Personalized Skin Protection: The Impact of Skin Pigmentation On Melanoma Formation

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Melanoma is the fifth most common cancer in the US. Although it accounts for less than two percent of all skin cancer cases, it causes a large majority of skin cancer deaths. Due to socioeconomic changes within the last century, exposing skin to sunlight, often unprotected, has become socially well-accepted; many light-skinned peoples, who are at higher risk of sustaining ultraviolet damage leading to melanoma, tan for cosmetic and recreational reasons. The first sunscreen ingredients were developed in the early 1930s, yet lifetime risk of developing melanoma has steadily increased since then, resulting in an almost 30-fold increase in lifetime melanoma risk (Figure 1).

It is well known that the risk of developing cutaneous melanoma varies between ethnicities. As shown by the Center for Disease Control and Prevention (CDC) in 2011, melanoma incidence in the US is about 3-5 times higher in Caucasians than in American Indians and Asians/Pacific Islanders, and about 15-25 times higher than in Hispanics and African Americans. Overall, the annual incidence rate of melanoma is 1 per 100,000 in blacks, 4 per 100,000 in Hispanics, and 25 per 100,000 in non-Hispanic whites.

Pheomelanin vs. Eumelanin

Clearly, melanin levels play a major part in skin cancer risk. Skin and hair color are determined by the relative amounts of pheomelanin and eumelanin in human hair follicles and epidermises. Pheomelanin, a yellow-to-red pigment, differs from black-to-brown eumelanin in its synthesis pathway, involving

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While multiple factors are responsible for the phenotypic expression of hair, eye and skin color, melanin is the primary contributing chromophore. It is produced in two forms called eumelanin and pheomelanin, and the relative quantity of each dictates the color of our skin and hair, our ability to tan, and our propensity to burn after UV exposure.

The factors controlling the balance between eumelanin and pheomelanin appear to be complex, but we know that the MC1R gene plays a major role. Various polymorphisms in this gene result in decreased eumelanin and increased pheomelanin synthesis, resulting in red hair, freckles and fair skin, all recognized melanoma risk factors. It turns out that people with these traits are at heightened risk for melanoma not only with UV exposure, but also without UV exposure. Dr. David Fisher and colleagues at Harvard Medical School have been exploring the underpinnings for these UV-independent melanomas in fair-skinned individuals.

In this issue of The Melanoma Letter, Dr. Fisher and his coauthor, Dr. Elisabeth Roider, reveal that along with high levels of pheomelanin, the “redheaded” MC1R polymorphisms are associated with decreased levels of glutathione, a major antioxidant. This reduced glutathione in turn predisposes cells to oxidative stress. Logic would suggest that diligent use of sunscreens and exogenous antioxidants might counteract the damaging effects of low glutathione and high eumelanin levels. However, paradoxically, in people with the mutant

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L-cysteine and its final oligomer structure, which incorporates benzothiazine and benzothiazole units, instead of the DHI (5,6-dihydroxyindole) and DHICA (5,6-dihydroxyindole-2-carboxylic acid) involved in eumelanin synthesis.6

Red hair has been associated with polymorphisms in the melanocortin 1 receptor (MC1R), a seven transmembrane G-protein coupled receptor expressed in the melanocytes of the skin and hair follicles. MC1R variant alleles are associated with red pheomelanin-rich hair in humans.5 Pigment formation primarily depends on the activity of different MC1R downstream proteins and the cellular availability of cysteine (Figure 2).6,7

As 80 percent of all individuals with red hair and pale skin carry loss-of-function polymorphisms in both MC1R alleles, the activity of MC1R’s downstream regulated genes in these people is low, resulting in the characteristic orange-red pheomelanin synthesis. This lower-functioning melanin is less effective than eumelanin in shielding against UVB or UVA exposure. Recognition of this pheomelanin-dependent oncogenic process of melanoma development via ROS called into question the currency of UVB-dependent mutagenesis, but also by reactive oxygen species (ROS), with pheomelanin likely playing an important role. Dysplastic nevi have been shown to carry higher pheomelanin levels and express more reactive oxygen radicals than normal human melanocytes.4 It has also been posited that the common somatic BRAF (V600E) mutation may occur via the action of oxygen radicals.15

**Melanomagenesis Without UV**

In 2012, Mitra, et al16 showed that pheomelanin synthesis even promotes melanoma formation in a UV radiation-independent context. Using a conditional allele of the melanoma oncoprotein BRAF V600E in red-haired mice carrying an inactivating mutation in the MC1R gene, Mitra’s team observed a high incidence of invasive melanomas compared to genetically matched black mice (MC1R wild-type). Introduction of an albino allele, which deleted all pigment production, actually conferred protection against melanoma development in the UVR-free context. Correspondingly, skin of BRAF-mutant red animals showed significantly higher oxidative DNA and lipid peroxidation damage than skin from genetically matched albino-red mice—all in the complete absence of any measurable UVB or UVA exposure. Recognition of this pheomelanin-dependent oncogenic process of melanoma development via ROS called into question the currency and effectiveness of sunscreen and other accepted forms of sun protection, as well as clinical screening methods and melanoma therapies, in those with mutant MC1R.

**How Does Pheomelanin Contribute to Melanoma?**

In 2013, Morgan, et al suggested that pheomelanin might either enhance ROS generation directly or deplete major antioxidants.17 Subsequently, Panzella, et al investigated the effect of red human hair pheomelanin (RHP) on cellular redox systems, exploring autoxidation of GSH (glutathione), the most important cellular antioxidant, and NADH (nicotinamide adenine dinucleotide), a central component of the respiratory chain.18 They showed that GSH and NADH, considered critical indices of metabolic state and intracellular redox
levels, were significantly decreased by pheomelanin. As it did not affect superoxide dismutase (SOD) and catalase (CAT), they assumed that pheomelanin does not produce significant levels of ROS measured with a variety of methods, including electron paramagnetic resonance (EPR) and spin trapping or colorimetric assays, but can induce depletion of GSH and NADH by a UV- and ROS-independent pathway. They assumed that pheomelanin can serve as a redox catalyst accepting H-atoms from the substrates and transferring electrons to oxygen.

Operation of a direct H-atom transfer to pheomelanin was confirmed by EPR analysis, showing a selective decrease in the pheomelanin signal in purified red hair pigment following incubation with excess GSH. This raised the hypothesis of pheomelanin as a “living” polymer and biocatalyst, which may trigger autoreactive prooxidant processes. In this mechanistic scheme, ROS would be produced from reoxidation of pheomelanin by oxygen but would be immediately utilized in the redox cycle. Nevertheless, it has not been fully determined whether MC1R exerts its oncogenicity via pheomelanin only or also by controlling different redox genes, such as the base excision repair enzymes 8-oxoguanine DNA glycosylase (OGG1) and the DNA damage repair enzyme apurinic apyrimidinic endonuclease 1 (APE-1/Ref-1). In addition, the prooxidant activity of pheomelanin on UV-induced liposomal lipid peroxidation has been suspected to originate from pheomelanin-metal complexes.

The oncogenic effect of oxidative stress on cancer development has been shown for multiple tumors, such as liver, lung, breast, and skin cancers. ROS are short-lived entities that are continuously generated at low levels during the course of normal aerobic metabolism, likely playing the role of a second messenger. Skin exposure to ionizing and UV radiation, and/or xenobiotics, generates ROS in excessive quantities that may overwhelm cutaneous antioxidant reserves. This may expose the nucleus and other cellular organelles in the cytosol to high levels of oxidative stress. In skin, uncontrolled release of ROS is involved in

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**Figure 2. Pigment synthesis pathway and involved genes in melanocytes.**

Pigment synthesis is stimulated by binding of alpha-melanocyte-stimulating hormone (α-MSH) to MC1R on melanocytes. MC1R activates cyclic adenosine monophosphate (cAMP) production, which in turn activates cAMP response element-binding protein (CREB)-mediated transcriptional activation of the microphthalmia-associated transcription factor (MITF). The melanocytic master transcriptional regulator MITF controls transcription of the pigmentation genes tyrosinase and tyrosinase-related proteins 1 and 2 (TYRP1 and TYRP2), which mediate pigment synthesis and the eu- to pheomelanin ratio.

- **alpha-melanocyte-stimulating hormone:** α-MSH
- **Agouti signaling protein:** ASIP
- **Melanocortin 1 receptor:** MC1R
- **G-proteins:** GP
- **Adenylate cyclase:** AC
- **cAMP response element-binding protein:** CREB
- **Tyrosinase:** TYR
- **Tyrosinase-related protein 1:** TYRP1
- **Tyrosinase-related protein 2:** TYRP2
the pathogenesis of a number of human skin disorders, especially cutaneous neoplasia. Within cells, ROS induce cell cycle alterations, DNA structural alterations including DNA strand breaks, DNA-protein crosslinks, and alterations of the mitogen-activated protein kinase (MAPK) pathway, as well as effects on lipid peroxidation and modulation of transcription factors such as activator protein 1 (AP-1) and nuclear factor KB (NF-kB). Additional effects on the tumor microenvironment and immune system were alterations in Th1 and Th2 response patterns as well as changes in dendritic cell surface markers.21

What Can Sunscreens Do?

Sunlight reaching the Earth’s surface is composed of differing wavelengths of the electromagnetic spectrum, ranging from infrared to visible and UV light. The UVB and UVA spectrum, between 280 nm and 400 nm, seems to be the most damaging light to our skin. UVA, with its longer wavelengths, can penetrate into deeper layers of the skin than UVB, with more rays penetrating the basal epidermis cells where melanocytes are located. Mathematical models predict that regular sunscreen use may greatly reduce the lifetime incidence of nonmelanoma skin cancers, and studies have clearly shown this with cutaneous squamous cell carcinoma—yet results with respect to melanoma incidence have been somewhat more complex.

Even though comprehensive review of all studies from 1966 to 2003 found no evidence that sunscreen increases melanoma risk,24,25 the question of how efficient sunscreens really are in melanoma prevention was not fully demonstrated until 2010, when a randomized controlled human trial showed that sunscreens can indeed prevent melanoma formation.26 Green and colleagues demonstrated a ~50% reduction in melanoma incidence after long-term follow-up of 1,621 Australian individuals randomized to daily SPF 16 sunscreen application to head, neck, arms, and hands. In this study, performed at a low-latitude township in Australia, the control group was also allowed “discretionary” use of sunscreen, but was not guided concerning type or application frequency. Overall, this clinical study demonstrated that sunscreen application can help prevent melanoma formation, but also highlighted the need for consistent rather than intermittent use.26 In 2014, Viros, et al showed that a broad-spectrum sunscreen applied on mice expressing BRAF(V600E) delayed the onset of UVR-driven melanoma, but only provided partial protection.27

Although both studies validated public health campaigns promoting sunscreen protection for individuals at risk of melanoma, it is becoming clear that current sunscreens and prevention guidelines might not be sufficient to fully prevent the disease. An ideal sunscreen would protect against 100 percent of all UVR wavelengths and offer long-term protection without reapplication, but such a sunscreen does not yet exist.28

A factor traditionally used, and broadly publicized, to evaluate sunscreen efficacy is the sun protection factor (SPF), defined as the ratio of UVR required to produce minimal erythema/sunburn with sunscreen, compared to without sunscreen. It is important to understand that the SPF endpoint is not a measure of skin cancer risk, but of propensity to induce skin erythema. Overall, an individual’s protection against sunburn depends on that individual’s capacity to generate erythema, a clinical feature usually mediated by UVB exposure. But no matter how high the SPF value is, the efficacy of a sunscreen also depends on the amount applied, the frequency of reaplication, and the Fitzpatrick skin phototype of the individual. For example, in calculating SPF the US Food and Drug Administration (FDA) uses a dose of 2.2 mg/cm² of exposed skin, applying the sunscreen 30 minutes before UV exposure. However, in practice, most people do not follow these guidelines, usually applying only one-quarter of the recommended dose.29,30

Furthermore, it is becoming clear that UVA plays a significant role in melanoma formation. It may potentiate the carcinogenic effects of UVB and is known to stimulate generation of ROS within the skin. So far only three UVA absorbers are FDA-approved in the US, whereas seven are available in Europe. Among the UVA filters approved in Europe are three – Tinosorb S, Tinosorb M, and Mexoryl SX – whose safety has been studied for years in Europe, and which are between 3.8 and 5.1 times more UVA-protective than the maximum allowable concentration of avobenzene, the most common UVA filter in US products.31

Some years ago US sunscreen makers began seeking FDA approval to use some of these compounds, but approval has not yet been granted. Newer sunscreen products attempt to filter both UVA and UVB spectra. However, half of all sunscreens advertised to offer “broad spectrum” UVA/UVB protection provide only low or medium UVA protection.32 It is therefore important to continue emphasizing the importance of additional sun safety measures, such as sun avoidance, shade, clothing, and sunglasses.

Another challenge of uncertain relevance is the impact of sunscreens themselves on ROS formation in skin. Mostly limited to a small number of solution-phase and in vitro studies, para-aminobenzoic acid (PABA) and 2-phenyl-benzimidazole-5-sulfonic acid (PBSA) have been described to induce both singlet oxygen (1O2) and thymine-dimer formation.33-35 Solution-phase studies found that octylmethoxycinnamate, octocrylene, and PABA all produce 1O2 in phosphate-buffered saline.33,35

In 2006, Hanson, et al used the fluorescent ROS indicator dihydroorhodamine (DHR) to measure generation of oxidative stress after topical application of oxybenzone, octocrylene, and octinoxate on human ex vivo skin and an epidermal skin model.36 Two-photon fluorescence microscopy revealed that even though early time points showed decreased ROS formation after 60 minutes of incubation, oxidative stress increased. In addition, in vitro experiments using zinc oxide nanoparticles showed increases of oxidative stress and decreased viability in melanoma cells.37,38

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Overall, in most real-life situations, it seems very likely that the net effect of sunscreen use is to reduce net free radical formation due to its UV-filtering benefits. But several concerns would suggest that even greater efficacy might be achieved through certain considerations. As discussed above, cell-intrinsic pheomelanin has been associated with increased ROS production. For individuals who tan easily in response to UV exposure, their pheomelanin/eumelanin ratio is predicted to be increased by consistent sunscreen usage. This would suggest that there may be a “ceiling” to the magnitude of protection offered by UV filters, determined in part by intrinsic melanin pigments within an individual’s skin. Additionally, formation of free radicals by the UV-absorbing chemical species, still likely preferable to UV exposure, may nonetheless limit the magnitude of protection afforded by these agents. Clearly, additional detailed studies are needed to more fully understand mechanisms of action and any limitations of highly used skin care products, as well as to develop agents that optimally protect against both UV and oxidative genotoxic damage.

What Does This Mean for Physicians and Patients?

Since the Australian SunSmart campaign with its slogan “slip/slop/slap/slide” was introduced in 1988, many people have been convinced to slip on a long-sleeved top, slop on sunscreen, slap on a hat, seek shade and slide on sunglasses. What has changed since then?

As sunlight remains one of the top risk factors for development of cutaneous melanoma, rigorous sun protection remains the main goal. This includes avoidance of direct sun exposure in peak hours, seeking shade, use of covering clothes, a broad-brimmed hat, and sunglasses. While sunscreen use remains a vital recommendation for skin cancer prevention, we might rethink the concept of sunscreens as being equivalent to physical sun protection. Sunscreen ingredients remain chemicals with the potential risk—as any other skin care product—to increase oxidative damage in skin. Optimizing both the safety and efficacy of these ingredients is not a trivial task, as their effects may vary for individuals of different skin phototypes.

Current evidence suggests that naturally pigmented skin (not pigmented by UV exposure) is one of the best available skin cancer “sunscreens,” providing up to a 50-fold decrease in the risk of basal and squamous cell carcinomas, and a 12-fold decrease in the risk of melanoma39—benefits not to date achieved or measurable with sunscreen usage. Natural skin color in darker-pigmented individuals of skin type > 2, not resulting from UV exposure, may offer significant protection from typical daily sunlight exposure through its direct shielding and antioxidant features. However, the situation is very different in individuals with pale skin types 1 and 2. This population, with poor tanning dose/response to UV resulting in oncogenic mutational events, bears a greatly increased risk of developing melanoma.

Antioxidants vs. Pheomelanin

The concept of adding antioxidants into sunscreens is attractive yet potentially controversial. While antioxidants diminish oxidative stress through their direct chemical actions, they may also increase cellular cysteine levels due to sparing of endogenous cellular antioxidant consumption. This could be important because cysteine is one of the key ingredients in the pheomelanin synthesis pathway. Therefore, addition of antioxidants might indeed decrease ROS at early time points due to direct chemical quenching, but later switch the pigmentation pathway from eumelanin towards pheomelanin synthesis, thereby indirectly contributing to pheomelanin’s prooxidative effect. It has been shown that cysteine deprivation promotes eumelanogenesis in human melanoma cells,40 and that correspondingly, oral administration of gluthatione induces skin lightening/depigmentation.41 Such effects are, however, complex, and are likely to depend significantly on chemical details of the antioxidant species, the skin’s intrinsic antioxidant features, and the skin’s pigmentation status. Further research on sunscreens is needed to develop non-ROS-generating products that are both safe and efficient. Possibilities include introducing chemical step-down processes, finding non-cysteine-modifying antioxidants, or discovering a safe way to induce skin pigmentation. Further investments by manufacturers and researchers alike in sunscreen research and enhanced approval processes are also greatly needed. This includes efforts to promote faster FDA approval of safe, effective sunscreens.

In the meantime, sunscreen use remains strongly recommended, for prevention not only of melanoma but of squamous cell carcinoma and benign skin damage including photoaging. Furthermore, skin type 1 and 2 individuals should minimize sun exposure and avoid tanning, and should utilize physical sun protection as much as possible. All individuals, especially those at high-risk, should regularly visit an experienced physician trained in skin examination and practice rigorous self-examination routinely. Overall, personalizing skin cancer protection and early melanoma detection based on an individual’s skin type might help to stem the relentless morbidity and mortality of melanoma.

References


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MC1R variant, these products may in fact contribute to further damage. Exogenous antioxidants increase cysteine levels, which may tilt melanin production away from protective eumelanin towards the less protective, inherently damaging pheomelanin. And sunscreens, by allowing unaware vulnerable individuals to stay in the sun longer without burning, may lead to mounting oxidative stress, inadequately counteracted by these individuals’ low glutathione levels. Thus, especially for those with the redheaded MC1R variant, clothing and shade may need to be the primary forms of sun protection, with sunscreen serving a secondary protective role. At the same time, people of all skin phenotypes and colors must be apprised that skin can be UV-damaged even without sunburn, and that sunscreens are best used as necessary, complementary protection, but never as an excuse to stay out longer.

As these new insights are refined and reinforced, and as the mechanisms involved in melanomagenesis are better elucidated, skin cancer prevention strategies will be increasingly tailored for individuals of different phenotypes and genotypes.

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