Amelanotic acral melanomas: Clinicopathological, *BRAF* mutation, and *KIT* aberration analyses

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Background: Amelanotic acral melanoma (AAM) is very rare and difficult to diagnose both clinically and pathologically. Complete-type AAM shows no black to brown pigmentation in the lesion, whereas incomplete-type AAM shows focal or subtle pigmentation. AAM has been the subject of few investigations.

Objectives: We analyzed the clinicopathological features, *BRAF* mutations, and *KIT* aberrations in 35 Korean AAM cases.

Methods: We included 28 cases of complete-type and 7 cases of incomplete-type AAM.

Results: In all, 26 AAMs (45.7%) were located on the feet of patients, 21 of which (82.9%) showed ulceration. Sixteen cases developed in subungual areas. Nodular melanoma was the most common histopathological subtype (63.6%). The most frequent cell types affected were epithelioid and spindled. HMB-45 staining was strongly positive in 66.7% of AAMs; 4 (12.1%) were negative for HMB-45, and 3 of these were complete-type AAMs. Of 33 total patients, *BRAF* mutations were detected in 2 AAM cases, and *KIT* aberrations were present in 11 cases (33.3%). Four cases (12.1%), all of which were complete-type AAMs, had *KIT* mutations. *KIT* aberrations were weakly correlated with c-kit staining. Twenty patients were TNM stage I or II, and mean survival was 30.14 ± 4.54 months.

Limitations: The study is limited by the small number of patients.

Conclusion: Physicians should be aware of rare and hard-to-diagnose AAMs. We expect that tyrosine kinase inhibitors would be effective for *KIT*-mutated patients with complete-type AAMs. (J Am Acad Dermatol 2013;69:700-7.)

Key words: acral melanoma; amelanotic acral melanoma; amelanotic melanoma; *BRAF* mutation; HMB-45; *KIT* mutation; prognosis.

A melanotic melanoma is a subtype of melanoma with little or no black to brown pigmentation that represents 2% to 8% of all melanomas.[1] Clinical diagnosis can be difficult because clinical diagnostic features routinely associated with melanomas, such as asymmetry, irregular borders, and color variegation, are rarely present.[2] However, true amelanotic melanoma is rare; often some pigmentation is present at the periphery of the lesion.[3] In amelanotic melanomas, a subtle pigmentation around the melanoma can be helpful for the diagnosis of melanoma, and it is more easily visualized by dermoscopy.[2] Therefore, amelanotic melanomas can be divided into 2 subtypes; the pure or complete type, which does not produce any discernible eumelanin, so there is no pigmentation at all, and the hypomelanotic or incomplete type,

Abbreviations used:

AAM:	amelanotic acral melanoma
AJCC:	American Joint Committee on Cancer
PCR:	polymerase chain reaction

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which produces low levels of eumelanin, so that focal or subtle pigmentation is present.[2,4] Acral melanoma, the occurrence of melanoma on acral sites, such as palms, soles, and subungual areas, is the most common type of melanoma in Asians and typically has a poor prognosis.[5] Amelanotic acral melanoma (AAM) is rare and a diagnostic challenge to physicians because it often mimics benign diseases such as calluses, warts, tinea pedis, nonhealing ulcers, ingrown toenails, and nail lichen planus.[6-8]

In this study, we reviewed 35 cases of AAM in Koreans, analyzed the clinicopathological features, and performed immunohistochemical staining, including staining for Melan-A, S100, HMB-45, and c-kit. In addition, we performed *BRAF* and *KIT* sequencing to detect mutations and *KIT* gene amplification in the AAM lesions.

CAPSULE SUMMARY

- Amelanotic acral melanomas are difficult to diagnose clinically and pathologically.
- Subungually located and ulcerated nodular melanomas were common, and HMB-45 staining was sometimes negative. *BRAF* mutations and *KIT* aberrations were detected mostly in complete-type amelanotic acral melanomas.
- Amelanotic acral melanomas should be considered in clinical and upcoming target therapeutic settings.

PATIENTS AND METHODS

Patients and tumor tissue samples

We examined formalin-fixed, paraffin-embedded specimens from 35 Korean patients with AAMs who were treated at Chonnam National University Hospital and Chonnam National University Hwasun Hospital (Gwangju, Korea) between 1994 and 2012. AAMs were defined as melanomas located on the palms, soles, and subungual areas, which clinically showed no or little black pigmentation. Histopathologically, AAMs could manifest as acral lentiginous melanoma, nodular melanoma, superficial spreading melanoma, or other forms. Clinicopathological data, including age, sex, anatomic site of the lesion(s), Breslow thickness, ulceration, TNM staging according to the recent American Joint Committee on Cancer (AJCC) system,[9] and survival, were evaluated. The study protocol was approved by the institutional review board of Chonnam National University Hwasun Hospital.

Immunohistochemical staining for Melan-A, S100, HMB-45, and c-kit

Immunohistochemical staining was performed using LSAB2 System-HRP (DakoCytomation, Carpinteria, CA). Samples from 33 patients with AAMs were formalin fixed and paraffin embedded. Sections of 4-μm thickness were deparaffinized in xylene and rehydrated through graded ethanol. For c-kit and Melan-A antibody staining, antigen retrieval was performed. To block endogenous peroxidase activity, the sections were fixed for 5 minutes in methanol containing 3% hydrogen peroxide. The sections were incubated for 60 minutes at 4°C with monoclonal antibodies against Melan-A (diluted 1:100; Dako, Glostrup, Denmark), S100 (diluted 1:1000; Dako, Glostrup, Denmark), HMB-45 (diluted 1:200; Dako, Glostrup, Denmark), or c-kit (CD117; diluted 1:300; Dako, Glostrup, Denmark), followed by incubation for 30 minutes with a secondary antibody conjugated to biotinylated peroxidase. Immunostaining was visualized by incubation with chromogen solution for 2 minutes, and sections were counterstained with hematoxylin. Negative control staining was performed by omitting the primary antibody. The intensity of the immunoreactivity for c-kit was estimated using a scale of negative, mild, moderate, and strong. The staining was graded independently by 2 dermatopathologists.

Mutation sequencing of *BRAF* and *KIT*

Tumor-rich areas were extracted from 5 to 10 paraffin sections (10-μm thickness) containing representative portions of each tumor block. Genomic DNA was extracted from the formalin-fixed, paraffin-embedded sections using a QIAamp DNA FFPE tissue kit (Qiagen, Hilden, Germany). Polymerase chain reaction (PCR) and DNA sequencing were

Table I. Demographic data of patients (n = 35)

Mean age, y	66.8
Sex, no. (%)	
Male	24 (68.6)
Female	11 (31.4)
Amelanotic subtype, no. (%)	
Complete	28 (80.0)
Incomplete	7 (20.0)
Site of lesion, no. (%)	
Foot	26 (74.3)
Plantar	18 (69.2)
Subungual	8 (30.8)
Hand	9 (25.7)
Palmar	1 (11.1)
Subungual	8 (88.9)