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ORIGINAL ARTICLE

PTEN Promoter Hypermethylation Is Associated with Breslow Thickness in Acral Melanoma on the Heel, Forefoot, and Hallux

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Background: Acral melanoma occurs on glabrous skin or the nail apparatus and is distinct from ultraviolet-related melanoma due to differing genetic alteration patterns. Although the pathogenesis of acral melanoma is not well understood, mechanical stress is thought to induce acral melanoma. The incidence of gene mutation 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. **Objective:** To investigate the relationship between clinical/genetic factors and mechanical stress in acral melanoma. **Methods:** A retrospective review of 52 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 the tumors. **Results:** The heel (34/52, 65.4%) was the most common anatomical tumor site. Mutations in *BRAF* (6/48, 12.5%), *NRAS* (6/49, 12.2%), and the *TERT* promoter (4/33, 12.1%), along with *KIT* amplification (3/37, 8.1%) and *PTEN* promoter hypermethylation (12/48, 25.0%) were observed in the tumors. On the forefoot, heel, and hallux, *PTEN* promoter hypermethylation was significantly associated with Breslow thickness ($p=0.001$) and ulceration rate ($p=0.042$). On the midfoot and lesser toes, there was no significant difference in Breslow thickness or ulceration rate regardless of *PTEN* promoter hypermethylation ($p>0.05$). **Conclusion:** *PTEN* promoter hypermethylation is associated with Breslow thickness and tumor ulceration on the forefoot, heel, and hallux in acral melanoma in Korean patients. **(Ann Dermatol 33(1) 18～25, 2021)**

-Keywords-
Acral melanoma, Malignant melanoma, Mechanical stress, Methylation, Phosphatase and tensin homolog

INTRODUCTION

Cutaneous melanoma, which is a heterogeneous group of tumors, is subdivided into four types: chronic sun damage-induced melanoma (CSD), non-CSD, mucosal melanoma, and acral melanoma[1]. Among them, acral melanoma occurs on glabrous skin or the nail apparatus and is distinct from ultraviolet (UV)-related melanoma due to differing genetic alteration patterns[2,3]. Acral melanoma mostly displays histologic features of acral lentiginous melanoma (>80%)[4]. Acral melanoma accounts for 2% to 8% of melanomas in Caucasians, whereas it accounts for more than 40% of melanomas in Asians[5]. Acral melanoma is the most common type of melanoma in the Korean population[4].

Melanoma is one of cancer that harbors the highest somatic mutational burdens among all solid malignancies[6]. Acral melanoma is genetically distinct from other cuta-

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neous melanomas. Molecular genetics research has demonstrated several mutational differences between UV-related melanoma and non-UV related melanoma. Acral melanomas have a low mutational burden, differing mutated genes, and a differing type of genetic alteration[3]. UV-related mutational signatures, C>T or CC>TT, are typically not detected in acral melanomas[2]. *BRAF* mutations (14.8%) and *NRAS* mutations (13.3%) are less frequently observed in acral melanomas than in other cutaneous melanomas (45%~60% and 15%~25%)[7]. *TERT* promoter mutations are reported in 33%~65% of cutaneous melanomas, but in less than 10% of acral melanomas[8]. Acral melanomas show recurrent genomic copy number alterations, which include gains of *TERT* (5p15), *KIT* (4q12), *CCND1* (11q13), and *AURKA* (20q13)[2]. These results suggest that acral melanomas may have different genetic causal pathways than UV-related melanomas[9].

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

Besides genetic factors, other factors, such as mechanical stress, are considered factors for non-UV related melanoma[12-14]. Several reports have shown a high incidence of acral melanomas on the weight-bearing portion of the sole[15-17], and more oral mucosal melanomas have occurred on the hard palatal mucosa and maxillary gingiva than on other areas of the oral mucosal surface[14]. From studies on sole pressure while standing and walking[18,19], the heel and forefoot showed the highest peak pressures. The little toes and midfoot are relatively low-pressure areas. Also, recent studies have shown that more acral melanomas occur on the heel than on any other areas of the plantar surface[12,13]. Based on the relationship between acral melanoma and pressure, we investigated whether there are genetic or clinical factors associated with pressure. We evaluated mutations in *BRAF*, *NRAS*, and in the *TERT* promoter, along with *KIT* amplification and *PTEN* promoter hypermethylation in primary tumors on the plantar surface in Korean acral melanoma patients. We assessed the association of these genetic/epigenetic alterations with tumor status, mechanical stress, and clinical characteristics.

MATERIALS AND METHODS

This study included 52 acral melanoma patients. A retrospective review of 52 patients diagnosed with acral melanoma was carried out. Medical records and mutation analysis results were reviewed from acral melanoma patients who were treated at Severance Hospital and Yonsei Cancer Hospital from 2002 to 2012. We selected 52 patients with genetic and epigenetic alteration results for analysis. Clinical data that included age, sex, body mass index, tumour-node-metastasis stage, Breslow thickness, and ulceration (both macroscopic and microscopic) were collected. From the genetic and epigenetic alteration results, mutations in *BRAF*, *NRAS*, and the *TERT* promoter, along with *KIT* amplification and *PTEN* promoter hypermethylation were reviewed. This retrospective study was approved by the Institutional Review Board of Gangnam Severance Hospital (IRB no. 2019-0487-001). The requirement of informed consent was exempted.

All lesions with clinical pictures were standardized as a composite image and plotted by location according to pressure (Fig. 1). We divided the sole into 4 regions (heel, midfoot, forefoot, and toes), and each region was subdivided into various areas (center, medial, lateral, lesser

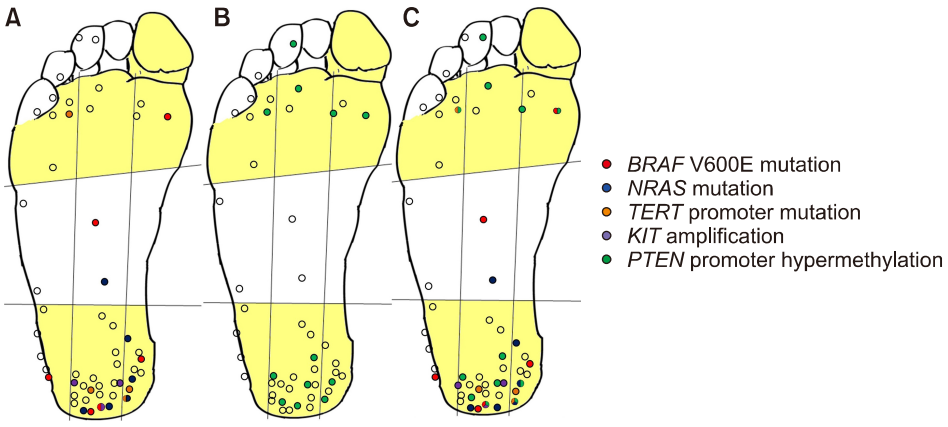

Fig. 1. Anatomical mapping of genetic and epigenetic alterations in acral melanoma on the glabrous foot. Heel, forefoot, and hallux are yellow-colored and the centers of the lesions are plotted (n=52). Each dot is color-coded according to the mutation type (A) or *PTEN* promoter hypermethylation (B). (C) Both mutations and *PTEN* promoter hypermethylation are shown in each dot.

toes, hallux) based on previous pedobarographic pressure studies[18-20]. The forefoot included the metatarsal lesion and transverse arch. The midfoot included the cuboid bone and the medial and lateral arches. The heel included the calcaneus and talus bones. From previously published literature[15-17] and in consideration of pedobarographic pressure[20] and the 3 arches (2 longitudinal and 1 transverse), we divided plantar foot into two areas (heel, forefoot, hallux vs. midfoot, lesser toes) (Fig. 1). The staging was determined according to the American Joint Committee on Cancer (AJCC) 8th guidelines for melanoma.

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

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′
PTE promoter		F:	5′-GGATGTGGGTGTTTGTGTAATTA-3′
		R:	5′-biotin-AATTCCCACTCCCCAATAATAAC-3′
		S:	5′-TTTGTGTAATTAGTTTTTTA-3′

F: forward primer, R: reverse primer, S: sequencing primer, GAPDH: glyceraldehyde-3-phosphate dehydrogenase.

Sequencing analysis was performed using PyroMark Q24 software ver. 1.0.10 (Qiagen) in allele quantification analysis mode.

KIT amplification was analyzed as previously described[21]. *KIT* copy number was assessed by quantitative real-time PCR using glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as the control gene (*KIT* exon 17 and *GAPDH* primers are listed in Table 1). PCR reactions were performed in the Rotor-Gene 2000 Real-Time Cycler (Corbett Research, Mortlake, Australia) using the QuantiTect SYBR Green PCR Kit (Qiagen) with a 20 μl total volume and 100 ng of genomic DNA. Relative copy numbers were calculated by the $\Delta\Delta$Ct method, where Ct is the threshold cycle for amplification. For each sample, ΔCt for *KIT* vs. *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, USA) using the formula $\Delta\Delta$Ct=ΔCt (test sample)−ΔCt (reference pool). Relative DNA copy number was calculated using the formula $2^{-\Delta\Delta Ct}$.

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 of the *PTEN* gene transcription start site, were analyzed. CpG islands were identified within the core promoter region. For primer design, DNA sequences were converted *in silico* to the methylated form of CpG as follows: CG motifs were converted to YG with Y equalling either C/T or G/A, and then 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 the pyrosequencer PyroMark Q24 version 1.0.10 software (Qiagen). The primer sequences are listed in Table 1. Fifty nanogram of bisulfite-treated DNA was used in the PCR reaction with 200 nmol/L forward and reverse primers. PCR conditions for *PTEN* were 95°C for 15 minutes; 50 cycles of 95°C for 40 seconds, 55°C for 40 seconds, and 72°C for 40 seconds; and 72°C for 10 minutes. PCR reactions included 0.5 U of Amplitaq Gold (Applied Biosystems, Austin, TX, USA). The percentage methylated fraction (C/T ratio) was automatically calculated. Each site was 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 the C was unmethylated in the genomic DNA sample. Intermediate C/T percentages denote partial methylation in the sample. The methylation values were calculated as the peak height methylated/(peak height methylated+peak height unmethylated)×100.

All statistical analyses were performed using SPSS ver.

23.0 software (IBM Corp., Armonk, NY, USA). Categorical data were described using frequencies and percentages. Continuous data, such as age, were described using mean ± standard deviation or median (range) for normally distributed data. A chi-square test or Fisher exact test was used to differentiate the rates of different groups. Differences in measurement data between two groups were evaluated by unpaired t-test or Mann–Whitney test. Hierarchical clustering was performed to identify *PTEN* promoter "hypermethylated" and "hypomethylated" samples. All statistical analyses were two-sided, and significance was assigned at $p < 0.05$.

RESULTS

Baseline clinical characteristics

Fifty-two acral melanoma patients were analyzed (Table 2). There were 26 males and 26 females with a sex ratio of 1:1. The median age was 65 years (range, 37～89 years). Ulceration was present in 17 patients (39.5%), with a mean Breslow thickness of 2.99 mm. There was no significant relationship between high body mass index (BMI) and acral melanoma on the heel, forefoot, and hallux ($p > 0.05$; Table 2). The mutation rate was 12.5% for *BRAF* (6/48), 12.2% for *NRAS* (6/49), and 12.1% for the *TERT* promoter (4/33). The gene amplification rate for *KIT* was 8.1% (3/37). *PTEN* promoter hypermethylation (12/48) was also observed in 25.0% of patients. The center of each acral melanoma lesion was plotted on the composite image. Each dot is color-coded according to the gene alteration type (Fig. 1A) or *PTEN* promoter hypermethylation (Fig. 1B). All *BRAF* mutations (n = 6) were *BRAF* V600E. In the 6 patients with *NRAS* mutations, a mutation in codon 61 was identified in 2 patients. In the other four patients, mutations resulting in G12R in one patient and G13R in 3 patients were also detected. A G > A transition in the *TERT* promoter was found at position −124 bp (relative to the ATG start site) in 4 patients.

Anatomical mapping of acral melanoma

Among the 52 patients, the vertical distribution of lesions were as follows: 34 lesions (65.4%) on the heel, 4 lesions (7.7%) on the midfoot, 9 lesions (17.3%) on the forefoot, and 5 lesions (9.6%) on the toes. The heel (n = 34, 65.4%) was the most common site. The parallel distribution of lesions was as follows: 12 lesions (23.1%) on the medial side, 14 lesions (26.9%) on the lateral side, and 26 lesions (50.0%) in the central region. The anatomic mapping of site distribution is shown in Fig. 1C.

Analysis of the relationship between clinical/genetic factors and pressure

To analyze the relationship with pressure, the foot was divided into a pressure prone area and a lesser pressure prone area. Heel, forefoot, and hallux are yellow-colored on Fig. 1. We also analyzed the relationship between clinical/genetic factors and pressure. Mutations in *BRAF*, *NRAS*, and the *TERT* promoter and *KIT* amplification profiles showed

Table 2. Baseline clinical characteristics of acral melanoma patients

Variable	Total	Heel, forefoot and hallux	Midfoot and lesser toes	*p*-value
Patient	52 (100.0)	43 (82.7)	9 (17.2)	
Median age, yr (range)	65 (37～89)	63 (37～89)	67 (37～80)	0.341
Female sex	26 (50.0)	22 (51.2)	4 (44.4)	0.725
BMI, kg/m^2 (n = 51)	24.04 ± 3.14	24.36 ± 2.85	22.37 ± 3.90	0.675
≤ 23	20	15 (35.7)	5 (55.6)	
23 < BMI ≤ 25	10	9 (21.4)	1 (11.1)	
> 25	21	18 (42.9)	3 (33.3)	
Ulceration (n = 43)				0.071
Yes	17	16 (47.1)	1 (11.1)	
No	26	18 (52.9)	8 (88.9)	
Thickness, mm (n = 48)	2.99 ± 2.93	3.34 ± 3.15	1.37 ± 0.93	0.088
Gene alteration				
BRAF (n = 48)	6 (12.5)	5	1	
NRAS (n = 49)	6 (12.2)	5	1	
TERT promoter (n = 33)	4 (12.1)	4	0	
KIT amplification (n = 37)	3 (8.1)	3	0	
PTEN promoter hypermethylation (n = 48)	12 (25.0)	11	1	

Values are presented as number (%), mean (range), mean ± standard deviation, or number only.

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

Category	*BRAF*		*NRAS*		*KIT* amplification		*TERT* promoter	
	Yes	No	Yes	No	Yes	No	Yes	No
Heel, forefoot, hallux								
Breslow thickness								
≤1	1 (20.0)	9 (27.3)	0	10 (29.4)	0	9 (33.3)	1 (33.3)	7 (28.0)
>1.0~2.0	1 (20.0)	10 (30.3)	1 (25.0)	10 (29.4)	2 (50.0)	6 (22.2)	1 (33.3)	2 (8.0)
>2.0~4.0	0	6 (18.2)	2 (50.0)	4 (11.8)	0	2 (7.4)	0	6 (24.0)
>4	3 (60.0)	8 (24.2)	1 (25.0)	10 (29.4)	1 (66.6)	10 (37.0)	1 (33.3)	10 (40.0)
p-value	0.239		0.295		0.325		0.543	
Ulceration								
Yes	2 (66.7)	13 (43.3)	2 (50.0)	13 (44.8)	2 (66.6)	10 (43.5)	0	11 (52.4)
No	1 (33.3)	17 (56.7)	2 (50.0)	16 (55.2)	1 (25.0)	13 (56.5)	2 (100.0)	10 (47.6)
p-value	0.579		>0.999		0.425		0.478	
Midfoot, lesser toes								
Breslow thickness								
≤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)	1 (12.5)	0	2 (33.3)	0	3 (75.0)
>2.0~4.0	1 (100)	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-value	0.222		0.556		NA		NA	
Ulceration								
Yes	0	1 (12.5)	0	1 (12.5)	0	0	0	0
No	1 (100)	7 (87.5)	1 (100)	7 (87.5)	1 (100)	6 (100)	1 (100)	4 (100)
p-value	>0.999		>0.999		NA		NA	

Values are presented as number (%).

no association with Breslow thickness or ulceration rate on both groups ($p>0.05$; Table 3). However, *PTEN* promoter hypermethylation showed an association with Breslow thickness ($p=0.001$; Table 4) and ulceration ($p=0.042$; Table 4) on the heel, forefoot, and hallux. In the presence of *PTEN* promoter hypermethylation, Breslow thickness was increased only on the heel, forefoot, and hallux. In the midfoot and lesser toes, there were no significant differences in Breslow thickness or ulceration rate associated with *PTEN* promoter hypermethylation ($p>0.05$; Table 4).

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

Category	*PTEN* promoter hypermethylation		
	Yes	No	*p*-value
Heel, forefoot, hallux			
Breslow thickness			0.001*
≤1	0	9 (33.3)	
>1.0~2.0	1 (11.1)	8 (29.6)	
>2.0~4.0	0	6 (22.2)	
>4	8 (88.9)	4 (14.8)	
Ulceration			0.042*
Yes	6 (75.0)	7 (30.4)	
No	2 (25.0)	16 (69.6)	
Midfoot, lesser toes			
Breslow thickness			0.556
≤1	0	2 (25.0)	
>1.0~2.0	0	4 (50.0)	
>2.0~4.0	1 (100)	2 (25.0)	
>4	0	0	
Ulceration			0.125
Yes	1 (100)	0	
No	0	7 (100)	

Values are presented as number (%). *Statistically significant ($p<0.05$).

DISCUSSION

In the present study, we investigated whether there are genetic or clinical factors associated with pressure in Korean acral melanoma patients. We discovered that *PTEN* promoter hypermethylation was associated with foot melanoma thickness and ulceration rate only on the heel, forefoot, and hallux. We found that 25.0% of acral melanoma patients had *PTEN* promoter hypermethylation. The hypermethylation rate (12/48, 25.0%) of Korean acral melanoma patients is consistent with the rate found in a previous Korean study (31/158, 19.6%)[11]. We checked the association between *PTEN* promoter hypermethylation and Breslow

thickness, and there was no significant correlation (p= 0.238), which is also consistent with the previous study[11]. Somatic mutation rates of acral melanoma patients were also similar to other studies[23]. No other somatic alterations (mutations in *BRAF*, *NRAS*, and the *TERT* promoter, *KIT* amplification) were associated 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 have suggested that mechanical stress is a possible factor in promoting acral melanoma, particularly on the sole[24]. Several reports have shown a high incidence of acral melanoma on the weight-bearing portion of the soles[15-17,25]. Sheen et al.[25] recently showed that acral melanomas tended to develop on weight-bearing areas, and the distribution pattern was not associated with clinical and prognostic factors. Our study show that heel (34/52, 65.4%) was the most common anatomical site of tumors and there was no significant relationship between high pressure area and other clinical factors (sex, age, BMI, Breslow thickness, ulceration), which is consistent results with previous study[25].

We aimed to evaluate the role of long-term mechanical stress in acral melanoma by analyzing clinical and genetic factors on the heel, forefoot, and hallux. There was no significant difference in Breslow thickness or ulceration rate on the heel, forefoot and hallux compared to the midfoot and lesser toes. Our data showed that tumor thickness and ulceration rate were only related to *PTEN* promoter hypermethylation on the heel, forefoot, and hallux.

There are various types of mechanical stresses, which include pressure, friction, shearing forces, and stretching[24]. The relationship between mechanical stress and disease has been studied in Hidradenitis suppurativa, callus, and diabetic foot ulcers, but little has been reported in acral melanoma. Friction primarily applies to the epidermis, which causes intertrigo and hidradenitis suppurativa[26], whereas shear injury affects deeper skin layers and causes pressure ulcers. Shear stress is defined as force per unit area exerted parallel to the sole plane while walking[27]. When standing, the pressure acts perpendicular to the plane and extends across the entire skin layer (epidermis, dermis, subcutaneous tissue)[24]. In acral melanoma patients, standing pressure and shear stress mainly affect the foot.

Physiologically, shear stress elicits an increase in cutaneous microvascular reactivity and endothelial function[27]. In the tumor microenvironment, shear stress activates transforming growth factor-β (TGF-β) signalling, inducing the epithelial to mesenchymal transition[28]. TGF-β signalling, which is associated with tissue fibrosis and the tumor microenvironment have predominant roles by stimulating the non-canonical hedgehog pathway in the epithelial-mesenchymal transition. This stimulation is important in melanoma invasion and metastasis. However, how macroscopic mechanical forces regulate cell fate through genetic/epigenetic alterations remains unclear in acral melanoma. Further prospective investigation of mechanical stress and genetic/epigenetic alterations will be needed.

PTEN, a tumor-suppressor gene, is implicated in cellular differentiation, reproduction, and apoptosis, as well as in cellular adhesion and mobility. Multiple studies have shown reduced, but not absent, PTEN in melanoma[29,30]. Recent studies have shown that complete or partial loss of PTEN in melanoma is associated with poor overall survival[31,32]. However, PTEN loss cannot be fully explained by genetic alteration. In the New York University and the Cancer Genome Atlas (TCGA) melanoma cohorts, *PTEN* mutations and deletions were relatively uncommon in melanoma[32]. *PTEN* promoter hypermethylation has also been associated with loss of PTEN in melanoma. In the TCGA and Korean melanoma cohorts, *PTEN* promoter methylation was a significant negative prognostic marker of survival for melanoma patients[7,11]. Our study showed that *PTEN* promoter methylation is associated with increased Breslow thickness and higher ulceration rates in acral melanomas. This result was limited to the heel, forefoot, and hallux. Based on these clinical results, additional studies will be needed to investigate whether epigenetic alterations of *PTEN*, such as hyper- or hypomethylation and histone modifications, are associated with PTEN loss in acral melanoma. In selected acral melanoma patients, induction of tumor PTEN expression above a threshold level might suppress Akt activity and tumor growth and promote anti-tumor immunity to improve patient survival[33].

There are a number of limitations of this study. First, as a retrospective single-center study, the sample size is small. Further large-scale and population-based analysis containing more samples, especially on midfoot and lesser toes, is needed to clearly show the relationship between mechanical stress and genetic/epigenetic alterations. Second, the methodology used to analyze weight bearing area was different from other studies. However, there is no study clarifying where is weight bearing area of sole. In this study, sole was divided into two parts considering pedobarography results of non-melanoma patients while walking and standing. In future study, measuring pedobarography of acral melanoma patients may help to consider not only weight bearing portion of sole, but also the external pressure details such as personal walking habits, pressure caused by shoes, and pressure when lying down during sleep.

In conclusion, we have characterized genetic/epigenetic

alterations and the relationship with pressure in 52 acral melanoma patients. We have shown that about 25% of acral melanoma patients harbor *PTEN* promoter hypermethylation. Moreover, epigenetic alterations, like *PTEN* promoter hypermethylation, are related to increased Breslow thickness and higher ulceration rates on the heel, forefoot, and hallux in acral melanoma patients. These results could help identify the possible role of mechanical stress in promoting acral melanoma. Further prospective investigation of molecular alterations will be needed to understand the relationship between mechanical stress and genetic/epigenetic alterations.

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CONFLICTS OF INTEREST

The authors have nothing to disclose.

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DATA SHARING STATEMENT

Research data are not shared.

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