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## Perspective

# Etiologic Pathogenesis of Melanoma: A Unifying Hypothesis for the Missing Attributable Risk

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Melanoma is the eighth most common malignancy in the United States and has shown rapid increases in its incidence rate over the past two decades, especially in early-stage disease (1–4). A recent analysis of data from the Surveillance Epidemiology and End Results Program indicates that the incidence of melanoma increases with age, with somewhat different patterns in men and women (3). Genetic and environmental interactions clearly play a role in melanoma and may explain in part the variations in age-specific incidence rates and the differences between the rates in men and women.

The etiology of malignant melanoma has been attributed to a great extent to the role of UV light exposure as the most important risk factor for melanoma in those with phenotypic susceptibility (5–10). The increase in the risk of melanoma by age has also been attributed to exposure to environmental agents in addition to UV light where the onset of melanoma depends on latency periods between the onset of environmental exposure and tumor occurrence and multiple other factors. Most epidemiological studies are examining the effect of known risk factors identified and quantified through case-control and association studies. However, the epidemiological studies usually do not measure effectively synergism between two or more possible etiologic factors in gene/environment and gene/gene studies and provide very little information regarding mechanisms of disease etiology.

The pathogenesis of human melanocyte transformation remains incompletely understood. Many non-UV-related factors have been proposed as contributing to the risk of melanoma, but no coherent etiologic picture has emerged (10, 11). Most notably and relevant to our proposal is that many epidemiological studies have shown an increased risk of melanoma in many occupations associated with the electronic and chemical industries (12–16). We propose that the attributable risk not explained by UV light exposure or genetic risk can largely be understood by using a model that proposes that increases in reactive oxygen

species are central to the pathogenesis of melanocyte transformation and melanoma progression (Fig. 1). We review here our studies and those of others that suggest that changes in the melanin from antioxidant to pro-oxidant are a critical early pathogenic event. We further discuss the consequences of this alteration for the melanocyte, how such changes may explain most of the epidemiological observations that have been postulated as exogenous risk factors for melanoma, and the consequences of these modulations for the (chemo) prevention and possible treatment of human melanoma.

A unique biological feature of the melanocyte is its production of melanin, a molecule with a diversity of complex redox and free-radical properties and interactions, including an interaction with oxygen (17, 18). Over the past five years we have performed a series of experiments that have led us to postulate that redox-recycling of melanin and its precursors is an etiologically important and possibly unifying explanation for many of the seemingly unconnected epidemiological observations that have been associated with melanoma risk. We first demonstrated that exogenous oxidative stress elicited a signal for generation of reactive oxygen species in melanocytes and melanoma cells that was not seen in nonpigmented cells (19). Paradoxically, the addition of superoxide dismutase to melanoma cultures markedly enhanced this signal whereas no such effect of the enzyme was noted in melanocyte cultures. Of great interest was that the intracellular levels of superoxide anion in melanoma cells compared with normal human melanocytes were markedly increased. These and other findings suggested that the pigment melanin had been converted to a pro-oxidant (19, 20).

We explored the possibility of melanin being the source of the pro-oxidant response in more detail using a synthetic melanin model system based on dihydroxyindole (21, 22), the major precursor of melanin. Here we observed that oxidation of the synthetic melanin increased its affinity for metal ions, attributable to a unique tautomer of the oxidized catecholic monomer termed a quinone-imine. Therefore, it results that metal ion uptake promotes the oxidation and redox cycling of the pigment (21). Even binding of divalent Zn, a redox inactive metal ion, produces a strong pro-oxidant response from the pigment as detected by electron paramagnetic resonance spin traps and an assay for hydroxyl radical generation (21); the pro-oxidant effect is even further enhanced by the redox active bio-metal Cu(II). We also have observed a similar response in cultured melanoma cells using both molecular probes of oxidative stress as well as by electron paramagnetic resonance spin trapping (20, 23). These observations have led us to develop a series of lipophilic chelators for therapeutic indications (23). Many metals and other substances such as polycyclic hydrocarbons (e.g., pesticides, herbicides, dyes) avidly bind melanin and have been measured therein (24).

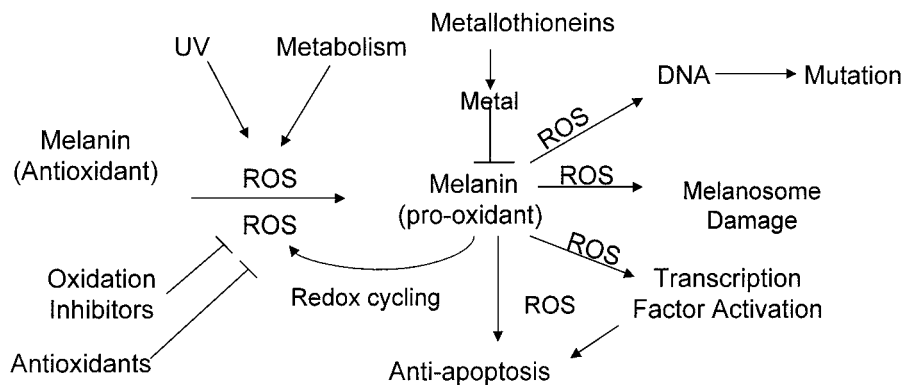
We propose that the following sequence of events occurs during melanoma pathogenesis (Fig. 1). Melanin, normally an antioxidant, is oxidized by reactive oxygen species generated by

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**Fig. 1** A model for chemoprevention of early melanoma progression. Oxidation of melanin leads progressively to generation of a redox-active tautomer (quinone-imine), intracellular redox cycling (enhanced by metals or other substances bound by melanin) with melanosomal and DNA damage, transcription factor activation and enhancement, and activation of the natural antiapoptotic (drug-resistant) phenotype of the melanocyte. Antioxidants include a number of cellular antioxidants (ascorbic acid,  $\alpha$ -tocopherol, and glutathione) whereas upstream inhibitors of oxidation might include such drugs as inhibitors of cholesterol synthesis or inhibitors of mitochondrial activity. Metal uptake into cells is regulated by metallothioneins, and polymorphisms should contribute to differential uptake and risk. ROS, reactive oxygen species.

UV, normal metabolic processes, or inflammatory responses, and the pro-oxidant quinone-imine content is increased. This organic chelator serves as a nidus for accumulation of metals or other chemicals (e.g., polycyclic hydrocarbons). Alternatively, some unusual exposure to metal ions or melanin-binding chemicals may likewise increase the pro-oxidant response of melanin. Redox cycling occurs and a build-up of reactive oxygen species gradually results. Initially this build-up is dampened by cellular antioxidants, but they are eventually depleted. Picardo *et al.* (25, 26) have convincingly demonstrated that depletion of cellular antioxidants is a prominent feature during melanoma pathogenesis. The recent demonstration in two large randomized cardiovascular trials that two lipid-lowering medications are associated with a decreased incidence of melanoma is relevant here (27, 28). Although the two compounds, lovastatin and gemfibrozil, work through different molecular mechanisms (3-hydroxy-3-methylglutaryl-CoA reductase and peroxisome proliferator-activated receptor- $\alpha$ , respectively), both should lower intracellular oxidative stress by decreasing downstream intracellular-reactive oxygen species generation.

Increasing oxidative stresses eventually leads to loss of control over melanosomal regulation and compartmentalization. Qualitative and quantitative abnormalities of melanomas during melanoma progression have been noted for some time (29, 30); however, the functional consequences of the altered melanosome during melanoma pathogenesis has not been appreciated nor studied despite the extensive functional studies of melanosomes in nonmalignant disease states such as the Griselli and Hermansky-Pudlak syndromes (31, 32). In this regard the recent description of an individual with a CDKN2A/p16 homozygous mutation and multiple nevi attributed to a concomitant glucose-6-phosphate deficiency (which produces intercellular oxidative stress) is of considerable interest (33). Consistent also with our overall hypothesis is that dietary antioxidants appear to have a protective effect against melanoma development (34). However, although antioxidants should be protective against oxidative stress early in pathogenesis, they could enhance melanoma cell survival once antioxidant deficiency occurs secondary to the

constitutive oxidative stress; that is, repletion of antioxidants in this setting may further protect the fully transformed melanocyte (Fig. 1; Ref 35).

Our hypothesis provides a biological framework from which to further explore etiologic considerations in human melanoma. We postulate the following: heavy metals and other redox-active compounds that bind melanin play a cocarcinogenic role in melanoma pathogenesis. These metals could include Cu, Fe, Mn, and Co. (A number of other rarer metals including Sc, La, In, Al, Zn, and Cd also found in melanin can increase the semiquinone radical and would produce a similar oxidative stress). The uptake of metals into cells is specifically regulated by a family of enzymes known as the metallothioneins (36–38). Polymorphisms of the isoenzymes have been little studied, and a molecular epidemiological study of these enzymes and possible polymorphisms in individuals at risk for melanoma should be done. Recently, overexpression of one of these enzymes has been associated with a poor prognosis in patients with melanoma (39). Nonmetals of particular interest that also bind melanin would include organic amines, polychlorinated biphenyls, pesticides, herbicides, and dyes (12–16), and further study of risk for melanoma using agricultural health data bases might be informative. Advances in occupational epidemiology and the ability to measure metals at low concentrations in individual cells in tissue (e.g., inductively coupled plasma spectrometry, inductively coupled plasma-mass spectrometer; Refs. 40 and 41), should allow more direct assessment of this issue than has been possible in the past. Additionally, further studies of UV, metal, and melanin interactions, both in reconstituted chemical systems and in cultured melanocytes or partially transformed melanoma cells (e.g., nevus or radical growth phase melanoma cells) should be particularly informative.

The increased intracellular-reactive oxygen species generation should lead to DNA damage. The recent demonstration that BRAF is an early, if not the earliest mutation that leads to immortalization, provides a specific target for exploration (42) as well as the Rb and p53 genes, which are frequently mutated during melanoma pathogenesis, although later in the carcino-

genic cascade (43). Recently, the prospects for chemoprevention of human melanoma have been reviewed (44); most of the interventions proposed were based on generalized observations of signaling alterations and lacked a specific etiologic or pathogenic rational. We propose that focusing on the earliest stages of pathogenesis in the context of a hypothesis with a logical mechanistic basis may be more productive when the mutational damage to the melanocyte is minimal.

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