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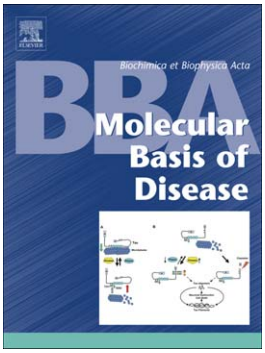

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MC1R SIGNALING. INTRACELLULAR PARTNERS AND PATHOPHYSIOLOGICAL IMPLICATIONS.

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Abbreviations: AC, adenylyl cyclase; ACTH, adrenocorticotropic hormone; ARRB, β-arrestin; ASIP, agouti signal protein; CBD103, dog β-defensin-3; CREB, cAMP responsive-element binding protein; EDN1, endothelin 1; ER, endoplasmic reticulum; ERK, extracellular signal-regulated protein kinase; Fsk, forskolin; GPCR, G protein-coupled receptor; GRK, G protein-coupled receptor kinase; HBD3, human β-defensin-3; il, intracellular loop; KSR1, Kinase Suppressor of Ras 1; MAPK, mitogen-activated protein kinase; MC, melanocortin; MCR, melanocortin receptor; MITF, Microphthalmia transcription factor; αMSH, α melanocyte-stimulating hormone; NF2, neurofibromin 2 (merlin); NHM, normal human melanocyte; NLS, nuclear localization signal; PDE, phosphodiesterase; PGC-1α, peroxisome proliferator-activated receptor γ coactivator-1α; POMC, proopiomelanocortin; RHC, red hair color; ROS, reactive oxygen species; RTK, receptor tyrosine kinase; UV, ultraviolet; UVR, ultraviolet radiation; WT, wild-type.

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ABSTRACT

The melanocortin-1 receptor (MC1R) preferentially expressed in melanocytes is best known as a key regulator of the synthesis of epidermal melanin pigments. Its paracrine stimulation by keratinocyte-derived melanocortins also activates DNA repair pathways and antioxidant defenses to build a complex, multifaceted photoprotective response. Many MC1R actions rely on cAMP-dependent activation of two transcription factors, MITF and PGC1α, but pleiotropic MC1R signaling also involves activation of mitogen-activated kinases and AKT. MC1R partners such as β-arrestins, PTEN and the E3 ubiquitin ligase MGRN1 differentially regulate these pathways. The *MC1R* gene is complex and polymorphic, with frequent variants associated with skin phenotypes and increased cancer risk. We review current knowledge of signaling from canonical MC1R, its splice isoforms and natural polymorphic variants. Recently discovered intracellular targets and partners are also discussed, to highlight the diversity of mechanisms that may contribute to normal and pathological variation of pigmentation and sensitivity to solar radiation-induced damage.

Keywords: Melanocortin 1 receptor; Melanocytes; Pigmentation;

Photoprotection; Signaling; Melanoma

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1- Introduction

Almost 25 years ago, two groups independently reported the molecular cloning of a cDNA coding for a G protein-coupled receptor (GPCR) expressed in melanocytes that was able to bind several melanocortin peptides including α melanocyte-stimulating hormone (αMSH) and adrenocorticotropic hormone (ACTH) [1, 2]. The paper by Roger Cone's group also reported a highly related receptor specific for ACTH, preferentially expressed in the adrenal cortex. Both receptors were found to stimulate cAMP signaling upon activation by melanocortin (MC) peptides. The description of 3 related integral membrane proteins rapidly ensued to complete the five-membered subfamily of the Gs protein-coupled melanocortin receptors (MCRs) (recently reviewed by [3]). These were named MC1R to MC5R according to the order of their identification, and the melanocytic receptor with high affinity for αMSH was termed MC1R.

The demonstration that a mouse Mc1r mutation causing a premature truncation of the protein was associated with a yellow coat color, whereas gain-of-function mutations resulting in constitutive or hyperactive signaling to the cAMP pathway were causally related with a darker coat [4] paved the way to the current concept of *MC1R* as a major determinant of cutaneous pigmentation. Human epidermis is a pluristratified epithelium where pigment-producing cells, the melanocytes located in the basal layer, are surrounded by keratinocytes, the majority type of epidermal cells. Keratinocytes and melanocytes are in tight physical and functional contact. Melanocytes synthesize melanin pigments within specific organelles called melanosomes, and transfer them to keratinocytes through prominent dendritic processes. Upward movement of melanosome-loaded basal layer keratinocytes disperses melanins throughout the skin to ensure an even distribution of the pigment. Mammalian melanins are of two types, whose amount and ratio determine the pigmentation phenotype: the black-brown eumelanins, and the yellowish-reddish sulfur-containing feomelanins [5]. Activation of MC1R by keratinocyte-derived MC agonists not only switches the melanin biosynthesis

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pathway from basal feomelanogenesis to an activated state where eumelanin synthesis prevails, thus leading to darker pigmentation, but also promotes pigment transfer to keratinocytes. In fact, the MC1R is now considered a master regulator of most if not all processes involved in pigment production and distribution throughout the skin [6]. Accordingly, the MC1R is centrally located in a hub of paracrine interactions determining a complex phenotype and provides a unique example of bi-directional intercellular cooperation.

On the other hand, the local concentration of the paracrine effectors that control MC1R expression and activity are tightly regulated by environmental factors [7]. The effect of ultraviolet radiation (UVR) in triggering the production and release of MC1R agonists from cutaneous keratinocytes is particularly relevant since it plays a central role in the tanning response of sun-exposed human skin [8]. Moreover, studies performed during the last decade have firmly established that MC1R is a key player not only in UVR-induced tanning, but also in pigmentation-independent responses to UVR, including induction of antioxidant defenses and DNA repair mechanisms (reviewed by [9]). Thus, MC1R signaling provides an outstanding model for the role of GPCRs in determining complex physiological adaptive responses to environmental factors.

Of note, the *MC1R* gene seems to have evolved more rapidly than other members of the MCR subfamily [10] or, on a broader scale, the GPCR superfamily of cell surface receptors. Extensive genetic studies have shown that it is highly polymorphic. Given that many alleles are associated with skin and hair pigmentation phenotypes [11], functionally relevant natural variants are fairly easy to detect, identify and analyze in terms of structure-function-phenotype associations. Moreover, the *MC1R* genotype is a key determinant of the degree of sensitivity to sun-induced skin damage and certain variants are well-established genetic risk factors for a number of diseases, including melanoma [12-18]. Therefore, the study of MC1R provides clues to understand the role

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of GPCRs as determinants of normal human variation and genetic susceptibility to certain diseases.

This review is focused on MC1R signaling and regulation and will consider newly identified interactions with potentially relevant intracellular effector proteins. Since MC1R is best known for its role in human pigmentation and cutaneous biology, these will be the main physiological processes considered here. The continuously growing repertoire of MC1R non-pigmentary, non-melanocytic actions involving a variety of cell types and pathological conditions is beyond the scope of this review and has been covered elsewhere [19-21].

2- Gene structure and expression

The human *MC1R* gene (MIM# 155555, Ensembl ID ENSG00000258839) mapping to the murine *Extension* locus [1, 2, 22] is preferentially expressed in melanocytes, although low levels of expression are also detected in many non-melanocytic cells such as human keratinocytes, fibroblasts, and immune cells [19]. Compared with most GPCR genes, the structure of *MC1R* is quite complex, as it displays several splice variants and a high degree of polymorphism. *MC1R* was first reported as intronless [23], but further analysis established that it actually comprises 4 exons and yields several transcripts as a result of intra- and intergenic splicing. The major transcript, with a 951 nucleotides (nt) coding region (ID ENST00000555147, named MC1R-001), contains exons 2, 3 and 4, with retention of two unspliced intervening sequences between exons 2–3 and 3–4 (Fig 1A). This transcript encodes for a 317 amino acid integral membrane protein with all the structural characteristics of a family A GPCR [24] which is now considered the canonical form of the protein (Consensus sequence CDS 56011.1). In this isoform, the N-terminal fragment, cytosolic tail and all transmembrane fragment-connecting loops are short, compared with most GPCRs, which may have

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functional consequences (Figure 1B and 1C). Tan and coworkers reported an alternative spliced MC1R form designated MC1R-002 (ID ENST00000555427), which contains exons 1–4 and results in a 1149 nt-long reading frame encoding for a 382 amino acids protein [25]. This isoform is identical to MC1R-001 up to Ser316, followed by an additional 66 amino acids C-terminal extension (Fig 1B and 1C). This difference, even if involving an intracellular region not directly interacting with extracellular ligands, affects its agonists binding properties [25]. Another splice variant, named MC1R-350, was isolated from cultured normal human melanocytes (NHMs) and skin sections [26]. This isoform shares with MC1R-001 and MC1R-002 the sequence up to Cys315, but shows a different 35 amino acids C-terminal extension (Fig 1C). One more reported transcript, named MC1R-003 (ENST00000539976), lacks a functional open reading frame.

FIGURE 1

The 16q24 region containing the *MC1R* gene is a highly packed area with short intergenic regions between *MC1R* and its upstream and downstream neighbors [23]. This dense packing and the presence of an unusual and inefficient polyadenylation signal in human *MC1R* apparently promote intergenic splicing to the downstream *Tubulin beta III* (*TUBB3*) gene, particularly in UVR-exposed melanocytes [27, 28], and two intergenic splice products have been described [27]. One of the resulting chimeric proteins contains *MC1R* exons 3 and 4 fused to *TUBB3* exons 3, 4 and 5 (Fig 1A). This transcript (also called Iso1 and RP11-566K11.2-001 transcript, ID ENST00000556922) encodes for a 797 amino acids in-frame fusion chimera corresponding to the first 366 residues of MC1R-002 and most of the TUBB3 sequence (Fig 1B). The other intergenic splice variant, named Iso2, arises by out-of-frame fusion of *MC1R* exon 3 and exon 3

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of *TUBB3* (Fig 1A), yielding a 432 amino acids protein where the first 316 residues match the MC1R-001 sequence. The remaining 116 C-terminal residues share no homology with known proteins (Fig 1B) [27]. Within the subgroup of the MCRs, such an extensive transcript diversity seems distinctive of *MC1R*. *MC3R* and *MC4R* express a single transcript (Ensembl ID ENST00000243911 and ENST00000299766, respectively), corresponding to the consensus sequence of the receptor. However, in addition to the transcript coding for the full length canonical isoform (ENST00000327606 and ENST00000324750, respectively), both *MC2R* and *MC5R* express one alternative transcript where the coding sequence is incomplete in its 3' end, leading to shorter polypeptides of 123 and 173 amino acids respectively (ENST00000399821 and ENST00000589410). These small proteins should be completely inactive because of early truncation.

A recent study confirmed the occurrence of MC1R-TUBB3 intergenic splice transcripts in human melanocytic cells, even in resting cells without previous exposure to UVR or treatment with MC1R agonists [29]. Expression of Iso1 and Iso2 chimeric mRNA and the canonical MC1R-001 transcript was compared in a panel of human melanoma cell lines and a human epidermal melanocytic cell line. Iso1 and Iso2 transcripts were found in all the cell lines tested, although at much lower levels than canonical MC1R-001 transcripts. The chimeric proteins were expressed in heterologous HEK293T cells and human melanoma cells, for functional characterization. Iso1 and Iso2 proteins were expressed at lower steady-state levels than MC1R-001, suggesting a shorter intracellular half-life. This was confirmed by estimation of their rates of decay in cycloheximide-treated cells. In keeping with decreased intracellular stability, it was found that Iso1 and Iso2 expressed in HEK293T cells were strongly retained in intracellular compartments, as shown by extensive co-localization with the endoplasmic reticulum (ER) marker calnexin and low cell surface expression. Thus, the shorter half-life of the chimeric proteins might likely be related with recognition by the ER-resident

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protein quality control system, leading to their proteolytic removal. Consistent with a reduced plasma membrane expression, the chimeric proteins activated very weakly the cAMP pathway after stimulation with a αMSH analog. Interestingly, co-immunoprecipitation experiments showed that the chimeric isoforms heterodimerized efficiently with MC1R-001. This association apparently impaired forward trafficking of the canonical form, as suggested by high co-localization in intracellular locations and decreased number of αMSH binding sites on the cell surface.

Human *MC1R* is highly polymorphic (recently reviewed by [30]), and the number of newly identified and characterized alleles keeps growing continuously [31, 32]. Overall, around 200 nonsynonymous coding region variants in virtually all the structural regions of the protein have been described (Fig 1C). Some of these variants show large differences in allelic frequency in different populations. For instance, the R163Q variant is extremely common in Asian populations [33-35], but rare in individuals of European descent, with some exceptions [36]. The differential distribution of *MC1R* allelic variants in people of different ancestry may be useful to determine the population origin of individuals for forensic and legal medicine purposes [37]. Finally, some 50 polymorphisms in 3' and 5' untranslated regions have been found, whose potential effects on *MC1R* gene expression or mRNA processing and/or stability remain unknown.

3- MC1R downstream signaling pathways

Most GPCRs can engage multiple signaling pathways, not only through promiscuous coupling to more than one type of G protein, but also through G protein-independent mechanisms such as those relying on ligand-induced interaction with β-arrestins or other scaffolding proteins (recently reviewed by [38]). For the MC1R, pleiotropic signaling to the cAMP, the p38 stress-activated kinase and the extracellular signal-

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regulated protein kinases 1 and 2 (ERK1/2) is well documented, and there is also evidence for involvement of AKT (Fig 2). The complexity of MC1R downstream signaling is further enhanced by: i) the variety of MC1R activatory and inhibitory natural ligands [39], ii) the crosstalk between MC1R-triggered signaling modules [40], iii) the independent regulation of each pathway by intracellular proteins [41], and iv) the potentially biased effects of single amino acid substitutions in the signaling properties of the many natural coding region variants [42].

3.1- MC1R natural ligands.

Human MC1R and to a lesser extent mouse Mc1r exhibits significant agonist-independent functional coupling to the cAMP pathway [43, 44]. This constitutive signaling is further enhanced upon paracrine/autocrine activation by MCs (mostly αMSH and ACTH) released by skin keratinocytes and melanocytes (reviewed in [7]). Production and release of these natural MC1R ligands is under the control of environmental and internal cues. Importantly, in skin keratinocytes, expression of the *POMC* gene encoding for the precursor of αMSH and ACTH is activated by the p53 tumor suppressor. UVR-induced DNA damage in keratinocytes leads to stabilization and increased activity of p53, which activates transcription of *POMC* gene, resulting in increased paracrine stimulation of MC1R [8]. This provides a mechanism accounting for the darkening of UVR-exposed skin known as tanning.

MC1R signaling is negatively regulated by the **A**gouti **Si**gnal **P**rotein (ASIP), a small secreted protein which behaves as an inverse agonist since it competes with MCs for binding with MC1R and it decreases constitutive signaling to the cAMP pathway. A developmentally regulated pulse of ASIP secretion within the hair follicle is responsible for a transient inhibition of MC1R signaling, with production of yellowish pheomelanins instead of dark eumelanins. This leads to a dark-light banding pattern of dorsal hair color in mice (the pigment-type switch, reviewed by [39]). Whereas the major role of ASIP as a determinant of pigmentation in the mouse has been known for long, the

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association of the protein with human skin pigmentation traits has been established recently [45, 46]. A peptide expressed in the adrenal glands and the central nervous system, termed AgRP (for **Ag**outi **R**elated **P**rotein) due to its structural similarity to ASIP, is a potent antagonist of MC3R and MC4R, but it lacks obvious pigmentary effects presumably because of inability to bind MC1R with high affinity [47].

Analysis of a mutation in domestic dogs leading to black furs identified β-defensin103 (CBD103) as a high affinity Mc1r ligand [48]. This small secreted protein belongs to a family of cationic antimicrobial peptides, some of which are produced in the skin. The human orthologue (HBD3) was shown to inhibit αMSH-mediated increases in cAMP and in the activity of the rate-limiting melanogenic enzyme, tyrosinase (Tyr) in NHMs [49]. HBD3 also inhibits other MC1R responses such as phosphorylation of ATR [50]. Careful biochemical and functional studies further showed that in spite of previous reports that HBD3 might be a MC1R partial agonist, this peptide actually acts as a neutral antagonist with affinity for MC1R and MC4R, which is also able to displace ASIP [51, 52].

3.2- The cAMP pathway.

Most physiological effects of MC1R on melanocytes, notably including induction of eumelanogenesis, appear mediated by the cAMP pathway [53, 54]. The αMSH-MC1R complex activates the Gs protein, followed by Gsα-dependent stimulation of adenylyl cyclase (AC) and increased cAMP levels (Fig 2). The ensuing activation of PKA and PKA-dependent phosphorylation of CREB proteins leads to transcriptional activation of a master regulator of melanocyte biology, the Microphthalmia-associated transcription factor (MITF) [55]. This transcription factor upregulates expression of a set of melanogenic enzymes, mainly the rate-limiting Tyr and the Tyr-related proteins Tyrp1 and Tyrp2/Dct [55-58]. MITF is also involved in cell cycle control [59, 60] and is essential for survival of melanoblasts during embryonic development [61]. Moreover, by regulating the expression of Rab27a, a small GTPase involved in melanosome

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transport [62, 63], MITF also contributes to the control of the distribution of melanosomes and their transfer to keratinocytes [64]. Therefore, by increasing MITF activity, the cAMP pathway stimulates most of the processes responsible for pigment production in melanocytes and melanin distribution throughout the skin. Several studies have shown that expression of the *MC1R* gene in human and mouse melanocytes is upregulated by αMSH or the AC activator forskolin (Fsk), apparently in a MITF-dependent manner, thus giving rise to a positive feedback loop under certain physiological conditions such as exposure of the skin to UVR [26, 65, 66].

FIGURE 2

Recently, another important transcriptional regulator has been shown to be targeted by the cAMP pathway in melanocytes. The peroxisome proliferator-activated receptor γ coactivator-1α (PGC-1α) is a member of a small family of transcriptional coactivators which interact with many nuclear receptors and transcription factors and are critically involved in the regulation of mitochondrial biogenesis, liver and brown adipose tissue metabolism, and detoxification of reactive oxygen species (ROS) (reviewed by [67-69]). αMSH and cAMP were shown to increase strongly PGC-1α expression both in melanoma cells and NHMs, by two complementary mechanisms involving transcriptional activation of *PGC-1α* gene expression and stabilization of the PGC-1α protein. This post-translational effect most likely resulted from direct phosphorylation of PGC-1α by PKA. PGC-1α and the related PGC-1β stimulated MITF and Tyr expression, as well as melanin pigment production [70]. Most notably, MITF was shown to increase PGC-1α expression in a subset of human melanoma tumors [71, 72]. Consistent with the known physiological actions of PGC-1α, this led to increased mitochondrial energy metabolism, resistance to oxidative stress and ROS-inducing

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drugs and poor prognosis [72]. This series of investigations firmly established PGC-1α as a new and important downstream target of MC1R signaling via the cAMP pathway, and highlighted a second positive feedback loop in this pathway (Fig 2), whereby MITF expression is stimulated by PGC-1α as a result of paracrine activation of cAMP signaling downstream of MC1R, followed by MITF activation of PGC-1α expression [73].

The mechanisms responsible for downregulation of cAMP signaling and for the control of the two positive feedback loops triggered by cAMP downstream of the MC1R have been investigated. The *Phosphodiesterase 4D3* gene (*PDE4D*) is a transcriptional target of cAMP, via MITF [74]. Accordingly, increased PDE4D activity limits cAMP accumulation. Moreover, prolonged exposure to agonists leads to homologous desensitization of MC1R [75]. In addition, as discussed below MC1R activates ERK signaling. Phosphorylation of MITF by these Ser/Thr protein kinases leads to MITF protein degradation [76, 77]. It has also been shown that αMSH switches expression of *MC1R* transcripts from the canonical MC1R-001 form encoding for the fully active receptor to the chimeric MC1R-TUBB3 transcripts Iso 1/2 [27, 29]. The functional coupling of the MC1R-TUBB3 chimeras to the cAMP pathway has been analyzed [29]. Consistent with their reduced plasma membrane expression, the chimeric proteins were shown to activate very weakly the cAMP pathway after stimulation with a saturating concentration of a synthetic melanocortin peptide agonist. Thus, diversion of a fraction of *MC1R*-derived mRNA towards inactive intergenic splice isoforms might fine-tune a potentially dangerous positive feedback loop. In summary, prolonged cAMP signaling would be attenuated by the concerted action of mechanisms acting to achieve a MC1R isoform switch, to decrease cAMP synthesis and increase its hydrolytic degradation, and to increase the rate of MITF proteolysis.

It is well established that, in addition to Gs, Gq and G11 mediate specific physiological responses to MCs acting through various MCRs, including MC4R [78]. These

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observations (recently reviewed by [3]), together with the well-established role of Gq and G11 in determining hair and skin pigmentation in mice [79] suggest that MC1R might engage more than one G protein-coupled pathway. This is further underscored by reports of MC1R-dependent but cAMP-independent effects of ASIP [80]. However, attempts to demonstrate activation of the Gq effector PKC downstream of MC1R have been unsuccessful [81]. Thus, the cAMP pathway remains the sole G protein-dependent pathway unambiguously shown to be activated by MC1R.

3.3- cAMP-dependent activation of p38 in melanocytes and melanoma cells.

The p38 mitogen-activated protein kinases (MAPKs) are activated by growth factors, GPCRs, inflammatory cytokines or a wide variety of environmental stresses notably including UVR, to regulate differentiation, proliferation, and senescence or death in specific cell types [82-85]. In B16 mouse melanoma cells, αMSH in the low nM range and Fsk were shown to activate p38, suggesting that this effect was mediated by cAMP signaling downstream of MC1R [86]. p38 activation was associated with differentiation and decreased proliferation of melanoma cells. Activation of p38 downstream of αMSH-stimulated Mc1r in B16 cells has been confirmed by others [87]. In agreement with these results, it has been reported that treatment of COLO 853 human melanoma cells with αMSH induced time- and concentration-dependent increases in the activatory phosphorylation of p38 MAPK, along with decreased phosphorylation of the retinoblastoma protein pRB, and accumulation of cells in the G1 phase [88]. Moreover, treatment of human melanocytes with either αMSH or Fsk increased the levels of active p38, in agreement with a cAMP-dependent mechanism of p38 activation in NHMs [89]. Furthermore, stimulation of melanocytes with αMSH prior to exposure to UVR increased p38 phosphorylation levels above those obtained after UVR alone in wild-type (WT) MC1R human melanocytes, indicating a synergistic effect of UVR and αMSH on p38 signaling.

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The effects of cAMP stimulation downstream of MC1R on p38 activity may differ in a cell type-specific mode. MC1R expression is not restricted to melanocytes and has been observed in other cell types including keratinocytes, fibroblasts, monocytes, dendritic cells and endothelial cells [90-92]. Of note, it has been shown that activation of MC1R by αMSH leads to cAMP- and PKA-dependent inhibition of p38 in leukocytes [93]. In keeping with this observation, Li and Taylor showed that MC1R expression is required for αMSH suppression of LPS-activated inflammatory activity in macrophages, through inhibition of NF-κB activation and p38 phosphorylation [94]. These reports highlight two important aspects, namely that MC1R can activate cAMP signaling in non-melanocytic cells, and that the outcome of the crosstalk of the cAMP and p38 MAPK pathways is dependent on the cellular context.

3.4- G protein-independent activation of extracellular signal-regulated protein kinases ERK1 and ERK2.

The mitogen-activated protein kinase (MAPK) module involving the ERK1 and ERK2 Ser/Thr kinases is a major intracellular signaling pathway that controls cell proliferation and differentiation [95, 96]. Canonical ERK signaling is triggered by cell surface tyrosine kinase receptors (RTKs) and involves the sequential activation of RAS, RAF and MEK isoforms. This leads to translocation of active ERKs to the nucleus, where they regulate by phosphorylation the activity of various transcription factors such as c-Fos, nuclear receptors, c-Myc, Elk1, ATF2, AP-1 and others (reviewed by [97, 98]). Regulation of this signaling module is crucial for melanocyte proliferation and differentiation, and activating mutations in *N-RAS* and *B-RAF* are very frequent in melanoma (~ 25% for *N-RAS* and ~ 60% for *B-RAF*) [99-103]. Within melanocytes ERK1 and ERK2 can phosphorylate MITF [76]. ERK1/2-dependent MITF phosphorylation on Ser73 enhances its transcriptional activity, but also targets MITF for ubiquitination. This process involves the ubiquitin-conjugating enzyme hUBC9 and leads to MITF degradation by the proteasome [77]. The resulting decrease in MITF

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levels downregulates the melanogenic enzymes and inhibits melanogenesis upon prolonged stimulation of the ERKs.

In B16 mouse melanoma cells, both stimulation of Mc1r with MCs or combined treatment with an AC activator and a PDE inhibitor to increase cAMP intracellular levels led to ERK activation by cAMP-dependent but PKA-independent activation of N-RAS and B-RAF [104]. However, several lines of evidence suggested that in human melanocytic cells MC1R coupling to the ERK pathway is a cAMP-independent process. On one hand, incubation with Fsk failed to induce ERK activation in NHMs and melanoma cells [105]. On the other hand, blunting cAMP production downstream of MC1R signaling using the potent AC inhibitor 2',5'-dideoxyadenosine did not impair ERK activation in HBL human melanoma cells stimulated with the synthetic αMSH analog NDP-MSH [81]. This study also showed induction of a rapid and transient increase in tyrosine phosphorylation in human melanoma cells expressing WT MC1R challenged with NDP-MSH, but not with Fsk, suggesting the cAMP-independent transactivation of an RTK. Further analysis identified this RTK as cKIT, a receptor crucial for melanogenesis, proliferation, migration, and survival of melanocytes. Pharmacological or siRNA-mediated inhibition of cKIT signaling abolished ERK activation downstream of MC1R, and MC agonists failed to activate the ERKs in melanoma cells lacking detectable expression of cKIT. In addition, functional coupling of MC1R to the ERK module in heterologous cellular systems lacking cKIT was strongly dependent on co-expression of cKIT. Overall, these observations established a causal link between cKIT transactivation downstream of MC1R and ERK activation in human melanocytic cells (Fig 2), and delineated a new cAMP-independent noncanonical pathway of MC signaling [81, 106].

Other members of the MCR family have been shown to activate the ERK signaling cascade (recently reviewed by [3]). A recent study by Simamura and colleagues showed that all 5 MCRs are expressed in erythroblasts at different levels during cellular

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differentiation. At least 3 of them, namely MC1R, MC2R, and MC5R, were found to contribute to erythroblast differentiation in a non-redundant fashion through activation of ERK signaling, as well as STAT5 and AKT [107]. ERK activation downstream of the MCRs was required for erythroid enucleation, the critical step for terminal differentiation in erythropoiesis. These observations demonstrated that several MCR subtypes can be expressed in individual cell types in a developmentally regulated manner to co-ordinately regulate MC-modulated complex differentiation programs.

3.5- Activation of PI3K/AKT signaling downstream of MC1R.

The phosphoinositide-3-kinase (PI3K)-AKT (also known as protein kinase B, PKB) pathway regulates a variety of important processes leading to cell cycle progression and proliferation, cell migration and survival [108, 109]. Activated RTKs bind to and activate PI3K, which catalyses the conversion of phosphatidylinositol-(3,4)-bisphosphate (PIP_2) lipids to phosphatidylinositol-(3,4,5)-trisphosphates (PIP_3). These lipid messengers activate 3-phosphoinositide-dependent protein kinase 1 (PDK1) and AKT to trigger cell survival and proliferation. The PI3K-AKT pathway is negatively regulated by the phosphatase and tensin homologue deleted on chromosome 10 (PTEN), a PIP_3 phosphatase which terminates PI3K signaling by hydrolysis of the lipid messenger. The balance of PI3K and PTEN activity, which determines the strength and duration of AKT activation, is frequently lost in many types of cancer, including melanoma [103].

Cheng and co-workers demonstrated that αMSH binding to MC1R expressed in retinal pigment epithelium (RPE) reduced H_2O_2-induced RPE cell damage and apoptosis through activation of AKT and mTOR pathways. This might account for αMSH-mediated survival of RPE cells under conditions promoting oxidative stress [110]. Moreover, AKT activation downstream of αMSH has been demonstrated in UV-irradiated melanocytes and related with the induction of anti-apoptotic pathways [111]. Treatment of NHMs with αMSH prior to UV irradiation resulted in AKT activation as

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measured by phosphorylation of Bad, a known AKT substrate (Fig 2), and blocked the reduction in anti-apoptotic Bcl2 expression caused by UVR. Interestingly, these pro-survival effects of αMSH were further induced in combination with endothelin-1, another keratinocyte-derived paracrine regulator of melanocytes [111].

In addition, AKT signaling might be involved in resistance of melanoma cells to vemurafenib, an inhibitor of mutant B-RAF employed in the targeted chemotherapy of B-RAF-mutated melanomas [112].

4- Intracellular MC1R partners and noncanonical signaling

A number of intracellular proteins that appear to interact physically with MC1R have been identified (Fig 3), and the outcome of these interactions has been investigated. These include the cytosolic β-arrestins (ARRBs), PTEN and the E3 ubiquitin ligase Mahogunin Ring Finger-1 (MGRN1). In addition, two recent high throughput proteomic studies identified a number of potential MC1R interactors [113, 114]. These presumed partners still wait for confirmation and analysis of the functional consequences of their association with MC1R.

4.1- ARRBs.

Functional coupling of GPCRs to their cognate G proteins is almost invariably modulated by the ARRBs which bind to the cytosolic side of agonist-GPCR complexes and terminate a first wave of G protein-dependent signaling by displacing the Gα subunit. GPCR-bound ARRBs orchestrate the formation of endocytic vesicles to trigger receptor internalization (recently reviewed by [115]) and may mediate the assembly of functional signaling complexes responsible for the activation of several MAPKs in a second wave of G protein-independent signaling (reviewed in [116, 117].

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The mechanisms of MC1R desensitization have been investigated. Co-transfection of *MC1R* and *GRK2* or *GRK6* genes in heterologous cells led to impaired MC-dependent activation of the cAMP pathway. Agonist-stimulated cAMP production decreased in melanoma cells enriched with GRK6 and increased after stable expression of a dominant negative GRK2 mutant. However, only GRK6 inhibited MC1R agonist-independent constitutive signaling [75]. Expression of GRK6, but not GRK2, increased the rate of internalization of agonist-receptor complexes either in heterologous cellular systems or in melanoma cells, whereas a GRK6 dominant-negative mutant inhibited receptor sequestration away from the cell surface [118]. Accordingly, although it appeared that MC1R homologous desensitization can be mediated by GRK2 and GRK6, the effect of these GRKs on MC1R signaling are not equivalent and GRK6 seems mainly responsible for MC1R desensitization (Figure 3). Attempts were made to determine the Ser/Thr residues in MC1R phosphorylated by GRK6. Mutagenesis studies pointed to Thr308 and Ser316, two residues located in the short MC1R cytosolic C-terminal extension since a T308D/S316D mutant mimicking their phosphorylated state was constitutively desensitized and associated with endocytic vesicles, whereas a T308A/S316A mutant was resistant to desensitization and internalization [118]. Of note, T308 and S316 are conserved in mouse and human MC1R and T308 is conserved in all human MCRs except MC5R.

FIGURE 3

Upon phosphorylation by GRKs, GPCRs most often recruit members of the small family of cytosolic ARRBs. Most cell types express two ubiquitous non-visual ARRBs, β-arrestin1 (ARRB1) and β-arrestin2 (ARRB2). ARRB1 and ARRB2 are highly homologous proteins that promiscuously bind to most GPCRs and apparently perform

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partially redundant functions, since the phenotype of mice lacking either one of the ARRBs is mild, whereas simultaneous knockout of both ARRB genes is lethal. However, recent evidence suggests that uncoupling and/or internalization of several GPCRs is specifically performed by either ARRB1 or ARRB2, with little or no effect of the other [119, 120]. Abrisqueta and coworkers recently analyzed the interaction of nonvisual ARRBs with MC1R [41]. They found that both ARRB1 and ARRB2 were able to interact with MC1R, as shown by co-immunoprecipitation and co-elution in size exclusion chromatography columns. This interaction occurred not only in heterologous systems overexpressing MC1R and ARRBs, but also in human melanoma cells at physiological levels of expression. Interestingly, MC1R-ARRB interactions could be detected in the absence of agonist stimulation, and for a GRK-resistant MC1R mutant (T308A/S316A) unable to undergo phosphorylation of the C-terminal residues implicated in the regulation of receptor internalization. Following stimulation with NDP-MSH, the association of MC1R and ARRB2 was long-lived [41]. Thus, MC1R can be classified as a Class B GPCR [121]. Of note, even though both ARRB1 and ARRB2 were able to bind MC1R, the functional output of such interaction was isoform-specific. ARRB2 inhibited agonist-dependent cAMP production and stimulated receptor internalization, whereas ARRB1 lacked significant effects (Fig 3). On the other hand, neither ARRB1 nor ARRB2 had any effect on ERK activation downstream of MC1R, consistent with a cAMP-independent mode of coupling. Given that the two ARRB isoforms share a common fold and extensive sequence identity, and that the intracellular fragments of the MC1R are short, suggesting substantial overlap of binding surfaces, a potential competition of the ARRBs for MC1R was investigated. It was found that ARRBs bound MC1R in a competitive and mutually exclusive fashion and that, by competing with the inhibitory ARRB2, high levels of ARRB1 might paradoxically increase signaling to the cAMP pathway. These data suggested a new mechanism of MC1R functional regulation based on the relative expression of inhibitory ARRB2 and functionally neutral ARRB1 [41]. Moreover, they showed that the activation of the ERK

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and cAMP signaling pathways downstream of the MC1R can be independently regulated.

4.2- PTEN

As discussed above, there is some evidence that MC1R can trigger the AKT pathway upon activation by αMSH. RTKs including cKIT are functionally connected with AKT [122] and MC1R has been shown to transactivate cKIT signaling [81]. Thus, AKT activation by αMSH might be at least partially carried out via an MC1R/cKIT/PI3K pathway. In keeping with this observation, the PI3K inhibitor LY294002 was shown to reduce the anti-apoptotic effect of αMSH on human melanocytes [111]. In addition to this pathway, a UVR-dependent mechanism of regulation of AKT activity involving the direct interaction of MC1R with PTEN has been reported [123]. It was shown that UVR induced inactivation of PTEN and activation of AKT in human epidermal cells with a WT *MC1R* genotype, and that depletion of MC1R acted synergistically to further promote AKT activation. This effect of MC1R on AKT activity appeared to rely on a UVR-triggered physical association of MC1R and PTEN, detected by co-immunoprecipitation and size exclusion chromatography. It was postulated that following UV irradiation, the αMSH-MC1R complex recruits PTEN in an interaction, thus preventing its ubiquitination by the E3 ubiquitin ligase WWP2 and its subsequent proteasomal degradation. The resulting stabilization of PTEN downregulated AKT signaling. Accordingly, two complementary MC1R-dependent mechanisms may operate in melanocytes to regulate AKT activity. On one hand, transactivation of cKIT would lead to AKT activation via PI3K. On the other hand, PTEN stabilization upon formation of a MC1R-PTEN complex would limit PIP_3-dependent activation of PI3K to fine-tune the extent of AKT signaling.

4.3- MGRN1

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The RING Finger domain-containing protein MGRN1 (Mahogunin Ring Finger 1) was identified by positional cloning of mahoganoid (md) [124], a coat color mutation with pleiotropic and complex effects including alterations in expression and activity of mitochondrial proteins in the brain [125] followed by spongiform neurodegeneration with features of prion diseases [126]. Mutant mice also exhibit abnormal patterning of the left-right axis with congenital heart defects [127]. Mice homozygous for the *md* loss-of-function mutation in the *MGRN1* gene show a coat color similar to mice with gain-of-function mutations at the *Extension* locus coding for Mc1r. The human and mouse *MGRN1* genes consist of 17 and 18 exons, respectively. Exon 10 in human and mouse MGRN1 codes for a RING Finger domain, a hallmark of E3 ubiquitin ligases [128] which catalyze the conjugation of ubiquitin (Ub) units to target proteins. A number of proteins that may be ubiquitinated *in vivo* by MGRN1 have been reported, including TSG101 [129] and the α isoform of tubulin [130]. Given that many GPCRs undergo agonist-dependent ubiquitination [131], the possible functional interactions of MC1R and MGRN1 were analyzed.

The *MGRN1* genes yield 4 isoforms that share exon 10 encoding for the RING Finger domain, by alternative splicing of exons 12 and 17 [132, 133]. All MGRN1 isoforms inhibited with similar potency signaling from MC1R and MC4R to cAMP when co-transfected with the MCRs in heterologous cells or when they were transfected in human melanoma cells expressing endogenous MC1R. Inhibition of MC1R functional coupling to the cAMP pathway was independent on MC1R ubiquitination since it was also observed for a ubiquitination-incompetent MC1R mutant where all intracellular Lys residues were mutated to Arg. Notwithstanding, a later report provided evidence that MGRN1 can ubiquitinate MC2R [134]. Further analysis located the site of action of MGRN1 on the cAMP pathway upstream of AC and showed that MGRN1 co-immunoprecipitated efficiently with MC1R and MC4R, suggesting a physical interaction of the proteins. This interaction appeared to be competitive with respect to Gs, since

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forced expression of its Gsα subunit alleviated inhibition of MC1R signaling by MGRN1 and decreased co-immunoprecipitation with the MCRs. These observations suggested a new mechanism of regulation of MC1R and MC4R signaling involving competition between MGRN1 and Gs for binding to the receptors (Fig 3), that may well be specific for this subfamily of GPCRs given that MGRN1 lacked any effect on the β2-adrenergic receptor [133].

Two MGRN1 transcripts contain exon 12, which encodes for a short peptide sequence bearing a canonical bipartite nuclear localization signal (NLS) consisting of 2 blocks of basic amino acids separated by a short intervening sequence [135]. However, these isoforms were found in a cytosolic subcellular localization when expressed in cells lacking MCRs, thus showing that the NLS was cryptic in the absence of MC signaling. Interestingly, exon 12-containing isoforms, but not the isoforms lacking the NLS were shuttled to the nucleus upon co-transfection with MC1R or MC4R, or when transfected in melanocytic cells expressing endogenous MC1R (Fig 3). Thus, the molecular interaction between MGRN1 and MC1R or MC4R, and likely other MCRs, triggered a modification of MGRN1 resulting in activation of its NLS. Ubiquitination is a common mechanism of regulation of transcription factors and other nuclear proteins. Accordingly, it has been postulated that MGRN1 might provide a novel and still unexplored pathway for MCR signaling from the cell surface to the nucleus [133].

4.4- Other potential partners of MC1R.

Two recent studies identified several potential MC1R interactors [113, 114] (compiled at the BioGRID repository, https://thebiogrid.org/). At least two of them may prove to be relevant in the light of their known properties. These are the Kinase Suppressor of Ras 1 (KSR1) and the product of the neurofibromatosis type 2 gene, neurofibromin 2 (NF2, also known as merlin and schwannomin). KSR1 is a scaffolding protein of the ERK signaling pathway, which binds to MEK in the cytoplasm of resting cells (reviewed by [136]). Following activation of RAS downstream of RTKs, the KSR-MEK complex

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moves to the plasma membrane, where it interacts with RAF and ERK to bring in close proximity the 3 kinases of the RAF/MEK/ERK pathway, thus allowing for the cascade of activatory phosphorylations to proceed. A potential interaction between human KSR1 and MC1R [114] might therefore be somehow related with the cAMP-independent activation of the ERK pathway downstream of MC1R in human melanocytes, although such connection remains speculative.

On the other hand, interaction of MC1R and NF2 has also been reported. NF2 is a key component of the Hippo signaling pathway, whose description is beyond the scope of this review and can be found elsewhere [137]. NF2 acts as a tumor suppressor frequently mutated in neurofibromatosis type 2, a disease associated with formation of nervous system tumors, mostly schwannomas, meningiomas, and ependymomas (reviewed by [138]). Involvement of Hippo signaling in melanoma is likely [139], and NF2 inactivatory mutations or reduced expression have been found in at least 5% of melanomas [140]. Moreover, aberrant Hippo signaling may promote invasion and increase melanoma invasive properties [141] and, consistent with this, inactivation of NF2 has been shown to enhance migration, invasion and cell proliferation in melanomas [142]. Importantly, inactivation of NF2 might not only contribute to a more aggressive phenotype in melanoma, but might also confer resistance to BRAF inhibitors in BRAF-mutated melanomas [143]. Accordingly, a MC1R-NF2 interaction might be an important factor modulating the properties of melanocytes and melanoma cells, particularly if it contributes to regulate the levels of the NF2 protein, as shown for PTEN [123].

In any case, these interactions remain to be confirmed and analyzed for their effects on melanocyte and melanoma cell biology. Of note, the size of the cytosolic fragments of MC1R is small, thus suggesting that at least some of its potential intracellular partners should bind the receptor in a competitive and mutually exclusive manner, as already shown for ARRB1 and ARRB2. Accordingly, the dynamic aspects of the MC1R

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interactome and their regulation by extracellular cues, including UVR, might prove to be important.

5- The RHC variants and their role in melanoma susceptibility

Soon after the identification of coat color-associated mouse mutations in *Mc1r* [22], Valverde and coworkers [144] first reported the association of human *MC1R* variants frequent in European population with a phenotype characterized by red hair, light skin, high number of freckles and sun sensitivity (the **R**ed **H**air **C**olor, RHC, phenotype). Since then, a number of studies have firmly established that i) *MC1R* gene polymorphisms are a major determinant of the normal variation of human pigmentation [145], ii) polymorphic variants leading to nonsynonymous substitutions are frequent not only in light skinned population of European descent but also in southern European population with significantly darker cutaneous pigmentation [146], and iii) whereas nonsynonymous variants are infrequent in dark-skinned African populations, synonymous variants such as ACA → ACG T314T are found in these populations at a relatively high frequency compared with Caucasians [10, 147, 148]; iv) extensive genetic studies strongly suggest occurrence of purifying selection at the *MC1R* gene in Africans, whereas some positive selection might contribute to the higher frequency of certain RHC alleles in Caucasians [149]. This is in accordance with the hypothesis that migration from Africa to northern latitudes involved the transition towards progressively less pigmented phenotypes favoring the formation of active vitamin D under conditions of limited exposure to UVR [150].

According to their penetrance for the RHC phenotype, *MC1R* variants have been classified into strong “R” alleles, weaker “r” forms, and pseudoalleles with no significant effect on the phenotype [151]. Even though both R and r variants are hypomorphic in activation of the cAMP pathway, they often display a certain residual activity [42, 106,

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152-157]. This residual coupling is below 50% for penetrant RHC variants such as R151C or R161W overexpressed in heterologous cellular systems, and even smaller for other R variants such as D294H or D84E. Moreover, MC-dependent activation of the cAMP pathway is barely detectable in NHMs homozygotes or compound heterozygotes for RHC variants [89, 158]. On the other hand, genetic studies have shown the occurrence of gene dosage effects, whereby the effects of carrying variant MC1R alleles are additive [159, 160]. In addition, MC1R forms dimeric species during its post-translational processing, and this event appears critical for efficient anterograde trafficking to the cell surface. At least the major RHC variants R151C, R160W and D294H efficiently heterodimerize with the WT form to modify its trafficking and functional properties [156], thus allowing for a pseudodominant-negative behavior [159]. Therefore, dominant-negative as well as dosage effects, together with the retention of different levels of residual activity contribute to extensive variation of pigmentation in humans.

On the other hand, many RHC alleles were shown to retain efficient signaling to the ERKs in spite of being clearly hypomorphic in activation of the cAMP cascade [81, 106]. Conversely, p38 activation by αMSH was severely compromised in RHC variant melanocytes [89]. This is consistent with a crucial role of cAMP in p38 activation by αMSH as opposed to the cAMP-independent mode of activation of the ERKs. Accordingly, most RHC variants are best described as imbalanced signaling forms rather than as loss-of-function mutants. The biased effect of the RHC mutations on cAMP versus ERK signaling may suggest that, *in vivo*, ERK activation has a minor effect on MC1R-dependent pigmentation in human skin, as compared with activation of the cAMP pathway. In keeping with this possibility, for most natural variants found at a frequency high enough to allow for meaningful association analyses, the degree of functional impairment in activating cAMP synthesis grossly correlates with penetrance [30, 42], and treatment with pharmacological cAMP-elevating agents such as Fsk

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increases pigmentation *in vivo* [161]. Of note, the MC1R-TUBB3 chimeras behave as R variants in terms of signalling, as their functional coupling to the cAMP pathway is dramatically compromised, but activation of the ERKs remains efficient [29].

According to the cause of functional impairment, RHC variants can be classified as those with reduced cell surface expression and those with normal plasma membrane density but decreased functional coupling. The study of the first group of variants led to a model for the regulation of anterograde trafficking of the MC1R that may apply to other MCR, and that was previously reviewed [30]. The second major group of RHC alleles comprises those that reach the cell surface at an apparently normal rate but are unable to transduce the signal provided by extracellular MCs. Receptor variants correctly trafficked but unable to bind MC agonists such as C289R [162] and variants that bind agonists with reasonable affinity but do not undergo the transition to an active conformation competent for Gs protein activation such as D294H [163] have been described. These variants can be expressed on the cell surface at densities even higher than WT, most likely as a result of inefficient recognition by the GRKs and the internalization machinery.

The RHC variants not only display altered functional coupling to the cAMP pathway, but also show aberrant interactions with other MC1R partners. It has been shown that the rate of internalization of the R alleles R151C and R160W expressed in heterologous systems was higher than WT, suggesting a more efficient interaction with the GRK/ARRB machinery responsible for MC1R endocytosis [118]. Moreover, the major RHC variants R151C, R160W and D294H bound with PTEN less effectively than WT. Accordingly, they afforded a smaller protection against ubiquitin-dependent degradation of the phosphatase. This may favor AKT signaling in variant melanocytes, a phenomenon that may contribute to their association with an increased risk of malignant transformation [123].

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Genetic epidemiology studies firmly established the association of RHC variants with increased risk of melanoma. Initial reports based on studies performed with a limited number of cases and controls and sequencing of the complete *MC1R* open reading frame [14, 15] were extended and confirmed by powerful genome-wide association studies and meta-analyses [12, 13, 16-18]. A similar association exists with nonmelanoma skin cancer [14, 16, 23, 164-167]. However, no association with somatic mutations in the major melanoma driver genes BRAF and NRAS has been documented [168, 169] in spite of initial reports. Mechanistically, the association of RHC alleles and skin cancer risk can be partially explained by a pigmentation-dependent effect whereby a higher content of photoprotective eumelanin pigments in carriers of two WT alleles would act as a shield protecting skin cells against mutagenic UVR. Moreover, as opposed to eumelanins, feomelanins preferentially synthesized in the absence of robust MC1R signaling to the cAMP pathway are not only poorly photoprotective, but they might even behave as photosensitizers [170-172].

However, carrying *MC1R* allelic variants also increases melanoma risk in dark-skinned population of European origin and an association with skin cancer risk persists after taking into account pigmentation effects, thus pointing to pigment-independent actions of MC1R [16, 33, 146, 164, 166, 173]. These non-pigmentary effects of MC1R are most likely related with its ability to induce antioxidant defenses and DNA damage repair (recently reviewed by [9]). In this respect, it has been shown that skin cell co-cultures established from carriers of *MC1R* variants showed de-regulated expression of a large number of genes, notably including genes related to cellular management of oxidative stress and DNA damage [174]. Moreover, several studies demonstrated that αMSH can prevent UVR-induced DNA damage and apoptosis in melanocytes. Interestingly, these protective responses were impaired by MC1R antagonists ASIP and HBD3 [50, 175].

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The MC1R-dependent DNA protective responses triggered by αMSH have been investigated. At least part of these responses appear to depend on a potentiation of activation of p53, a major sensor of DNA damage. Indeed, Kadekaro et al showed that pretreatment with nanomolar concentrations of αMSH before exposure to UVR resulted in accumulation of p53 above the levels achieved by irradiation with UVR in the absence of the hormone [176]. αMSH also increased activatory p53 phosphorylation at Ser15 with nuclear translocation of the protein, resulting in enhanced transcriptional activity as shown by higher levels of the p53 targets p21 and GADD45. These effects were mimicked by Fsk, suggesting their dependency on efficient cAMP signaling, and were not detected in compound MC1R heterozygote melanocytes (*MC1R* genotype R160W/D294H). Activation of p53 resulted in reduction of the production of H_2O_2 following exposure to UVR, which could be accounted for by increased expression of antioxidant enzymes, mostly catalase. Interestingly, a reduction of phosphorylated histone H2AX (γ-H2AX) indicative of decreased number of DNA repair foci was also observed, along with an induction of enzymes involved in base excision repair (BER) such as 8-oxoguanine DNA glycosylase and apurinic apyrimidinic endonuclease 1 [176]. Consistent with decreased production of H_2O_2, hormone-treated melanocytes were also protected against generation of DNA strand breaks, as estimated by comet assays. Further studies from the same laboratory showed that αMSH also enhanced the repair of cyclobutane pyrimidine dimers and rescued the decrease in the damage sensing protein XPC in UV-irradiated melanocyte cultures [177]. The hormone also potentiated a sustained activatory phosphorylation of the upstream kinases ataxia telangiectasia and Rad3-related protein (ATR) and ataxia telangiectasia mutated (ATM) and their downstream kinases Chk1 and Chk2. Again, the effects were apparently dependent on a WT *MC1R* genotype, as they were less evident in MC1R-variant melanocytes. However, in this study the variants expressed in the melanocyte cultures were not specified, and given the functional non-equivalence of variant MC1R alleles, a residual DNA repair activity of some of them cannot be ruled out. Phosphorylation of

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ATR downstream of MC1R has also been shown to be involved in nucleotide excision repair (NER) by Jarrett et al, who further demonstrated the involvement of PKA and the relevance of this activatory phosphorylation for recruitment of the NER factor XPA to UVR-induced damage sites [175]. More recently, this group found another unexpected connection of components of the cAMP signaling pathway with MC1R-dependent DNA damage responses. It was shown that ATR interacts with and promotes phosphorylation of the A-kinase-anchoring protein 12 (AKAP12) [178]. This interaction increased upon UV irradiation and resulted in translocation of the AKAP12-ATR complex from the cytosol to the nucleus, where it contributed to the recruitment of XPA to damaged sites in DNA to promote NER. Interestingly, activatory phosphorylation of ATR by PKA was potentiated by AKAP12, suggesting a scaffolding role for the protein to optimize ATR activation. Overall, these observations suggest a pathway whereby elevation of cAMP downstream of MC1R results in ATR phosphorylation by PKA in an AKAP12-dependent manner, AKAP12 phosphorylation by activated ATR and stable AKAP12-ATR interaction. This complex moves to the nucleus to interact with UVR-induced photoproducts and promote recruitment of XPA and other NER factors [178]. The role of cAMP in triggering this pathway would account for the requirement of a WT *MC1R* genotype for activation of NER by αMSH, given the impaired functional coupling of most variant MC1R alleles to the cAMP-synthesizing machinery.

It has also been shown that MC1R signaling upon stimulation of B16 mouse melanoma cells and NHMs with αMSH rapidly, and transiently, induced the transcription of the NR4A subfamily of orphan nuclear receptors [179]. Further analysis showed that NR4A was recruited to novel nuclear foci following UV irradiation, in a process dependent on p38 and PARP signaling, and participated in the NER pathway [180, 181]. αMSH also prevents UV-induced DNA damage in keratinocytes by enhancing UV-induced DNA repair via a XPA-dependent and melanogenesis-independent mechanism [182]. Accordingly, signaling downstream of the MC1R upregulates the expression and/or

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activity of a number of components of DNA repair pathways suggesting the possibility of synergistic interactions to increase the robustness of induction of DNA repair by the melanocortin peptides, not only in melanocytes but also in keratinocytes.

The data summarized above provide overwhelming evidence showing that a WT *MC1R* genotype confers protection against skin cancer. Indeed, *MC1R* is considered a major melanoma susceptibility gene, that contributes significantly to the melanoma burden of Caucasian populations in spite of its low-moderate penetrance, by virtue of the high frequency of hypomorphic variants. Rather counterintuitively, there is also evidence of association of a WT *MC1R* genotype with poor prognosis of melanoma patients, and population-based case-control studies point to a small survival benefit for patients carrying *MC1R* variants [183, 184]. These association studies have been very recently confirmed in an animal model using B16 mouse melanoma cells engineered to synthesize and secrete the natural MC1R antagonist ASIP, injected in syngeneic C57BL/6 mice. In this model, it has been shown that ASIP-expressing B16 cells formed tumors that grew less rapidly and were associated with a 20% longer survival, compared with control cells. It was also shown that upon co-injection of ASIP-secreting and control melanoma cells, ASIP-producing tumors were able to inhibit MC1R in adjacent tumors derived from control cells with a significant survival benefit [185]. The molecular bases of the paradoxical association of variant *MC1R* alleles with better melanoma-specific survival have not been systematically investigated to date. However, the available data on the effects of MC1R on melanocyte biology underscore several possibilities. On one hand, it has been reported that persistent inhibition of MC1R resulted in a slower rate of growth in an animal model [185]. On the other hand, provocative recent findings indicated that oxidative stress inhibited formation of distant metastases by human melanoma cells in immunocompromised mice and that circulating melanoma cells underwent oxidative stress that limited their viability in the bloodstream [186]. Accordingly, one interesting possibility is that the induction of

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antioxidant defenses downstream of MC1R signaling may reduce oxidative stress in circulating melanoma cells to increase the likelihood of colonization of distant organs. In this scenario, the two-edged sword behaviour of a *MC1R* genotype, with a protective action against melanomagenesis and an adverse effect on survival of melanoma patients, would be dependent on the same MC1R-triggered pathways and targets.

6- Perspectives

During the last two decades, our view of MC1R as a structurally simple GPCR preferentially associated with the regulation of mammalian pigmentation has been dramatically transformed. On one hand, we now realize that the *MC1R* gene is complex owing to the occurrence of multiple exons as well as intra- and intergenic alternative splicing events. Future studies will clarify the function of the various alternative splicing isoforms, in terms of the regulation of their relative abundance by internal (e.g. MC peptides) or external (e.g. UVR) stimuli. The signaling properties of the encoded proteins will also be fully characterized and compared in order to assess their physiological relevance. Moreover, most of the myriad of polymorphisms described to date have not yet been analyzed for function. In particular, the functional significance of SNPs in the 3' or 5' noncoding regions, if any, as well as the possible effects of synonymous changes in the open reading frame on mRNA levels, stability or processing remain to be determined.

On the other hand, recent research on the multiple roles played by MC1R in melanocyte biology has considerably enlarged the scope of the field. There is no doubt that MC1R is much more than the receptor transducing the melanogenic signal provided by keratinocyte-derived MCs, but probably because the pigmentation-related effects of MC1R signaling are the most conspicuous, they also remain the best known and understood at the molecular level. However, it can be anticipated that the next

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years will see a shift of interest towards the molecular mechanisms of non-pigmentary actions of the αMSH/MC1R system, not only in melanocytes but also in the many different cell types where MC1R is expressed. For instance, the metabolic effects of MC signaling in melanocytes, underscored by the recently uncovered connection between MC1R and PGC1α, will be further analyzed and studies will be likely extended to cell types expressing other MCRs. The precise nature of the DNA repair mechanisms controlled by MC1R will be established, and the contribution of the various signaling pathways activated downstream of the receptor to these nonpigmentary responses will be assessed.

Several laboratories have demonstrated the physical association of MC1R intracellular fragments with a variety of protein partners such as ARRBs, PTEN and MGRN1, and more recently KSR1 and NF2. The functional consequences of these interactions are still poorly understood or unknown, but they are potentially far-reaching in the light of the known roles of the MC1R partners. Moreover, given that the MC1R is a small GPCR with short intracellular loops and cytosolic extension, the question arises as to the possibility of simultaneous association with more than one partner, as opposed to competitive binding. This likely competition is highlighted by the mutually exclusive interaction with ARRB1 and ARRB2. In this respect, the scaffold properties of the ARRBs are slightly different, thus leading to formation of different GPCR-based complexes known as “signalosomes” [187]. Therefore, the competitive binding of ARRBs raises the possibility that association of MC1R with a specific ARRB can direct the formation of a subset of the available ARRB scaffolds in a context-dependent manner. Moreover, the interaction of MC1R and PTEN is apparently promoted by UVR. Thus, a dynamic signalosome whose specific composition and properties might be modulated in response to environmental cues can be envisaged, and this attractive possibility deserves being tested.

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Overall, these studies should lead to a better understanding of the pathophysiological implications of WT and variant MC1R signaling. In particular, the dual role of a WT genotype, which protects against melanoma but is associated with a poorer prognosis, needs to be clarified in order to ensure safe and effective clinical applications of MC1R ligands in melanoma protection and in a variety of photodermatoses.

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Figure legends

Figure 1. Structure of the MC1R gene and MC1R isoforms. (A) Exon organization of MC1R splice variants (MC1R-001, MC1R-002 and MC1R-350) and MC1R-TUBB3 intergenic transcripts, Iso1 and Iso2. Exons of all MC1R derived transcripts are depicted in colored boxes and the number of nucleotides in the corresponding open reading frames (ORF) is shown below. (B) Structural domains of *MC1R* gene-derived proteins. Note the conservation of the 7 transmembrane domains (TM) and most of the canonic MC1R C-terminal cytosolic extension in all the proteins, including the intergenic MC1R-TUBB3 chimeras. The chimeric Iso1 form also conserves most of the TUBB3 sequence, with all its relevant domains. (C) Amino acid sequence, coding sequence polymorphisms and possible arrangement of transmembrane regions in MC1R isoforms. The sequences correspond to transcript MC1R-001 (ID number ENST00000555147), MC1R-002 (ID ENST00000555427) and MC1R-350. Polymorphic positions for which no reliable association studies are available are indicated in blue. Positions of R and r variants are shown in red and orange, respectively. Residues shown in gray correspond to indels and black circles with white lettering followed by broken arrows to premature stop codons. Positions where both an indel and a point mutation have been found are shown as blue circles hatched in white. Ser/Thr residues presumably phosphorylated are highlighted with a red border. The two Asn residues glycosylated in WT MC1R-001 are indicated with a green border. The sequence of the three proteins is identical up to Cys315. For MC1R-350 and MC1R-002, only the sequence of their specific cytosolic extensions is shown (pink and green, respectively).

Figure 2. Signalling pathways triggered by MC1R agonists. Upon agonist binding, MC1R activates AC via the Gsα protein, thus triggering cAMP synthesis and PKA activation. Active PKA catalytic subunits phosphorylate and activate multiple targets, such as the transcription factors CREB and PGC1α, which increase the rate of transcription of the *MITF* gene. In turn, the MITF protein stimulates PGC1α expression. cAMP signaling also leads to activation of p38 kinase. Agonist-activated MC1R also transactivates cKIT to trigger the NRAS-BRAF-MEK-ERK cascade. Active ERKs can phosphorylate MITF to increase its transcriptional activity and its proteasome-dependent degradation. In addition, AKT activation downstream of αMSH has been demonstrated, probably via cKIT.

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Figure 3. Intracellular partners of MC1R. The scheme depicts proteins that establish stable interactions with MC1R as shown by co-immunoprecipitation experiments and/or size exclusion chromatography. The proposed outcome of such interaction is also indicated. Agonist-bound MC1R is desensitized by GRK2 or GRK6-dependent phosphorylation and ARRB2 recruitment, followed by sequestration in endocytic vesicles and recycling to the cell surface. MGRN1 binding inhibits MC1R signaling via the cAMP cascade by competition with Gsα. On the other hand, it has been postulated that upon UV irradiation the αMSH-MC1R complex recruits PTEN. This would protect PTEN from ubiquitination and degradation, thereby downregulating AKT signaling.

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References

[1] V. Chhajlani, J.E. Wikberg, Molecular cloning and expression of the human melanocyte stimulating hormone receptor cDNA, FEBS letters, 309 (1992) 417-420.

[2] K.G. Mountjoy, L.S. Robbins, M.T. Mortrud, R.D. Cone, The cloning of a family of genes that encode the melanocortin receptors, Science, 257 (1992) 1248-1251.

[3] A.R. Rodrigues, H. Almeida, A.M. Gouveia, Intracellular signaling mechanisms of the melanocortin receptors: current state of the art, Cellular and molecular life sciences : CMLS, 72 (2015) 1331-1345.

[4] L.S. Robbins, J.H. Nadeau, K.R. Johnson, M.A. Kelly, L. Roselli-Rehfuss, E. Baack, K.G. Mountjoy, R.D. Cone, Pigmentation phenotypes of variant extension locus alleles result from point mutations that alter MSH receptor function, Cell, 72 (1993) 827-834.

[5] M. d'Ischia, K. Wakamatsu, F. Cicoira, E. Di Mauro, J.C. Garcia-Borron, S. Commo, I. Galvan, G. Ghanem, K. Kenzo, P. Meredith, A. Pezzella, C. Santato, T. Sarna, J.D. Simon, L. Zecca, F.A. Zucca, A. Napolitano, S. Ito, Melanins and melanogenesis: from pigment cells to human health and technological applications, Pigment cell & melanoma research, 28 (2015) 520-544.

[6] J.L. Rees, The genetics of sun sensitivity in humans, American journal of human genetics, 75 (2004) 739-751.

[7] A. Slominski, D.J. Tobin, S. Shibahara, J. Wortsman, Melanin pigmentation in mammalian skin and its hormonal regulation, Physiological reviews, 84 (2004) 1155-1228.

[8] R. Cui, H.R. Widlund, E. Feige, J.Y. Lin, D.L. Wilensky, V.E. Igras, J. D'Orazio, C.Y. Fung, C.F. Schanbacher, S.R. Granter, D.E. Fisher, Central role of p53 in the suntan response and pathologic hyperpigmentation, Cell, 128 (2007) 853-864.

ACCEPTED MANUSCRIPT

[9] Z.A. Abdel-Malek, V.B. Swope, R.J. Starner, L. Koikov, P. Cassidy, S. Leachman, Melanocortins and the melanocortin 1 receptor, moving translationally towards melanoma prevention, Archives of biochemistry and biophysics, 563 (2014) 4-12.

[10] B.K. Rana, D. Hewett-Emmett, L. Jin, B.H. Chang, N. Sambuughin, M. Lin, S. Watkins, M. Bamshad, L.B. Jorde, M. Ramsay, T. Jenkins, W.H. Li, High polymorphism at the human melanocortin 1 receptor locus, Genetics, 151 (1999) 1547-1557.

[11] V. Maresca, E. Flori, M. Picardo, Skin phototype: a new perspective, Pigment cell & melanoma research, 28 (2015) 378-389.

[12] C.I. Amos, L.E. Wang, J.E. Lee, J.E. Gershenwald, W.V. Chen, S. Fang, R. Kosoy, M. Zhang, A.A. Qureshi, S. Vattathil, C.W. Schacherer, J.M. Gardner, Y. Wang, D.T. Bishop, J.H. Barrett, M.E.L.I. Geno, S. MacGregor, N.K. Hayward, N.G. Martin, D.L. Duffy, Q.M. Investigators, G.J. Mann, A. Cust, J. Hopper, A. Investigators, K.M. Brown, E.A. Grimm, Y. Xu, Y. Han, K. Jing, C. McHugh, C.C. Laurie, K.F. Doheny, E.W. Pugh, M.F. Seldin, J. Han, Q. Wei, Genome-wide association study identifies novel loci predisposing to cutaneous melanoma, Hum Mol Genet, 20 (2011) 5012-5023.

[13] F. Chatzinasiou, C.M. Lill, K. Kypreou, I. Stefanaki, V. Nicolaou, G. Spyrou, E. Evangelou, J.T. Roehr, E. Kodela, A. Katsambas, H. Tsao, J.P. Ioannidis, L. Bertram, A.J. Stratigos, Comprehensive field synopsis and systematic meta-analyses of genetic association studies in cutaneous melanoma, Journal of the National Cancer Institute, 103 (2011) 1227-1235.

[14] T. Dwyer, J.M. Stankovich, L. Blizzard, L.M. FitzGerald, J.L. Dickinson, A. Reilly, J. Williamson, R. Ashbolt, M. Berwick, M.M. Sale, Does the addition of information on genotype improve prediction of the risk of melanoma and nonmelanoma skin cancer

ACCEPTED MANUSCRIPT

beyond that obtained from skin phenotype?, American journal of epidemiology, 159 (2004) 826-833.

[15] J.S. Palmer, D.L. Duffy, N.F. Box, J.F. Aitken, L.E. O'Gorman, a.C. Green, N.K. Hayward, N.G. Martin, R.a. Sturm, Melanocortin-1 receptor polymorphisms and risk of melanoma: is the association explained solely by pigmentation phenotype?, American journal of human genetics, 66 (2000) 176-186.

[16] E. Pasquali, J.C. Garcia-Borron, M.C. Fargnoli, S. Gandini, P. Maisonneuve, V. Bagnardi, C. Specchia, F. Liu, M. Kayser, T. Nijsten, E. Nagore, R. Kumar, J. Hansson, P.A. Kanetsky, P. Ghiorzo, T. Debniak, W. Branicki, N.A. Gruis, J. Han, T. Dwyer, L. Blizzard, M.T. Landi, G. Palmieri, G. Ribas, A. Stratigos, M.L. Council, P. Autier, J. Little, J. Newton-Bishop, F. Sera, S. Raimondi, M.S.S. Group, MC1R variants increased the risk of sporadic cutaneous melanoma in darker-pigmented Caucasians: a pooled-analysis from the M-SKIP project, International journal of cancer, 136 (2015) 618-631.

[17] S. Raimondi, F. Sera, S. Gandini, S. Iodice, S. Caini, P. Maisonneuve, M.C. Fargnoli, MC1R variants, melanoma and red hair color phenotype: a meta-analysis, International journal of cancer, 122 (2008) 2753-2760.

[18] P.F. Williams, C.M. Olsen, N.K. Hayward, D.C. Whiteman, Melanocortin 1 receptor and risk of cutaneous melanoma: a meta-analysis and estimates of population burden, International journal of cancer, 129 (2011) 1730-1740.

[19] M. Bohm, T.A. Luger, D.J. Tobin, J.C. Garcia-Borron, Melanocortin receptor ligands: new horizons for skin biology and clinical dermatology, The Journal of investigative dermatology, 126 (2006) 1966-1975.

[20] T. Brzoska, T.A. Luger, C. Maaser, C. Abels, M. Bohm, Alpha-melanocyte-stimulating hormone and related tripeptides: biochemistry, antiinflammatory and

ACCEPTED MANUSCRIPT

protective effects in vitro and in vivo, and future perspectives for the treatment of immune-mediated inflammatory diseases, Endocrine reviews, 29 (2008) 581-602.

[21] A. Catania, Neuroprotective actions of melanocortins: a therapeutic opportunity, Trends in neurosciences, 31 (2008) 353-360.

[22] L.S. Robbins, J.H. Nadeau, K.R. Johnson, M.A. Kelly, L. Roselli-Rehfuss, E. Baack, K.G. Mountjoy, R.D. Cone, Pigmentation phenotypes of variant extension locus alleles result from point mutations that alter MSH receptor function., Cell, 72 (1993) 827-834.

[23] A.G. Smith, N.F. Box, L.H. Marks, W. Chen, D.J. Smit, J.R. Wyeth, G.A. Huttley, S. Easteal, R.A. Sturm, The human melanocortin-1 receptor locus: analysis of transcription unit, locus polymorphism and haplotype evolution, Gene, 281 (2001) 81-94.

[24] V. Katritch, V. Cherezov, R.C. Stevens, Structure-function of the G protein-coupled receptor superfamily, Annual review of pharmacology and toxicology, 53 (2013) 531-556.

[25] C.P. Tan, K.K. McKee, D.H. Weinberg, T. MacNeil, O.C. Palyha, S.D. Feighner, D.L. Hreniuk, L.H. Van Der Ploeg, D.J. MacNeil, A.D. Howard, Molecular analysis of a new splice variant of the human melanocortin-1 receptor, FEBS letters, 451 (1999) 137-141.

[26] F. Rouzaud, G.E. Costin, Y. Yamaguchi, J.C. Valencia, W.F. Berens, K.G. Chen, T. Hoashi, M. Bohm, Z.A. Abdel-Malek, V.J. Hearing, Regulation of constitutive and UVR-induced skin pigmentation by melanocortin 1 receptor isoforms, FASEB journal : official publication of the Federation of American Societies for Experimental Biology, 20 (2006) 1927-1929.

ACCEPTED MANUSCRIPT

[27] M. Dalziel, M. Kolesnichenko, R.P. das Neves, F. Iborra, C. Goding, A. Furger, Alpha-MSH regulates intergenic splicing of MC1R and TUBB3 in human melanocytes, Nucleic acids research, 39 (2011) 2378-2392.

[28] M. Dalziel, N.M. Nunes, A. Furger, Two G-rich regulatory elements located adjacent to and 440 nucleotides downstream of the core poly(A) site of the intronless melanocortin receptor 1 gene are critical for efficient 3' end processing, Molecular and cellular biology, 27 (2007) 1568-1580.

[29] C. Herraiz, C. Olivares, M. Castejon-Grinan, M. Abrisqueta, C. Jimenez-Cervantes, J.C. Garcia-Borron, Functional Characterization of MC1R-TUBB3 Intergenic Splice Variants of the Human Melanocortin 1 Receptor, PloS one, 10 (2015) e0144757.

[30] J.C. Garcia-Borron, Z. Abdel-Malek, C. Jimenez-Cervantes, MC1R, the cAMP pathway, and the response to solar UV: extending the horizon beyond pigmentation, Pigment cell & melanoma research, 27 (2014) 699-720.

[31] S. Guida, N. Bartolomeo, P.T. Zanna, C. Grieco, I. Maida, S. De Summa, S. Tommasi, M. Guida, A. Azzariti, C. Foti, R. Filotico, G. Guida, Sporadic melanoma in South-Eastern Italy: the impact of melanocortin 1 receptor (MC1R) polymorphism analysis in low-risk people and report of three novel variants, Archives of dermatological research, 307 (2015) 495-503.

[32] M. Shahzad, J. Sires Campos, N. Tariq, C. Herraiz Serrano, R. Yousaf, C. Jimenez-Cervantes, S. Yousaf, Y.M. Waryah, H.A. Dad, E.M. Blue, N. Sobreira, F. Lopez-Giraldez, G. University of Washington Center for Mendelian, T. Kausar, M. Ali, A.M. Waryah, S. Riazuddin, R.S. Shaikh, J.C. Garcia-Borron, Z.M. Ahmed, Identification and functional characterization of natural human melanocortin 1 receptor mutant alleles in Pakistani population, Pigment cell & melanoma research, 28 (2015) 730-735.

ACCEPTED MANUSCRIPT

[33] M.R. Gerstenblith, A.M. Goldstein, M.C. Fargnoli, K. Peris, M.T. Landi, Comprehensive evaluation of allele frequency differences of MC1R variants across populations, Human mutation, 28 (2007) 495-505.

[34] S.A. Savage, M.R. Gerstenblith, A.M. Goldstein, L. Mirabello, M.C. Fargnoli, K. Peris, M.T. Landi, Nucleotide diversity and population differentiation of the melanocortin 1 receptor gene, MC1R, BMC genetics, 9 (2008) 31.

[35] I. Yuasa, K. Umetsu, S. Harihara, A. Kido, A. Miyoshi, N. Saitou, B. Dashnyam, F. Jin, G. Lucotte, P.K. Chattopadhyay, L. Henke, J. Henke, Distribution of two Asian-related coding SNPs in the MC1R and OCA2 genes, Biochemical genetics, 45 (2007) 535-542.

[36] Z. Hatvani, V. Brodszky, M. Mazan, D. Pinter, J. Harsing, V. Toth, B. Somlai, S. Karpati, Genotype analysis in Hungarian patients with multiple primary melanoma, Experimental dermatology, 23 (2014) 361-364.

[37] S. Masui, M. Nakatome, R. Matoba, Variants of the melanocortin 1 receptor gene (MC1R) and P gene as indicators of the population origin of an individual, International journal of legal medicine, 123 (2009) 205-211.

[38] C. Walther, S.S. Ferguson, Minireview: Role of intracellular scaffolding proteins in the regulation of endocrine G protein-coupled receptor signaling, Molecular endocrinology, 29 (2015) 814-830.

[39] W.P. Walker, T.M. Gunn, Shades of meaning: the pigment-type switching system as a tool for discovery, Pigment cell & melanoma research, 23 (2010) 485-495.

[40] N. Dumaz, R. Marais, Integrating signals between cAMP and the RAS/RAF/MEK/ERK signalling pathways. Based on the anniversary prize of the Gesellschaft fur Biochemie und Molekularbiologie Lecture delivered on 5 July 2003 at the Special FEBS Meeting in Brussels, The FEBS journal, 272 (2005) 3491-3504.

ACCEPTED MANUSCRIPT

[41] M. Abrisqueta, C. Herraiz, A.B. Perez Oliva, B.L. Sanchez-Laorden, C. Olivares, C. Jimenez-Cervantes, J.C. Garcia-Borron, Differential and competitive regulation of human melanocortin 1 receptor signaling by beta-arrestin isoforms, Journal of cell science, 126 (2013) 3724-3737.

[42] C. Herraiz, F. Journe, G. Ghanem, C. Jimenez-Cervantes, J.C. Garcia-Borron, Functional status and relationships of melanocortin 1 receptor signaling to the cAMP and extracellular signal-regulated protein kinases 1 and 2 pathways in human melanoma cells, The international journal of biochemistry & cell biology, 44 (2012) 2244-2252.

[43] I.J. Jackson, P.S. Budd, M. Keighren, L. McKie, Humanized MC1R transgenic mice reveal human specific receptor function, Hum Mol Genet, 16 (2007) 2341-2348.

[44] J. Sanchez-Mas, C. Hahmann, I. Gerritsen, J.C. Garcia-Borron, C. Jimenez-Cervantes, Agonist-independent, high constitutive activity of the human melanocortin 1 receptor, Pigment cell research / sponsored by the European Society for Pigment Cell Research and the International Pigment Cell Society, 17 (2004) 386-395.

[45] F. Liu, M. Visser, D.L. Duffy, P.G. Hysi, L.C. Jacobs, O. Lao, K. Zhong, S. Walsh, L. Chaitanya, A. Wollstein, G. Zhu, G.W. Montgomery, A.K. Henders, M. Mangino, D. Glass, V. Bataille, R.A. Sturm, F. Rivadeneira, A. Hofman, I.W.F. van, A.G. Uitterlinden, R.J. Palstra, T.D. Spector, N.G. Martin, T.E. Nijsten, M. Kayser, Genetics of skin color variation in Europeans: genome-wide association studies with functional follow-up, Human genetics, 134 (2015) 823-835.

[46] P. Sulem, D.F. Gudbjartsson, S.N. Stacey, A. Helgason, T. Rafnar, M. Jakobsdottir, S. Steinberg, S.A. Gudjonsson, A. Palsson, G. Thorleifsson, S. Palsson, B. Sigurgeirsson, K. Thorisdottir, R. Ragnarsson, K.R. Benediktsdottir, K.K. Aben, S.H. Vermeulen, A.M.

ACCEPTED MANUSCRIPT

Goldstein, M.A. Tucker, L.A. Kiemeney, J.H. Olafsson, J. Gulcher, A. Kong, U. Thorsteinsdottir, K. Stefansson, Two newly identified genetic determinants of pigmentation in Europeans, Nature genetics, 40 (2008) 835-837.

[47] M.M. Ollmann, B.D. Wilson, Y.K. Yang, J.A. Kerns, Y. Chen, I. Gantz, G.S. Barsh, Antagonism of central melanocortin receptors in vitro and in vivo by agouti-related protein, Science, 278 (1997) 135-138.

[48] S.I. Candille, C.B. Kaelin, B.M. Cattanach, B. Yu, D.A. Thompson, M.A. Nix, J.A. Kerns, S.M. Schmutz, G.L. Millhauser, G.S. Barsh, A -defensin mutation causes black coat color in domestic dogs., Science (New York, N.Y.), 318 (2007) 1418-1423.

[49] V.B. Swope, J.A. Jameson, K.L. McFarland, D.M. Supp, W.E. Miller, D.W. McGraw, M.A. Patel, M.A. Nix, G.L. Millhauser, G.F. Babcock, Z.A. Abdel-Malek, Defining MC1R regulation in human melanocytes by its agonist α-melanocortin and antagonists agouti signaling protein and β-defensin 3., The Journal of investigative dermatology, 132 (2012) 2255-2262.

[50] S.G. Jarrett, E.M. Wolf Horrell, M.C. Boulanger, J.A. D'Orazio, Defining the Contribution of MC1R Physiological Ligands to ATR Phosphorylation at Ser435, a Predictor of DNA Repair in Melanocytes, The Journal of investigative dermatology, 135 (2015) 3086-3095.

[51] M.A. Nix, C.B. Kaelin, R. Palomino, J.L. Miller, G.S. Barsh, G.L. Millhauser, Electrostatic Similarity Analysis of Human beta-Defensin Binding in the Melanocortin System, Biophysical journal, 109 (2015) 1946-1958.

[52] M.A. Nix, C.B. Kaelin, T. Ta, A. Weis, G.J. Morton, G.S. Barsh, G.L. Millhauser, Molecular and functional analysis of human beta-defensin 3 action at melanocortin receptors, Chemistry & biology, 20 (2013) 784-795.

ACCEPTED MANUSCRIPT

[53] J. D'Orazio, D.E. Fisher, Central role for cAMP signaling in pigmentation and UV resistance, Cell cycle, 10 (2011) 8-9.

[54] C.I. Rodriguez, V. Setaluri, Cyclic AMP (cAMP) signaling in melanocytes and melanoma, Archives of biochemistry and biophysics, 563 (2014) 22-27.

[55] C. Levy, M. Khaled, D.E. Fisher, MITF: master regulator of melanocyte development and melanoma oncogene, Trends in molecular medicine, 12 (2006) 406-414.

[56] N.J. Bentley, T. Eisen, C.R. Goding, Melanocyte-specific expression of the human tyrosinase promoter: activation by the microphthalmia gene product and role of the initiator, Molecular and cellular biology, 14 (1994) 7996-8006.

[57] C. Bertolotto, K. Bille, J.P. Ortonne, R. Ballotti, Regulation of tyrosinase gene expression by cAMP in B16 melanoma cells involves two CATGTG motifs surrounding the TATA box: implication of the microphthalmia gene product, The Journal of cell biology, 134 (1996) 747-755.

[58] K. Yasumoto, K. Yokoyama, K. Shibata, Y. Tomita, S. Shibahara, Microphthalmia-associated transcription factor as a regulator for melanocyte-specific transcription of the human tyrosinase gene, Molecular and cellular biology, 14 (1994) 8058-8070.

[59] S. Carreira, J. Goodall, I. Aksan, S.A. La Rocca, M.D. Galibert, L. Denat, L. Larue, C.R. Goding, Mitf cooperates with Rb1 and activates p21Cip1 expression to regulate cell cycle progression, Nature, 433 (2005) 764-769.

[60] S. Carreira, J. Goodall, L. Denat, M. Rodriguez, P. Nuciforo, K.S. Hoek, A. Testori, L. Larue, C.R. Goding, Mitf regulation of Dia1 controls melanoma proliferation and invasiveness, Genes & development, 20 (2006) 3426-3439.

ACCEPTED MANUSCRIPT

[61] K. Opdecamp, A. Nakayama, M.T. Nguyen, C.A. Hodgkinson, W.J. Pavan, H. Arnheiter, Melanocyte development in vivo and in neural crest cell cultures: crucial dependence on the Mitf basic-helix-loop-helix-zipper transcription factor, Development, 124 (1997) 2377-2386.

[62] A.N. Hume, L.M. Collinson, A. Rapak, A.Q. Gomes, C.R. Hopkins, M.C. Seabra, Rab27a regulates the peripheral distribution of melanosomes in melanocytes, The Journal of cell biology, 152 (2001) 795-808.

[63] A.N. Hume, D.S. Ushakov, A.K. Tarafder, M.A. Ferenczi, M.C. Seabra, Rab27a and MyoVa are the primary Mlph interactors regulating melanosome transport in melanocytes, Journal of cell science, 120 (2007) 3111-3122.

[64] T. Passeron, P. Bahadoran, C. Bertolotto, C. Chiaverini, R. Busca, G. Valony, K. Bille, J.P. Ortonne, R. Ballotti, Cyclic AMP promotes a peripheral distribution of melanosomes and stimulates melanophilin/Slac2-a and actin association, FASEB journal : official publication of the Federation of American Societies for Experimental Biology, 18 (2004) 989-991.

[65] F. Rouzaud, J.P. Annereau, J.C. Valencia, G.E. Costin, V.J. Hearing, Regulation of melanocortin 1 receptor expression at the mRNA and protein levels by its natural agonist and antagonist, FASEB journal : official publication of the Federation of American Societies for Experimental Biology, 17 (2003) 2154-2156.

[66] M.C. Scott, I. Suzuki, Z.A. Abdel-Malek, Regulation of the human melanocortin 1 receptor expression in epidermal melanocytes by paracrine and endocrine factors and by ultraviolet radiation, Pigment cell research / sponsored by the European Society for Pigment Cell Research and the International Pigment Cell Society, 15 (2002) 433-439.

ACCEPTED MANUSCRIPT

[67] S. Austin, J. St-Pierre, PGC1alpha and mitochondrial metabolism--emerging concepts and relevance in ageing and neurodegenerative disorders, Journal of cell science, 125 (2012) 4963-4971.

[68] J.T. Rodgers, C. Lerin, Z. Gerhart-Hines, P. Puigserver, Metabolic adaptations through the PGC-1 alpha and SIRT1 pathways, FEBS letters, 582 (2008) 46-53.

[69] J.A. Villena, New insights into PGC-1 coactivators: redefining their role in the regulation of mitochondrial function and beyond, The FEBS journal, 282 (2015) 647-672.

[70] J. Shoag, R. Haq, M. Zhang, L. Liu, G.C. Rowe, A. Jiang, N. Koulisis, C. Farrel, C.I. Amos, Q. Wei, J.E. Lee, J. Zhang, T.S. Kupper, A.A. Qureshi, R. Cui, J. Han, D.E. Fisher, Z. Arany, PGC-1 coactivators regulate MITF and the tanning response, Molecular cell, 49 (2013) 145-157.

[71] R. Haq, J. Shoag, P. Andreu-Perez, S. Yokoyama, H. Edelman, G.C. Rowe, D.T. Frederick, A.D. Hurley, A. Nellore, A.L. Kung, J.A. Wargo, J.S. Song, D.E. Fisher, Z. Arany, H.R. Widlund, Oncogenic BRAF regulates oxidative metabolism via PGC1alpha and MITF, Cancer cell, 23 (2013) 302-315.

[72] F. Vazquez, J.H. Lim, H. Chim, K. Bhalla, G. Girnun, K. Pierce, C.B. Clish, S.R. Granter, H.R. Widlund, B.M. Spiegelman, P. Puigserver, PGC1alpha expression defines a subset of human melanoma tumors with increased mitochondrial capacity and resistance to oxidative stress, Cancer cell, 23 (2013) 287-301.

[73] Z. Ronai, The masters talk: the PGC-1alpha-MITF axis as a melanoma energizer, Pigment cell & melanoma research, (2013).

[74] M. Khaled, C. Levy, D.E. Fisher, Control of melanocyte differentiation by a MITF-PDE4D3 homeostatic circuit, Genes & development, 24 (2010) 2276-2281.

ACCEPTED MANUSCRIPT

[75] J. Sanchez-Mas, L.A. Guillo, P. Zanna, C. Jimenez-Cervantes, J.C. Garcia-Borron, Role of G protein-coupled receptor kinases in the homologous desensitization of the human and mouse melanocortin 1 receptors, Molecular endocrinology, 19 (2005) 1035-1048.

[76] M. Wu, T.J. Hemesath, C.M. Takemoto, M.A. Horstmann, A.G. Wells, E.R. Price, D.Z. Fisher, D.E. Fisher, c-Kit triggers dual phosphorylations, which couple activation and degradation of the essential melanocyte factor Mi, Genes & development, 14 (2000) 301-312.

[77] W. Xu, L. Gong, M.M. Haddad, O. Bischof, J. Campisi, E.T. Yeh, E.E. Medrano, Regulation of microphthalmia-associated transcription factor MITF protein levels by association with the ubiquitin-conjugating enzyme hUBC9, Experimental cell research, 255 (2000) 135-143.

[78] Y.Q. Li, Y. Shrestha, M. Pandey, M. Chen, A. Kablan, O. Gavrilova, S. Offermanns, L.S. Weinstein, Gq/11alpha and Gsalpha mediate distinct physiological responses to central melanocortins, The Journal of clinical investigation, 126 (2016) 40-49.

[79] C.D. Van Raamsdonk, G.S. Barsh, K. Wakamatsu, S. Ito, Independent regulation of hair and skin color by two G protein-coupled pathways, Pigment cell & melanoma research, 22 (2009) 819-826.

[80] T. Hida, K. Wakamatsu, E.V. Sviderskaya, A.J. Donkin, L. Montoliu, M. Lynn Lamoreux, B. Yu, G.L. Millhauser, S. Ito, G.S. Barsh, K. Jimbow, D.C. Bennett, Agouti protein, mahogunin, and attractin in pheomelanogenesis and melanoblast-like alteration of melanocytes: A cAMP-independent pathway, Pigment Cell and Melanoma Research, 22 (2009) 623-634.

ACCEPTED MANUSCRIPT

[81] C. Herraiz, F. Journe, Z. Abdel-Malek, G. Ghanem, C. Jimenez-Cervantes, J.C. Garcia-Borron, Signaling from the human melanocortin 1 receptor to ERK1 and ERK2 mitogen-activated protein kinases involves transactivation of cKIT, Molecular endocrinology, 25 (2011) 138-156.

[82] A. Cuenda, S. Rousseau, p38 MAP-kinases pathway regulation, function and role in human diseases, Biochimica et biophysica acta, 1773 (2007) 1358-1375.

[83] V.L. Lowes, N.Y. Ip, Y.H. Wong, Integration of signals from receptor tyrosine kinases and g protein-coupled receptors, Neuro-Signals, 11 (2002) 5-19.

[84] M.J. Marinissen, J.S. Gutkind, G-protein-coupled receptors and signaling networks: emerging paradigms, Trends in pharmacological sciences, 22 (2001) 368-376.

[85] E.F. Wagner, A.R. Nebreda, Signal integration by JNK and p38 MAPK pathways in cancer development, Nature reviews. Cancer, 9 (2009) 537-549.

[86] K. Smalley, T. Eisen, The involvement of p38 mitogen-activated protein kinase in the alpha-melanocyte stimulating hormone (alpha-MSH)-induced melanogenic and anti-proliferative effects in B16 murine melanoma cells, FEBS letters, 476 (2000) 198-202.

[87] H. Chung, J.H. Lee, D. Jeong, I.O. Han, E.S. Oh, Melanocortin 1 receptor regulates melanoma cell migration by controlling syndecan-2 expression, The Journal of biological chemistry, 287 (2012) 19326-19335.

[88] K.S. Smalley, T.G. Eisen, Differentiation of human melanoma cells through p38 MAP kinase is associated with decreased retinoblastoma protein phosphorylation and cell cycle arrest, Melanoma research, 12 (2002) 187-192.

ACCEPTED MANUSCRIPT

[89] R.A. Newton, D.W. Roberts, J.H. Leonard, R.A. Sturm, Human melanocytes expressing MC1R variant alleles show impaired activation of multiple signaling pathways, Peptides, 28 (2007) 2387-2396.

[90] A. Catania, S. Gatti, G. Colombo, J.M. Lipton, Targeting melanocortin receptors as a novel strategy to control inflammation, Pharmacological reviews, 56 (2004) 1-29.

[91] J.L. Curry, W. Pinto, B.J. Nickoloff, A.T. Slominski, Human keratinocytes express functional alpha-MSH (MC1-R) receptors, In vitro cellular & developmental biology. Animal, 37 (2001) 234-236.

[92] T.A. Luger, T. Brzoska, T.E. Scholzen, D.H. Kalden, C. Sunderkotter, C. Armstrong, J. Ansel, The role of alpha-MSH as a modulator of cutaneous inflammation, Annals of the New York Academy of Sciences, 917 (2000) 232-238.

[93] S.W. Yoon, S.H. Goh, J.S. Chun, E.W. Cho, M.K. Lee, K.L. Kim, J.J. Kim, C.J. Kim, H. Poo, alpha-Melanocyte-stimulating hormone inhibits lipopolysaccharide-induced tumor necrosis factor-alpha production in leukocytes by modulating protein kinase A, p38 kinase, and nuclear factor kappa B signaling pathways, The Journal of biological chemistry, 278 (2003) 32914-32920.

[94] D. Li, A.W. Taylor, Diminishment of alpha-MSH anti-inflammatory activity in MC1r siRNA-transfected RAW264.7 macrophages, Journal of leukocyte biology, 84 (2008) 191-198.

[95] C. Cohen, A. Zavala-Pompa, J.H. Sequeira, M. Shoji, D.G. Sexton, G. Cotsonis, F. Cerimele, B. Govindarajan, N. Macaron, J.L. Arbiser, Mitogen-actived protein kinase activation is an early event in melanoma progression, Clinical cancer research : an official journal of the American Association for Cancer Research, 8 (2002) 3728-3733.

ACCEPTED MANUSCRIPT

[96] M. Katz, I. Amit, Y. Yarden, Regulation of MAPKs by growth factors and receptor tyrosine kinases, Biochimica et biophysica acta, 1773 (2007) 1161-1176.

[97] L.O. Murphy, J. Blenis, MAPK signal specificity: the right place at the right time, Trends in biochemical sciences, 31 (2006) 268-275.

[98] F. Zassadowski, C. Rochette-Egly, C. Chomienne, B. Cassinat, Regulation of the transcriptional activity of nuclear receptors by the MEK/ERK1/2 pathway, Cellular signalling, 24 (2012) 2369-2377.

[99] N. Dhomen, R. Marais, BRAF signaling and targeted therapies in melanoma, Hematology/oncology clinics of North America, 23 (2009) 529-545, ix.

[100] I.V. Fedorenko, G.T. Gibney, V.K. Sondak, K.S. Smalley, Beyond BRAF: where next for melanoma therapy?, British journal of cancer, 112 (2015) 217-226.

[101] E. Hodis, I.R. Watson, G.V. Kryukov, S.T. Arold, M. Imielinski, J.P. Theurillat, E. Nickerson, D. Auclair, L. Li, C. Place, D. Dicara, A.H. Ramos, M.S. Lawrence, K. Cibulskis, A. Sivachenko, D. Voet, G. Saksena, N. Stransky, R.C. Onofrio, W. Winckler, K. Ardlie, N. Wagle, J. Wargo, K. Chong, D.L. Morton, K. Stemke-Hale, G. Chen, M. Noble, M. Meyerson, J.E. Ladbury, M.A. Davies, J.E. Gershenwald, S.N. Wagner, D.S. Hoon, D. Schadendorf, E.S. Lander, S.B. Gabriel, G. Getz, L.A. Garraway, L. Chin, A landscape of driver mutations in melanoma, Cell, 150 (2012) 251-263.

[102] M. Krauthammer, Y. Kong, B.H. Ha, P. Evans, A. Bacchiocchi, J.P. McCusker, E. Cheng, M.J. Davis, G. Goh, M. Choi, S. Ariyan, D. Narayan, K. Dutton-Regester, A. Capatana, E.C. Holman, M. Bosenberg, M. Sznol, H.M. Kluger, D.E. Brash, D.F. Stern, M.A. Materin, R.S. Lo, S. Mane, S. Ma, K.K. Kidd, N.K. Hayward, R.P. Lifton, J. Schlessinger, T.J. Boggon, R. Halaban, Exome sequencing identifies recurrent somatic RAC1 mutations in melanoma, Nature genetics, 44 (2012) 1006-1014.

ACCEPTED MANUSCRIPT

[103] E. Shtivelman, M.Q. Davies, P. Hwu, J. Yang, M. Lotem, M. Oren, K.T. Flaherty, D.E. Fisher, Pathways and therapeutic targets in melanoma, Oncotarget, 5 (2014) 1701-1752.

[104] R. Busca, P. Abbe, F. Mantoux, E. Aberdam, C. Peyssonnaux, A. Eychene, J.P. Ortonne, R. Ballotti, Ras mediates the cAMP-dependent activation of extracellular signal-regulated kinases (ERKs) in melanocytes, The EMBO journal, 19 (2000) 2900-2910.

[105] A. Tada, E. Pereira, D. Beitner-Johnson, R. Kavanagh, Z.A. Abdel-Malek, Mitogen- and ultraviolet-B-induced signaling pathways in normal human melanocytes, The Journal of investigative dermatology, 118 (2002) 316-322.

[106] C. Herraiz, C. Jimenez-Cervantes, P. Zanna, J.C. Garcia-Borron, Melanocortin 1 receptor mutations impact differentially on signalling to the cAMP and the ERK mitogen-activated protein kinase pathways, FEBS letters, 583 (2009) 3269-3274.

[107] E. Simamura, T. Arikawa, T. Ikeda, H. Shimada, H. Shoji, H. Masuta, Y. Nakajima, H. Otani, H. Yonekura, T. Hatta, Melanocortins contribute to sequential differentiation and enucleation of human erythroblasts via melanocortin receptors 1, 2 and 5, PloS one, 10 (2015) e0123232.

[108] P. Mellor, L.A. Furber, J.N. Nyarko, D.H. Anderson, Multiple roles for the p85alpha isoform in the regulation and function of PI3K signalling and receptor trafficking, The Biochemical journal, 441 (2012) 23-37.

[109] K.M. Vasudevan, L.A. Garraway, AKT signaling in physiology and disease, Current topics in microbiology and immunology, 347 (2010) 105-133.

[110] L.B. Cheng, L. Cheng, H.E. Bi, Z.Q. Zhang, J. Yao, X.Z. Zhou, Q. Jiang, Alpha-melanocyte stimulating hormone protects retinal pigment epithelium cells from

ACCEPTED MANUSCRIPT

oxidative stress through activation of melanocortin 1 receptor-Akt-mTOR signaling, Biochemical and biophysical research communications, 443 (2014) 447-452.

[111] A.L. Kadekaro, R. Kavanagh, H. Kanto, S. Terzieva, J. Hauser, N. Kobayashi, S. Schwemberger, J. Cornelius, G. Babcock, H.G. Shertzer, G. Scott, Z.A. Abdel-Malek, alpha-Melanocortin and endothelin-1 activate antiapoptotic pathways and reduce DNA damage in human melanocytes, Cancer research, 65 (2005) 4292-4299.

[112] M. Krayem, F. Journe, M. Wiedig, R. Morandini, F. Sales, A. Awada, G. Ghanem, Prominent role of cyclic adenosine monophosphate signalling pathway in the sensitivity of (WT)BRAF/(WT)NRAS melanoma cells to vemurafenib, European journal of cancer, 50 (2014) 1310-1320.

[113] A.R. Kristensen, J. Gsponer, L.J. Foster, A high-throughput approach for measuring temporal changes in the interactome, Nature methods, 9 (2012) 907-909.

[114] L. Zhou, J. Lyons-Rimmer, S. Ammoun, J. Muller, E. Lasonder, V. Sharma, E. Ercolano, D. Hilton, I. Taiwo, M. Barczyk, C.O. Hanemann, The scaffold protein KSR1, a novel therapeutic target for the treatment of Merlin-deficient tumors, Oncogene, 35 (2016) 3443-3453.

[115] P.Y. Jean-Charles, V. Rajiv, S.K. Shenoy, Ubiquitin-Related Roles of beta-Arrestins in Endocytic Trafficking and Signal Transduction, Journal of cellular physiology, (2016).

[116] L.M. Luttrell, S. Maudsley, L.M. Bohn, Fulfilling the Promise of "Biased" G Protein-Coupled Receptor Agonism, Molecular pharmacology, 88 (2015) 579-588.

[117] A.K. Shukla, K. Xiao, R.J. Lefkowitz, Emerging paradigms of beta-arrestin-dependent seven transmembrane receptor signaling, Trends in biochemical sciences, 36 (2011) 457-469.

ACCEPTED MANUSCRIPT

[118] B.L. Sanchez-Laorden, C. Jimenez-Cervantes, J.C. Garcia-Borron, Regulation of human melanocortin 1 receptor signaling and trafficking by Thr-308 and Ser-316 and its alteration in variant alleles associated with red hair and skin cancer, The Journal of biological chemistry, 282 (2007) 3241-3251.

[119] S.M. DeWire, S. Ahn, R.J. Lefkowitz, S.K. Shenoy, β-Arrestins and Cell Signaling, Annual Review of Physiology, 69 (2007) 483-510.

[120] L.M. Luttrell, D. Gesty-Palmer, Beyond desensitization: physiological relevance of arrestin-dependent signaling., Pharmacological reviews, 62 (2010) 305-330.

[121] R.H. Oakley, S.A. Laporte, J.A. Holt, M.G. Caron, L.S. Barak, Differential affinities of visual arrestin, beta arrestin1, and beta arrestin2 for G protein-coupled receptors delineate two major classes of receptors, The Journal of biological chemistry, 275 (2000) 17201-17210.

[122] J. Guo, W. Jie, Z. Shen, M. Li, Y. Lan, Y. Kong, S. Guo, T. Li, S. Zheng, SCF increases cardiac stem cell migration through PI3K/AKT and MMP2/9 signaling, International journal of molecular medicine, 34 (2014) 112-118.

[123] J. Cao, L. Wan, E. Hacker, X. Dai, S. Lenna, C. Jimenez-Cervantes, Y. Wang, N.R. Leslie, G.X. Xu, H.R. Widlund, B. Ryu, R.M. Alani, K. Dutton-Regester, C.R. Goding, N.K. Hayward, W. Wei, R. Cui, MC1R is a potent regulator of PTEN after UV exposure in melanocytes, Molecular cell, 51 (2013) 409-422.

[124] L.K. Phan, F. Lin, C.A. LeDuc, W.K. Chung, R.L. Leibel, The mouse mahoganoid coat color mutation disrupts a novel C3HC4 RING domain protein, The Journal of clinical investigation, 110 (2002) 1449-1459.

ACCEPTED MANUSCRIPT

[125] K. Sun, B.S. Johnson, T.M. Gunn, Mitochondrial dysfunction precedes neurodegeneration in mahogunin (Mgrn1) mutant mice, Neurobiology of aging, 28 (2007) 1840-1852.

[126] L. He, X.-Y. Lu, A.F. Jolly, A.G. Eldridge, S.J. Watson, P.K. Jackson, G.S. Barsh, T.M. Gunn, Spongiform degeneration in mahoganoid mutant mice., Science (New York, N.Y.), 299 (2003) 710-712.

[127] C.D. Cota, P. Bagher, P. Pelc, C.O. Smith, C.R. Bodner, T.M. Gunn, Mice with mutations in Mahogunin ring finger-1 (Mgrn1) exhibit abnormal patterning of the left-right axis., Developmental dynamics : an official publication of the American Association of Anatomists, 235 (2006) 3438-3447.

[128] M.B. Metzger, J.N. Pruneda, R.E. Klevit, A.M. Weissman, RING-type E3 ligases: master manipulators of E2 ubiquitin-conjugating enzymes and ubiquitination, Biochimica et biophysica acta, 1843 (2014) 47-60.

[129] B.Y. Kim, J.A. Olzmann, G.S. Barsh, L.S. Chin, L. Li, Spongiform neurodegeneration-associated E3 ligase Mahogunin ubiquitylates TSG101 and regulates endosomal trafficking, Molecular biology of the cell, 18 (2007) 1129-1142.

[130] D. Srivastava, O. Chakrabarti, Mahogunin-mediated alpha-tubulin ubiquitination via noncanonical K6 linkage regulates microtubule stability and mitotic spindle orientation, Cell death & disease, 5 (2014) e1064.

[131] J.E. Kennedy, A. Marchese, Regulation of GPCR Trafficking by Ubiquitin, Progress in molecular biology and translational science, 132 (2015) 15-38.

[132] P. Bagher, J. Jiao, C. Owen Smith, C.D. Cota, T.M. Gunn, Characterization of Mahogunin Ring Finger-1 expression in mice, Pigment cell research / sponsored by the

ACCEPTED MANUSCRIPT

European Society for Pigment Cell Research and the International Pigment Cell Society, 19 (2006) 635-643.

[133] A.B. Perez-Oliva, C. Olivares, C. Jimenez-Cervantes, J.C. Garcia-Borron, Mahogunin ring finger-1 (MGRN1) E3 ubiquitin ligase inhibits signaling from melanocortin receptor by competition with Galphas, The Journal of biological chemistry, 284 (2009) 31714-31725.

[134] S.N. Cooray, L. Guasti, A.J. Clark, The E3 ubiquitin ligase Mahogunin ubiquitinates the melanocortin 2 receptor, Endocrinology, 152 (2011) 4224-4231.

[135] A. Lange, R.E. Mills, C.J. Lange, M. Stewart, S.E. Devine, A.H. Corbett, Classical nuclear localization signals: definition, function, and interaction with importin alpha, The Journal of biological chemistry, 282 (2007) 5101-5105.

[136] F. Witzel, L. Maddison, N. Bluthgen, How scaffolds shape MAPK signaling: what we know and opportunities for systems approaches, Frontiers in physiology, 3 (2012) 475.

[137] F.X. Yu, K.L. Guan, The Hippo pathway: regulators and regulations, Genes & development, 27 (2013) 355-371.

[138] A.M. Petrilli, C. Fernandez-Valle, Role of Merlin/NF2 inactivation in tumor biology, Oncogene, 35 (2016) 537-548.

[139] J.E. Kim, G.J. Finlay, B.C. Baguley, The role of the hippo pathway in melanocytes and melanoma, Frontiers in oncology, 3 (2013) 123.

[140] A.B. Bianchi, T. Hara, V. Ramesh, J. Gao, A.J. Klein-Szanto, F. Morin, A.G. Menon, J.A. Trofatter, J.F. Gusella, B.R. Seizinger, et al., Mutations in transcript isoforms of the neurofibromatosis 2 gene in multiple human tumour types, Nature genetics, 6 (1994) 185-192.

ACCEPTED MANUSCRIPT

[141] F. Nallet-Staub, V. Marsaud, L. Li, C. Gilbert, S. Dodier, V. Bataille, M. Sudol, M. Herlyn, A. Mauviel, Pro-invasive activity of the Hippo pathway effectors YAP and TAZ in cutaneous melanoma, The Journal of investigative dermatology, 134 (2014) 123-132.

[142] L.B. Murray, Y.K. Lau, Q. Yu, Merlin is a negative regulator of human melanoma growth, PloS one, 7 (2012) e43295.

[143] O. Shalem, N.E. Sanjana, E. Hartenian, X. Shi, D.A. Scott, T.S. Mikkelsen, D. Heckl, B.L. Ebert, D.E. Root, J.G. Doench, F. Zhang, Genome-scale CRISPR-Cas9 knockout screening in human cells, Science, 343 (2014) 84-87.

[144] P. Valverde, E. Healy, I. Jackson, J.L. Rees, a.J. Thody, Variants of the melanocyte-stimulating hormone receptor gene are associated with red hair and fair skin in humans., Nature genetics, 11 (1995) 328-330.

[145] J.L. Rees, Genetics of hair and skin color, Annual review of genetics, 37 (2003) 67-90.

[146] A.J. Stratigos, G. Dimisianos, V. Nikolaou, M. Poulou, V. Sypsa, I. Stefanaki, O. Papadopoulos, D. Polydorou, M. Plaka, E. Christofidou, H. Gogas, D. Tsoutsos, O. Kastana, C. Antoniou, A. Hatzakis, E. Kanavakis, A.D. Katsambas, Melanocortin receptor-1 gene polymorphisms and the risk of cutaneous melanoma in a low-risk southern European population., The Journal of investigative dermatology, 126 (2006) 1842-1849.

[147] R.M. Harding, E. Healy, A.J. Ray, N.S. Ellis, N. Flanagan, C. Todd, C. Dixon, A. Sajantila, I.J. Jackson, M.A. Birch-Machin, J.L. Rees, Evidence for variable selective pressures at MC1R, American journal of human genetics, 66 (2000) 1351-1361.

ACCEPTED MANUSCRIPT

[148] P.R. John, K. Makova, W.H. Li, T. Jenkins, M. Ramsay, DNA polymorphism and selection at the melanocortin-1 receptor gene in normally pigmented southern African individuals, Annals of the New York Academy of Sciences, 994 (2003) 299-306.

[149] C. Martinez-Cadenas, S. Lopez, G. Ribas, C. Flores, O. Garcia, A. Sevilla, I. Smith-Zubiaga, M. Ibarrola-Villaba, M. Pino-Yanes Mdel, J. Gardeazabal, D. Boyano, A. Garcia de Galdeano, N. Izagirre, C. de la Rua, S. Alonso, Simultaneous purifying selection on the ancestral MC1R allele and positive selection on the melanoma-risk allele V60L in south Europeans, Molecular biology and evolution, 30 (2013) 2654-2665.

[150] N.G. Jablonski, G. Chaplin, Colloquium paper: human skin pigmentation as an adaptation to UV radiation, Proceedings of the National Academy of Sciences of the United States of America, 107 Suppl 2 (2010) 8962-8968.

[151] D.L. Duffy, N.F. Box, W. Chen, J.S. Palmer, G.W. Montgomery, M.R. James, N.K. Hayward, N.G. Martin, R.a. Sturm, Interactive effects of MC1R and OCA2 on melanoma risk phenotypes, Human Molecular Genetics, 13 (2004) 447-461.

[152] P.A. Frandberg, M. Doufexis, S. Kapas, V. Chhajlani, Human pigmentation phenotype: a point mutation generates nonfunctional MSH receptor, Biochemical and biophysical research communications, 245 (1998) 490-492.

[153] R.A. Newton, S.E. Smit, C.C. Barnes, J. Pedley, P.G. Parsons, R.A. Sturm, Activation of the cAMP pathway by variant human MC1R alleles expressed in HEK and in melanoma cells, Peptides, 26 (2005) 1818-1824.

[154] A. Ringholm, J. Klovins, R. Rudzish, S. Phillips, J.L. Rees, H.B. Schiöth, Pharmacological characterization of loss of function mutations of the human melanocortin 1 receptor that are associated with red hair, Journal of Investigative Dermatology, 123 (2004) 917-923.

ACCEPTED MANUSCRIPT

[155] B.L. Sanchez-Laorden, C. Herraiz, J.C. Valencia, V.J. Hearing, C. Jimenez-Cervantes, J.C. Garcia-Borron, Aberrant trafficking of human melanocortin 1 receptor variants associated with red hair and skin cancer: Steady-state retention of mutant forms in the proximal golgi, Journal of cellular physiology, 220 (2009) 640-654.

[156] B.L. Sánchez-Laorden, J. Sánchez-Más, E. Martínez-Alonso, J.a. Martínez-Menárguez, J.C. García-Borrón, C. Jiménez-Cervantes, Dimerization of the human melanocortin 1 receptor: functional consequences and dominant-negative effects., The Journal of investigative dermatology, 126 (2006) 172-181.

[157] H.B. Schiöth, S.R. Phillips, R. Rudzish, M.a. Birch-Machin, J.E. Wikberg, J.L. Rees, Loss of function mutations of the human melanocortin 1 receptor are common and are associated with red hair., Biochemical and biophysical research communications, 260 (1999) 488-491.

[158] M.C. Scott, K. Wakamatsu, S. Ito, A.L. Kadekaro, N. Kobayashi, J. Groden, R. Kavanagh, T. Takakuwa, V. Virador, V.J. Hearing, Z.A. Abdel-Malek, Human melanocortin 1 receptor variants, receptor function and melanocyte response to UV radiation, Journal of cell science, 115 (2002) 2349-2355.

[159] K.A. Beaumont, S.L. Shekar, R.A. Newton, M.R. James, J.L. Stow, D.L. Duffy, R.A. Sturm, Receptor function, dominant negative activity and phenotype correlations for MC1R variant alleles, Human Molecular Genetics, 16 (2007) 2249-2260.

[160] T.H. Wong, J.L. Rees, The relation between melanocortin 1 receptor (MC1R) variation and the generation of phenotypic diversity in the cutaneous response to ultraviolet radiation, Peptides, 26 (2005) 1965-1971.

[161] J.A. D'Orazio, T. Nobuhisa, R. Cui, M. Arya, M. Spry, K. Wakamatsu, V. Igras, T. Kunisada, S.R. Granter, E.K. Nishimura, S. Ito, D.E. Fisher, Topical drug rescue strategy

ACCEPTED MANUSCRIPT

and skin protection based on the role of Mc1r in UV-induced tanning, Nature, 443 (2006) 340-344.

[162] A.B. Perez Oliva, L.P. Fernendez, C. Detorre, C. Herraiz, J.A. Martinez-Escribano, J. Benitez, J.A. Lozano Teruel, J.C. Garcia-Borron, C. Jimenez-Cervantes, G. Ribas, Identification and functional analysis of novel variants of the human melanocortin 1 receptor found in melanoma patients, Human mutation, 30 (2009) 811-822.

[163] B.L. Sanchez-Laorden, J. Sanchez-Mas, M.C. Turpin, J.C. Garcia-Borron, C. Jimenez-Cervantes, Variant amino acids in different domains of the human melanocortin 1 receptor impair cell surface expression, Cellular and molecular biology, 52 (2006) 39-46.

[164] M.T. Bastiaens, J.a. ter Huurne, C. Kielich, N.a. Gruis, R.G. Westendorp, B.J. Vermeer, J.N. Bavinck, Melanocortin-1 receptor gene variants determine the risk of nonmelanoma skin cancer independently of fair skin and red hair., American journal of human genetics, 68 (2001) 884-894.

[165] J. Han, P. Kraft, G.A. Colditz, J. Wong, D.J. Hunter, Melanocortin 1 receptor variants and skin cancer risk, Int.J.Cancer, 119 (2006) 1976-1984.

[166] C. Kennedy, J. Ter Huurne, M. Berkhout, N. Gruis, M. Bastiaens, W. Bergman, R. Willemze, J.N. Bouwes Bavinck, Melanocortin 1 receptor (MC1R) gene variants are associated with an increased risk for cutaneous melanoma which is largely independent of skin type and hair color, Journal of Investigative Dermatology, 117 (2001) 294-300.

[167] D. Scherer, R. Kumar, Genetics of pigmentation in skin cancer--a review, Mutation research, 705 (2010) 141-153.

ACCEPTED MANUSCRIPT

[168] Z. Garcia-Casado, V. Traves, J. Banuls, M. Niveiro, E. Gimeno-Carpio, A.I. Jimenez-Sanchez, M. Moragon, J.A. Onrubia, V. Oliver, R. Kumar, E. Nagore, BRAF, NRAS and MC1R status in a prospective series of primary cutaneous melanoma, The British journal of dermatology, 172 (2015) 1128-1131.

[169] E. Hacker, C.M. Olsen, M. Kvaskoff, N. Pandeya, A. Yeo, A.C. Green, R.M. Williamson, J. Triscott, D. Wood, R. Mortimore, N.K. Hayward, D.C. Whiteman, Histologic and Phenotypic Factors and MC1R Status Associated with BRAF(V600E), BRAF(V600K), and NRAS Mutations in a Community-Based Sample of 414 Cutaneous Melanomas, The Journal of investigative dermatology, 136 (2016) 829-837.

[170] D. Mitra, X. Luo, A. Morgan, J. Wang, M.P. Hoang, J. Lo, C.R. Guerrero, J.K. Lennerz, M.C. Mihm, J.A. Wargo, K.C. Robinson, S.P. Devi, J.C. Vanover, J.A. D'Orazio, M. McMahon, M.W. Bosenberg, K.M. Haigis, D.A. Haber, Y. Wang, D.E. Fisher, An ultraviolet-radiation-independent pathway to melanoma carcinogenesis in the red hair/fair skin background, Nature, 491 (2012) 449-453.

[171] A. Napolitano, L. Panzella, G. Monfrecola, M. d'Ischia, Pheomelanin-induced oxidative stress: bright and dark chemistry bridging red hair phenotype and melanoma, Pigment cell & melanoma research, 27 (2014) 721-733.

[172] L. Panzella, L. Leone, G. Greco, G. Vitiello, G. D'Errico, A. Napolitano, M. d'Ischia, Red human hair pheomelanin is a potent pro-oxidant mediating UV-independent contributory mechanisms of melanomagenesis, Pigment cell & melanoma research, 27 (2014) 244-252.

[173] M.T. Landi, P.A. Kanetsky, S. Tsang, B. Gold, D. Munroe, T. Rebbeck, J. Swoyer, M. Ter-Minassian, M. Hedayati, L. Grossman, A.M. Goldstein, D. Calista, R.M. Pfeiffer,

ACCEPTED MANUSCRIPT

MC1R, ASIP, and DNA repair in sporadic and familial melanoma in a Mediterranean population, Journal of the National Cancer Institute, 97 (2005) 998-1007.

[174] J.A. Puig-Butille, M.J. Escamez, F. Garcia-Garcia, G. Tell-Marti, A. Fabra, L. Martinez-Santamaria, C. Badenas, P. Aguilera, M. Pevida, J. Dopazo, M. del Rio, S. Puig, Capturing the biological impact of CDKN2A and MC1R genes as an early predisposing event in melanoma and non melanoma skin cancer, Oncotarget, 5 (2014) 1439-1451.

[175] S.G. Jarrett, E.M. Wolf Horrell, P.A. Christian, J.C. Vanover, M.C. Boulanger, Y. Zou, J.A. D'Orazio, PKA-mediated phosphorylation of ATR promotes recruitment of XPA to UV-induced DNA damage, Molecular cell, 54 (2014) 999-1011.

[176] A.L. Kadekaro, J. Chen, J. Yang, S. Chen, J. Jameson, V.B. Swope, T. Cheng, M. Kadakia, Z. Abdel-Malek, Alpha-melanocyte-stimulating hormone suppresses oxidative stress through a p53-mediated signaling pathway in human melanocytes, Molecular cancer research : MCR, 10 (2012) 778-786.

[177] V. Swope, C. Alexander, R. Starner, S. Schwemberger, G. Babcock, Z.A. Abdel-Malek, Significance of the melanocortin 1 receptor in the DNA damage response of human melanocytes to ultraviolet radiation, Pigment cell & melanoma research, 27 (2014) 601-610.

[178] S.G. Jarrett, E.M. Wolf Horrell, J.A. D'Orazio, AKAP12 mediates PKA-induced phosphorylation of ATR to enhance nucleotide excision repair, Nucleic acids research, 44 (2016) 10711-10726.

[179] A.G. Smith, N. Luk, R.A. Newton, D.W. Roberts, R.A. Sturm, G.E. Muscat, Melanocortin-1 receptor signaling markedly induces the expression of the NR4A nuclear receptor subgroup in melanocytic cells, The Journal of biological chemistry, 283 (2008) 12564-12570.

ACCEPTED MANUSCRIPT

[180] K. Jagirdar, K. Yin, M. Harrison, W. Lim, G.E. Muscat, R.A. Sturm, A.G. Smith, The NR4A2 nuclear receptor is recruited to novel nuclear foci in response to UV irradiation and participates in nucleotide excision repair, PloS one, 8 (2013) e78075.

[181] K. Yin, R.A. Sturm, A.G. Smith, MC1R and NR4A receptors in cellular stress and DNA repair: implications for UVR protection, Experimental dermatology, 23 (2014) 449-452.

[182] L. Dong, J. Wen, E. Pier, X. Zhang, B. Zhang, F. Dong, N. Ziegler, M. Mysz, R. Armenta, R. Cui, Melanocyte-stimulating hormone directly enhances UV-Induced DNA repair in keratinocytes by a xeroderma pigmentosum group A-dependent mechanism, Cancer research, 70 (2010) 3547-3556.

[183] J.R. Davies, J. Randerson-Moor, K. Kukalizch, M. Harland, R. Kumar, S. Madhusudan, E. Nagore, J. Hansson, V. Hoiom, P. Ghiorzo, N.A. Gruis, P.A. Kanetsky, J. Wendt, D. Pjanova, S. Puig, P. Saiag, D. Schadendorf, N. Soufir, I. Okamoto, P. Affleck, Z. Garcia-Casado, Z. Ogbah, A. Ozola, P. Queirolo, A. Sucker, J.H. Barrett, R. van Doorn, D.T. Bishop, J. Newton-Bishop, Inherited variants in the MC1R gene and survival from cutaneous melanoma: a BioGenoMEL study, Pigment cell & melanoma research, 25 (2012) 384-394.

[184] N.J. Taylor, A.S. Reiner, C.B. Begg, A.E. Cust, K.J. Busam, H. Anton-Culver, T. Dwyer, L. From, R.P. Gallagher, S.B. Gruber, S. Rosso, K.A. White, R. Zanetti, I. Orlow, N.E. Thomas, T.R. Rebbeck, M. Berwick, P.A. Kanetsky, G.E.M.S. Group, Inherited variation at MC1R and ASIP and association with melanoma-specific survival, International journal of cancer, 136 (2015) 2659-2667.

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[185] R.G. Kansal, M.S. McCravy, J.H. Basham, J.A. Earl, S.L. McMurray, C.J. Starner, M.A. Whitt, L.M. Albritton, Inhibition of melanocortin 1 receptor slows melanoma growth, reduces tumor heterogeneity and increases survival, Oncotarget, (2016).

[186] E. Piskounova, M. Agathocleous, M.M. Murphy, Z. Hu, S.E. Huddlestun, Z. Zhao, A.M. Leitch, T.M. Johnson, R.J. DeBerardinis, S.J. Morrison, Oxidative stress inhibits distant metastasis by human melanoma cells, Nature, 527 (2015) 186-191.

[187] K.A. DeFea, Beta-arrestins as regulators of signal termination and transduction: how do they determine what to scaffold?, Cellular signalling, 23 (2011) 621-629.

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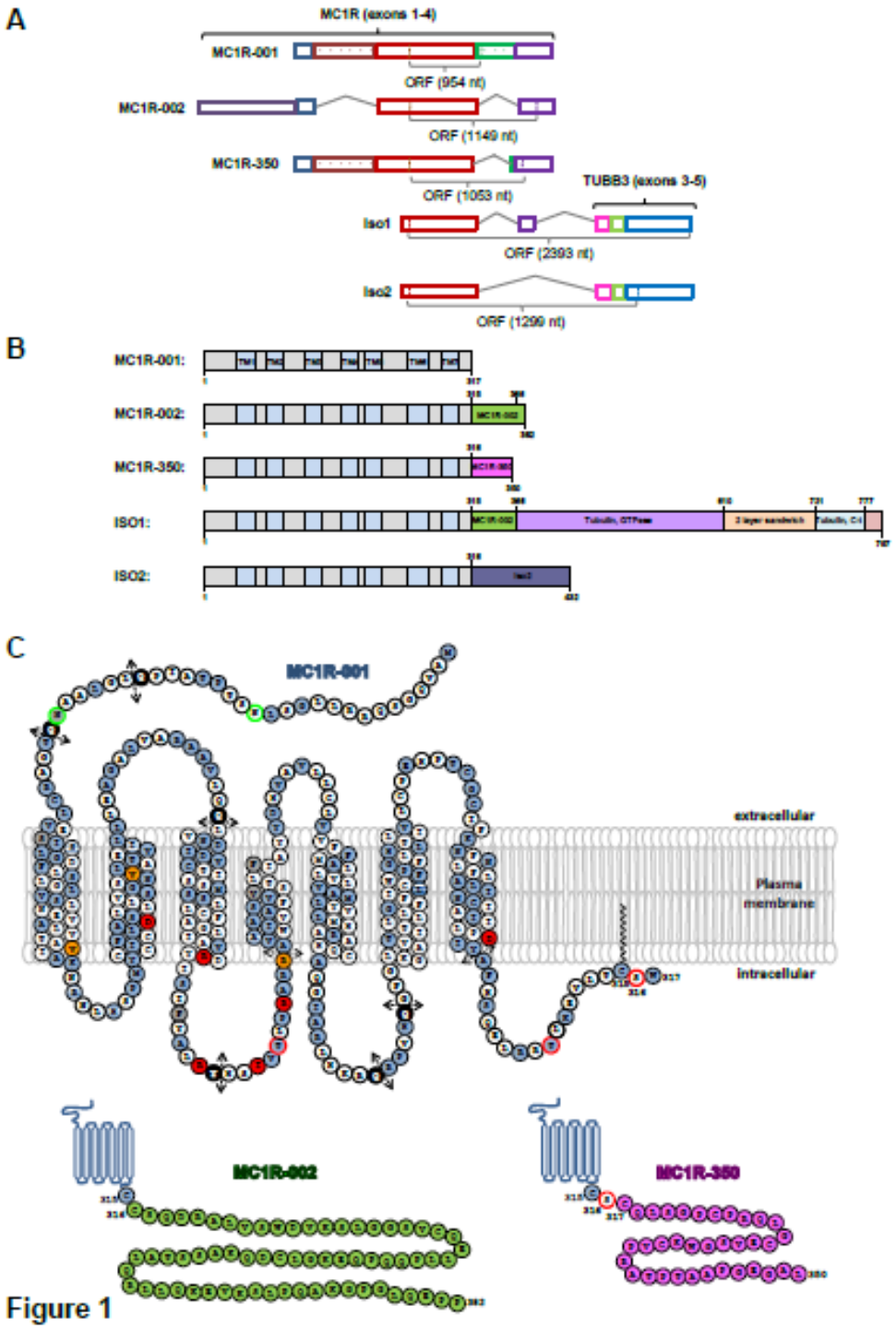

Figure 1

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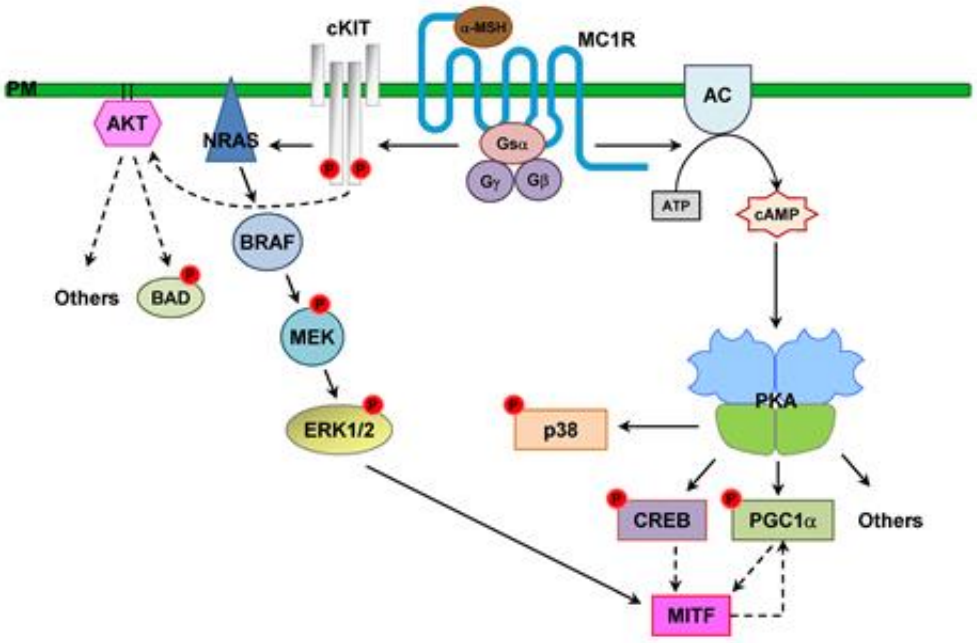

Figure 2

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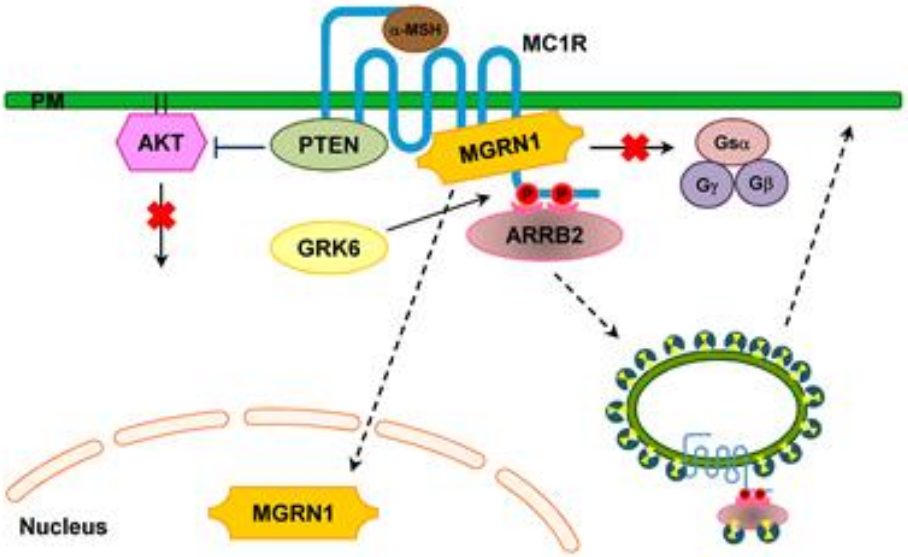

Figure 3

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Highlights

MC1R signaling is key for cutaneous homeostasis and photoprotection

The MC1R gene is complex due to polymorphism and intergenic splicing to TUBB3 gene

Intergenic splicing to the TUBB3 gene yields hypomorphic MC1R-TUBB3 chimeric proteins

Wild type MC1R stimulates photoprotective eumelanogenesis and activates DNA repair

For variant MC1R, altered signaling and aberrant interactions increase melanoma risk

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