

REVIEW

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# Alpha-melanocyte stimulating hormone ( $\alpha$ -MSH): biology, clinical relevance and implication in melanoma

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## Abstract

Alpha-melanocyte stimulating hormone ( $\alpha$ -MSH) and its receptor, melanocortin 1 receptor (MC1R), have been proposed as potential target for anti-cancer strategies in melanoma research, due to their tissue specific expression and involvement in melanocyte homeostasis. However, their role in prevention and treatment of melanoma is still debated and controversial. Although a large body of evidence supports  $\alpha$ -MSH in preventing melanoma development, some preclinical findings suggest that the  $\alpha$ -MSH downstream signalling may promote immune escape and cancer resistance to therapy. Additionally, in metastatic melanoma both MC1R and  $\alpha$ -MSH have been reported to be overexpressed at levels much higher than normal cells. Furthermore, targeted therapy (e.g. BRAF inhibition in BRAF<sup>V600E</sup> mutant tumours) has been shown to enhance this phenomenon. Collectively, these data suggest that targeting MC1R could serve as an approach in the treatment of metastatic melanoma. In this review, we explore the molecular biology of  $\alpha$ -MSH with particular emphasis into its tumor-related properties, whilst elaborating the experimental evidence currently available regarding the interplay between  $\alpha$ -MSH/MC1R axis, melanoma and antitumor strategies.

**Keywords** Melanoma,  $\alpha$ -MSH, MC1R, Melanoma resistance, Anticancer strategies

## Introduction

Melanocortins are peptidic pituitary hormones produced by the cleavage and posttranslational modifications of pro-opiomelanocortin hormone (POMC). The family of melanocortins includes Adrenocorticotropic Hormone (ACTH), Melanocyte Stimulating Hormone

(MSH) and endorphins, that activate five forms of membrane receptors called Melanocortin Receptors (MCRs) with different affinities. MSH consists of the three forms  $\alpha$ -,  $\beta$ - and  $\gamma$ -MSH. Among them  $\alpha$ -MSH is well-characterized and first described for its melanin-inducing activity in frogs.  $\alpha$ -MSH is a 13 amino acid neuropeptide secreted by melanocytes and keratinocytes after ultraviolet light exposure and it is responsible of the melanin synthesis, being the main actor of skin pigmentation [1–3]. Moreover, it has been shown that  $\alpha$ -MSH and analogues have anti-inflammatory and antimicrobial properties, activating melanocortin receptors (MCR) signaling [4, 5].  $\alpha$ -MSH binds to four out of five MCR subtypes (MC1R, MC3R, MC4R, MC5R), regulating several downstream cascades in different cell types. Notably, in melanocytes MC1R is highly expressed and the binding of  $\alpha$ -MSH promotes the expression

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of melanogenesis enzyme genes via Adenyl Cyclase (AC)/cyclic AMP (cAMP)/Protein Kinase A (PKA) pathway. Beyond melanin synthesis, the  $\alpha$ -MSH/MC1R axis controls a plethora of important processes such as DNA damage repair, reduction of free radical production and cell proliferation among others. For the broad spectrum of properties, the use of  $\alpha$ -MSH or its synthetic analogs has been proposed for several pathologic conditions. The primary target cell for  $\alpha$ -MSH is the melanocyte, in which, despite the proven efficacy in the prevention of melanoma development, its role in malignant melanoma, and in particular in metastatic stage disease still remains underinvestigated [6].

## 2- Molecular biology of $\alpha$ -MSH

### $\alpha$ -MSH production and melanocortin receptors

Human POMC gene is located on chromosome 2p23.3 and it is expressed in a variety of tissues but broadly in testis, pancreas and fat tissue. The early encoded protein undergoes extensive posttranslational processing via prohormone convertases cleavage, in order to produce at least ten active peptides mainly synthesized in corticotroph cells of the anterior pituitary. Among them, ACTH is essential for physiologic steroidogenesis whereas in other tissues such as placenta and epithelium, proteolytic cleavage gives rise to peptides with roles in energy homeostasis, melanocyte stimulation, and immune modulation. These include several distinct melanotropins (or melanocortins):  $\alpha$ -,  $\beta$ - and  $\gamma$ -MSH. All forms of MSHs bind to four well characterised G-Protein Coupled Receptor (GPCR) subtypes: Melanocortin Receptors (MC1R, MC3R, MC4R, and MC5R), whereas MC2R is specific for the binding with ACTH [7–10].

MC1R is an intron less gene encoding seven pass transmembrane GPCR, preferentially expressed on cell membrane of melanocytes and mainly recognized as the key regulator of the synthesis of epidermal melanin pigments [11, 12]. MC1R gene is polymorphic and frequent variants are associated not only with hair/skin phenotypes but also with increased melanoma risk [13–16]. MC1R is also the target of the  $\alpha$ -MSH antagonists Agouti protein and Agouti related protein (Agrp), both responsible for the inhibition of eumelanin production in favour of pheomelanin [17].

MC3R and MC4R genes encode the GPCRs for MSH and ACTH and are expressed in tissues other than the adrenal cortex and melanocytes. Studies suggest a functional role of MC3R and MC4R in the regulation of energy homeostasis and food intake. Mutations of this receptors have been correlated to susceptibility to obesity and anorexia in humans [18–22]. Evidence suggests that MC5R plays a key role in the regulation of sexual behaviour,

thermoregulation and exocrine secretion (sebogenesis) but also in immune reaction and inflammatory response via cAMP signal transduction [23, 24].

### $\alpha$ -MSH regulation of melanocyte function: MC1R/cAMP signaling cascade

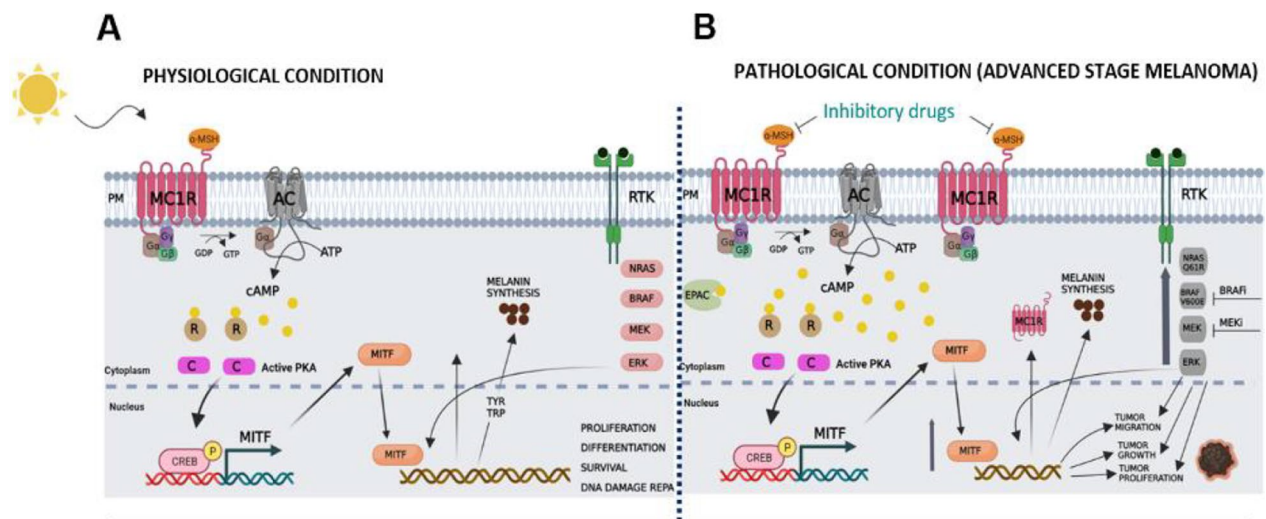
MC1R plays a key role in cutaneous homeostasis and photoprotection as it is coupled to the stimulatory G protein  $G\alpha$  which in turn activates AC switching on the cAMP/PKA pathway [25].

PKA phosphorylates the transcription factor cAMP Response Element Binding Protein (CREB) that stimulates the Microphthalmia inducing Transcription Factor (MiTF) which in turn promotes the expression of melanogenesis enzyme genes Tyrosinase (TYR), Tyrosinase Related Protein 1 and 2 (TRP1,TRP2) and Dopachrome Tautomerase (DCT) [26, 27]. MiTF coordinates a broad range of biological processes including cell survival, differentiation, proliferation, migration, invasion, senescence, metabolism, and DNA damage repair (Fig. 1).

$\alpha$ -MSH stimulated MC1R triggers the production of both free radicals (ROS) and brown/black eumelanin, acting as a filter against UV. MC1R polymorphisms are associated with pigmentary phenotypes such as Red-Hair-Colour (RHC) and light skin [28, 29]. Patients carrying these variants show a reduced ability to produce eumelanin and therefore pheomelanin synthesis prevails. Pheomelanin acts as a photosensitizer and these patients are more susceptible to skin cancer development, both by UV- dependent and independent mechanisms [30].

### Other pathways connected with $\alpha$ -MSH/MC1R signal

The Mitogen-Activated Protein Kinase (MAPK) signal transduction cascades are highly conserved regulators of cell proliferation, differentiation and survival which are activated by signals as cytokines, growth factors and other stress inducers. The most widely studied MAPK pathway is the RAS/RAF/MEK/ERK cascade that controls melanogenesis and it is aberrantly activated in 90% of human cutaneous melanomas as well as in several type of cancers. Gain of Function (GoF) mutations in N-RAS and B-RAF are common drivers for melanoma development (~25% for N-RAS and ~60% for B-RAF) as they are responsible for dysregulated cell cycle and proliferation [31–35]. Multiple stimuli such as growth factors, cytokines, viruses, GPCR ligands and oncogenes can sequentially activate the ERK pathway and result in ERK1/2 phosphorylation that regulates different transcription factors, including c-FOS, cJUN, ELK-1, c-MYC, and ATF-2 controlling cell growth, migration, and differentiation [36]. Noteworthy, ERK1/2 can phosphorylate MiTF decreasing its protein levels and leading to a negative regulation of melanogenic enzymes, inhibiting melanogenesis process. In human melanocytic cells ERK activation



**Fig. 1** **A** Physiological condition. **B** Pathological condition (advanced stage melanoma). In physiological condition melanocytes express a membrane receptor (MC1R) that controls the melanin synthesis process. **A** Upon UV exposure, alpha-melanocyte stimulating hormone ( $\alpha$ -MSH) is released by keratinocytes: the binding of  $\alpha$ -MSH to MC1R activates Adenyl Cyclase (AC) that stimulates cAMP production and the activation of Protein Kinase A (PKA). PKA phosphorylates the transcription factor CREB that stimulates the transcription factor MITF which in turn promotes the expression of melanogenesis enzyme genes TYR, TRP1 and DCT. In our working hypothesis **B** in advanced stage of melanoma, tumour cells overexpress MC1R and BRAF inhibitor treatment significantly increase this MC1R expression via MITF-dependent pathways, leading to enhanced ligand binding on the cell surface. As a consequence, the cAMP/PKA pathway is aberrantly altered and might promote tumour migration, growth and proliferation. PM: Plasmatic Membrane; Ga, G $\beta$ , G $\gamma$ : G proteins; CREB: cAMP Response Element Binding protein; RTK Tyrosine Kinase Receptor. This figure was created with [www.BioRender.com](http://www.BioRender.com)

145 upon  $\alpha$ -MSH binding to MC1R is a cAMP-independent  
 146 process, it occurs through a transactivation mechanism of  
 147 the Tyrosine Kinase Receptor (RTK) c-KIT and plays an  
 148 important role in melanogenesis [37–39].

149 Another pathway linked to  $\alpha$ -MSH/MC1R axis is PI3K/  
 150 AKT, an intracellular signal transduction cascade that,  
 151 through the phosphorylation of several downstream  
 152 substrates, is involved in cellular functions such as cell  
 153 growth, proliferation, and differentiation. The key mol-  
 154 ecules involved in this signalling pathway are RTKs,  
 155 phosphatidylinositol 3-kinase (PI3K), phosphatidylinositol-  
 156 4,5-bisphosphate (PIP2), phosphatidylinositol-3,4,5-  
 157 triphosphate (PIP3) and AKT/protein kinase B. The  
 158 binding of RTK with growth factors and various stimuli  
 159 activates PI3K which in turn phosphorylates PIP2 leading  
 160 to the production of the second messenger PIP3 that reg-  
 161 ulates metabolic processes by recruiting signaling pro-  
 162 teins, including AKT/Protein kinase B (PKB) [40]. PTEN  
 163 (Phosphatase and TENSin homolog) is a phosphatase  
 164 responsible for the conversion of PIP3 to PIP2, acting as  
 165 an antagonist of the PI3K/AKT response. Investigating  
 166 the interaction between MC1R and PI3K/PTEN signal-  
 167 ing, it has been shown that upon  $\alpha$ -MSH binding, MC1R  
 168 interacts with PTEN and, by preventing its degradation,  
 169 inactivates AKT. It has also been shown that RHC MC1R  
 170 allelic variants have an impaired ability to interact with  
 171 PTEN, thus increasing AKT signaling and predisposing

172 melanocytes to melanomagenesis [41]. Studies with a  
 173 synthetic analog of  $\alpha$ -MSH revealed that the stimulation  
 174 of RHC MC1R variants activates DNA repair pathways  
 175 through a cAMP-independent mechanism mediated  
 176 by AKT activation [42]. On the other hand, it has been  
 177 shown that the binding of  $\alpha$ -MSH to MC1R activates  
 178 DNA repair and antioxidant signals in a cAMP-depend-  
 179 ent manner with decreased AKT phosphorylation [43].  
 180 Moreover an interplay between  $\alpha$ -MSH/MC1R and Per-  
 181 oxisome Proliferator-Activated Receptor- $\gamma$  (PPAR- $\gamma$ ) has  
 182 been reported [44]. Briefly,  $\alpha$ -MSH induces the release  
 183 of calcium ( $\text{Ca}^{2+}$ ) from endoplasmic reticulum (ER) by a  
 184 phospholipase C (PLC) dependent mechanism and  $\text{Ca}^{2+}$   
 185 efflux is connected with the translocation of PPAR $\gamma$  into  
 186 the nucleus, where it promotes the transcription of target  
 187 genes involved in lipid metabolism, adipogenesis, main-  
 188 tenance of metabolic homeostasis, inflammation and  
 189 anticancer effects in a variety of human tumours [45].

### $\alpha$ -MSH/MC1R: range of action

#### Maintenance of cell integrity and DNA damage repair.

#### MC1R polymorphism

190 In physiologic conditions, the main role of  $\alpha$ -MSH is to  
 191 protect skin from UV exposure by coordinating the pro-  
 192 duction of eumelanin. However, both in melanocytes and  
 193 keratinocytes, several studies have established that the  
 194  $\alpha$ -MSH/MC1R-cAMP axis is also involved in additional  
 195  
 196  
 197

198 responses, like antioxidant defences and DNA damage  
199 repair [42, 46]. UV radiation and melanin synthesis pro-  
200 cess are sources of ROS among which hydrogen peroxide  
201 ( $H_2O_2$ ), that is able to injure all cell compartments [47].  
202 After UV exposure, human melanocytes stimulate the  
203 generation of  $H_2O_2$  with a concomitant decrease in the  
204 activity of catalase, the enzyme most involved in  $H_2O_2$   
205 neutralization [48]. Therefore, it has been shown that  
206 treatment with  $\alpha$ -MSH protects melanocytes from oxida-  
207 tive stress since  $\alpha$ -MSH through MC1R induces both the  
208 activation and overexpression of catalase, reducing  $H_2O_2$   
209 production [49, 50].

210 Exposure to UV radiation is considered the most com-  
211 mon environmental risk factor for skin melanoma. The  
212 high prevalence of polymorphisms of MC1R, with more  
213 than 300 variants, makes it the best-established suscepti-  
214 bility gene for cutaneous melanoma [25, 51]. The associa-  
215 tion between some MC1R polymorphisms and red hair,  
216 freckles, and inability to tan (the RHC phenotype) was  
217 first reported in 1995 by Valverde et al. [52]. An exten-  
218 sive body of research shows that inactivating variants of  
219 MC1R are the main contributors to the increased risk of  
220 melanoma development, because the functions of UV  
221 protection and DNA damage repair are lost. According  
222 to their penetrance RHC MC1R alleles have been classi-  
223 fied as high (R) or low (r) variants. “R” variants include  
224 D84E, R142H, R151C, R160W, and D294H and people  
225 carrying these variants MC1R have the highest risk of  
226 developing melanoma and non-melanoma skin cancers  
227 whereas “r” variants: V60L, V92M, and R163Q showed a  
228 weaker association with the RHC phenotype [52–56].

229 In keratinocytes, the canonical  $\alpha$ -MSH/MC1R-cAMP-  
230 PKA pathway enhances Nucleotide Excision Repair  
231 (NER) activity: PKA directly phosphorylates the DNA  
232 damage sensors Ataxia Telangiectasia Mutated (ATM)  
233 and Rad3 related (ATR) which actively recruits the key  
234 NER protein Xeroderma Pigmentosum complementation  
235 group A (XPA) to sites of nuclear UV damage, thus accel-  
236 erating the clearance of UV-induced lesions and reducing  
237 the mutagenesis rate [57].

238 It has been reported that  $\alpha$ -MSH-MC1R axis can  
239 induce cutaneous carcinogenesis other than melanoma.  
240 Regarding Non-Melanoma Skin Cancers (NMSCs), it  
241 must be highlighted that carriers of two MC1R variant  
242 alleles have a higher risk of developing NMSC than the  
243 WT. However, it is not clear whether MC1R variants  
244 confer a relevant contribution in the genesis of skin car-  
245 cinomas [58].

#### 246 **Anti inflammatory and immunomodulatory properties**

247 In addition to its effects on melanocytes,  $\alpha$ -MSH has  
248 potent anti-inflammatory effects when administered  
249 systemically or locally [59]. Its immunomodulating

250 properties rely mainly on the binding with MC1R that  
251 is also expressed on monocytes, macrophages, and den-  
252 dritic cells (DCs).  $\alpha$ -MSH downregulates the produc-  
253 tion of pro-inflammatory cytokines IL-1, IL-6, TNF- $\alpha$ ,  
254 IL-2, IFN- $\gamma$ , IL-4, IL-13 and in contrast, anti-inflamma-  
255 tory IL-10 production is upregulated. At the molecular  
256 level,  $\alpha$ -MSH affects several pathways implicated in the  
257 regulation of transcription factors such as NF $\kappa$ B thus  
258 modulating inflammatory cell proliferation, activity and  
259 migration. NF $\kappa$ B regulates the transcription of genes  
260 involved in cell survival, and inhibition of NF $\kappa$ B activa-  
261 tion has been considered as a strategy for the treatment  
262 of melanoma [60–63].  $\alpha$ -MSH was discovered to be an  
263 ancient natural antimicrobial agent against two repre-  
264 sentative pathogens *Staphylococcus A.* and *Candida A.*,  
265 enhancing the local inflammatory reaction. It has been  
266 described that the candidacidal activity is mostly based  
267 on increasing intracellular cAMP levels that interferes  
268 with microbial regulatory pathway thus reducing fungal  
269 viability and germ tube formation [64].

270 From an oncological perspective, in human melanoma  
271 cells, an anti-inflammatory and anti-invasive effects of  
272  $\alpha$ -MSH have been reported [65].

#### 273 **Broad spectrum of $\alpha$ -MSH applications**

274 The pivotal role of  $\alpha$ -MSH in stimulating skin pigmen-  
275 tation and protecting from UV damage led to propose  
276 its topical application as strategy to improve a “sunless  
277 tanning” both for cosmetic purpose and mostly as skin  
278 cancer prevention. Therefore, by boosting the  $\alpha$ -MSH/  
279 MC1R-cAMP/PKA pathway activation and MiTF tran-  
280 scription, melanogenesis and DNA damage repair appa-  
281 ratus are enhanced [66].

282 Moreover, studies revealed that  $\alpha$ -MSH and synthetic  
283 analog peptides could be resolute for other conditions  
284 as Hypoactive Sexual Desire Disorders (HSDD) or be  
285 neuroprotective against cerebral ischemia/reperfusion  
286 injury as well as neovascularization inhibition [67–69].  
287 Additionally  $\alpha$ -MSH was found to be involved in appe-  
288 tite regulation (suppressor), in the pathogenesis of rest-  
289 less legs syndrome and in insulin resistance/sensitivity  
290 [70–73].

#### 291 **$\alpha$ -MSH/MC1R and cancer**

##### 292 **Melanoma**

293 Cutaneous malignant melanoma arises from melano-  
294 cytes, the pigment producing cells, and remains a chal-  
295 lenging disease due both to difficult early diagnoses and  
296 to the tendency to metastasize quickly to lymph nodes  
297 and distant organs such as liver, lung and brain. Although  
298 melanoma accounts for only about 10% of skin cancers it  
299 is responsible for the vast majority of deaths [74, 75].



300 Mortality is correlated with the stage at diagnosis and,  
301 to date, the management of metastatic disease remains a  
302 relevant clinical issue. Genetic mutations in oncogenes  
303 and tumour suppressor genes affecting the RAS-RAF-  
304 MEK-ERK signalling pathway (MAPK) are the main  
305 drivers in most cutaneous melanomas. A common muta-  
306 tion found in melanoma patients is BRAF<sup>V600E</sup> whereas  
307 tumours bearing NRAS mutations are less frequent but  
308 more aggressive and associated with shorter survival  
309 [76]. The MAPK cascade leads to activation of ERK1  
310 and ERK2 which translocate into the nucleus to regulate  
311 MiTF, cMYC and other transcription factors to sustain  
312 cell cycle progression, tumor invasion, metastasis and  
313 immune evasion [77].

314 The BRAF<sup>V600E</sup> mutation is found only in about 50% of  
315 melanoma and this fact limits the use of BRAF inhibitors  
316 (BRAFi). Moreover, most of patients in BRAFi therapy for  
317 metastatic melanoma relapses early after an initial par-  
318 tial response. The development of drug resistance within  
319 some metastatic clones causes the relapse of disease.

#### 320 MC1R overexpression in melanoma

321  $\alpha$ -MSH/MC1R/cAMP axis converges to the regulation of  
322 MiTF expression with a pivotal role for homeostasis but  
323 when impaired in melanoma environment it takes a role  
324 in tumor progression and survival. It has been reported  
325 that MiTF is a factor that supports melanoma stem cells  
326 properties [27, 78, 79].

327 Many studies showed increased levels of MC1R expres-  
328 sion on the surface of most melanomas (either primary or  
329 metastatic tissues) but not in carcinoma cell lines making  
330 it a valuable marker of melanoma cells [80, 81]. Moreo-  
331 ver, the tumor itself overproduces  $\alpha$ -MSH, leading to an  
332 autocrine hyperproliferative process, described in mela-  
333 noma metastases [82].

#### 334 EPAC in melanoma

335 cAMP regulates a wide range of physiologic processes in  
336 melanocyte homeostasis mainly by acting through the  
337 canonical PKA-CREB pathway. During melanoma initia-  
338 tion the system might switch and impaired cAMP signal-  
339 ing might sustain the tumor environment in a way that  
340 need to be explored deeply. However Rodriguez et al.  
341 showed that topical application of forskolin that directly  
342 activates AC, increases the level of cAMP, speeding mela-  
343 noma tumor development in BRAF<sup>V600E</sup>/PTEN mouse  
344 model of melanoma and stimulating the proliferation  
345 of mouse and human primary melanoma cells in vitro.  
346 Although the process was cAMP-driven, an alternative  
347 downstream effector called Exchange Protein directly  
348 Activated by cAMP (EPAC) is involved. EPAC has been  
349 identified in 1998 and it acts as a guanine nucleotide  
350 exchange factor for the GTPase Ras family: RAP1 and

351 RAP2 [83]. Modulating different signaling pathways,  
352 EPAC is involved in several cellular processes such as  
353 cell proliferation, migration, apoptosis and adhesion in  
354 various tissues [84]. In addition it has been shown that  
355 MC1R-cAMP-EPAC cascade promotes DNA repair by  
356 increasing the nuclear translocation of XPA protein in  
357 keratinocytes [57]. On the other hand, EPAC has shown  
358 to have a pro-metastatic role as it acts by activating ERK  
359 pathway and  $\alpha_v\beta_3$  integrin through RAP1 thus promoting  
360 tumorigenesis and migration in human lung cancer cells  
361 but also by influencing other signalling cascades in cells  
362 derived from human metastatic melanomas, in human  
363 melanoma samples and melanoma cell lines [85–87]. The  
364 current hypothesis is that EPAC could have a different  
365 function during different stages of melanoma progres-  
366 sion, with EPAC-RAP1 axis showing both a pro-survival  
367 role in primary melanoma and an anti-survival role in  
368 metastatic melanoma. Hence, it could be speculated that  
369 proliferation is inhibited during metastasis promoting an  
370 invasive phenotype [88, 89].

#### 371 $\alpha$ -MSH-based strategies in melanoma treatment

372 The MC1R receptor is recognized to play a key role in  
373 melanocyte, melanosome, and melanoma cell (patho)  
374 physiology. Regarding metastasis, overexpression levels  
375 of MC1R, and MSH production by the neoplastic tissue  
376 itself are well-established data in the scientific literature.  
377 In this way, metastasis creates and self-maintains an  
378 autocrine loop that stimulates the growth, proliferation  
379 and invasiveness of the neoplasm, with the possibility of  
380 recurrence at metastatic sites, progression and dissemi-  
381 nation, creating new metastatic sites and thus making the  
382 patient life-threatening.

383 This mechanism may also play a role in resistance to  
384 targeted therapy against mutated B-RAF (V600E B-RAF)  
385 where this phenomenon is described to be enhanced,  
386 suggesting that MC1R activation may contribute to the  
387 development of cancer resistance to dabrafenib. For these  
388 reasons, our group among others posits MC1R inhibition  
389 as a possible strategy to counteract this autocrine loop  
390 that intervenes in metastatic disease.

391 MC1R potentially constitutes an ideal target for design  
392 of novel anticancer drugs both for its involvement in mel-  
393 anocytic pathophysiology and for its high levels of tissue-  
394 specific expression in melanoma cells.

395 At present, many works have shown promising results  
396 using the tissue specificity of MC1R for melanocytic tis-  
397 sues as an antitumor strategy.

398 Liu and collaborators reported that the immunotoxin  
399  $\alpha$ -MSH-PE38KDEL, constructed by connecting the  
400  $\alpha$ -MSH gene to PE38KDEL (a mutated and truncated  
401 form of a bacterial toxin), showed in vitro high cytotox-  
402 icity on MC1R positive melanoma cell lines, promoting

apoptosis via Erk1/2/MITF/TYR signaling modulation in a MC1R-dependent manner [90]. They demonstrated that MC1R is essential for the immunotoxin-mediated cytotoxicity, promoting melanoma cell apoptosis inhibiting MITF and TYR expression. In fact, the overexpression of MITF or TYR abolishes  $\alpha$ -MSH-PE38KDEL induction of apoptosis in mouse melanoma B16-F10 cell line. The authors demonstrated that the same pathway modulation significantly inhibited the *in vivo* tumor-forming ability of B16-F10 cells, when injected into athymic BALB-C nude mice.

Other works, using radionuclide- $\alpha$ MSH analogs conjugates, depicted interesting results in a theranostic setting. These studies, conducted in melanoma-bearing mouse models, demonstrated the high specificity of those molecules for MC1R, with a good bioavailability and renal clearance. In *in vivo* preclinical experimental animal model bearing mouse B16F1 or B16F10 melanoma radiolabelled peptides targeting MC1R, the radionuclide- $\alpha$ MSH analogs conjugates are able to selectively and specifically kill melanoma cells, sparing healthy cells and normal tissue. These studies are reviewed and summarised in two recent works [91, 92]. Shi and collaborators considered studies about molecular probes for melanoma theranostics targeting either MC1R or melanine. These MC1R targeted radiotracers, displaying a good tumor uptake and retention, could potentially be used for imaging of MC1R expressing melanoma in clinic. These imaging probes could be transformed into therapeutic radiopharmaceuticals through radiolabeling with beta- or alpha emitters. [91].

These novel sensitive and specific MC1R targeted radiotracers can overcome the actual limitation of (18)F FDG PET (I.e poor selectivity for distinguishing tumor from inflammatory tissue and low sensitivity in the detection of both nodal and lung and brain metastases) [92]. Furthermore, in a potential clinical application, cytotoxic radiation generated by therapeutic radionuclides could help treat remnant metastatic deposits, in an adjuvant setting, after surgical excision of the tumor.

Notheworthy, the group of Cachin reported the results of a multicenter phase III clinical trial [93]. This trial evaluated the accuracy of a new benzamide-derivative melanin targeted radiotracer, the (123)I-BZA2 radiopharmaceutical. This trial was prematurely closed after the enrollment of 87 patients, because of the low sensitivity of the radioconjugate in comparison to (18)F FDG when considering both a patient-based and a lesion-based analyses. However, (123)I-BZA2 demonstrated higher specificity than (18)F FDG for diagnosis of melanoma metastasis in a lesion-based analysis.

Further clinical studies are needed to validate the results of promising pre-clinical works.

## Conclusions and perspectives

In this study we reviewed and summarised the molecular biology of  $\alpha$ -MSH/MC1R, their range of action beyond pigmentation, the role of  $\alpha$ -MSH/MC1R axis in melanoma and the MC1R targeting therapeutic strategies that have been proposed for melanoma.

$\alpha$ -MSH is the key hormone for melanocytic metabolism. It is not only the main actor of skin pigmentation but it displays also anti-inflammatory and anti-microbial properties. Among melanocortin receptors, melanocytes mainly express MC1R, whose binding with  $\alpha$ -MSH promotes both the production of eumelanin, through the activation of AC/cAMP/PKA pathway, and melanocytic proliferation, survival and migration.

In summary, this review shows the ambivalence in the relationship between  $\alpha$ -MSH and its membrane receptor. In physiological condition, the intracellular pathways elicited by this bond ensure skin pigmentation, DNA repair and anti-microbial and inflammatory defense. On the other hand, in pathological conditions, the overstimulation of the  $\alpha$ -MSH/MC1R axis can lead to survival, uncontrolled proliferation, and invasion of cancer cells in metastatic melanoma. Moreover, some reports showed that synthetic alpha-MSH analogues (MC1R agonists) could lead to proliferation of melanocytic cells in predisposed patients, representing an increased risk for atypical naevi and melanoma development [94, 95]. Notheworthy, Kansal et al., reported that the inhibition of MC1R diminishes melanoma growth and increases survival of mice bearing melanoma [96]. Being overexpressed in metastatic melanoma, and particularly in targeted therapy-resistant clones, MC1R could represent a molecular target for metastatic melanoma and its inhibition a molecular strategy to delay resistance.

## Abbreviations

AC	Adenylyl Cyclase	491
ACTH	Adrenocorticotropic Hormone	492
Agrp	Agouti related protein	493
$\alpha$ -MSH	Alpha-melanocyte stimulating hormone	494
ATM	Ataxia Telangiectasia Mutated	495
BRAF <sup>i</sup>	BRAF inhibitors	496
Ca <sup>2+</sup>	Calcium	497
cAMP	Cyclic AMP	498
Dcs	Dendritic cells	499
DCT	Dopachrome Tautomerase	500
EPAC	Exchange Protein directly Activated by cAMP	501
ER	Endoplasmic reticulum	502
GoF	Gain of Function	503
GPCR	G-Protein Coupled Receptor	504
HSDD	Hypoactive Sexual Desire Disorders	505
IFN- $\gamma$	Interferon-gamma	506
MAPK	Mitogen-Activated Protein Kinase	507
MC1R	Melanocortin 1 receptor	508
MCRs	Melanocortin Receptors	509
MiTF	Microphthalmia inducing Transcription Factor	510
NER	Nucleotide Excision Repair	511
Nf $\kappa$ B	Nuclear factor kappa-light-chain-enhancer of activated B cells	512

514	PI3K	Phosphatidylinositol 3-kinase
515	PIP2	Phosphatidylinositol-4,5-bisphosphate
516	PIP3	Phosphatidylinositol-3,4,5-triphosphate
517	PKA	Protein Kinase A
518	PLC	Phospholipase C
519	POMC	Pro-opiomelanocortin hormone
520	PPAR-γ	Peroxisome Proliferator-Activated Receptor-γ
521	PTEN	Phosphatase and TENSin homolog
522	RHC	Red-Hair-Colour
523	RTK	Tyrosine Kinase Receptor
524	TYR	Tyrosinase
525	TNF-α	Tumor Necrosis Factor alpha
526	TRP	Tyrosinase Related Protein
527	XPA	Xeroderma Pigmentosum complementation group A

### Acknowledgements

528 The authors thank Associazione Piccoli Punti and Mr Nicolò Socal for their sup-  
529 port during the preparation and the revision of the manuscript.  
530

### Author contributions

531 LDO and NP were responsible for conceiving the ideas. All authors wrote  
532 different parts of the manuscript. All authors read and approved the final  
533 manuscript.  
534

### Funding

535 Open access funding provided by Università degli Studi di Padova within the  
536 CRUI-CARE Agreement. The fellowship of NP was supported by the "5 × 1000"  
537 IOV grant.  
538

### Availability of data and materials

539 Not applicable.  
540

### Declarations

541

### Ethics approval and consent to participate

542 Not applicable.  
543

### Consent for publication

544 Not applicable.  
545

### Competing interests

546 The authors declare that they have no competing interests.  
547  
548

549 Received: 3 May 2023 Accepted: 1 August 2023

550

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