Invited Review

Hormonal Regulation of the Repair of UV Photoproducts in Melanocytes by the Melanocortin Signaling Axis†

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ABSTRACT

Melanoma is the deadliest form of skin cancer because of its propensity to spread beyond the primary site of disease and because it resists many forms of treatment. Incidence of melanoma has been increasing for decades. Although ultraviolet radiation (UV) has been identified as the most important environmental causative factor for melanoma development, UV-protective strategies have had limited efficacy in melanoma prevention. UV mutational burden correlates with melanoma development and tumor progression, underscoring the importance of UV in melanogenesis. However, besides amount of UV exposure, melanocyte UV mutational load is influenced by the robustness of nucleotide excision repair, the genome maintenance pathway charged with removing UV photoproducts before they cause permanent mutations in the genome. In this review, we highlight the importance of the melanocortin hormonal signaling axis on regulating efficiency of nucleotide excision repair in melanocytes. By understanding the molecular mechanisms by which nucleotide excision repair can be increased, it may be possible to prevent many cases of melanoma by reducing UV mutational burden over time.

MELANOMA—A CANCER OF MELANOCYTES

Although melanoma accounts for less than a tenth of all skin cancers, it is responsible for approximately three quarters of skin cancer deaths because of its aggressive nature, tendency to metastasize and to resist anticancer therapies (1). Whereas the more common skin malignancies—basal cell carcinoma and squamous cell carcinoma—derive from epidermal keratinocytes, melanoma arises from melanocytes, which are neural crest-derived cells characterized by their ability to produce melanin pigments (2). In the skin, melanocytes can be found in the dermis within hair follicles where they impart pigmentation to hair as it grows and more superficially in the interfollicular epidermis where they produce melanin that accumulates in the epidermis (Fig. 1). Epidermal melanin functions to absorb UV radiation in the skin; therefore, the more melanin that is found in the skin, the darker the complexion and the more resistant the skin is to UV damage (3). When epidermal or dural melanocytes undergo malignant degeneration to become melanoma, they tend to retain their melanin-producing ability, which is why the majority of melanomas are darkly pigmented lesions. Furthermore, many melanomas arise from benign nevi, underscoring the importance of early detection efforts to catch the disease as early as possible based on the A-B-C-D-E model of carefully assessing moles for asymmetric shape, border irregularities, color changes, growth in diameter or evolution of appearance over time. When detected early—before metastatic spread—melanoma can usually be cured by surgical excision alone. Survival rates plummet, however, once the disease has spread to regional lymph nodes or distant organs. Fortunately, the great majority of melanomas are detected and excised before systemic spread, and overall 5-year survival rates currently exceed ninety percent (4).

MELANOMA—EPIDEMIOLOGY

U.S. melanoma incidence has risen faster than any other cancer in the last several decades, growing at an estimated 3% yearly (Fig. 2). The most recent data from the National Cancer Institute’s Surveillance, Epidemiology and End Results (SEER) program suggest that an estimated 76,380 Americans will be diagnosed with melanoma and the disease will kill 10,130 in 2016 alone. Roughly one in every fifty Americans will now be diagnosed with melanoma at some point in his/her life (5). The underlying reasons for the rising incidence of melanoma are likely to be multifactorial (increasing age of the population, better detection methods and awareness, more recreational UV exposure, etc.) and represent a critical research question in need of clarification. Although melanoma can happen at any age, it most commonly occurs after adolescence with incidence peaking in the fifth decade of life. Nonetheless, melanoma often affects people in their prime and represents the second most common cancer in people aged 15–29. Currently, it is estimated that over one million Americans have been diagnosed with melanoma at some point in their lives (6).
MELANOMA—RISK DETERMINANTS

Inherited and environmental factors heavily influence melanoma risk (Table 1; please see (7) for a comprehensive review). Genetic risk factors include number and nature of nevi as well as prior diagnosis of melanoma in the family. Some genes that are known to influence melanoma risk include CDKN2A, CDK4, MITF and variants in pigmentation loci or DNA repair genes (8–11). Other inherited traits that are controlled by multiple loci are number and nature of nevi (12) and immunocompetence of T-cell responses (13).

A wealth of epidemiologic and molecular data points to ultraviolet radiation (UV) as the most important environmental risk factor for melanoma (14). Many melanomas arise from nevi on sun-exposed anatomic areas of the body (15–17), melanoma incidence increases with geographic proximity to the equator where sun-exposed anatomic areas of the body (15

UV energy increases with geographic proximity to the equator where sun-exposed anatomic areas of the body (15

Table 1. Melanoma risk factors. Various genetic and environmental risk factors have been linked with increased melanoma incidence (recently reviewed in (7)).

<table>
<thead>
<tr>
<th>Inherited risk factors</th>
<th>Environmental risk factors</th>
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<tr>
<td>Family or personal history of melanoma and nonmelanoma skin cancer</td>
<td>UV exposure, especially blistering sunburns early in life</td>
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<tr>
<td>Large number of nevi</td>
<td>UV-rich geographic locations</td>
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<td>Nevi of large size</td>
<td>Indoor artificial UV exposure</td>
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<td>Dysplastic nevi</td>
<td>Psoralen, UV light therapy</td>
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<td>Melanocortin 1 receptor (MC1R) function</td>
<td>Pharmacologic immunosuppression</td>
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<tr>
<td>T-cell immunodeficiency</td>
<td>Various chemicals, heavy metals possible</td>
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<tr>
<td>Defective DNA repair (e.g. xeroderma pigmentosum)</td>
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<tr>
<td>Fair skin complexion</td>
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The International Agency for Research on Cancer (IACR) classifies UV as a “Group 1” carcinogen, meaning that there is irrefutable evidence that UV causes cancer in humans (24). Unlike many other carcinogens that are chemical in nature, UV consists of photons whose wavelength and energy fall between ionizing radiation (X-rays) and visible light (Fig. 3). The sun is the natural source of UV, although significant UV exposure can be obtained by artificial UV sources in the form of tanning beds (25). As water vapor and particles in the atmosphere can absorb or deflect UV photons, UV in sunlight becomes attenuated when it travels through the atmosphere. Thus, solar UV energy is strongest in those places on Earth at the equator where the sun hits the Earth directly and at high altitudes where the land reaches highest into the sky. UV can be subdivided by wavelength and energy into UV-A (315–400 nm; 3.10–4.43 eV photon–1), UV-B (280–315 nm; 3.94–4.43 eV photon–1) and UV-C (200–280 nm; 4.43–12.4 eV photon–1). In most places on Earth, ambient sunlight consists of >90% UV-A with the remainder UV-B because atmospheric ozone effectively absorbs shorter-wavelength UV-C energy.

UV energy is highly bioactive, exerting physicochemical changes that damage and/or cross-link a variety of cellular macromolecules such as lipids, RNA, DNA and protein (26). Using a murine model with “humanized skin,” Noonan et al. showed that melanomas could be initiated by UV-A or UV-B radiation (27); therefore, melanocyte carcinogenesis can be promoted across the UV spectrum. Longer wavelength UV promotes
the formation of singlet oxygen ($^1\text{O}_2$) which oxidizes molecules in lipids, amino acids as well as nucleotide bases in RNA and DNA (28). In general, these changes interfere with the function of macromolecules and in many cases lead to their premature destruction (29). While lipids, RNA and protein are renewable molecules that can be replaced when damaged, DNA cannot simply be replaced and must be repaired (30). Indeed, the repair of DNA must happen in a timely manner because many of the UV changes interfere with the function of polymerases and/or cause errant base pairing which yields RNAs and proteins of inappropriate sequence and function. More serious, however, is errant base pairing during cell division. In this manner, unrepaired UV-induced DNA changes introduce mutations into the genome of daughter cells. Oxidation-mediated changes to nucleotide bases, for the most part, are dealt with by the base excision repair (BER) pathway wherein specific oxidatively generated lesions are recognized and removed from the DNA by lesion-specific glycosylases without breaking the sugar-phosphate backbone to generate an apurinic site which is then filled in using the undamaged sister strand as a template.

UV photons also promote the formation of covalent base changes in DNA at sites of neighboring pyrimidines by breaking existing C5/C6 bonds in cytosines or thymines. Wavelengths in the UV-B range generate cyclobutane pyrimidine dimers (CPDs) and to a lesser extent pyrimidine (6-4) pyrimidone photoproducts (6-4PPs), whereas those in the UV-A range promote CPD formation rather than 6-4PPs. UV-A photons also efficiently promote photoisomerization of 6-4PPs into Dewar valence isomers (Fig. 4) (31). All of these photolesions block transcription and DNA replication, activate cell cycle checkpoints, stimulate mutagenesis and chromosomal rearrangements and can promote apoptosis. If left unrepaired, photolesions cause transition mutations (e.g. CC-to-
TT) known as “UV-signature mutations” which are found in abundance in melanoma isolates (32). Indeed, the high degree of UV-signature mutations in melanoma is among the strongest pieces of molecular evidence linking UV with the disease (21–23).

Recently, a paradigm-shifting insight was made regarding how photoproduts form after UV exposure. It had long been observed that although UV photoproducts can be identified in cells immediately following UV exposure, their levels increase for some time after the UV exposure ends. Brash et al. termed these lesions “dark CPDs” and reported that they arise when UV-induced reactive oxygen and nitrogen species combine to excite an electron in fragments of melanin that creates a quantum triplet state that has the energy of a UV photon. Triplet-state excite an electron in fragments of melanin that creates a quantum chemical excited intermediate. This process, known as CPDs by the transfer of triplet-state energy from oxidized melanin that gives rise to a chemically excited intermediate. This process, known as "dark CPD" formation, can occur for several hours after UV exposure and may account for the majority of all UV photoproducts.

![Figure 5](image-url) Dark CPD formation. UV can induce delayed formation of CPDs by the transfer of triplet-state energy from oxidized melanin that gives rise to a chemically excited intermediate. This process, known as “dark CPD” formation, can occur for several hours after UV exposure and may account for the majority of all UV photoproducts.

**MELANOCORTIN 1 RECEPTOR (MC1R) AND CAMP SIGNALING**

Melanocytes are long-lived cells that must, as a result of their anatomic location in the skin, cope with environmental insults such as UV radiation. They must not only survive following UV exposure, but they must also respond physiologically to ramp up melanin production to protect the skin against further UV damage. Much of their ability to respond appropriately to UV is regulated by the melanocortin 1 receptor (MC1R), a G-protein-coupled receptor located in the melanocyte extracellular membrane (34). This transmembrane protein spans the extracellular membrane seven times and transmits signals from ligands that interact with MC1R’s extracellular domains to the interior of the cell (Fig. 6) (35). The MC1R is centrally placed in a broader melanocortin signaling axis that regulates a variety of melanocyte UV responses (36) and melanoma risk (37). When appropriately...
activated, the MC1R engages adenylyl cyclase and intracellular levels of the second messenger cAMP increase (38). In melanocytes, higher cytoplasmic cAMP levels drive melanin production (39,40), increase melanocyte resistance to UV-mediated apoptosis (41,42) and enhance genomic stability to prevent UV mutagenesis (Fig. 7) (43–48).

Although there is evidence for ligand-independent MC1R signaling (49), its ability to promote cAMP signaling increases following interaction between MC1R and either of two high-affinity agonistic ligands: alpha-melanocyte stimulating hormone (α-MSH) or adrenocorticotropic hormone (ACTH) (50). Both are derived from pro-opiomelanocortin (POMC), a hormone propeptide expressed in the epidermis and pituitary (51,52). Interaction between MC1R and α-MSH or ACTH leads to adenylyl cyclase activation and an accumulation of cAMP (53). Higher cAMP levels promote the activity of cAMP-activated protein kinase (protein kinase A; PKA) and upregulate levels and activity of two key melanocyte transcription factors: cAMP response element-binding protein (CREB) and microphthalmia transcription factor (MITF) (54). Together, CREB and MITF increase transcription of melanin biosynthetic enzymes such as tyrosinase and dopachrome tautomerase, ultimately resulting in upregulation of melanin synthesis (50). Melanin produced by melanocytes is transferred to keratinocytes where it accumulates in the epidermis to protect deep layers of the skin from UV damage. In this way, MC1R directly controls the adaptive tanning response which is critical for cutaneous UV protection (40,55). MC1R activation also enhances melanocytes’ ability to resist UV damage, as will be discussed in detail below.

Not only can MC1R signaling be enhanced by ligand–receptor interactions with either α-MSH or ACTH, it can also be inhibited by physical interactions with either of two physiologic antagonists: human beta-defensin 3 (βD3) or agouti signaling protein (ASIP) (48,56). βD3 acts as a neutral antagonist, interfering with melanocortin binding to MC1R and preventing cAMP increases in the presence of α-MSH or ACTH (57). In contrast, ASIP is a negative agonist, not only inhibiting melanocortin-mediated MC1R activation but also downregulating ligand-independent MC1R signaling (58). Whereas ASIP is not thought to be physiologically relevant in human skin (59), βD3 is robustly expressed in a variety of circumstances including inflammation and infection (60–63). Thus, regulation of melanocyte cAMP levels and the functional downstream consequences of melanin production and DNA repair may be dynamic and multifaceted, being regulated by ligand-independent signaling as well as positive and negative ligands.

NUCLEOTIDE EXCISION REPAIR

DNA repair is essential for maintaining the integrity of the genome, which when faulty contributes to mutagenesis, genetic instability and carcinogenesis. Nucleotide excision repair is the primary system for removing UV-induced damage such as CPDs and 6-4PPs. The xeroderma pigmentosum complementation group proteins (XPs) play a critical role in coordinating and promoting nucleotide excision repair (reviewed in (64–66)). In elegant in vitro experiments, the Wood and Sancar laboratories independently reported the molecular requirements for reconstitution of nucleotide excision repair in vitro (67,68). In global

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**Figure 7.** Melanocortin–MC1R signaling axis. Melanocortins such as α-MSH and ACTH, produced in the pituitary and the epidermis, act as high-affinity agonistic ligands to the MC1R, a G, protein-coupled receptor on the surface of melanocytes. As a result, adenylyl cyclase is activated and cytoplasmic levels of the second messenger cAMP increase. This leads to activation of cAMP-dependent protein kinase (protein kinase A; PKA) and upregulation of the cAMP responsive binding element (CREB) and microphthalmia (Mitf) transcription factors. Together, CREB and Mitf stimulate melanin production through increased expression of melanin biosynthetic enzymes such as tyrosinase. Through increased production and accumulation of UV-blocking melanin pigments, the skin is better protected against UV insults. In addition to stimulating melanin production, MC1R/cAMP signaling also enhances the ability of melanocytes to resist and recover from UV damage by boosting nucleotide excision repair (nucleotide excision repair), the genome maintenance pathway charged with the removal of mutagenic UV photolesions.
genomic repair (GG-nucleotide excision repair), the recognition of helical distorting lesions is achieved by XPC-RAD23B (69), and in some cases UV-DDB (70). Initial events in lesion recognition remain somewhat unclear with several studies supporting a model of initial recognition by XPC followed by binding of TFIIH, XPA and RPA (71–74), but it is generally accepted that the XPC/HHR23B complex is recruited to photodamage by interactions with the undamaged complementary strand (64). In transcription-coupled repair (TC-nucleotide excision repair), a blocked RNA polymerase acts as the damage recognition signal. TC-nucleotide excision repair and GG-nucleotide excision repair differ in damage recognition, but subsequent steps converge into one repair pathway (Fig. 8). After damage recognition, there is recruitment of transcription factor II H (containing XPB and XPD) leading to strand separation to facilitate binding of other nucleotide excision repair factors including XPA, replication protein A (RPA), XPG and excision repair cross-complementation group 1 (ERCC1)-XPF (75,76). Once ERCC1-XPF is positioned on DNA via its interaction with XPA (77), it cuts the damaged strand 5’ to the lesion (77), followed by XPG incising 3’ to the damage (78). DNA is restored to its original form (with preservation of original sequence) by the action of DNA polymerases (e.g. Polδ and Polκ) and associated factors using the undamaged complementary strand as a template (79). Failure to fill the gap leads to activation of cellular damage responses (80).

**XERODERMA PIGMENTOSUM**

The importance of nucleotide excision repair in cancer resistance is best illustrated by considering the natural history of patients with XP, a rare UV hypersensitivity syndrome caused by homozygous defects in certain components of the pathway: XPA, ERCC1, ERCC3 (XP-B), XPC, ERCC2 (XP-D), DDB2 (XPE), ERCC4 (XP-F), ERCC5 (XP-G) and POLH (81). Patients

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**Figure 8.** A simplified overview of nucleotide excision repair. UV photodamage is repaired by the nucleotide excision repair genome maintenance pathway. In TC-nucleotide excision repair, a blocked RNA polymerase acts as the damage recognition signal, whereas in GG-nucleotide excision repair, XPC initiates repair by locating the damage and recruiting the subunits of a large complex called transcription factor II H (containing XPB and XPD). These proteins initiate strand separation, enabling other nucleotide excision repair factors to bind, including XPA, replication protein A (RPA), XPG and excision repair cross-complementation group 1 (ERCC1)-XPF. Once ERCC1-XPF is positioned on DNA via its interaction with XPA, it incises the damaged strand 5’ to the lesion, followed by XPG performing the 3’ incision. Repair is completed by polymerases in combination with proliferating cell nuclear antigen that replace the gap using the undamaged complementary strand for fidelity. Please see the following excellent recent reviews for more detail about nucleotide excision repair (46, 65, 66).
with XP demonstrate hyper UV sensitivity and develop characteristic skin changes including pigmentary abnormalities, capillary telangiectasias and atrophy on UV-exposed anatomic sites at very early ages (82). Premalignant lesions and skin cancers develop at high frequency and much sooner than in unaffected persons. Melanomas, basal cell carcinomas and squamous cell carcinomas typically develop before the second decade of life, many years before the general population (83). In addition, XP-associated skin cancers frequently demonstrate “UV-signature mutations,” clearly indicating the importance of nucleotide excision repair in resisting cancers caused by UV (84).

**MELANOCORTIN 1 RECEPTOR AND NUCLEOTIDE EXCISION REPAIR**

The melanocortin signaling axis controls not only skin pigmen
tary responses but also directly regulates melanocyte DNA repair (46,47,85,86). When activated and functional, MC1R/cAMP signaling boosts the ability of melanocytes to rid their DNA of UV photoproducts. MC1R-mediated signaling influences nucleotide excision repair at the level of gene transcription as well as through the post-translational modifications of repair factors. Much of the downstream transcriptional effects of MC1R-cAMP stimulation in melanocytes are controlled by MITF. By combining high-throughput sequencing (ChIP-seq) and RNA sequencing analyses, MITF was identified to target several nucleotide excision repair genes; loss of Mitf resulted in downregulation of the crucial nucleotide excision repair components XPA, RPA, DNA ligase I and DNA polymerase delta (Pol δ) (87). In another large-scale gene expression study, Mc1r loss of function in C57BL/6J mice was further associated with reduced XPAB1 gene expression in neonatal whole skin (88). Although transcriptional activation of the expression of repair factors may position melanocytes in the skin to cope with future UV insults, the melanocortin signaling cascade also facilitates more immediate repair responses through post-translational modifications of key proteins in the UV damage response pathway. Our data establish an interaction between MC1R/cAMP signaling and the ataxia telangiectasia mutated and Rad3-related (ATR) protein. ATR function is critical to UV DNA damage signaling (89), cell survival (90) and is linked with nucleotide excision repair (91).

We recently reported that a critical molecular event linking MC1R signaling to DNA repair is a cAMP-dependent phosphorylation event on ATR (46). When cAMP levels are induced either by MSH-MC1R interactions or pharmacologically with adenylyl cyclase activation by forskolin, PKA becomes activated

![Figure 9](https://example.com/figure9.png)

**Figure 9.** Summary figure of PKA-ATR-XPA model. In melanocytes, enhancement of nucleotide excision repair by cAMP is dependent on a post-translational modification of ATR. MC1R activation by melanocortins results in PKA-induced phosphorylation of ATR at the S435 residue. This event promotes binding between XPA and ATR in the nucleus, and together, ATR and XPA localize to UV photodamage in an accelerated and enhanced manner. MC1R agonists α-MSH or ACTH activate PKA-mediated ATR phosphorylation and nucleotide excision repair, whereas MC1R antagonists ASIP or β3 inhibit this repair-enhancing pathway. In this way, genomic stability and susceptibility to UV mutagenesis in melanocytes may be hormonally influenced.
and phosphorylates ATR on its serine 435 (S435) residue. This post-translational modification causes ATR to associate with the key nucleotide excision repair factor XPA (46). Together, XPA and ATR-pS435 co-localize to sites of UV-induced DNA damage in an accelerated and enhanced manner (Fig. 9). Loss of S435 within ATR prevents PKA-mediated ATR phosphorylation, disrupts ATR–XPA binding, delays recruitment of XPA to UV-damaged DNA and elevates UV-induced mutagenesis in melanocytes. Our data suggest that PKA modification promotes nuclear entry of an ATR–XPA complex to “prime” DNA damage responses in melanocytes immediately upon UV exposure (46). Indeed, S435 is part of a PKA target sequence within ATR’s predicted nuclear localization sequence (425-DGISPKRRLSSSLNPSKRPAP), suggesting that its phosphorylation might impact ATR’s nuclear localization, possibly through interactions with nuclear importins (92). Interestingly, PKA-directed nuclear localization of DNA-PK, another PIKK family member, has also been reported (93). Our data suggest that PKA may be an important regulator of nuclear import of key nucleotide excision repair factor(s) during DNA repair. Alternatively, PKA-mediated phosphorylation of ATR at S435 may optimize nucleotide excision repair through enhanced intranuclear interactions with XPA to facilitate transport and/or assembly of nucleotide excision repair factors at sites of UV damage. By a variety of possible mechanisms, it is clear that PKA-mediated modification of ATR may “prime” melanocytes to better cope with UV injury.

Melanocortin signaling also impacts nucleotide excision repair through other mechanisms. For example, the nucleotide excision repair proteins, DDB1, DDB2 and XPC have all been shown to be upregulated following cAMP activation (94). Of note, it appears that Melanocortin signaling also impacts nucleotide excision repair responses in melanocytes immediately upon UV exposure (46). Indeed, S435 is part of a PKA target sequence within ATR’s predicted nuclear localization sequence (425-DGISPKRRLSSSLNPSKRPAP), suggesting that its phosphorylation might impact ATR’s nuclear localization, possibly through interactions with nuclear importins (92). Interestingly, PKA-directed nuclear localization of DNA-PK, another PIKK family member, has also been reported (93). Our data suggest that PKA may be an important regulator of nuclear import of key nucleotide excision repair factor(s) during DNA repair. Alternatively, PKA-mediated phosphorylation of ATR at S435 may optimize nucleotide excision repair through enhanced intranuclear interactions with XPA to facilitate transport and/or assembly of nucleotide excision repair factors at sites of UV damage. By a variety of possible mechanisms, it is clear that PKA-mediated modification of ATR may “prime” melanocytes to better cope with UV injury.

HORMONAL REGULATION OF NUCLEOTIDE EXCISION REPAIR BY MC1R AGONISTS AND ANTAGONISTS

Having previously established that MC1R-enhanced nucleotide excision repair is dependent on PKA-mediated phosphorylation of ATR on S435, recruitment of XPA and accelerated localization to nuclear UV photodamage (46), we sought to determine the influence of physiologic MC1R ligands on ATR-pS435 accumulation and nucleotide excision repair function (48). To study the kinase activity of the MC1R–cAMP–PKA axis, high-throughput methodologies were developed. ATR-pS435 was detected in vitro using a 14-mer peptide (consisting of residues 428–441 of ATR) using a phospho-specific (ATR-pS435) antibody. In melanocytes with intact MC1R signaling, the ATR-pS435–XPA axis is heavily influenced by agonists and antagonists of MC1R, with melanocyte genomic stability regulated by MC1R signaling status and concentrations of MC1R ligands in the local milieu. Enzyme kinetic studies revealed higher Vmax and lower Km values for forskolin-mediated ATR-pS435 compared with z-MSH-mediated ATR-pS435 (48). These different kinetic properties suggest an increased “cAMP load” may enhance the capability of PKA to recognize ATR-S435 and/or impact how strongly PKA binds with the S435 substrate.

In primary human melanocytes and MC1R-transfected HEK293 cells, either z-MSH or ACTH enhanced ATR-pS435, XPA’s association with UV-damaged DNA and optimized nucleotide excision repair. In contrast, either ASIP or β3 interfered with ATR-pS435 generation, impaired XPA–DNA interaction and impeded DNA repair. Interestingly, ASIP downregulated basal levels of ATR-pS435, consistent with it being an MC1R inverse agonist capable of downregulating ligand-independent MC1R signaling. β3, however, had no impact on constitutive levels of ATR-pS435, supporting its function as a neutral MC1R antagonist instead (48).

To elucidate the functional effect of MC1R ligands on DNA repair, the oligonucleotide retrieval assay that quantifies repair by PCR-based amplification was adapted (97). In this assay, the presence of photoproduc(s) interferes with the DNA polymerase; therefore, the amount of amplification across the oligonucleotide is proportional to clearance of photolesions by nucleotide excision repair. We adapted this method by directly UV-irradiating the oligonucleotide (instead of a chemically generated single CPD lesion) which resulted in more CPDs and 6-4PPs (48). We observed that nucleotide excision repair responses were regulated by MC1R status and ligand interactions and that they correlated with ATR-pS435 accumulation and XPA–DNA binding (48). Thus, z-MSH promoted nucleotide excision repair, while ASIP and β3 blocked z-MSH-mediated enhancement of repair. ASIP blunted repair of UV-induced DNA damage to a greater extent than β3, which is explained by the fact that ASIP has a greater ability to inhibit ATR-pS435 generation than β3. Together, these findings support the hypothesis that MC1R/cAMP signaling controls melanocytic nucleotide excision repair through downstream PKA-mediated ATR phosphorylation on S435. Furthermore, these data raise the possibility that dysregulated expression of ASIP or β3 in the skin may impair DNA repair responses in melanocytes to heighten UV mutagenesis and melanoma risk.

MC1R AS A MELANOMA RISK DETERMINANT

It has been known for many years that inherited signaling defects in MC1R correlate with melanoma risk (37,98–100). While MC1R loss certainly yields a UV-sensitive fair complexion phenotype, it is clear that melanocyte genome stability is reduced as a result of MC1R loss. Indeed, a recent study established a role for germline loss-of-function MC1R variants in increasing the melanoma risk (105,106). When MC1R signaling is blunted, melanocytes with intact MC1R signaling, the ATR–XPA complex to “prime” DNA damage responses in melanocytes immediately upon UV exposure (46). Indeed, S435 is part of a PKA target sequence within ATR’s predicted nuclear localization sequence (425-DGISPKRRLSSSLNPSKRPAP), suggesting that its phosphorylation might impact ATR’s nuclear localization, possibly through interactions with nuclear importins (92). Interestingly, PKA-directed nuclear localization of DNA-PK, another PIKK family member, has also been reported (93). Our data suggest that PKA may be an important regulator of nuclear import of key nucleotide excision repair factor(s) during DNA repair. Alternatively, PKA-mediated phosphorylation of ATR at S435 may optimize nucleotide excision repair through enhanced intranuclear interactions with XPA to facilitate transport and/or assembly of nucleotide excision repair factors at sites of UV damage. By a variety of possible mechanisms, it is clear that PKA-mediated modification of ATR may “prime” melanocytes to better cope with UV injury.

Melanocortin signaling also impacts nucleotide excision repair through other mechanisms. For example, the nucleotide excision repair proteins, DDB1, DDB2 and XPC have all been shown to be upregulated following cAMP activation (94). Of note, it appears that MC1R wild-type melanocytes regulate DDB2 protein levels through a p38 MAPK-associated signaling. Recently, melanocortin-enhanced DNA repair was shown to be influenced by increased levels of XPC and H2AX, potentially promoting the formation of DNA repair complexes (47). Other components of the UV DNA damage repair response also are impacted by MC1R and include the NR4A superfamily of nuclear receptors (86,95,96). NR4A is recruited to sites of nuclear DNA damage together with XPC and XPE. Taken together, these studies provide a mechanistic view of MC1R as a master orchestrator of nucleotide excision repair in melanocytes.
production of a brown/black UV-blocking pigment known as eumelanin, there is production of a pigment variant known as pheomelanin, which is a red/blonde melanin bioaggregate that is less able to block UV radiation and actually promotes free radical formation (107). Indeed, pheomelanin is an independent melanoma risk factor (108); therefore, MC1R deficiency in fluences mutagenic risk not only because of diminished eumelanin formation, but also because of increased pheomelanin production. MC1R deficiency also results in less effective nucleotide excision repair (43,46–48); therefore, MC1R defects contribute to melanoma development not only by permitting more UV damage in melanocytes but also by blunting the ability of melanocytes to repair that damage.

**TRANSLATIONAL IMPLICATIONS**

The MSH–MC1R signaling axis is an innate melanocyte protective pathway recruited after UV exposure to help melanocytes recover from damage and protect the skin against further UV damage. Persons with inherited defects in MC1R signaling function suffer higher melanoma risk because of suboptimal melanization and defective DNA repair, each of which leads to

**Figure 10.** MC1R function and UV physiologic responses. Strength of MC1R signaling correlates with melanization, UV sensitivity, robustness of melanocyte DNA repair. Individuals with inherited loss-of-function MC1R polymorphisms have heightened melanoma risk, in part, because their melanocytes accrue more UV-signature mutations over time.

**Figure 11.** Pharmacologic strategies to mimic MC1R signaling in melanocytes. Raising cAMP levels in melanocytes enhances UV protection through enhanced melanin production and improved melanocyte genome stability. MC1R-specific approaches include melanocortin analogs or, theoretically, inhibitors of MC1R antagonists such as βD3, but would be expected to be of benefit only for individuals with intact MC1R signaling. Pharmacologic induction of cAMP could be achieved by activation of adenylyl cyclase or through inhibition of phosphodiesterases and would be independent of MC1R function.
accumulation of UV-induced mutations known to be causative for melanoma. Because MC1R-mediated melanization and enhanced genome maintenance are dependent on cellular cAMP levels, it is possible to pharmacologically stimulate MC1R responses to protect MC1R-impaired individuals from UV-induced mutagenesis by manipulating melanocyte cAMP levels. There are two fundamental approaches to achieve this: (1) those that specifically target melanocytes by taking advantage of the fact that MC1R expression is limited to melanocytes in the skin and (2) those that raise cAMP through MC1R-independent mechanisms (Fig. 11). Targeting approaches that limit cAMP induction in melanocytes is important because of the potential for off-target effects of cAMP stimulation on other cells (e.g., keratinocytes) and systems (immune system, circulatory system, etc.). Therefore, cutaneous application of agents that mimic melanocortin agonists or that inhibit MC1R antagonists would be expected to increase cAMP levels only in melanocytes because of selective action on MC1R (44). Although these approaches offer melanocyte specificity, neither melanocortin analogs nor inhibitors of MC1R antagonists would be expected to offer benefit to individuals with inherited MC1R signaling defects. As these persons are among the most melanoma-prone in the general population, MC1R-independent mechanisms of raising melanocyte cAMP levels seem more appropriate. Our group has used forskolin, a powerful activator of adenyl cyclase, to show that topical induction of cAMP rescues melanization (40,109,110) and optimizes nucleotide excision repair (46) in a humanized mouse model of the MC1R-defective human. Similarly, others have rescued melanization in this model by interfering with phosphodiesterases (111), the enzymes responsible for degrading cAMP. Global induction of cAMP in the skin would overcome MC1R defects and enhance melanocyte UV resistance, but this approach lacks melanocyte specificity. While more research is required to understand the risks and benefits of different therapeutic approaches aimed at augmenting MC1R signaling, it is now clear that it is possible to reduce short- and long-term UV injury to the skin by rationally targeting the innate melanocortin signaling axis UV-protective pathway.

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