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Cross talk between protein kinase A and androgen signaling pathway

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Summary

Protein kinases are enzymes that add phosphate group to proteins to modify their function. These proteins regulate signal transduction pathways, essential for many biological processes such as cell cycle, cell signaling, protein and enzyme regulation, etc. There are 518 protein kinases, divided in to 7 main families. Protein kinase A (PKA) is a member of AGC family of protein kinases. It is activated by activation of G protein-coupled receptors (GPCR) and plays an important role in many cellular pathways including androgen signaling. Steroid hormones such as androgens primarily function through a genomic pathway, binding to cytosolic androgen receptors (AR) and initiating changes in transcription of target genes. Androgens also functions through a non-genomic pathway which is rapid and mediated by membrane receptors. It manifests its effects by activation of cellular signal transduction pathways such as PKA, Protein kinase C, and mitogen activated kinase, and does not involve transcription. In this review, we have analyzed the interaction between androgen signaling pathways and PKA, and have highlighted how each of these pathways complements and strengthens the function of the other. PKA plays an important role in complete activation of nuclear AR and in turn PKA can be activated by androgens. The complex interaction between the two pathways plays a critical role in development and progression of prostate cancer (PCa). Though the exact role of each pathway is not completely understood yet simultaneous inhibition of both pathways could prove to be beneficial for PCa patients.

Key words: Androgen signaling, non-genomic signaling, prostate cancer, protein kinase A.

Introduction:

Protein kinase is an enzyme that chemically adds phosphate group to target proteins, altering their activity. This process is known as phosphorylation. The phosphorylated target proteins activate signal-transduction pathways, which play significant role in many biological processes (Cheetham, 2004; Kondapalli et al., 2005). A total of 518 proteins have been identified in human genome and they constitute about 2% of all human genes (Manning et al., 2002). These are divided among 7 main families. The entire repertoire of kinases in the genome is known as Kinome.

In this review we are going to focus on protein kinase A (PKA), a member of the AGC group and its interaction with steroid hormones such as androgens.

Protein kinase A

PKA was discovered by Krebs in 1968 (Taylor et al., 2002). It is activated as a result of the hormone binding to a protein called G protein-coupled receptor (GPCR). When a hormone binds to a GPCR, transmembrane proteins called G-proteins are activated, which further activate adenylate cyclase converting ATP to cyclic adenosine monophosphate (cAMP). cAMP in turn activates PKA because of which PKA is also called cAMP dependent kinase. PKA pathway is involved in regulation of many cellular processes such as cell cycle, proliferation, differention and regulation of microtubule dynamics, assembly and disassembly of nuclear envelope, condensation and decondensation of chromatin (Tasken and Aandahl, 2004). It has several functions in the body including regulation of glycogen, sugar and lipid metabolism. PKA phosphorylates proteins that have the Arg-Arg-X-Serine motif, thus activating/deactivating the proteins. The effects of PKA activation vary with cell type. Recent research shows that PKA also interacts with steroid hormones such as androgens. This interaction is important for the functioning of many cells and tissues. Here we analyze the interaction of PKA with steroid hormones such as androgens, and evaluate its role in diseases such as PCa.

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Table 1. Kinome

Name	Description
AGC	Contains PKA, PKG, and PKC
CAM kinases	Contains Ca2+/ CAM-dependent protein kinase
CK1	Contains Casein kinase 1
CMGC	Contains CDK, MAPK, GSK3, CLK Kinase
STE	Contains homologs of yeast sterile 7, 11, 20 kinases; MAP kinase
PTK	Contains protein tyrosine kinase
PTKL	Contains rotein tyrosine kinase-like group
RGC	Contains receptor guanylate kinase

Genomic and non-genomic androgen signaling

Androgens play an important role in male sexual differentiation and maturation (Heinlein and Chang, 2002). Androgens such as testosterone and its metabolite DHT (5-alpha-dihydrotestosterone) bring about their biological functions by binding to the androgen receptor (AR). AR is a ligand-dependent transcription factor which is a member of the nuclear receptor superfamily. It is found in a complex with heat shock proteins in the cell. After binding with androgen, AR dissociates from heat shock proteins, forms a dimer, translocates to the nucleus where it binds to a specific DNA sequence known as androgen responsive element (ARE) to modulate transcription of target genes (Lee and Chang, 2003). This is known as genomic mode of androgen action and takes hours to manifest. The genomic pathway of androgen is well documented in various studies. In addition to the long term genomic mode of action which results in modulation of transcription of specific genes, androgens also carry out non-genomic signaling. The non- genomic mode of androgen signaling induces the activation of various cellular signaling cascades (Heinlein and Chang, 2002) often with the help of secondary messengers and protein kinases (Culig et al., 2003). This type of signaling is rapid as it does not involve gene transcription. Various cases of non-genomic action of androgen have been observed.

In Sertoli cells plasma membraneis permeabe to un-conjugated testosterone but impermeable to albumin-conjugated testosterone. Arrival of testosterone at Sertoli cell leads to rapid rise of intracellular concentration of Ca^{2+} (Liebererr and Grosse, 1994; Gorczynska and Handelsman, 1995). The increase in the level of calcium is used in initiation and maintenance of normal spermatogenesis by testosterone (T) through nongenomic mode (Gorczynska and Handelsman, 1995). Similarly, in murine T cells, BSA-testosterone induces a rapid rise in Ca^{2+} ions through nonvoltage-gated Ca^{2+} channels. The testosterone-induced import of Ca^{2+} is not inhibited by cyproterone demonstrating non-involvement of nuclear AR (Benten et al., 1999). In rat osteoblasts, both influx of Ca^{2+} from outside and the release of Ca^{2+} from intracellular stores are seen. Ca^{2+} influx occurs through voltage-gated Ca^{2+} channels. Release of Ca^{2+} is induced by binding of testosterone to membrane AR, leading to activation of phospholipase C causing hydrolysis of phophotidylinositol 4,5-bisphosphate. Also, exposure of cells to BSA-testosterone-FITC (fluorescein isothiocynate) causes the outer membrane of the cells to fluorescence indicating the presence of membrane receptors capable of specific binding to androgens. Taken together this indicates a GPCR-mediated non-genomic mechanism of androgen action in these cells (Lieberherr and Grosse, 1994).

In cardiovascular cells, testosterone induces relaxation of aorta and coronary arteries. Coronary arteries and aortic rings contracted with prostaglandin were treated with testosterone at various concentrations. Relaxation of contracted arteries and aorta were seen at testosterone concentration 1-10µmol/L and 10-100µmol/L, respectively. In these cells testosterone activates potassium channels (Bk^{ca}) by stimulating rise in Ca^{2+} ions by activation of cyclic-guanosine monophosphate (cGMP) and protein kinase G (PKG) (Yue et al., 1995; Costarella et al., 1996; Chou et al., 1996). Exposure of testosterone also increases vesicular resistance and

blocks the effects of vasodilatory agents (Ceballos et al., 1999; Rubio-Gayosso et al., 2002).

In LNCaP cells, androgen evokes a GPCR-mediated nongenomic pathway to activate PKA. In these cells, increase in PKA activity was observed with increasing concentration of testosterone. The PKA activation was not inhibited by nuclear AR antagonist biculatamide, indicating that the activation is nuclear AR-independent. Also, the activation of PKA can be attenuated by using SiRNA against Gα subunit of G protein. Taken ogether, an androgen-sensitive GPCR-mediated PKA activation mechanism seems to exist in the prostate cells that also contributes to the classical nuclear AR-mediated signaling by androgens. Inhibition of PKA activation along with standard AR-targeted therapies can reduce tumor cell proliferation in PCa patients (Bagchi et al., 2008).

Androgens also mediate some non-genomic actions via their structural properties. Androgen acquire additional charges from sulfate residues to penetrate in to lipid-protein complex of cell membrane, leading to decrease in membrane flexibility and modulating the actions of enzymes required for ATP hydrolysis. This is important in brain function regulation (Zylinska et al., 1999).

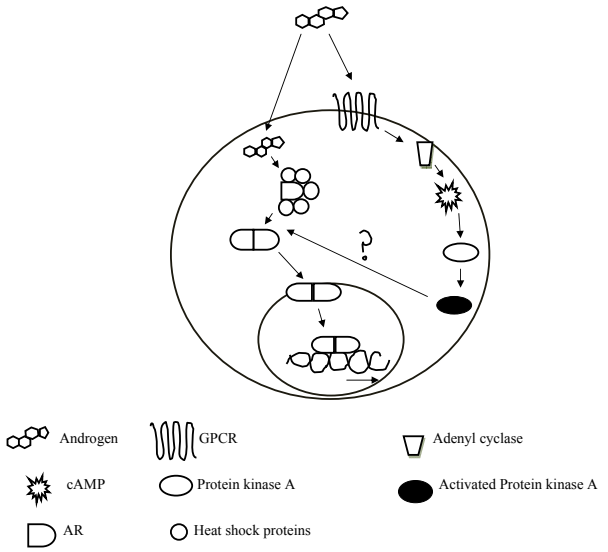

Fig. 1: Genomic and non-genomic modes of androgen signaling.

Classically, androgen binds to cytosolic AR causing its dissociation from heat shock proteins such as HSP70, HSP20. A dimer of AR is formed which translocates to nucleus and transcription initiates. In the non-genomic mode, androgen activates a GPCR, which causes activation of adenylate cyclase and cAMP. cAMP activation leads to the activation of PKA. PKA activation contributes to AR signaling.

Crosstalk between PKA and androgen signaling

For several years, the mechanisms of actions of lipid-soluble hormones and water-soluble hormones were assumed to be distinct. However, various studies in the recent past have indicated a definite interaction and dialogue between the two.

In LNCaP cells, AR is activated in the absence of androgen via activation of PKA by forskolin (FSK) (Sadar, 1999). These cells, containing functional AR and prostate specific gene (PSA), were treated with FSK (1uM) for 16 hr following which total RNA was extracted and global gene expression was analyzed using Affymetrix gene-chips. FSK caused activation of various genes including AR and PSA (Wang et al., 2006).

Studies have shown that PKA can also induce nuclear localization of AR. LNCaP cells were treated with isoproterenol (ISO), a PKA activator or R1881 (a synthetic androgen). Both ISO and R1881 could stimulate PSA expression and translocation of AR to the nucleus. In the presence of H89, a known inhibitor of PKA, attenuation of PSA expression and translocation was seen. This demonstrates that inhibiting PKA activation also inhibits activation and signaling by nuclear AR (Kasbohm et al., 2005).

Aberrant nuclear AR signaling and PKA activation are responsible for the transition of the androgen-sensitive PCa to androgen-insensitive PCa or castration-resistance prostate cancer (CRPC). In CRPC state AR gets activated by activation of PKA in the absence of androgen (Nazareth et al., 1996; Sadar, 1999; Bagchi et al., 2008). Each of the two isoforms of PKA, PKA-I and PKA-II, have two different regulatory units RI and RII and these in

turn have four different subunits RIα, RIβ, RIIα and RIIβ. Regulation of PKARIα seems to be linked with regulation of AR. Down-regulation of AR results in decrease of PKARIα protein levels, causing inhibition of PKA activity; also the down-regulation of PKARIα results in inhibition of AR signaling. This indicates that both pathways are linked to each other (Desiniotis et al., 2010). Simultaneous targeting of AR and PKA signaling pathways together can be of higher benefit for treatment of CRPC. In fact, recent research has shown that the combination treatment of AR and PKARIα with oligodeoxynucleotide (ODN) results in inhibition of prostate cancer and also CRPC (Eder et al., 2013).

Prostate cancer

PCa is one of the major health problems in Western countries. It is the second leading cause of male cancer death (Jemal et al., 2005). Androgens play a major role in the normal growth and development of prostate glands and in progression of prostate cancer (de Winter JA et al., 1994). Initially, PCa depends on androgen for proliferation which can be treated by surgical removal of tumor (Anscher, 2004). The hormonal therapies lead to temporary shrinkage of the tumor but it reappears in the form of androgen-independent (AI) PCa. In AI PCa, AR gets activated even in the presence of low levels of androgens (Feldman et al., 2001).

The PKA signaling pathway may contribute to the progression of PCa (Sarwar et al., 2014). Activation of PKA by various hormones including androgens occurs via signaling cascade involving GPCR-mediated cAMP activation. The activation of PKA may aid in the progression of prostate cancer from an androgen-dependent to -independent state (Bagchi et al., 2008).

The inhibition of PKA activation, together with standard AR targeted therapies, can be of greater use in the treatment of patients with PCa (Bagchi et al., 2008). In fact it has been shown that simultaneous knockdown of PKARIα and AR using siRNA caused significant reduction in LNCaP cell proliferation (Desiniotis et al., 2010).

Discussion and Conclusions

PKA, a member of AGC group, is involved in regulation of many important cellular processes. In this review interaction of PKA with androgen signaling pathway has been analyzed. Recent research has revealed that PKA is required for complete activation of AR. Also PKA activation can be triggered by androgens (Bagchi et al., 2008). Simultaneous inhibition of PKA and androgen signaling might help in the treatment of prostate cancer. Although PKA is required for complete activation of AR, the exact role of PKA in enhancement of androgen signaling is not known. Two phosphorylation sites present in the amino terminal domain of AR may be the target of PKA phosphorylation (Sadar, 1999), yet this observation has not been supported by other studies. Also, studies have indicated that PKA enhancement of AR transcription does not co-relate with increase in AR expression and stabilization (Kim et al., 2005). Studies need to be performed to elucidate the step(s) in AR signaling pathway which are modulated by activation of PKA. It is known that AR signaling occurs in multiple steps such as dimerization, translocation to the nucleus, modulation of transcription and activation of PKA may potentiate any of these steps. Involvement of PKA in AR signaling may be responsible for androgen-independent PCa. Identification of the step where PKA is involved in AR signaling would help in designing drugs and therapeutics for PCa.

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