

**For PASPCR Commentary (September 2006)**

**PATHOGENESIS AND CHEMOPREVENTION  
OF HUMAN CUTANEOUS MELANOMA: REDOX GONE WRONG**

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**Why did we become interested in the current approach?**

Our laboratory has investigated the potential experimental therapeutics of human melanoma for quite some time including examining the role of tumor stem cells (before it became popular) and the activity of retinoids, antiestrogens, gamma-interferon and others. This current commentary represents one in a series in which the history and contributions of my laboratory have been detailed (1,2). This current commentary details some alternative concepts regarding the etiology and pathogenesis of human melanoma that has evolved from our investigators into redox modulation over the past decade.

In the early nineties we were one of the clinical units of the randomized trial to prevent lung cancer in heavy smokers using  $\beta$ -carotene and retinol (CARET) and accrued over 4,000 of the total 19,000 participants. We, as well as the field of chemoprevention, were shocked when the final results came in: the participants taking  $\beta$ -carotene had more, rather than fewer, lung cancers and cardiovascular events (a), a result similar to the ATBC trial conducted in Europe (b). These results prompted me to learn more about oxidation and I took a very informative sabbatical for 6 months in the laboratory of Helmut Sies (Dusseldorf), who was and remains one of the best chemists in the arena of oxidation/antioxidation. Working among a large group of chemists was indeed a humbling experience!

**What evidence have we and others generated that intracellular oxidative stress is important in the pathogenesis of human melanoma?**

Shortly after returning home I began thinking about the biology of human melanoma again and was struck by the diverse and vast literature regarding melanin synthesis and its role in benign pigmentary diseases such as albinism and vitiligo. Melanin synthesis represents a complex series of tightly regulated steps involving the consumption of oxygen and superoxide and the production and utilization of hydrogen peroxide, all carefully orchestrated within the well-organized organelle, the melanosome. Although it had been known for some time that melanosomes become progressively disordered during melanogenesis(c) neither the etiology of this disruption nor its functional consequences had been explored.

With this realization there were many avenues we could have pursued. The first question we posed was: how do normal melanocytes and melanoma cells handle a peroxide stress? The results were surprising; melanocytes efficiently abrogated the peroxide stress while melanoma cells were seriously impaired in their ability to do so and generated higher levels of reactive oxygen species (ROS); of great interest was that when superoxide dismutase (SOD) was added an enhanced intracellular oxidative response occurred in melanoma cells that did not occur in melanocytes (3).

It would be fair to say that the better part of the next eight years was spent trying to explain this phenomenon, which we have explored in a large number of studies involving the use of sophisticated molecular probes to assess intracellular oxidation (4), the determination of the relationship of level of intracellular hydrogen peroxide and superoxide anion to NF $\kappa$ b and AP-1 expression (5-9), redox characterization of



**Why do we think metals are important in the etiology and pathogenesis of melanoma?**

There are three major lines of evidence that suggest that some substances that bind melanin contribute to the etiology and pathogenesis of human melanoma.

- (1) Our experimental evidence in which we demonstrate that certain transition metals result in redox recycling and the generation of ROS in oxidized melanin and in melanosomes prepared from melanoma cells but not melanocytes.
- (2) The elegant work of Pavel and colleagues in which metals are implicated in the formation of dysplastic nevi (d,e)
- (3) The studies of Picardo and colleagues on the role of antioxidants in melanocytes of patients with melanoma (f,g).
- (4) An extensive (and largely forgotten) epidemiologic literature that implicates certain metals and polychlorophylbiphenyls (PCB) in the etiology and pathogenesis of melanoma (h,i).

**What are the appropriate models in which to test our overall hypothesis?**

This is a very difficult question. To date an in vitro model that simulates well the human disease has not been developed despite many attempts by many investigators to do so. For the past five years our group has been developing an in vitro chemoprevention model system and settled on using cells from a radical growth phase melanoma to measure the effects of candidate chemoprevention agents (14). We will extend this model to study the effect of UVR-B and metals on various parameters of transformation. Recently, we have also completed some difficult studies with human melanocytes and

have found that UVR-B plus certain metals result in the generation of cells that phenotypically resemble melanocytes from dysplastic nevi in culture. We will continue to characterize this system as well as to explore its utility as a platform for assessment of chemoprevention agents.

There have been many murine and transgenic models proposed for the study of human melanoma. Although many of these models are useful for studying various biologic, genetic, and molecular properties of melanoma cells, we (and many others) consider the HGF/SF transgenic model of Noonan and Merlino as the best in its representation of human melanoma, both in terms of its response to UVR and in the tumors that form which closely resemble human melanoma histologically and in their growth patterns (j,k). We will therefore use this model as the major source of animal experiments that will begin in the near future.

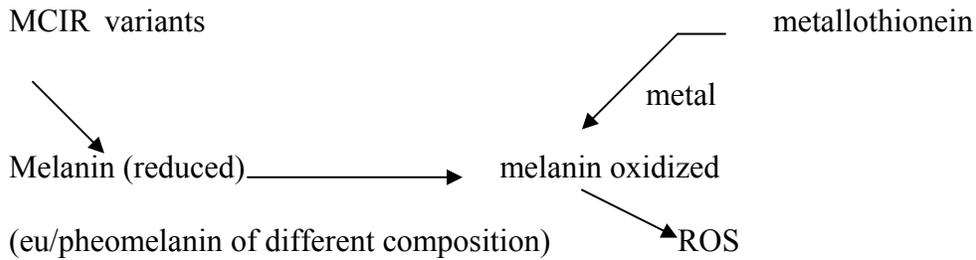
### **So what about ultraviolet radiation?**

It should be explicitly clear that we have not gone off the deep end and abandoned UVR as important in the pathogenesis of melanoma. Extensive epidemiologic observations support a complex role for sunlight in melanoma etiology and pathogenesis. However, a striking feature of melanoma is the inability to detect thymine dimers or other classical UVR induced mutations in primary or metastatic melanomas, even in genes of interest. This finding suggests either that UVR-B is not involved, that the UVR-effect is indirect (via  $\sqrt$   $H_2O_2$  generation or via an effect on signaling pathways), functions via a hit and run genomic mechanism (i.e. like a virus?), or works through some other as yet undiscovered mechanism. Alternatively, we favor the explanation that UVR effects its outcome via oxidation of

melanin (and perhaps critical other macromolecules) in susceptible individuals and the establishment and ongoing maintenance of redox-cycling with low grade ROS generation resulting in diffuse genomic damage. We propose that two major intrinsic contributors to susceptibility to UVR are the types of melanin formed by individuals and the capacity to uptake metals, a property which is very heterogenous.

For quite some time there has been abundant controversy regarding the relative role of UVR-A (320-400 nm) and UVR-B (290-320 nm). In the past few years genetic analysis of primary melanomas has shown that the relationship of UV exposure, B-Raf mutations and melanoma is complex (l) and recently these interactions have been further explored by assessing the role of the MC1R gene on melanoma susceptibility (m). Since MC1R isoforms undoubtedly have a downstream effect on the type of melanin formed, (not explored as far as we know) we speculate that the MC1R isoform effect on susceptibility that is seen is a reflection of redox events involving the particular constellation of eu- and pheomelanins formed in response to melanin and UVR. Metals and other substances bind melanin, especially its oxidized form, setting up redox cycling. Noteworthy in the epidemiology literature of melanoma is a great deal of relevant information about the influence of melanin-binding substances, such as heavy metals and PCBs and risk for melanoma(h,i).

Metal uptake is closely regulated by an extensive family of genes called metallothioneins. We postulate that susceptible individuals have a different range of metallothionein isoforms; to date this potential phenomenon has not been characterized. Our working model then would be expanded:



### **Why are melanomas rare in Black and White Albinos?**

One of the most puzzling clinical observations in dermatology about melanoma is the following: non-melanoma skin cancers occur with high frequency in black (and white) albinos and yet the development of cutaneous melanoma is rare (n,o). Why is this? Melanocytes are still present but they do not make melanin. We propose that without melanin melanomas do not occur. If so, then why don't darkly pigmented individuals develop melanoma at a high frequency? There are many well-established (and thought out) answers to this latter question that have been addressed by many others including the role of eumelanosomes (versus pleomelanosomes), the differences in intracellular distribution of melanosomes in people with different ethnic backgrounds, and most recently the differential role of keratinocytes obtained from different ethnic skin in determining melanocyte responses to UVR.

There are, of course, many potential confounders of our overall proposition. Our work is based on the central hypothesis: oxidation of melanin in susceptible individuals (MCIR? and metallothionein? variants) by UVR is one of the earliest pathogenic events in the evolution of melanoma and the conversion of melanin from an anti-oxidant to a

pro-oxidant with ongoing generation of ROS leads to widespread genomic damage and widespread altered cellular responses. (This current commentary has been on pathogenesis; in a future commentary for the Society of Melanoma Research, we will explore the relevance of our findings to the phenomenon of drug resistance) and the development of new drugs for the treatment of melanoma ( 13-19).

We will continue to provide experimental support for this new central hypothesis and to study the effect of candidate chemoprevention compounds in the models described. Eventually an appropriate clinical chemoprevention trial in patients at high risk for melanoma development will be undertaken (20). It's going to be a long journey, but we need to start somewhere because all other non-surgical therapeutic approaches to this disease except perhaps interferon in a limited subset of up patients have failed (with apologies to my hard-working immunological colleagues).

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