Specific Dermoscopy Patterns and Amplifications of the Cyclin D1 Gene to Define Histopathologically Unrecognizable Early Lesions of Acral Melanoma In Situ

Maki Yamaura, MD; Minoru Takata, MD, PhD; Atsushi Miyazaki, MD; Toshiaki Saida, MD, PhD

Objective: To define early lesions of acral melanoma in situ that cannot be recognized histopathologically.

Design: A retrospective review of the clinical, dermoscopic, and histopathological findings.

Setting: University department of dermatology.

Patients: Thirty-three patients with melanocytic lesions on acral volar skin that were clinically suspected of being early melanomas.

Main Outcome Measures: Fluorescent in situ hybridization studies to detect the cyclin D1 gene amplification in proliferating melanocytes, which is a characteristic genetic aberration recently found in acral melanoma.

Results: Seventeen of 33 lesions were histopathologically diagnosed as either melanoma in situ (8 lesions) or benign melanocytic nevi (9 lesions). Amplification of the cyclin D1 gene was observed in 2 (25%) of the 8 melanomas in situ. None of the 9 nevi showed the amplification. The remaining 16 lesions were, however, difficult to classify histopathologically because most of them showed only a slight increase of nonatypical melanocytes in the basal cell layer of the epidermis. On dermoscopic examination, 9 of these 16 lesions exhibited the parallel ridge pattern that has been reported to be highly specific to melanoma in situ, and 4 (44%) of them had amplifications of the cyclin D1 gene. Amplifications were not found in any of the remaining 7 lesions that showed dermoscopic patterns suggestive of melanocytic nevi.

Conclusions: Cyclin D1 gene amplification detected by fluorescent in situ hybridization identified a very early progression phase of acral melanoma that precedes histopathologically defined melanoma in situ. The present study also indicates the specificity of the parallel ridge pattern on dermoscopy to detect melanomas on acral volar skin at such a very early developmental phase.

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To improve the prognosis of patients with malignant melanoma, the early detection and correct diagnosis of lesions at the in situ phase are mandatory. In nonwhite populations, such as the Japanese, the diagnosis of pigmented skin lesions on acral sites, especially on the palms and soles, is important because nearly half of all cutaneous melanomas affect these sites. In the past 2 decades, our group has characterized the clinical and histopathological features of malignant melanomas affecting the soles of the feet and has proposed several clinical and histopathological guidelines for the early detection of plantar melanomas. More recently, we and others introduced dermoscopic evaluation in the diagnosis of the pigmented lesions on acral volar skin and found that the recognition of several dermoscopic patterns is immensely helpful in differentiating melanomas from benign melanocytic nevi. These guidelines and criteria are quite effective in detecting early melanomas affecting acral volar skin. In the course of these investigations, however, we confronted diagnostic problems with several pigmented lesions on the palms and soles. These lesions showed clinical features suggestive of melanoma in situ, such as late onset, a large size (maximum diameter exceeding 7 mm), and dermoscopic findings of the parallel ridge pattern (PRP). However, histopathological examinations of these cases revealed only a slight increase of melanocytes, with no (or minimal) cytological atypia in the basal cell layer of the epidermis. Similar cases were reported previously in Japanese populations and were diagnosed as atypical melanosis of the foot. Although these lesions may represent a very early stage of melanoma in situ, currently there is no evidence for this.

Recently, Bastian et al performed comparative genomic hybridization analy-
A total of 33 pigmented lesions on acral volar skin, excised under the suspicion of being early melanomas according to the clinical guideline proposed by Saida, were first classified into 3 groups based on the histopathological features (Figure 1 and Table). Eight lesions (group 1) showed the typical histopathological features of melanoma in situ (Figure 2), and 9 lesions (group 3) showed the features of benign melanocytic nevi (5 junctional, 2 dermal, and 2 compound). A total of 30 pigmented lesions on the acral volar skin of patients at the Department of Dermatology, Shinshu University Hospital, Matsumoto, Japan. The guideline recommends surgical excision of acral melanocytic lesions for histopathological evaluation when a lesion has 1 of the following clinical or dermoscopic features: (1) a maximum diameter exceeding 7 mm, (2) a highly irregular shape and/or color, or (3) a PRP revealed on dermoscopic examination. We obtained written informed consent from each patient. We included in the study 3 referral cases in which lesions suspected to be early melanomas had been excised at other hospitals. We excluded from the study cases of invasive acral melanomas, which are easily diagnosed clinically, as well as cases of congenital melanocytic nevi. Dermoscopic examinations were performed as described previously. The excised specimens were fixed in 10% buffered formalin and embedded in paraffin. All the pathology slides were evaluated using the standard criteria, and the difficult cases were reviewed by an expert dermatopathologist (T.S.).

**METHODS**

**PATIENTS AND LESIONS**

From 1997 to 2004, following Saida’s guideline that encouraged the detection of early melanoma on the soles, we excised
tional nevi, 2 compound nevi, and 2 dermal nevi). In these 17 cases, the dermoscopic findings were mostly concordant with the histopathological diagnoses. All 8 cases designated as melanoma in situ showed either the PRP or irregular diffuse pigmentation, both of which are highly specific to melanoma in situ on acral volar skin.9,10 Nine nevi showed benign dermoscopic patterns such as a parallel furrow pattern, a fibrillar or filamentous pattern, or a lattice-like pattern.5,7

However, the remaining 16 lesions (group 2) were difficult to classify histopathologically; 13 showed only a slight increase of nonatypical melanocytes in the basal cell layer of the epidermis (Table, cases 9–

<table>
<thead>
<tr>
<th>Case No./Sex/Age</th>
<th>Site</th>
<th>Histopathological Diagnosis</th>
<th>Dermoscopic Pattern</th>
<th>CCND1/CEP Ratio Melanocytes</th>
<th>CCND1/CEP11 Ratio &gt;2.5*</th>
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<tbody>
<tr>
<td>1/M/69</td>
<td>Finger</td>
<td>25.0 x 21.0</td>
<td>Melanoma in situ</td>
<td>PRP</td>
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<td>PRP</td>
<td>2.0†</td>
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<td>3/F/73</td>
<td>Toe</td>
<td>6.7 x 6.2</td>
<td>Melanoma in situ</td>
<td>PRP</td>
<td>1.01</td>
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<td>4/F/52</td>
<td>Toe</td>
<td>8.5 x 7.1</td>
<td>Melanoma in situ</td>
<td>DP</td>
<td>1.11</td>
</tr>
<tr>
<td>5/F/80</td>
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<td>15.1 x 11.0</td>
<td>Melanoma in situ</td>
<td>PRP</td>
<td>1.00</td>
</tr>
<tr>
<td>6/F/73</td>
<td>Finger</td>
<td>12.7 x 9.8</td>
<td>Melanoma in situ</td>
<td>PRP</td>
<td>1.17</td>
</tr>
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<td>Melanoma in situ</td>
<td>NE</td>
<td>1.34</td>
</tr>
<tr>
<td>8/M/54</td>
<td>Sole</td>
<td>35.0 x 35.0</td>
<td>Melanoma in situ</td>
<td>NE</td>
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Group 2B

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<tr>
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<th>Site</th>
<th>Histopathological Diagnosis</th>
<th>Dermoscopic Pattern</th>
<th>CCND1/CEP Ratio Melanocytes</th>
<th>CCND1/CEP11 Ratio &gt;2.5*</th>
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<tr>
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<td>15.4 x 10.2</td>
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<td>PRP</td>
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<td>10/F/60</td>
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<td>6.0 x 5.9</td>
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<td>1.92†</td>
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<tr>
<td>11/F/51</td>
<td>Sole</td>
<td>22.0 x 11.0</td>
<td>Undetermined</td>
<td>PRP</td>
<td>1.88†</td>
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<tr>
<td>12/F/73</td>
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<td>22.0 x 12.0</td>
<td>Undetermined</td>
<td>PRP</td>
<td>1.72†</td>
</tr>
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<td>13/M/66</td>
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<td>1.27</td>
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<td>14/F/57</td>
<td>Palm</td>
<td>10.0 x 6.0</td>
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<td>PRP</td>
<td>1.03</td>
</tr>
<tr>
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<td>8.0 x 6.2</td>
<td>Undetermined</td>
<td>PRP</td>
<td>1.03</td>
</tr>
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</table>

Group 3

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<th>Site</th>
<th>Histopathological Diagnosis</th>
<th>Dermoscopic Pattern</th>
<th>CCND1/CEP Ratio Melanocytes</th>
<th>CCND1/CEP11 Ratio &gt;2.5*</th>
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<tbody>
<tr>
<td>25/F/14</td>
<td>Sole</td>
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<td>Compound nevus</td>
<td>FFP</td>
<td>1.15</td>
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<td>Sole</td>
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<td>Compound nevus</td>
<td>LLP</td>
<td>1.08</td>
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<tr>
<td>27/F/55</td>
<td>Sole</td>
<td>9.0 x 7.0</td>
<td>Intradermal nevus</td>
<td>NC</td>
<td>1.07</td>
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<tr>
<td>28/F/14</td>
<td>Sole</td>
<td>11.0 x 6.0</td>
<td>Intradermal nevus</td>
<td>LLP</td>
<td>1.06</td>
</tr>
<tr>
<td>29/F/29</td>
<td>Sole</td>
<td>8.5 x 5.0</td>
<td>Junctional nevus</td>
<td>FFP</td>
<td>1.20</td>
</tr>
<tr>
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<td>Sole</td>
<td>7.6 x 11.2</td>
<td>Junctional nevus</td>
<td>FFP</td>
<td>1.05</td>
</tr>
<tr>
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<td>8.0 x 5.0</td>
<td>Junctional nevus</td>
<td>CDP</td>
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<td>FFP</td>
<td>0.98</td>
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<tr>
<td>33/F/31</td>
<td>Sole</td>
<td>8.0 x 4.0</td>
<td>Junctional nevus</td>
<td>FFP</td>
<td>1.11</td>
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</tbody>
</table>

Abbreviations: CDP, crista-dotted pattern; CEP11, Central Enumeration Probe for chromosome 11 (Vysis Inc, Downers Grove, IL); DP, irregular diffuse pigmentation; FFP, fibrillar or filamentous pattern; FISH, fluorescence in situ hybridization; LLP, lattice-like pattern; NC, not classified; NE, not examined; PFP, parallel furrow pattern; PRP, parallel ridge pattern.

† Bold-faced numbers indicate CCND1 amplification. See the “FISH to Detect the CCND1 Amplification” subsection of the “Methods” section for criteria of amplification.
findings; 9 lesions (group 2A) showed PRP, and the remaining 7 lesions (group 2B) showed patterns suggestive of melanocytic nevi such as a parallel furrow pattern, a fibrillar or filamentous pattern, or a lattice-like pattern (Table and Figure 1).

Amplification of \textit{CCND1} was detected with FISH in 2 (25%) of 8 lesions in group 1 (Figure 2) and 4 (44%) of 9 lesions in group 2A (Figure 3). No lesions in groups 2B or 3 showed \textit{CCND1} amplification. In 2 cases in group 1 (cases 1 and 2) with amplifications, about half of the melanocytes had more than 2.5 times more \textit{CCND1} probe signals than reference signals (Table and Figure 2). In case 9 in group 2A, nearly all melanocytes showed amplifications. In cases 10, 11, and 12 in group 2A, about 25% to 37% of melanocytes showed a \textit{CCND1} copy number increase. The findings in case 10 were most intriguing because the maximum diameter of a light brown macule on the sole of a 60-year-old woman was only 6 mm. However, the dermoscopic examination revealed the typical PRP. Although the histopathological findings showed only a slight increase of nonatypical melanocytes in the basal layer of the epidermis, amplification of \textit{CCND1} was observed with FISH in the epidermal melanocytes (Figure 3).

In this study, 33 melanocytic lesions on acral volar skin clinically or dermoscopically suspected of being early melanoma were histopathologically reviewed. Although half of the lesions were unambiguously classified as either melanoma in situ or benign melanocytic proliferations such as melanocytic nevus and lentigo simplex (groups 1 and 3), the remaining lesions (group 2) posed problems in histopathological diagnosis. Despite their atypical clinical features, such as a large size and a variegated color and/or irregular shape, most of these lesions showed minimal histopathological changes that did not provide evidence of malignancy. These lesions were essentially the same as those reported previously by Nogita et al.\textsuperscript{9} They distinguished these lesions from acral melanoma in situ by the lack of histopathological evidence of malignancy and designated them as “atypical melanosis of the foot” with undetermined biological behavior. However, we identified amplification of \textit{CCND1}, a genetic hallmark of acral melanoma,\textsuperscript{11} in proliferating melanocytes in a subset of such lesions, which strongly suggests that such atypical pigmented lesions on acral volar skin represent a very early
Figure 3. Case 10, a 60-year-old woman. A, Light brown macule on the sole of her foot. Maximal diameter of this lesion is only 6 mm. B, Dermoscopic image showing the typical parallel ridge pattern (original magnification ×20). C, Histopathologic image showing a very slight increase of healthy-looking melanocytes along the basal layer (hematoxylin-eosin, original magnification ×200). D, Fluorescence in situ hybridization image showing the CCND1 amplification in a melanocyte (white arrow) within the basal cell layer of the epidermis. Clustered and multiple red CCND1 signals are seen (inset).
associated with overexpression of the CCND1 protein. Although CCND1 is a well-known growth promotor, it may also function as a survival factor for tumor cells.13 It is thus speculated that neoplastic melanocytes harboring an increased gene dosage of CCND1 may preferentially survive and proliferate in the microenvironment provided by epidermal keratinocytes in the rete ridges of the crista profunda intermedia.

The frequency of CCND1 amplification in group 2A was 4 (44%) of 9 lesions, which was equal to the reported frequency of 44% in acral melanomas.11,12 It was reported11,12 that all acral melanomas appeared to have at least 1 gene amplification and that, in addition to 11q13, which contains the CCND1, chromosome regions including 4q12, 5p12, and 12q14 were also found to be amplified. These regions contain known oncogenes, such as platelet-derived growth factor receptor-α, human telomerase reverse transcriptase, and cyclin-dependent kinase 4. Therefore, it would be interesting to determine whether the amplifications of these oncogenes (other than CCND1) exist in group 2A cases that lacked CCND1 amplification. This would further verify the specificity and usefulness of the PRP in the diagnosis of early melanoma on acral volar skin. Because the histopathological findings did not provide convincing evidence of malignancy in the lesions classified as group 2A cases, dermoscopic observations might be superior to histopathological methods in detecting early lesions of melanoma.

Very recently, Soyer et al.23 pointed out the limitations of histopathological methods in the diagnosis of melanoma and emphasized the importance of a combined approach of histopathological and dermoscopic evaluation. The present investigation also shows that histopathological methods may not be the gold standard in the classification of melanocytic lesions on the acral volar skin, particularly in the diagnosis of an early evolving phase of acral melanoma, and emphasizes the importance of dermoscopic evaluation in the diagnosis of pigmented skin lesions. Furthermore, this study also highlights the usefulness and importance of molecular techniques in the field of dermatopathology, as we and others have already shown in other settings.24-26

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