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Review

Investigation of cAMP microdomains as a path to novel cancer diagnostics ☆

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ABSTRACT

Understanding of cAMP signaling has greatly improved over the past decade. The advent of live cell imaging techniques and more specific pharmacologic modulators has led to an improved understanding of the intricacies by which cAMP is able to modulate such a wide variety of cellular pathways. It is now appreciated that cAMP is able to activate multiple effector proteins at distinct areas in the cell leading to the activation of very different downstream targets. The investigation of signaling proteins in cancer is a common route to the development of diagnostic tools, prognostic tools, and/or therapeutic targets, and in this review we highlight how investigation of cAMP signaling microdomains driven by the soluble adenylyl cyclase in different cancers has led to the development of a novel cancer biomarker. Antibodies directed against the soluble adenylyl cyclase (sAC) are highly specific markers for melanoma especially for lentigo maligna melanoma and are being described as "second generation" cancer diagnostics, which are diagnostics that determine the 'state' of a cell and not just identify the cell type. Due to the wide presence of cAMP signaling pathways in cancer, we predict that further investigation of both sAC and other cAMP microdomains will lead to additional cancer biomarkers. This article is part of a Special Issue entitled: The role of soluble adenylyl cyclase in health and disease.

1. Introduction

Cyclic adenosine monophosphate (cAMP) is one of the most ancient signaling molecules present from bacteria to man. In mammals, cAMP controls a wide range of cellular processes and is present in every cell type and organ. cAMP is synthesized from ATP by a class of enzymes called adenylyl cyclases (ACs), which are encoded by 10 different genes (ADCY1–10) [1]. ACs 1–9 encode for proteins with a fairly similar structure in that all of them are transmembrane proteins (tmACs) and reside principally at the plasma membrane and endosomes making these ACs well suited to respond to extracellular signals. tmACs provide an important link between hormonal (e.g., melanocortin stimulating hormone) signals and intracellular processes. In many ways, tmACs function to coordinate cells within a tissue. Most tmACs are principally regulated by G protein coupled receptors via direct stimulation by heterotrimeric G proteins either by direct interaction between tmACs and the Gαs subunit or β/γ subunits [1]. Regulation of tmACs can be divided into four groups: Group 1, Ca^{2+}/calmodulin-stimulated AC1, AC3, and AC8; Group 2, Gα-stimulated and Ca^{2+}-insensitive AC2, AC4, and AC7; Group 3, Gαi/Ca^{2+}/PKA-inhibited, AC5 and AC6; and Group 4, forskolin/Ca^{2+}/ Gα-insensitive, AC9 [1]. The more recently identified AC (ADCY10) is also called the soluble adenylyl cyclase (sAC), which unlike the tmACs has no membrane spanning motifs and therefore is free to localize to multiple locations within a cell of which the best characterized are the nucleus and mitochondria [2,3]. sAC is primarily regulated by changes in bicarbonate [4] and calcium ions [5]. Bicarbonate ion functions to both increase the Vmax of the enzyme and alleviate substrate, ATP, and inhibition [5]. The ability to sense bicarbonate allows sAC to function as a pH sensor [2,6]. Calcium functions to decrease the Km for MgATP [5]. Whereas most proteins have a Km for MgATP that far exceeds the normal resting levels of ATP in the cell, ~1–3 mM (e.g., tmACs have a Km for MgATP in tens to hundreds of micromolar [7]), sAC's Km in the presence of calcium is approximately 1–3 mM. The elevated Km for MgATP enables sAC to sense changes in metabolism [8,9]. In addition to regulation by bicarbonate, calcium and ATP, the sAC protein contains a P loop [10], a heme binding domain [11] and other predicted protein domains and phosphorylation sites that may provide

Abbreviations: cAMP, Cyclic adenosine monophosphate; sAC, Soluble adenylyl cyclase; tmAC, Transmembrane adenylyl cyclase; PDE, Phosphodiesterase; PKA, Protein kinase A; AKAP, A kinase anchoring protein; EPAC, Exchange protein activated by cAMP; GEF, Guanine nucleotide exchange factor; GAP, GTPase-activating proteins; HMB, Human melanoma black; MITF, Microphthalmia transcription factor

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additional regulatory mechanisms. As a pH and metabolic sensor, sAC is poised to function as an intrinsic sensor of cellular health.

Since sAC and the nine different tmACs each respond to distinct signals yet produce the same second messenger, cAMP, it is important for the cell to respond specifically to each source of cAMP. The cell has at its disposal three families of cAMP effector proteins, an entire family of cAMP catabolizing enzymes, and a family of scaffolding proteins allowing the cell to establish spatially and temporally separate cAMP signaling domains (microdomains) capable of inducing a wide variety of downstream cascades. cAMP microdomains were first appreciated in the 1970s by the groups of Keely, Hayes, Brunton, and others when they recognized that different tmAC activating hormones (e.g., β-adrenergic receptor and prostaglandin E1 agonists) all led to cAMP elevation but each induced unique cellular events in cardiomyocytes, e.g., only β-adrenergic stimulation induced increased contractility and glycogen metabolism [12].

In the following sections, we will review the role of exchange protein activated by cAMP, protein kinase A, and A kinase anchoring proteins in cAMP signaling and how investigations of each have contributed to our understanding of cAMP microdomains. For the purpose of brevity, we have chosen not to review the vast literature of cyclic AMP gated ion channels, which lie at the plasma membrane and are an important link between cAMP signaling and ion transport, and phosphodiesterases, which catabolize cAMP into AMP and can be localized to many different areas of the cell. Finally, we will discuss cAMP signaling in cancer, specifically melanoma, and how a better understanding of cAMP microdomains in cancer may improve cancer diagnostics.

2. Exchange protein activated by cAMP

The exchange protein activated by cAMP (EPAC) family of effector proteins was discovered coincidently by two laboratories in 1998. One group initiated a database search to determine how cAMP could activate the small G protein Rap1 in a PKA-independent manner [13] while the other group found both EPAC1 (cAMP-GEF-I) and EPAC2 (cAMP-GEF-II) in a differential display screen for novel cAMP binding proteins [14]. Small G proteins cycle between a GDP bound inactive state and a GTP bound active state. Proteins such as guanine nucleotide exchange factors (GEFs) facilitate the exchange of GDP for GTP whereas GTPase-activating proteins (GAPs) help G proteins convert GTP back to GDP. Because small G proteins have a very slow intrinsic GTPase activity, restricted access to GAPs leads to prolonged activation of the proteins, explaining why Ras activating mutations block association of Ras with GAPs. Therefore, proper control of the Rap1 and Rap2 cascades require both spatial and temporal control over the GEFs and GAPs. EPAC can have multiple splice variants and is broadly expressed depending on developmental stage and disease [15]. EPAC1 is highly expressed in the heart, kidneys, blood vessels, adipose tissue, central nervous system, ovaries, and uterus [14,16] with multiple hematopoietic cell types. EPAC2, however, is mostly expressed in the central nervous system, adrenal gland, and pancreas with no detectable expression in hematopoietic cells [17]. EPACs are multidomain proteins with regulatory domains at the N terminus and the catalytic GEF domain at the C terminus. Both EPAC genes encode for cAMP binding domains (cAMP-B domain in EPAC1 and both cAMP-A and B domains in EPAC2), a disheveled-Egl-10-pleckstrin (DEP) domain that is responsible for plasma membrane localization, a Ras exchange motif (REM) domain that assists in catalysis, a Ras association (RA) domain that allows for interaction with GTP bound G proteins (e.g., Ras), and the CDC25HD domain that provides GEF activity [17]. Binding of cAMP to the cAMP-A and/or B domains along with interaction with small G proteins via the RA domain can alter the location of EPAC proteins and control when and where Rap1 is activated [15,18]. Interestingly, EPAC1 is known to associate with Ran-GTP, which is thought to control its nuclear localization. Nuclear EPAC1 is thought to control the DNA damage response and the DNA damage-responsive DNA-protein kinase [17]. EPAC controls nuclear export of histone deacetylase (HDAC)-4 and -5 [19,20]. The presence of EPAC in the nucleus predicts that a source of cAMP should exist to regulate its activity and/or localization. sAC was shown to localize to the nucleus in numerous cell types by cellular fractionation and immunohistochemistry [3] and later confirmed by FRET based imaging [21]. sAC moves in and out of the nucleus of certain cells during specific inflammatory diseases (e.g., psoriasis), infectious diseases (e.g., human papillomavirus infection), and malignancy (e.g., squamous cell carcinoma and melanoma) discussed in more detail below [3,22,23]. Since sAC is known to regulate Rap1 in an EPAC-dependent manner it is plausible that sAC is a nuclear source of cAMP regulating EPAC in the nucleus. However, it is important to note that some groups have found that activation of tmACs can also lead to a rise in nuclear cAMP as measured by a EPAC-FRET probe [21].

3. Protein kinase A

Protein kinase A (PKA) is one of the first characterized and best known cAMP effector proteins. It is a broad specificity serine/threonine kinase that consists of two catalytic (C) and two regulatory (R) subunits. This tetrameric holoenzyme is activated when two molecules of cAMP bind to each R subunit resulting in a confirmational change and release of active C subunit [24]. There are three C subunits (α, β, and γ). Cα and Cβ are ubiquitously expressed but Cγ is expressed mainly in the testis [25]. There are four R subunits which fall into two categories: RI (α and β) and RII (α and β), and all isoforms are able to bind cAMP. Each isoform has unique protein domains, which target isoforms to distinct areas of the cell, and therefore, are subject to different regulation and downstream substrates [24–26]. Regulatory subunits are not functionally redundant. The two major stable and well-folded domains are the dimerization/docking domain at the N terminus and the two tandem cAMP binding domains at the C-terminus. The D/D domain is a four-helix bundle that binds to the amphipathic helix motif characteristic of A kinase anchoring proteins (AKAPs), which will be discussed in more depth in the next section [27]. A number of studies have identified PKA at distinct locations throughout the cell including the mitochondria [13], the nucleus [28], and the centriole [29], in addition to the plasma membrane. Localization of PKA is driven by the association of PKA with AKAPs.

4. A kinase anchoring proteins

AKAPs are a large and diverse group of proteins defined by their ability to bind PKA [25]. There are 43 known genes, which encode the AKAP family of proteins [30] and many of these genes produce mRNAs that are alternatively spliced thereby producing >70 distinct AKAP proteins. As mentioned above, AKAPs are able to bind to the R subunit of PKA via an amphipathic helix consisting of 14–18 amino acids that bind to the D/D domain on R subunit [31]. Originally AKAPs were thought to only bind to RII subunits but it is now accepted that RI subunits can also bind albeit with much lower affinity [31,32]. AKAPs are found throughout the cell in many different organelles and associated with different proteins. This allows AKAPs to tether PKA to different areas of the cell thereby directing cAMP signaling to specific targets. While AKAPs are defined by the ability to bind PKA they can also form multimolecular signaling complexes, which can include other cAMP signaling proteins such as PDEs and EPAC, and/or proteins from other signaling cascades such as MAPK signaling proteins [33]. These AKAP defined microdomains have been well established in cardiac myocytes and the role of AKAPs in these cells and others has been reviewed previously [33,34]; however, we will illustrate specific examples of how AKAPs define cAMP microdomains to highlight how cAMP can signal in unique areas of the cell.