Primary Melanoma of the Skin and Cutaneous Melanomatous Metastases

Comparative Histologic Features and Immunophenotypes

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Abstract

Through careful clinicopathologic correlation, we identified 37 metastatic melanomas in the skin, all of which had intraepidermal components. These were compared with 43 microscopically similar primary melanomas with a predetermined panel of immunostains in general use in surgical pathology, including bcl-2 protein, mutant p53 protein, Ki-67 (MIB-1), proliferating cell nuclear antigen (PCNA), α-isoform actin, and CD117 (c-kit protein). There was no significant difference in bcl-2 or α-isoform actin staining patterns of primary vs secondary cutaneous melanomas. The expression of Ki-67 generally was higher in metastatic melanomas than in primary lesions, and the same was true of mutant p53 protein labeling; however, some overlap was observed. CD117 staining was retained in 65% of metastatic melanomas (24/37) when they originated from ocular primary tumors; nevertheless, that marker was lost in virtually all of the other metastatic melanocytic neoplasms, whereas primary melanomas demonstrated consistent reactivity for c-kit protein. Although they are not definitive, these trends in immunoreactivity could facilitate the process of distinguishing the multiple primary melanoma syndrome from melanomatous metastases to the skin. That undertaking is best approached with circumspection, because clinicopathologic discriminators for this diagnostic separation are still imperfect.

Metastatic melanomas to the skin can imitate the microscopic appearance of primary melanocytic tumors virtually perfectly.1-5 This phenomenon has been acknowledged only relatively recently; series of cases addressing the problem have appeared only during the past 25 years. Before that time, several microscopic features of melanocytic lesions, such as intraepidermal growth, the lack of a “grenz” zone in the dermis, and involvement of dermal appendages, were touted as useful in identifying such tumors as primary.6 In particular, Allen and Spitz7 were responsible, in 1953, for establishing the adage that dermoepidermal junctional melanocytic atypia was diagnostic of an origin in the skin surface. Their conclusion was accepted as valid until 1978, when Kornberg and colleagues8 presented a series of unquestionably metastatic cutaneous melanomas, defined by compelling clinical findings, in which epidermotropic melanocytic growth perfectly replicated the appearance of primary neoplasms. Since then, other reports have solidified their observations.1,6

It is recognized that secondary melanomas in the skin cannot be distinguished reliably from lesions arising there, because they potentially share literally all of the histologic features of primary melanocytic tumors. As summarized by White and Hitchcock,6 these characteristics include an ability for epidermotropic growth beyond the lateral confines of a dermal component, formation of epidermal collarettes around nodules in the corium, intravascular tumor cell aggregates, tumor symmetry, nevoid cytologic features, apparent dermal “maturation,” and an ability for solely intraepidermal growth with the appearance of in situ melanoma.

In recent years, it has been noted that there are, however, potential biochemical differences between such tumors. For
example, metastases of melanoma might lack CD117, which is present more often in primary lesions.9-12 Likewise, secondary melanomatous tumors manifest higher proliferation indices, different p53 and histocompatibility-related proteins, amplified apoptotic inhibitors such as bcl-2, augmented protease production, and dissimilar integrin or adhesion molecule profiles compared with primary melanomas.13 In the light of those facts, the present study was initiated to reassess whether histologic features, selected immunohistologic markers in general use in surgical pathology, or both might permit more confident separation of malignant primary and secondary melanomatous neoplasms of the skin.

Materials and Methods

We retrospectively selected 80 cases of malignant melanoma from the archival tissue files in the Department of Pathology, University of Virginia, Charlottesville. These were chosen based on their known clinicopathologic features and comprised 37 tumors that were metastatic to the skin and 43 histologically similar primary melanomas of the skin and uveal tract, all of which were judged to be in the vertical growth phase morphologically. All specimens had been processed routinely for pathologic assessment. Selection criteria for secondary lesions included epidermal involvement, a lack of obscuring pigment or inflammation among the lesional cells, and detailed clinical information on the appearances of the tumors and their biologic evolution. Whenever they were available, sections of primary and secondary melanomas from the same case were reviewed in evaluations of cutaneous metastases. However, slides and blocks from the primary lesions often were inaccessible because most of the patients with metastatic skin lesions had been referred from other institutions.

Sections stained with H&E were reexamined in each case, and detailed notes were made of the histologic characteristics of the lesions. Paraffin sections were cut at 4 µm, mounted on charged glass slides, and stained with monoclonal antibodies to CD117, mutant p53 protein, Ki-67/MIB-1, bcl-2 protein, α-isoform actin, and proliferating cell nuclear antigen (PCNA) using the avidin-biotin-peroxidase complex technique and an automated immunostaining device (Ventana, Tucson, AZ). Microwave-mediated epitope retrieval in citrate buffer was used uniformly, following an accepted protocol.14 Grading of immunoreactivity was undertaken independently by several of us (P.M.G.-K., E.L.H., and M.R.W.) with respect to the intensity and number of labeled cells in each section. Appropriate positive and negative control sections were included for each determinant studied.

Standard χ² analysis was used to compare each immunohistochemical analyte between primary and metastatic lesional groups. This was accomplished by using an Internet-based calculator (located at http://www.graphpad.com/calculators/chisquared2.cfm).

Results

In cases in which the primary site was known, 31 (84%) of 37 intracutaneous metastases were found clinically to be near the primary melanoma anatomically Image 1 and Image 2. In the remaining cases, they appeared at distant skin sites. The majority (22/37 [59%]) of 37 secondary intracutaneous tumors showed sharp delimitation histologically, and 19 (51%) were symmetrical. Atypical melanocytes were present at the dermoepidermal junction in all cases, and tumor cells filled the papillary dermis in 9 (24%) of 37 cases. Pagetoid epidermal involvement by tumor was apparent in only 1 (3%) of the secondary lesions Image 3; similarly, the width of a dermal component was more limited than epidermal growth in the same proportion of cases. The lesions appeared to “mature” in the dermis, with a progressive decrease in cellular and nuclear volume, in 3 (8%) cases. Nevoid cytologic features similarly were noted, at least partially, in 3 cases (8%). None of the metastatic tumors showed the presence of an epidermal collarette. Of the metastatic tumors, 3 (8%) virtually perfectly recapitulated the appearance of in situ (intraepidermal) melanoma, with no

### Table 1

<table>
<thead>
<tr>
<th>Antibody/Reagent</th>
<th>Commercial Source</th>
<th>Clone</th>
<th>Dilution</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD117</td>
<td>DAKO, Santa Barbara, CA</td>
<td>104D2</td>
<td>1:40</td>
</tr>
<tr>
<td>p53 (mixture)</td>
<td>Oncogene Science, Boston, MA, and DAKO</td>
<td>D01 and D07</td>
<td>1:160 and 1:240</td>
</tr>
<tr>
<td>Ki-67</td>
<td>AMAC, Westbrook, ME</td>
<td>MIB-1</td>
<td>1:200</td>
</tr>
<tr>
<td>PCNA</td>
<td>Novocastra, Newcastle upon Tyne, England</td>
<td>PC10</td>
<td>1:400</td>
</tr>
<tr>
<td>α-isoform actin</td>
<td>DAKO</td>
<td>1A4</td>
<td>1:400</td>
</tr>
<tr>
<td>bcl-2</td>
<td>DAKO</td>
<td>124</td>
<td>1:40</td>
</tr>
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PCNA: proliferating cell nuclear antigen.
dermal growth whatsoever. Only 1 (3%) showed angiolymphatic invasion in the corium.

The 43 primary melanomas demonstrated morphologic features of the vertical growth phase, as described in previous publications. Other findings in such lesions included atypical junctional melanocytic proliferation in 38 cases (88%), haphazard intraepidermal cell growth in 36 cases (84%), atypical cells filling the papillary dermis in 33 cases (77%), apparent vertical maturation of tumor cells in the dermis in 9 cases (21%), sharp lateral delimitation of the tumors in 10 cases (23%), lesional symmetry in 7 cases (16%), an intraepidermal “shoulder” of melanocytic proliferation in 6 cases (14%), formation of an appendageal collarette in 5 cases (12%), and nevoid cytologic characteristics in 5 cases (12%).

The immunolabeling patterns, expressed as the mean number of immunopositive cells per case, for the tumors in this series are shown in Table 2. p53 protein staining was intense in 5% or more of the neoplastic cells in cases that were scored as positive, and it was nuclear in localization in all cases. Ki-67 stains showed moderate to
strong nuclear labeling in the tumor cells Image 6; PCNA preparations exhibited comparable findings. CD117 (c-kit) staining was cytoplasmic with cell-membranous accentuation Image 7, whereas labeling for bcl-2 protein was variably intense and cytoplasmic. Only 1 case of intracutaneous amelanotic metastatic melanoma was immunostained for S-100 protein, HMB-45, and tyrosinase, to confirm its melanocytic nature. All other cases showed diagnostic features of metastatic melanoma both architecturally and cytologically, with discernible melanin production, and, therefore, no confirmatory immunostains were performed in those cases. There were no discernible differences in the qualitative aspects of immunostaining in intraepidermal and dermal tumor components for any of the markers assessed.

A statistical comparison of CD117 immunoreactivity in primary and secondary lesions demonstrated a marked skewing of positivity toward primary lesions using an immunolabeling threshold of 10%, but only when tumors originating in the eye were excluded from evaluation ($P < .0001$) Figure 1. Various numeric thresholds were assessed for comparisons of mutant p53 and Ki-67. When a level of immunoreactivity of 25% or more was used in that context for mutant p53, a statistically significant difference was achieved ($P = .0235$) Figure 2. Similarly, in reference to Ki-67 and a threshold of 15% immunoreactivity, results between primary and metastatic tumors were significant ($P = .003$) Figure 3. Comparisons of bcl-2 protein, $\alpha$-isoform actin, and PCNA did

<table>
<thead>
<tr>
<th>Marker</th>
<th>CD117</th>
<th>p53</th>
<th>Ki-67</th>
<th>PCNA</th>
<th>Actin</th>
<th>bcl-2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary melanomas</td>
<td>21</td>
<td>9</td>
<td>6</td>
<td>50</td>
<td>1</td>
<td>37</td>
</tr>
<tr>
<td>Metastatic melanomas</td>
<td>4</td>
<td>54</td>
<td>21</td>
<td>89</td>
<td>3</td>
<td>32</td>
</tr>
</tbody>
</table>

PCNA, proliferating cell nuclear antigen.
* Data are given as percentages, which refer to the mean labeling indices of each tumor group (number of immunopositive cells per 100 tumor cells) for the listed markers.
Discussion

Although the microscopic dissimilarities between them may be few, the clinical distinction between primary and metastatic melanomas in the skin has considerable importance with regard to management issues. All primary melanomas need to be excised with a suitable margin of uninvolved tissue, whereas metastases in the skin cannot be cured surgically. The present study was designed to examine histologic findings and patterns in the expression of immunohistologic markers that might be used to separate such lesions from one another.

Histologic characteristics of the lesions we studied were closely similar. Indeed, in an individual case setting, they would not be reliable as indicators of the primary or secondary status of any given melanomatous skin tumor. This conclusion reiterates that of White and Hitchcock.6

The most novel differences in observed immunophenotypes were found in reference to CD117/c-kit expression. The c-kit protein (also known as stem cell factor receptor) is a transmembrane receptor tyrosine kinase that is involved in melanocytic development during embryologic development. Normal melanocytes, melanocytic nevi, in situ melanomas, and invasive radial growth phase melanomas typically can be...
labeled for CD117, but its expression has been relatively infrequent in previous studies on metastatic melanomas. The present analysis also supports the premise that secondary deposits of melanoma generally lose CD117. Only 4% of the tumor cells in metastatic intracutaneous lesions from primary melanomas of the skin in this series were positive for c-kit. Interestingly, however, we found that the cutaneous metastases from ocular melanomas expressed CD117 in the majority of cases; 65% (24/37) manifested strong immunostaining for that marker. The biologic significance of this observation, if any, remains to be seen. At this point, it would be unwarranted to suggest that therapeutic anti-c-kit agents such as imatinib mesylate should or could be used for CD117+ melanomas in the absence of data from prospective therapeutic trials.

Immunostains for putatively mutant p53 protein also were used in our assessment to examine their potential value as a discriminator between primary and metastatic melanomas. Previous reports have demonstrated that mutant p53 is significantly more common in the vertical growth phase components of invasive melanomas, ie, in the tumor cells of such lesions that have acquired metastatic potential. In contrast, aberrant p53 protein isoforms are observed infrequently in radial growth phase (nonmetastasizing) melanomas. Our observations support this construct. The mean level of mutant p53--positive tumor cells in primary melanomas was 9% compared with 54% in metastatic intracutaneous lesions.

Ki-67 antigen has been used widely in surgical pathology as an index of cellular proliferation, and, therefore, one could justifiably hypothesize that intracutaneous melanomatous metastases might demonstrate more Ki-67 labeling than primary tumors do. In general terms, that premise has support from the results of previous studies, although Ki-67 in secondary melanoma in the skin has not been studied specifically heretofore. It also was confirmed in this study, because metastatic melanomas showed a mean labeling index of 21% compared with 6% in primary melanomas.

Another proliferation marker is represented by PCNA. Although data on that protein in melanomas have not been as consistent in showing increased expression during tumor progression, the majority of published information on that point supports such a relationship. We, likewise, found that the mean number of PCNA-positive tumor cells in metastatic melanomas was 89% compared with 50% in primary tumors, but there was much more overlap in labeling for that protein than for Ki-67 among the 2 study groups. The information reported herein on both Ki-67 and PCNA seems promising on its face; nevertheless, labeling indices for those 2 proteins are affected greatly by a number of variables, including tissue preservation, processing methods, and immunohistologic technique. In the light of that reality, it is difficult to suggest that either marker would individually prove definitive for making a diagnosis of metastatic melanoma.

Similar comments apply to our results for bcl-2 protein and α-isoform actin, which demonstrated comparable labeling in both primary and metastatic intracutaneous melanomas. Hence, even though bcl-2 protein is conceptualized as a promoter of cellular “immortality” in melanocytic proliferations, it does not seem to differ at an immunohistologic level in the cells of melanomas at different points of their biologic evolution. In regard to actin, Bishop et al reported that metastatic melanomas often acquired immunoreactivity for that filament protein; however, we were unable to corroborate their observation.

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**Figure 3**: A, Ki-67 index in metastatic intracutaneous melanoma. B, Ki-67 index in primary intracutaneous melanoma.
This analysis was focused on immunohistologic markers that are familiar to virtually all anatomic pathologists. Several other proteins could have been included that have been associated with acquisition of metastatic potential by melanomas, including various metalloproteinases and integrins, cellular adhesion molecules, nm23, dipeptidyl-aminopeptidase IV, and class II histocompatibility molecules. However, those cell products have not been proven to separate primary from secondary melanomas consistently in individual cases, either singly or in combination with one another, and they generally are not available to most hospital practitioners. A practical approach to the problem at hand is only partially provided by the results of this study. First, it can be concluded, as other authors have done, that conventional morphologic features are not reliable in the distinction of primary cutaneous melanomas from melanomatous metastases in the skin. Second, immunohistochemical studies with commonly used markers might provide biasing information toward one interpretation or the other in reference to CD117, mutant p53, and Ki-67. Nevertheless, there is an overlap in the immunoreactivity for each of those 3 markers among primary and metastatic lesions; thus, they are truly helpful in this context only when all of them favor one of the two diagnoses.

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References


