Pathology of Melanoma
David E. Elder

Abstract
The purposes of pathologic examination of a lesion suspected of being a malignant melanoma are to provide an accurate diagnosis of melanoma (or not), and to provide prognostic information useful in the clinical management of the patient. In the near future, pathologic attributes will also likely be used to predict responses to therapy, as a guide to the selection of specific therapeutic agents such as "small molecule" inhibitors of signaling pathways.

Accurate Diagnosis of Melanoma
Like other cancers, most malignant melanomas evolve through stages of tumor progression. Clinically, many melanomas begin as a pigmented patch of skin which evolves to become a palpable plaque, and enlarges as if it were along the radii of an imperfect circle. This stage or "phase" of progression has been termed the "radial growth phase" (RGP) based on this clinical analogy (1, 2). Many melanomas, at the time of diagnosis, have progressed to the next phase of progression or "vertical growth phase" (VGP), in which a tumor papule appears, often within the confines of a premalignant RGP or sometimes de novo in clinically normal skin. This papule enlarges to become a nodule and may become ulcerated. Histologically, as well as clinically, melanomas can be categorized as "nontumorigenic" or "tumorigenic." Metastasis is very rare in nontumorigenic melanomas, whereas melanomas with a tumorigenic compartment may have competence for metastasis (3–5).

Whereas most melanomas are diagnosed rapidly, reproducibly, and accurately by routine pathology, there are troublesome subsets of cases in which an accurate, agreed on diagnosis may be impossible to achieve (6, 7). We believe that in such cases, it is best to express uncertainty directly, rather than sweeping all doubt "under the rug." Patients deserve to understand their differential diagnosis, and therapy should be designed with that differential diagnosis in mind. As such, we may use a descriptive diagnosis such as "superficial (or intraepithelial) atypical melanocytic proliferation of uncertain significance," with a statement as to the differential diagnosis. Treatment of these lesions should be based on excision with, at a minimum, a pathologically confirmed clear margin around the scar of the procedure and any residual lesion. Tumorigenic lesions that may simulate melanomas include Spitz nevi, cellular nodules in congenital nevi, deep penetrating nevi, cellular blue nevi, pigmented epithelioid melanocytomas, and others (8). In such cases, we may use a descriptive diagnosis such as "melanocytic tumor of uncertain potential," again with a statement as to the differential diagnosis that includes information as to the microstaging factors that might be applicable if the lesion is a melanoma. Treatment may include a consideration of sentinel lymph node (SLN) sampling and, possibly, adjuvant therapy.

Tumorigenic and Mitogenic (VGP) Melanoma
The original observations that melanomas commonly progress from a nontumorigenic "RGP" to a tumorigenic "VGP" are supported by many observations. First, the excellent survival of patients whose melanomas lacked VGP has been documented (3, 9). The metastasis rate in patients with melanomas that are confined to the RGP is of the order of 1% to 2%, and in our experience, and that of others, virtually all such cases have had regression within the RGP lesion (10). It is likely that this regression may have incorporated a small focus of melanoma with competence for metastasis, which metastasized prior to the occurrence of the regression in the primary tumor.

Biological evidence for progression from RGP to VGP includes differences in cell culture behavior between the two compartments. The success rate of establishing permanent cell lines from RGP lesions is only 10% of that for biologically late primary or metastatic melanomas, and in consequence, only a few cell lines are available. The cells are immortal but show reduced or no proliferation in soft agar and immunodeficient mice when compared with the VGP (11). Additional evidence has come from molecular studies; for example, by comparative genomic hybridization, there are shared chromosomal abnormalities between the RGP and the VGP compartments (11–13). In addition, it has been shown by microdissection and sequencing studies that the same mutated oncogenes are
shared by the VGP, RGP, and associated nevus compartments of a primary melanoma (14).

The working definition of the VGP that was established in early studies includes the properties of “tumorigenicity” and “mitogenicity.” Thus, early VGP was defined as: first, the presence of a cluster of cells in the dermis that is larger than the largest cluster in the epidermis (tumorigenicity, Fig. 1), and/or second, the presence of any dermal mitoses (mitogenicity). Either of these two properties suggests that the melanoma has now acquired the capacity for growth not only in the epidermis but also in the dermis (4, 9). Prognosis has long been known to correlate with melanoma thickness as measured microscopically, which forms the basis of the American Joint Committee on Cancer (AJCC) staging system that is currently in use (15).

In a recent study of expression of the proliferation marker Ki-67 in 402 AJCC stage I “thin” invasive melanomas, it was shown that proliferation of melanoma cells in RGP melanomas slows as they enter the dermis, and then increases with the onset of tumorigenic VGP (16). The mean dermal Ki-67 expression in 171 invasive RGP tumors was 3.5%, whereas in 199 tumorigenic VGP melanomas, the expression was 13%. Similar findings were shown in terms of mitoses. These were absent in all of the invasive RGP melanomas by definition, whereas they were present in 54% of the tumorigenic VGP melanomas. Thus, in the evolution of primary melanomas, there is a shift in the pattern of proliferation from a predominantly epidermal pattern in the RGP to an epidermal and dermal pattern of proliferation in the VGP (16).

In another study of prognosis in thin melanomas, which are considered to have a good prognosis, a tree-structured analysis of 10-year metastasis defined four risk groups: high risk, men with VGP lesions that had a mitotic rate (MR) >0, and for whom the 10-year metastasis rate was 4% (2-7%; n = 411). Survival curves differed significantly among the four groups (P < 0.001; ref. 17).

In addition to its role as a predictor of metastasis-free survival, it has also been recently shown that the property of mitogenicity is useful as a predictor of the likelihood of SLN involvement in patients with AJCC stage I melanomas. Among 181 patients with thin melanomas who underwent lymphatic mapping and sentinel lymphadenectomy, the overall SLN positivity rate was 5%. All patients with a positive SLN had an MR of >0, i.e., were “mitogenic.” On the basis of a classification tree, patients with an MR >0 and tumor thickness ≥0.76 mm were identified as a higher-risk group, with an SLN positivity rate of 12.3% (18). Thus, in patients with thin melanomas, mitogenicity was a significant predictor of SLN positivity that may be used to risk-stratify and select patients, supporting findings from a similar study by Sondak et al. (19).

These findings show that two major properties of melanoma, i.e., tumorigenicity and proliferation rate, as judged by mitotic rate or Ki-67 expression, are critical determinants of prognosis in thin melanomas. In particular, the property of mitotic rate (determined as the number of mitoses per square millimeter usually from a “hotspot” if any is present on the sides of the lesion) has been reported in multiple studies from many institutions (16, 17, 19–32) and is a simple attribute that can be readily and reproducibly determined by any pathologist using standard equipment (25). This attribute, therefore, should be recorded in pathology reports, and in particular, the simple property of mitogenicity, i.e., the presence of any mitoses in the dermis, should, in the opinion of this author, be taken into consideration as a modifying attribute for AJCC stage 1 and used in making decisions regarding indications for lymphatic mapping and sentinel lymphadenectomy.

**Microscopic Morphology and Clinicopathologic Subtypes of the RGP**

The microscopic features of the RGP or “nontumorigenic compartment” of the melanoma are confined to the epidermis and the papillary dermis. Any lesional cells that are present in the dermis are, by definition, “nonmitogenic” and “nontumorigenic.” There are often two, but not necessarily mutually exclusive, major patterns of proliferation of melanoma cells in the epidermis—a pattern of extensive, high-level “pagetoid” proliferation of uniformly atypical melanocytes, extending throughout the layers of the epidermis seen in the common melanomas of the superficial spreading type (Fig. 1), and a “lentiginous” pattern of continuous basal proliferation of uniformly atypical melanocytes, seen in lentigo maligna and acral-lentiginous melanomas. These patterns correlate broadly with site, with chronic versus intermittent sun exposure, and with molecular findings that likely reflect differences in pathogenesis of the lesions (1, 13, 33–36).

**Molecular studies in melanoma.** High throughput molecular studies in melanoma have been done by comparative genomic hybridization (CGH), fluorescent in situ hybridization, and by RNA expression profiling. Comparative genomic hybridization is a technique in which DNA copy numbers from tumors are compared with standard controls. Amplifications and deletions of individual genes can then be confirmed by fluorescent in situ hybridization (CGH), which is a technique in which DNA copy numbers from tumors are compared with standard controls. Amplifications and deletions of individual genes can then be confirmed by fluorescent in situ hybridization (CGH). In addition to its role as a predictor of metastasis-free survival, it has also been recently shown that the property of mitogenicity is useful as a predictor of the likelihood of SLN involvement in patients with AJCC stage I melanomas. Among 181 patients with thin melanomas who underwent lymphatic mapping and sentinel lymphadenectomy, the overall SLN positivity rate was 5%. All patients with a positive SLN had an MR of >0, i.e., were “mitogenic.” On the basis of a classification tree, patients with an MR >0 and tumor thickness ≥0.76 mm were identified as a higher-risk group, with an SLN positivity rate of 12.3% (18). Thus, in patients with thin melanomas, mitogenicity was a significant predictor of SLN positivity that may be used to risk-stratify and select patients, supporting findings from a similar study by Sondak et al. (19).

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hybridization done on sections of the tumors. The comparative genomic hybridization technique was first used in melanoma by Trent’s group at the University of Michigan, in a study that showed clonal derivation of the VGP from the RGP components of three tumors (12). In more recent studies using array-based comparative genomic hybridization, the use of this technique has been shown for helping to make the diagnostically important distinction between atypical Spitz nevi and “spitzean” melanomas (37–39). These studies have also shown important genomic alterations common to the various subsets of melanomas, including cyclin D amplification, which is most frequent in acral melanomas (40, 41), and BRAF amplification, which seems to be most important in melanomas on intermittently sun-exposed skin (superficial spreading melanomas; refs. 36, 42, 43).

Various techniques have been used for profiling the expression of genes at the mRNA level. At present, the most common of these is the use of high-density oligonucleotide arrays. In an early microarray study, a cluster of aggressive expression of genes at the mRNA level. At present, the most significant genomic alteration common to the various subsets of melanomas, including cyclin D amplification, which is most frequent in acral melanomas (40, 41), and BRAF amplification, which seems to be most important in melanomas on intermittently sun-exposed skin (superficial spreading melanomas; refs. 36, 42, 43).

Unsupervised hierarchical clustering accurately separated nevi from primary melanomas, and metastases were compared (46). In another recent study, the gene expression profiles of a series of nevi, primary melanomas, and metastases were compared (46). Unsupervised hierarchical clustering accurately separated nevi from primary melanomas. The metastatic melanomas exhibited two dichotomous patterns of gene expression that unexpectedly reflected gene expression differences that were apparent after comparing the radial and vertical phases of a large primary melanoma. Further analysis of the genes involved in these differences will lead to an improved molecular understanding of the pathogenesis of melanoma and to new approaches to diagnosis. However, at the present time, histopathology remains the gold standard for diagnosis and prognosis.

Open Discussion

Dr. Slingluff: The father of one of the 20-year-olds I treated called me with a concern about his insurance rates. One pathologist had called the lesion a primary melanoma and one had called it a dysplastic nevus. We performed a sentinel node biopsy. Some melanocytic cells were apparent in the sentinel node, but there was disagreement about whether they represented a melanoma metastasis or a cell rest. The father asked for a note saying that this was not melanoma. How do you suggest dealing with that when there’s a record that says melanoma at some institutions and not at others?

Dr. Elder: Obviously, if the patient has a diagnosis of melanoma, the insurance company has a right to rate him based on that. You’re probably a little less likely to have an insurance problem with a diagnosis of melanocyte tumor of uncertain malignant potential with a note that says, “Cannot rule out melanoma, treat for the worst case scenario.”

Dr. Kirkwood: The biggest risk factor for this disease is age. Increasingly, very young patients arise with spitzoid lesions that defy diagnosis. How should age be factored into the threshold for diagnosis of melanoma, especially in the spitzoid lesions that plague us increasingly? Why would cellularly identical lesions in a 2-year-old and a 30-year-old only be called melanoma in the 30-year-old?

Dr. Elder: If it’s strictly identical, it should probably be called melanoma in both. But in lesions where there is really a diagnostic dilemma, age certainly factors in. There’s a nice paper by Vollmer that gives a table of the probability of a diagnosis based on age, with a certain prior probability of diagnosis. If it’s a 50/50 call, the table will take you down a curve. If it’s a 2-year-old, then it’s 90% benign; if it’s a 40-year-old, it’s 90% malignant, just based on the prior probabilities of knowing the patient’s age (47).

Dr. Kirkwood: We have a 2-year-old coming in next week for a sentinel node biopsy. We now think that the morbidity caused by sentinel node biopsy is so low that the chance to illuminate the lesion makes the process worth it.

Dr. Sondak: We are in the process of reviewing our results with over 60 children with melanoma treated over the past decade or so. Our youngest child with a sporadic melanoma is 4 ½ years old. At 2 years, I would be suspicious of that diagnosis if the patient didn’t have a congenital nevus. There’s so much we don’t know. Our biggest enemy is the fear of expressing uncertainty—if pathologists aren’t sure what the lesion is, they should tell us and let us help the patient and their family make an informed decision.

Dr. Atkins: What is the role of gender? It’s surprising that it came out so powerful in your database, when it didn’t come out in the AJCC database as being that important. Is it different for different types of melanoma? Is there some component of either testosterone or estrogen that’s important here?

Dr. Elder: Gender has been an important prognostic attribute in our model since the first complete model we did back in 1989. It’s not surprising that it is apparent again in the same, although much greatly expanded, basic data set. The attributes that we look at are vastly different from the attributes that were looked at in the AJCC model.

Dr. Ross: In the AJCC model, the incidence of ulceration in thin melanomas was 6% or 8%, whereas in the University of Pennsylvania database, it is only 1.6%. It’s probably a different group of patients. Gender may be relatively more important than other factors, but it may get trumped by other important factors such as tumor thickness or sentinel node positivity.

Dr. Elder: But gender still plays a role, and we don’t know why. It’s certainly not just based on the fact that women have their melanomas on the leg, which is a prognostically more favorable site.

Dr. Sondak: You implied that we understand some of these survival factors that promote tumors progressing from radial to vertical growth phase. What are some examples of those survival factors?

Dr. Elder: There was a study performed in Meenhard Helmy’s lab by Mei Yu Hsu with radial growth phase–like cell lines. They were nontumorigenic in mice and were derived from the radial growth phase of complex primaries. So they’re different from the usual cell lines, which would be tumorigenic in mice. In the skin reconstruct model, these cell lines mixed up nicely with the keratinocytes and they line up along the basal layer. They look like in situ melanomas. They don’t invade the
References


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