Shedding Light on Melanocyte Pathobiology In Vivo

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Abstract
Cutaneous malignant melanoma is rapidly increasing in the developed world and continues to be a challenge in the clinic. Although extensive epidemiologic evidence points to solar UV as the major risk factor for melanoma, there is a significant gap in our knowledge about how this most ubiquitous environmental carcinogen interacts with the largest organ of the mammalian body (skin) at the microenvironmental and molecular level. We review some recent advances that have started to close this gap. Cancer Res; 72(7); 1591–5. ©2012 AACR.

Introduction
Cutaneous malignant melanoma remains one of the few malignancies that exhibit positive rates of increase in the developed world (1). In the past few decades, the incidence of melanoma has accelerated to increasingly become a major disease of young women. In fact, according to the latest Surveillance Epidemiology and End Results data (1975–2008), melanoma is now the most prevalent cancer in women under the age of 40, surpassing even breast cancer. The major etiologic risk factor for melanoma is thought to be exposure to intermittent erythogenic doses of UV, especially during childhood and early adolescence years (2). We experimentally validated these UV-associated risks in the hepatocyte growth factor/scatter factor transgenic (HGF/SF-Tg) mouse model, which features constitutive signaling through the receptor tyrosine kinase MET (3). We also produced in vivo experimental evidence that sunscreen, when applied as directed, inhibits melanoma (4). Notably, in the albino HGF/SF-Tg mouse, UVB was highly melanomagenic, but UVA was not (5). Unfortunately, the molecular mechanisms underlying UVB-driven melanoma-genes have remained elusive.

The UV spectrum of sunlight is divided into 3 regions: UVA (320–400 nm wavelength), UVB (290–320 nm), and UVC (200–290 nm; there is no standard definition for these wavebands, and one may find in the scientific literature other definitions such as that of the Commission Internationale d’Eclairage, which recommended 100–280 nm for UVC, 280–315 nm for UVB, and 315–400 nm for UVA). Terrestrial UVC is biologically irrelevant, as it is almost completely absorbed by the stratospheric ozone layer. Both UVA and UVB reach the earth’s surface and have deleterious effects on nucleic acids and proteins. UVB is considered to be more carcinogenic than UVA, as it directly causes 2 types of DNA lesions: cyclobutane pyrimidine dimers (CPD), formed between adjacent thymine (T) or cytosine (C) residues, and 6-pyrimidine 4-pyrimidone photoproducts (6). The CPDs are more abundant, more carcinogenic, and less efficiently repaired. These UVB-induced lesions give rise to DNA mutations hallmarking by C—T and CC—TT transitions, the so-called “UVB signature mutations” (6). On the other hand, UVA mutates DNA indirectly, thought to be mediated through generation of reactive oxygen species via absorption by endogenous photosensitizers (6).

There is a clear association between UV-induced DNA damage and skin cancer. In nonmelanoma skin cancer, for example, squamous cell carcinoma and basal cell carcinoma, UVB signature DNA mutations have been found in several genes and are considered to play an essential role (7). The most well-characterized example is the tumor suppressor p53, which exhibits UVB signature mutations in a large majority of nonmelanoma skin cancer and seems to be an initiating event (7). In contrast, although several melanoma susceptibility genes have been identified, no definitive UVB-induced driver mutations have been observed in melanoma. CDKN2A, a bona fide melanoma susceptibility gene, harbors mutations at dipyrimidine sites, but many of these genetic alterations are not UVB signature mutations. C—T transition mutations in CDKN2A are also observed in cancers of internal organs that are protected from sun exposure, for example, gastric adenocarcinomas, calling into question a central role of these mutations in UV-induced melanomagenesis (8). BRAF, a part of the RAS signaling pathway and critical for the cellular response to growth signals, has been shown to be mutated in a majority of melanomas, but these mutations are not UVB signature mutations (9). Similarly, mutations in NRAS are found in melanomas but do not carry UVB signatures (9). Although a small percentage of melanomas have UVB signature mutations in p53, these mutations are considered late events during the progression of melanoma to a higher grade and are not involved in the initiation stages. Thus, evidence so far for the presence of UV-generated signature mutations in melanoma that could be ascertained as the driver mutations has been less than compelling (9). UVB exposure is undoubtedly mutagenic, and signature mutations are starting to be uncovered; for

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example, UVB-induced mutations in the PTEN gene have been reported in melanomas arising in patients with xeroderma pigmentosum (10), and whole-genome and exome sequencing methodologies have revealed large-scale UV-type mutational signatures in melanoma tumors and cell lines (11, 12). Nevertheless, strong evidence that UV can also induce immunosuppression and inflammation (1, 13) has fueled the notion that these processes work in concert with DNA damage in the initiation and/or progression of melanoma. Support is strong for the notion that UV is a complete carcinogen, acting with respect to melanoma as both an initiator, through genotoxicity, and a promoter, through immunosuppression.

It is well described that UV initiates a profound and immediate p53-dependent stress response in the skin, as well as a variety of inflammatory mediators and paracrine factors, notably, but not exclusively, from keratinocytes, which profoundly alter melanocyte function. Ultimately, the combined effect of these stress responses is a rapid onset of cell-cycle arrest and DNA repair mechanisms. Although several studies have examined the immediate genomic response of UV-induced stress in several skin cell types, the long-term persistent response in melanocytes beyond the initial 48 hours had not been investigated. We hypothesized that novel clues to the molecular mechanism(s) underlying UV-induced melanomagenesis would be found within the persistent genomic response of melanocytes to UV radiation.

We recognized that any relevant analysis of the UV response by melanocytes had to be done in situ, within their natural physiologic and morphologic microenvironment. The considerable technical challenge was to devise a means to study a cell type in vivo that constitutes a tiny fraction of the cellular milieu of the mammalian skin and bears no exclusive cell surface markers. To circumvent this problem, we developed a mouse model in which melanocytes can be both imaged in vivo and highly purified by virtue of tetracycline-inducible, melanocyte-specific GFP expression (Dct-GFP mice; refs. 14, 15). The faithful and rapid on-demand GFP labeling of melanocytes within the Dct-GFP mouse provided an invaluable tool to begin to explore melanocyte and melanoma biology in vivo.

Key Findings

Our previous studies had shown that UVB, but not UVA, was melanomagenic in our albino HGF/SF-Tg mouse model (5). We reasoned that waveband-specific tumorigenicity could be explained by differential cellular and/or molecular responses of melanocytes to different wavelengths. We therefore used the Dct-GFP mouse to examine the responses to UVB versus UVA irradiation of melanocytes residing within their natural morphologic and physiologic microenvironment. These studies showed that the answers to many of the questions about the role of UV in melanomagenesis lie not only in the penchant of UV for inducing DNA mutations, but also in its ability to provoke interactions between melanocytes and elements of the microenvironment to regulate remodeling of UV-damaged skin.

It was clear that UVB was far more effective at inducing gene expression response in melanocytes than UVA, consistent with their respective melanomagenic potential in the HGF/SF-Tg mouse model. UVB induced melanocyte activation, characterized by increased proliferation and migration toward the epidermis. GFP-labeled melanocytes were isolated by fluorescence-activated cell sorting and subjected to microarray analysis of gene expression. In addition to an expected early stress response that waned, a delayed response was seen, which was largely comprised of IFN-γ response genes. Antibody-mediated blockade of IFN-γ, but not IFN-α, abolished the UVB-induced melanocyte activation, suggesting that the IFN-γ-induced gene signature was responsible for the activation of melanocytes. Immunohistochemical and flow cytometric analyses identified a subset of infiltrated macrophages as the source of IFN-γ. When the UV-recruited skin macrophages were isolated and admixed with melanoma cells transplanted in syngeneic mice, tumor growth was significantly enhanced. The biologically active agent responsible for this effect was found to be IFN-γ, as the enhanced growth was mitigated by systemic anti–IFN-γ antibody treatment.

Erythemal UVB induces overtly contrasting microenvironmental responses in neonatal and adult skins. Neonatal mouse skin is characterized by robust macrophage infiltration, whereas the adult mouse skin exhibits a rapid, but short-lived, neutrophil influx, severe enough to disrupt cycling hair follicles (16). These qualitative differences coincide with the fact that UVB is melanomagenic in neonatal mice, but not in adults (3).

We have proposed that the UVB-induced melanomagenic mechanisms operate within the immunoediting paradigm proposed by Dunn and colleagues (17). The UVB-activated mutant melanocytes and/or their progenitors may be able to evade immune-mediated elimination and persist during an extended equilibrium phase before evolving into clinically significant melanoma (Fig. 1). UVB-induced melanocytic proliferation might aid in the fixation of UVB-induced mutations as well as accumulation of non–UVB-type mutations. In addition, migration of melanocytes outside of their privileged niche could facilitate neonate-specific UVB-associated long-term tolerance to melanocytic antigens by promoting aberrant interactions between melanocytes and their inflammatory microenvironment. Moreover, inflammation-associated epigenetic alterations in transformed cells and macrophages can set forth enduring biologic effects.

The most notable discovery from our analysis of in vivo UV-irradiated melanocytes was that, rather than deterring melanomagenesis, physiologically relevant levels of IFN-γ actually promoted survival of melanocytes within this hostile inflammatory microenvironment, as well as melanoma cells within tumors. Antibody-mediated systemic blockade experiments showing the importance of IFN-γ in UVB-induced melanocyte activation and mouse melanoma development strongly support the notion that the cellular effects of IFN-γ are context dependent and can be antitumorigenic or protumorigenic (18).

Significance and Implications

Immunosuppressive cell networks and factors clearly play a significant role in the failure of antitumor immune responses and currently available therapies to eradicate melanoma. We propose that the same immunoevasive and/or survival
mechanisms noted in UVB-irradiated neonatal skin can be exploited by melanoma and likely contribute to the selection of the aggressive and therapeutically resistant melanoma phenotype. The importance of IFN-$\gamma$ noted in UVB-induced melanocyte activation in the neonatal skin fuels speculation that IFN-$\gamma$ signaling is a critical early step in subsequent susceptibility to melanomagenesis. It is noteworthy that the IFN "survival" signature seen in the UVB-activated melanocytes (15) includes several genes known to be involved in immune evasion, including nonclassic MHC class I b antigens. For example, H2-T23 (Qa-1; mouse homolog of HLA-E) plays a vital role in suppression of natural killer (NK) and cytotoxic T lymphocytes, via binding with the CD94/NKG2 family of receptors, leading to T-cell anergy (19). Several MHC I subclass H2-Q genes (functional homologs of HLA-G) also exhibited UVB-induced upregulation in melanocytes (15). Indeed, the human MHC class I b molecules HLA-G and HLA-E have been implicated in the immune escape of human melanoma (20, 21). The UVB-induced melanocyte survival signature also features the complement isoforms C4A and C4B, which are involved in suppression of systemic autoimmunity (22). CTLA4 is another potent immune evasion facilitator highly upregulated in neonatal melanocytes in response to UVB, as well as in transplanted melanoma cells admixed with macrophages isolated from UVB-irradiated neonatal skin (15). CTLA4 negatively regulates T-cell immune response via auto-attenuation of effector T-cell activation, as well as by positive regulation of the suppressor function of regulatory T cells (T$^{\text{reg}}$; ref. 23). Interestingly, CTLA4 has been shown to play a role in T$^{\text{reg}}$-mediated immunosuppression induced by UV radiation (24). CTLA4-blocking monoclonal antibody, considered a prototypical immunotherapeutic agent, has been reported to induce long-term regression of metastatic melanoma and improved survival of patients (25).

Melanocytes are built for enhanced survival to withstand both UV exposure ensuring the continued synthesis of melanin and the chemical stresses associated with the synthesis of melanin itself. Therefore, the discovery of the upregulation of a variety of immune evasion molecules in melanocytes in the aftermath of UVB irradiation has important implications.
These elements may play an integral role in further protecting melanocytes from eradication by the UVB-induced inflammatory response, otherwise designed to remodel all damaged portions of the skin. Melanoma could take advantage of this built-in circuitry to develop into one of the most evasive cancers. The fact that this circuitry converges on IFN-\(\gamma\) epitomizes the importance of this pathway to melanocytic survival mechanisms. At the same time, it represents an exciting new class of potential antimelanoma preventive and/or therapeutic targets.

IFN-\(\gamma\)-associated survival mechanisms operational in neonatal melanocytes may be recapitulated in melanoma, contributing to the selection of more aggressive and therapeutically resistant phenotypes. The relevance of this finding to human melanoma is supported by detection of macrophage-associated IFN-\(\gamma\) expression in most human melanoma samples examined (15). Moreover, a clinical trial has shown that IFN-\(\gamma\) may have adverse effects on patients with melanoma with respect to relapse and mortality (26). We have provided evidence that IFN-\(\gamma\) signaling can facilitate melanoma progression, a significant discovery considering that high-dose IFN-\(\alpha\) is used as an adjuvant in melanoma treatment regimens, although often with limited success.

We observed that a substantial proportion of human melanomas harbor IFN-\(\gamma\)-producing macrophages, consistent with the proposed role for infiltrating macrophages within the IFN-\(\gamma\)-driven protumorigenic microenvironment created in UVB-irradiated skin. Although strong data implicate lymphocytic infiltration in primary melanoma as a favorable prognostic marker (27), little is known about the prognostic significance of macrophage infiltration. The prospect of validating IFN-\(\gamma\)– macrophages as a new and simple cellular marker of poor prognosis deserves further investigation. It is noteworthy that IFN-\(\gamma\) has already been implicated in serum as an independent prognostic indicator for disease recurrence in patients with melanoma (28). Moreover, recently Cho and colleagues have shown that IFN-\(\gamma\) decreases the susceptibility of B16 melanoma cells to CD8\(^+\) T-cell–mediated cytolysis (29).

Protumorigenic inflammatory elements represent promising targets of anticancer therapeutics. We have discovered IFN-\(\gamma\) to be the driver of novel cellular and/or molecular inflammatory mechanisms that may underlie the initiation, immunoevasion and/or survival, and outgrowth of UVB-induced melanoma. These studies strongly suggest that IFN-\(\gamma\) and/or IFN-\(\gamma\)R, or its downstream pathway members, represent not only promising prognostic markers, but may also prove to be efficacious therapeutic targets for human melanoma. Our findings also raise the likely possibility that tools designed to enable study of the biology and pathobiology of melanocytes in their natural microenvironment will continue to generate novel mechanistic and therapeutic insights into melanogenesis.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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