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Melanoma and Redox: “A riddle wrapped in a mystery inside an enigma”*

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I very much liked the title of the SMR article authorized by David Fisher in the last issue of this series, “Dissecting the melanoma enigma: learning from the details”. The title in our contribution continues the “enigma” theme

We have recently proposed: a new paradigm for thinking about melanoma pathogenesis based initially on attempting to unravel the redox function of melanin, a Byzantine molecule with an uncertain structure; indeed, with something for anyone who looks. Although melanin has classically been referred to as a polymer, it probably is not. Following a sabbatical in 1995 (see 1, for reasons totally unrelated to melanin or melanoma) in a redox chemistry laboratory, my laboratory began to explore the phenomenon of redox metabolism in normal melanocytes and melanoma cells.

Our initial experiments produced the surprising result that human melanoma cells generated an oxidative burst in response to an oxidative stress (low concentrations of hydrogen peroxide) while in melanocytes the initial burst was abrogated and then suppressed in contrast to that seen in melanoma cells in which the response persisted (2). There was no easy way to explain this finding but we postulated that the melanin in melanoma cells was different than that in melanocytes and that when oxidized became a superoxide generator. Using electroparamagnetic measurements and spin traps our group has demonstrated in melanoma cells that our initial hypothesis was probably correct (3,4). We have also shown that the response of redox-sensitive transcription factors (NF-KB and AP-1) was different in melanoma cells compared to melanocytes (5-7) in that: (1) The family members were different and in the case of NF-KB were mutated and (2) The response to redox stress resulted in further up regulation of the transcription factors in melanoma cells while down regulation occurred in melanocytes.

* With apologies to Winston Churchill's famous statement "Russia is a riddle wrapped in a mystery inside an enigma". ¹

These series of studies led us to propose a new paradigm for the pathogenesis of melanoma which has both preventive and therapeutic implications (8). The rationale goes like this: Epidemiologic observations attribute ultraviolet irradiation as etiologic in about 40-50% of cases. However, classical UV-mutations have not been documented in melanomas, either overall or in likely relevant genes. Either UV is working indirectly, the process is "hit and run" or UV is a surrogate for an as yet unidentified risk factor(s). A series of experimental studies suggest that reactive oxygen species play a role in the pathogenesis of melanoma. Our initial data indicates that oxidation of melanin, which is normally an antioxidant, and its "transformation" to a pro-oxidant is an essential early feature of melanoma pathogenesis. Based on this observation, we proposed that:

- (1) Either melanin oxidation is a primary event or a genetic abnormality leading to this condition is fundamental (e.g, mutation of NADPH oxidase or of a melanosomal structural protein);
- (2) Oxidized melanin provides a unique pathway for the development of drugs targeted to the putative quinone-imine (a chelator) in oxidized melanin; These leads provide new and varied opportunities to develop chemoprevention and treatment strategies for the management of human melanoma;
- (3) Oxidation of mitochondrial DNA leads to unique respiratory chain mutations that convey a unique drug resistance phenotype; and
- (4) Activation of the multifunctional protein, apurinic-apyridinidic endonuclease/redox-effector factor – 1 (APE, Ref-1), as an adaptive stress response that keeps transcription factors in a reduced state(discussed below).

A consequence of this process is that the use of antioxidants as preventive agents may be useful, but as therapeutic agents may lower the oxidative stress level in melanoma cells and actually enhance survival. A considerable amount of epidemiologic data also suggests that substances that bind melanin (e.g. heavy metals) are co-factors in melanoma etiology (9). However, experimental evidence needs to be developed to bolster this theory and more epidemiologic studies need to be done since most of the extensive data is old.

Another important, but forgotten, consideration is that the melanosomes in melanoma cells are abnormal (10). The functional consequence of these abnormalities are largely unexplored despite the fact that there are many benign pigmentary defects that are secondary to melanosomal redox problems (11). Of

specific interest are the recent findings that dysplastic nevi have abnormal melanosomes and exhibit an oxidative stress phenotype (12, 13).

We have developed a therapeutic strategy of enhancing the oxidative stress and producing cell killing by overriding the adaptive protective response (14). We have identified disulfiram (the alcohol aversion drug-and chelator) as a potent inducer of apoptosis in melanoma cells at nanomolar concentrations (15, 16). Although we are not entirely sure why DSF is so active we postulate that it may be a combination of its effect on oxidizing the mitochondrial transition pore and the appearance of mitochondrial DNA mutations that accumulate in melanoma cells (17). We are just beginning to explore the functional consequences of these mutations. Based on these initial findings, Pat Farmer in our Department of Chemistry, has been synthesizing a series of lipophilic chelators (18) which are being screened in vitro for therapeutic activity.

All of this work has caused us to think about how melanoma cells live and survive under constant oxidative stress: These considerations have led us to the remarkable multi-functional protein APE/REF-1 which was discovered about 15 years ago (review 19) and is being explored as a therapeutic target in brain tumors (20). This protein both serves an essential function as the critical enzyme in DNA base excision repair and also reduces a wide variety of nuclear transcription factors thereby facilitating their DNA binding and transcriptional activation. We have recently reported our extensive findings in melanoma (21): APE/Ref-1 is markedly up regulated and we have found, using three-dimensional modeling that the phenolic antioxidant resveratrol fits nicely into a deep druggable pocket in the redox-regulation domain. We are preparing to combine high through-put screening, three-dimensional modeling, and interactive chemical synthesis to identify new structures.

Our overall strategy to explore the redox features of melanocytes and melanoma cells nearly ten years ago has taken us to new and unexpected places. Our long term strategy is to develop rational combination therapy based on the principles that our basic studies are elucidating. By the way, we have three open postdoc positions: molecular basis of DSF action, functional consequences of mitochondrial DNA mutations, and molecular biology of AP-1/APE-Ref-1. Contact Pfarmer@uci.edu for the first and flmeyske@uci.edu for the last two.

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