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Melanocortins and the melanocortin 1 receptor, moving translationally towards melanoma prevention

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ABSTRACT

Beginning in the last decade of the twentieth century, the fields of pigment cell research and melanoma have witnessed major breakthroughs in the understanding of the role of melanocortins in human pigmentation and the DNA damage response of human melanocytes to solar ultraviolet radiation (UV). This began with the cloning of the melanocortin 1 receptor (*MC1R*) gene from human melanocytes and the demonstration that the encoded receptor is functional. Subsequently, population studies found that the *MC1R* gene is highly polymorphic, and that some of its variants are associated with red hair phenotype, fair skin and poor tanning ability. Using human melanocytes cultured from donors with different *MC1R* genotypes revealed that the alleles associated with red hair color encode for a non-functional receptor. Epidemiological studies linked the *MC1R* red hair color variants to increased melanoma risk. Investigating the impact of different *MC1R* variants on the response of human melanocytes to UV led to the important discovery that the MC1R signaling activates antioxidant, DNA repair and survival pathways, in addition to stimulation of eumelanin synthesis. These effects of MC1R were absent in melanocytes expressing 2 *MC1R* red hair color variants that result in loss of function of the receptor. The importance of the MC1R in reducing UV-induced genotoxicity in melanocytes led us to design small peptide analogs of the physiological MC1R agonist α-melanocortin (α-melanocyte stimulating hormone; α-MSH) for the goal of utilizing them for melanoma chemoprevention.

Introduction

Since the early 1990s, there have been major advances in understanding the role of melanocortins and the melanocortin 1 receptor (MC1R)[1] in human pigmentation, and their significance of the MC1R/melanocortin axis in the UV response of human melanocytes. Despite the established role of melanocortins, mainly α-melanocortin (α-MSH), as the physiological regulator of integumental pigmentation in many vertebrate species, including fish, amphibians, reptiles and mammals, their significance for human pigmentation remained elusive until the1990s [35,37,97]. In poikilotherms, such as amphibians and reptiles, melanocortins stimulate rapid color change within minutes, allowing for camouflage in order to blend with the environment and evade predators, and to adjust body temperature in accordance with the climate [82,103,104,127]. The controversy about the role of melanocortins in human pigmentation stemmed from the assumption that melanocortins are endocrine factors that are secreted by the intermediate lobe of the pituitary gland, which is vestigial in humans. Absence of measurable serum levels of melanocortins in humans raised doubt about their putative endocrine functions [121]. Additionally, no direct effect of melanocortins on primary cultures of human melanocytes could be demonstrated using the original culture conditions that were not permissive for responsiveness to melanocortins. The first medium described for culturing human melanocytes included cholera toxin, an irreversible activator of adenylate cyclase, which like α-MSH, activates the cAMP pathway [29,87]. In the 1960s, the late Aaron B. Lerner and his associates reported that injection of crude preparations of melanocortins into human subjects resulted in skin darkening [67,68]. Such an effect, however, could not be definitively attributed to a direct effect on melanocytes, as it could be indirectly elicited by stimulating the synthesis of other melanogenic factor(s) by keratinocytes or other

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[1] *Abbreviations used:* MC1R, melanocortin 1 receptor; α-MSH, α-melanocortin; ACTH, adrenocorticotropic hormone; POMC, pro-opiomelanocortin; ASIP, agouti signaling protein; HBD3, human β-defensin 3; RHC, red hair color; XP, xeroderma pigmentosum; HGF, hepatocyte growth factor; DDR, DNA damage response.

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skin cell types. The cloning of the MC1R gene from cultured human melanocytes, the demonstration that the encoded receptor is functional, and the findings that melanocortins, mainly α-MSH and adrenocorticotropic hormone (ACTH), are synthesized in the epidermis by keratinocytes and melanocytes, supported the relevance of melanocortins and the MC1R in regulating human melanocytes, and thereby human pigmentation [2,20,45,79,99,112].

It is now recognized that the melanocortins/MC1R axis is a principle regulator of human pigmentation, and that the *MC1R* is an important melanoma susceptibility gene. In this review, we provide an overview of the role of MC1R agonists and antagonists in regulating human cutaneous pigmentation via controlling the synthesis of eumelanin and pheomelanin, the significance of *MC1R* in the diversity of human pigmentation, and the emerging importance of melanocortins and MC1R in regulating the DNA damage response of melanocytes to UV and in determining the predisposition to melanoma. We also discuss how the current knowledge about the MC1R/melanocortin axis can be translated into developing melanoma chemoprevention strategies based on using melanocortin analogs that target the MC1R.

Melanocortins and the melanocortin receptors

The melanocortins α-, β-, and γ-MSH are a family of peptides, and together with β-lipotropic hormone and β-endorphin are derived from one large precursor protein, pro-opiomelanocortin (POMC), which is processed by various pro-convertase enzymes [73,102]. Pro-opiomelanocortin is synthesized in human skin, mainly by keratinocytes and melanocytes in the epidermis [23,90,113]. Epidermal cells also express pro-convertases, thus can process POMC into bioactive derivatives, including α-MSH, ACTH, and β-endorphin [11,20,85,90,124,131]. In addition to α-MSH and ACTH, β-endorphin has mitogenic and melanogenic effects, which are mediated by activating the η-opiate receptor expressed on human melanocytes [60]. This provides unequivocal evidence that POMC derivatives function as autocrine/paracrine regulators of melanocytes. Expression of POMC has been found to be further stimulated upon UV irradiation of the skin *in vivo*, thus lending support for a physiological role for POMC-derived peptides in regulating the response of melanocytes to UV [23,113].

The melanocortins α-, β-, and γ-MSH and ACTH share sequence homology (Met-Glu-His-Phe-Arg-Trp) that accounts for some of their overlapping pharmacological properties and effects [97]. These peptides bind with different affinities to 5 different melanocortin receptors (MCRs), which belong to the large family of G protein-coupled receptors with 7 transmembrane domains, and are encoded by separate genes [3]. These MCRs are coupled to G_s protein, thus signal mainly by activating adenylate cyclase to increase cAMP formation. The significance of the cAMP pathway in MC1R signaling is supported by the findings that forskolin, a direct adenylate cyclase activator, and isobutylmethylxanthine, an inhibitor of phosphodiesterases, the enzymes that degrade cAMP, mimic the effects of melanocortins on melanocytes [1,25,101]. α-MSH can bind to 4 MCRs, namely MC1, MC3, MC4, and MC5R. Both α-MSH and ACTH are full agonists of the human MC1R, the only melanocortin receptor expressed on the cell surface of human melanocytes [2,112]. However, the human MC1R has a lower affinity for β-MSH than α-MSH or ACTH, and least affinity for γ-MSH [112]. The main agonist of the neuronal receptor MC4R is α-MSH, while the agonist for MC3R is γ-MSH. ACTH is the sole agonist for MC2R that is expressed in the adrenals and regulates steroidogenesis [79]. While the human MC1R binds ACTH and α-MSH with equal affinity, the MC2R does not recognize α-MSH as a ligand. That ACTH is an agonist of MC1R explains the hyperpigmentation associated with Addison's disease, which results from overproduction of ACTH [95]. Evidence for the role of POMC-derived melanocortins in regulating pigmentation was provided by the findings that mutations that disrupt *POMC* gene expression result in red hair phenotype due to lack of ligand of MC1R, in addition to metabolic disorders, including adrenal insufficiency and obesity, resulting from lack of activation of MC2R and MC4R, respectively [65,81].

The MC1R, key regulator of eumelanin and pheomelanin synthesis

Mammalian melanocytes synthesize two forms of the pigment melanin, eumelanin, the dark brown/black pigment, and pheomelanin, the yellow–red pigment. It is established that α-MSH is the main stimulator of eumelanin synthesis [51]. Administration of α-MSH to mice increased eumelanin synthesis within hair follicles, resulting in black coat color [35]. The *extension* locus in mice codes for the melanocortin 1 receptor (mc1r) [89,105]. Expression of functional mc1r is required for the effect of α-MSH on mouse follicular melanocytes, since the *recessive yellow* (*e*/*e*) mutation, a loss-of-function mutation in *extension*, results in a yellow coat color due to inhibition of eumelanin synthesis [89,120]. The ortholog of *extension* in humans is the *MC1R* [79]. In the mouse, a second genetic locus that negatively regulates eumelanin synthesis is the *agouti* locus, which codes for a soluble protein, agouti signaling protein (ASIP) that acts as an inverse agonist for the mc1r [16,66,77,114] Agouti signaling protein blocks the binding of α-MSH, and thereby inhibits eumelanin synthesis, allowing only for the synthesis of pheomelanin [12,71,114]. In the mouse, expression of ASIP is very tightly regulated in a temporal fashion. In the wild type mouse, hairs are characterized by a banding pattern, with a proximal and a distal black band, interrupted by a yellow band resulting from expression of *agouti* and the switch from eumelanin to pheomelanin synthesis [16,105]. Stimulation with α-MSH can overcome the inhibitory effect of *agouti*, resulting in black hairs. Loss-of-function mutation in *extension* (*e*/*e*), or activating mutation in *agouti* result in a similar pigmentary phenotype, namely yellow coat color of mice [89,105,120]. The latter mutation, which causes over expression of ASIP, also results in a hyperphagic and obese mouse, due to activation of the mc4r by high concentrations of ASIP that can cross the blood/brain barrier [54,71,130].

The human *Agouti* gene was cloned in 1994, and functional studies revealed that similar to its mouse ortholog, it codes for an antagonist of the MC1R that competes with α-MSH for binding to the MC1R [66,114] (Fig. 1). Human melanocytes, unlike mouse melanocytes, synthesize both eumelanin and pheomelanin at any one time [47]. Analysis of eumelanin and pheomelanin levels in skin biopsies or primary melanocyte cultures derived from donors with different pigmentary phenotypes showed that eumelanin content is the main determinant of constitutive pigmentation in human skin, as it correlates directly with total melanin and visual pigmentation [43,125]. In some cases, melanocytes of donors with different pigmentary phenotypes displayed different eumelanin contents but similar pheomelanin contents, thus underscoring the role of eumelanin, rather than pheomelanin, in determining skin pigmentation. Treatment of cultured human melanocytes with melanocortins increased eumelanin synthesis, and thus the ratio of eumelanin to pheomelanin [48] (Fig. 1). Biochemical studies revealed that stimulation of eumelanin synthesis requires high levels and activities of the melanogenic enzymes tyrosinase, TRP-1 and TRP-2, and treatment with purified ASIP inhibited the levels of these enzymes [2,7,62,91]. These results led to the conclusions that eumelanin synthesis has more stringent requirements than pheomelanin synthesis, and that pheomelanin production occurs