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Malignant Melanoma and Melanocortin 1 Receptor

A. A. Rosenkranz[1,2,3], **T. A. Slastnikova**[1], **M. O. Durymanov**[1,2], and **A. S. Sobolev**[1,2,3]

A. A. Rosenkranz: aar@igb.ac.ru

[1]Institute of Gene Biology, Russian Academy of Sciences, ul. Vavilova 34/5, 199334 Moscow, Russia; fax: +7 (499) 135-4105

[2]Faculty of Biology, Lomonosov Moscow State University, Leninsky Gory 1-12, 119234 Moscow, Russia; fax: +7 (495) 939-4309; info@mail.bio.msu.ru

[3]Targeted Delivery of Pharmaceuticals “Translek” LLC, ul. Vavilova 34/5, 199334 Moscow, Russia; translek@genebiology.ru

Abstract

The conventional chemotherapeutic treatment of malignant melanoma still remains poorly efficient in most cases. Thus the use of specific features of these tumors for development of new therapeutic modalities is highly needed. Melanocortin receptor-1 (MC1R) overexpression on the cell surface of the vast majority of human melanomas, making MC1R a valuable marker of these tumors, is one of these features. Naturally, MC1R plays a key role in skin protection against damaging ultraviolet radiation by regulating eumelanin production. MC1R activation is involved in regulation of melanocyte cell division. This article reviews the peculiarities of regulation and expression of MC1R, melanocytes, and melanoma cells, along with the possible connection of MC1R with signaling pathways regulating proliferation of tumor cells. MC1R is a cell surface endocytic receptor, thus considered perspective for diagnostics and targeted drug delivery. A number of new therapeutic approaches that utilize MC1R, including endoradiotherapy with Auger electron and α- and β-particle emitters, photodynamic therapy, and gene therapy are now being developed.

Keywords

melanoma; melanocortin receptor-1; α-melanocyte-stimulating hormone; receptor; endocytosis; cancer therapy; diagnostics

Malignant melanoma is one of the most aggressive and therapy-resistant types of cancer. In 2011, more than 8,700 new cases of melanoma diagnosed and 3,368 deaths caused by this cancer were documented in Russia. In contrast to many other cancers, the mortality caused by melanoma is still increasing, with almost 30% increment in registered deaths during the last decade in Russia [1]. The same tendency takes place in other countries [2, 3]. This lack of progress in the treatment of malignant melanoma is caused in part by the high invasiveness of melanomas, thus making surgical treatment lowly effective. Conventional

Correspondence to: A. A. Rosenkranz, aar@igb.ac.ru.

chemotherapy of this cancer is also inefficient [4]. Median survival of patients with non-cutaneous metastasis is less than one year, with a 5-year survival not more than 10% even in developed countries [5]. Thus, the search for specific markers of this disease, especially specific surface markers [6] and altered signaling pathways, for development of immunotherapy and different types of targeted therapy [4, 7] is of great interest.

Melanoma

Melanomas develop as a result of malignant transformation of pigment cells called melanocytes, which are localized in the epidermis immediately over the basal membrane, rarely in derma and choroid. Skin melanocytes compose 10–25% of basal layer cells of the epidermis [8] and are the cells producing melanin in response to ultraviolet radiation with subsequent melanin accumulation in melanocytes and surrounding keratinocytes [9]. Primary melanomas are usually revealed in skin, but ocular localization of melanoma also occurs quite often (around 10% of cases), and rarely melanomas localize on mucous membranes [10]. In its early stage of development (the stage of radial growth) the tumor remains localized, thus surgical treatment yields good results. As soon as the cells able to penetrate into deeper layers of skin appear, the tumor switches to a vertical growth stage known for its high metastatic potential and poor prognosis [11].

Malignant transformation of melanocytes to melanoma is associated with production of growth factors and expression of receptors that are absent on and are not produced by normal melanocytes. This list of growth factors includes basic fibroblast growth factor, transforming growth factors alpha and beta, platelet-derived growth factor, interleukins, growth hormone, granulocyte-macrophage colony-stimulating factor, stem cell factor, and some other [12]. Melanoma is able to produce not only the different growth factors and cytokines, but also their receptors, thus enabling self-regulation of tumor cell behavior.

The most frequent genetic alterations revealed in melanoma cells are: amplification of apoptosis inhibitor Bcl-2 (>90% cases), activation of one of the key cell cycle regulators mTor (mammalian target of rapamycin, 67–77%), mutagenic constant activation of MAPK/ERK-signaling pathway regulator BRAF (v-Raf murine sarcoma viral oncogene homolog B1, 66%) kinase [13], enhanced expression of adaptor protein NEDD9 (neural precursor cell expressed, developmentally down-regulated 9) promoting cell invasion (50–60%) [14, 15], and loss of CDKN2A (cyclin-dependent kinase inhibitor 2A) [15]. While attempts to inhibit the first two factors were poorly effective clinically, the use of BRAF inhibitors for melanoma treatment seems to be encouraging [16, 17]. Also, attention of researchers specializing in investigation and treatment of melanomas has being focused for more than three decades on α-melanocyte-stimulating hormone (α-MSH) receptor expression on melanoma cells [18]. The specific interest is that the expression of this receptor, a member of a melanocortin receptors family (MC1R, melanocortin 1 receptor), is usually upregulated in melanoma cell lines as well as in melanomas [19– 25]. The fact that immunocytochemical staining of slides of different tissues reveals enhanced MC1R expression in human melanomas that is significantly higher than in any healthy tissue [23] is also important for diagnostics and potential treatment. However, the diagnostics relying on MC1R becomes somewhat complicated due to the usual secretion of proopiomelanocortin peptide gene

products (adrenocorticotropic hormone, melanocyte-stimulating hormones, β-endorphin, etc.) by melanoma cells, and moreover their production rate usually correlates with the melanoma stage [11, 21]. Endogenous ligands to MC1R that are presented in tissues can affect the interactions of diagnostic drugs because of their competition for the receptor as well as a decrease in cell surface receptors due to the internalization of hormone–receptor complexes.

α-MSH also stimulates the division of normal melanocytes when they are affected by mitogens like basic fibroblast growth factor and phorbol esters [26]. The ability of α-MSH to enhance proliferation and to increase (in some cases) the number of cell surface receptors can seemingly lead to stimulation of melanoma growth. An increase in α-MSH and MC1R expression in keratinocytes and fibroblasts probably promotes the development of an early melanoma stage [27].

Melanocortin 1 receptor

Receptor for α-MSH (MC1R) belongs to the melanocortin receptors group, which also includes four other receptors with 40–60% homology. All these receptors can bind to adrenocorticotropic hormone (ACTH) and all except one (MC2R) can bind to α-MSH. The other melanocyte-stimulating peptides (β- and γ-MSH) also can bind to MC1R and MC(3–5)R evoking a biological response; however, their affinity for these receptors is significantly lower. MC2R is the specific receptor for ACTH only [28]. Melanocortin receptors with their polypeptide chain passing through the plasma membrane seven times belong to the huge G-protein coupled receptors (GPCR) family that interact with G-proteins. Melanocortin receptors are the smallest GPCR members, having short *N*- and *C*-termini and extracellular second loop. All five MCRs are functionally connected to adenylate cyclase, and their action is mediated mostly by cAMP-dependent signaling pathway activation [28]. The activation of the MC1R–signaling pathway in melanocytes stimulates tyrosinase activity, which is the rate-limiting enzyme of melanin production [29] (Fig. 1, see color inset). Melanin accumulates in specialized cellular organelles called melanosomes, which are transported along dendritic spines to the extracellular space to be seized there by neighboring keratinocytes. One melanocyte contacts and provides with melanin approximately 35 keratinocytes [30]. Ultraviolet radiation induced MC1R activation in skin is mediated by an increase in synthesis and release of melanocortin peptides by melanocytes and keratinocytes [31–33]. In response to ultraviolet radiation, keratinocytes also produce higher amount of endothelin-1, which is a paracrine mitogen for melanocytes that enhances MC1R expression in these cells [34, 35]. α-MSH-mediated response of melanocytes lasts a long time, as the hormone leads to enhancement of the receptor mRNA synthesis, obviously via increasing cAMP level [35], thus resulting in higher level of MC1R expression [36–38]. Similar effects can be seemingly observed in some cases in melanoma cells [38, 39]. Moreover, melanoma can significantly enhance MC1R expression in neighboring cells [40].

MC1R endocytosis and intracellular distribution

Like the other GPCR, newly synthesized MC1R is transported to the surface of its expressing cells. Interestingly, the cell surface melanocortin receptors exist in pre-organized

homo- and heterodimeric forms. Moreover, this dimerization does not depend on ligand–receptor binding [41]. MC1R dimerization is mediated by both noncovalent interactions and formation of disulfide bonds between monomers [42]. Apparently, heterodimerization between mutant and normal MC1R forms can be responsible for dominant-negative action of some MC1R alleles [43].

Incubation of cells with MC1R agonists promotes internalization of the ligand–receptor complexes [44], which is one of the ways to decrease the activity of the agonist-stimulated processes. In the case of MC1R, response desensitization is realized via two members of the GPCR kinases (GRK) family – GRK2 and GRK6 [45, 46]. Agonist binding to MC1R results not only in activation of the receptor itself, but it also activates its kinases. Due to this fact, the initial hormone-induced cAMP level increase in cells goes through a maximum and starts decreasing. While one of these kinases (GRK2) can decrease constant MC1R activity, which does not depend on agonist activation [45], the other (GRK6) mediates agonist-dependent internalization of the receptor via phosphorylation of its cytoplasmic *C*-terminus amino acids [46]. Recent findings reveal that this internalization is regulated by binding of arrestins to the cytoplasmic part of the receptor [47]. Arrestin-β-1 (ARRB1) and arrestin-β-2 (ARRB2) are involved in this process. ARRB2 promotes MC1R endocytosis and inhibits agonist-dependent cAMP production, while ARRB1 does not affect these processes but does compete with ARRB2 for binding to MC1R [47] (Fig. 1).

Immunochemical staining of melanoma cells also reveals perinuclear localization of MC1R in these cells and keratinocytes, neighboring melanoma [40, 48], indicating the endocytosis of α-MSH–receptor complexes. Interestingly, immunocytochemical staining of placental trophoblasts for MC1R demonstrates generally intranuclear staining [49]; therefore, under these conditions MC1R can be transported actively to the nucleus.

Interaction of MC1R with intracellular regulators

In addition to interaction with $G\alpha_s$ and subsequent activation of adenylate cyclase, MC1R interplays also with a number of other intracellular proteins. Like the other melanocortin receptors, MC1R can interact with the small accessory protein MRAP (melanocortin-2-receptor accessory protein) and with its homolog MRAP2. MC1R interaction with these transmembrane proteins decreases cAMP production, mediated by melanocyte-stimulating ligands, but in contrast to some other melanocortin receptors (first of all, MC2R) it does not affect the transport to the plasma membrane of newly synthesized MC1R [50].

Additionally, MC1R can interact with a number of other cellular regulatory proteins, thus affecting the receptor-mediated signaling. One of these proteins is attractin, a coreceptor for the interaction of the α-MSH antagonist ASIP (agouti signal protein) with MC1R [51]. Another is ubiquitin E3 ligase with RING-domain (real interesting new gene) – MGRN1 (mahogunin RING finger-1). This intracellular protein decreases agonist receptor activation by competing with the MC1R effector $G\alpha_s$ mediating adenylate cyclase activation [52] (Fig. 1).

Regulation of MC1R expression

The regulation of MC1R expression in mouse and human melanoma cells is quite different. Agonist-mediated internalization of melanocortin receptors into B16 mouse melanoma cells results in the appearance of labeled agonist in the lysosomal fraction, which can be revealed as early as 30 min after the start of incubation [44]. The endocytosed receptor in these cells does not recycle to the cell surface, with its mRNA production downregulation lasting for a long period of time. Agonists-mediated downregulation of cell surface receptors is prominent in mouse melanoma cell lines (B16, Cloudman S91 melanoma). Incubation of human melanoma cell lines with agonists either has negligible effect, or it results finally in highly increased number of their surface receptors due to enhanced MC1R expression [39, 53].

Generally, the *MC1R* gene expression regulation scheme in melanocytes and melanoma cells clearly includes the following steps: melanocortin peptides stimulate $G\alpha_s$-connected MC1R, resulting in activation of adenylate cyclase, enhancement of cAMP production, activation of protein kinase A, and CREB (cAMP-responsive element-binding protein) phosphorylation. Phosphorylated CREB binds to CRE (cAMP-responsive element) of microphthalmia transcription factor promoter, provoking protein MITF (microphthalmia transcription factor) expression. MITF, in turn, interacts with M-boxes of a number of other promoter areas resulting in activation of synthesis of MC1R, tyrosinase, tyrosinase-related proteins 1 and 2, and probably some other proteins. In turn, an increased level of MC1R in the cell can enhance the above-mentioned cascade. MITF-driven activation of gene expression leads to chromogenesis, proliferation, and inhibition of apoptosis of the melanocytes [12, 54]. The release of melanocortin peptides may be another factor providing positive feedback during activation of melanocytes via MC1R, because human melanocyte melanosomes contain both prohormone convertases, which are necessary for proopiomelanocortin cleavage, and its cleavage products – MSH and ACTH [55].

Recently, another possible mechanism of MC1R regulation on the transcriptional level by intergenic splicing was revealed [56]. The authors pointed out the complicated atypical polyadenylation site of the *MC1R* gene that makes intergenic splicing between *MC1R* and the closely downstream located β-III-tubulin (*TUBB3*) gene possible. As a result, in addition to normal MC1R, two variants of MC1R–TUBB3 are produced, with only one of them located on the cell surface. α-MSH can shift the ratio of these splice variants to MC1R–TUBB3 that are significantly less potent for increasing cAMP level.

MC1R expression in tissues

MC1R expression, as well as the expression of all melanocortin receptors in normal tissues, is rather low, with the most prominent expression found in melanocytes. Other melanocortin receptors are expressed mostly in the central nervous system (MC3R, MC4R), adrenal cortex (MC2R), gastrointestinal tract (MC3R), lymphocytes, and exocrine glands (MC5R) [57].

In normal human skin, MC1R can be detected immunohistochemically in the melanocytes of hair follicles, sweat and sebaceous glands, but not in the keratinocytes and most

interfollicular epidermal melanocytes. When a tumor appears, the keratinocytes adjacent to primary melanoma are stained intensively with anti-MC1R antibodies [40, 58] with a gradient in immunocytochemical staining increasing towards the tumor [40].

Among the skin cells primarily neighboring melanoma melanocytes, keratinocytes [59] and vascular endothelial skin cells [60, 61] were shown to express MC1R. It is worth noting that in most cases MC1R expression level in normal skin cells is at least one order of magnitude lower than in melanocytes, and it clearly has no physiological significance [62]. Moreover, in most cases with MC1R being detected only by RT-PCR, the radioligand method fails to reveal any melanocortin receptor [62]. In other tissues, MC1R is present in scattered neurons of gray substance in the brain, on macrophages and monocytes, Leydig cells in testicle, lutein cells, placenta trophoblasts, and also on astrocytes [59]. Immunohistochemical staining demonstrates that MC1R expression level in melanoma is much higher than in all normal tissues [23]. Still significantly less than in melanoma, but relatively high MC1R staining was revealed in the adrenal medulla. In monocytes, MC1R expression can increase in response to different cytokines, but it also remains much less than its expression on melanoma cells [23]. Thus, the summarized data available to date [19–25] clearly demonstrates that MC1R can be regarded as melanoma marker. Moreover, due to its endocytic surface receptor nature, MC1R can also be regarded as a promising agent for targeted intracellular drug delivery into melanoma cells. The elucidation of the interrelationships between MC1R expression and melanoma development has great importance.

Role of MC1R in melanoma formation, development, and prevention

MC1R is a key factor in skin protection from ultraviolet radiation, thus playing several roles in the melanocyte transformation process. On one hand, as mentioned above, MC1R activation is obviously involved in proliferation of melanoma cells, and on the other hand the same activation as well as elevated α-MSH production, are factors protecting melanocytes and other cells from ultraviolet radiation due to induction of melanin synthesis, which absorbs light and also acts as an antioxidant [9]. Therefore, normal functioning of this pathway is the main factor preventing melanoma formation. Alterations in this functioning result in change in eumelanin (brown/black melanin subtype) production by melanocytes. This change shifts eumelanin to pheomelanin (yellow/red melanin pigment subtype) ratio determining, in particular, skin color. *MC1R* gene polymorphism is seemingly one of the key factors determining the variety of human skin pigmentation [63]. At least 85 allelic *MC1R* gene variants are known to date [64, 65]. Some of these mutations, especially those determining red hair, are connected with an increased risk for melanoma [43, 65, 66].

MC1R expression level is connected with melanoma cell migration: the higher the level, the higher the migration ability of cells is [67]. MC1R decreases the activity of stress response by p38 MAPK kinase, increasing the expression of syndecane-2, which is involved in increasing melanoma cell motility. On the contrary, the addition of α-MSH decreases the cell mobility.

Additionally, MC1R activation, as already mentioned, evokes an increase in MITF expression in melanocytes, which is involved in realization of the RAS/RAF/MEK/ERK-signaling pathway and promotes cell proliferation and survival [13, 68]. It is worth mentioning that MC1R and the RAS/RAF/MEK/ERK-pathway are also connected more directly (Fig. 2; see color insert). MC1R activation can result not only in increase in cAMP level, but also in transactivation of stem cell growth factor receptor c-KIT, probably via Src-kinase [69], which in turn leads to activation of extracellular-regulated kinases ERK1 and ERK2 [69, 70]. Several MC1R variants possessing red hair-causing mutations loose the ability for hormone-mediated cAMP-level increase but retain the ability for activation of ERK [70, 71].

Summarizing the available data, one can conclude that MC1R expression on melanoma cells is not only a peculiarity reflecting the origin of these tumors, but also is directly connected with activation of cell division and metastasizing ability. The higher the expression of this receptor, the more frequent cell division occurs, leading to accumulation of cells overexpressing MC1R within the tumor. Thus, ligands for this receptor can be regarded as suitable components for the design of diagnostic agents and systems for targeted delivery to melanoma cells. Melanocortin receptors, and MC1R in particular, are notable for the existence of a number of natural agonists and antagonists regulating the functioning of the effector systems associated with these receptors.

Natural ligands for melanocortin receptors

Four different natural ligands (ACTH and α-, β-, and γ-MSH) activating melanocortin receptors and initiating the whole chain of subsequent intracellular processes originate from the same progenitor polypeptide, proopiomelanocortin, having a molecular weight more than 30 kDa [72]. Posttranslational cleavage of this peptide results in a large number of biologically active peptides – melanocortins, β-lipotropic hormone, and β-endorphin. The formation of different proopiomelanocortin derivatives is tissue-specific and depends on the activity of prohormone convertases. Prohormone convertase 1 activity promotes formation of ACTH and β-lipotropin along with the *N*-terminal peptide. Further proteolysis of ACTH by prohormone convertase 2 yields α-MSH. β-Lipotropin cleavage gives γ-lipotropin, which is cleaved in turn to produce β-MSH and β-endorphin. Proteolytic cleavage of the *N*-terminal proopiomelanocortin fragment gives γ-MSH [28]. All the melanocortin peptides contain the same conservative sequence His-Phe-Arg-Trp. The somewhat longer peptide Met-Glu-His-Phe-Arg-Trp-Gly, which is a part of α- and β-MSH and of ACTH, is apparently important for activation of melanogenesis. The *C*-terminal tripeptide of α-MSH determines its antiinflammatory and immunosuppressive effects [72, 73]. However, the receptors determining these effects of α-MSH differ from the receptors inducing melanogenesis in melanocytes in both the effective α-MSH concentration range and amino acid sequences responsible for the specific effect. These data suggest the presence on immune cells of an additional receptor different from MC1R that mediates effects of α-MSH on the immune system [72, 73]. A possible intracellular pathway for implementation of this effect of α-MSH is inhibition of NF-κB nuclear translocation via inhibition of I-κB phosphorylation by its kinase, with subsequent prevention of proteasomal degradation of I-κB [73].

Production of melanocortin peptides is highest in the anterior pituitary; however, these peptides are widely produced also in other tissues (adrenal glands, intestinal neurons, skin), where their function is rather paracrine than endocrine [73]. Melanomas are characterized by enhanced expression of proopiomelanocortin. This can probably be attributed to upregulated expression of corticotropin-releasing hormone and its receptor in these tumors [21]. However, there can be another explanations, as proopiomelanocortin expression is also regulated by other pathways, for example via the JAK2/STAT3-regulatory pathway, which is activated by leukemia inhibitory factor [74].

α-MSH is a 13-amino-acid peptide. Its amino acid sequence is the same for many mammals – human, mouse, cattle, etc. α-MSH can bind to four human melanocortin receptors (except for MC2R), but it has the highest affinity and specificity for MC1R, possessing 260, 5500, and 47,500 times lower affinities for MC3R, MC4R, and MC5R, respectively. It is also worth noting that human MC1R needs lower α-MSH concentrations for its activation than does mouse MC1R [36].

The presence of the natural melanocortin receptor antagonists, agouti signaling protein (ASIP) and agouti-related protein (AGRP), is an interesting feature of the melanocortin receptors system. The former binds to MC1R and MC4R, while the latter is an antagonist of MC3R–MC5R activation. ASIP is a concurrent inhibitor of α-MSH on melanocytes, and it enhances acute inflammatory reactions [72]. As already mentioned above, this ligand also has another receptor – attractin; binding to this receptor also affects skin pigmentation [75]. Another antagonist of α-MSH capable of MC1R–binding and preventing cAMP level increase in melanocytes and the subsequent biological effects is β-defensin 3 (BD3) [66, 76].

Development of diagnostic and therapeutic agents based on α-MSH and its analogs

The identical sequence of α-MSH (Ac-Ser-Tyr-Ser-Met-Glu-His-Phe-Arg-Trp-Gly-Lys-Pro-Val-NH_2) shared by human, mouse, and a number of other mammals is one of the advantages of utilizing this hormone for drug development. Therefore, α-MSH is both a useful model and a perspective agent for subsequent therapeutic use for targeted drug delivery into cancer cells. This ligand has already been extensively used for development of different agents for specific delivery into melanoma cells (Table 1).

The amount of MC1R on cultivated human and mouse melanoma cells varies from a few hundreds to tens of thousands [90–92], with up to a few thousand particularly on human melanoma cells [91, 92], which is significantly less than the amount of most surface proteins. Therefore, when exploiting this receptor in therapeutic applications, the selectivity of delivery and efficacy of affecting the intracellular targets are the guarantees for successful therapy of melanoma *in vivo*. As an example of enhanced efficacy, one can mention the delivery of a photosensitizer that generates a large amount of reactive oxygen species upon irradiation in the nucleus, which is the most vulnerable compartment of the cell. Selective transport into the nuclei of the necessary type of target cells can be achieved by creation of multifunctional modular transporting systems [93, 94]. Exploiting this principle for delivery of the photosensitizer bacteriochlorin *p* into mouse melanoma cells made it possible to

achieve more than two orders of magnitude enhanced cytotoxicity of the photosensitizer for mouse melanoma B16-F1 cells [84, 85].

It is worth noting that the ability to bind the receptor by itself may be insufficient for action on tumor cells *in vivo*. For example, the interaction of gold nanoparticles coated with polyethylene glycol modified with MC1R–binding peptides with transplanted melanoma differed depending on the ability of the peptide to initiate receptor-mediated endocytosis [95]. Nanoparticles conjugated with an MC1R agonist peptide that is an α-MSH analog demonstrated better tumor retention due to receptor-mediated internalization, than similar nanoparticles with a peptide derived from ASIP relative protein, which resulted in only binding without subsequent internalization of nanoparticles. Agonist-coated particles not only internalized inside the cells, but were also subjected to transcytosis and better tissue penetration, finally yielding 2-fold enhanced accumulation in the tumor.

Considering that metastasizing melanoma is the most dangerous melanoma type, the synthesis of α-MSH analogs and radiotherapeutics based on these peptides is one of the most intensively studied areas in the development of melanoma diagnostic tools. A number of recent review articles [96–99] have been devoted to this field. Up to now, many variants based on α-MSH and its biologically stable synthetic analog Ac-[Nle^4, DPhe^7]-α-MSH (NDP-MSH), which binds to all melanocortin receptors except for MC2R with similar affinity, has been synthesized [100]. Among them are linear and cyclic (for fixation the most favorable conformation for receptor binding) peptides conjugated with chelators for ^{111}In [101–110], ^{99m}Tc [101, 109, 111–115], and ^{67}Ga [116] for diagnostics using single photon emission computed tomography (SPECT), and also for ^{18}F [117–119], ^{68}Ga [110, 116, 120, 121], ^{64}Cu [122, 123], and ^{86}Y [122] for positron emission tomography (PET) diagnostics. Gene therapy using Na-I symporter (NIS) is another approach exploiting MC1R–specific ligands [124]. MC1R–specific peptide inclusion into polyplexes – nanoparticles consisting of a polycation and transferred plasmid DNA, results in significantly improved mouse melanoma transfection *in vivo*, enabling melanoma cells to accumulate intravenously injected ^{123}I, an isotope suitable for SPECT.

MC1R–mediated delivery can be exploited not only for diagnostics. There have been several studies on therapy of experimental mouse melanomas to date that point to the feasibility of this approach (Table 2).

Radionuclides emitting sufficiently high linear energy transfer (LET) radiation, specifically α-particles with very high LET along with the short range limited up to few cell diameters, demonstrate high therapeutic efficacy in model experiments [126]. β-Emitters can be useful for the treatment of larger tumors, but these may also damage neighboring healthy tissues. Auger electron emitters characterized by high LET along with ultra-short (usually less than 0.5 μm) range in tissues can be useful for eliminating single melanoma cells and micrometastasis if delivered into the nuclei of target cells [130]. Moreover, non-isotope methods of melanoma treatment based on MC1R selectivity, such as photodynamic therapy and suicide gene therapy, have therapeutic potential as well. For example, the photosensitizer bacteriochlorin *p* conjugated to modular transporter DTox-HMP-NLS-MSH, delivered into the cell nuclei of experimental mouse melanomas, inhibits tumor growth by

80–98% and increases survival 1.6–2.5-fold after a course of photodynamic therapy [86, 87]. Incorporation of a peptide highly specific for MC1R into a synthetic gene-delivering construct resulted in significantly improved melanoma suicide therapy using polyplexes carrying the Herpes simplex virus thymidine kinase gene [128].

Much experience in development of highly selective ligands for a specific type of melanocortin receptor, either consisting of natural amino acids [129, 131] or containing various synthetic analogs [100, 132–134] has accumulated to date. The use of these ligands can enhance the specificity of delivery into melanoma cells and decrease side effects.

Summarizing the data about melanocortin receptors and their expression in melanoma cells available to date, one can conclude that MC1R is a specific marker of melanoma cells and, in some cases, of the cells neighboring this tumor. MC1R expression in melanomas is usually higher than in any normal tissues, and ligands to this receptor can internalize specifically into the cells, thus creating the prerequisites for designing effective targeted diagnostic and therapeutic agents based on ligands for MC1R.

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Abbreviations

ACTH	adrenocorticotropin
AGRP	agouti-related protein
α-MSH	α-melanocyte-stimulating hormone
ARRB1	arrestin-beta-1
ARRB2	arrestin-beta-2
ASIP	agouti signal protein
Bcl-2	apoptosis regulator from B-cell lymphoma 2
BD3	β-defensin 3
BRAF	v-Raf murine sarcoma viral oncogene homolog B1
CDKN2A	cyclin-dependent kinase inhibitor 2A
CRE	cAMP-responsive element
CREB	cAMP-responsive element-binding protein
ERK	extracellular signal-regulated kinases
GPCR	G-protein coupled receptors
GRK	GPCR kinase
I-κB	nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor

LET	linear energy transfer
MAPK	mitogen-activated protein kinases
MC1R	melanocortin 1 receptor
MGRN1	mahogunin RING finger-1 (ubiquitin E3 ligase with RING-domain)
MITF	microphthalmia transcription factor
MRAP	melanocortin-2-receptor accessory protein
mTor	mammalian target of rapamycin
NEDD9	neural precursor cell expressed, developmentally down-regulated 9
NF-κB	nuclear factor kappa-light-chain-enhancer of activated B cells
NIS	Na-I symporter
PKA	protein kinase A
RING	real interesting new gene
Sdc2	syndecane-2
TUBB3	β-III-tubulin

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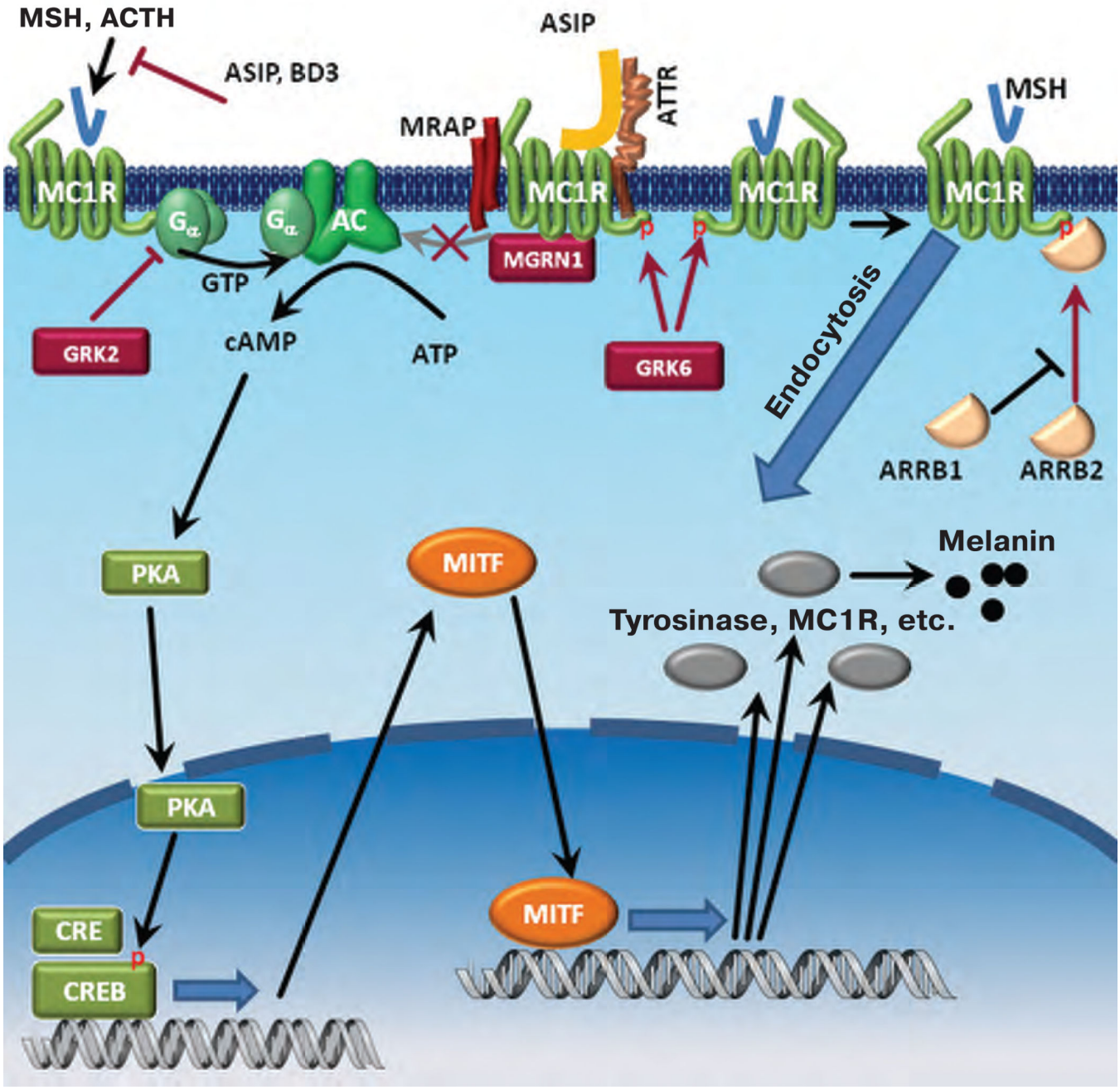

Fig. 1.

Scheme of participation of MC1R in regulation of melanogenesis. ACTH or α-, β-, or γ-melanocyte-stimulating hormone binding evokes cAMP level increase due to the activation of adenylate cyclase (AC), the induction of MITF, which in turn promotes the expression of a number of proteins including tyrosinase and hence the production of melanin. This pathway is inhibited by extracellular regulators, ASIP via MC1R binding and attractin (ATTR) and β-defensin 3 (BD3), as well as by a variety of intracellular regulatory proteins (MRAP, MGRN1, GRK2). In addition, due to GRK6 phosphorylation and subsequent ARRB2 binding, activated MC1R undergoes endocytosis. In turn, the effect of GRK6 is

controlled by competition with ARRB1, which also binds to MC1R but does not induce endocytosis.

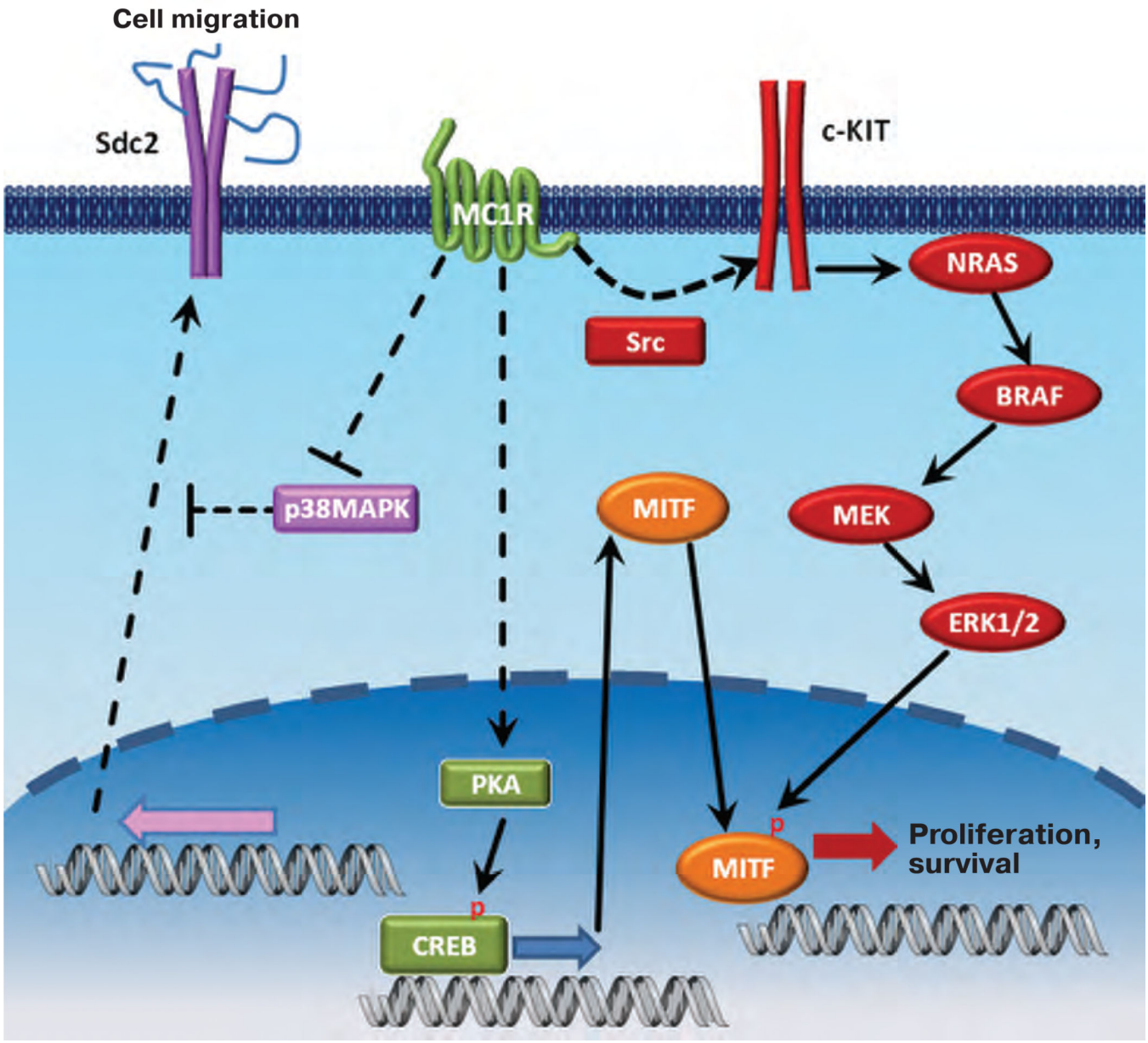

Fig. 2.

Scheme of the impact of MC1R on development of melanoma. MC1R can affect the microphthalmia-associated transcription factor via protein kinase A (PKA), as well as via oncogenic signaling pathway RAS/RAF/MEK/ERK by Src-dependent c-KIT activation [69]. Additionally, MC1R promotes melanoma cell migration due to enhancement of syndecane-2 (Sdc2) expression [67].

Table 1

Constructions for delivery into melanoma cells based on natural α-MSH

Name, composition	Type	Active principle	Reference
DAB_{389}-MSH	recombinant	diphtheria toxin ADP-ribosyltransferase	[20, 77, 78]
α-MSH-melphalan	synthetic	melphalan	[79, 80]
α-MSH-HPMA-copolymer-doxorubicin	synthetic	doxorubicin	[81]
Liposomes with lipopeptide MSH-C4A2	synthetic	various	[82]
HGM–pCH110-17m–pLyP101	recombinant/synthetic	delivered gene	[83]
Modular nanotransporter DTox-HMP-NLS-MSH	recombinant/synthetic	photosensitizers	[84–87]
Nanoparticles containing PLL and PGA-MSH	synthetic	delivered gene	[88]
HFt-MSH	recombinant/synthetic	fluorophores for diagnostics	[89]

Note: DAB389-MSH, recombinant protein with ligand domain of diphtheria toxin substituted with α-MSH; HPMA, N-(2-hydroxypropyl) methacrylamide; MSH-C4A2, α-MSH with two aliphatic chains (C16) connected via aspartate and tetramethylene bridge; HGM, GAL4 DNA-binding domain and α-MSH chimeric protein; pCH110-17m, plasmid with GAL4-binding site; pLy101, conjugate of polylysine with nuclear localization signal peptide; DTox-HMP-NLS-MSH, nanotransporter containing translocating domain of diphtheria toxin, *E. coli* hemoglobin-like protein, optimized large T-ag SV40 virus nuclear localization signal, and α-MSH; PLL, polylysine; PGA, polyglycolic acid; HFt, human ferritin.

Table 2

Therapeutic efficacies of pharmaceuticals utilizing MC1R ligands for treatment of experimental tumors

Name	Active principle	Maximal effect	Reference
^{188}Re-(Arg^{11})CCMSH	^{88}Re – β-particle emitter	1.8-fold increase in mean life span of mice	[125]
^{212}Pb[DOTA]-Re(Arg^{11})CCMSH	^{212}Pb – α-particle emitter	3.4-fold increase in mean life span of mice, 45% surviving animals	[126]
^{177}Lu-DOTA-Re(Arg^{11})CCMSH	177Lu – β-particle emitter	1.2-fold increase in mean life span of mice	[127]
Modular nanotransporter DTox-HMP-NLS-MSH	photosensitizer	2.7-fold increase in mean life span of mice	[86, 87]
Polyplex PEI-PEG-MC1sp-peptide, with thymidine kinase of herpes simplex virus	phosphorylated ganciclovir derivative	3.6-fold increase in mean life span of mice	[128]

Note: ^{188}Re-(Arg^{11})CCMSH – ^{188}Re-[Cys^{3,4,10}, DPhe^{7}, Arg^{11}]-α-MSH$_{3–13}$, peptide cyclized by rhenium via 3, 4, and 10 cysteine residues, truncated (3–13), and modified by α-MSH at amino acids indicated by the upper indexes; DOTA, 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid, a chelator; PEI, polyethyleneimine; PEG, polyethylene glycol; MC1sp, peptide with sequence (SSIISHFRWGKPV) highly specific for MC1R [129].