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Review

Cutaneous Biology

Diverse effects of cAMP signaling in melanoma support the role of distinct cAMP microdomains in melanomagenesis, metastasis, and resistance to therapy

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Abstract

Introduction

Cyclic adenosine monophosphate (cAMP) is a second messenger signalling molecule, present from bacteria to man, involved in many cellular functions. Pathways mediated by cAMP signalling are widespread in the body. cAMP plays a role in both normal physiology and in various diseases such as cancer. In melanoma, cAMP signalling can either activate or inhibit growth thereby leading to conflicting results. In many cases the same cAMP signalling proteins such as melanocortin 1 receptor (MC1R), protein kinase A (PKA), and phosphodiesterases (PDEs) are involved in both growth stimulation and inhibition begging the question how cAMP can seemingly lead to disparate cellular events in melanoma via the same signalling cascade. Recently, it has become clear that cAMP signals in discrete microdomains capable of responding to distinct upstream signals and, via identical protein machinery, can induce unique cellular events. The complex role of cAMP signalling in melanoma supports an important role for cAMP microdomains in melanoma biology. The aim of this review was to discuss how diverse effects of cAMP signalling in melanoma support the role of distinct cAMP microdomains in melanomagenesis, metastasis, and resistance to therapy.

Conclusion

Further research is warranted to elucidate the impact of contextual cues and intrinsic cell type differences on the role of cAMP signaling in melanoma, in order to better understand melanomagenesis, metastasis, and resistance and to help identify new therapeutic targets.

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Introduction

Cyclic adenosine monophosphate (cAMP) is a signalling molecule present from bacteria to man. In mammalian cells, there are ten different enzymes called adenylyl cyclases (AC) that produce cAMP from ATP. ADCYs 1-9, transmembrane ACs (tmACs), synthesize cAMP subsequent to ligand binding a seven-transmembrane G protein couple receptor (GPCR). ADCY10, soluble adenylyl cyclase, is not G protein responsive but instead is regulated by calcium, bicarbonate and adenosine triphosphate (ATP).[1,2] Many effector proteins of cAMP have been identified, notably Protein Kinase A (PKA), exchange proteins activated by cAMP (EPAC) and cyclic nucleotide-gated channels. Pathways mediated by cAMP signalling are widespread in the body, with different functions depending on the location, context and cellular milieu. The pathway is turned off when cAMP is hydrolysed to AMP by a class of enzymes called phosphodiesterases (PDEs), which are expressed in many tissues and cell types.

Mutations affecting cAMP signalling pathways have been implicated in a variety of genetic diseases with associated malignancies and in some cases acquired cancer. For example, inactivating mutations in the PRKRA1A gene on chromosome 17q22-24, which codes for the alpha 1-regulatory subunit of PKA, have been observed in the majority of Carney Complex cases (a hereditary disease characterized by cardiac myxoma, endocrine disorders and skin pigmentation).[3] Activating mutations of the GNAS1 gene, which codes for the Gs alpha subunit of the heterotrimeric G protein, result in constitutive activation of tmACs and are associated with McCune-Albright syndrome (characterized by polyostotic fibrous dysplasia, gonadotropin-independent precocious puberty, and café-au-lait macules).[4] In addition, inactivating mutations of PDEs are associated with both prostate and testicular cancer[5,6] suggesting that these two cancer types are supported by an elevation of cAMP.

However, in other cancers with known mutations in cAMP regulatory proteins, the association between cAMP and the development of cancer is less clear. For example, in melanoma, cAMP signalling can be either activated or inhibited with conflicting results. In this review we will discuss the current understanding of cAMP signalling in melanoma (Figure 1).

Evidence supporting an inhibitory role for cAMP in melanoma

In this section, we will discuss how cAMP inhibits melanoma growth and in concert how loss of cAMP signalling can enhance melanomagenesis. Several cAMP pathway proteins can inhibit melanomagenesis. The first of these is the melanocortin 1 receptor (MC1R), a G protein coupled receptor (GPCR) that signals through cAMP to mediate melanin synthesis. Strong MC1R activity is associated with the production of black/brown eumelanin, which is effective at absorbing ultraviolet radiation (UV) and reactive oxygen species (ROS).[7] Polymorphisms at the MC1R gene, as are found in individuals with red hair and fair skin, lead to less cAMP synthesis and are

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associated with production of red/yellow pheomelanin, which has weak photoshielding capacity relative to eumelanin and can lead to the generation of ROS.[8] Thus, decreased MC1R/cAMP signalling may be associated with increased melanoma risk and elevated cAMP levels would be expected to prevent melanomagenesis.[9] In agreement with this hypothesis, a meta-analysis found that MC1R mutations, in addition to affecting pigmentary pathways, may also play a role in melanomagenesis.[10]

As a further confirmation that loss of MC1R activity enhances melanomagenesis, one study found that in mice carrying an inactivating mutation in the MC1R gene (a model for the human red hair/fair skin phenotype) which limited the epidermis to only pheomelanin synthesis supported melanomagenesis via pheomelanin-dependent production of oxidative DNA and lipid damage in a UV-independent manner.[11]

Since multiple lines of evidence suggest that loss of MC1R activity enhances melanomagenesis, the expectation is that activation of MC1R activity would inhibit melanoma. Consistent with that hypothesis, MC1R activation and subsequent cAMP synthesis directly inhibits melanoma cell proliferation by delaying progression from G2 into M phase via phosphorylation of cdc25B, a cyclin-dependent kinase 1-activating phosphatase.[12]

cAMP-dependent pathways can also inhibit the mitogen activated protein kinase (MAPK) pathway, a pathway important for both proliferation and differentiation. The prototypical MAPK pathway is initiated by tyrosine kinase receptor stimulation of small G-proteins (e.g, RAS) followed by the activation of several downstream protein kinases (RAF, MEK and ERK). Three distinct mammalian RAF proteins—ARAF, BRAF and CRAF—each perform a different function, both physiologically and in cancer. In normal melanocytes, BRAF but not CRAF transducers the signal from RAS to MEK because CRAF is inhibited by cAMP–dependent PKA. This inhibitory

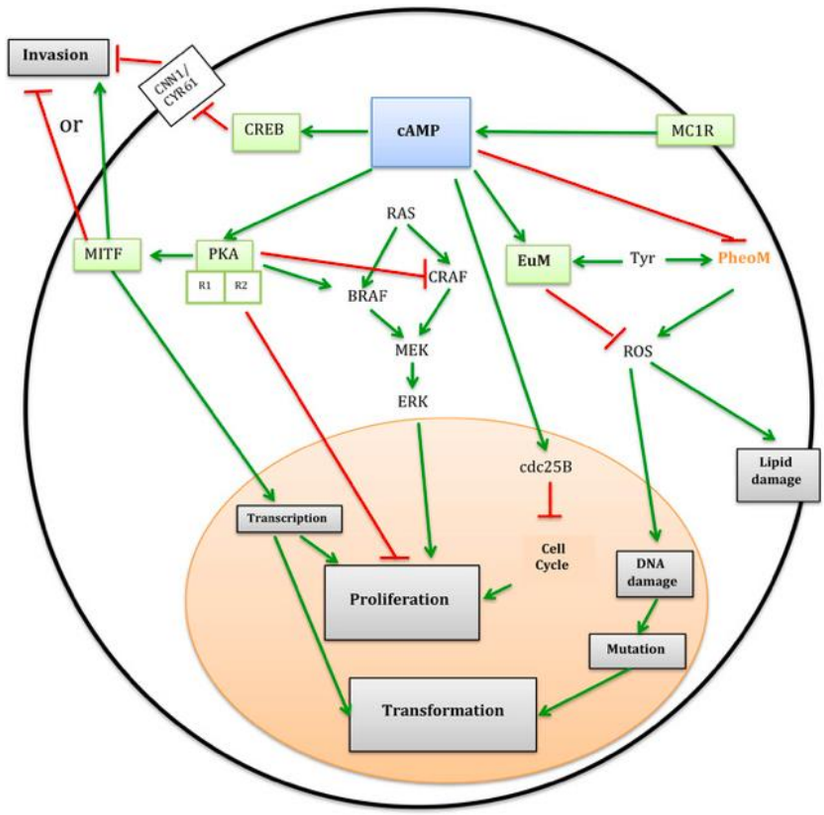

Figure 1: The role of cAMP in melanoma: promotor and suppressor of melanomagenesis. ATP is converted to cAMP by adenylylcyclases and phosphodiesterases metabolize cAMP to AMP. Activation is shown with green arrows and inhibition with red arrows. PheoM is pheomelanin, EuM is eumelanin, Tyr is tyrosinase, BRAF is the serine/threonine-protein kinase B-raf, CRAF is the serine/threonine-protein kinase C-raf, MEK is the extracellular signal-regulated kinase or the mitogen-activated protein kinase and ERK is the extracellular signal-regulated kinase and RAS represents the protein superfamily of small GTPases.

cAMP pathway is hijacked by melanoma. In RAS-mutated melanoma, CRAF rather than BRAF is utilized to activate MEK/ERK and this switch in RAF utilization is due to a disruption of cAMP signaling, most likely from the activation of PDE4. In other words, a loss of cAMP promotes melanoma growth in RAS-mutated melanoma.[13,14]

In another example, in NRAS mutated melanoma, reactivation of the cAMP pathway can inhibit proliferation and induce apoptosis of tumour cells[15] and in human oral malignant melanoma cells, inhibition of PDE2A2 (the only PDE splice variant in these cells), and subsequent elevation of cAMP levels, results in inhibition of DNA synthesis and reduction of cell proliferation (via G2/M checkpoint arrest).[16] The importance of cAMP signalling in promoting melanoma growth suggests this pathway may be a therapeutic target for melanoma.[17]

There are multiple mechanisms aside from CRAF/BRAF switching in which cAMP via activation of PKA can suppress melanoma growth. PKA has multiple distinct functions in the cell due to its varied localization. PKA is composed of four subunits, two catalytic and two regulatory (R1 and R2). PKA is targeted to different areas of the cell by binding of the regulatory subunit to a class of proteins called A-kinase anchoring proteins (AKAPs), which are present throughout the cell in different organelles. R1 containing PKAs tend to influence cell proliferation, while R2 PKAs are mostly involved in differentiation. Human melanomas express an altered ratio of

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the two types of PKA regulatory subunits, with higher-than-normal levels of R1 as compared to R2. Restoring R2 activity via a R2 subunit selective cAMP analogue led to diminished proliferation and increased apoptosis of melanoma cells.[18]

Finally melanoma with intrinsic resistance to BRAF inhibitors, including BRAFV600E, NRAS mutant, and BRAF wild-type melanomas, often have a deficiency in cAMP synthesis and restoring cAMP levels sensitizes these cells to BRAF inhibitors via PKA-dependent phosphorylation of CRAF.[19]

Evidence supporting the growth of melanoma by cAMP signalling

Contrary to the findings reviewed above, multiple investigators have demonstrated that some melanomas favour elevated cAMP signalling. Some MC1R variants found in melanoma cells are associated with elevated cAMP signalling, through non-canonical pathways, and with cell proliferation.[20]

Furthermore, cAMP may play a role in promoting melanoma drug resistance. Garraway and colleagues recently demonstrated that of 110 genes identified as conferring resistance to MAPK pathway inhibitors, GPCRs, the adenylyl cyclase gene ADCY9, and the catalytic subunit of PKA alpha were identified as conferring resistance to all MAPK-pathway inhibitors examined.

Taken together, these findings suggest that signalling through GPCRs and activation of the AC-cAMP-PKA axis may confer resistance to MAPK inhibitors in melanoma. Expression of transcription factors downstream of the MAPK and cAMP pathways (e.g. microphtalmia-associated transcription factor (MITF)) resulted in resistance. Treatment with a combination of MAPK-pathway and histone deacetylase inhibitors suppressed cAMP-mediated resistance and MITF expression.[21] MITF amplification can be more prevalent in metastatic melanoma as compared to local disease, and is correlated with decreased overall patient survival. Reduction of MITF activity can sensitize melanoma cells to chemotherapeutic agents.[22] Despite its role in promoting proliferation, transformation and metastasis, however, the role of MITF in invasion is unclear; it has shown to both promote and inhibit invasion.[23,24]

Beyond melanomagenesis and drug resistance, cAMP signalling is associated with melanoma invasion and metastasis. Activation of cAMP response element binding protein (CREB), a known target of PKA, can promote metastasis by down-regulating cysteine-rich protein 61 (CCN1/ CYR61), an extracellular matrix-associated protein that can regulate a variety of cellular activities, including adhesion, motility, survival, proliferation and angiogenesis. [25]

Finally, Calipel and colleagues demonstrated that inhibition or deletion of PKA reduced BRAF activity, ERK1/2 signalling, and cell proliferation in BRAF wild type uveal melanoma cells but did not affect BRAF mutant uveal melanoma cells. This finding suggests that PKA and thus cAMP may have a key role in promoting BRAF/ERK signalling in BRAF wild type uveal melanoma cell growth. [26]

Discussion

The authors have referenced some of their own studies in this review. The protocols of these studies have been approved by the relevant ethics committees related to the institution in which they were performed.

The role of cAMP signalling in melanoma is more complex than once thought. One explanation for how this second messenger seemingly has multiple disparate cellular functions is that cAMP microdomains consisting of AC, PKA, and PDE resides in multiple distinct locations throughout the cell and respond to their own unique upstream activators.[27,28,29] While inhibition of specific isoforms of PDE may result in cAMP elevation, the downstream effects will vary widely depending on the microdomain in which this elevation has occurred.

Variations in site of tumour origin, melanoma cell genotype, and site of metastasis may also play a role. For example, cAMP may function differently in the course of melanomagenesis than it does in the development of resistance after therapeutic pressure has been exerted, or during metastasis.

Conclusion

Further research is warranted to elucidate the impact of contextual cues and intrinsic cell type differences on the role of cAMP signalling in melanomagenesis to help identify new therapeutic targets.

Conflict of interests

JH Zippin is a founder of CEPBiotech which commercializes antibodies directed against the soluble adenylyl cyclase.

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