Participation of the Melanocortin-1 Receptor in the UV Control of Pigmentation

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The cloning of the *melanocortin-1 receptor (MC1R)* gene from human melanocytes and the demonstration that these cells respond to the melanocortins α-melanocyte stimulating hormone (α-MSH) and adrenocorticotropic hormone (ACTH) with increased proliferation and melanogenesis have renewed the interest in investigation the physiological role of these hormones in regulating human pigmentation. α-Melanocyte stimulating hormone and ACTH are both synthesized in the human epidermis, and their synthesis is upregulated by exposure to ultraviolet radiation (UVR). Activation of the MC1R by ligand binding results in stimulation of cAMP formation, which is a principal mechanism for inducing melanogenesis. The increase in cAMP is required for the pigmentary response of human melanocytes to UVR, and for allowing them to overcome the UVR-induced G1 arrest. Treatment of human melanocytes with α-MSH increases eumelanin synthesis, an effect that is expected to enhance photoprotection of the skin. Population studies have revealed more than 20 allelic variants of the *MC1R* gene. Some of these variants are overexpressed in individuals with skin type I or II, red hair, and poor tanning ability. Future studies will aim at further exploration of the role of these variants in MC1R function, and in determining constitutive human pigmentation, the response to sun exposure, and possibly the susceptibility to skin cancer.
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CUTANEOUS PIGMENTATION, PHOTOPROTECTION, AND SKIN CANCER RISK

Exposure to ultraviolet rays (UVR) from solar radiation is known be the main etiologic factor for skin cancers (Epstein, 1983; Sober *et al*, 1991). Chronic exposure to the sun is known to result in photocarcinogenesis and photoaging (Epstein, 1983; Sober *et al*, 1991). The incidence of skin cancer is increasing at an alarming rate, and is accentuated by the depletion of the ozone layer that limits the penetration of UVR through the earth's atmosphere (Redman, 1987). The risk for skin cancer is much higher in individuals with fair skin and blue eyes, who have a poor tanning ability (skin type I or II individuals) than in individuals with dark skin who tan efficiently when exposed to UVR (skin types III–VI) (Epstein, 1983; Sober *et al*, 1991; Pathak *et al*, 1980; Kaidbey *et al*, 1979). The presence of high melanin content in the latter skin types is known to limit the penetration of UVR through the epidermal layers, and thus reduce the photodamaging effects of sun exposure (Kaidbey *et al*, 1979). Melanin is synthesized and packaged within melanosomes in epidermal melanocytes, and melanosomes from each melanocyte are then transferred to the surrounding keratinocytes (Pathak *et al*, 1980). The rate of melanin synthesis by melanocytes and the rate of delivery of melanosomes to keratinocytes are two important determinants of constitutive skin color. Interest in investigating the regulation of human cutaneous pigmentation stems primarily from the importance of melanin in photoprotection (Morison, 1985; Menon and Haberman, 1977). The photoprotective effect of melanin has been attributed to its ability to filter and attenuate solar radiation by scattering, as well as by absorption and dissipation as heat (Pathak *et al*, 1980, 1995; Kaidbey *et al*, 1979). Microscopic examination of different skin types revealed that in skin type I, melanized melanosomes are degraded in the lower epidermal layers, while in dark skin, melanosomes remain intact even in the horny layer (Pathak *et al*, 1971). The presence of melanosomes in the epidermis of dark skinned individuals has recently been shown to confer photoprotection, because melanosomes form supranuclear caps that reduce the extent of DNA photoproducts by limiting the amount of UVR that reach nuclear DNA (Kobayashi *et al*, 1998).

EUMELANIN *VERSUS* PHEOMELANIN IN HUMANS

Human epidermal melanocytes synthesize both eumelanin and pheomelanin. The presence of cysteine in pheomelanin accounts for its reddish-yellowish color that distinguishes it from the dark brown eumelanin (Prota *et al*, 1967, 1998). In mouse hair follicles, eumelanin synthesis is associated with increased expression of the melanogenic enzymes tyrosinase, tyrosinase-related protein (TRP)-1, and TRP-2, whereas pheomelanin synthesis is associated with a reduction in tyrosinase and absence of TRP-1, TRP-2, and P protein (Kobayashi *et al*, 1995; Lamoreux *et al*, 1995). The ratio of eumelanin to pheomelanin differs dramatically in different skin phenotypes, being highest in skin types V and VI, and lowest in skin types I and II (Hunt *et al*, 1995a). Not only total melanin content (Kaidbey *et al*, 1979),

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but the ratio of eumelanin to pheomelanin seem to dictate the extent of photoprotection conferred by total melanin content in the epidermis. Earlier studies showed that pheomelanin was more photolabile than eumelanin (Menon *et al*, 1985). Irradiation of pheomelanin was found to generate considerable amounts of superoxide, while irradiation of eumelanin did not. In fact, eumelanin behaved as a pseudo-dismutase, thus reducing cellular oxidative stress. The addition of irradiated pheomelanin to cells resulted in their lysis, a response thought to be caused by the generation of hydrogen peroxide from superoxide, and the possible formation of singlet oxygen and hydroxyl radicals (Menon *et al*, 1983). Another factor that contributed to cell lysis is membrane lipid peroxidation and the oxidation of cysteine in membrane proteins that caused alteration of their structure (Chedekel and Zeise, 1988).

In mammalian melanocytes, the synthesis of eumelanin is stimulated by α-melanocyte stimulating hormone (α-MSH) (Geschwind *et al*, 1972; Hunt *et al*, 1995b). Treatment of human melanocytes with α-MSH stimulates the synthesis of eumelanin without significantly altering total melanin, thus resulting in an increased eumelanin to pheomelanin ratio. Because UVR stimulates the synthesis of α-MSH and the related hormone adrenocorticotropic hormone (ACTH) in epidermal melanocytes and keratinocytes, it is expected that UV exposure will increase eumelanin formation (Schauer *et al*, 1994; Chakraborty *et al*, 1996).

In human epidermal melanocytes, as well as in mouse follicular melanocytes, eumelanin synthesis is stimulated by the activation of the melanocortin 1 receptor (MC1R), the receptor for α-MSH that is coded for by the *extension* locus (Tamate and Takeuchi, 1984; Mountjoy *et al*, 1992; Robbins *et al*, 1993; Hunt *et al*, 1995b). In the mouse, the switch between eumelanin and pheomelanin synthesis in follicular melanocytes is primarily regulated by the *extension* locus and the *agouti* locus that codes for a soluble factor, agouti signaling protein (ASP) (Tamate and Takeuchi, 1984; Silvers, 1958, 1979; Yen *et al*, 1994). The availability and processing of proopiomelanocortin (POMC), the precursor for all melanocortins, is also important for stimulation of eumelanin formation (Eipper and Mains, 1980; Wakamatsu *et al*, 1997). Recently, two mutations in the *POMC* gene were identified in two unrelated children. These mutations resulted in red hair, as well as obesity and adrenal insufficiency associated with deficiency of POMC products, particularly α-MSH and ACTH (Krude *et al*, 1998). In the mouse, loss of function mutations at the *extension* locus result in a yellow coat color due to inability of follicular melanocytes to respond to α-MSH with eumelanin synthesis (Tamate and Takeuchi, 1984; Robbins *et al*, 1993). Also, mutations at the *agouti* locus that result in excessive and ectopic synthesis of ASP result in yellow coat color and obesity, due to competitive inhibition of α-MSH binding to the MC1R and MC4R (the receptor for α-MSH in the hypothalamus), respectively, by ASP (Lu *et al*, 1994; Yang *et al*, 1997; Ollmann *et al*, 1998). Inhibition of MC1R activity by ASP results in blocking of eumelanin production and stimulation of pheomelanin synthesis. We have shown that human melanocytes respond to α-MSH and ACTH with stimulation of melanin formation and increased proliferation (Abdel-Malek *et al*, 1995; Suzuki *et al*, 1996). Recently, we demonstrated that human melanocytes respond to purified recombinant human or mouse ASP with a reduction in basal tyrosinase activity and complete abrogation of the mitogenic and melanogenic effects of α-MSH, changes known to be associated with induction of pheomelanin synthesis (Kobayashi *et al*, 1995; Suzuki *et al*, 1997). As in the case of mouse follicular melanocytes, ASP acted as a competitive inhibitor of α-MSH binding to the human MC1R.

THE PHYSIOLOGIC EFFECTS OF α-MSH

α-Melanocyte stimulating hormone has been known for decades to be the physiologic stimulator of integumental pigmentation of many vertebrate species, including fish, reptiles, amphibians, and mammals (Sawyer *et al*, 1983; Sherbrooke *et al*, 1988). In animals that undergo rapid color change α-MSH stimulates the dispersion of preexisting melanin-containing melanosomes, resulting in skin darkening. In the mouse, the most widely used animal model to study the regulation of mammalian pigmentation, α-MSH induces *de novo* synthesis of eumelanin within follicular melanocytes (Geschwind *et al*, 1972; Levine *et al*, 1987). Gain of function mutations in the α-MSH receptor as in the homozygous tobacco mouse have been characterized and shown to result in increased ability to synthesize eumelanin, whereas loss of function mutations result in yellow coat color, due to the synthesis of pheomelanin (Tamate and Takeuchi, 1984; Robbins *et al*, 1993).

α-MSH belongs to the family of melanocortins, which include β-, γ-MSH, and ACTH (Smith *et al*, 1998). All of these bioactive peptides, together with β-lipotropic hormone and β-endorphin, are derived from a large precursor peptide, POMC (Rubinstein *et al*, 1978; Liotta *et al*, 1980). This peptide is classically known to be synthesized in the pituitary gland and to be processed by two main enzymes, prohormone convertase (PC) 1 and PC2 (Bloomquist *et al*, 1991; Lindberg, 1991). The former enzyme results in the formation of full length ACTH[1–39], and the latter cleaves ACTH into α-MSH[1–13]. The entire 13 amino acid sequence that makes up the structure of α-MSH is included within the structure of ACTH. It is now recognized that POMC is synthesized and differentially processed in many different tissues and organs, including various brain regions, the hypothalamus, placenta, gonads, gastrointestinal tract, and the skin (O'Donohue and Dorsa, 1982; Can *et al*, 1998). The presence of α-MSH-like peptides in human skin was reported many years ago (Thody *et al*, 1983). Recently, this was substantiated by the demonstration that cultured human epidermal keratinocytes and melanocytes synthesize α-MSH and ACTH (Schauer *et al*, 1994; Chakraborty *et al*, 1996). The presence of POMC as well as the enzyme PC1 in human epidermis *in situ* was also demonstrated (Wakamatsu *et al*, 1997). This suggests that melanocortins are potential paracrine/autocrine factors that regulate specific epidermal functions in human skin.

In addition to its best-described role as a regulator of skin pigmentation, α-MSH and related hormones serve many other functions. In several animal models α-MSH has been shown to be a potent inhibitor of the pyrogenic effects of interleukin-1 (IL-1) (Murphy *et al*, 1983; Robertson *et al*, 1988). This initial observation led to many studies describing the systemic, as well as the cutaneous anti-inflammatory effects of α-MSH, which include inhibition of antibody formation by B-lymphocytes, and suppression of contact hypersensitivity (Rheins *et al*, 1989; Luger *et al*, 1998). The immunosuppressive effects of α-MSH in the skin are mediated, at least in part, by stimulation of IL-10 synthesis, and by inhibition of the synthesis of IL-1, IL-6, and tumor necrosis factor-α (Luger *et al*, 1998). Other effects of α-MSH and related peptides include trophic effects on the adrenals during fetal development, stimulation of neuroneal regeneration following nerve injury and increased neurite formation, regulation of gonadal function, participation in the regulation of feeding behavior, and stimulation of memory and learning (Beckwith *et al*, 1977; Pigache and Rigter, 1981; Silman *et al*, 1981; Verhaagen *et al*, 1986; Seeley *et al*, 1997).

THE MELANOCORTIN RECEPTORS

The pleiotropic effects of α-MSH are mediated by binding to one of five melanocortin receptors (MCR) that are expressed by different types of target cells (Mountjoy *et al*, 1992; Chhajlani *et al*, 1993; Roselli-Rehfuss *et al*, 1993; Gantz *et al*, 1993a, b; Labbé *et al*, 1994). These receptors have been cloned and found to be coded for by five distinct genes. The five MCR represent a distinct family of G-protein coupled receptors with seven transmembrane domains. Binding of ligand to these receptors activates adenylate cyclase and stimulates cAMP formation. The MCR differ in their tissue distribution and in their relative affinities for the various melanocortin peptides. The first of these receptors, the MC1R, was cloned from mouse Cloudman melanoma cells and normal human melanocytes (Mountjoy *et al*, 1992). The MC2R was cloned from the adrenal medulla (Chhajlani *et al*, 1993); this receptor is the ACTH receptor, and among the melanocortin peptides, it recognizes ACTH with the highest affinity. The MC3, MC4, and MC5R were subsequently cloned (Roselli-Rehfuss *et al*, 1993; Gantz *et al*, 1993a, b; Labbé *et al*, 1994). The MC3R seems to be selective for γ-MSH, and is expressed mainly in the hypothalamus, the placenta, and gastrointestinal tract. The MC4R is expressed widely

in the brain, and participates in the regulation of food intake. Disruption of function of the MC4R in the mouse results in obesity (Huszar *et al*, 1997). The MC5R was recently shown to be important for the regulation of exocrine gland function, and disruption of this receptor in the mouse results in defects in water repulsion and thermoregulation, due to reduced lipid production by sebaceous glands (Chen *et al*, 1997).

MELANOCORTINS, MC1R, AND HUMAN CUTANEOUS PIGMENTATION

The physiologic role of α-MSH in the regulation of integumental pigmentation of many vertebrate species has been recognized for many years; however, its role in the regulation of human cutaneous pigmentation remained controversial and is still not very well understood. Decades ago, Lerner and McGuire demonstrated that injection of human volunteers with high concentrations of α- and β-MSH, or with α-MSH and ACTH, resulted in skin darkening (Lerner and McGuire, 1961, 1964). Years later, Levine *et al* reported that injection of human subjects with a superpotent analog of α-MSH, namely [Nle^4, D-Phe^7] α-MSH, increased skin pigmentation, especially in anatomical sites that are habitually exposed to the sun (Levine *et al*, 1991). These reports suggested that melanocortins induce human skin darkening, but did not provide a solid evidence for a direct response of human melanocytes to these hormones. Some *in vitro* studies on cultured human melanocytes failed to demonstrate any pigmentary effect of α-MSH, and based on this, it was inferred that these cells do not express receptors for this hormone (Halaban *et al*, 1993). Those results were challenged by the cloning of the MC1R and the demonstration that this receptor is expressed on normal human epidermal melanocytes (Donatien *et al*, 1992; Mountjoy *et al*, 1992). The pharmacologic characterization of the human MC1R revealed that it is more sensitive to α-MSH and to ACTH than the mouse MC1R (Mountjoy, 1994). Subsequently, it was shown that treatment of cultured human melanocytes with α-MSH increases melanogenesis. Another report, however, concluded that α-MSH stimulates human melanocyte proliferation, but has no effect on melanogenesis (De Luca *et al*, 1993). We have reported that α-MSH is both mitogenic and melanogenic for human melanocytes *in vitro* (Abdel-Malek *et al*, 1995). Moreover, we showed that these cells respond equally well to α-MSH and ACTH. In a later report, we demonstrated that the MC1R expressed on human epidermal melanocytes binds α-MSH and ACTH with the same affinity, has a lower affinity for β-MSH, and least affinity for γ-MSH (Suzuki *et al*, 1996). The differential ability of the above four melanocortins to bind and activate the MC1R on human melanocytes was corroborated by their respective stimulatory effects on tyrosinase activity, and hence melanogenesis, as well as proliferation (Suzuki *et al*, 1996).

The above described *in vitro* studies were the first to demonstrate a direct effect of melanocortins on normal human melanocytes, suggesting a role for these hormones in the regulation of human cutaneous pigmentation. Subsequent studies revealed that activation of the MC1R on human epidermal melanocytes stimulated eumelanin formation (Hunt *et al*, 1995b). In this respect, the human MC1R is homologous to its mouse counterpart that is known to induce eumelanin synthesis in follicular melanocytes. Epidermal human melanocytes are known to express a low number (≈1000 binding sites per cell) of MC1R (Donatien *et al*, 1992). As mentioned earlier, the human MC1R is more sensitive to α-MSH and ACTH than its mouse counterpart, which has led to the conclusion that the human MC1R has evolved to be supersensitive to melanotropins, and that ACTH might play a physiologic role in regulating human pigmentation (Mountjoy, 1994; Suzuki *et al*, 1996). We have found that activation of human MC1R by ligand binding results in prolonged increase (for at least 24 h) in the synthesis of the second messenger cAMP (Suzuki *et al*, 1996). In contrast, in mouse melanoma cells, stimulation of cAMP formation by α-MSH treatment persisted for less than 2 h (Wong *et al*, 1974). We have found that the human MC1R mRNA level is upregulated by brief treatment with either α-MSH or ACTH (Suzuki *et al*, 1996). We also found that the MC1R does not undergo desensitization, as evidenced by the ability of human melanocytes to respond to continued and prolonged treatment with these hormones. These properties of the human MC1R might compensate for the low level of expression of the MC1R on human melanocytes and explain the sensitivity of these cells to α-MSH and ACTH.

We have found that, unlike human melanocytes, cultured human keratinocytes lack the expression of the MC1R (unpublished results). Using northern blot analysis, we could detect MC1R mRNA in melanocytes using 5 μg total RNA. In the same experiments, we could not detect any MC1R mRNA in keratinocytes, even when using a 10-fold higher amount of total RNA. Also, by receptor binding assays, we could not find any specific binding of α-MSH to keratinocytes. Furthermore, cAMP radioimmunoassays did not reveal any increase in cAMP levels in keratinocytes following treatment with α-MSH, γ-MSH, or ACTH. The results of these various assays indicate that human keratinocytes differ from human melanocytes in that they do not constitutively express functional MCR.

It has been reported that human endothelial cells, antigen-presenting cells, and macrophages express MC1R, as determined by RT-PCR analysis, and immunostaining using an antibody for MC1R (Hartmeyer *et al*, 1997; Bhardwaj *et al*, 1997). These receptors were found to be activated by subpicomolar concentrations of α-MSH, whereas the minimal effective dose of α-MSH that is required for activation of the MC1R expressed on melanocytes is 0.1 nM. Hence, it is important to pharmacologically characterize the putative MC1R on cells other than melanocytes, using competitive binding assays and cAMP radioimmunoassays.

The involvement of melanocortins in the UV-induced melanogenic response has long been proposed. Exposure of mouse Cloudman melanoma cells to UVR has been shown to increase α-MSH binding and to upregulate the expression of the α-MSH receptor (Pawelek *et al*, 1992; Chakraborty *et al*, 1995). Irradiation of brown guinea pigs with UVR resulted in increased pigmentation, an effect that was significantly augmented by the topical application of α-MSH to the irradiated sites (Bolognia *et al*, 1989). Evidence for a role of melanocortins in the responses of human melanocytes to UVR was suggested by the observations that UVR increases the serum levels of α-MSH, and stimulates the synthesis of α-MSH and ACTH by human keratinocytes and melanocytes (Altmeyer *et al*, 1986; Schauer *et al*, 1994; Chakraborty *et al*, 1996). We have been investigating the possible role of α-MSH in the UV-induced response of normal human melanocytes (Im *et al*, 1998). We found that in the absence of this hormone or any other agent that activates the cAMP pathway, human melanocytes respond to UVR with a decrease, rather than an increase, in tyrosinase activity, and with a profound reduction in the amount of tyrosinase. The melanogenic effect of UVR was only obvious when UV-irradiated melanocytes were treated with α-MSH or any other cAMP inducer. These results underscore the importance of the cAMP pathway in stimulating melanogenesis. Among the known regulators of human melanocytes, α-MSH and ACTH are the best known to activate the cAMP signaling pathway. Therefore, we propose that these hormones are pivotal for the melanogenic response to UVR.

We have reported that a single irradiation of human melanocytes with UVR resulted in their arrest in G1 (Barker *et al*, 1995). We also found that UVR resulted in a dose-dependent increase in cell death. Treatment of melanocytes with α-MSH immediately after UV irradiation resulted in their partial recovery from G1 arrest, but did not reduce the extent of cell killing (Im *et al*, 1998). At sublethal doses of UVR, we found that treatment of irradiated melanocytes with α-MSH significantly enhanced their proliferative rate, and completely reversed the growth inhibitory effect of UVR (unpublished results). We conclude, based on these results, that the increase in epidermal melanocyte activity and number that is observed in sun-exposed skin *in situ*, can be attributed at least partially to α-MSH and ACTH. Irradiation of melanocytes in the absence of α-MSH with a lethal dose of UVBR resulted in a prolonged increase in the accumulation of the tumor suppressor protein p53, enhanced expression of the cyclin-cdk inhibitor p21, and reduction of the level of the apoptotic inhibitor Bcl2 (Garcia *et al*, 1992; Ullrich *et al*, 1992; El-Deiry *et al*, 1993; Reed, 1994; Im *et al*, 1998). Because p53 is a cell cycle checkpoint, accumulation of p53 and the resulting induction of p21 are expected

to arrest cells in G1 (Ullrich *et al*, 1992; El-Deiry *et al*, 1993; Harper *et al*, 1993). Treatment of melanocytes immediately after UV irradiation with α-MSH did not reverse these effects, but induced melanocyte proliferation despite the high levels of p53 and p21 (Im *et al*, 1998). These findings suggest that α-MSH induces the proliferation of irradiated melanocytes by activating a p53 independent pathway. The observation that α-MSH stimulated the proliferation of UV-irradiated melanocytes but did not reduce the lethal effects of UVR indicates that this hormone is mainly a mitogen, and not a survival factor for these cells.

We hypothesize that the response of human melanocytes to UVR is mediated to a large extent by a network of paracrine factors that are induced in the epidermis upon UV exposure. These factors include interleukin-1, α-MSH, ACTH, endothelin-1, and basic fibroblast growth factor (Kupper *et al*, 1987; Halaban *et al*, 1988; Imokawa *et al*, 1992; Schauer *et al*, 1994; Chakraborty *et al*, 1996). Interleukin-1 is known to be a primary cytokine, and to induce the synthesis of endothelin-1 by keratinocytes, and of α-MSH and ACTH by keratinocytes and melanocytes (Chakraborty *et al*, 1996; Imokawa *et al*, 1992). After UV exposure, keratinocyte-derived factors regulate the proliferation and melanization of melanocytes, whereas the melanocyte protects the keratinocytes from photodamage by increasing the rate of synthesis and transfer of eumelanin containing melanosomes. In our studies on the regulation of the MC1R, we found that in the absence of mitogens, UVR resulted in a dose-dependent reduction in the MC1R mRNA level in epidermal melanocytes (unpublished results). Treatment with α-MSH immediately after UV exposure resulted in reversing the inhibitory effect of UVR and in upregulating the mRNA level for MC1R. Interestingly, we found that the MC1R mRNA level is upregulated by endothelin-1 (Tada *et al*, 1998) and basic fibroblast growth factors (unpublished results), two keratinocyte-derived factors that act as paracrine mitogens for human melanocytes. The synthesis of these two factors by keratinocytes is stimulated by UVR (Imokawa *et al*, 1992; Halaban *et al*, 1988). Based on this, we propose that activation of MC1R expression is central to the paracrine regulation of human melanocytes and their response to UVR, and is an important determinant of the extent of photoprotection in the epidermis.

MC1R ALLELIC VARIANTS

There is increasing evidence that mutations in the *MC1R* gene and low eumelanin to pheomelanin ratio in the epidermis are associated with poor tanning ability and increased risk for skin cancer (Valverde *et al*, 1995). In the populations so far studied, variants of the *MC1R* gene have been identified and found to be expressed in ver 80% of individuals with red (pheomelanogenic) hair (Valverde *et al*, 1995). These individuals have skin type I or II phenotype, tend to burn easily, and tan poorly when exposed to the sun. Most of the mutations so far identified are clustered in a region of 42 amino acids spanning the second transmembrane domain of the MC1R. Recently, eight MC1R variants were identified in an Irish population. Three of these variants, namely Arg151Cys, Arg160Trp, and Asp294His, were significantly over represented in individuals with red hair (Smith *et al*, 1998). The presence of any variant together with Arg151Cys was strongly associated with individuals who have freckles and a poor tanning response. The presence of Asp294His variant was also common in red headed Dutch individuals. The possible association of MC1R variants with melanoma has been investigated by comparing the frequency of variants in the second and seventh transmembrane domains in 43 melanoma cases and 44 controls (Valverde *et al*, 1996). Forty-six per cent of the melanoma patients were carriers of variant alleles, compared with only 18 per cent in the controls. The Asp84Glu variant was detected in 23% of melanoma tumors but not in any of the controls. The possible association between MC1R variants and the risk for melanoma needs to be further investigated, because some variants might prove useful as markers to predict the risk for this disease.

About 20 allelic variants of the human MC1R have been identified so far. Whether or not these variants impair the function of the MC1R has not been fully investigated. The consequence of only two point mutations, Arg151Cys and Val 92Met, on the function of the MC1R has been determined. Expression of the Arg151Cys variant in heterologous cells revealed that this mutant receptor had a normal binding affinity for α-MSH, but was totally deficient in activating adenylate cyclase, as monitored by lack of stimulation of cAMP formation (Frändberg *et al*, 1998). It was reported that the Val92Met substitution generated a NSP1 restriction site and unique restriction fragments of 359 and 724 bp. This mutation was found to have no significant effect on the binding or signaling of the MC1R (Koppula *et al*, 1997); however, in another study, this variant receptor was reported to have a reduced binding affinity for α-MSH (Xu *et al*, 1996). We and others (Jonathan Rees, personal communication) did not find a correlation between Val92Met variant expression and hair color, skin type, and loss of MC1R function. Therefore, we presume that this variant might have a minimal effect that cannot be detected in the human populations that have been studied so far.

To gain a better understanding of the significance of the MC1R gene mutations for the MC1R function and eumelanin formation, we compared the responses of various epidermal melanocyte cultures to α-MSH and the genetic sequences of their MC1R gene. We conducted dose–response experiments to determine the stimulatory effects of α-MSH on cAMP formation, tyrosinase activity, and proliferation. We tested five melanocyte cultures, each derived from a single skin type V or VI donor, and found that the minimal effective dose of α-MSH in all of the above assays was 0.1 nM. The cAMP data obtained from these experiments were consistent with the results obtained using the cloned wild-type MC1R expressed in heterologous cells. In five melanocyte cultures that were established from skin type I or II donors, one culture had a diminished response to α-MSH and demonstrated a shift to the right (by one or two logs) in the dose–response curves in all three assays. We are pursuing the genetic analysis of the *MC1R* to determine the cause for the loss of function of the MC1R in these melanocytes. Binding assays will be carried out in order to determine whether or not the affinity of the MC1R for its ligands is reduced. The response of melanocytes harboring mutated MC1R to UVR is also being investigated, in order to determine the influence of the MC1R variants on the extent of DNA damage in melanocytes.

SUMMARY

The demonstration that human epidermal melanocytes express functional MC1R and respond to α-MSH and ACTH with increased proliferation and melanogenesis, has put to rest a long lasting controversy about the role of α-MSH in the regulation of human pigmentation. Recent genetic studies clearly indicate that normal expression of the MC1R and its ligands is essential for the synthesis of eumelanin. Assuming that eumelanin is superior to pheomelanin in its photoprotective properties suggests that melanotropins are likely to play an important role in photoprotection of the skin against sun-induced DNA damage. This is supported by the association between the MC1R variants and poor tanning ability, and possibly melanoma formation. Allelic variants of the MC1R have been identified and found to be strongly associated with the red hair phenotype. The ramifications of these variants on the function of the MC1R need to be rigorously evaluated.

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