Recent Advances in Melanoma Biology

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ABSTRACT

The incidence and mortality rates of melanoma have increased at annual rates of 2%-3% for the last 30 years. Disseminated disease is largely refractory to cytotoxic chemotherapy and is almost universally fatal. Several recent advances in melanoma biology offer new strategies for potentially treating this aggressive malignancy. This review focuses on three significant advances involving tumor initiation, etiology, and progression. New experimental models reveal a direct role for UV-B light in initiating melanomas in human skin. Studies on E- and N-cadherin elucidate the importance of local homeostatic mechanisms in regulating tumor progression. Finally, several discoveries concerning apoptotic mechanisms in melanoma suggest strategies for future treatments. The Oncologist 2004;9:182-187

INTRODUCTION

The increase in melanoma incidence in U.S. Caucasians is among the greatest for any cancer. From 1973 to 1994, melanoma incidence rates increased 120.5% [1]. It was estimated that 7,400 people in the U.S. would die from melanoma in 2003 [2]. Recent Surveillance, Epidemiology, and End Results (SEER) Program data reveal a 1.91% lifetime risk for Caucasian men to be diagnosed with melanoma and a 1.37% risk for Caucasian women [3].

In addition to the rising incidence, mortality rates have also increased, though at a slower rate. The mortality rate due to melanoma increased 38.9% from 1973 to 1994 [1]. While early, localized disease is effectively treated with wide excision, metastatic disease is almost universally fatal. The median survival time for patients with disseminated melanoma is 8.1 months with only approximately 2% surviving for 5 years [4]. Treatments for advanced disease are inadequate. Chemotherapy with cytotoxic agents has a low cure rate of 1% and significant toxicity. Similar cure rates
have been experienced with interleukin-2 therapy. In light of the rising incidence and high mortality rate in advanced disease, several countries now recognize melanoma as a top public health priority. This article reviews selected recent advances in the understanding of melanoma biology that involve the initiation, progression, and programmed cell death processes.

**UV Light and Melanoma**

UV light has been implicated in the genesis of several forms of cutaneous malignancies: squamous cell carcinoma, basal cell carcinoma, and melanoma. Epidemiological studies reveal a strong association between melanoma formation and sunlight exposure. Whereas squamous and basal cell carcinomas appear to be linked to total lifetime sun exposure, melanoma development is most closely associated with intense, intermittent exposure [5-9]. A history of sunburn is often used as a surrogate measure for intense intermittent exposure. The odds ratios for increased risk of melanoma due to sunburns in adult life, adolescence, and in childhood were 1.91, 1.73, and 1.95, respectively. In addition, the locations of melanomas suggest causation by intermittent sun exposure. Melanomas occur relatively less frequently in areas that are continuously exposed to sunlight, like the face, hands, and arms, and more frequently in sun-protected areas receiving intermittent exposure, like the trunk in men and the backs of legs in women [8].

Experimental studies support the epidemiologic evidence implicating sun exposure in causing melanoma. Intense intermittent exposure apparently does not give melanocytes time to synthesize melanin to protect themselves from UV irradiation. This irradiation leads to DNA mutations. Although UV-A light is more abundant in sunlight than UV-B light, the latter is responsible for intense intermittent exposure. The odds ratios for increased risk of melanoma due to sunburns in adult life, adolescence, and in childhood were 1.91, 1.73, and 1.95, respectively. In addition, the locations of melanomas suggest causation by intermittent sun exposure. Melanomas occur relatively less frequently in areas that are continuously exposed to sunlight, like the face, hands, and arms, and more frequently in sun-protected areas receiving intermittent exposure, like the trunk in men and the backs of legs in women [8].

Animal experimental systems support a role for UV light in melanoma causation. For example, 70 weeks of exposure to UV irradiation alone caused 5 of 13 Monodelphis domestica opossums to develop melanocytic tumors [16]. Administration of a topical carcinogen, 7,12-dimethylbenz(a)anthracene (DMBA), and UV irradiation induced melanomas in hairless and newborn mice [17]. A single exposure of UV-B or UV-A light was sufficient to induce melanomas in a cross between platyfish and swordtail fish [18]. One new animal model reinforces epidemiological findings that childhood sunburn is a significant risk factor for developing melanoma. Noonan and colleagues found that a single high dose of UV radiation was sufficient to produce melanoma-like tumors in neonatal, but not adult, mice if the skin cells expressed a growth factor that stimulates melanocytes [19, 20]. Chin and colleagues established a second elegant genetic model of murine melanoma in which the INK4a gene deletion cooperates with Ras overexpression [21]. In a more recent development of the model driven by H-Ras activation and loss of p19ARF function, UV light exposure resulted in a marked acceleration in melanoma genesis [22]. UV-radiation-induced melanomas showed a strict reciprocal relationship between cyclin-dependent kinase (Cdk)6 amplification and p16INK4a loss, which is consistent with the actions of UV light along the Rb pathway. Although these animal models provide important insights into the role of UV light in melanoma causation, they differ from human skin in critical and obvious ways. Correlating animal models with human disease is difficult because of the unique architecture of murine skin. In mice, melanocytes are in the dermis and hair follicle and rarely in the epidermis, whereas in human skin, melanocytes are confined to the basal layer of the epidermis.

Until 1998, a direct causal relationship between UV-B light and melanoma in humans had not been established. RAG-1 immune-deficient mice with grafted newborn human foreskin have proven such a relationship [23]. Seventy-three percent of UV-B radiation-treated xenografts manifested melanocytic hyperplasia, while one graft treated with both DMBA and UV-B light developed a human malignant melanoma. This was the first experimental system to provide evidence that UV-B radiation and an exogenous carcinogen could produce human malignant melanoma de novo.

A similar model induced human melanoma using a combination of UV-B light and overexpression of an endogenous growth factor, basic fibroblast growth factor (bFGF) (Fig. 1) [15]. Numerous local stressors may stimulate increases in cytokine production. Such stressors include trauma, infection, and other causes of inflammation. Sunburn clinically reflects an overdose of UV light leading to inflammation with an increase in cytokine production. While the mechanisms for this model of melanoma
B-raf in Melanoma

A fortuitous discovery in 2002 has electrified the melanoma field because it opens new avenues for melanoma diagnosis and therapy [24, 25]. Davies et al. [26] detected an activating mutation in the B-raf protooncogene in 60%-70% of melanoma cell lines and tissues. The B-raf<sup>V600E</sup> missense mutation represents over 80% of the B-raf alleles described to date [27, 28], resulting in constitutive and maximal activation of B-Raf kinase activity. Thus, activation of the mitogen-activated protein kinase (MAPK) pathway in melanoma occurs through multiple mechanisms: A) mutation in the B-raf gene; B) stimulation by the endogenous growth factors, bFGF and hepatocyte growth factor [28]; and C) exogenous stimulation by insulin-like growth factor-I [29] and by adhesion receptor signaling (see below).

B-raf mutations have been detected in nonmalignant nevi [30], suggesting that B-Raf is insufficient for transformation. The results also indicate that nevi already have some characteristics of malignant cells, even if only 1 in 10,000 actually progresses to melanoma. B-raf mutations occur rarely in non-sun-exposed melanomas such as acral lentiginous melanoma, vulvar melanoma, and ocular melanoma [25]. However, there are no classical UV-radiation-induced signature mutations in the gene, suggesting that other mechanisms are important for the etiology of these genetic aberrations. The enzymatic nature of the B-Raf kinase has now spurred an intense effort of investigation, because a small molecule inhibitor of the Abi and Kit tyrosine kinases, imatinib mesylate (Gleevec<sup>™</sup>, STI-571) induced dramatic responses in chronic myelogenous leukemia (CML) and gastrointestinal stromal tumor (GIST) cases. The results in CML and GIST are highly encouraging, and there is much hope that related inhibitors of B-Raf will be effective in melanoma therapy. Several major drug companies have initiated drug screening and preclinical studies. One B-Raf inhibitor, BAY 43-9006, is already in clinical trials of melanoma patients [25]. We expect, in the next few years, a multitude of exciting new therapeutic approaches in melanoma.

Progression: The E-Cadherin to N-Cadherin Switch

Clinical and pathological features of melanoma suggest that it often progresses along five distinct steps [31, 32]. The first step consists of structurally normal melanocytes forming common acquired and congenital nevi. Dysplastic nevi (moles) with structural and architectural atypia define the second step. The third step is called the radial growth phase (RGP) primary melanoma. Cells from RGP lesions can individually invade the dermis but have no capacity to metastasize. The vertical growth phase (VGP), the fourth step in progression, involves primary melanoma cells that have invaded the dermis as a large cluster of cells and have metastatic potential. The final step is metastatic melanoma to distant organ sites.

The development of melanoma may be seen as a disruption of normal homeostatic mechanisms in the skin. Such mechanisms control when and how cells proliferate, differentiate, and undergo apoptosis. Disruption of these homeostatic controls can lead to progression of melanoma in which adhesion molecules, such as E-cadherin and N-cadherin, play key roles [33-35]. The development of melanoma is associated with the loss of E-cadherin and the appearance of N-cadherin (Fig. 2).

Cadherins are cell-surface glycoproteins that promote calcium-dependent cell-cell adhesion, and they are expressed in developmental-, cell-, and tissue-specific manners. The major adhesion mediator between keratinocytes and normal melanocytes is E-cadherin, which disappears during melanoma progression [36]. While normal melanocytes express E-cadherin, this molecule is not found on nevus or melanoma cells [33, 35]. The loss of E-cadherin likely plays a crucial role in tumor progression. Cells that have lost epithelial differentiation, as manifested by the loss of functional E-cadherin, show increased mobility and invasiveness. Keratinocytes can no longer control melanoma cells that have lost E-cadherin. When melanoma cells are forced to express E-cadherin and are cocultured with keratinocytes, they dramatically change: melanomas adhere to keratinocytes, no longer express invasion-related molecules, and lose their invasive capacities [37]. Instead of E-cadherin, melanoma cells express N-cadherin, which allows them to change cellular partners. Melanoma cells adhere through N-cadherin to fibroblasts and endothelial cells. N-cadherin is a survival factor for melanoma cells as they migrate through the dermis [38]. Melanoma cells establish gap junctions with fibroblasts; gap junctions are small channels for electrolyte transport. N-cadherin is also a major adhesion receptor when melanoma cells adhere to each other; coreceptors such as MCAM facilitate cluster formation.

Along with the upregulation of N-cadherin and the increased association of melanoma cells with stromal fibroblasts and endothelial cells, occurs a major shift in expression of cell surface receptors on malignant cells. Major changes in expression levels occur for cell-cell and cell-matrix adhesion receptors. One of the most important molecules is the vitronectin receptor ανβ3. It is a multifunctional receptor binding not only vitronectin but over ten other matrix proteins, and
it is also involved in cell-cell adhesion [39]. Overexpression of β3 integrin in melanoma cells leads to highly increased invasiveness and tumorigenicity [40]. Signaling through the αvβ3 receptor in melanoma cells occurs through the MAPK proliferation pathway, whereas signaling for the other major adhesion receptor, MCAM or CD146, occurs through the survival AKT pathway [41]. Thus, adhesion receptors cooperate with receptor tyrosine kinases for activation of the major proliferation and survival pathways [42]. Antagonists for the αvβ3 receptor are currently in clinical trials [43].

Figure 1. Photograph (A) and histologic section (B) depicting UV-light-induced melanocytic lesions in human skin. A) An abdominal human skin graft on a severe combined immunodeficient (SCID) mouse developed this melanocytic lesion following 4 weeks of injections of bFGF/Ad5 (once weekly) and UV-B irradiation (30-50 mJ/cm², three times each week). B) Histologic section (hematoxylin and eosin stained, magnification 200×) of a lentiginous form of malignant melanoma in a human skin graft on a SCID mouse. The graft had received a total of seven injections of bFGF/Ad5 and 26 UV-B irradiations. Note the hyperplastic atypical melanocytic cells (arrows) in the epidermis in a dense lentiginous growth pattern. Photographs courtesy of Carolla Berking.

Figure 2. Altered cadherin expression during melanoma progression. The progression of melanoma along with its accompanying change in cadherin expression is illustrated. A) Keratinocytes and melanocytes both express E-cadherin. Through E-cadherin expression, keratinocytes dictate melanocyte behavior. B) While keratinocytes continue to express E-cadherin, early melanomas begin to lose expression of E-cadherin and escape keratinocyte control. C) More advanced melanomas begin to express N-cadherin and may interact with other cells that express N-cadherin, like fibroblasts and endothelial cells.
predominantly target tumor-infiltrating endothelial cells. In melanoma, we expect dual targeting for normal and malignant cells.

**CONCLUSION**

Recent advances in the understanding of the biology of melanoma promise to provide not just keys to further knowledge per se, but also clues to novel and more effective therapies. Models employing UV-B light to induce melanoma in human skin promise to lead to significant advancements in prevention. Scientists can now better dissect the specific mechanisms of UV-B-radiation-induced melanoma genesis as well as observe key steps in tumor initiation and progression. Findings from studies of the B-raf gene illustrate that broad screening approaches have a value effective treatments. Apoptotic pathways should also offer hope for new and possible therapeutic interventions. Increased knowledge about apoptotic pathways should also offer hope for new and effective treatments.

**REFERENCES**


