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16 New Perspectives on Melanoma Pathogenesis and Chemoprevention

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Abstract

Epidemiologic studies implicate ultraviolet radiation (sunlight) as an etiologic agent for the pathogenesis of melanoma. However, the experimental evidence is less convincing. We present information from recent experimental findings that elevation of reactive oxygen species follows from melanin serving as a redox generator, and that this may play an important role in the etiology and pathogenesis of cutaneous melanoma. These observations offer a new paradigm for the development of preventive (and therapeutic) approaches to this disease.

Introduction

The incidence of melanoma has increased rapidly in the last few decades. Fortunately, early detection and prompt surgical intervention has increased 5-year survival in 1970 from 50% to nearly 90% today. Nevertheless, the morbidity from this disease is considerable and the years of productive life lost still high. Epidemiologic and experimental data has confirmed that ultraviolet light radiation (UVR) is etiologic for most cases of non-melanoma skin cancer. However, the relationship and correlation of UVR and melanoma is much more complex. Its definitive involvement in the direct etiology and pathogenesis of the disease is problematic and requires a better mechanistic understanding and/or reconsideration of its involvement (Berwick et al. 2005).

Epidemiologic data suggests that "at best" UVR accounts for only 40%–50% of the attributable risk for melanoma. The data is not simple, and unlike non-melanoma skin cancer, the risk is not correlated with cumulative UVR exposure, but rather, intermittent exposure, especially serious sunburns earlier in life. Also, intensive "sun sense" campaigns have not led to a decrease in the incidence of melanoma, although evidence does suggest that regular exposure to sunlamps may indeed be harmful.

Implicating UVR is also difficult at a molecular level as UVR-signature mutations, although uniformly found in non-melanoma skin cancer, are rarely detected in benign nevi, dysplastic nevi, or in primary or advanced melanoma cells. Furthermore, direct transformation of human melanocytes with UVR has not been successfully accomplished, despite many attempts to do so. Recent studies of the distribution of BRAF mutations in primary melanomas suggest that there are at least two pathways: one in which chronic sun damage is not associated with BRAF mutation and a second in which BRAF mutations occurred in melanomas that developed in skin that was not sun-damaged . (Curtin et al. 2005). In neither case were classical UVR mutations in the BRAF gene evident. However, the development of a transgenic mouse model that, after UVR exposure at the neonatal stage, does lead to melanomas that simulate the human disease pathologically may help elucidate the etiologic and pathogenic role of this carcinogen (Ha et al. 2005; Wolnicka-Glubisz and Noonan 2006). However, this model is highly engineered (hepatocyte growth factor and stem cell factor have been introduced) and its direct relevance to understanding the pathogenesis of human melanoma remains to be established.

In toto, this data suggests several distinct possibilities: UVR's transforming effect on DNA is mediated by reactive oxygen species (ROS) or other molecules; UVR works indirectly to transform melanocytes via a paracrine effect; UVR works in concert with a yet to be identified co-carcinogen; or that the epidemiologic results are spurious and UVR is not involved at all in melanoma pathogenesis.

Developing a New Paradigm for Melanoma Etiology and Pathogenesis

The adverse results of the β-carotene and lung cancer prevention trials in which this nutrient (although at pharmacologic doses) led to more, rather than fewer, lung cancers in heavy smokers (Omenn et al. 1994) led me to reconsider the role of antioxidants in carcinogenesis, especially in melanoma genesis. One of the unique features of melanocytes is that they produce the unique differentiation product melanin whose major function has always been presumed to be protection against UVR. There are several unique features about melanin and its synthesis that merit comment:

- Hydrogen peroxide is generated and consumed during the synthesis of melanin.
- Melanin synthesis occurs in a complex and poorly understood complex organelle, the melanosome, which has many lysosomal properties.
- Melanin functions as an antioxidant in normal melanocytes.

For some time, it has been recognized that abnormalities of melanin synthesis lead to a range of benign pigmentary diseases. There is also available considerable descriptive data that has suggested melanosomes are abnormal in melanoma cells and became progressively deranged during the pathogenic process (Rhodes et al. 1988). However, the functional consequences of these abnormalities for transformation have been largely ignored.

Redox Status of Melanocytes and Melanoma Cells

We initially asked a simple question: How do melanocytes respond to oxidative stress? (Meyskens et al. 1997). The conclusions from our studies are summarized as follows, and illustrated in Fig. 1. Melanoma were exposed to a low dose of H_2O_2 generated by adding titered amounts of glucose oxidase to the medium, and with a fixed dose of glucose, a predictable amount of H_2O_2 was generated. (Using UVR-B as the source of ROS was too complex as it produces many cellular effects including direct DNA damage.) The intracellular oxidative response was determined by luminol-enhanced chemiluminescence, a crude signal for superoxide/peroxide flux. The results were surprising, in that no fluorescence signal was apparent in several non-melanin-containing cell lines. In normal human melanocytes (NHM), a small signal was initially generated, but rapidly suppressed over a few minutes. In contrast, a large and continuous chemiluminescence response was seen in all melanoma cell lines tested. The signal was quickly abrogated by added exogenous catalase in both melanocytes and melanoma cells. However, when exogenous superoxide dismutase was added, the luminescence signal was mildly decreased in melanocytes but greatly enhanced in all melanoma cells tested. We postulated that melanoma cells contain a potential generator of superoxide anion that was not found in melanocytes or other cells.

Further studies using various redox-sensing probes indicated that melanoma cells have increased intracellular ROS at all stages of the cell cycle compared to melanocytes, implicating that the elevation was largely due to superoxide anion (Meyskens et al. 2001). Subsequently, an electrochemical model of eumelanin (dihydroxyindole polymerized on a graphite surface) (Gidanian et al. 2002) and was used to measure ROS generation, as measured by spin trapping molecules of superoxide and hydroxyl radicals. It was demonstrated that exposure of synthetic eumelanin to oxygen led to that the generation of ROS, markedly enhanced by the addition of transition metals (Farmer et al. 2003). Previous speciation characterization had suggested that an

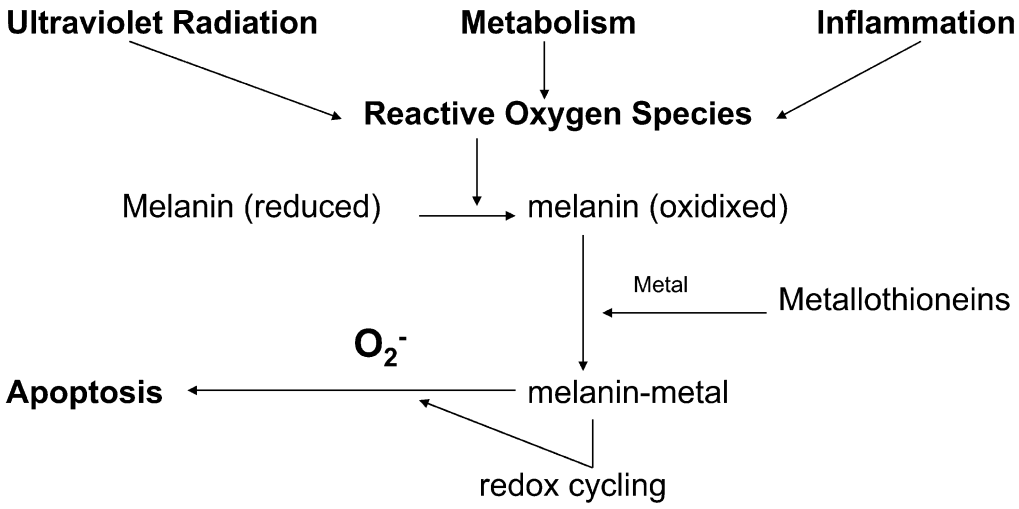

Fig. 1 The altered state of melanin during melanomagenesis. Melanin is normally in a reduced antioxidant state. Exposure to ROS leads to an oxidized condition which facilitates binding of transition metals (and other compounds such as polyphenol chlorinated biphenyls). Metal uptake into the cell is closely regulated by a family of metallothioneins. This situation sets up the oxidized melanin as a redox generator, which leads to increasing intracellular oxidative stress, a widespread adaptive response including TF upregulation, cell death, a high rate of cellular turnover and secondary intrinsic drug resistance (see Meyskens et al. 2001)

oxidized species within the melanin, a quinone imine, could serve as a powerful metal chelator (Spoganicz 2002), and this might then enhance the pro-oxidant generation of ROS. Importantly, the ROS generated by the synthetic melanin behaved in the same manner as the melanoma cell lines, in that exogenous catalase quenched the signal but superoxide dismutase greatly enhanced the signal (Farmer 2003); and using the EPR and DNA clipping assays, the similar phenomenon as described above was demonstrated with intact melanoma cells, reconfirming the initial luminescence experiments. These results suggested that melanin in melanocytes became pro-oxidant during the transformation process; utilizing the idea that metals may enhance this behavior, we have designed a number of lipophilic chelators (Farmer et al. 2005) as candidate chemotherapeutic drugs.

Part and parcel of our observations has been documentation of the constitutive upregulation of redox-sensitive transcription factors-TFs (AP-1 and NF-KB) in melanomas (Meyskens et al. 1999; McNulty et al. 2001, 2004; Yang et al. 2004; Yang and Meyskens 2005). A surprising result was that enhancement of oxidative stress led to further TFs upregulation. We therefore wondered what addition of an antioxidant would do and fortuitously chose PDTC as the antioxidant. Much to our surprise, PDTC produced apoptosis in the melanoma cells, even at very low concentrations. With the realization that PDTC is both an antioxidant and chelator, we tested a series of chelators on melanoma cell growth. Antioxidants had little effect but transition metal chelation produced a strong apoptotic effect. One such compound, disulfiram, was readily available, as it has been used for over 50 years, as an anti-alcohol aversion drug and was found to be active as an antimelanoma therapy at nanomolar concentrations (Cen et al. 2002, 2004). Based on these and other studies, we have now initiated a phase II trial of disulfiran plus arsenical trioxide for patients with advanced melanoma disease.

Based on these findings, we postulated that the etiology and pathogenesis of melanoma is a redox-driven process that offered opportunities for understanding the etiology and patho-

genesis of the disease as well as providing entry points and targets for developing new therapies (Meyskens et al. 2004).

A remarkable feature of this phenomenon is that melanosomes themselves become more and more abnormal and melanin particles are released extracellularly during the progression of melanoma genesis (Jimbow et al. 1989; Rhodes et al. 1988). Our preliminary data indicate that these abnormal melanosomes are a potent source of intra- and extracellular superoxide anion.

Epidemiologic Evidence for Metal Involvement in Melanin Etiology and Pathogenesis

There is a considerable amount of occupational epidemiology that suggests that high exposure to metals or polyphenol chlorinated biphenyls (PCPs) is a risk factor for melanoma (Austin and Reynolds 1986; Loomis et al. 1997). Relative risks for printers, lithographers, electrical utility, and semiconductor workers are consistently elevated (1.5–4.0), and in many studies a dose and time exposure effect are evident (i.e., Loomis et al. 1997). Dietary studies also implicate excessive alcohol ingestion as a risk factor (RR above 2.0) (Millen et al. 2204), probably working through its metabolite acetaldehyde, which results in increased ROS. In contrast, broad dietary studies suggest that dietary antioxidants are protective against melanoma development (RR = 0.7).

No genetic studies of metallothioneins that regulate metal uptake or their polymorphisms and melanoma risk have yet been reported. However, one large study of this enzyme has shown that MT expression is an unfavorable prognostic sign and can identify those thin melanomas (<1.5 mm) that are aggressive (Weinlich et al. 2005).

Upregulation of Transcription Factors in the Pathogenesis of Melanoma

We have shown that AP-1 and NF-kB are elevated early in the pathogenic process, are protective against apoptosis-inducing events, and respond to redox stress by further upregulation in response to increased ROS. We have recently focused on the multifunctional protein apurinic/apyrimidinic endonuclease/redox effector 1 (APE/Ref-1), which functions in both the third step in base excision repair and also reduces a wide range of TFs so that they can bind to DNA sites and effect their action (Yang et al. 2005). We have recently shown that the polyphenoic antioxidant resveratrol binds to the redox pocket of Ref-1 and slows melanoma growth. We are in the process of designing a number of inhibitors based on this lead compound and by using an iterative structure–function approach and three-dimensional software modeling.

Conclusions

Our studies of the role of reactive oxygen species in melanoma genesis have led us to several conclusions. During the process of transformation and progression of the cutaneous melanocyte:

1. Melanin is converted from an antioxidant to a pro-oxidant, takes on properties of a metal chelator, becomes a redox generator, and produces large amounts of superoxide anion.
2. Intracellular oxidative stress increases markedly during pathogenesis and produces a cascade of adaptive responses with transcriptional factor activation including AP-1, Ref-1, and APE/Ref-1 that lead to drug resistance.
3. An understanding of these events provides a new scientific basis for developing novel preventive (see Meyskens et al. 1994) and therapeutic approaches (see Meyskens et al. 2001) to melanoma management (see Meyskens 2003).

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