Importance

The improved knowledge of clinical, morphologic, and epidemiologic heterogeneity of melanoma in the context of multiple primary and familial melanomas may improve prevention, diagnosis, and prognosis of melanoma.

Objective

To characterize reflectance confocal microscopy (RCM) morphologic patterns of melanomas in multiple primary and familial melanomas.

Design, Setting, and Participants

In this cross-sectional, retrospective study, patients in a hospital-based referral center were recruited from March 1, 2010, through August 31, 2013; data analysis was conducted from September 1, 2013, through May 31, 2014. Consecutive primary melanomas, documented by dermoscopic and confocal examination, from multiple primary and familial melanomas with known CDKN2A mutational status were studied.

Main Outcomes and Measures

Epidemiologic, genetic, dermoscopic, and histologic data were evaluated according to an RCM morphologic classification: dendritic cell, round cell, dermal nest, combined, and nonclassifiable types.

Results

Fifty-seven melanomas from 50 patients (28 women [56%] and 49 white patients [98%]) were included: 23 dendritic cell (40%), 21 round cell (37%), 2 dermal nests (4%), 2 combined (4%), and 9 nonclassifiable (16%). The median (SD) age of the participants was 53.0 (16.9) years (interquartile range, 41.8-71.2 years), and the median (SD) age at the first melanoma was 46.0 (17.1) years (interquartile range, 35.8-61.5 years). Dendritic cell melanoma was characterized by older age at diagnosis, phenotypes 2 and 3, more intense solar exposure, and moderate to severe solar lentigines; it was the most prevalent confocal type in facial lesions and was associated with the lentigo maligna histologic subtype. Round cell melanomas were identified more often in the familial context and in individuals with phototype 1 skin types; RCM features, such as junctional thickening, dense dermal nests, and nucleated cells within papillary dermis, were more frequently found in this subtype. Dermal nest and combined melanoma were associated with the absence of pigmented network on dermoscopy and thicker tumors on histologic analysis. Nonclassifiable type was associated, by RCM, with the absence of pagetoid cells on confocal examination and lower frequency of marked atypia on melanocytes in the basal cell layer; it presented with lower ABCD Total Dermoscopy Scores and RCM scores compared with the other types. CDKN2A mutation carriers may develop any RCM type of melanoma.

Conclusions and Relevance

Different routes to develop melanoma can be identified according to RCM morphologic classification, with dendritic cell melanomas being associated with chronic sun damage and round cell melanoma with early age at onset and phototype 1 in the context of multiple primary and familial melanomas. The morphologic expression of melanomas via dermoscopy and confocal examination varies according to differences in tumor stage and biological behavior.

Published online August 31, 2016.

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Currently, morphologic analysis-based melanoma classification is insufficient to provide enough information about causative factors or to select patients for specific treatments. In fact, refining classification of melanomas into morphologic subtypes that correlate with phenotypical characteristics or prognostic factors may enhance knowledge about the disease and create a model for future investigations with molecular analysis and/or therapy stratification.

In vivo reflectance confocal microscopy (RCM) is a non-invasive imaging technique that allows for the en face visualization of microscopic structures and cellular detail in the epidermis and superficial dermis at histopathologic resolution. Furthermore, integrating dermoscopy and confocal techniques is useful for distinguishing early-stage melanoma that lacks frankly malignant features, especially in high-risk patients during follow-up, adding to morphologic information about the biology of the lesions. The use of in vivo RCM added a new dimension in melanoma knowledge because the entire surface of the melanoma can be analyzed at histologic resolution. Not only the tumor but also patient characteristics may influence the morphologic findings. In fact, CDKN2A germline mutations and genetic variants in MC1R may influence dermoscopic features. Among Spanish carriers of CDKN2A mutations, the presence of 2 MC1R red hair color variants was associated with melanomas with a less suspicious clinical and dermoscopic appearance.

Recently, 4 distinct melanoma phenotypes on RCM were described as follows: dendritic cell, round cell, dermal nest, and combined melanomas. The authors found that melanomas with a predominant population of dendritic cells were thinner by Breslow index, and melanomas typified by roundish melanocytes or dermal nests were smaller but thicker by Breslow index. The purpose of this study was to correlate morphologic RCM patterns with clinical data, genetic variants, dermoscopic features, and histologic criteria in the context of patients with multiple primary and familial melanomas.

Methods

Patient and Melanoma Selection

A cross-sectional, retrospective, hospital-based study was performed that included patients treated and followed up at the Melanoma Unit of the Hospital Clinic of Barcelona, Spain, from March 1, 2010, through August 31, 2013; data analysis was conducted from September 1, 2013, through May 31, 2014.

The inclusion criteria were patients undergoing periodic follow-up at the Melanoma Unit with a history of at least 2 melanomas (multiple primary melanoma) or hereditary familial melanoma (≥2 patients affected by melanoma in first- or second-degree relatives or at least 1 primary melanoma and at least 1 first- or second-degree relative with pancreatic cancer). Primary melanomas proven by histopathologic examination (any histologic subtype, Breslow Index ≤3 mm) and documented by photographic dermoscopic and confocal examination and known CDKN2A status (wild type or mutated) were included. Tumors thicker than 3 mm were excluded to avoid outliers that could modify the interpretation of results.

Because this study is focused on morphologic classification based on confocal microscopy of primary melanomas, we wanted to avoid the more advanced tumors because losing primary morphologic features and acquiring more heterogeneity could obscure the results of our study. Patients with genetic conditions, such as Li-Fraumeni syndrome, xeroderma pigmentosum, and albinism, and patients undergoing systemic treatment for advanced melanoma were excluded. Clinical data were collected for each patient, and tumor characteristics were also registered.

The study was approved by the ethical committee of Hospital Clinic of Barcelona. Written informed consent was obtained from each patient, and data were deidentified.

Dermoscopic Analysis

Dermoscopic images were captured using digital cameras (Olympus Camedia [Olympus America Inc], Canon G7 [Canon Inc], and/or Nikon Coolpix 4500 [Nikon Corp]) equipped with a polarized dermatoscope (DermLitePhoto; 3 GEN LLC) and with a epiluminescence microscope system (Mole Max II; Derma Medical Systems). Dermoscopic evaluation was based on the pattern analysis and ABCD Total Dermoscopy Score (TDS) as follows: (A) asymmetry, (B) borders, (C) colors, and (D) different structural components (structureless areas, pigment network, branched streaks, dots, and globules). A TDS less than 4.75 is indicative of a benign melanocytic lesion, a TDS of 4.75 through 5.45 is suggestive but not diagnostic of melanoma, and a TDS greater than 5.45 should be considered melanoma.

Confocal Analysis

Confocal examinations were performed by Vivascope 1500 (Lucid Inc). Distinct melanoma types based on RCM were classified as recently proposed by Pellacani et al: (1) dendritic cell melanomas, with a predominantly dendritic cell population and rings and/or thin meshwork pattern; (2) round cell melanomas, with predominantly large, roundish cells with a tendency to aggregate into nests forming a meshwork pattern; (3) dermal nest melanomas, with predominantly large aggregates of cells in the papillary dermis; (4) combined melanomas, with a combination of the 3 previous confocal patterns; and (5) nonclassifiable melanoma, without any of the previous patterns.
On the basis of the presence of major or minor criteria of malignancy, the lesion was classified according to the Pellacani and Barcelona confocal microscopy total score.\textsuperscript{14,15} According to the Pellacani score, the cutoff for malignancy was a score equal to or greater than 5. According to the Barcelona algorithm, the cutoff for malignancy was a score equal to or greater than 1.

**Genetic Study**

The study only included patients for whom \textit{CDKN2A} and \textit{MCIR} sequencing results were available. DNA samples were obtained from peripheral blood lymphocytes of all patients by standard methods, and molecular analysis was conducted at the Molecular Genetics Department of the Hospital Clinic, Barcelona, Spain. Specific mutations in \textit{CDKN2A} and polymorphisms in \textit{MCIR} were studied as previously described.\textsuperscript{16-20} \textit{MCIR} polymorphisms were classified as red hair variants or non-red hair variants. Variants that produce no change in amino acid sequence (synonymous changes) were considered as wild type for statistical analysis.

**Statistical Analysis**

Continuous variables were analyzed and reported as mean (SD) and median (interquartile range). The analysis of the association between categorical variables and outcomes used the Pearson $\chi^2$ test. The Fisher exact test was applied when the expected frequency in the 2 x 2 table is below 5 and the t test was used in the comparison of quantitative variables. A $\kappa$ value for the evaluation of the interobserver reproducibility was calculated based on the independent evaluation of 2 masked readers (T.C.G., I.A.) who analyzed dermoscopy and confocal images of 10 preselected cases. The differences are considered significant at $P < .05$. The calculations were made using Haploview (Broad Institute of MIT and Harvard) and SPSS statistical software, version 16.0 (SPSS Inc).

**Results**

Fifty-seven melanomas from 50 patients (28 women [56%] and 49 white patients [98%]) were included in the study. The median (SD) age of the participants was 53.0 (16.9) years (interquartile range, 41.8-71.2 years), and the median (SD) age at the first melanoma was 46.0 (17.1) years (interquartile range, 35.8-61.5 years). The most prevalent histologic subtype was superficial spreading melanoma (43 of 53 [81%]), followed by lentigo maligna melanoma (6 of 53 [11%]), nodular melanoma (3 of 53 [6%]), and nevoid melanoma (1 of 53 [2%]). According to the American Joint Committee on Cancer classification, 31 lesions (54%) were in situ melanomas, 23 (40%) were stage I, and 3 (5%) were stage II. Forty patients (80%) had multiple primary melanoma, and the 10 individuals (20%) with a single melanoma were members of families with hereditary cancer syndrome. Ten patients (20%) were \textit{CDKN2A} mutation carriers.

The mean (SD) Breslow thickness was 0.79 (0.75) mm, and the median thickness was 0.52 mm. The mean (SD) general TDS was 6.2 (0.7), the mean (SD) Barcelona general RCM score was 1.0 (1.0), and the mean (SD) Pellacani general RCM score was 5.0 (1.7). Melanomas arising in patients with multiple primary melanoma had a significantly lower mean (SD) TDS than single melanomas developed in a familial context (6.0 [0.6] vs 7.0 [0.4], $P < .001$).

The classification of melanomas according to morphologic subtypes via confocal analysis resulted in 23 dendritic cell types (40%), 21 round cell types (37%), 2 dermal nest types (4%), 2 combined types (4%), and 9 nonclassifiable types (16%) (Table 1 and Figure 1). The description of the data of the 3 less...
Table 2. Association Between Reflectance Confocal Microscopy Dendritic Cell and Round Cell Melanoma Types and Clinical and Epidemiologic Findings

<table>
<thead>
<tr>
<th>Variable</th>
<th>Dendritic Cell</th>
<th>Round Cell</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female</td>
<td>12/23 (52.2)</td>
<td>15/21 (71.4)</td>
<td>.19</td>
</tr>
<tr>
<td>Age, mean (SD), y</td>
<td>60.4 (17.5)</td>
<td>49.9 (15.6)</td>
<td>.04a</td>
</tr>
<tr>
<td>Age at first CM, mean (SD), y</td>
<td>53.6 (17.5)</td>
<td>43.8 (14.8)</td>
<td>.053</td>
</tr>
<tr>
<td>Multiple CMs</td>
<td>19/23 (82.6)</td>
<td>16/21 (76.2)</td>
<td>.72</td>
</tr>
<tr>
<td>Familial CMs</td>
<td>8/23 (34.8)</td>
<td>13/21 (61.9)</td>
<td>.07</td>
</tr>
<tr>
<td>Phototype</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>0/23 (0)</td>
<td>5/21 (23.8)</td>
<td>.02a</td>
</tr>
<tr>
<td>2</td>
<td>13/23 (56.5)</td>
<td>9/21 (42.9)</td>
<td>.36</td>
</tr>
<tr>
<td>3</td>
<td>10/23 (43.5)</td>
<td>6/21 (28.6)</td>
<td>.30</td>
</tr>
<tr>
<td>4</td>
<td>0/23 (0)</td>
<td>0/21 (0)</td>
<td>NA</td>
</tr>
<tr>
<td>5</td>
<td>0/23 (0)</td>
<td>1/21 (4.8)</td>
<td>.48</td>
</tr>
<tr>
<td>Nevus count &gt;100</td>
<td>8/22 (36.4)</td>
<td>8/16 (50.0)</td>
<td>.40</td>
</tr>
<tr>
<td>Solar exposure, y</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;10</td>
<td>9/23 (39.1)</td>
<td>10/21 (47.6)</td>
<td>.57</td>
</tr>
<tr>
<td>10-18</td>
<td>11/23 (47.8)</td>
<td>7/21 (33.3)</td>
<td>.33</td>
</tr>
<tr>
<td>&gt;18</td>
<td>10/23 (43.5)</td>
<td>2/20 (10.0)</td>
<td>.02a</td>
</tr>
<tr>
<td>Solar lentigo, &lt;10</td>
<td>7/18 (38.9)</td>
<td>1/15 (6.7)</td>
<td>.046b</td>
</tr>
</tbody>
</table>

Abbreviations: CM, cutaneous melanoma; NA, not applicable.

* Data are presented as number/total number (percentage) of patients unless otherwise indicated.

a P < .05 (Pearson χ², Fisher exact, and t tests).

Additional notes:
- The interobserver κ index was moderate to very good for single confocal features included in RCM melanoma scores, ranging from 0.458 to 1.000 (eTable 1 in the Supplement).
- The descriptive epidemiologic and clinical database between the 2 most prevalent types of melanoma on RCM were dendritic type and round cell types, as seen in Table 2.
- The dendritic cell melanomas were located mainly on the trunk (10 of 23 [44%]) and extremities (7 of 23 [31%]). Although not the most frequent location, the dendritic cell type was the most prevalent confocal type in facial lesions (5 of 6 lesions [83%]). Most of the dendritic cell melanomas were between 5 and 10 mm. The most common histologic subtype found in dendritic cell tumors was superficial spreading melanoma (18 of 23 [78%]). However, the confocal pattern of dendritic cell melanoma was significantly associated with lentigo maligna histologic subtype compared with other RCM types (5 of 6 [83%] vs 1 of 6 [17%], P = .03). The mean (SD) tumor thickness in the dendritic cell type was 0.5 (0.1) mm. More than half (13 of 23 [57%]) of dendritic cell lesions were in situ melanomas. Regarding dermoscopic features, dendritic cell melanomas had an atypical pigmented network and atypical dots and globules (22 of 23 [96%]) (Figure 2). A multicomponent pattern was seen in 5 of 23 lesions (22%). The mean (SD) TDS of the dendritic cell type was 6.3 (0.8), which was not statistically different from the general score (mean [SD], 6.1 [0.6]; P = .58). The Barcelona confocal score achieved a mean (SD) value of 1.0 (1.0), and the mean (SD) Pellacani score was 4.9 (1.7).
- With confocal microscopy, dendritic cell melanomas frequently exhibited pagetoid cells larger than 20 μm (23 of 23 [100%] vs 21 of 34 [62%], P = .001) and pleomorphic cellular populations (20 of 23 [87%] vs 16 of 34 [47%], P = .002). Dendritic cell melanomas also presented a smaller nucleus of less than 10 μm (19 of 23 [83%] vs 11 of 34 [32%], P < .001) and a
Round cell melanomas were identified more often in a familial context (13 of 21 [61.9%]) than dendritic cell melanomas (8 of 23 [34.8%], \( P = .07 \)). Phototype 1 was more prevalent than in the dendritic cell type (5 of 21 [24%] vs 0 of 23, \( P = .02 \)), and 8 of 16 patients (50%) in this group had more than 100 nevi (Table 2). The most common location of round cell melanomas was on the trunk (13 of 21 [62%]). Most round cell melanomas (13 of 21 [62%]) were between 5 and 10 mm in diameter and were of the superficial spreading histologic subtype (15 of 17 [88%]) (Table 1). Most round cell melanomas (13 of 21 [62%]) were in situ, and the mean (SD) tumor thickness was 0.6 (0.5) mm (Figure 3). Only 3 round cell melanomas (14%) occurred in a CDKN2A mutation carrier. The frequency of any MCIR variant allele in this melanoma type was 80%. The most prevalent dermoscopic findings in round cell melanomas were atypical pigment network (17 of 21 [81%]) and atypical dots and globules (21 of 21 [100%]) (Figure 2). The global dermoscopic pattern was classified as multicomponent in 5 of 21 lesions (24%). The TDS presented a mean (SD) value of 6.4 (0.6). The mean (SD) RCM Barcelona score of 1.4 (0.7) was statistically higher than other groups (mean [SD], 0.7 [1.1]; \( P = .006 \)). The mean (SD) round cell Pellacani score was 5.4 (1.1). Single confocal features, such as junctional thickening, dense dermal nests, and nucleated cells within papillary dermis, were more frequently found in round cell melanomas (17 of 21 [81%] vs 16 of 36 [44%], \( P = .007 \); 8 of 21 [38%] vs 4 of 36 [11%], \( P = .02 \); and 14 of 21 [67%] vs 13 of 36 [36%], \( P = .03 \), respectively) (eTable 2 in the Supplement).

No significant association was found between the presence of CDKN2A mutations and a specific confocal melanoma type being any RCM melanoma type present in CDKN2A carriers. The presence of at least 1 MCIR variant was associated with widespread pagetoid cells within the epidermis compared with wild-type status (12 of 42 [29%] vs 0 of 14, \( P = .03 \)).
Discussion

In the present study performed on multiple primary and familial melanomas, we stratified melanomas into the 5 confocal types previously described; it was possible to associate specific phenotypic features with some types of melanomas. Dendritic cell melanomas appeared in older patients with a history of a more intense solar exposure and moderate to severe solar lentigines as previously described by Bassoli et al.4 Round cell melanomas had a predisposition for fair-skinned individuals and tended to occur with a family history of melanoma. However, in this study, the association with a high nevus count also and tended to occur with a family history of melanoma.

Cell melanomas had a predisposition for fair-skinned individuals.21

Unspecific pattern are probably very early melanomas in which lesions had a multicomponent pattern, probably reflecting lesion evolution with 1 of the 3 previous patterns. Finally, lesions with unsppecific pattern are probably very early melanomas in which the pattern is as yet undefined.21

Mean Barcelona score was higher in round cell type, probably because of the higher frequency of atypical nucleated dermal cells. These findings support the behavior of round cell melanomas to aggregate into clusters at the dermal-epidermal junction and papillary dermis and also to spread as isolated cells into the dermis. The dendritic cell melanomas, adjusting for tumors of similar stage and thickness, may take a longer time for single cells to aggregate into nests and to disseminate into the dermis, possibly representing slow-growing melanomas as previously proposed.11

Considering TDS, RCM score, and tumor thickness, it is possible that the combined and nonclassifiable melanomas represent opposite ends of a morphologic spectrum. In fact, milder morphologic expression in dermoscopy and confocal examination of nonclassifiable type is characteristic of early-stage tumors, which are frequently difficult to diagnose by in vivo techniques.

CDKN2A mutation carriers may develop any RCM type of melanoma, even those less represented as dermal nests. It is possible that the low sample size was not able to demonstrate any differences in the distribution between different types, if they actually exist.

Conclusions

Specific phenotypic features of patients with multiple primary and familial melanomas were associated with some types of melanomas based on confocal microscopy classification. Morphologic expression of melanomas under dermoscopy and confocal examination may be associated with differences in tumor stage and biological behavior. Future studies are necessary to enhance our knowledge of the interaction of multiple coexisting causal factors that drive melanoma development.
History of Podophyllin

Ismaël Maatouk, MD

Podophyllotoxin is the principal active compound from the resin mixture known as podophyllin. It is obtained from one of the 3 species of Podophyllum: P. peltatum (from North America), P. hexandrum (from Taiwan), or P. pleianthum (from Taiwan). Podophyllum peltatum is an indigenous, North American, herbaceous perennial flowering in May and bearing fruit in late summer or fall. Other common names include May apple, mandrake, Indian apple, wild lemon, and duck’s foot.

One of the first recorded medicinal uses of this agent, mentioned in the pre-Conquest English medical book the Leech Book of Bald (AD 900-950), was in a salve for cancer. Catesby, in his Natural History of the Carolinians (1731), described the May apple and noted that the root is “said to be an excellent emetic and is used as such in the Carolinas.” Jacques Cartier reported usage of this agent both as a mortal poison and as a topical antitode for snake venom. The root was considered both a medicine and a poison by the North American Indians, and it was used as a suicide agent among the Iroquois. John Uri Lloyd stated that Podophyllum was used by the Cherokees for deafness and as an anthelmintic, and by the Wyandottes and Southern Indians as a cathartic.

The early colonists learned of the medicinal properties of the root from the Indians, and it was used as a cathartic in the first United States Pharmacopoeia (1820). It was listed until the 12th revision (1942), from which it was dropped. Stories of the new American drug spread to England and the continent of Europe. The resin, podophyllin, was first separated from Podophyllum by John King in 1835. With the preparation of podophyllin on a commercial scale in 1850 by Merrell, the use of the resin supplanted that of the crude Podophyllum.

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With the increased production of podophyllin, reports of its toxic properties appeared in the literature: pain in the eyes; hyperemia of the iris, cornea, mucous membranes, and eyelids; and erythematous eruptions of the scrotum. Moreover, oral and parenteral administration of podophyllin has been followed by serious results. Topical podophyllin was introduced in 1942 and is still accepted today as an effective treatment for condyloma acuminate.

Author Affiliation: Department of Dermatology, Keserwan Medical Center affiliated with AUBMC, Beirut, Lebanon.

Corresponding Author: Ismaël Maatouk, MD, Department of Dermatology, Keserwan Medical Center affiliated with AUBMC, Beirut, Lebanon (ismaelmaatouk@gmail.com).

1. Lloyd JU. Origin and History of All the Pharmacopæial Vegetable Drugs. Christchurch, New Zealand: Caxton Press, 1929.

NOTABLE NOTES

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