Focus on melanoma

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Introduction, epidemiology, and risk factors

Over the past 50 years, the incidence of melanoma in most developed countries has risen faster than any other cancer type. Once a rare cancer, incidence rose dramatically between the 1930s and 1970 (~10%/year, doubling every ten years), slowing to its present rate of ~5%/year over the last 30 years (Figure 1) (Berwick and Halpern, 1997; Oliveria et al., 2001). Mortality rates have also risen substantially, although at a slower rate. In the U.S., 1 in 82 women and 1 in 58 men will develop melanoma. Unlike the majority of cancers, melanoma incidence is not strongly dependent on age, and melanoma is one of the most common causes of cancer and cancer deaths between the ages of 20–35. Thus, by afflicting young and middle-aged adults, the socioeconomic and psychological impact is great.

Melanoma provides one of the best examples of how genetics and environment interact in the pathogenesis of cancer. Incidence is strongly affected by race and geographic location (Balch et al., 2002). Melanoma is predominantly a disease of populations with lighter pigmentation (i.e., Caucasians) and incidence is 5- to 20-fold lower in populations with darker skin color (e.g., Africans, East Asians, Hispanics) (see Figure 1). Within Caucasian populations, incidence is influenced by proximity to the equator, suggesting a link to solar exposure. For instance, melanoma incidence in the southern U.S. is two to three times higher than the northern U.S. Studies of migrants further emphasize the role of environment, based on retained high-risk when migrants moved from high incidence to lower incidence areas.

Melanocytic nevi (moles), benign clusters of melanocytes, have drawn special attention as potential precursor lesions. However, Caucasians have an average of 15–35 benign common nevi per person, and the risk of an individual benign common nevus becoming melanoma is extremely small. In addition, only ~10%–20% of primary melanomas are associated with nevi, suggesting that the large majority of primary melanomas do not arise from nevi. On the other hand, so-called “atypical nevi” are a marker for increased risk for melanoma. For instance, persons with >100 flagrant atypical nevi and a family history of melanoma have a very high probability (lifetime risks approaching 100%) of developing melanoma. However, none of the clinical diagnostic criteria for atypical nevi are precise or highly reproducible. Atypical nevi are prevalent in Caucasian populations (as high as 18% of individuals). There is a desperate need for more reliable, molecularly defined markers for these “atypical” lesions. An undisputed precursor lesion is lentigo maligna, a benign pigmented lesion occurring on heavily sun-exposed skin usually in elderly individuals. It is estimated that 5%–30% of lentigo maligna lesions progress to invasive lentigo maligna melanoma, linking chronic sun exposure to this special type of melanoma.

Genetic factors play a dominant role in risk for melanoma (Polsky et al., 2001b). In addition to skin pigmentation, other heritable risk factors include family history of melanoma, density and type of nevi, and propensity to sunburn but not tan. With regard to environmental factors, several lines of epidemiologic evidence point to an increased relative risk related to sun exposure: (1) higher incidence at lower latitudes for Caucasian populations, and (2) occurrence of melanoma most frequently on skin sites that are exposed to the sun during recreation (e.g., not protected by bathing suits). A confounding observation is that persons who work outdoors, with the heaviest sun exposure, have a lower incidence of melanoma than white-collar workers and professionals who work indoors. One proposed explanation is the “intermittent sun exposure” hypothesis, suggesting that the risk comes from exposure of untanned skin to relatively short bursts of high intensity sun (e.g., sunburns during winter vacation in Florida) (Houghton and Viola, 1981). The action spectra of solar radiation implicated in melanoma are not clear—there are arguments for both the mutagenic UV-B radiation (sunburn and tanning wavelengths) and the proinflammatory, melanocyte activation effects of UV-A (tanning wavelengths) (Kripke, 1991; Wang et al., 2001). In addition, UV radiation induces immune suppression in the skin, theoretically interfering with immunological mechanisms of cancer surveillance (Kripke, 1991). Perhaps the clearest evidence for a relationship between UV exposure and melanoma comes from a genetic experiment of nature, a rare set of heritable diseases called xeroderma pigmentosum that are characterized by molecular defects in nucleotide excision repair of UV-B-induced DNA damage. Patients with xeroderma pigmentosum developed large numbers of nonmelanomas skin cancers by early adulthood (Lynch et al., 1967), and a subset are at extraordinarily high risk (>1000-fold) for melanoma. However, the relationship of sun exposure to melanoma is complex and mechanisms are uncertain.

Natural history and precursor lesions

Melanoma arises from normal pigment cells, the neural crest-derived melanocytes, located on the basement membrane of epithelial surfaces (Figure 2). A major function of melanocytes is the synthesis, storage, and transfer of melanin pigments to surrounding epithelial cells. Melanin in melanocytes is synthesized in special endocytic vesicles called melanosomes (Vijayasaradhi et al., 1995). Primary melanoma cells generally retain these differentiation functions to make and store pigment in melanosomes. The presence of pigment, melanosome-specific proteins by immune staining, and melanosomes by electron microscopy can be used diagnostically to distinguish melanoma from other types of cancer. Most melanomas arise on the skin (>90%), particularly sun-exposed sites on the limbs, trunk, and face. Notably, melanomas in persons with dark skin arise at a much higher frequency in non-sun exposed sites, including mucous membranes, nailbeds, palms, and soles of feet. Approximately 3%–5% of melanomas arise from melanocytes in the uveal tract of the eye (not to be confused with retinal pigmented epithelial cells), and rarely from noncuta-

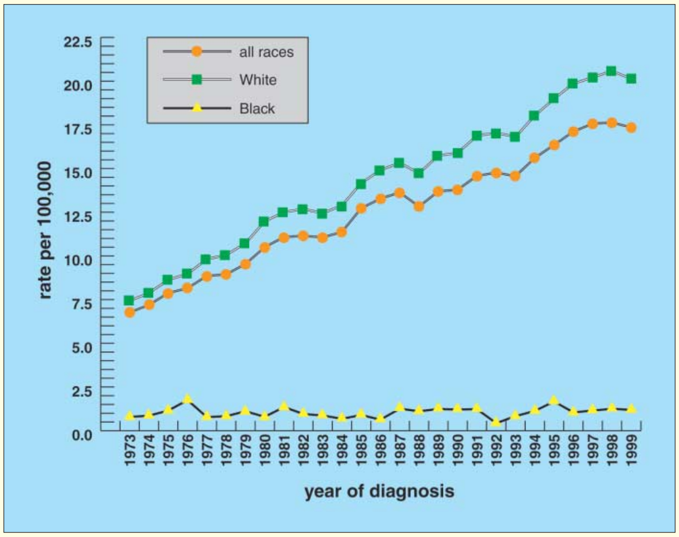

Figure 1. Age-adjusted incidence rates in the United States, by race, 1973–1999, from the SEER database (http://www.seer.cancer.gov)

neous epithelial surfaces including mucous membranes of the sinuses and oropharynx, esophagus, rectum, and vagina. Approximately 5% of melanoma cases first appear as metastatic lesions with no known primary site. In most cases, these probably represent complete regression of an initial cutaneous primary lesion (through perhaps immunological or other host mechanisms), with no effect of melanoma regression at the primary site on the relatively poor prognosis of metastases, emphasizing differences in the biology of primary versus metastatic lesions. Several lines of evidence have clearly demonstrated that the immune system can recognize melanoma cells. In fact, much of our knowledge of cancer immunity in humans comes from studies of melanoma (Houghton et al., 2001). Immune therapies including interferons and interleukin-2 are standard treatments for advanced melanoma, although not very effective (Balch et al., 2002).

Diagnosis, staging, and conventional therapy

The large majority of persons diagnosed with melanoma are cured (>85%) due to diagnosis at early stages of tumor progression. On the other hand, melanoma can be highly malignant, metastasizing to almost any organ in the body; melanoma diagnosis at later stages of tumor progression is associated with a poor prognosis. Typical clinical characteristics of melanoma skin lesions include asymmetry, irregularity of borders, variability in color, and diameter >6 mm (the so-called "ABCD's" of melanoma). However, even the most experienced clinicians have difficulty diagnosing pigmented lesions correctly, and clinical accuracy of diagnosis rarely exceeds 60%. Pathological examination must be used to establish a diagnosis.

Invasion and metastases are the two cardinal characteristics of cancer, and form the basis for pathologic diagnosis and staging melanoma. The well-defined progression of malignant transformation from normal melanocytes to metastatic melanomas provides an excellent model for malignant transformation, invasion and metastases (Figure 2). Primary melanoma progresses generally through two phases (Figure 2): (1) the radial growth phase characterized by horizontal spreading of transformed melanocytic cells within the epidermis, and small nests of invasive cells limited to the upper part of the dermis, and (2) vertical growth phase characterized by invasion of melanoma cells into the deeper dermis and underlying subcutaneous tissues. Generally only vertical phase melanoma lesions are associated with metastasis, while pure radial growth phase melanomas almost never metastasize. These observations suggest that invasion is required for metastasis, and in fact outcome is determined by the depth of invasion, the predominant prognostic factor. Staging of primary melanomas is based on the depth of (Clark et al., 1969) invasion into the dermis, using either Clark's method (based on anatomic skin markers) or Breslow's method (direct measurement of depth of invasion from the epidermis). The TNM classification is used to determine stage (T, primary tumor; N, regional lymph nodes; M, metastases). In situ (stage 0) melanomas are noninvasive, and have not broken the integrity of the epidermal basement membrane (these lesions are not included in melanoma statistics). Stage I (generally ≤2.0 mm depth) and stage II (>2.0 mm depth) melanomas are localized primary tumors and are treated by surgical excision. Stage III melanoma is characterized by regional spread through lymphatic vessels, and is treated by surgery with or without adjuvant therapy using interferon-α2b. In stage IV melanoma, distant metastases are disseminated through hematogenous spread. In addition to surgery and radiation therapy, systemic therapy (chemotherapy and immunotherapy) is used, but induces complete remissions in only a small proportion of patients.

Key genes and pathways involved in sporadic and familial melanoma

Loss of cell cycle regulation

Alterations of chromosomes 1, 6, 7, and 10 are prevalent in melanomas, but appear to be acquired late in tumor progression. Other genetic observations have implicated alterations in the Rb pathway for cell cycle regulation as an early step in the pathogenesis of melanoma. Frequent deletions in the 9p21 region in primary melanoma lesions as well as in melanoma cell lines led to the eventual identification of *p16INK4A* (within the *CDKN2A/MTS1* locus) as the candidate tumor suppressor by linkage analysis (Cannon-Albright et al., 1992; Fountain et al., 1992). Mutations in the *INK4A* region account for only ~20% of familial melanoma kindreds, implying that other genes are responsible in the majority of familial melanomas. Although the *CDKN2A* locus also encodes p14ARF (which influences cell cycle through the p53 pathway rather than the Rb pathway), evidence supporting a role of *ARF* in melanoma is currently lacking. Penetrance of *CDKN2A* germline mutations in melanoma susceptibility in predominantly Caucasian populations varies according to geographic location—for instance, 0.58 in Europe and 0.91 in Australia by age 80 (Bishop et al., 2002). In three melanoma families a *CDK4* mutation (R24C) has been identified, but such mutations are rare in melanoma family kindreds (Zuo et al., 1996). *p16INK4A* inhibits the G1-S transition by blocking the activity of cyclin-dependent kinases 4 and 6 (CDK4 and CDK6) that regulate early phosphorylation of the Rb protein. Binding of p16INK4A to CDK4 is blocked by the R24C mutation, and knock-in mice expressing a mutant CDK4 R24C allele develop melanomas, establishing a role for the Rb pathway (Sotillo et al., 2001). Somatic mutations in *p16INK4A* are relatively uncommon in sporadic (nonfamilial) melanomas (ranging from 0%–28%). Epigenetic inactivation of *p16INK4A* via methylation of the promoter region has been observed in a small proportion of cases. In a preliminary study upregulation of *Id-1*, a transcriptional repressor of *p16INK4A*, has been detected in

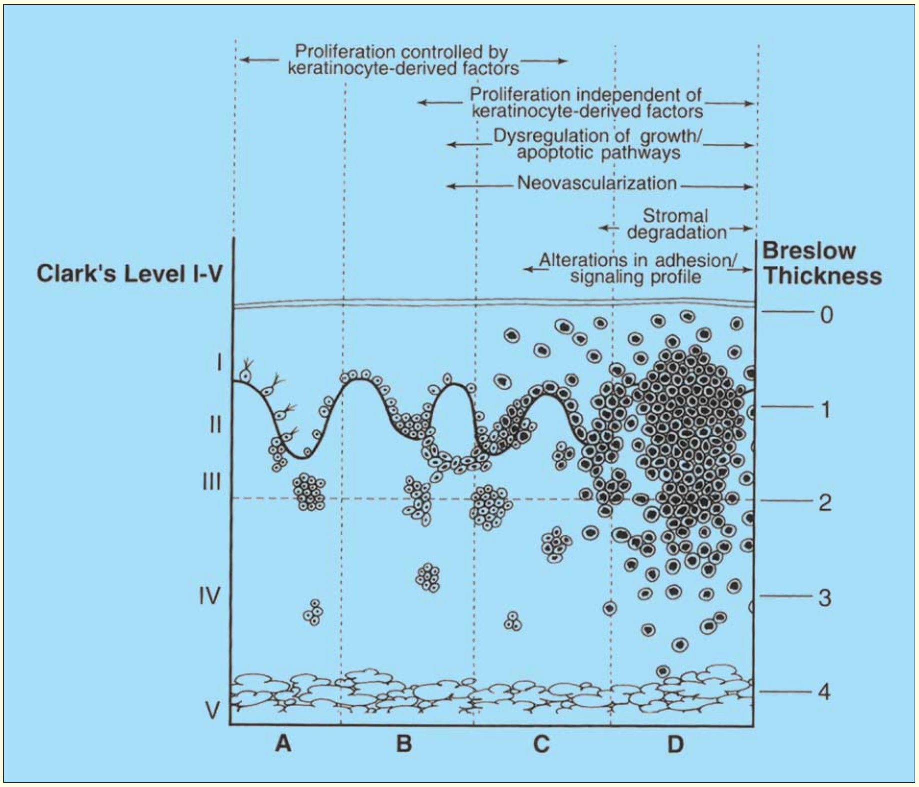
Proliferation controlled by keratinocyte-derived factors
Proliferation independent of keratinocyte-derived factors
Dysregulation of growth/ apoptotic pathways
Neovascularization
Stromal degradation
Alterations in adhesion/ signaling profile
Clark's Level I-V
Breslow Thickness
I
II
III
IV
V
0
1
2
3
4
A
B
C
D

Figure 2. Primary melanoma arises from melanocytes

A vertical section of skin is illustrated, including epidermis (level I at the left margin), dermis (levels II–IV) and subcutaneous tissue (level V). **A:** Column shows normal melanocytes arranged individually at the epidermal-dermal junction or in small organized clusters of benign nevus. **B:** Proliferation of atypical melanocytes, characteristic of atypical nevi. **C:** An early melanoma in radial growth phase. **D:** An advanced, invasive melanoma in vertical growth phase. Depth of invasion, determined by Clark's level and Breslow thickness, is the major determinant of prognosis. A model of biologic changes corresponding stages of melanoma progression is presented at the top of the figure. Reprinted from Polsky et al. (2001b) with permission of Elsevier Science.

early melanomas, providing an alternative mechanism to silence *p16INK4A* (Polsky et al., 2001c). Interestingly, amplification of *cyclin D1* has been seen, but only in melanomas occurring on relatively sun protected, acral (hands, feet) surfaces (Sauter et al., 2002). Alterations of the p53 tumor suppressor pathway remain poorly defined. Unlike many other common forms of cancer, *p53* mutations are infrequent in melanomas. However, overexpression of the p53 inhibitor HDM2 is found in early melanomas, suggesting concomitant dysregulation of the p53 pathway (Polsky et al., 2001a). In support of a potential role for the p53 pathway, mice expressing mutant ras alleles in a p53-deficient background develop melanomas (Bardeesy et al., 2001). In metastatic melanomas, inactivation of *APAF-1*, an effector of apoptosis downstream of p53, is common and provides one explanation for the resistance of melanoma to cytotoxic drug therapy (Soengas et al., 2001).

Activation through the Ras pathway

Although mutations in *RAS* family genes in melanoma specimens are uncommon (<10%), data from mouse models support the role of this important pathway in tumorigenesis (Chin et al., 1999; Bardeesy et al., 2000). Overexpression of the mutant Ha-Ras oncoprotein has been shown to induce malignant transformation of normal human melanocytes (Albino et al., 1992). Furthermore, transgenic mice induced to express mutant *Ha-Ras* in cutaneous melanocytes developed invasive melanomas when placed on a *CDKN2*-deficient background although mutant ras alone was insufficient to induce full transformation (Chin et al., 1999). Interestingly, maintenance of growing tumors depended on continued expression of mutant *Ha-Ras*. Experimental models have demonstrated that signaling via the bFGF receptor, upstream of ras, contributes to melanoma tumorigenesis. Recently, a report using human material suggested that activating mutations in *BRAF* are very frequent in melanomas, detected in 59% of melanoma cell lines and 6 of 9 melanoma specimens (Davies et al., 2002). *BRAF* encodes a cytoplasmic serine/threonine kinase in the RAS pathway (RAS→RAF→MEK→ERK→MAP kinase) that is regulated by RAS binding. If confirmed in a broader panel of melanoma specimens (including primary lesions), BRAF would become a legitimate target for experimental therapy of melanoma. Another report suggested that overexpression of *RhoC*, a member of the *Rho* family of GTP-hydrolyzing proteins, was important in the switch from localized melanoma to metastatic disease (Clark et al., 2000). These reports have provided evidence in human material that alterations of the Ras pathway are important in human melanoma.

Missing fingerprints for UV mutations

UV-B induces cyclobutane pyrimidine dimers, primarily thymidine dinucleotides. Lesions not repaired by nucleotide excision repair can lead to GC→AT transitions, leaving a mutagenic fingerprint. In nonmelanoma skin cancers, mutations with a UV-B signature are prevalent in the tumor suppressor gene *p53*. UV signature mutations in melanoma specimens have not been identified with any frequency in *Ink4a* or *BRAF*. These observations have provided little evidence for mutagenic effects of UV radiation in the pathogenesis of melanoma. On the other hand, the epidemiologic evidence for a role for solar exposure in Caucasian populations is compelling. Furthermore, in an experimental model, sunburn UV-B irradiation of neonatal mice expressing hepatocyte growth factor from a transgene induced a marked increase in melanocytic lesions (including those characteristic of in situ melanomas, a potential precursor lesion of invasive melanoma) compared to irradiated older mice (Noonan et al., 2001). It is possible that other UV irradiation during childhood/adolescence is important and that target genes are waiting to be discovered, but one must begin to think more seriously about nonmutagenic mechanisms such as immune suppression, UV induction of melanocyte growth factors by inflammatory cells or damaged keratinocytes, or UV production of mutagenic oxidative radicals during inflammation or melanogenesis.

Strategies for experimental therapies

Because systemic therapies for advanced melanoma are marginally effective, experimental therapies are an option for patients with advanced disease if they are eligible for protocols. To date, the search for active therapies against melanoma has been largely empiric. However, as pathways in melanoma pathogenesis are revealed, new opportunities for targeted therapies are appearing. The recent success of the kinase inhibitor STI-571 in treating chronic myelogenous leukemia provides a rationale for targeting appropriate tyrosine and serine/threonine kinases in melanoma. These include drugs that target receptor tyrosine kinases (e.g., FGF receptor) and molecules in the ras pathway, including MAP kinase inhibitors, farnesyl transferase inhibitors, and ansamycins (which destabilize folding of kinases in the endoplasmic reticulum). Melanoma cells resist apoptosis, particularly mediated through the mitochondrial pathway. One mechanism involves deficiencies in Apaf-1, through deletion of one allele and inactivation of the other allele by methylation. Apaf-1 methylation can be inhibited by 5-azacytidine, reverting resistance to apoptosis through the p53 pathway (Soengas et al., 2001). These observations suggest that demethylating agents could be combined with cytotoxic drugs to overcome resistance. Other antiapoptotic molecules expressed in melanoma include bcl-2, survivin, and livin. Antisense strategy against bcl-2 in combination with chemotherapy is in clinical trials.The immune system of melanoma patients can recognize melanocyte-specific proteins, particularly melanosome proteins and certain proteins expressed in germ cells that are extinguished in somatic cells but reexpressed in melanomas (sometimes called cancer-testes antigens). These molecules are targets for active immunotherapy (therapeutic vaccination).

Future challenges

Much remains to be learned about the pathogenesis of melanoma. How do different receptors and signaling pathways regulate uncontrolled growth, invasion, and metastases? What is the role of solar exposure in the etiology of melanoma? As genetics and cell biology reveal pathways and key molecules, new targets for prevention and therapy will appear. Mouse models that recapitulate human melanoma and the relevant pathways should allow better preclinical models for screening and developing new classes of therapeutic agents.

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