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MC1R functions, expression and implications for targeted therapy

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Abstract (100)

The G-protein-coupled melanocortin 1 receptor MC1R is expressed in melanocytes and has a pivotal role in human skin pigmentation, with reduced function human genetic variants exhibiting a red hair phenotype and increased melanoma pre-disposition. Beyond its role in pigmentation, MC1R is increasingly recognized as promoting UV-induced DNA damage repair. Consequently, there is mounting interest in targeting the MC1R for therapeutic benefit. However, whether MC1R expression is restricted to melanocytes or is more widely expressed remains a matter of debate. Here, we review MC1R function and highlight that unbiased analysis suggests its expression is restricted to melanocytes, granulocytes and the brain.

Text

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 (Sturm and Duffy, 2012; Pavan and Sturm, 2019), and their functions defined in the production of melanin, the structural integrity of melanosomes and their transport and transfer to keratinocytes, a pivotal role is played by the melanocortin 1 receptor (*MC1R*) gene (Abdel-Malek 1999) that 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.

MC1R belongs to a sub-family of G protein-coupled receptors (GPCRs), that bind melanocortins (MCs) to control several key physiological and behavioural traits (Roulin and Ducrest, 2011; Yang and Harmon, 2017). Specifically, MC1R exhibits a high affinity for α melanocyte-stimulating hormone (αMSH) (Mountjoy et al., 1992), a peptide hormone derived by proteolytic cleavage from pro-opiomelanocortin (POMC) (Drouin and Goodman, 1980). Of note, human MC1R, unlike its mouse counterpart, can also recognize adrenocorticotropic hormone (ACTH), in addition to α-MSH as a full agonist with similar affinity to α-MSH (Suzuki et al., 1996). Although MC1R can signal via several distinct pathways, most studies have focused on its ability to increase cyclic AMP (cAMP) levels which is mediated through MC1R interaction with the Gs protein that interacts with adenylate cyclase (Rodrigues et al., 2015). Significantly, the MC1R gene shows a 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 (Elfakir et al., 2010; Tagliabue et al., 2015; Pellegrini C et al., 2019; Guida et al., 2019; Caini et al., 2020; Guida et al., 2021). 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 red pheomelanin,

thereby providing cells with photo-protective and anti-oxidant 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. Here 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 inter- 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 C315 (García-Borrón et al., 2005; Wolf Horrell et al 2016; Chen et al 2017) (Figure 1A, B)

Human MC1R expression is regulated by a single promoter partly controlled by the microphthalmia-associated transcription factor MITF, a key regulator of melanocyte biology (Goding and Arnheiter, 2019), that binds a CACGTG E-box motif (Figure 1C), showing results of MITF Chromatin-immunoprecipitation-sequencing performed 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; Moro et al., 1999; Miccadei et al., 2008). At the 3' end of the MC1R gene, an atypical polyadenylation site enables intergenic splicing between MC1R and the downstream β-III-tubulin (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 via cAMP but a retention of ERK signaling (Dalziel et al., 2011; Herraiz et al., 2015). However, although the mRNAs corresponding to these chimeric MC1R–TUBB3 chimeric 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 (Herraiz et al., 2011a; Sanchez-Laorden et al., 2007; Chen S et al., 2017). MC1R dimerization occurs constitutively and both the WT receptor and its allelic variants can homo- or heterodimerize, leading to different functional consequences (Sánchez-Laorden BL et al., 2006; Sánchez-Laorden BL et al., 2007). An additional contribution to decreased internalization is given by MC1R N-glycosylation (Herraiz et al., 2011a), while palmitoylation at Cysteine 315 (C315) contributes to receptor structure, stability, membrane localization, and interaction with partner proteins (Chen et al., 2017; Chen et al., 2019). Phosphorylation at Threonine-157 is critical for receptor trafficking, while phosphorylation at Threonine-308 and Serine-316 influence receptor desensitization and internalization (Sanchez-Laorden et al., 2007; Sanchez-Laorden et al., 2009). Of note, however, care needs to be taken in interpretation of experiments performed by some studies in non-melanocytes such as melanoma cells or HEK293 cells (human embryonic kidney 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. (Swope et al., 2012) provided evidence for differential expression of GCPR kinases (GRKs) in human melanocytes versus 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 J 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 keratinocytes and melanocytes, primarily αMSH and to a lesser extent 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 (Patel et al., 2010; Jackson et al., 2005). The human inverse agonist is encoded by the *Agouti* (*ASIP*) gene, cloned by Wilson et al. (Wilson et al., 1995), and its effects on human melanocytes firstly reported by Suzuki et al (Suzuki et al., 1997). Additionally, 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 Agouti Signal Protein (ASP) (Walker and Gunn, 2010) and AgRP (for Agouti Related Protein). In cell culture, pre-treatment with MC1R antagonists blocks the responsiveness to αMSH, suggesting that antagonists can induce receptor internalization, inhibit its recycling, and/or have prolonged receptor occupancy (Swope et al., 2012; Suzuki et al., 1997).

MC1R polymorphisms

MC1R is highly polymorphic, with around variants 200 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 (Valverde et al., 1995; Box et al., 1997). 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, though outside of Africa the prevalence of *MC1R* polymorphisms appear to reflect a neutral selection (Harding et al, 2000), facilitating ultraviolet (UV) stimulated production in the skin of vitamin D which is essential for bone health and 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). In 1995, Valverde et al. (Valverde et al., 1995) described the association between some *MC1R* LOF polymorphisms and red hair, fair skin, and sun sensitivity (the Red Hair Color, RHC, phenotype), an association confirmed by many subsequent studies (Smith et al., 1998, Rees, 2000, Beaumont et al., 2008). Genetic epidemiology studies firmly established the association of *MC1R* RHC variants with melanoma (Ichii-Jones F et al., 1998; Williams et al., 2011; Guida et al., 2015; Tagliabue et al., 2018) and non-melanoma skin cancers (Tagliabue et al., 2015; Joshi et al., 2018). 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 (Palmer et al., 2000; Kennedy et al., 2001; Scott et al., 2002; Mitra et al., 2012). 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 pseudo-alleles showing no significant effect on phenotype (Duffy et al., 2004). Both RHC and r polymorphisms generate hypomorphic proteins, impairing 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 include those with reduced cell surface expression, such as R151C, R160W, D84E (Beaumont et al 2005; Beaumont et al., 2007; Morgan et al., 2018), involving the regulation of anterograde trafficking of the MC1R or cAMP coupling impairment (García-Borrón et al., 2014; Beaumont et al., 2007), and those with normal surface expression, such as D294H and R142H, but decreased functional coupling (Newton et al 2005; Beaumont et al., 2007) related to an inability to bind the MCs (Sánchez Más J et al., 2002; Scott et al., 2002; Beaumont et al., 2005). 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 WT (Scott et al., 2002; Kadekaro et al., 2010).

MC1R: pigmentary and non-pigmentary 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 non-pigmentary roles as a regulator of antioxidant defenses, DNA-repair mechanisms and genome integrity (Abdel-Malek et al., 2014; Swope et al., 2014; Li et al., 2021). Supplementary Fig. 1

Pigmentary functions and the cAMP pathway

The human epidermis primarily comprises keratinocytes 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 αMSH from cutaneous keratinocytes (Cui et al., 2007) inducing a tanning response in skin during which melanosomes, the pigment-containing organelles synthesized in melanocytes, are transferred to keratinocytes to protect against UV-induced DNA damage. Mechanistically, keratinocyte-derived αMSH activates the MC1R GPCR, leading to stimulation of adenylyl cyclase (AC) and ultimately to increased cAMP levels. The consequent activation of protein kinase A (PKA) leads to phosphorylation of the cAMP-response element binding protein 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 Tyrosinase (TYR) and the Tyr-related proteins TYRP1 and DCT as well as regulating cell cycle progression (Levy et al., 2006; Carreira et al., 2005; Carreira et al., 2006; Loercher et al., 2005; Goding and Arnheiter, 2019). 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 keratinocytes (Passeron et al., 2004; d'Ischia et al., 2015).

In addition to regulating CREB, the cAMP pathway in melanocytes also targets another transcriptional regulator, the peroxisome proliferator-activated receptor γ coactivator-1α (PGC-1α) whose expression is also activated by MITF (Ferretta et al 2016; Vazquez et al 2013; Haq 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 reactive oxygen species (ROS) (Villena, 2015; Maresca et al., 2015). The link between these two pathways provides an interesting connection between pigmentation, anti-oxidant activity and metabolism and indeed, in addition to mitochondrial biogenesis, MITF has recently been identified as a key regulator of the TCA cycle (Louphrasitthiphol

et al., 2019) and fatty acid desaturation (Vivas-Garcia et al., 2020). Thus MC1R, through MITF, may play a key role in coordinating melanocyte metabolism.

Additionally, MITF contributes to a negative feedback loop downstream from MC1R signaling by downregulating cAMP signaling by increasing expression of phosphodiesterase 4D3 gene (PDE4D), a transcriptional target of cAMP via MITF (Khaled et al., 2010), limiting cAMP accumulation.

Non-pigmentary roles of MC1R

In addition to its well-characterized function in pigmentation, MC1R plays a key role in pigmentation-independent responses to UV radiation, such as the antioxidant response and induction of DNA repair mechanisms (Abdel-Malek Z et al 2000; Abdel-Malek et al., 2014), although the underlying mechanisms by which the 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 via MC1R signaling to the ataxia telangiectasia-mutated and Rad3-related (ATR) pathway (Jarrett et al., 2015). Indeed, in human melanocytes, α-MSH signaling via MC1R was shown to activate both DNA damage sensors ATR and ATM, as well as 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 (Strub

et al., 2011; Seoane et al., 2019) and activate an anti-oxidant response (Louphrasitthiphol et al., 2019). Consistent some non-pigmentary roles for MC1R being mediated by MITF, recent evidence also assigns MC1R a protective role in chromosome stability and centromere integrity, again mediated via MITF (Li et al., 2021).

Activation of NER in UV-irradiated melanocytes is also mediated through cAMP signaling to p53. Like MITF, p53 plays an important role in melanocytes as it counteracts oxidative damage, and maintains homeostasis of melanocytes (Kadekaro et al., 2012). To support this hypothesis, impaired MC1R signaling in melanocytes exposed to UV radiation 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 (Hauser et al., 2006; Jarrett et al., 2014; Jarrett et al., 2015; Cassidy et al., 2015; Kadekaro et al., 2010). As a consequence, compared to 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 (Robles-Espinoza et al., 2016; Johansson et al., 2017).

Additional non-pigmentation-related effects of MC1R have been attributed to PTEN, PI3K/AKT signaling, mitogen-activated protein kinases, and c-KIT (Herraiz et al., 2009; Cao et al., 2013). Interestingly, activation of the ERK1/2 by stress signals such as αMSH and by p38 signaling, mimicking the effects of UV-light exposure, seem to be mediated by the crosstalk with c-KIT and is not affected by cAMP levels (Smalley and Eisen, 2000; Herraiz et al., 2011b; Castejón-Griñán et al., 2018). By contrast, activation of AKT downstream from PI3K signaling appears to promote 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 (Cao et al., 2013) who revealed that UV exposure triggers an association between MC1R WT, but not RHC variants, and PTEN, a negative regulator of PI3K signaling, to prevent PTEN degradation and suppress PI3K signaling. Hyperactivation of PI3K signaling in primary melanocytes can lead to increased senescence, but in BRAFV600E mutated melanoma, MC1R mutation leading to elevated PI3K signaling can promote oncogenic transformation (Cao et al., 2013).

αMSH has also been reported to antagonize the effects of pro-inflammatory 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; since events leading to down-regulation of MITF can trigger inflammatory signaling and MMP expression (Carreira et al., 2006; Giuliano et al., 2010; Vivas-Garcia et al., 2020), MITF up-regulation should suppress these activities.

Targeting the MC1R

Since 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-wild-type mice by increasing eumelanin deposition and by improving NER (Bautista et al., 2020). Other studies also revealed that raising cAMP levels using forskolin in in vitro and in animal models can decrease UV-induced DNA damage and melanoma incidence in an 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., 2017; Chen et al., 2019). Since 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 since palmitoylation is a commonly used to regulate many signaling molecules (Wang et al., 2020). As such, targeting de-palmitolyation may lead to effects well beyond MC1R signaling.

Importantly, one clinically successful approach to targeting MC1R is the use of an αMSH analogue, afamelanotide. This has proven to be effective and safe in the treatment of photosensitivity related to erythropoietic protoporphyria, a rare disease that may be associated to acute phototoxicity (Langendonk et al., 2015). Consequently, based on 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 erythropoietic protoporphyria.

Other MC1R agonists have been developed to exploit the role of MC1R WT in increasing pigmentation, anti-oxidant defenses and DNA repair (Mowlazadeh Haghighi et al., 2018; Jackson et al., 2019; Koikov et al., 2021). For example, based on 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 EC50. Moreover, Koikov et al (2021) were able to generate a tripeptide agonist with greater than 100,000-fold selectivity for MC1R over other melanocortin receptors. Based on amino acid sequence and affinity with the human-receptor, additional applications of MC1R agonists have been proposed such as vitiligo treatment, and as anti-aging and anti-greying hair agents (Almeida Scalvino et al., 2018; Jackson et al., 2019; Koikov et al., 2021).

MC1R and non-melanocytic 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 keratinocytes, fibroblasts, endothelial cells, immune system cells) has been widely reported (Hill et al., 2006; Hiramoto et al., 2010; Muffley et al., 2011; Luo et al., 2013; Bohm and Stegemann, 2014; Kleiner et al., 2016) and related to anti-inflammatory effects, immune response, response to burn injuries, collagen synthesis and scar formation. At first sight therefore it seems that expression of MC1R would be widespread with pleiotropic effects and important implications for targeted therapies. However, while expression of MC1R in melanocytes has been validated, with strong genetic evidence to back up its role in melanocyte pigmentation, many studies in non-melanocyte cell types lack the key controls necessary to make definitive conclusions. For example, while semi-quantitative reverse transcriptase-polymerase chain reaction (RT-PCR) at high cycle numbers can detect MC1R mRNA in non-melanocytic cells, quantitative RT-PCR revealed a low level of MC1R expression in keratinocyte and fibroblast cell cultures, as compared to the much higher levels of MC1R transcripts found in melanocytes (Roberts et al., 2006). Consistent with the low level of mRNA in non-melanocytic cells, MC1R protein expression at the cell surface was not detected (Roberts et al., 2006). Similarly, while addition of αMSH to melanocytes leads to a robust increase in cAMP levels that is dependent on the receptor, a direct comparison between the effects of αMSH on non-melanocytes versus melanocytes, the use of control non-functional peptides, or control cells lacking or depleted or mutated for the MC1R receptor 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, frequently absent from many studies of MC1R function in non-melanocytes. For those studies where suitable controls were used, the levels of *MC1R* expression in non-melanocyte cell types is very low compared to 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 non-melanocytes, we

interrogated data curated at the Human Protein Atlas (www.proteinatlas.org). Based on single cell sequencing of human skin as reported by (Sole-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-seq from multiple tissue types again revealed 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 erythropoietic protoporphyria treated with afamelanotide reported, in addition to increased skin pigmentation, nausea and headache (Langendonk et al., 2015), suggesting the *MC1R* could be expressed in the brain. Consistent with this, *MC1R* mRNA expression can be detected in the cerebellum, dysfunction of which can cause nausea, as well as other brain regions (Figure 2C). Whether in cell types expressing low to intermediate levels of *MC1R*, the mRNA is translated into MC1R protein 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, that facilitate the immune response.

Conclusions

Our knowledge of *MC1R* gene, its regulation, protein structure and associated functions (Supplementary Fig. 1) have increased substantially over the past 30 years. While 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 physio-pathology. However, increasing evidence based partly on single cell sequencing studies suggests a physiological role in the skin for MC1R in melanocytes only, as compared to non-melanocytic skin cells, though 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 αMSH analogs for therapeutic purposes.

Data Availability Statement

No datasets were generated or analyzed during the current study.

Conflict of interest

The authors declare no conflict of interest.

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Figure legends

Figure 1. Structure of the MC1R. **A.** Sequence of the MC1R with locations of specific variants indicated in orange and the R151C variant indicated. **B.** Left: 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. Colours 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 correct insertion into the plasma membrane indicated by the two disks; Right: Potential structure of the MC1R with locations of variants, including R151C, indicated in orange. **C**. MITF binding to the human *MC1R* locus. University of Santa Cruz (UCSC) browser screenshot showing 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.

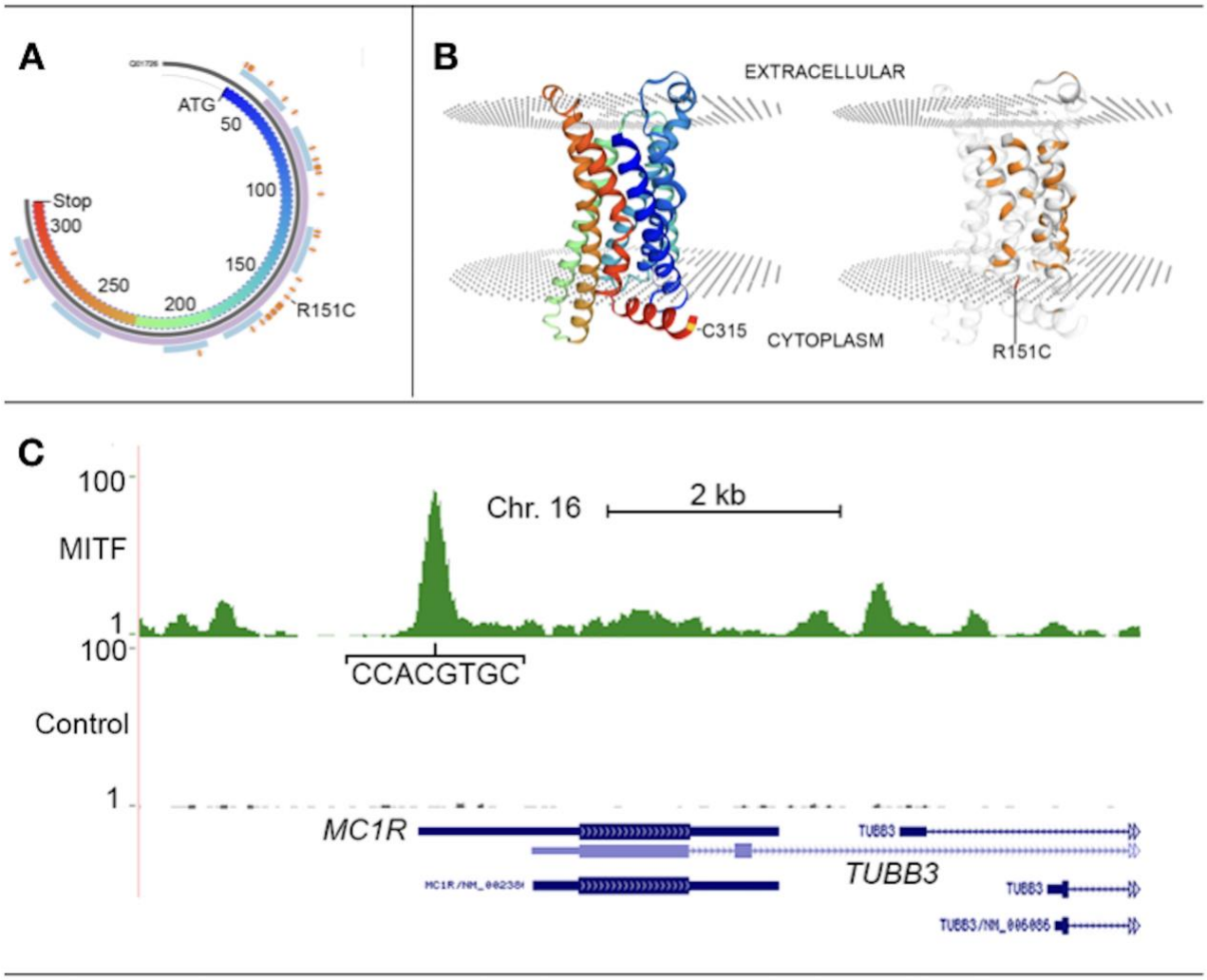
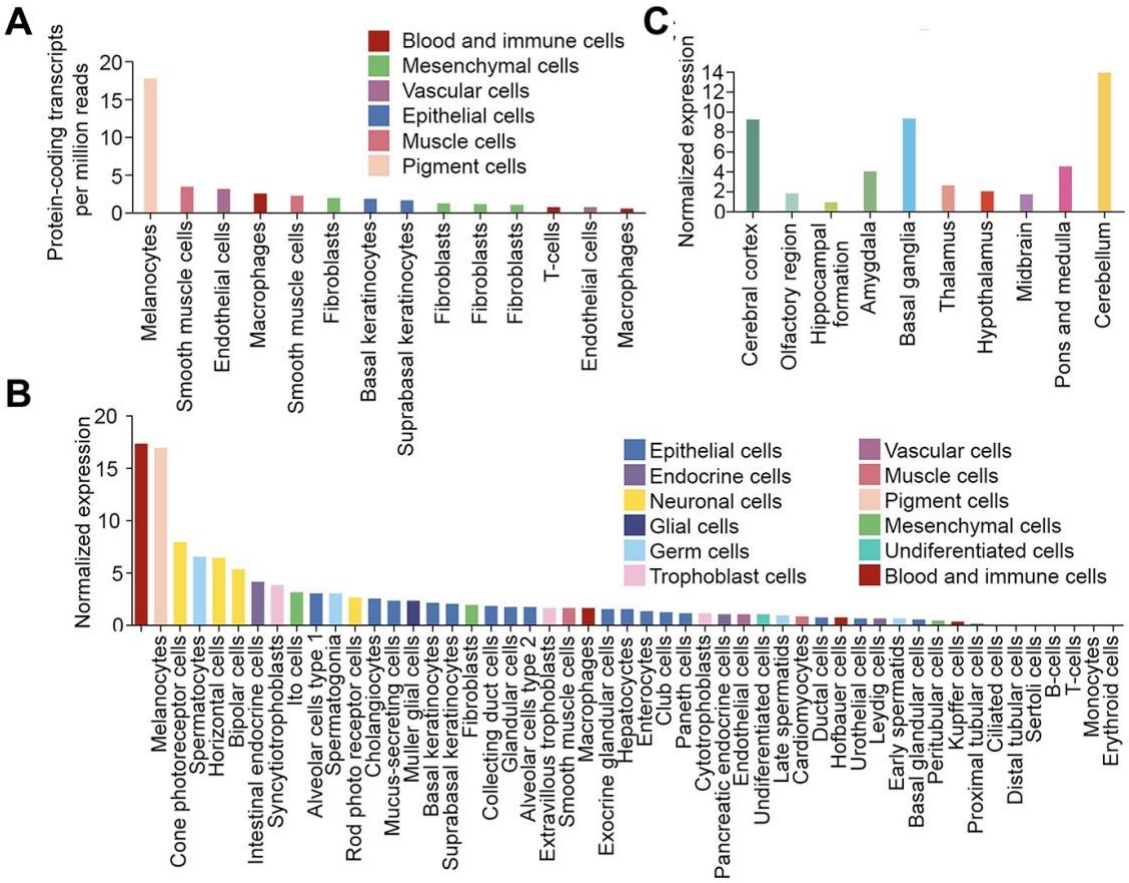


Figure 2. Expression of *MC1R*. **A**. Single cell RNA-seq data showing expression of MC1R mRNA from different cell types from human skin. Multiple notations of a single cell type such as fibroblasts indicates that each fibroblast population was distinct as delineated by the bioinformatic clustering analysis. **B.** Single cell RNA seq data showing normalized expression of MC1R from multiple tissue types. **C.** RNA-seq showing 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



Supplementary Fig 1. Summary of the biological functions of the MC1R.