Title
Redox regulation in human melanocytes and melanoma.

Journal
Pigment cell research / sponsored by the European Society for Pigment Cell Research and the International Pigment Cell Society, 14(3)

ISSN
0893-5785

Authors
Meyskens, F L, Jr
Farmer, P
Fruehauf, J P

Publication Date
2001-06-01

Peer reviewed

PIGMENT CELL RES 14: 148–154. 2001
Printed in Ireland—all rights reserved

ISSN 0893-5785

Invited Review

Redox Regulation in Human Melanocytes and Melanoma

FRANK L. MEYSKENS JR.[1], PATRICK FARMER[2] and JOHN P. FRUEHAUF[1]

[1]*Department of Medicine (Hematology/Oncology), Chao Family Comprehensive Cancer Center, University of California, Irvine, Orange County, California*
[2]*Department of Chemistry, Chao Family Comprehensive Cancer Center, University of California, Irvine, Orange County, California*
**Address reprint requests to Dr. Frank L. Meyskens, Jr., Chao Family Comprehensive Cancer Center, University of California, Irvine, 101 The City Drive, Rt. 81, Bldg. 23, Room 406, Orange County, CA 92868-3298. E-mail: FLMeyske@uci.edu*

Received 30 January 2001; in final form 22 February 2001

The human melanocyte is continuously exposed to intrinsic and extrinsic sources of reactive biochemical species, but is finely tuned via the intrinsic anti-oxidant and radical properties of melanin to suppress the build-up of an altered redox phenotype. We propose that this control is lost during melanomagenesis and inappropriate redox-sensitive transcriptional factor activations occur which result in enhancement of an anti-apoptotic phenotype in the transformed cell. This conceptual framework offers testable steps to determine the role of redox alterations in the carcinogenic evolution, prevention and treatment of melanoma and other diseases of the melanocyte.

Key words: Transcription factor, NF-κB, AP-1, Reactive oxygen species, Glutathione, Superoxide anion

INTRODUCTION

The melanocyte, which is uniquely poised to accomplish its primary function of delivering melanin to keratinocytes, is under continuous low-grade oxidative insult (Fig. 1). Within the melanocyte the synthesis of its sine qua non-product (melanin) results in the generation of hydrogen peroxide. If inappropriately processed within the melanosome, this molecule can lead to the generation of hydroxyl radicals and other ROS. Melanin itself can serve as both an anti-oxidant and pro-oxidant depending on its redox state, the presence of metal ions, and potentially its state of aggregation. The implications of these diverse sources of generalized oxidative stress for melanocytes and their potential consequences during or contributing to carcinogenesis of this cell has been little explored. In this review, we emphasize the role of redox in melanocyte transformation and melanoma cell proliferation. A growing literature suggests that a review of redox regulation by melanin stimulating hormone (MSH) and in such diseases as vitiligo would offer new insights into normal melanocyte regulation as well.

The following topics as they relate to redox control will be covered in this review: melanogenesis, melanin synthesis and glutathione (GSH), melanin as an anti-oxidant and cellular pro-oxidant, response of melanin to ultraviolet (UV) light, anti-oxidant levels and melanomagenesis, redox regulation of transcription factors, and a conceptual framework for the pathogenesis of melanoma based on altered redox control.

In response to UV light an inflammatory response is generated involving the production of massive amounts of various cytokines and growth factors by keratinocytes, a vigorous inflammatory/immunologic host response, and in some cases angiogenesis. Each one of these responses generates or stimulates the production of reactive oxygen or nitrogen species. Additionally, UV light interacts directly with biochemical constituents of the melanocyte to generate intracellular reactive oxygen species (ROS), hydrogen peroxide and/or superoxide anion. The sum total of these alterations is that melanocytes are subjected to an panoply of redox changes with secondary effects on melanin synthesis and a variety of signaling cascades.

We suggest that alterations in these processes are fundamentally involved in the pathogenesis of melanoma and perhaps other pathologic states. These observations encum-

Abbreviations – GST, glutathione-*S*-transferase; GSH, glutathione (reduced); ROS, reactive oxygen species; UV, ultraviolet light; EPR, electronic paramagnetic resonance

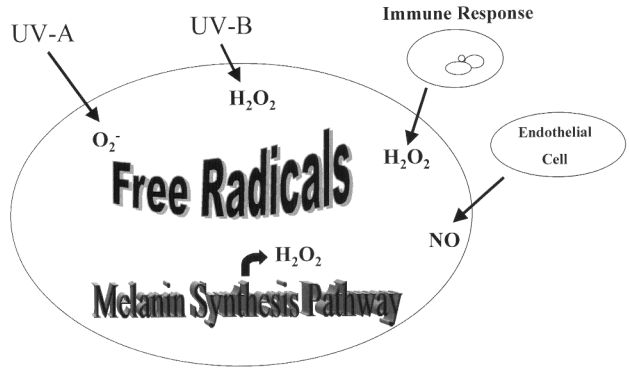

Fig. 1. The natural condition of the melanocyte assures exposure to multiple sources of oxidative stress.

ber the seeds of novel therapeutic strategies for melanoma, as well as harboring etiologic implications.

Melanogenesis

Melanins are polymeric pigments formed from successive oxidations of tyrosine. Two general types are known (Fig. 2), black eumelanins formed by the polymerization of dihydroxyindole precursors and red/brown pheomelanins, that are colored as a result of cysteine incorporation during oxidation (1). In melanocytes, tyrosinase catalyzes two successive reactions, hydroxylation of tyrosine and oxidation of the product, L-dopa. It has been postulated that tyrosine may protect melanocytes from the cytotoxic effects of superoxide anion (2). The product of dopa oxidation cyclizes to a 5,6-dihydroxyindole intermediate, which is highly reactive and upon further oxidation gives rise to eumelanin polymers by a radical-coupling pathway. Pheomelanins are thought to be engendered by covalent linkage of dopaquinone (DQ) with cysteine, which results in the incorporation of a benzothiazine monomer into the polymer (3).

The chemical structure of these pigments is not well defined because of a variety of coupling modes and precursors available. The details of the polymeric structure and bonding patterns have been difficult to characterize, and likely vary in subtle ways. Although often presented as separate forms, eumelanin and pheomelanin are rather qualitative descriptions of a wide variety of native melanins, likely co-polymers with both indolic (eu-) or benzothiazine (pheo-) subunits. Chemical analysis has shown that natural melanins are composed mainly of dihydroxyindole subunits, with a much lower percentage of benzothiazine subunits even in red and blond-colored samples (4, 5).

Melanin Synthesis and GSH

The regulation of GSH metabolism is complex and the reduced form of GSH interacts with the melanin synthesis pathway in a complex manner (Fig. 3). GSH is involved in one of the initial steps in pheomelanin synthesis via its conjugation to DQ by glutathione-*S*-transferase (GST), and in the reduction of hydrogen peroxide evolved during both eumelanin and pheomelanin synthesis via GSH-dependent peroxidase (6).

GSH levels influence melanin synthetic pathways through the formation of adducts with DQ, an unstable intermediate in melanin synthesis produced by tyrosinase action on tyrosine and dopa (7–10). Like cysteinyldopa, glutathionyldopa formation favors pheomelanin over eumelanin synthesis. In addition to neutralizing this highly reactive orthoquinone, GSH metabolism protects melanocytes from the toxic effects of hydrogen peroxides formed during melanin synthesis (6, 11, 12). GSH metabolism, therefore, appears to be critically important to the maintenance of melanocyte and melanoma cell viability.

Interruption of GSH Metabolism and Cytotoxicity to Melanoma Cells

GSH, in conjunction with GST, is also involved in the detoxification of therapeutic agents, particularly alkylating agents (13–15). Therefore, disruption of GSH metabolism should adversely affect melanoma cell viability, and increase

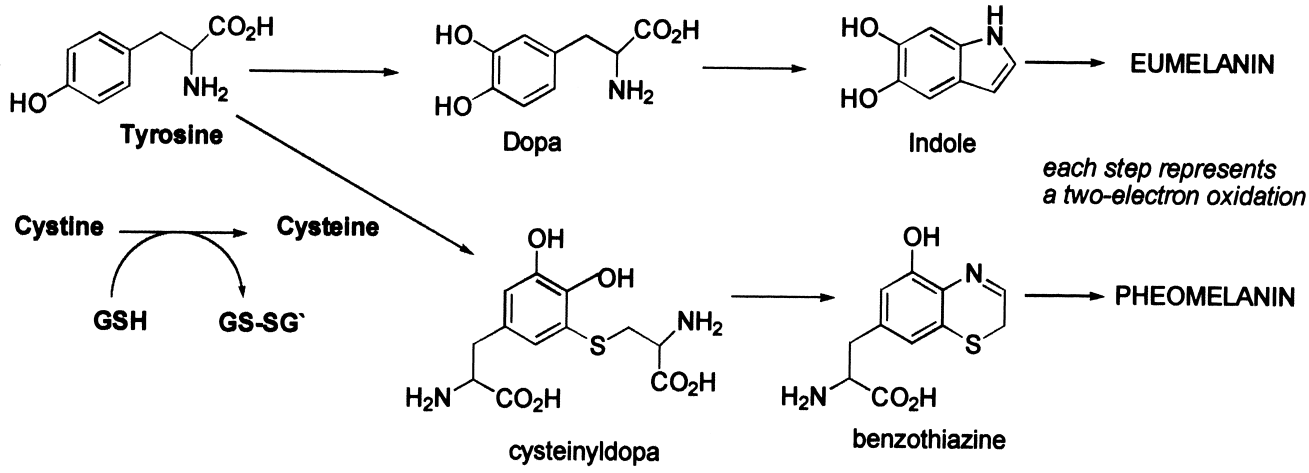

Fig. 2. The major synthetic pathways of melanin synthesis and its relationship to GSH. GSH levels in the cytoplasm are related to cysteine levels but GSH does not directly participate in melanin synthesis as it does not get through the melanosomal membrane.

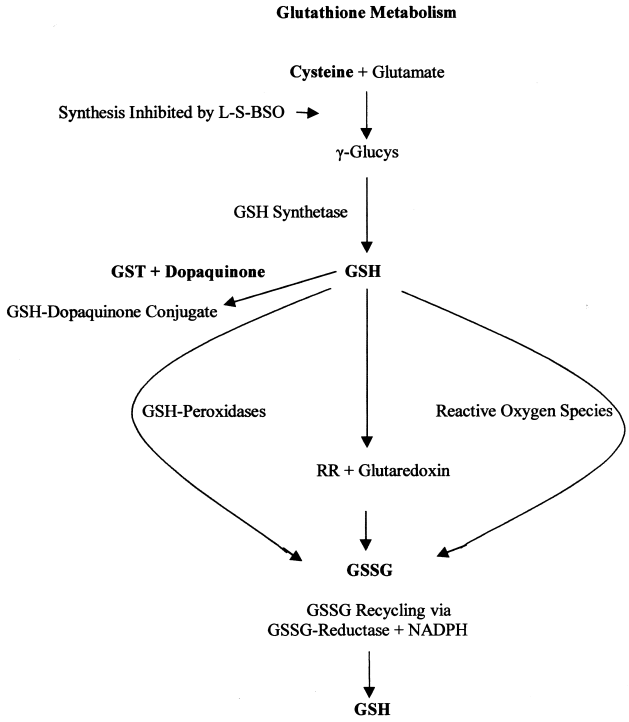

Fig. 3. The regulation of GSH metabolism. GST, glutathione-*S*-transferase; RR, ribonucleotide reductase.

melanoma response to alkylator therapy. Glutathione peroxidase is the primary means for converting peroxides to water. If neutralization of these molecules can be blocked by depletion of GSH, peroxides should accumulate and a vicious cytotoxic cycle would be created.

L-Buthionine-*S*-sulfoximine (BSO) is a selective, irreversible inhibitor of γ-glutamylcysteine synthetase (GCS), the enzyme involved in the first step in GSH synthesis (16, 17). Inhibition of GSH synthesis results in depletion of intracellular GSH over time as GSH-dependent processes consume GSH. The use of BSO as a way to enhance intracellular oxidative stress may provide a new approach to melanoma treatment (18, 19). In one study involving melanoma, a patient with metastatic melanoma treated with standard dose melphalan and infusional BSO was noted to have a partial response after course two (19). After four additional cycles a complete response was attained which was durable for 18 months.

An additional and highly significant aspect of GSH metabolism involves its participation in the synthesis of deoxyribonucleotide precursors of DNA (20, 21). Ribonucleotide reductase catalyses the first unique and rate-limiting step of DNA synthesis by reduction of the 2′ OH group of ribose. Either thioredoxin or glutaredoxin may function as ribonucleotide reductase co-factors by donating reducing equivalents. GSH is utilized by glutaredoxin, while NADPH is used by thioredoxin. GSH is oxidized to GSSG in this reaction, with GSSG recycled back to GSH by glutathione reductase and NADPH (22). In cells that utilize glutaredoxin as the primary ribonucleotide reductase cofactor, DNA synthesis is dependent on adequate levels of GSH. Tumors dependent on the glutaredoxin system have been found to be sensitive to BCNU, which blocks GSH recycling, and to GSH depletion by BSO (23–26).

Schallreuter et al. have found that human melanoma specimens could be distinguished on the basis of their glutaredoxin vs. thioredoxin dependence (23, 24). Amelanotic melanomas tended to have higher levels of thioredoxin and were sensitive to Fotemustine, while those that were melanotic contained more glutaredoxin than thioredoxin, and were Fotemustine resistant. This group has also found that reduced thioredoxin inhibits tyrosinase activity, possibly contributing to the amelanotic character of thioredoxin rich melanomas. These studies indicate that DNA synthesis in some malignant melanomas may be dependent on glutaredoxin, and therefore, sensitive to GSH depletion by BSO. In fact, our data indicate that melanotic melanomas are indeed more sensitive to BSO than amelanotic tumors (26). We have found that melanoma cell lines show significant DNA synthesis inhibition after BSO, which becomes stoichiometrically related to GSH levels in the presence of BCNU, which cripples GSH recycling. The ability of BCNU to inhibit both glutathione reductase and thioredoxin reductase makes it particularly well suited to act in combination with BSO for attacking DNA synthesis. BSO may therefore act both by increasing free radical damage to melanomas and by blocking the formation of deoxynucleotides necessary for repair and synthesis of DNA.

Melanin as an Anti-oxidant

A large body of work has been undertaken on the interaction of melanins with oxygen, hydroxyl radicals, and superoxide (27). The ability of melanin to neutralize ROS has been well documented. Quinol/quinone redox transformations of DHI monomers are the source of this anti-oxidant reactivity (Fig. 4). Benzoquinol, the simplest para-substituted dihydroxybenzene, can both oxidize and reduce superoxide via electron transfer reactions (28), thus, acting as a superoxide dismutase by catalyzing the disproportionation of superoxide to peroxide and oxygen. Similar SOD activity has been demonstrated for isolated melanins (29).

We have reported direct evidence of the ability of cellular melanins to mediate oxidative stress (30). In these studies, cells from various sources were exposed to oxidative stress resulting from generation of H_2O_2 by glucose/glucose oxidase. For all cells tested, an initial build-up of extracellular peroxide was seen, but melanocytes were found to vary significantly, proportional to their melanin content, in their

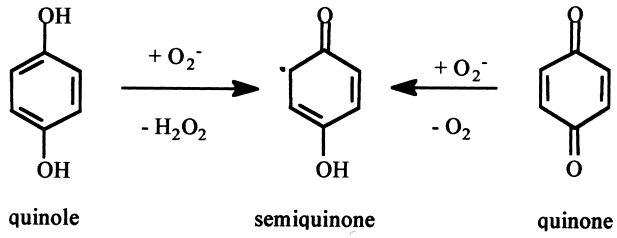

Fig. 4. The mediation of the anti-oxidant activity of melanoma by quinol/quinone transformation.

ability to inhibit this initial build-up. After the initial period, all normal melanocytes were able to neutralize the peroxide to comparable levels, but melanoma cells showed dramatically less ability to neutralize the extracellular peroxide that resulted in a continued build-up over extended periods. Interestingly, the addition of SOD during this time resulted in a 3-fold increase in peroxide, implying that large amounts of superoxide were being generated by the melanoma cells under peroxide stress.

Further evidence of the protective anti-oxidant ability of melanins is found in its use by pathogenic black fungus and bacteria (31). Melanin formation in the cell wall of the pathogen is cited as a virulence factor aiding the protection of the pathogen from normal respiratory burst and microbicidal defenses of the host. For example, the melanin production by *E. dermatitidis* was demonstrated to protect the yeast from being killed in the phagolysosome, even after phacocytosis and oxidative burst by neutrophils (32).

Still, the presence of melanin does not completely negate susceptibility to oxidative stress. Excess hydrogen peroxide decomposes melanin in vitro (33). In vivo it induces higher tyrosinase levels in melanocytes (34). The cytotoxic effect of catechol derivatives towards melanogenic cells has been linked to hydrogen peroxide formation (35).

Melanin as a Cellular Pro-oxidant

A unique feature of melanogenesis is that ROS are generated during the oxidative polymerization (36, 37), the implications of which have not been explored. When the pheomelanin pathway is inhibited, the eumelanin pathway is favored, and 2-fold greater levels of peroxide are produced. As the peroxides formed during melanin synthesis are toxic to the cell, blocking the pheomelanin pathway may be advantageous. Thus, 'factors modulating the levels of H_2O_2 in melanocytes and melanoma cells play critical roles in directing the course of melanogenesis and influencing the potential cytotoxicity of the biosynthetic pathways' (36, 37).

Melanins are known to absorb metal ions in vivo (38); these metal ions dramatically affect the redox status of the polymer. The absorbance of cationic metal ions can affect the quinole/semiquinone/quinone redox state by inducing the deprotonation of the quinole form; e.g., Zn^{2+} binding greatly stabilizes the EPR-active semiquinone radical of dihydroxyindoles (39). The common bio-metals, Fe and Cu, may themselves be involved in specific redox reactivities with melanin and oxygen species. Melanin initiated lipid peroxidation is accelerated by the presence of Fe ions (40). This reactivity is thought to involve Fenton chemistry, in which a redox-active metal mediates the formation of reactive hydroxyl (OH) radicals from melanin-derived superoxide. Fe-induced pro-oxidant behavior has been implicated in the neurodegeneration associated with Parkinson's disease (41). Likewise, diffusible melanin-related metabolites are susceptible to such effects. Dihydroxyindole was found to be pro-oxidant at low concentrations relative to Fe because of reduction of the metal by semiquinone produced by autooxidation (42). Recently, it has also been reported that the aggregate state of melanin affects the redox state (43), which may have substantial implications during the processing of melanin precursors during melanosome biogenesis (44, 45).

Response of Melanin to UV light

UV light has been generally accepted to lead to altered ROS levels in mammalian cells (46), although this phenomenon has been little studied in melanocytes. It is generally accepted that melanin is produced to shield the skin against photodamage. UV radiation is the main physiological stimulus for human skin pigmentation; recent work suggests this process may be mediated by nitric oxide activation of guanylate cyclase, in manner similar to the initiation of endothelial cell relaxation (47). It has been well characterized that UV irradiation of both eumelanin and pheomelanin can lead to the production of superoxide and peroxide from reaction of the photo-excited pigment with oxygen (48–50), but the photo-yields are small compared with the ability of melanin itself to attenuate such ROS. Melanoma cells, even with low levels of endogenous anti-oxidants such as GSH, are relatively resistant to photo-induced stress (51). Similarly, the sensitivity of human melanomas to photodynamic therapy has been shown to decrease with the melanin content of the cells; the melanin acts as a mediator of both oxidative and irradiative stress (52).

Because of the susceptibility of fair-skinned sun-exposed individuals to melanomagenesis, it has been postulated that this cancer results from the effect of UV radiation on pheomelanin substituents or precursors (5). However, the exact nature of the photobiological response is unknown, as is the specific chromophore that initiates it. Prota has found that photoanalysis of 5-*S*-cysteinyldopa by UVA (320–400 nm) leads to oxygen-dependent oxidation producing the quinone (and subsequent oxidative products such as the benzothiazines) and presumably superoxide (53). Irradiation with UVB (280–320 nm) results in desulfurization by cleavage of the C–S bond, yielding dopa as the main product. Previously Koch and Chedekel had suggested the potential mutagenic effects of the radical photodecomposition products of UVB irradiation of 5-*S*-cysteinyldopa (54).

Anti-oxidant Levels and Melanomagenesis

Nearly all cancers have some imbalance in anti-oxidant levels compared with the cell of origin (55). Levels of anti-oxidant enzymes such as MnSOD, catalase, and glutathione peroxidase are typically decreased, as are levels of non-enzymatic small molecular weight anti-oxidants such as GSH and vitamins E, C, and A (56). It was noted over a decade ago that normal cutaneous fibroblasts, keratinocytes, and melanocytes exhibited disparate anti-oxidant enzyme activities in culture (56). Although abnormalities in chromosome 6 and the *MnSOD* gene have been demonstrated in some melanomas (57), this is uncommon and unlikely to provide a genetic or generic basis for the altered anti-oxidant phenotype. Picardo and his colleagues have followed up this observation and found that the anti-oxidant phenotype of normal epidermal melanocytes from patients with melanoma fell into one of two categories: a) the same as melanocytes from normal donors or b) a phenotype resembling melanoma cells: elevated vitamin E and polyunsaturated fatty acids and a lowered catalase and unchanged SOD activity (58–60). Remarkably, a similar anti-oxidant pattern was also seen when uveal melanocytes and uveal melanoma cell cultures were compared (61).

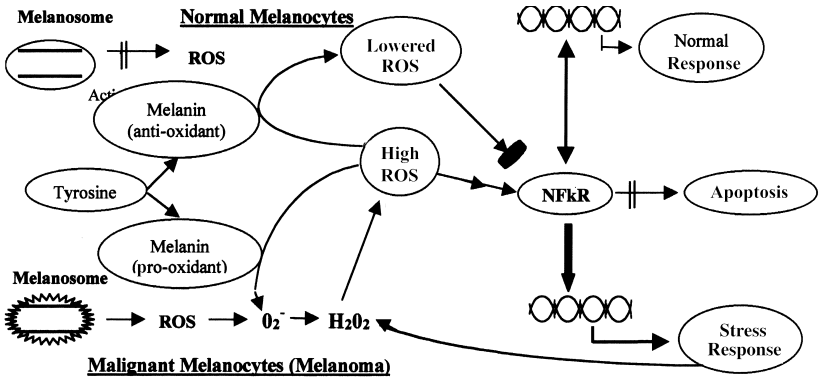

Fig. 5. Proposed evolution of redox changes during melanocyte carcinogenesis.

Increased extracellular production of superoxide anion in melanoma cells has been demonstrated (62). We have shown that intracellular levels of this ROS are probably elevated also, both indirectly using chemiluminescent probes following exogenous oxidant stress (30) and more directly under basal conditions using fluorescent molecular probes (63). In the former situation melanoma cells were exposed to exogenous hydrogen peroxide and ROS measured by luminol-enhanced chemiluminescence. Melanocytes were able to suppress the response while melanoma cells were not. Further, the addition of superoxide dismutase unexpectedly increased the signal in melanoma cells, while having no effect on melanocytes in this situation. This result suggested that under conditions of oxidant stress, melanin in melanoma cells functioned as a pro-oxidant and generated superoxide anion. We propose that continuously elevated levels of intracellular oxidants leads to an altered redox state with complex compensatory mechanisms. One would anticipate that the anti-oxidant enzymes would increase, but this does not seem to occur, as these enzymes are low in melanoma cells. However, these changes have not been measured throughout melanocyte progression so it is possible that these enzymes are elevated early on during carcinogenesis. This is not an unreasonable expectation as pre-neoplasias in many organs have increased enzymatic or molecular changes that are subsequently depressed after the cell is completely transformed (64).

Redox Regulation of Transcription Factors

The major redox-sensitive transcription factors in mammalian cells are NF-κB, AP-1, and thioredoxin (65). We have previously shown that jun and fos regulation is dysregulated in melanoma cells (65). Although these early studies did not explore the mechanistic basis for this phenomenon, we further examined the complex effects of low and high dose UV light B on AP-1 and NF-κB binding in human melanocytes (66). We have recently studied the NF-κB and AP-1 binding in melanoma cells and melanocytes (67, 68). Under basal conditions, NF-κb binding was markedly elevated in melanoma cells and despite this high level was further increased in the presence of a peroxidative stress. In contrast, AP-1 binding was unaffected in melanocytes and decreased in melanoma cells. The molecular basis for these responses is unclear, but we have recently shown that constitutive overexpression of RelA may underlie the abnormality in NFκB regulation in melanoma cells (68). Others have suggested that abnormal IκBα processing may also play a role (69). NF-κB/relA activity has also been demonstrated to contribute to tumorigenicity, angiogenesis, and metastasis of human melanoma cells implanted in nude mice (70). Haycock has also shown that MSH can inhibit activation of NF-κB in human melanocytes and melanoma cells (71). Of related interest is the observation that α-MSH modulates nitric oxide production in human melanocytes (72).

Conceptual Framework for Pathogenesis of Melanoma Based on Altered Redox Control

We propose the following scenario (Fig. 5). The process of melanin synthesis is a highly regulated process that results in the formation of a biopolymer that normally functions as an anti-oxidant. This results in efficient sequestration of ROS generated by endogenous melanin synthesis, cellular interaction with UVA and UVB, and superoxide anion and peroxides produced by host immune and angiogenic responses (e.g., to UV or in response to antigenic changes during carcinogenesis). In melanocytes this process is under tight control and the lowering of intracellular ROS or inhibition of an increase allows turning off of NF-κB activation and other redox-sensitive transcription factors and abrogation of the stress response. It has been well established by others that the process of melanin synthesis becomes dysregulated during carcinogenesis, with differential polymerization of selected melanins and disruption of melanosomal organization (73–75). We propose that at some point early in pathogenesis, melanin becomes progressively more oxidized and begins to function as a pro-oxidant. Additionally, we speculate that as the cell becomes transformed, the disorganization of melanosomal structure leads to less efficient handling of the generated ROS within the matrix and 'leaking' of radicals to the cytoplasm and the production of further intracellular oxidative stress. This leads to NF-κB activation and other transcription factor alterations and a downstream stress response including an anti-apoptotic effect. (Alternatively, this might occur in ageing normal melanocytes and lead to premature cell death with hair graying and vitiligo as the outcome.) However, as a result of either continuous high level of ROS, protein modification secondary to high levels of ROS or a primary mutation in the Rel system (possibly induced by ROS), the NF-κB pathway remains activated and an anti-apoptotic milieu

maintained. We further propose that these alterations are at the heart of the remarkable changes in genotype and phenotype that occur during melanocyte carcinogenesis. Each of these steps is testable, and others and we are beginning to examine this candidate pathogenic mechanism.

Acknowledgements – Supported in part by CA62203 from the National Cancer Institute. We thank Sandy Schroeder for excellent administrative assistance.

REFERENCES

1. Prota G. Regulatory mechanisms of melanogenesis: Beyond the tyrosinase concept. J Invest Dermatol 1993;100:156S–161S
2. Valverde P, Manning P, Todd C, McNeil CJ, Thody AJ. Tyrosinase may protect human melanocytes from the cytotoxic effects of the superoxide anion. Exp Dermatol 1996;5:247–253
3. Napolitano A, Costantini C, Crescenzi O, Prota G. Characterisation of 1,4-benzothiazine intermediates in the oxidative conversion of 5-*S*-cysteinyldopa to pheomelanins. Tet Lett 1994;35(34):6365
4. Ito S. High-performance liquid chromatography (HPLC) analysis of eu- and pheomelanin in melanogenesis control. J Invest Dermatol 1993;100:166S–171
5. Vincensi MR, d'Ischia M, Napolitano A, Procaccini EM, Riccio G, Monfrecola G, Santoianni P, Prota G. Phaeomelanin vs. eumelanin as a chemical indicator of ultraviolet sensitivity in fair-skinned subjects at high risk for melanoma: A pilot study. Melanoma Res 1998;8:53–58
6. Prota G. Melanins and Melanogenesis. San Diego: Academic Press Inc; 1992
7. Ito S, Palumbo A, Prota G. Tyrosinase-catalyzed conjugation of dopa with glutathione. Experientia 1985;41:960–961
8. Carstam R, Hansson C, Lindbladh C, Rosman H, Rosengren E. Dopaquinone addition products in cultured human melanoma cells. Acta Derm Venereol (Stockh) 1987;67:100–105
9. Benedetto JP, Ortonne JP, Voulot C, Khachadourian C, Prota G, Thivolet JJ. Role of thiol compounds in mammalian melanin pigmentation. Part 2. Glutathione and related enzymatic activities. J Invest Dermatol 1982;79:422–424
10. Agrup G, Falck B, Rosman H, Rosengren AM, Rosengren E. Glutathionyldopa in malignant melanoma. Acta Derm Venereol (Stockh) 1977;57:221–222
11. Hochstein P, Cohen G. The cytotoxicity of melanin precursor. Ann NY Acad Sci 1963;100:876–886
12. Benedetto JP, Ortonne JP, Voulot C, Khachadourian C, Prota G, Thivolet JJ. Role of thiol compounds in mammalian melanin pigmentation. Part 1. Reduced and oxidized glutathione. J Invest Dermatol 1981;77:402–405
13. Wang YW, Teicher BA, Shea TC et al. Cross-resistance and glutathione-*S*-transferase-pi levels among for human melanoma cell lines selected for alkylating agent resistance. Cancer Res 1989;49:6185–6192
14. Hansson J, Berhane K, Castro VM, Jungnelius U, Mannervik B, Ringborg U. Sensitization of human melanoma cells to the cytotoxic effect of melphalan by the glutathione transferase inhibitor ethracrynic acid. Cancer Res 1991;51:94–98
15. Tew K. Glutathione-associated enzymes in anticancer drug resistance. Cancer Res 1994;54:4313–4320
16. Griffith OW, Meister A. Potent and specific inhibition of glutathione synthesis by buthionine sulfoximine (*S*-n-butyl homocysteine sulfoximine). J Biol Chem 1979;254:7558–7560
17. Griffith OW. Mechanism of action, metabolism and toxicity of buthionine sulfoximine and its higher homologs, potent inhibitors of glutathione synthesis. J Biol Chem 1982;257:13704–13712
18. Odwyer PJ, Hamilton TC, Young RC. Depletion of glutathione in normal and malignant cells in vivo by buthionine sulfoximine: Clinical and biochemical results. J Natl Cancer Inst 1992;84:264–267
19. Baily HH, Mulcahy KD, Tutsch RZ, Arzoomanian D et al. Phase I clinical trial of intravenous L-buthionine-sulfoximine and melphalan: An attempt at modulation of glutathione. J Clin Oncol 1994;12:194–205
20. Holmgren A. Glutathione-dependent synthesis of deoxynucleotides. J Biol Chem 1979;254:3664–3671
21. Holmgren A. Regulation of ribonucleotide reductase. Curr Top Cell Reg 1981;19:47–76
22. Holmgren A, Aslund F. Glutaredoxin. Methods Enzymol 1995;252:283–292
23. Schallreuter KU, Gleason FK, Wood JM. The mechanism of action of the nitrosourea anti-tumor drugs on thioredoxin reductase, glutathione reductase and ribonucleotide reductase. Biochim Biophys Acta 1990;1054:14–20
24. Schallreuter KU, Wood JM. Sensitivity and resistance in human metastatic melanoma to the new chloroethylnitrosourea anti-tumor drug Fotemustine. Biochim Biophys Acta 1991;1096:277–283
25. Fruehauf, JP. BCNU-mediated glutathione depletion and inhibition of glutathione reductase, ribonucleotide reductase, and DNA synthesis: novel mechanisms of antineoplastic activity. Thesis PhD, Rush University, 1996.
26. Fruehauf JP, Zonis S, Al-Bassam M, Kyshtoobayeva A, Dasgupta G, Milovanovic T, Parker R, Buzaid AC. Melanin content and down regulation of glutathione-*S*-transferase contribute to the action of L-buthionine-*S*-sulfoximine on human melanoma. Chem Biol Interact 1998;111–112:277–305
27. Sarna T, Swarz HM, Scott G, eds. Interactions of Melanin with Oxygen (and Related Species) in Atmospheric Oxidation and Antioxidants, vol. III. Oxford: Elsevier Science Publishers; 1993. pp. 129–165
28. Misra HR, Fridovich I. The univalent reduction of oxygen by reduced flavins and quinones. J Biol Chem 1972;247:188–192
29. Bustamante J, Bredeston L, Malanga G. Role of melanin as a scavenger of active oxygen species. Pigment Cell Res 1993;6:348–353
30. Meyskens FLJr, Van Chau H, Tohidian N, Buckmeier J. Luminol-enhanced chemiluminescent response of human melanocytes and melanoma cells to hydrogen peroxide stress. Pigment Cell Res 1997;10:184–189
31. Jacobson ES, Jenkins ND, Todd JM. Relationship between superoxide dismutase and melanin in a pathogenic fungus. Infect Immun 1994;62:4085–4086
32. Schnitzler N, Peltrche-Llacsahuanga H, Bestier N, Zundorf J, Lutticken R, Haase G. The effect of melanin and carotenoids of exophiala (Wangiella) dermatitidis on phagocytosis, oxidative burst, and killing by human neutrophils. Infect Immun 1999; 94–101.
33. Korytowski W, Sarna T. Bleaching of melanin pigments: role of copper ions and hydrogen peroxide in autooxidation and photooxidation of synthetic dopa-melanin. J Biol Chem 1990;265:12410–12416
34. Karg E, Odh G, Wittbjer A, Rosengren E, Rorsman H. Hydrogen peroxide as an inducer of elevated tyrosinase level in melanoma cells. J Invest Dermatol 1993;100:209S–213S
35. Karg E, Rosengren E, Rorsman H. Hydrogen peroxide as a mediator of dopac-induced effects on melanoma cells. J Invest Dermatol 1991;96:224–227
36. Nappi AJ, Vass E. Hydrogen peroxide generation associated with the oxidation of the eumelanin precursors 5,6-dihydroxyindole and 5,6-dihydroxyindole-2-carboxylic acid. Melanoma Res 1996;6:341–349
37. Nappi AJ, Vass E, Frey F, Carton Y. Superoxide anion generation in Drosophila during melanotic encapsulation of parasites. Eur J Cell Biol 1995;68:450–456
38. Sarzanini C, Mentasti E, Abollino O, Fasano M. Metal ion content in sepia-officinalis melanin. Marine Chem 1992;39:243
39. Kalyanaraman B, Korytowski W, Pilas B, Sarna T, Land EJ, Truscott TG. Reaction between ortho-semiquinones and oxygen: Pulse radiolysis electron spin resonance, and oxygen uptake studies. Arch Biochem Biophys 1988;266:277–284
40. Sotomatsu A, Tanaka M, Hirai S. Synthetic melanin and ferric ions promote superoxide anion-mediated lipid peroxidation. FEBS Lett 1994;342:105–108
41. Ben-Shachar D, Youdim Moussa BH. Iron, melanin and dopamine interaction: relevance to Parkinson's disease. Prog Neuro Psychopharmacol Biol Psychiatry 1993;17:139–150
42. Novellino L, Napolitano A, Prota G. 5,6-Dihydroxyindoles in the Fenton reaction: A model study of the role of melanin precursors in oxidative stress and hyperpigmentary processes. Chem Res Toxicol 1999;12:985–992

43. Tosk JM, Macknet AL, Chin C, Dulan S, Karimpour M, Makary M, Torrey RR. The relationship between redox activity and aggregation state of L-DOPA melanin. Pigment Cell Res 1999;7 (supplement):29, OP9
44. Setaluri V. Sorting and targeting of melanosomal membrane proteins: signals, pathways and mechanisms. Pigment Cell Res 2000;13:128–134
45. Jimbow K, Hua C, Gomez PF, Hirosaki K, Shinoda K, Salopek TG, Matsusaka H, Jin HY, Yamashita T. Intracellular vesicular trafficking of tyrosinase gene family protein in eu- and pheo-melanosome biogenesis. Pigment Cell Res 2000;13 (supplement 8):110–117
46. Bertling CJ, Lin F, Girotti AW. Role of hydrogen peroxide in the cytotoxic effects of UVA/B radiation on mammalian cells. Photochem Photobiol 1996;64:37–142
47. Romero-Gaillet C, Aberdam E, Clement M, Ortonne J-P, Ballotti R. Nitric oxide produced by ultraviolet-irradiated keratinocytes stimulates melanogenesis. J Clin Invest 1997;99:635–642
48. Felix CC, Hyde JS, Sarna T, Sealy RC. Melanin photoreactions in aerated media: Electron spin resonance evidence for production of superoxide and hydrogen peroxide. Biochem Biophys Res Commun 1978;84:335–341
49. Sarna T, Sealy RC. Photoinduced oxygen consumption in melanin systems. Action spectra and quantum yields for eumelanin and synthetic melanin. Photochem Photobiol 1984;39:69–74
50. Korytowski W, Pilas B, Sarna T, Kalyanaraman B. Photoinduced generation of hydrogen peroxide and hydroxyl radicals in melanins. Photchem Photobiol 1987;45:185–190
51. Applegate LA, Scaletta C, Labid F, Vile GF, Frenk E. Susceptibility of human melanoma cells to oxidative stress including UVA radiation. Int J Cancer 1996;67:430–434
52. Hadjur C, Richard JJ, Parat MO, Jardon P, Favier AA. Photodynamic effects of hypericin on lipid--peroxidation and antioxidant status in melanoma cells. Photochem Photobiol 1996;64:375–381
53. Costantini C, d'Ischia M, Palumbo A, Prota G. Photochemistry of 5-*S*-cysteinyldopa. Photochem Photobiol 1994;60:33–37
54. Koch WH, Chedekel MR. Photoinitiated DNA damage by melanogenic intermediates in vitro. Phytochem Photobiol 1986;44:703–710
55. Oberley TD, Oberley LW. Antioxidant enzyme levels in cancer. Invited review. Histol Histopathol 1997;12:525–535
56. Yohn J, Norris D, Yrastorza D, Buno I, Leff J, Hake S, Repine J. Disparate antioxidant enzyme activities in cultured cutaneous fibroblasts, keratinocytes and melanocytes. J Invest Dermatol 1991;97:405–410
57. Church SL, Grant JW, Ridnour LA, Oberley LW, Swanson PE, Meltzer PS, Trent JM. Increased manganese superoxide dismutase expression suppresses the malignant phenotype of human melanoma cells. Proc Natl Acad Sci USA 1993;90:3113–3117
58. Grammatico P, Maresca V, Roccella F, Rocella M, Biondo L, Catricala C, Picardo M. Increased sensitivity to peroxidizing agents is correlated with an imbalance of antioxidants in normal melanocytes from melanoma patients. Exper Dermatol 1998;8:205–212
59. Picardo M, Grammatico P, Roccella F, Rocella M, Grandinetti M, Del Porto G, Passi S. Imbalance in the antioxidant pool in melanoma cells and normal melanocytes from patients with melanoma. J Invest Dermatol 1996;107:322–326
60. Picardo M, Maresca V, Eibenschutz L, De Bernardo C, Rinaldi R, Grammatico P San gallicano Dermatological Institute, Rome, Italy, *Chair of Medical Genetics, University 'La Sapienza,' Rome, Italy. Correlation between antioxidants and phototypes in melanocytes cultures. A possible link of physiologic and pathologic relevance. J Invest Dermatol 1999;113:424–425
61. Blasi MA, Maresca V, Roccella M, Roccella F, Sansolini T, Grammatico P, Balestrazzi E, Picardo M. Antioxidant pattern in uveal melanocytes and melanoma cell cultures. Invest Ophthal Vis Sci 1999;40:3012–3060
62. Bittinger F, Gonzalez-Garcia JL, Klein CL, Brockhausen C, Offner F, Kirkpatrick CJ. Production of superoxide by human malignant melanoma cells. Melanoma Res 1998;8:381–387
63. Spillane TJ, Buckmeier JA, Tohidian NB, Chau HV, Meyskens FL, Jr. Famous ions promote an anti-apoptotic pathway in human metastatic melanoma cells. Pigment Cell Res 1999;7 (supplement):49
64. Blot WJ. Preventing cancer by disrupting progression of precancerous lesions. J Natl Cancer Inst 2000;92:1868–1869
65. Yamanishi DT, Buckmeier JA, Meyskens FL, Jr. Expression of c-jun, jun-b and c-fos protooncogenes in human primary melanocytes and metastatic melanomas. J Invest Dermatol 1991;97:349–353
66. Meyskens FL, Jr, Buckmeier J, Tohidian N. Regulation of the redox-sensitive transcription factors NF-κB and AP-1 by ultraviolet light-B in human melanocytes. In: Hori Y, Hearing VJ, Nakayama J. Melanogenesis and Malignant Melanoma: Biochemistry, All Biology, Molecular Biology, Pathophysiology, Diagnosis and Treatment. Amsterdam: Elsevier; 1996. pp. 25–34
67. Meyskens FL, Jr, Buckmeier JA, McNulty SE, Tohidian NB. Activation of nuclear factor-κB in human metastatic melanoma cells and the effect of oxidative stress. Clin Cancer Res 1999;5:1197–1202
68. Meyskens FL, Jr, McNulty S, Tohidian N, Buckmeier J. Understanding regulation of NF-κB as a basis for melanocytic transformation and melanoma cell therapeutic resistance. Pigment Cell Res 1999;7 (supplement):93 (abs)
69. Shattuck-Brandt RL, Richmond A. Enhanced degradation of IκB-α contributes to endogenous activation of NF-κB in Hs294T melanoma cells. Cancer Res 1997;14:3032–3039
70. Huang S, DeGuzman A, Bucana CD, Fidler IJ. Nuclear Factor-κB Activity correlates with growth, angiogenesis and metastasis of human melanoma cells in nude mice. Clinical Cancer Res 2000;6:2573–2581
71. Haycock JW, Wagner M, Morandini R, Ghanen G, Rennie IG, MacNeil S. Melanocyte-stimulating hormone inhibits NF-κB activation in human melanocytes and melanoma cells. J Invest Dermatol 1999;113:560–566
72. Isatmali M, Graham A, Szatkowski D, Ancans J, Manning P, McNeil CJ, Graham AM, Thody AJ. melanocyte-stimulating hormone modulates nitric oxide production in melanocytes. J Invest Dermatol 2000;114:520–526
73. Jimbow K. Current update and trends in melanin pigmentation and melanin biology. Keio J Med 1995;44:9–18
74. Jimbow K, Horikoshi T, Takahashi H, Akutsu Y, Maeda K. Fine structural and immunohistochemical properties of dysplastic melanocytic nevi: comparison with malignant melanoma. J Invest Dermatol 1989;92:304S–309S
75. Takahashi H, Horikoshi T, Jimbow K. Fine structure characterization of melanosomes in dysplastic nevi. Cancer 1985;56:111–123