Chapter 7
Melanocortin 1 Receptor (MC1R) as a Global Regulator of Cutaneous UV Responses: Molecular Interactions and Opportunities for Melanoma Prevention

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Abstract UV radiation is a pervasive environmental agent that affects the skin in complex ways. It benefits human health by its contribution to the biosynthesis of vitamin D in the skin, however it also is a major carcinogen responsible for millions of skin cancers diagnosed each year. One of the most important physiologic responses recruited with UV exposure is the melanocortin signaling axis. This pathway, initiated by melanocortins such as melanocyte stimulating hormone (α-MSH) or adrenocorticotropic hormone (ACTH), is dependent on the signaling function of the melanocortin 1 receptor (MC1R), a G_s protein-coupled cell surface receptor found on melanocytes in the skin. MC1R mediates its downstream UV-protective responses through activation of adenylyl cyclase and production of the second messenger cAMP. In melanocytes, cAMP stimulation leads to improved survival and UV-defensive sequelae. Here, we review how MC1R signaling protects melanocytes from UV-induced malignant degeneration, focusing on recent insights into molecular links between MC1R signaling and the nucleotide excision repair (NER) genome maintenance pathway. Finally, we highlight how insights into the MC1R UV protective response may facilitate the development of rational melanoma-protective strategies.

Keywords Melanocyte · Melanoma · UV radiation · Mutation · MC1R · Melanocortin · Skin cancer · ATR · cAMP · PKA · DNA repair

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7.1 Introduction

Diagnosed in almost 70,000 Americans per year, melanoma is the most lethal skin cancer and claims the lives of nearly 10,000 people each year in the US (Simard et al. 2012). Like other skin cancer, melanoma has a clear association with UV and incidence increases with age due to the cumulative effects of mutations over time. Melanoma, which is derived from melanocytes, accounts for approximately 4 % of skin tumors but accounts for about three quarters of all deaths by skin cancer (Narayanan et al. 2010). Melanoma metastasizes relatively quickly and once it has spread is difficult to control. In contrast, the keratinocyte-derived skin malignancies (basal cell carcinomas and squamous cell carcinomas) are diagnosed more often but are much less deadly because most remain confined to their primary site of origin and are amenable to local control measures such as surgical resection. Because melanoma incidence has been increasing for many years (Linos et al. 2009), our research focuses on understanding early events in melanoma carcinogenesis in order to reduce melanoma morbidity and mortality through the development of rational interventions. Abundant molecular and epidemiologic data link melanoma to UV (Hodis et al. 2012; Lawrence et al. 2013; Shain et al. 2015), and it is estimated that UV causes nearly 65 % of melanomas (Pleasance et al. 2010). Risk of melanoma is also heavily influenced by skin complexion, with more darkly pigmented individuals being relatively protected from disease. A major reason why darkly pigmented people are protected from melanoma is the abundance of melanin in the epidermis which acts as a "built-in sunblock" to physically interfere with UV penetration into the skin. However it is now evident that certain pigment-regulating genes also influence melanocytic UV responses in melanin-independent ways that heavily influence UV mutagenesis, which is the focus of this chapter. Since UV signature mutations are frequently found in melanoma, it follows that resistance to UV-induced DNA changes is of paramount importance to melanoma risk.

7.2 The Skin

The great majority of melanomas are thought to initiate in the skin. The largest organ of the body, the skin accounts for approximately 16 % of total body mass. Skin is a complex tissue made up of two primary layers—the epidermis and the dermis—along with numerous specialized cells and structures of epithelial, mesenchymal, glandular and neurovascular components. The epidermis, derived from embryonic ectoderm, is the outermost layer of the skin and serves as the main protective barrier for the body's interaction with the external environment. The epidermis is critically important to resisting environmental stressors such as foreign microorganisms, chemical and physical agents and UV. The epidermis is mainly

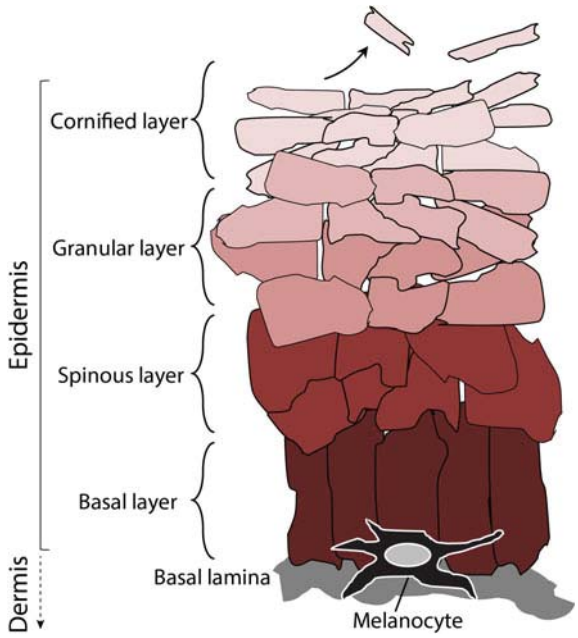

Fig. 7.1 Structure of the epidermis. The outermost layer of the skin is the epidermis, a self-renewing tissue composed mainly of keratinocytes in various stages of terminal differentiation. Nascent keratinocytes are produced in the basal layer (stratum basale). As they move outward through the epidermis, displaced by newly formed cells below, they differentiate into cytokeratin-rich cells linked to each other by tight junctions to form an effective physico-chemical barrier layer. Keratinocytes receive melanin pigments made from melanocytes, the pigment producing cells in the skin

made up of keratinocytes, which are epithelial cells organized into distinct layers defined largely by intercellular associations, nucleation and differential cytokeratin expression (Fig. 7.1). Keratinocytes are the most abundant cells in the epidermis and are characterized by their expression of cytokeratins and formation of desmosomes and tight junctions that together make the skin highly resistant to environmental stressors and pathogens. The dermis, derived from mesoderm, underlies and supports the epidermis and harbors cutaneous structures including hair follicles, nerves, sebaceous glands and sweat glands. Dermal immune cells and fibroblasts actively participate in many physiologic cutaneous responses. The dermis is separated from the epidermis by a basement membrane known as the basal layer which is where epidermal melanocytes are positioned. Epidermal keratinocytes, derived from keratinocyte stem cells in the basal layer, differentiate as they move outward toward the surface of the skin, accumulating cytokeratins, losing their nuclei and adhering to each other. As they mature, keratinocytes also accumulate melanin pigments that block incoming UV radiation from penetrating the epidermis to reach cells deep in the skin.

Although the majority of epidermal melanin may be in keratinocytes, it is not made by keratinocytes. Rather, melanin is exclusively synthesized in melanocytes which are neural crest-derived dendritic cells found in dermal hair follicles as well as in the interfollicular epidermis in the basal layer. Melanocytes intimately associate with several maturing keratinocytes by way of their dendritic processes. Through this interaction, keratinocytes and melanocytes signal each other through contact-dependent and paracrine interactions and melanin pigments are passed from melanocytes to keratinocytes. It has been estimated that an epidermal melanocyte may be functionally associated with as many as fifty keratinocytes in what has been termed an "epidermal melanin unit" (Nordlund 2007). In melanocytes, pigment is made in discreet intracellular organelles known as melanosomes which are membrane bound and contain the necessary enzymes and ion channels needed for melanogenesis. As melanin accumulates, melanosomes are transported away from perinuclear regions of the melanocytes along dendrites for eventual transfer to keratinocytes in the epidermal melanin unit. Rather than being released by exocytosis into the extracellular milieu, melanin is transferred to keratinocytes still packaged in melanosomes that are actively exported from melanocytes and taken up intact by neighboring keratinocytes (Yamaguchi and Hearing 2009). Once inside the keratinocytes, melanosomes accumulate in a cell-polarized manner to "shield" keratinocyte nuclei by accumulating on the side of the cell facing the outside of the skin where UV would enter. Interestingly, most inherited pigmentary defects are caused not by melanocyte deficiency but rather by mutations in melanogenic enzymes such as tyrosinase that causes oculocutaneous albinism type 1 (Scherer and Kumar 2010). Thus, the total number of melanocytes in the skin is similar regardless of an individual's complexion.

7.3 Melanocytes

Melanocytes are pigment producing cells derived from melanoblasts in embryonic development. After leaving the neural crest which develops in paravertebral/spinal locations, melanoblasts migrate through the mesenchyme to position themselves in dermal hair follicles and in the basal layer of the epidermis (Sommer 2011). Melanoblast survival and differentiation into melanocytes is dependent upon cell signaling events including stem cell factor-cKit and endothelin-endothelin B receptor interactions (Grichnik et al. 1998; Hou et al. 2004) as well as expression of the microphthalmia (Mitf) transcription factor (Widlund and Fisher 2003). Developmental defects in these signaling pathways lead to pigmentation defects such as piebaldism and Waardenburg syndrome. Cutaneous melanocytes in the basal layer at the epidermal/dermal junction play a major role in pigmentation of the skin. Indeed melanocytes are the only pigment-producing cell in the skin. Outside the skin, melanocytes are found in the leptomeninges, cochlea, retinal pigment

epithelium, substantia nigricans and locus coerulus where they serve important homeostatic mechanisms to these tissues. The great majority of melanomas, however, are thought to derive from melanocytes in the basal layer of the epidermis. Because they are long-lived terminally differentiated cells, melanocytes are highly susceptible to environmental carcinogens including UV radiation, heavy metal and chemicals. Accordingly, because of their position in the skin, melanocytes must be able to repair DNA damage to prevent mutations and carcinogenesis lest they undergo carcinogenic transformation. To that end, melanocytes have innate and inducible protective mechanisms to prevent and repair environmental-induced damage to preserve genomic maintenance. We and others have observed that the melanocortin signaling axis, discussed in depth below, significantly enhances the ability of melanocytes to resist UV damage and mutagenesis (Bohm et al. 2005; Abdel-Malek et al. 2006, 2009; Hauser et al. 2006; Song et al. 2009; Kadekaro et al. 2012; Jagirdar et al. 2013; Jarrett et al. 2014, 2015; Swope et al. 2014).

7.4 Melanin

Perhaps more than anything else, melanocytes are recognized for their production of melanin pigments. These pigments are physiologically critical to UV resistance since they are able to absorb UV energy and in so doing, prevent UV photons from entering the deep layers of the skin (Miyamura et al. 2007). Melanin is a heterogeneous bioaggregate made up of pigmented chemical species derived from the amino acid tyrosine. It exists in two major forms: (1) a brown/black pigment known as eumelanin that is abundantly expressed in the skin of individuals with dark UV-protected skin complexion, and (2) a reddish/blonde sulfated pigment called pheomelanin that is the main melanin species in fair-skinned UV-sensitive individuals (Slominski et al. 2004). Eumelanin and pheomelanin are both formed from the sequential oxidation and cyclization of tyrosine, with their synthetic pathways diverging after the formation of DOPAquinone. Since the amount of epidermal eumelanin is among the most important determinants of UV sensitivity and skin cancer risk, much attention has been made on the physiologic factors that regulate its production. Melanocytic eumelanin production is largely regulated by the amount of cAMP second messenger in melanocytes which is largely determined by the signaling activity of the melanocortin 1 receptor (MC1R) (Nasti and Timares 2015). Thus, fair-skinned people who are almost always UV-sensitive and have high risk of skin cancer have a high incidence of inherited loss-of-function polymorphisms in the MC1R and as a result express low levels of epidermal eumelanin. Skin cells of such individuals receive much more UV than those of darker-skinned individuals who, because of more epidermal UV-blocking eumelanin, have built-in "sunblock" in their skin.

7.5 Skin Complexion

Epidermal pigmentation is a multigenic phenotype and is among the most important determinants of UV sensitivity and cancer risk. Largely determined by epidermal eumelanin levels, skin pigmentation directly predicts how much cellular damage will be realized by UV. Many genes regulate basal pigmentation, with many associated with melanin synthesis or melanosome structure/function (Scherer and Kumar 2010). Many pigment-relevant genes were first identified through the study of color phenotype in mice and other model organisms. Since tyrosinase catalyzes the first two chemical reactions seminal for the production of either eumelanin or pheomelanin, its deficiency results in albinism wherein neither pigment is made to any significant degree. As a result, individuals with albinism are exceptionally sun-sensitive and burn with minimal doses of UV. In contrast, defects in other melanogenic enzymes cause dilutional pigmentary effects rather than total melanin loss. Finally, mutations in key melanocyte survival/differentiation regulators result in profound melanocyte-defect phenotypes such as piebaldism caused by loss-of-function of the c-Kit tyrosine kinase receptor (Tomita and Suzuki 2004).

7.6 Fitzpatrick Pigmentation Phenotype

The "Fitzpatrick Scale", developed in the 1970s by Dr. T.B. Fitzpatrick, to describe skin tone, is comprised of six pigmentation categories that describe a person's pigmentary phototype basted on skin color and sensitivity to UV radiation (Fig. 7.2). A semi-quantitative measurement of UV sensitivity is the "minimal erythematous dose" (MED) which reflects how much UV is required to result in skin inflammation. MED is generally calculated 24–48 h after UV exposure and is assessed using clinical indications of inflammation—erythema (redness) and edema (swelling)—as endpoints. Because eumelanin is an efficient UV blocker, more UV is generally needed to "burn" skin of dark pigmentation as compared to light-colored skin, and MED will accordingly be higher in dark-skinned individuals (Fig. 7.3).

The capacity to "tan" after UV exposure is an important physiologic cutaneous reaction to UV exposure. This adaptive pigmentary response is a well-regulated mechanism that is recruited into action following exposure to UV. It involves the proliferation of melanocytes and keratinocytes as well as increases in the production and accumulation of eumelanin in the epidermis. In this way, the skin protects itself against further UV insult. The tanning response is dependent on the function of the MC1R (D'Orazio et al. 2006), and people with inherited loss-of-function MC1R alleles tend to be particularly sun-sensitive. Thus, persons of skin phototypes I and II are quick to sunburn, tend not to be able to tan and are at higher risk for melanoma and other skin cancers when compared to people of higher Fitzpatrick phototype.

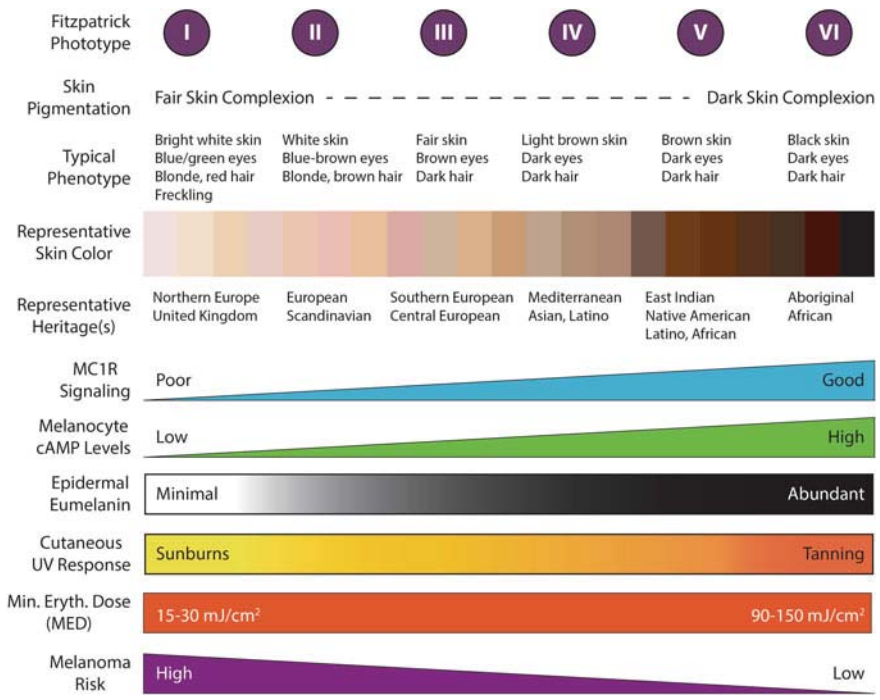

Fig. 7.2 Skin pigmentation, Fitzpatrick scale, MED and UV sensitivity. Skin complexion can be described by Fitzpatrick phototype, with individuals of least pigmentation having phototype I and persons of darkest complexion having phototype VI. Though pigmentation is determined by many genes, skin complexion and UV responses are heavily regulated by the MC1R signaling and epidermal eumelanin composition. Robust MC1R signaling leads to induction of cAMP in melanocytes which promotes eumelanin production responsible for a vigorous tanning response (adaptive pigmentation) and better protection from subsequent UV insults. Skin cancer risk, including melanoma, is heavily influenced by skin pigmentation and MC1R signaling

7.7 UV Radiation

UV radiation is a common and pervasive environmental carcinogen. Humans receive UV from ambient sunlight and increasingly from artificial UV sources such as indoor tanning devices. Though UV offers important health benefits including cutaneous production of vitamin D from cholesterol precursors (Holick 2008), excess exposure to UV causes many health consequences including photoaging, wrinkling, inflammation and cancer (Krutmann et al. 2012). UV is part of the electromagnetic spectrum, with wavelengths situated between the visible light and gamma radiation. Most ambient sunlight that strikes the Earth's surface is made up of a blend of UV-A and UV-B radiation in an approximate 9:1 ratio. UV-A has the longest wavelengths (315–400 nm) but the least energy of all UV. Nonetheless, UV-A can penetrate deeply into the skin, well into the dermis. UV-B radiation

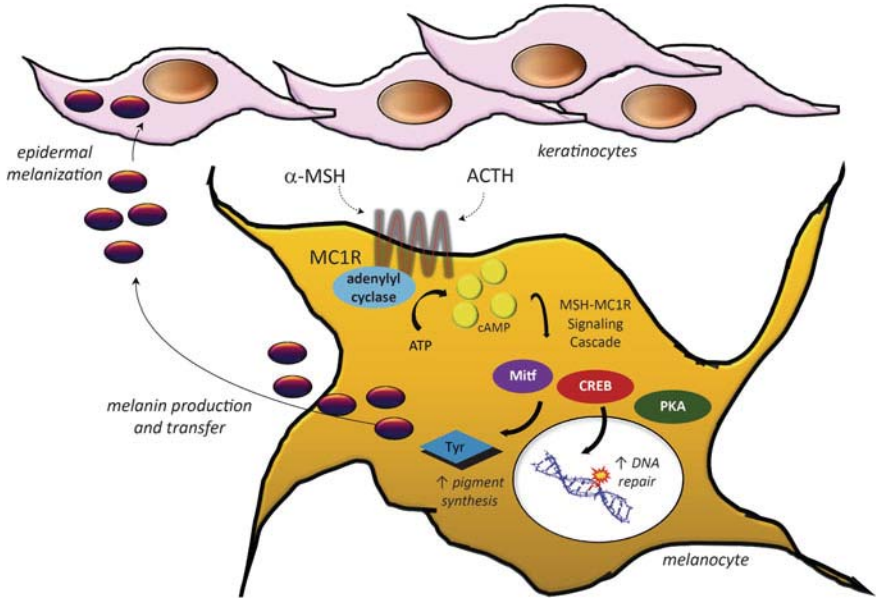

Fig. 7.3 Melanocortin—MC1R signaling axis. One of the most important ways in which the skin protects itself from UV damage is through increasing its melanin content after UV exposure. This process, commonly known as "tanning", is regulated by the melanocortin signaling axis. Melanocortins (α-MSH, ACTH) bind MC1R to activate adenylyl cyclase and stimulate cAMP production. cAMP activates the CREB and Mitf transcription factor network and increases the activity of PKA. Pigment enzymes such as tyrosinase are subsequently up-regulated and eumelanin production in melanosomes is increased. Melanin is transferred to neighboring keratinocytes to lay down a UV-protective layer of pigment in the epidermis. In the absence of effective MC1R signaling (e.g. in persons with loss-of-function MC1R alleles), these pathways are blunted and the skin does not tan effectively. Such individuals are also at higher risk for melanoma, in part because of sub-optimal DNA repair which permits the accumulation of UV signature mutations that contribute to malignant degeneration of melanocytes

(280–315 nm) is the highest energy component of ambient sunlight and can also penetrate into the skin and have significant physiologic impact on skin cells.

Indoor tanning and purposeful UV exposure represent an increasing risk factor for melanoma. With the successful marketing and increasing commercial availability of indoor tanning, the regular use of indoor tanning salons has skyrocketed in America. Whereas only 1 % of the US population had ever used a tanning bed 30 years ago, now over 25 % of Americans have used indoor tanning (Choi et al. 2010). The US tanning industry caters to nearly 30 million clients, employs roughly 100,000 workers and makes billions of dollars each year. Indeed, a significant percentage of the population regularly seeks out artificial UV in the form of indoor tanning with people starting to tan in adolescence and young adulthood. Because the UV administered from such sources can be up to ten times more powerful than ambient sunlight and can deliver both UVA and UVB radiation, indoor tanning

represents an increasingly important source of human UV exposure (Nilsen et al. 2011). Moreover, indoor tanning machines are poorly regulated and vary widely with respect to UV composition and strength. Indoor tanning is now unequivocally linked to increase risk of many forms of skin cancer including basal cell and squamous cell carcinomas, the most commonly diagnosed skin cancers. With respect to melanoma, lifetime risk increases by 75 % if people engage in artificial tanning before the age of 35 years (Fisher and James 2010). Most experts conclude the risks of indoor tanning and purposeful UV exposure far outweigh their potential health benefits. Indeed, decreasing UV radiation exposure may be the single best way to reduce incidence of melanoma and other skin cancers.

UV affects DNA directly through the absorption of UV energy by nucleotides in the double helix as well as indirectly through the generation of free radicals and subsequent oxidative damage to nucleotide bases in chromatin. The 5-6 double bond of pyrimidines seems especially vulnerable to UV-induced cleavage. When this occurs between neighboring cytosines or thymidines, abnormal covalent bonds can result to form two major "photolesions": [6,4]-photoproducts or cyclopyrimidine dimers. Each is highly mutagenic, mispairing with abnormal bases during replication to cause permanent base changes in the genome to yield "UV signature mutations" (particularly C-to-T transitions) (Brash 2015). Recently, UV photolesions have also been reported to be formed as a result of free radical damage even after UV exposure has ended—so called "dark photolesions" (Premi et al. 2015). A day's worth of sun exposure may result in up to 100,000 UV photolesions in every skin cell (Hoeijmakers 2009). Fortunately, melanocytes and other skin cells have a DNA repair pathway—nucleotide excision repair (NER)—to fix UV photodimers before they have the opportunity to result in a permanent somatic mutation in the genome.

7.8 Cutaneous Responses to UV

The skin responds to UV with a coordinated series of physiologic changes to limit cellular damage and to prevent further injury. Cutaneous response is proportional to the UV dose realized by the skin and is determined by strength of UV radiation, time of exposure and degree of photoprotection afforded by epidermal melanin. UV penetration into the skin elicits a variety of cellular events including release of cytokines, recruitment of immune cells, epidermal thickening and up-regulation of pigment synthesis to protect the skin from further UV insult. Above a certain dose threshold, UV induces inflammation, manifested by pain, erythema (redness) and edema (swelling). UV-induced inflammation, commonly referred to as "sunburn," is mediated by a variety of cytokines, vasoactive and neuroactive mediators in the skin. UV exposure causes an initial erythema that worsens over a period of 24–72 h due to vasodilation mediated in part by nitric oxide production by keratinocytes. Neutrophils infiltrate the dermis for several hours after UV exposure and promote inflammation through degranulation and production of free radicals.

The inflammatory response in the skin following exposure to UV radiation is complex, involving induction of pro-inflammatory cytokines and induction of an immunosuppressive phenotype. UV results in the production of TNF-alpha, IL-1β, and IL-10 by keratinocytes and IL-1α/β, IL-6, IL-8, and TNFα by melanocytes [reviewed in (Ullrich and Byrne 2012)]. These pro-inflammatory cytokines exert paracrine and autocrine effects locally and systemically. Above a certain UV threshold, keratinocytes die by apoptosis as manifested by the accumulation of "sunburn cells" (apoptotic keratinocytes) in UV-exposed skin (Lippens et al. 2009). UV induces a variety of other physiologic changes in the skin, including keratinocyte proliferation and stratum corneum thickening. The hyperkeratosis phenotype is a protective mechanism to prevent damage from subsequent UV radiation (Scott et al. 2012). Thus, UV causes a variety of physiologic changes in the skin, many of which lead to inflammation, photoaging and cancer.

7.9 Melanocortin—MC1R Signaling Axis

One of the most widely recognized effects of UV exposure on the skin is the up-regulation of melanin production, commonly referred to as "tanning". The tanning response occurs in two distinct stages: immediate pigment darkening and delayed tanning. The initial darkening is due to redistribution of pre-formed melanin granules while the delayed tanning response results from synthesis of new melanin pigment. The melanization response is largely under the control of the MC1R signaling cascade. There are three main known categories of MC1R ligands: positive melanocortin agonists adrenocorticotropic releasing hormone (ACTH) and alpha-melanocyte stimulating hormone (α-MSH), the negative agonist agouti signaling protein (ASIP), and the neutral antagonist beta-defensin 3 (βD3). MC1R agonists up-regulate melanin synthesis whereas MC1R antagonists either inhibit MC1R signaling directly or compete with MC1R agonists for MC1R binding.

The two major melanocortins—ACTH and α-MSH—are cleavage products from the pro-opiomelanocortin (POMC) protein. Besides basal POMC production by the pituitary, POMC can be made in the skin and UV exposure promotes its expression from keratinocytes in a cellular damage- and p53-dependent manner (Cui et al. 2007). The melanocortins bind to the MC1R with high affinity to promote a variety of survival and differentiation events in melanocytes. The MC1R is a G_s protein-coupled receptor (GPCR) and binding of MC1R by either ACTH or α-MSH activates adenylyl cyclase to induce cAMP second messenger generation. In turn, cAMP accumulation leads to a variety of signaling events including stimulation of the activity of protein kinase A (PKA) and increased expression and enhanced activity of the cAMP responsive binding element (CREB) and microphthalmia (Mitf) transcription factors (Yamaguchi and Hearing 2009). Many melanogenic biosynthetic enzymes and regulators are influenced by cAMP signaling and

the CREB and Mitf pathways including tyrosinase, dopachrome tautomerase, and pmel17. Similar to other GPCRs, the MC1R has seven transmembrane helical domains with an extracellular N-terminus and intracellular C-terminus (Garcia-Borron et al. 2005).

The MC1R is a highly polymorphic protein with many variants (Kennedy et al. 2001), some of which are associated with the red hair color (RHC) phenotype (Box et al. 1997). Of the alleles associated with the RHC phenotype, there are those that are strongly associated with RHC ('R' alleles: D84E, R151C, R160 W, and D294H) and those that are weakly associated with RHC phenotype ('RHC' alleles: V60L, V92M, R163Q) (Duffy et al. 2004). Mutations in MC1R affect either MC1R function or prevent relocation to the plasma membrane (Beaumont et al. 2005). Individuals with loss-of-function MC1R polymorphisms are highly susceptible to developing melanoma for at least three reasons: (1) they burn easily and accumulate a greater degree of UV damage, (2) they cannot repair UV damage as efficiently and are more susceptible to UV-induced mutations, and (3) they cannot tan and therefore do not protect their skin from future exposure to UV radiation. Pharmacologic manipulation of the MC1R signaling axis in individuals with defective MC1R signaling is a potential mechanism to prevent UV-induced damage and melanoma development.

7.10 MC1R Antagonists

There are two major proteins that bind to MC1R and antagonize MC1R signaling and downstream cAMP accumulation: agouti signaling protein (ASIP) and beta-defensin 3 (βD3). ASIP is a potent MC1R antagonist, decreasing basal MC1R signaling and antagonizing melanocortin effects on melanocytes by functioning as a competitive inhibitor to α-MSH (Sviderskaya et al. 2001). Binding of ASIP to MC1R causes a decrease in the pigment enzymes levels of tyrosinase related protein 1 and 2 and a decrease in tyrosinase activity, all of which diminishes eumelanin production (Suzuki et al. 1997). The effects of ASP (the mouse homolog to human ASIP) on pigment are evident in mice with the lethal yellow mutation in *ASP* that overexpress agouti and exhibit a blonde pheomelanotic coat color as a result (Jordan and Jackson 1998). Importantly, the effect of ASIP on mouse coat color is dependent on a functional MC1R confirming agouti's role as an MC1R regulatory ligand (Ollmann et al. 1998).

βD3, like ASIP, antagonizes melanocortin signaling through MC1R. βD3 is a member of the defensin family, a group of small antimicrobial proteins that exhibit innate antibacterial and antifungal properties (Arnett and Seveau 2011). In 2007, Barsh and coworkers determined that in addition to its role in the immune response, βD3 could affect pigmentation through interactions with MC1R. The group determined that black coat color of certain dog breeds was due to dominant

overexpression of the canine homolog of βD3 (CBD103) rather than loss-of-function MC1R mutations (Candille et al. 2007). Although the role of βD3 at MC1R remains somewhat controversial with one report suggesting it may act as a weak MC1R agonist (Beaumont et al. 2012), most experimental results suggest that βD3 functions as a neutral MC1R antagonist (Swope et al. 2012; Jarrett et al. 2015). Thus, unlike ASIP which down-regulates ligand-independent MC1R signaling, βD3 appears to act as competitive inhibitor for both the melanocortins and ASIP and therefore regulates skin pigmentation. The induction of βD3 following inflammatory stimuli is well established (Kaiser and Diamond 2000), however, the regulation following UV exposure is unclear. In vivo exposure of human subjects to UV radiation resulted in an increase in βD3 gene and protein expression (Glaser et al. 2009), however these results were not confirmed in an ex vivo setting suggesting the damage response initiated by UV radiation was not sufficient to induce βD3 expression (Wolf Horrell and D'Orazio 2014). The induction of the inflammatory response may be critical for the induction of βD3 following UV radiation which may be relevant in the setting of sunburns: if cutaneous βD3 expression is upregulated with UV exposure, it may compete with melanocortin-MC1R interactions and inhibit induction of MC1R-mediated UV protective pathways.

7.11 Nucleotide Excision Repair (NER)

Skin cells repair UV-induced DNA damage through a highly coordinated genome maintenance pathway known as the nucleotide excision repair (NER) pathway. The NER pathway repairs bulky DNA lesions that distort the double helical backbone and/or interfere with transcription (Nouspikel 2009). It repairs lesions with high fidelity, reliant on the undamaged complementary strand for specificity and invoking at least eight essential proteins that function coordinately to carry out NER. We understand NER's importance by observing the natural history of xeroderma pigmentosum (XP) patients who lack NER because of inherited homozygous loss of any one of the eight essential NER factors: *XPA*, *ERCC1*, *ERCC3 (XP-B)*, *XPC, ERCC2 (XP-D)*, *DDB2 (XP-E)*, *ERCC4 (XP-F)*, *ERCC5 (XP-G) and POLH*. Although rare, XP is a UV hypersensitivity syndrome largely characterized by progressive skin degenerative changes in UV-exposed areas. Because they lack NER and cannot reverse DNA photodamage, XP patients are profoundly UV sensitive, burning easily with minimal sun exposure and developing disfiguring skin changes in childhood including abnormal pigmentation, cutaneous telangiectasias, scarring and atrophy. Their risk of melanoma and other skin cancers is roughly 3 logs higher than unaffected patients, with premalignant and true skin cancers frequently occurring in childhood—decades before their peak in the general population—despite their best attempts to avoid UV (DiGiovanna and Kraemer 2012).

NER is a complex pathway regulated by many more proteins than simply the eight core NER factors, and the reader is referred elsewhere for an in-depth review of NER (Scharer 2013). In general, NER can conceptualized in discreet stages.

DNA damage is recognized either because it physically distorts the double helix or because it interferes with RNA polymerase in actively transcribed genes. In either case, a multiprotein repair complex is recruited to the damaged site where the damaged strand is isolated, unwound, stabilized and nicked on either side of the damage. A 25–30 mer oligo containing the damage is excised and the resultant gap is filled in by DNA polymerase using the undamaged complementary strand as a template. Finally, DNA ligase seals the nicks to restore the DNA to its original undamaged state in a manner designed to preserve fidelity of sequence (Fig. 7.4).

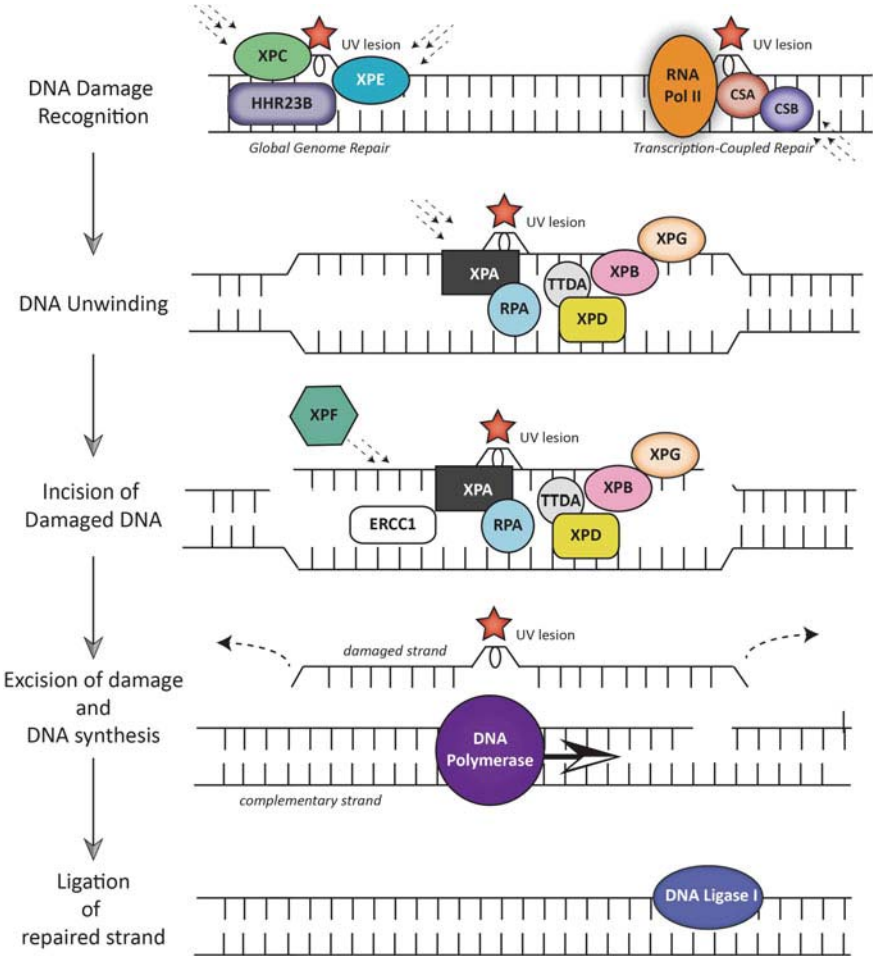

Fig. 7.4 Nucleotide excision repair (NER) pathway. At least eight core NER factors function in a coordinated manner to identify bulky DNA lesions, excise a 25–30 mer oligonucleotide harboring the damage and replace the excised region using the complementary strand to ensure fidelity of process. *Note* this is a highly simplified model of NER that does not show many accessory factors that regulate the repair process

While NER's importance to UV resistance and protection from malignancy is most clearly evident in XP patients, it is becoming evident that less penetrant NER polymorphisms may also impact cancer risk in the general population (Li et al. 2013). Since melanoma is heavily influenced by UV damage and mutagenesis, it follows that efficiency of NER is an important melanoma determinant.

7.12 MC1R and NER

NER efficiency is increased in melanocytes that have been stimulated by the MC1R/cAMP pathway. This was first demonstrated in human primary melanocyte lines wherein the clearance of UV photoproducts was improved by stimulating cells with melanocortins (Hauser et al. 2006). The benefit of MC1R was subsequently shown in whole skin of a congenic mouse model of humanized skin. Specifically, using *K14-Scf* transgenic mice that retain melanocytes in the interfollicular dermis because of constitutive expression of Kit ligand (stem cell factor), we found that repair of UV photoproducts was more efficient and more complete in *Mc1r*-intact (vs.-defective) animals. Moreover, when forskolin, a skin-permeable adenylyl cyclase stimulator, was applied to the skin of *Mc1r*-defective mice, their NER efficiency was greatly enhanced (Jarrett et al. 2014), showing that pharmacologic manipulation of cAMP in the skin could impact DNA repair of UV photolesions. These proof-of-concept experiments confirmed the important contribution of MC1R to NER in a genetically-defined system and, most importantly, suggested that NER can be pharmacologically boosted in Mc1r-defective UV-sensitive and melanoma-prone individuals.

Defining the mechanisms that link MC1R signaling to melanocytic DNA repair responses has been an active area of investigation. Recently, we reported that the key molecular event involved in MC1R-mediated augmentation of NER involves a post-translational modification of the global cell damage response protein "ataxia and rad3-related" (ATR) protein by PKA. We discovered that cAMP induction and activation of PKA, either through conventional melanocortin-MC1R interactions or through pharmacologic activation of adenylyl cyclase by forskolin, causes PKA to phosphorylate ATR on a serine at position 435. This event does not impact canonical ATR activation as defined by ATR-mediated phosphorylation of Chk1 and cell cycle arrest, but rather enhances the binding affinity of ATR for the xeroderma pigmentosum A (XPA) protein to facilitate NER (Fig. 7.5). With cAMP stimulation, XPA and pS435-ATR co-localize to UV photoproducts in a greatly accelerated and enhanced manner and NER is much more efficient and complete (Jarrett et al. 2014). Our group's efforts to determine how pS435-ATR is regulated and how it impacts NER are ongoing.

Melanocortin signaling may impact genome stability through other pathways beside PKA-mediated ATR phosphorylation and XPA recruitment. MC1R signaling, for example, promotes cellular antioxidant defenses, thereby endowing melanocytes with an improved ability to resist UV-mediated oxidative and free radical

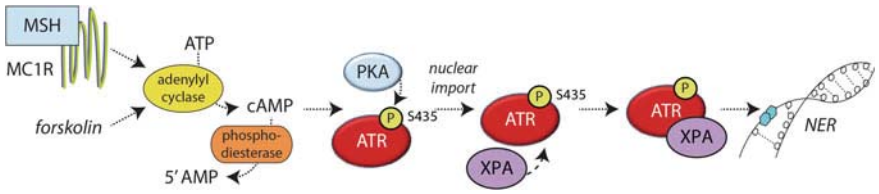

Fig. 7.5 MC1R augmentation of NER. Elevated levels of the second messenger cAMP can result from MC1R signaling (e.g. MSH), adenylyl cyclase activation (e.g. forskolin) or phosphodiesterase inhibition (e.g. rolipram). cAMP promotes the phosphorylation of ATR on the Ser 435 residue by PKA, which in turn facilitates ATR's nuclear import and association with the key NER factor XPA. Together, pS435-ATR and XPA translocate to sites of nuclear photodamage to accelerate NER in melanocytes

injury (Kadekaro et al. 2012). Furthermore, the cAMP signaling pathway may impact melanocytes by bolstering key DNA damage and repair factors besides XPA and ATR. The Abdel-Malek group found that MC1R stimulation promoted XPC expression, enhanced UV-induced phosphorylation of both ATR and ATM and increased phosphorylation of histone γH2AX (Swope et al. 2014). Smith and colleagues published that MC1R signaling resulted in increased expression of NR4A subfamily of nuclear receptors which were recruited to nuclear photodamage along with XPC and XPE (Jagirdar et al. 2013). Therefore the melanocortin-MC1R-cAMP signaling axis represents a multifaceted inducible pathway to protect melanocytes against UV injury and mutagenesis.

7.13 Future Directions: The Melanocortin-MC1R Axis as an Exploitable Melanoma Prevention Strategy

Inherited loss-of-function MC1R polymorphisms are common in the population, with some estimating up to 6–8 million Americans harboring double allele polymorphisms and millions more being hemizygous. Such persons tend to have fair complexions, are UV-sensitive (sunburn easily) and a four-fold or higher risk of melanoma (Kennedy et al. 2001). As we understand it, MC1R-mediated UV protection and melanoma resistance is proportional to the robustness of the cAMP response downstream of MC1R signaling. cAMP signaling positions melanocytes to resist UV damage and mutagenesis by stimulating production of melanin and by enhancing DNA repair through improving the efficiency of nucleotide excision repair (Fig. 7.6). In this way, persons with blunted MC1R signaling accumulate more UV damage because of diminished eumelanin production and less innate UV protection. Melanocytes in MC1R-defective individuals are also much less efficient at repairing UV photodamage because of a lack of the MC1R NER "boost". As a result, cells accumulate more UV-induced mutations and are more prone to carcinogenic transformation.

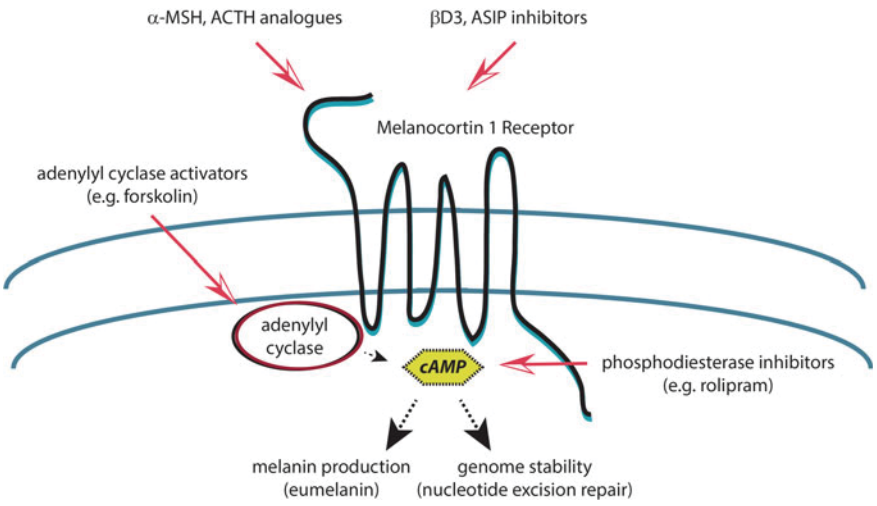

Fig. 7.6 Exploiting the MC1R-cAMP signaling axis for UV protection and melanoma prevention. Inducing cAMP in melanocytes, whether through up-regulating MC1R signaling with melanocortin analogs, activating adenylyl cyclase or inhibiting phosphodiesterases, would be expected to enhance pigment production and augment DNA repair, both of which would reduce UV-induced mutagenesis and cancer risk

Since a variety of pharmacologic strategies exist to impact cAMP signaling, it might be possible to reduce UV mutagenesis in melanocytes and reduce melanoma risk by exploiting the MC1R signaling axis. We previously showed that topical application of forskolin, a direct activator of adenylyl cyclase, rescued eumelanin production in fair-skinned and UV-sensitive *Mc1r*-defective animals (D'Orazio et al. 2006). This same animal model was used to show that topical application of rolipram, a phosphodiesterase-4 inhibitor, similarly rescued dark melanization of the skin (Khaled et al. 2010). In both cases, pharmacologic melanization of the skin protected against UV damage. Thus skin-permeable agents the increase cAMP in epidermal melanocytes are an effective way to mimic MC1R signaling in melanocytes and protect the skin against UV injury. Most recently, we published that topically-applied forskolin could also enhance clearance of UV photoproducts in the skin of these mice, essentially enhancing the level of repair to that of animals with intact Mc1r signaling (Jarrett et al. 2014). Together, these studies clearly prove the feasibility of pharmacologic manipulation of the cAMP signaling axis and subsequent protection of melanocytes against UV damage and mutagenesis.

Though applying general cAMP manipulators to the skin upregulates cAMP levels in epidermal melanocytes, this approach lacks specificity to induce signaling only in melanocytes. The effects of cAMP induction in other skin cells or in off-target tissues through systemic absorption are complex and likely to impede translational development. Melanocyte-directed approaches for cAMP stimulation would greatly enhance the potential translational appeal of this approach. To that end, targeted melanocyte-specific cAMP induction can be achieved through

melanocortin analogues such as those reported by Abdel-Malek and colleagues (Abdel-Malek et al. 2006). Melanocortin effects would be expected to be restricted to cells expressing melanocortin receptors such as MC1R on melanocytes. This approach, while offering better melanocyte specificity, requires MC1R signaling function to be intact in order for cells to respond to melanocortins by upregulating cAMP. Persons with inherited defects in MC1R signaling—the very individuals most at risk for UV sensitivity and melanoma development—would probably not benefit much from melanocortin therapy since MC1R signaling is impaired. For these individuals, perhaps the only way to trigger cAMP in melanocytes may be a global pharmacologic approach. Clearly much more mechanistic and feasibility work needs to be done to understand the risks and benefits of pharmacologic cAMP manipulation in melanocytes and in the skin. Overall, however, the broad melanocortin-MC1R signaling axis remains an attractive and potentially exploitable pathway for the development of novel melanoma prevention strategies in at-risk populations.

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