (16.7)	0	1 (25.0)
	> 4	0	0	0	0	0	0	0	0
			p=0.222		*p*=.556		NA		NA
	Ulceration - No. (%)								
	Yes	0	1 (12.5)	0	1 (12.5)	0	0	0	0
	No	1 (100.0)	7 (87.5)	1 (100.0)	7 (87.5)	1 (100.0)	6 (100.0)	1 (100.0)	4 (100.0)
			p>0.999		*p*>0.999		NA		NA

Table 4. *PTEN* promoter hypermethylation associated with Breslow thickness and ulceration in acral melanoma.

		PTEN promoter hypermethylation	
		YES	NO
Weight bearing	Breslow thickness – No. (%)		
	≤ 1	0	9 (32.1)
	1.0-2.0	1 (10.0)	8 (28.5)
	2.0-4.0	0	6 (21.4)
	> 4	9 (90.0)	5 (17.9)
			***p*=0.001**
	Ulceration – No. (%)		
	Yes	7 (77.8)	7 (30.4)
	No	2 (22.2)	16 (69.6)
			***p*=0.022**
Non weight bearing	Breslow thickness – No. (%)		
	≤ 1	0	2 (25.0)
	1.0-2.0	0	4 (50.0)
	2.0-4.0	1 (100.0)	2 (25.0)
	> 4	0	0
			p=0.556
	Ulceration – No. (%)		
	Yes	1 (100.0)	0
	No	0	7 (100.0)
		0	0
			p=0.125

Table 5. *PTEN* promoter hypermethylation associated with Breslow thickness and ulceration according to weight-bearing patterns.

		PTEN promoter hypermethylation							
		Type A		Type B		Type C		Type D	
		YES	NO	YES	NO	YES	NO	YES	NO
Weight bearing	Breslow thickness – No. (%)								
	≤ 1	0	9 (29.0)	0	7 (26.9)	0	6 (33.3)	0	4 (28.6)
	1.0-2.0	1 (10.3)	8 (25.8)	1 (11.1)	9 (34.6)	0	4 (22.2)	0	4 (28.6)
	2.0-4.0	0	6 (19.4)	0	5 (19.2)	0	5 (27.8)	0	4 (28.6)
	> 4	9 (90.0)	5 (16.1)	8 (88.9)	5 (19.2)	8 (100.0)	3(16.7)	7 (100.0)	2 (14.3)
			***p*=0.001**		***p*=0.001**		***p*=0.001**		***p*=0.002**
	Ulceration – No. (%)								
	Yes	7 (77.7)	7 (30.4)	7 (87.5)	6 (30.0)	6 (85.7)	4 (30.8)	6 (100.0)	3 (30.0)
	No	2 (22.2)	16 (69.6)	1 (12.5)	14 (70.0)	1 (14.2)	9 (69.2)	0	7 (70.0)
			***p*=0.022**		***p*=0.01**		*p*=0.057		***p*=0.011**
Non weight bearing	Breslow thickness – No. (%)								
	≤ 1	0	2 (25.0)	0	6 (54.5)	1 (20.0)	6 (40.0)	2 (25.0)	7 (43.8)
	1.0-2.0	0	4 (50.0)	0	2 (18.2)	3 (60.0)	4 (26.7)	4 (50.0)	3 (18.8)
	2.0-4.0	1 (100.0)	2 (25.0)	1 (50.0)	2 (18.2)	1 (20.0)	2 (13.3)	2 (25.0)	2 (12.5)
	> 4	0	0	1 (50.0)	1 (9.1)	0	3 (20.0)	0	4 (25.0)
			p=0.556		*p*=0.065		*p*=0.810		*p*=0.698
	Ulceration – No. (%)								
	Yes	1 (100.0)	0	1 (50.0)	1 (10.0)	2 (66.6)	3 (17.6)	2 (50.0)	4 (20.0)
	No	0	7 (100.0)	1 (50.0)	9 (90.0)	1 (33.3)	14 (82.4)	2 (50.0)	16 (80.0)
			p=0.125		*p*=0.318		*p* =0.14		*p*=0.251

IV. DISCUSSION

In the present study, we investigate whether there are genetic or clinical factors associated with pressure in Korean acral melanoma patients. We identified that *PTEN* promoter hypermethylation is associated with foot melanoma thickness and ulceration rate only on the weight bearing area. We found that 25.5% of acral melanoma patients were considered *PTEN* promoter hypermethylated. This hypermethylation rate (13/51. 25.5%) of Korean acral melanoma patients is consistent with the rate of the previous study in Korea.[11] (31/158, 19.6%)

Somatic mutation rates of acral melanoma patients were also similar to other studies.[23] No other somatic alteration (BRAF, NRAS, TERT promoter mutation, KIT amplification) showed association with Breslow thickness or ulceration rate in acral melanoma. These results are consistent with an analysis of 48 cases of acral melanoma in Brazil.[23]

Recently, several studies suggest that mechanical stress is a possible factor in promoting acral melanoma, particularly on the sole.[24] Several reports showed a high incidence of acral melanoma on the weight-bearing portion of the soles.[15-17] We tried to evaluate the role of long-term mechanical stress in acral melanoma by analysing clinical factors and genetic factors in the weight bearing area. There was no significant difference in Breslow thickness and ulceration rate according to weight bearing or not. Our data showed that tumour thickness and ulceration rate are related with only *PTEN* promoter methylation on the weight bearing area. The similar result was shown when weight-bearing areas were defined with other various methods containing the forefoot, heel, and hallux.

There are various types of mechanical stresses, including pressure, friction,

shearing forces, and stretching.[24] Hidradenitis supprativa, callus, and diabetic foot ulcer have been studied for the relationship between mechanical stress and disease, but not much has been studied in acral melanoma. Friction is mainly applied in the epidermis, which mainly causes intertrigo and hidradenitis supprativa,[25] whereas shear injury affects deeper skin layers and causes pressure ulcer. Shear stress which is defined as force per unit area exerted parallel to the sole plane while walking.[26] When standing, the pressure act perpendicular to the plane extends across the entire layer of the skin layer (epidermis, dermis, subcutaneous tissue).[24] In acral melanoma patients, standing pressure and shear stress are mainly considered to operate on foot.

Physiologically, shear stress elicits an increase in cutaneous microvascular reactivity and endothelial function.[26] In tumor microenvironment, shear stress activates TGF-β signaling inducing epithelial to mesenchymal transition.[27] Transforming growth factor-β signaling which is associated with tissue fibrosis and tumor microenvironment show predominant role via stimulating non-canonical hedgehog pathway in epithelial-mesenchymal transition, which is important in melanoma invasion and metastasis. However, how macroscopic mechanical forces regulate cell fate by affecting genetic/epigenetic alteration remained unclear in acral melanoma. Further prospective investigation of mechanical stress and genetic/epigenetic alteration will be needed.

PTEN, a tumour-suppressing gene, is implicated in cellular differentiation, reproduction and apoptosis, as well as cellular adhesion and mobility. Multiple studies showed reduced, not absent, PTEN in melanoma.[28,29] Recent studies showed complete or partial loss of PTEN level in melanoma are associated with worse overall survival.[30,31] PTEN loss cannot be fully explained by genetic alteration. In NYU and TCGA melanoma cohort, *PTEN* mutations and deletions are relatively uncommon in melanoma.[31] *PTEN* promoter hypermethylation has

also been associated with loss of PTEN in melanoma. In TCGA and Korean melanoma cohort, *PTEN* promoter methylation is a significant negative prognostic marker in survival of melanoma patients.[7,11] Our study showed *PTEN* promoter methylation is associated with deep Breslow thickness and higher ulceration rate also in acral melanoma. This result was limited to the weight bearing area of foot only. Based on these clinical results, additional studies will be needed to investigate whether epigenetic alterations of *PTEN*, such as hyper or hypomethylation and histone modification, are associated with PTEN loss in acral melanoma.

V. CONCLUSION

We have characterized genetic/epigenetic alteration and the relationship with pressure of 56 acral melanoma. We have shown that more than 25% of acral melanoma patients harbor *PTEN* promoter hypermethylation, and epigenetic alteration like *PTEN* hypermethylation are related with deep Breslow thickness and higher ulceration rate on weight bearing area of acral melanoma. These results could help in finding possible role of mechanical stress in promoting acral melanoma. Further prospective investigation of molecular alteration will be needed to understand the relationship between mechanical stress and genetic/epigenetic alteration.

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ABSTRACT (IN KOREAN)

발바닥 체중부하 부위에 발생하는 말단흑색종에서 PTEN 프로모터의 과메틸화와 Breslow Thickness의 연관성

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박해석

말단 흑색종은 손, 발의 털이 없는 피부에서 발생하며 조직학적으로 표피에서 방사형 성장 패턴을 나타낸다. 병인은 잘 알려져 있지 않지만, 말단부에 작용하는 물리적 압력이 말단 흑색종과 연관이 있는 것으로 생각된다. 말단 흑색종의 발생률, 유전자변이, 후성유전에 관한 연구가 여러 문헌에서 보고되고 있으나 이런 유전자의 변화와 피부흑색종의 물리적 압력과의 연관성은 아직 불명확하다. 따라서 이 연구에서는 말단 흑색종에서 임상/유전적 요인과 물리적 압력 사이의 관계를 조사하고자 하였다.

발의 말단흑색종으로 진단받은 56명의 환자를 대상으로 후향적 연구를 시행하였다. 환자의 임상적 특징, 종양의 상태, 종양의 위치 등을 조사하였으며, 종양 내의 BRAF 유전자 변이, NRAS 유전자

변이, TERT 유전자 프로모터 변이, KIT 유전자 증폭과 PTEN 프로모터의 메틸화에 대하여 조사하였다.

발뒤꿈치 (34/56, 60.7 %)가 종양의 가장 흔한 해부학적 부위였다. BRAF 변이 (6/52 11.5 %), NRAS 변이 (6/53 11.3 %), TERT 프로모터 변이 (4/37, 10.8 %), KIT 증폭 (4/41, 9.8 %) 및 PTEN 프로모터 과메틸화 (13/51, 25.5 %)가 관찰되었다. 발바닥의 체중 부하 영역에서 PTEN 프로모터 과메틸화가 있는 경우 Breslow thickness ($p = 0.001$)와 궤양 유발률 ($p = 0.022$)의 유의한 차이가 있었다. 비 체중 부하 영역에서는 PTEN promoter 과메틸화와 상관없이 Breslow thickness나 궤양 발생률의 유의미한 차이는 보이지 않았다. ($p > 0.05$)

따라서, 저자는 한국인의 말단흑색종에서 PTEN 프로모터의 과메틸화는 체중을 지지하는 발바닥의 Breslow thickness와 종양의 궤양 유발률과 관련이 있다고 보고하는 바이다.

핵심되는 말: 말단흑색종, 악성흑색종, 물리적압력, PTEN promoter 과메틸화