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Original article

Protein kinase A (PKA) pathway is functionally linked to androgen receptor (AR) in the progression of prostate cancer

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Abstract

Objectives: In the present study, we investigated whether the cyclic adenosine monophosphate (cAMP)-activated protein kinase A (PKA) pathway may regulate the expression of AR and prostate-specific antigen (PSA) and whether there is a correlation between the expression of cAMP/PKA-associated genes and androgen receptor (AR) in patients with prostate cancer (CaP).

Materials and methods: The functional studies were performed in LNCaP and PC3 cell lines. Data on the mRNA expression of sets of genes in human clinical samples, including prostate tissues from organ donors, prostate primary cancer, and metastatic cancer, were extracted from the National Center for Biotechnology Informations Gene Expression Omnibus (GEO) database. Statistical tests were applied.

Results: We showed that elevated levels of cAMP/PKA pathways induced an increased expression of AR and PSA proteins in LNCaP cells in the absence of androgen. A cAMP-associated phosphodiesterase-4 (PDE4) inhibitor, rolipram induced an up-regulation in AR expression, whereas a cAMP enhancer, forskolin increased PSA level without affecting AR expression. Forskolin treatment increased the level of PKA R1α in LNCaP cells, but remarkably inhibited R1α expression in aggressive PC3 cells. In patients with CaP, we found that the expression of genes encoding R1α and phosphodiesterase-4B was statistically significantly lower in the metastatic specimens than that in the primary CaP specimens or in the normal prostate tissues ($P < 0.01$) and was reversely correlated with *AR* expression. Conversely, *AR* and *PRKAR2B* mRNA expressions were significantly higher in metastatic lesions than those in the primary CaP specimens or in the normal prostate tissues ($P < 0.01$).

Conclusion: Our study revealed a novel mechanism to precisely define the functional and clinical interrelationship between the cAMP/PKA pathway and AR signaling in the development of androgen-independent growth of CaPs and metastasis progression.

Keywords: Rolipram; Forskolin; AR; cAMP/PKA pathways; CaP metastasis

1. Introduction

Prostate cancer (CaP) is one of the most common malignancies in men. The growth of cancer cells is highly dependent on androgens in the early stages. Androgen receptors (ARs) mediate the effects of hormones and play a central role in the formation and progression of tumors. Most primary CaPs exhibit elevated levels of androgen and are initially sensitive to androgen deprivation therapy (ADT). Despite therapy, the disease often progresses into a castration-resistant prostate cancer (CRPC) [1–3]. Although CRPC is no longer dependent on androgen stimulation, AR is still expressed in tumor cells and the disease becomes highly aggressive [3]. Thus, alterations in AR expression and its associated pathways may lead to the development of CRPC and CaP metastasis [4]. AR-mediated growth-promoting pathways are therefore important therapeutic targets in CRPC. A number of cellular pathways that crosstalk with AR signalings have been studied. It has been shown that the epidermal growth factor (EGF) pathway can trigger AR

Abbreviations: cAMP, cyclic adenosine monophosphate; PKA, protein kinase A; CaP, prostate cancer; CRPC, castration-resistant prostate cancer; PDE, phosphodiesterase; GEO, Gene Expression Omnibus.

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transcriptional activity in CaP cell lines [5,6]. The cytokine interleukin-6 (IL-6) and a family of neuropeptides, including bombesin- and gastrin-releasing peptides, have been shown to promote androgen-independent growth in several CaP cell lines [6,7].

One of the major intracellular signal transduction pathways – the cyclic adenosine monophosphate (cAMP)-activated protein kinase A (PKA) pathway – is implicated in CaP progression [8]. An elevated cAMP level leads to an increased expression of prostate-specific antigen (PSA) mRNA, and the rise in the PSA gene expression by PKA requires a functional AR, suggesting that cAMP and its downstream PKA pathways may regulate the expression and activity of AR [9]. However, the precise role of cAMP/PKA in the development of CRPC and the relationship between cAMP/PKA and AR signaling in CRPC are less explored.

The PKA pathway is critical for the fundamental cellular processes, including proliferation, differentiation, and apoptosis [10]. The synthesis of cAMP is mediated by adenylyl cyclase from adenosine triphosphate (ATP). The degradation of cAMP is mediated by a large family of cAMP-specific phosphodiesterases (PDEs) enzymes. Inhibition of PDEs, in particular by rolipram, a PDE4 inhibitor, leads to elevated levels of cAMP [8]. It is known that cAMP triggers the activation of downstream PKA, which facilitates the major cellular effects of cAMP [8]. PKA exists as a tetrameric holoenzyme consisting of 2 regulatory (R) and 2 catalytic (C) subunits forming a holoenzyme R_2C_2 [11]. The R subunits are the major intracellular receptors of cAMP. Binding of cAMP to the R subunits leads to the release of C subunits from the R-C complexes and allows the C subunits to phosphorylate downstream substrates [10,11]. The R subunits are further distinguished as R1α and R1β, and R2α and R2β, each of which are encoded by different genes like *PRKAR1A*, *PRKAR1B*, *PRKAR2A*, and *PRKAR2B*, respectively [11]. The C subunits, Cα, Cβ, and Cγ, are coded by the genes *PRKACA*, *PRKACB*, and *PRKACG*, respectively [10,11]. PKA exerts the effects of cAMP on several key transcriptional factors, and among them the cAMP response element (CRE)-binding protein 1 (CREB/CREB1) is the principle mediator [10,12]. The effects of cAMP/PKA on the cells are partly mediated by the transcriptional factor CREB1, which modulates the expression of a large number of genes involved in growth, survival, metabolism, reproduction, transport, and immune regulation [13,14]. Thus, cAMP/PKA pathways are recognized as the central signaling transduction pathways that are required for multiple cellular functions, including metabolism, cellular growth, differentiation, gene expression, and apoptosis [10].

Increasing evidence has shown that cAMP/PKA pathways may play a role in CaP progression. It has been shown that ARs can be activated by the PKA activator, forskolin in an androgen-independent manner in PC3 cells cotransfected with AR responsive reporter vectors and AR expression vectors [15]. Furthermore, activation of PKA by forskolin leads to the increased mRNA level of PSA in CaP cell lines [9]. Further, forskolin-induced increase in PSA mRNA expression was reversed by the addition of PKA inhibitor or by an AR antagonist, bicalutamide, in LNCaP cells [9]. Moreover, double knockdown of the AR and PKA subunit R1α induced a growth arrest of CaP cells with a better effect in comparison with what was achieved by a single knockdown of AR [16]. These data indicate that cAMP/PKA pathways may be the upstream mediators of ARs in CaP cells. However, the precise role of cAMP/PKA in the development of CRPC and the relationship between cAMP/PKA and AR signaling are less explored.

The aim of our study is therefore to investigate whether cAMP/PKA may be responsible for the activation of AR/PSA signaling during the progression of CRPC in the absence of androgen. We also assessed whether elevated levels of cAMP/PKA may sensitize CaP cells to low levels of androgen and thus promote progression of tumor cells from the androgen-dependent state to CRPC status. To further validate the correlation between the cAMP/PKA-associated regulators and AR expression, we utilized National Center for Biotechnology Information's Gene Expression Omnibus (GEO) database and analyzed the mRNA expression profiles of the sets of genes of interest from the prostate tissues of organ donors, and the tumor tissues and metastatic lesions from patients with CaP. Our findings for the first time showed that cAMP-dependent pathways regulate AR/PSA expression. In the aggressive CaP cells, cAMP-dependent pathways mediate the matrix metalloproteinase (MMP)-9 expression, suggesting that cAMP-dependent pathways may promote adaptation of CaP cells to a more aggressive phenotype in the absence of hormones. Our data from patient studies support our findings in cell line-based studies and suggest that cAMP/PKA may in part contribute to the progression of CRPC by increasing the levels of AR/PSA expression.

2. Materials and method

2.1. Cell lines

The PNT1A cell line, androgen-dependent cell line LNCaP, and the androgen-independent cell lines PC3, DU-145, and PC3M were purchased from the American Type Culture Collection (ATCC, Manassas, VA). The cell lines were maintained in RPMI 1640 medium supplemented with 10% fetal bovine serum, 1% penicillin-streptomycin, and 1 mM L-glutamine (Life Technologies, Paisley, UK) at 37 °C in a humidified atmosphere with 5% CO_2.

2.2. Treatment

For the treatment, rolipram, the cAMP-specific PDE4 inhibitor, and forskolin, the cell permeable cAMP enhancer (Calbiochem, Darmstadt, Germany) dissolved in 100% dimethyl sulfoxide (DMSO), were used. LNCaP or PC3