MC1R Functions, Expression, and Implications for Targeted Therapy

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The G protein-coupled MC1R is expressed in melanocytes and has a pivotal role in human skin pigmentation, with reduced function in human genetic variants exhibiting a red hair phenotype and increased melanoma predisposition. Beyond its role in pigmentation, MC1R is increasingly recognized as promoting UV-induced DNA damage repair. Consequently, there is mounting interest in targeting MC1R for therapeutic benefit. However, whether MC1R expression is restricted to melanocytes or is more widely expressed remains a matter of debate. In this paper, we review MC1R function and highlight that unbiased analysis suggests that its expression is restricted to melanocytes, granulocytes, and the brain.

Introduction

Human pigmentation plays a key role in skin physiology where different types and levels of pigmentation can affect skin cancer predisposition. Although many genes implicated in skin pigmentation have been identified (Pavan and Sturm, 2019; Sturm and Duffy, 2012) and their functions have been defined in the production of melanin, the structural integrity of melanosomes, and their transport and transfer to keratinocytes (KCs), a pivotal role is played by the *MC1R* gene (Abdel-Malek et al., 1999), which was first isolated from melanocytes almost 30 years ago (Mountjoy et al., 1992). In the intervening years, a substantial effort has been made to understand its role and regulation.

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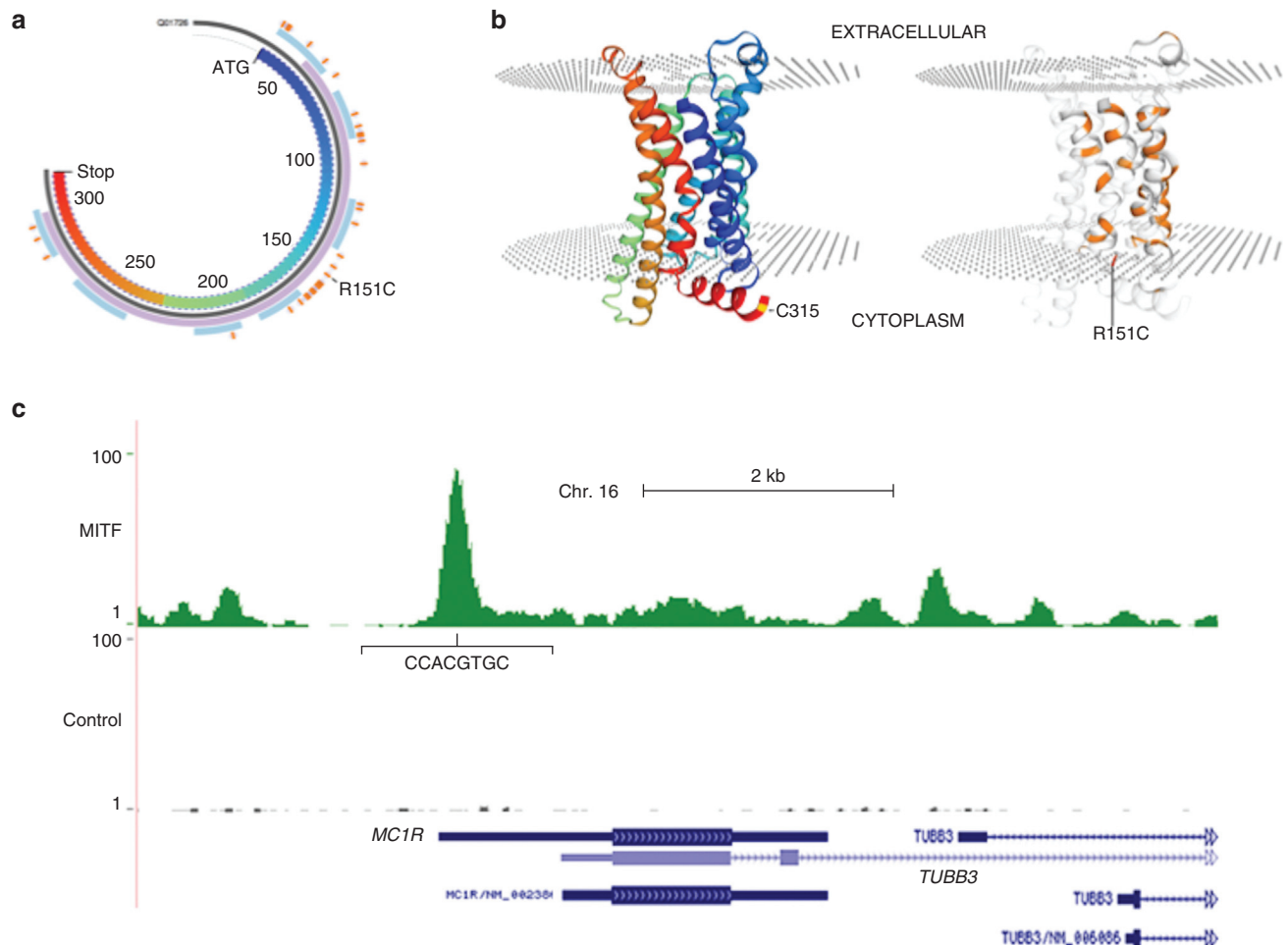
MC1R belongs to a subfamily of G protein-coupled receptors (GPCRs) that bind melanocortins to control several key physiological and behavioral traits (Roulin and Ducrest, 2011; Yang and Harmon, 2017). Specifically, MC1R exhibits a high affinity for α-melanocyte-stimulating hormone (α-MSH) (Mountjoy et al., 1992), a peptide hormone derived by proteolytic cleavage from pro-opiomelanocortin (Drouin and Goodman, 1980). Of note, human MC1R, unlike its mouse counterpart, can also recognize ACTH, in addition to α-MSH, as a full agonist with similar affinity to α-MSH (Suzuki et al., 1996). Although MC1R can signal through several distinct pathways, most studies have focused on its ability to increase cAMP levels, which is mediated through MC1R interaction with the G proteins that interact with adenylate cyclase (Rodrigues et al., 2015; Zanna et al., 2013). Significantly, the *MC1R* gene shows striking pleomorphism, with over 200 human variants described (Herraiz et al., 2017). Some variants are associated with a red hair color (RHC) phenotype, skin photoaging, and predisposition to skin cancers (Caini et al., 2020; Elfakir et al., 2010; Guida et al., 2021, 2019; Pellegrini et al., 2019; Tagliabue et al., 2015). The link between some reduced-function variants and the RHC phenotype highlights that one role of MC1R signaling is to increase the proportion of black eumelanin relative to that of red pheomelanin, thereby providing cells with photoprotective and antioxidant activities (Nasti and Timares, 2015; Swope and Abdel-Malek, 2018).

Whereas most MC1R functions have been related to melanocytes and their role in skin pigmentation, accumulating evidence suggests the expression and function of MC1R in other cell types. In this paper, we review the current knowledge concerning the established pigmentary and non-pigmentary roles of MC1R and evaluate the expression of MC1R in cells other than melanocytes.

MC1R gene structure, regulation, and activation

The human *MC1R* gene (16q24) was first isolated from melanocytes (Chhajlani and Wikberg, 1992; Mountjoy et al., 1992). Although intergenic and intragenic splicing has been described (Herraiz et al., 2015), the major transcript encodes a 317 amino acid integral membrane protein with the structural characteristics of the GPCRs, including an extracellular *N*-terminus, with an *N*-linked glycosylation site, seven transmembrane segments, and an intracellular *C*-terminal extension, including a key *C*-terminal palmitoylation site at cysteine 315 (C315) (Chen et al., 2017; García-Borrón et al., 2005; Wolf Horrell et al., 2016) (Figure 1a and b).

Human MC1R expression is regulated by a single promoter partly controlled by MITF, a key regulator of melanocyte biology (Goding and Arnheiter, 2019), which binds a CACGTG E-box motif (Figure 1c shows the results of MITF chromatin immunoprecipitation sequencing performed

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Figure 1. Structure of the MC1R. (**a**) The sequence of the MC1R with locations of specific variants indicated in orange and the R151C variant indicated. (**b**) Left: The likely structure of the MC1R based on its amino acid sequence aligned with a template provided by the 2.25Å structure of the human A2A adenosine receptor. Colors of structural features correspond to those of the amino acid sequence in **a**. Palmitoylation of C315, which is required for MC1R function, is likely to be necessary for a correct insertion into the plasma membrane indicated by the two disks; Right: The potential structure of the MC1R with locations of variants, including that of R151C, indicated in orange. (**c**) MITF binding to the human *MC1R* locus. UCSC browser screenshot showing the results of MITF chromatin immunoprecipitation sequencing (performed as described by Louphrasitthiphol et al. [2020]), including the bound CACGTG E-box sequence beneath the peak. The transcript reading through from *MC1R* to the *TUBB3* gene is also indicated. C315, cysteine 315; Chr., chromosome; kb, kilo base; UCSC, University of California Santa Cruz.

according to Louphrasitthiphol et al. [2020]), related to the core sequence of the melanocyte-specific regulatory MITF-regulatory element, the M-box (AGTCATGTGCT) (Lowings et al., 1992; Miccadei et al., 2008; Moro et al., 1999). At the 3′ end of the *MC1R* gene, an atypical polyadenylation site enables intergenic splicing between MC1R and the downstream *TUBB3* gene, a process stimulated by both α-MSH and p38 signaling, suggesting that intergenic splicing might be promoted by exposure to UV irradiation (Dalziel et al., 2011). As a consequence, in addition to wild-type (WT) MC1R, chimeric MC1R–TUBB3 receptors can be produced in humans (Figure 1c). However, the chimeric proteins produced show a reduced receptor functionality, most notably reduced cell surface expression and an inability to signal through cAMP but a retention of extracellular signal–regulated kinase (ERK) signaling (Dalziel et al., 2011; Herraiz et al., 2015). However, although the mRNAs corresponding to these chimeric MC1R–TUBB3 receptors have been

detected, the expression of the chimeric proteins in human skin has yet to be found.

After its synthesis in the endoplasmic reticulum and before MC1R is transported to the surface of its expressing cells, the receptor undergoes several modifications, including oligomerization, glycosylation, palmitoylation, and phosphorylation (Chen et al., 2017; Herraiz et al., 2011b; Sánchez-Laorden et al., 2007). MC1R dimerization occurs constitutively, and both the WT receptor and its allelic variants can homodimerize or heterodimerize, leading to different functional consequences (Sánchez-Laorden et al., 2007, 2006). An additional contribution to decreased internalization is given by MC1R *N*-glycosylation (Herraiz et al., 2011b), whereas palmitoylation at C315 contributes to receptor structure and stability, membrane localization, and interaction with partner proteins (Chen et al., 2019, 2017). Phosphorylation at threonine 157 is critical for receptor trafficking, whereas phosphorylation at threonine 308 and

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serine 316 influence receptor desensitization and internalization (Sánchez-Laorden et al., 2009, 2007). Of note, however, care needs to be taken in the interpretation of experiments performed by some studies in nonmelanocytes such as melanoma cells or human embryonic kidney (HEK) 293 cells (HEK cells that are in fact of neuronal origin) (Shaw et al., 2002). Components of the machinery that regulates processing, internalization, sensitization, and desensitization of GPCRs are differentially expressed in different cell types, which makes it necessary to use the physiological cells that express the particular receptor. For example, Swope et al. (2012) provided evidence for differential expression of GCPR kinases in human melanocytes versus in HEK cells. Moreover, the decrease of constitutive activation of MC1R signaling due to *MC1R* polymorphisms was detected in heterologous cells but not in cultured human melanocytes.

Unlike some other GPCRs, human MC1R exhibits agonist-independent constitutive activation of downstream signaling, which is abolished or decreased in the presence of MC1R polymorphisms that adversely affect MC1R function (Sánchez-Más et al., 2004), at least some of which decrease the key palmitoylation event on C315 (Chen et al., 2017). However, the presence of melanocortins released above all by KCs and melanocytes, primarily by α-MSH and to a lesser extent by ACTH, enhances paracrine-/autocrine-mediated MC1R signaling (Slominski et al., 2004). α-MSH can also shift the ratio of MC1R splice variants to MC1R—TUBB3, thereby reducing downstream signaling (Dalziel et al., 2011), suggesting that these chimeric receptors may play a role in the human facultative pigmentation system.

Notably, in both mice and humans, inverse or competitive agonists for MC1R have been described, with studies suggesting only a partial overlap between the binding of agonist and inverse agonists (Jackson et al., 2005; Patel et al., 2010). The human inverse agonist is encoded by the *ASIP* gene, cloned by Wilson et al. (1995), and its effects on human melanocytes were firstly reported by Suzuki et al. (1997). In addition, β-defensin 3 in humans can also bind MC1R, decreasing constitutive receptor signaling (Swope et al., 2012). In mice, a similar role is performed by ASP (Walker and Gunn, 2010) and AGRP. In cell culture, pretreatment with MC1R antagonists blocks the responsiveness to α-MSH, suggesting that antagonists can induce receptor internalization, inhibit its recycling, and/or have prolonged receptor occupancy (Suzuki et al., 1997; Swope et al., 2012).

MC1R polymorphisms

MC1R is highly polymorphic, with around 200 variants reported to date. Variants or polymorphisms of *MC1R* can be related to a decreased receptor function, resulting in a shift of melanin synthesis from eumelanin to the red—yellow and potentially mutagenic pheomelanin (Box et al., 1997; Valverde et al., 1995). Some variants show large differences in allelic frequency in different populations, following a variable selective pressure at the *MC1R* locus. In Africa, selection is in favor of functional MC1R. By contrast, loss of function (LOF) is tolerated in European and East Asian populations, although outside of Africa, the prevalence of *MC1R* polymorphisms appears to reflect a neutral selection (Harding et al., 2000), facilitating UV-stimulated production

of vitamin D in the skin, which is essential for bone health and for maintaining reproductive capacity and hence the preservation of the species (Harding et al., 2000).

MC1R gene polymorphisms are a major determinant of the normal variation in human pigmentation (Rees, 2003; Tagliabue et al., 2016). Valverde et al. (1995) described the association between some *MC1R* LOF polymorphisms and red hair, fair skin, and sun sensitivity (the RHC phenotype), an association confirmed by many subsequent studies (Beaumont et al., 2008; Rees, 2000; Smith et al., 1998). Genetic epidemiology studies firmly established the association of *MC1R* RHC variants with melanoma (Guida et al., 2015; Ichii-Jones et al., 1998; Tagliabue et al., 2018; Williams et al., 2011) and nonmelanoma skin cancers (Joshi et al., 2018; Tagliabue et al., 2015). These associations can be explained by a combination of poor photoprotection arising as a consequence of decreased eumelanin in the skin of individuals with *MC1R* RHC variants, combined with pigmentation-dependent but UV-independent carcinogenic stimuli as well as a pigmentation-independent effect of *MC1R* polymorphisms (Kennedy et al., 2001; Mitra et al., 2012; Palmer et al., 2000; Manganelli et al., 2021; Scott et al., 2002). Specifically, Mc1r $^{e/e}$ mouse skin exhibited increased oxidative damage to DNA and lipids, suggesting that pheomelanin itself contributes to a UV-independent mechanism to promote melanoma, an effect ablated if the Mc1r $^{e/e}$ mouse model had an additional albino allele leading to an absence of pheomelanin (Mitra et al., 2012). MC1R variants have therefore been classified, according to their penetrance for the RHC phenotype, into strong R or RHC alleles, weaker r forms, and pseudoalleles showing no significant effect on phenotype (Duffy et al., 2004). Both RHC and r polymorphisms generate hypomorphic proteins, impairing the activation of the cAMP pathway (Frändberg et al., 1998), although some residual activity may be observed (Herraiz et al., 2017).

Different MC1R RHC variants can be distinguished by their effects, and they include those with reduced cell surface expression, such as R151C, R160W, and D84E (Beaumont et al., 2007, 2005; Morgan et al., 2018), involving the regulation of anterograde trafficking of the MC1R or cAMP coupling impairment (Beaumont et al., 2007; García-Borrón et al., 2014), and those with normal surface expression, such as D294H and R142H, but decreased functional coupling (Beaumont et al., 2007; Newton et al., 2005) related to an inability to bind the melanocortins (Beaumont et al., 2005; Sánchez Más et al., 2002; Scott et al., 2002). The V60L, V92M, and R163Q r variants are expressed with normal or intermediate cell surface receptor levels (Beaumont et al., 2005), and their functional relevance is debatable, with some reports pointing to a minor signaling impairment, whereas others showed a behavior similar to that of WT (Kadekaro et al., 2010; Scott et al., 2002).

MC1R: pigmentary and nonpigmentary pathways

The MC1R is a pivotal regulator of pigment production and distribution throughout the skin (pigmentary function) (Rees, 2004) and more recently has also been identified as having nonpigmentary roles as a regulator of antioxidant defenses, DNA-repair mechanisms, and genome integrity

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(Abdel-Malek et al., 2014; Li et al., 2021; Swope et al., 2014) (Supplementary Figure S1).

Pigmentary functions and the cAMP pathway. The human epidermis primarily comprises KCs and melanocytes in tight physical and functional contact, the so-called epidermal–melanin unit. UV exposure triggers p53-dependent production and release of the MC1R agonist α-MSH from cutaneous KCs (Cui et al., 2007), inducing a tanning response in the skin during which melanosomes, the pigment-containing organelles synthesized in melanocytes, are transferred to KCs to protect against UV-induced DNA damage. Mechanistically, KC-derived α-MSH activates the MC1R GPCR, leading to the stimulation of adenylyl cyclase and ultimately to increased cAMP levels. The consequent activation of protein kinase A leads to phosphorylation of the CREB, a transcription factor that activates the promoter of the *MITF* gene (Bertolotto et al., 1998; Goding and Arnheiter, 2019; Price et al., 1998). In turn, MITF upregulates the mRNA expression of genes encoding melanogenic enzymes, such as *TYR* and *TYRP1* and dopachrome tautomerase gene, *DCT,* as well as those regulating cell cycle progression (Carreira et al., 2006, 2005; Goding and Arnheiter, 2019; Levy et al., 2006; Loercher et al., 2005). Therefore, the activation of MC1R switches melanin biosynthesis from basal yellowish–reddish sulfur-containing pheomelanin to an activated state where the black–brown eumelanin synthesis prevails, thus leading to darker pigmentation, but also promotes pigment transfer to KCs (d'Ischia et al., 2015; Passeron et al., 2004).

In addition to regulating CREB, the cAMP pathway in melanocytes also targets another transcriptional regulator, PGC-1α whose expression is also activated by MITF (Ferretta et al., 2016; Haq et al., 2013; Vazquez et al., 2013). PGC-1α interacts with many nuclear receptors and transcription factors, and it is the main positive regulator of mitochondrial biogenesis, increasing the capacity for cellular energy production, liver and brown adipose tissue metabolism, and detoxification of ROS (Maresca et al., 2015; Villena, 2015). The link between these two pathways provides an interesting connection between pigmentation, antioxidant activity, and metabolism, and indeed, in addition to mitochondrial biogenesis, MITF has recently been identified as a key regulator of the tricarboxylic acid cycle (Louphrasitthiphol et al., 2019) and fatty acid desaturation (Vivas-García et al., 2020). Thus MC1R, through MITF, may play a key role in coordinating melanocyte metabolism.

In addition, MITF contributes to a negative feedback loop downstream from MC1R signaling by downregulating cAMP signaling by increasing the expression of the *PDE4D* gene, a transcriptional target of cAMP through MITF (Khaled et al., 2010), limiting cAMP accumulation.

Nonpigmentary roles of MC1R. In addition to its well-characterized function in pigmentation, MC1R plays a key role in pigmentation-independent responses to UVR, such as the antioxidant response and induction of DNA-repair mechanisms (Abdel-Malek et al., 2014, 2000), although the underlying mechanisms by which MC1R defends against UV-induced damage still need further investigations. However, the MC1R-dependent UV response induces an abundance of genes associated with regulating the cell cycle, oncogenesis, and photoprotection (April and Barsh, 2007; Yin et al., 2014), and the cAMP pathway has been implicated in the development of epidermal thickness, a protective mechanism against UV injuries (Scott et al., 2012).

Importantly, the cAMP pathway enhances melanocyte nucleotide excision repair (NER) activity (Wolf Horrell et al., 2016), which is responsible for the removal of mutagenic UV photolesions. In part, this is mediated through MC1R signaling to ATR pathway (Jarrett et al., 2015). Indeed, in human melanocytes, α-MSH signaling through MC1R was shown to activate both DNA damage sensors ATR and ATM as well as the recruitment of XPC and XPA, the DNA damage recognition and verification proteins in NER, respectively (Swope et al., 2020).

Although MC1R-mediated activation of ATR signaling is independent of MITF activation by cAMP (Wolf Horrell et al., 2017), MITF itself can control genes implicated in DNA damage repair (Seoane et al., 2019; Strub et al., 2011) and activate an antioxidant response (Louphrasitthiphol et al., 2019). Consistent with some nonpigmentary roles for MC1R being mediated by MITF, recent evidence also assigns MC1R a protective role in chromosome stability and centromere integrity, again mediated by MITF (Li et al., 2021).

Activation of NER in UV-irradiated melanocytes is also mediated through cAMP signaling to p53. Similar to MITF, p53 plays an important role in melanocytes because it counteracts oxidative damage and maintains the homeostasis of melanocytes (Kadekaro et al., 2012). To support this hypothesis, impaired MC1R signaling in melanocytes exposed to UVR in vivo reduces the ability of surrounding cells to undergo p53-mediated cell cycle arrest and apoptosis (April and Barsh, 2007; Robinson et al., 2010).

Consistent with MC1R promoting DNA damage repair, an impaired NER pathway can be observed in subjects carrying *MC1R* LOF (Cassidy et al., 2015; Hauser et al., 2006; Jarrett et al., 2015, 2014; Kadekaro et al., 2010). As a consequence, compared with non-RHC carriers, MC1R RHC variants have been associated with a significant increase in somatic mutation burden in melanoma, including both C>T and non-C>T mutations, supporting the role of both UV-dependent and -independent events (Johansson et al., 2017; Robles-Espinoza et al., 2016).

Additional nonpigmentation-related effects of MC1R have been attributed to PTEN, phosphatidylinositol 3-kinase (PI3K)/protein kinase B (Akt) signaling, MAPKs, and c-KIT (Cao et al., 2013; Herraiz et al., 2009). Interestingly, activation of the ERK1/2 by stress signals such as α-MSH and by p38 signaling, mimicking the effects of UV-light exposure, seems to be mediated by the cross-talk with c-KIT and is not affected by cAMP levels (Castejón-Griñán et al., 2018; Herraiz et al., 2011a; Smalley and Eisen, 2000). By contrast, activation of Akt downstream from PI3K signaling appears to promote α-MSH—mediated survival of the retinal pigmented epithelium (Cheng et al., 2014). The role of MC1R in PI3K signaling was further substantiated by Cao et al. (2013) who revealed that UV exposure triggers an association between MC1R WT but not between RHC variants and PTEN, a negative regulator of PI3K signaling, to prevent PTEN

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degradation and suppress PI3K signaling. Hyperactivation of PI3K signaling in primary melanocytes can lead to increased senescence, but in BRAFV600E mutated melanoma, *MC1R* polymorphisms leading to elevated PI3K signaling can promote oncogenic transformation (Cao et al., 2013).

α-MSH has also been reported to antagonize the effects of proinflammatory cytokines (Hill et al., 2006) and to increase matrix metalloprotease (MMP) levels (Kiss et al., 1995). Perhaps at least in part, this may be related to the ability of MC1R to activate MITF expression; because events leading to the downregulation of MITF can trigger inflammatory signaling and MMP expression (Carreira et al., 2006; Giuliano et al., 2010; Vivas-García et al., 2020), MITF upregulation should suppress these activities.

Targeting the MC1R. Because impaired MC1R function is associated with the development of skin cancers, employing MC1R agonists and antagonists to regulate the receptor signaling might represent a therapeutic strategy (Koikov et al., 2021). In this respect, a recent study showed that topical application of forskolin, a skin permeable inducer of cAMP induction, induces UV resistance in *Mc1r*-heterozygous or *Mc1r*-WT mice by increasing eumelanin deposition and by improving NER (Bautista et al., 2020). Other studies also revealed that raising cAMP levels using forskolin in vitro and in animal models can decrease UV-induced DNA damage and melanoma incidence in MC1R-deficient conditions (D'Orazio et al., 2006; Jarrett et al., 2014).

An alternative strategy to rescue MC1R function and consequently potentially decrease melanoma risk is through MC1R palmitoylation (Chen et al., 2019, 2017). Because the RHC variants decrease palmitoylation of MC1R (Chen et al., 2017), small molecules that inhibit the depalmitoylation enzyme acyl-protein thioesterase 2 (APT2) can restore WT levels of palmitoylation and consequently MC1R function. APT2 inhibition reduced melanoma size and prolonged survival in $Mc1r^{R151C}$-mutant mice after UV irradiation on a $BRAF^{V600E}$ background (Chen et al., 2019). However, how clinically useful this approach might be needs to be carefully assessed because palmitoylation is commonly used to regulate many signaling molecules (Wang et al., 2020). As such, targeting depalmitolyation may lead to effects well-beyond MC1R signaling.
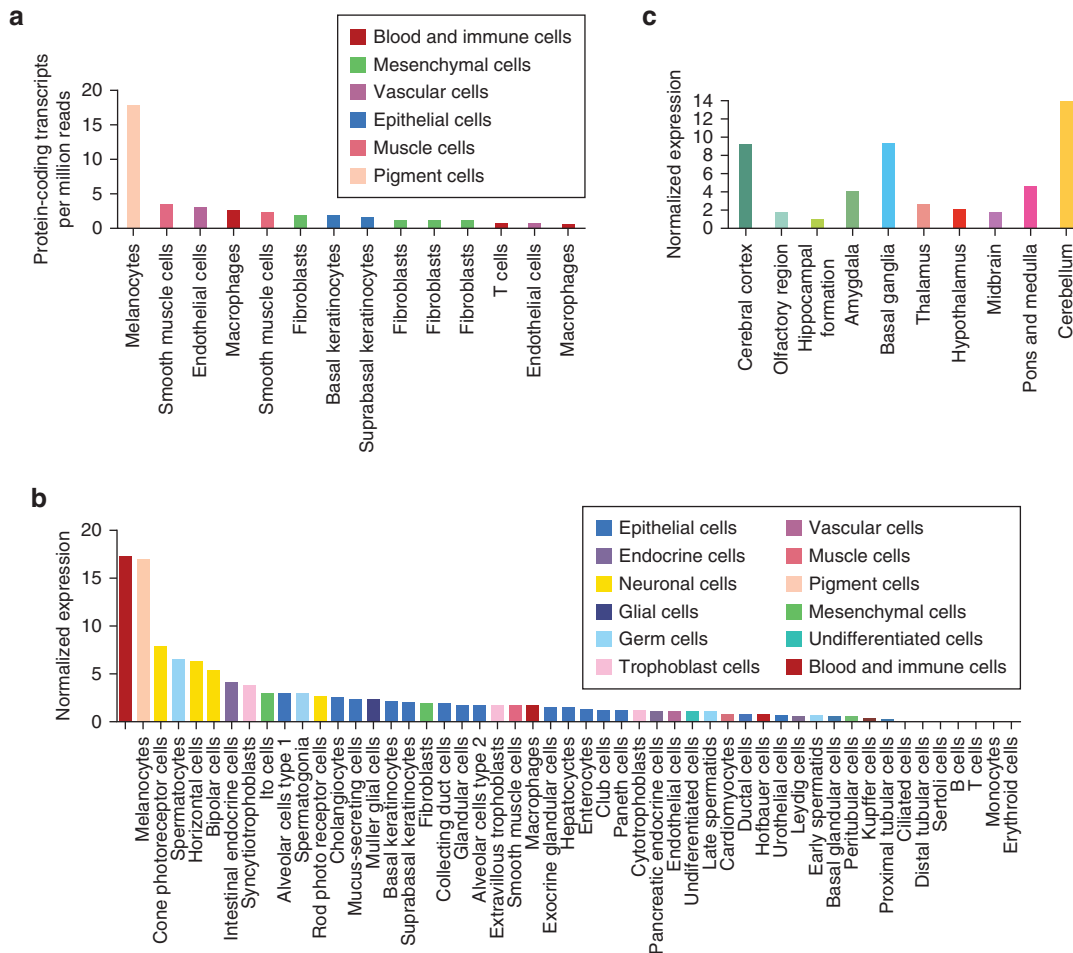
Importantly, one clinically successful approach to targeting MC1R is the use of an α-MSH analog, afamelanotide. This has proven to be effective and safe in the treatment of the photosensitivity related to erythropoietic protoporphyria (EPP), a rare disease that may be associated with acute phototoxicity (Langendonk et al., 2015). Consequently, on the basis of the rationale that increased skin pigmentation may protect against photosensitivities, afamelanotide administered through a slow-release subcutaneous implant is currently available to treat photosensitivity in patients with EPP.

Other MC1R agonists have been developed to exploit the role of MC1R WT in increasing pigmentation, antioxidant defenses, and DNA repair (Jackson et al., 2019; Koikov et al., 2021; Mowlazadeh Haghighi et al., 2018). For example, on the basis of using the His-Phe-Arg-Trp tetrapeptide as a pharmacore scaffold, Mowlazadeh Haghighi et al. (2018) were able to generate a tetrapeptide Ac-His-D-Phe(4-CF3)-Nle-Trp-NH2 as a potent and selective human MC1R agonist with a 10 nM half-maximal effective concentration. Moreover, Koikov et al. (2021) were able to generate a tripeptide agonist with >100,000-fold selectivity for MC1R over other melanocortin receptors. On the basis of amino acid sequence and affinity with the human receptor, additional applications of MC1R agonists have been proposed such as in vitiligo treatment and as antiaging and antigraying hair agents (Almeida Scalvino et al., 2018; Jackson et al., 2019; Koikov et al., 2021).

MC1R and nonmelanocytic cells
Given the genetic evidence for a role for MC1R in pigmentation, most knowledge of its function is related to melanocytes. However, the expression of MC1R in many different cells other than melanocytes (such as KCs, fibroblasts, endothelial cells, immune system cells) has been widely reported (Böhm and Stegemann, 2014; Hill et al., 2006; Hiramoto et al., 2010; Kleiner et al., 2016; Luo et al., 2013; Muffley et al., 2011) and related to anti-inflammatory effects, immune response, response to burn injuries, collagen synthesis, and scar formation. Therefore, at first sight, it seems that expression of MC1R would be widespread with pleiotropic effects and important implications for targeted therapies. However, although the expression of MC1R in melanocytes has been validated with strong genetic evidence to back up its role in melanocyte pigmentation, many studies in nonmelanocyte cell types lack the key controls necessary to make definitive conclusions. For example, whereas semiquantitative RT-PCR at high cycle numbers can detect *MC1R* mRNA in nonmelanocytic cells, quantitative RT-PCR revealed a low level of *MC1R* expression in KC and fibroblast cell cultures compared with the much higher levels of *MC1R* transcripts found in melanocytes (Roberts et al., 2006). Consistent with the low level of mRNA in nonmelanocytic cells, MC1R protein expression at the cell surface was not detected (Roberts et al., 2006). Similarly, although the addition of α-MSH to melanocytes leads to a robust increase in cAMP levels that is dependent on the receptor, a direct comparison between the effects of α-MSH on nonmelanocytes and the effects on melanocytes and the use of control nonfunctional peptides or control cells lacking or depleted or mutated for the MC1R are usually absent. Moreover, given that MC1R protein is expressed at low levels even in melanocytes, controls to determine the specificity of the antibodies used to detect MC1R expression are important but, again, are frequently absent from many studies of MC1R function in nonmelanocytes. For those studies where suitable controls were used, the levels of *MC1R* expression in nonmelanocyte cell types are very low compared with the levels in melanocytes, leading to questions concerning the physiological relevance of the low level of mRNA expression detected. To resolve the issue of whether *MC1R* is expressed in nonmelanocytes, we interrogated the data curated at the Human Protein Atlas (www.proteinatlas.org). On the basis of single-cell sequencing of human skin as reported by Solé-Boldo et al. (2020), *MC1R* was only expressed to high levels in melanocytes and granulocytes (Figure 2a). Examination of the broader expression pattern based on single-cell RNA sequencing from multiple tissue types again revealed

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Figure 2. Expression of MC1R. (**a**) Single-cell RNA-seq data showing the expression of *MC1R* mRNA from different cell types from human skin. Multiple notations of a single-cell type such as fibroblasts indicate that each fibroblast population was distinct as delineated by the bioinformatic clustering analysis. (**b**) Single-cell RNA-seq data showing the normalized expression of MC1R from multiple tissue types. (**c**) RNA-seq showing the relative expression of MC1R in the brain. Data used to generate the images can be found at https://swissmodel.expasy.org/repository/uniprot/Q01726?csm=CB67405A562C29B2. RNA-seq, RNA sequencing.

that the highest *MC1R* expression was found in melanocytes and granulocytes, with intermediate expression in cone photoreceptor cells and bipolar cells in the eye (Figure 2b). Other cell types expressed mostly low levels of *MC1R* mRNA. Importantly, some patients with EPP treated with afamelanotide reported nausea and headache in addition to increased skin pigmentation (Langendonk et al., 2015), suggesting that *MC1R* could be expressed in the brain. Consistent with this, *MC1R* mRNA expression can be detected in the cerebellum, a dysfunction of which can cause nausea, as well as in other brain regions (Figure 2c). Whether the mRNA is translated into MC1R protein in cell types expressing low-to-intermediate levels of *MC1R* with a physiologically relevant function in vivo remains to be determined. Nevertheless, the high mRNA expression in granulocytes suggests a potential role for MC1R signaling in this cell type that includes neutrophils, basophils, eosinophils, and mast cells, which facilitate the immune response.

Conclusions
Our knowledge of the *MC1R* gene, its regulation, protein structure, and associated functions have increased

substantially over the past 30 years. Whereas its role in human pigmentation has been largely deciphered, its non-pigmentary functions, including those related to DNA damage repair and chromosome stability, are likely to have an influence on the regulation of several aspects of skin physiopathology. However, increasing evidence based partly on single-cell sequencing studies suggests a physiological role in the skin for MC1R in melanocytes only, compared with that in nonmelanocytic skin cells, although it is likely that MC1R will be active in parts of the brain and in granulocytes. Whether there is a physiologically relevant link between MC1R-expressing cell type remains unclear, but future work that examines more precisely how, where, and when MC1R signals is especially important given the use of α-MSH analogs for therapeutic purposes.

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Conceptualization: SG, CRG; Data Curation: SG; Formal Analysis: SG; Supervision: GG, CRG; Validation: GG, CRG; Writing - Original Draft Preparation: SG, CRG; Writing - Review and Editing: SG, GG, CRG

CONFLICT OF INTEREST

The authors state no conflict of interest.

SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at www.jidonline.org, and at https://doi.org/10.1016/j.jid.2021.06.018.

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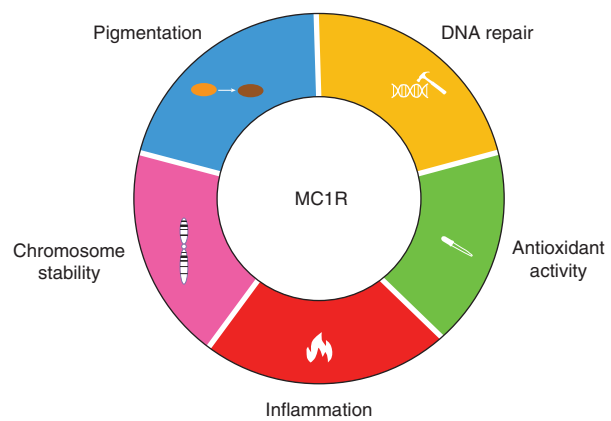
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SUPPLEMENTARY MATERIAL

Supplementary Figure S1. Summary of the biological functions of the MC1R.