Morphologic Features of Melanophages Under In Vivo Reflectance Confocal Microscopy

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Objectives: To determine morphologic features of melanophages under in vivo reflectance confocal microscopy (RCM) and to highlight morphologic features that are important in distinguishing melanophages from melanocytes.

Design: Consecutive retrospective study.

Setting: Referral center for pigmented lesions.

Patients: The study group retrospectively constituted 20 consecutive patients having biopsy-proven lichen planus–like keratoses that dermoscopically and histopathologically showed many melanophages and that had been imaged under RCM before biopsy.

Main Outcome Measures: The RCM characteristics of isolated dermal bright cells were scored blinded to dermoscopic features and histopathologic diagnosis.

Results: Under RCM, melanophages were significantly smaller than melanocytes (mean [SD] cell diameter, 13.6 [1.6] vs 18.2 [2.9] µm, P = .006). Nuclei (intracellular low-reflectance round-oval structures) were visible in only 16% (29 of 184) of the cells in melanophages vs 57% (28 of 49) of the cells in melanocytes (P < .001). When identified, nuclei were smaller in melanophages than in melanocytes (mean [SD] diameter, 3.2 [1.2] vs 6.4 [0.7] µm, P < .001). Compared with melanocytes, melanophages were significantly more ill defined (76% [140 of 184] vs 18% [9 of 49], P < .001), less round (23% [42 of 184] vs 69% [34 of 49], P < .001), and less dendritic (1% [2 of 184] vs 12% [6 of 49]) (P < .001).

Conclusion: Observed differences in morphologic features should enable distinction between melanophages and melanocytes under RCM, thereby improving the accuracy of skin lesion diagnosis using this technique.

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In vivo reflectance confocal microscopy (RCM) allows visualization of the upper layers of skin at cellular resolution. Melanin and melanosomes appear bright under reflectance at near-infrared wavelengths that are used in RCM. Recent studies have shown that identification and assessment of specific features of melanomas and nevi can improve the accuracy of melanocytic lesion diagnosis using RCM. These studies have examined only melanocytic lesions or selected images of cases. Therefore, their results cannot necessarily be directly extrapolated to normal clinical scenarios, in which pigmented lesions assessed under RCM include melanocytic and nonmelanocytic lesions. To our knowledge, none of the recent studies have determined if RCM can differentiate whether melanin-containing bright cells under RCM are melanocytes or pigment-laden macrophages (melanophages) on the basis of their morphologic features given by their reflectance signal.

When visualized histopathologically by light microscopy, melanophages measure 20 to 70 µm in diameter and contain a large vesicular nucleus (often with a single nucleolus) and phagocytized melanin in fine cytoplasmic granules. Under RCM, they appear as bright plump, oval, or star-shaped cells with no visible nucleus, and they may have ill-defined edges and may aggregate when particularly numerous in the upper dermis.

Under RCM, melanocytes in the superficial layers of the skin have a dark nucleus and bright cytoplasm and are frequently twice the size of keratinocytes. Atypia of melanocytes at the dermoepidermal junction under RCM is characteristic of melanoma. Marked melanocyte atypia under RCM is characterized by large (>50 µm) cell diameter, unusual cell shape (eg, triangular or star shaped), or the presence of large and eccentric nuclei. Papillary dermal melanocytes in melanoma appear as isolated round-oval refractive cells with a dark nucleus under RCM. One feature reported to be a robust criterion using RCM
for differentiating melanoma cells from nevus cells in the dermis is the presence of nucleated cells that are not aggregated in nests. However, distinguishing such cells from melanophages is important, and criteria for doing so have not been described to date. Furthermore, no systematic study describing the RCM features of melanophages has been previously published, to our knowledge. The objective of this study was to determine morphologic features of melanophages under in vivo RCM and to highlight morphologic features that are important in distinguishing melanophages from melanocytes.

**METHODS**

**RECRUITMENT**

The RCM images and histopathologic sections were obtained retrospectively from patients seen in a secondary care setting (Sydney Melanoma Diagnostic Centre, Camperdown, New South Wales, Australia) from September 27, 2005, to June 13, 2007. The study was approved by the Sydney South West Area Health Service Ethics Committee (Royal Prince Alfred Hospital zone) (protocol X05-0216), and informed and signed consent was obtained from all patients.

The objective of our study was to describe morphologic features of melanophages under RCM by examining defined cutaneous histopathologic entities that had been imaged using RCM before biopsy. For that purpose, patients were recruited retrospectively who had RCM-imaged lichen planus–like keratoses (LPLKs) with histologically confirmed dermal melanophages without increased melanocytes compared with normal skin. Control groups were identified by retrospectively recruiting patients having biopsy-proven LPLKs that dermoscopically and histopathologically showed many melanophages and that had been imaged under RCM before biopsy. The lesions were recognized under dermoscopy because they contained multiple blue-gray dots (granularity) in combination with areas of seborrheic keratoses or solar lentigo. Such lesions are often biopsied because they can mimic lentigo maligna. The patients underwent RCM of lesions, and then a biopsy specimen was obtained to confirm the diagnosis, as well as the presence of numerous melanophages and the absence of any underlying or associated melanocytic proliferation on routine histopathologic examination.

**STUDY GROUP**

The study group retrospectively constituted 20 consecutive patients having biopsy-proven LPLKs that dermoscopically and histopathologically showed many melanophages and that had been imaged under RCM before biopsy. The lesions were recognized under dermoscopy because they contained multiple blue-gray dots (granularity) in combination with areas of seborrheic keratoses or solar lentigo. Such lesions are often biopsied because they can mimic lentigo maligna. The patients underwent RCM of lesions, and then a biopsy specimen was obtained to confirm the diagnosis, as well as the presence of numerous melanophages and the absence of any underlying or associated melanocytic proliferation on routine histopathologic examination.

**CONTROL SUBJECTS**

**Control Group 1**

Control group 1 retrospectively constituted 25 consecutive patients who had RCM images obtained before biopsy and in whom histopathologic examination showed melanocytic lesions without increased melanophages compared with normal skin. All histopathologic slides were reviewed by a dermatopathologist (R.A.S.) to assure quality of the groups of lesions.

**Control Group 2**

Control group 2 retrospectively constituted 20 consecutive patients who had RCM images obtained before biopsy and in whom histopathologic examination showed nonmelanocytic lesions without increased melanophages compared with normal skin.

**Control Group 3**

To evaluate the RCM features of melanophages having other dermal inflammatory cell infiltrates, the RCM archival database was screened for inflammatory lesions. Fourteen patients with inflammatory disorders assessed using RCM between September 27, 2005, and June 13, 2007, were retrospectively identified, and the histopathologic findings were reviewed. Only 5 patients demonstrated a mixed dermal inflammatory cell (predominantly lymphocytic) infiltrate without increased melanophages compared with normal skin. These 5 patients were recruited as control group 3 to ensure that the nonmelanophage inflammatory infiltrate seen in most LPLKs was not confounding our measurements.

**RCM INSTRUMENT**

Before biopsy, RCM images were acquired using a reflectance confocal laser scanning microscope (Vivascope 1500; Lucid Inc, Henrietta, New York), which uses an 830-nm laser source. Instrument and acquisition procedures have been described previously. Each image corresponds to a horizontal section at a selected depth, with approximately a 500 × 500-µm field of view, a lateral resolution of 1.0 µm, and an axial resolution of 3 to 5 µm. Confocal sections, beginning at the stratum corneum and ending within the papillary dermis (stacks), were systematically recorded in the center of the lesion and in areas of interest for clinical diagnosis. The maximal optical penetration depth of the laser beam was 250 µm. More than 100 images per lesion were recorded (a minimum of 4 stacks in the center and 1 mosaic of at least 4 × 4 mm).

**RCM OBSERVATIONS**

The confocal images were scored retrospectively more than 1 year after acquisition by a single observer (P.G.) who was blinded to dermoscopic features and histopathologic diagnosis. The RCM images were viewed by opening codified folders containing all raw images acquired for the corresponding case without sorting. These folders were randomly mixed and then analyzed one by one in a blinded fashion.

Cells of the superficial dermis were defined as cells just under the honeycombed pattern layer (corresponding to the epidermis) with admixed vessels and reticulate structures (the latter corresponding to collagen fibers). The criteria analyzed were as follows: (1) Dermal bright cells (presence or absence). (2) Density, defined as minimal when fewer than 5 dermal bright cells were seen. If minimal density was found, cell characteristics were not recorded when they did not differ from the contingent of dermal bright cells in normal skin. If at least 5 dermal bright cells were seen, the cell characteristics were recorded for up to 10 cells. Cells were chosen randomly in the middle of the screen and then progressing centripetally until up to 10 cells were characterized on all raw images acquired in the superficial dermis. (3) Organization in nests (sparse, dense, or cerebriform) or a different type of aggregation. (4) Isolated cells (not organized in nests or aggregates). Cell characteristics recorded included maximum cell diameter in microns, cell shape (ill defined, round, or dendritic), maximum nucleus (hyporeflective intracellular round-oval structure) diameter in microns, and nucleus to cell ratio. (5) Visibility of the nucleus, defined as the number of visible nuclei divided by the number of cells recorded in each group. (6) Cell type predominance (melanophages, melanocyte, mixed cell type, or undefined cell type) according to the glossary by Scope et al.
MEASUREMENT OF HEMATOXYLIN-EOSIN–STAINED MELANOPHAGES IN LPLKs

Five melanophages were measured in the region of the histopathologic section with the highest melanophage density. The maximum diameter of melanophages in LPLKs was measured under light microscopy at ×400 magnification using an ocular micrometer.

STATISTICAL ANALYSIS

Statistical analysis was performed using commercially available software (STATA, release 9; StataCorp LP, College Station, Texas). Absolute and relative frequencies of observations in each group were obtained for each RCM feature already described. Descriptive statistics for continuous variables included means, medians, standard deviations, and interquartile ranges. Differences between groups were calculated using t test for comparison of the mean cell and nucleus diameters and nucleus to cell ratio and using χ² test of independence for comparison of the presence of dermal bright cells and the cell shape.

RESULTS

The cohort included 36 male and 34 female patients aged 12 to 82 years (mean age, 53 years). The study group comprised 20 patients with LPLKs. The melanocytic control group 1 comprised 6 patients with melanomas and 19 patients with nevi (15 dysplastic or atypical); among 25 patients, 9 lesions (4 lentigo maligna and 5 benign nevi) were junctional melanocytic, and 16 lesions were dermal or compound. The nonmelanocytic control group 2 comprised 20 patients (6 with nonpigmented basal cell carcinomas, 5 with ephelides, and 9 with other lesions [2 tricholem-
mal cysts, 2 scars, and 1 each of neurofibroma, seborrheic keratosis, dermatofibroma, trichoepithelioma, and granuloma). In the inflammatory infiltrate control group 3, histopathologic analysis showed moderate chronic (predominantly lymphoplasmacytic) inflammatory infiltrates in the superficial dermis; there were associated epidermal changes (spongiosis or hyperkeratosis and parakeratosis) in 2 patients, and there were small numbers of necrotizing granulomas in the dermis in a third patient. The RCM features are given for each group.

MELANOPHAGES WITHOUT MELANOCYTES
(LPLK-STUDY GROUP)

In the study group, dermal bright cells were seen under RCM in all 20 patients with LPLKs. There were at least 5 dermal bright cells seen using RCM in all but 1 patient (Figure 1). They were present in ill-defined sparse aggregates in 10 cases and in ill-defined dense aggregates in 2 cases (Figure 2). No sparse, dense, or cerebriform organization of melanocytic lesion nests was identified. The mean (SD) cell diameter of 184 isolated dermal bright cells was 13.6 (1.6) µm. Seventy-six percent (140 of 184) were ill defined, 23% (42 of 184) were round, and 1% (2 of 184) were dendritic. The mean nucleus to cell ratio was 0.25, with a mean (SD) nucleus diameter of 3.2 (1.2) µm. The nucleus was visible in 16% (29 of 184) of cells.

MELANOCYTIC LESIONS WITHOUT MELANOPHAGES (CONTROL GROUP 1)

Under RCM, dermal bright cells were seen in 48% (12 of 25) of patients in control group 1, with at least 5 dermal bright cells seen in 20% (5 patients). Therefore, dermal
bright cell characteristics were based on findings in 5 patients (3 dysplastic compound nevi, 1 lentigo maligna, and 1 superficial spreading melanoma). Dermal bright cells were organized as nests in 4 of 5 patients (dense in 2 patients, sparse in 1 patient, and cerebriform in 1 patient). The mean (SD) cell diameter of 49 isolated dermal bright cells was 18.2 (2.9) µm. Eighteen percent (9 of 49) were ill defined, 69% (34 of 49) were round, and 12% (6 of 49) were dendritic. The mean nucleus to cell ratio was 0.36, with a mean (SD) nucleus diameter of 6.4 (0.7) µm. The nucleus was visible in 57% (28 of 49) of cells (Figure 3).

**NONMELANOCYTIC LESIONS WITHOUT MELANOPHAGES (CONTROL GROUP 2)**

Scattered dermal bright cells were seen under RCM in 20% (4 of 20) of patients in control group 2. Findings in these patients contained minimal (<5) cells.

**NONMELANOCYTIC LESIONS WITHOUT MELANOPHAGES BUT WITH AN INFLAMMATORY INFILTRATE (CONTROL GROUP 3)**

Dermal bright cells were seen under RCM in 20% (1 of 5) of patients in control group 3. However, these were significantly smaller (maximum cell diameter, 6-10 µm; mean [SD] cell diameter, 6.8 [1.5] µm) than those seen under RCM in nonmelanocytic lesions with numerous melanophages (Figure 4).

**Differences Between Groups**

Differences between groups are summarized in the Table. The mean cell diameter was significantly smaller in melanophages than in melanocytes (P = .006). Compared with melanocytes, melanophages were significantly more ill defined (P < .001), less round (P < .001), and less dendritic (P = .001). The mean nucleus diameter (P < .001), visibility of the nucleus (P < .001), and nucleus to cell ratio (P = .02) were significantly less in melanophages compared with melanocytes.

Numerous melanophages (defined as in the glossary published previously13) were identified under RCM in all patients in the study group except for 1 patient in whom there were only 2 dermal bright cells seen. In contrast, a predominance of melanophages was not diagnosed using RCM in 12 melanocytic lesions in which dermal bright cells were seen.

**Measurement of Hematoxylin-Eosin–Stained Melanophages in the LPLK (Melanophage) Group**

The mean (SD) diameter of 100 hematoxylin-eosin–stained melanophages was 12.9 (3.8) µm. This was consistent with RCM measurements.

**Comment**

To our knowledge, the RCM characteristics of melanophages have not previously been studied systematically. To establish an accurate RCM diagnosis of a skin lesion with dermal bright cells, it is essential to distinguish melanocytes from melanophages. In contrast to melanocytes, melanophages have been described as “non-nucleated” cells under RCM.11 Although results of a previous histopathologic study12 suggested a large size range for melanophages (20-80 µm), the melanophages in our study as measured using RCM were significantly smaller, tending to be round and ill defined but not dendritic and with smaller and less visible nuclei compared with melanocytes.

Under RCM, the LPLK (melanophage) study group had high densities of dermal bright cells that were often aggregated. Because only typical LPLKs were included with many melanophages on dermoscopy and confirmed by histopathologic examination, the high den-

**Table. Comparison of Study Group and Control Groups**

<table>
<thead>
<tr>
<th>Experimental Group</th>
<th>Among Group Members, No. (%)</th>
<th>Among Dermal Bright Cells Seen</th>
<th>Cell Diameter, Mean (SD), µm</th>
<th>Cell Shape, No. (%)</th>
<th>Visible Nucleus, No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dermal Bright Cells Seen</td>
<td>≥5 Dermal Bright Cells Seen</td>
<td></td>
<td>Ill Defined</td>
<td>Round</td>
</tr>
<tr>
<td>Study Group (n=20)</td>
<td>20 (100)</td>
<td>19 (95)</td>
<td>13.6 (1.6)</td>
<td>140 (76)</td>
<td>42 (23)</td>
</tr>
<tr>
<td>Control Group 1 (n=25)</td>
<td>12 (48)</td>
<td>5 (20)</td>
<td>18.2 (2.9)</td>
<td>9 (18)</td>
<td>34 (69)</td>
</tr>
<tr>
<td>Control Group 2 (n=20)</td>
<td>4 (20)</td>
<td>0</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>Control Group 3 (n=5)</td>
<td>1 (20)</td>
<td>1 (20)</td>
<td>6.8 (1.5)</td>
<td>9 (90)</td>
<td>1 (10)</td>
</tr>
</tbody>
</table>

The mean (SD) nucleus diameters were 3.2 (1.2) µm for the study group (n=29) and 6.4 (0.7) µm for control group 1 (n=28). The mean (SD) nucleus to cell ratios were 0.25 (0.07) for the study group (n=29) and 0.36 (0.08) for control group 1 (n=28).
ers17), melanoma cells were usually larger and more poly-
form a detailed characterization of melanocytes in this 
spreading melanoma. Although we did not intend to per-
ictic compound nevi, 1 lentigo maligna, and 1 superficial 
cytic) cells in control group 1 were based on 3 dysplas-
sions14 (Figure 2).

The RCM features of isolated dermal bright (melano-
cytic) cells in control group 1 were based on 3 dysplastic 
compound nevi, 1 lentigo maligna, and 1 superficial 
spreading melanoma. Although we did not intend to per-
form a detailed characterization of melanocytes in this 
study (as they have been described previously by oth-
ers13), melanoma cells were usually larger and more poly-
morphous than nevus cells, and the larger standard 
deviation of dermal cells bright under RCM in control group 
1 reflects this heterogeneity. Of note, dermal bright nucle-
ated cells were seen under RCM in 1 lentigo maligna 
(Figure 5). However, the histopathologic features of this 

Figure 5. Control group 1 lesion. A, In vivo reflectance confocal microscopy of 
dermal bright cells in an advanced lentigo maligna. Dermal bright 
 melanoma cells are typically larger than melanophages, round, and well 
defined, with large diameter and visible nuclei (arrows); some are organized 
in dense nests (short arrows). The dermoepidermal junction is destroyed, so 
the distinction between dermal and epidermal bright cells is unclear. Bar 
indicates 50 µm. B, Histopathologic findings of large atypical melanocytes 
in dense nests (short arrows). The dermoepidermal junction is destroyed, so 
the distinction between dermal and epidermal bright cells is unclear. Bar 
indicates 50 µm. C, Large atypical melanocytes of dermal bright cells in an 
advanced lentigo maligna. Dermal bright 

In conclusion, this study characterizes dermal bright 
cells of melanophages as having a mean cell diameter of 
13.6 µm, with an ill-defined outline. Melanophages had a 
small nucleus (mean diameter, 3.2 µm), which was vis-
ble in only 16% (29 of 184) of cells. Such observations 
should aid in distinguishing between melanophages and 
 melanocytes, both of which appear as dermal bright cells 
under RCM, thereby improving the diagnostic accuracy 
of this technique.

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Menzies.
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sistance with the manuscript.

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