The Scaffolding Protein RACK1 Interacts with Androgen Receptor and Promotes Cross-talk through a Protein Kinase C Signaling Pathway*

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Anastasia C. Rigas‡, Daniel M. Ozanne‡, David E. Neal§, and Craig N. Robson‡¶

From the ‡Prostate Research Group, School of Surgical and Reproductive Sciences, University of Newcastle, Framlington Place, Newcastle upon Tyne NE2 4HH, United Kingdom and the §Oncology Centre, Addenbrooke Hospital, University of Cambridge, Cambridge CB2 3QQ, United Kingdom

The androgen receptor (AR), a member of the nuclear hormone receptor superfamily, functions as a ligand-dependent transcription factor that regulates genes involved in cell proliferation and differentiation. Using a C-terminal region of the human AR in a yeast two-hybrid screen, we have identified RACK1 (receptor for activated C kinase-1) as an AR-interacting protein. In this report we found that RACK1, which was previously shown to be a protein kinase C (PKC)-anchoring protein that determines the localization of activated PKC/II isoform, facilitates ligand-independent AR nuclear translocation upon PKC activation by indolactam V. We also observed RACK1 to suppress ligand-dependent and-independent AR transactivation through PKC activation. In chromatin immunoprecipitation assays, we demonstrate a decrease in AR recruitment to the AR-responsive prostate-specific antigen (PSA) promoter following stimulation of PKC. Furthermore, prolonged exposure to indolactam V, a PKC activator, caused a reduction in PSA mRNA expression in prostate cancer LNCaP cells. Finally, we found PKC activation to have a repressive effect on AR and PSA protein expression in androgen-treated LNCaP cells. Our data suggest that RACK1 may function as a scaffold for the association and modification of AR by PKC enabling translocation of AR to the nucleus but rendering AR unable to activate transcription of its target genes.

The androgen receptor (AR),1 a member of the steroid hormone nuclear receptor superfamily, is a ligand-dependent transcription factor that regulates gene expression required for proliferation and differentiation of cells within the prostate and also has a role in the development and progression of prostate cancer. In its inactive state, unliganded AR is associated with heat-shock proteins from which it dissociates upon binding of the ligand, dihydrotestosterone (DHT), which is generated from testosterone by membrane-bound 5-a-reductase. Following homodimerization, AR translocates to the nucleus where it interacts with coactivators or corepressors and binds to androgen response elements (AREs) located in the promoter region of target genes triggering their transcriptional activation or repression (1, 2).

There is evidence to support AR cross-talk with other cellular signal transduction pathways resulting in AR ligand-independent activation and modulation of AR transactivation. There is also mounting evidence that ligand-independent activation of AR may play a role in hormone refractory prostate cancer. cAMP-dependent kinase (PKA) has been reported to mediate ligand-independent AR activation and cross-talk between AR and PKA signaling pathways resulting in androgen-independent induction of prostate-specific antigen (PSA) gene expression (3, 4). In contrast, protein kinase C (PKC) negatively regulates AR-dependent transcription (5). AR activation in the absence of ligand has been reported in response to the peptide hormones epidermal growth factor (EGF), keratinoocyte growth factor (KGF), and insulin-like growth factor I (IGF-I), which serve as ligands for receptor-tyrosine kinases and activate downstream intracellular kinase cascades (6). These growth factors can activate transcription from an androgen-responsive reporter construct in the absence of ligand or synergistically in conjunction with androgens. Interleukin-6 (IL-6), a multifunctional cytokine, can also stimulate ligand-independent AR activation via mitogen-activated protein kinase (MAPK) and signal transducer and activator of transcription-3 (STAT-3) signaling pathways in LNCaP human prostate cancer cells (7). Moreover, steroid receptor coactivator-1 (SRC-1) has been shown to enhance ligand-independent activation of the N-terminal domain (NTD) of the AR by IL-6 via a pathway that is dependent on MAPK in LNCaP (8).

The mechanism by which these signaling pathways affect AR function is not known. They may modulate AR via phosphorylation of either AR itself or of AR coregulators. For example, activation of the HER2/Neu MAPK pathway results in AR phosphorylation, increasing its ability to recruit coregulators and enhancing AR transactivation (9). Pyk2 interacts with ARA55 protein and represses AR transactivation via phosphorylation of ARA55 (10). In turn, these alternative pathways may affect AR translocation to the nucleus or transcriptional activity via interaction with scaffolding proteins that orchestrate the precise compartmentalization of signal transduction components. It is thought scaffolding proteins cluster signaling proteins thus allowing a tight control of cellular pathways as well as cross-talk between different cascades.

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‡ To whom correspondence should be addressed: Prostate Research Group, School of Surgical and Reproductive Sciences, University of Newcastle, Framlington Place, Newcastle upon Tyne NE2 4HH, UK. Tel.: 44-191-222-7076; Fax: 44-191-222-8514; E-mail: c.n.robson@ncl.ac.uk.

¶ The abbreviations used are: AR, androgen receptor; RACK1, receptor for activated C kinase-1; PKC, protein kinase C; PSA, prostate-specific antigen; DHT, dihydroxytestosterone; IndoV, indolactam V; GF, GF109203X; GFP, green fluorescent protein; RFP, red fluorescent protein; RT-PCR, reverse transcriptase-polymerase chain reaction; SDM, steroid-depleted medium; CHIP, chromatin immunoprecipitation assays; TPA, 12-O-tetradecanoy phorbol-13 acetate; PBS, phosphate-buffered saline; SRC-1, steroid receptor coactivator-1.
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Caveolin, a major component of caveole membrane structures, has been implicated as a principal scaffold for many signal transduction pathways. In a study by Lu et al. (28), caveolin was shown to interact with AR and its overexpression potentiated ligand-dependent AR activation. Recently, we identified filamin, an actin-binding protein also thought to act as a scaffold, as an AR-interacting protein that facilitates ligand-dependent AR nuclear translocation (11). In filamin-deficient M2 cells AR remained cytoplasmic even after prolonged exposure to androgen. Previous studies have also identified filamin as an intercellular partner for caveolin (12). Furthermore, caveolin also appears to have a role in ligand-independent estrogen receptor α nuclear translocation (13).

The aim of this study was to identify novel AR-interacting proteins involved in movement or signaling from the cytoplasm to the nucleus. A yeast-two hybrid screen identified RACK1 (receptor for activated protein kinase C-1) as an AR-interacting protein. RACK1, a 36-kDa homologue of the β subunit of G proteins, is a member of the WD-40 family of proteins characterized by highly conserved internal WD-40 repeats (Trp-Asp) (14, 15). It was initially identified as a protein that interacts with activated PKC and acts as a shunting protein that coordinates the correct localization of βIIIPKC isoform for its function (16, 17). Due to its association with a large number of signaling proteins such as pPKC (18), Src (19), integrin subunit (20), phosphodiesterase PDE4D5 (21), STAT1 (22), IGF-I receptor (23, 24), a central role of RACK1 as a scaffolding, anchor or adaptor protein in multiple intracellular signal transduction pathways has been suggested. We have found RACK1 to have a role in ligand-independent AR movement and transactivation via the PKC signaling pathway suggesting a possible function for this scaffolding protein in AR cross-talk with other signaling pathways.

EXPERIMENTAL PROCEDURES

Materials—R8181 was purchased from PerkinElmer Life Sciences. Dihydrotestosterone, Indolactam V, and monoclonal anti-α tubulin antibody were purchased from Sigma. GF109203X was purchased from Calbiochem. Monoclonal anti-RACK1 antibody was purchased from Transduction Laboratories. Monoclonal anti-AR antibody was purchased from Santa Cruz Biotechnology.

Western Blotting—LNCaP cells were seeded in 6-well cell culture plates at densities of 1 × 10^6 cells/well. After 24 h, cells were washed in PBS and incubated for 48 h in SDM in the presence or absence of activator/inhibitor. Adherent cells were lysed directly using SDS sample buffer containing 10% β-mercaptoethanol. Samples were denatured and separated by SDS-PAGE, followed by transfer to nitrocellulose membrane. The membrane was probed with monoclonal anti-RACK1 antibody. Immunoreaction was visualized using enhanced chemiluminescence (ECL) reagents (Amersham Biosciences).

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Full-length RACK1 (corresponding to residues 1–326) was cloned into pEGFP-C1 (Clontech) to generate GFP–RACK1 (Clontech) to generate the GFP–RACK1 construct. The constructs were transfected into 293T cells and cell membranes were visualized using a Leica scanning laser confocal microscope.

Reverse Transcription PCR—Total RNA was isolated from LNCaP cells using TRIzol reagent according to the manufacturer’s recommendation (Invitrogen). Complementary DNA was synthesized from 2 μg of
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Fig. 1. Relative β-galactosidase activities of RACK1163–317, cotransformed with AR deletion constructs as indicated in a yeast two-hybrid LacZ assay. The AR deletion construct comprising the DNA-binding and steroid-binding domain (AR559–918) presented the highest activity in the presence of DHT followed by the AR deletion construct containing the steroid-binding domain alone (AR624–918). Other AR constructs used in the assay did not present a significant interaction above that observed with the empty vectors only. Bar chart shows the average activity observed from two independent experiments performed in triplicate.

RESULTS

RACK1 Is an AR-interacting Protein—Yeast two-hybrid screening using an AR construct AR559–918 comprising the DNA and ligand-binding domains (DBD and LBD respectively) as bait, identified RACK1 as an AR-interacting protein (Fig. 1). The clone isolated in the screen spanned a region of 154 amino acids from amino acids 163 to 317, designated RACK1163–317. The interaction of AR and RACK1 is enhanced in the presence of ligand, demonstrated by an approximate 3-fold increase in β-galactosidase activity in its presence. Co-translation of RACK1163–317, with AR deletion constructs in yeast revealed an interaction between RACK1163–317 and the AR fragment AR624–918 comprising the LBD alone upon treatment with ligand. The approximate 3-fold increase in β-galactosidase levels in the presence of 1 μM DHT suggested that additional residues in the DBD further contribute to the interaction or influence the conformational change upon ligand binding. These preliminary data imply that AR interacts with RACK1 in the presence of ligand.

RACK1 Interacts with the AR in Vitro and in Vivo—To confirm the data obtained from the yeast two-hybrid system, we sought to determine whether RACK1 could interact with AR in vitro. The interaction was analyzed using in vitro transcription and translation of the proteins labeled with [35S]methionine. Fig. 2A represents in vitro transcribed/translated protein products before immunoprecipitation. Fig. 2B demonstrates that RACK1163–317 interacts with full-length AR (amino acids 1–918), the DBD and LBD (amino acids 559–918) and LDB alone (amino acids 624–918). No interaction was observed between RACK1163–317 and the transactivation domain and DBD (amino acids 1–624). In agreement with the results from the
two-hybrid analysis the site of the RACK1 interaction on AR appeared to be around the LBD (amino acids 624–918).

Immunoprecipitation and subsequent Western analysis were used to confirm the interaction between RACK1 and AR in vivo. A rabbit polyclonal antibody was used to immunoprecipitate AR protein from prostate LNCaP cells prior to detection of RACK1 by Western blotting. LNCaP cells were also treated with androgen to study the influence of ligand on the interaction. As shown in Fig. 2C, a 36-kDa band, corresponding to RACK1 was observed in the absence (lane 4) and presence of ligand (lane 5) but was absent in control lanes omitting extract (lanes 2 and 3) or antibody (lane 1).

**Minimal Domain Mapping of AR-RACK1 Interaction**—The presence of WD-40 repeats, which have been implicated in protein–protein interactions, within RACK1 suggested that one of these motifs may be responsible for the interaction. The clone isolated in the two-hybrid system, RACK1<sub>163–317</sub>, contains five out of the seven WD repeat sequences. The minimal domain of RACK1 required to interact with AR was investigated by deleting residues from the C terminus of RACK1<sub>163–317</sub>. The deletion constructs were tested for their ability to interact with AR<sub>559–918</sub> in yeast. Resultant transformants were grown on media lacking adenine, leucine, and tryptophan selecting for both plasmids and for an interaction between the proteins encoded by these plasmids. Interaction was observed between AR<sub>559–918</sub> and all RACK1 deletion constructs with the exception of RACK1<sub>WD7</sub>.

**RACK1 Is Involved in Ligand-independent AR Nuclear Translocation**—To assess the functional significance of AR-RACK1 interaction, the role of RACK1 in AR movement to the nucleus was investigated by fluorescence (confocal) microscopy tagging the interacting proteins with GFP or red fluorescent protein (RFP). A recent report (17) proposed RACK1 to act as a PKC shuttling protein that moves βIIIPKC upon activation from one intracellular site to another. It has been previously demonstrated using a GFP-AR chimera that unliganded AR is cytoplasmic but rapidly translocates to the nucleus upon addition of ligand (27). This ligand-induced nuclear translocation was also observed in this study in COS7 cells (Fig. 4). In the absence of R1881, GFP-AR is predominantly cytoplasmic with background nuclear fluorescence (column 1, row 1). Following a 20-min exposure to steroid, GFP-AR is predominantly nuclear (column 1, row 2). We next determined the localization of RFP-RACK1 in COS7 cells. RFP-RACK1 was found to be cytoplasmic but also localized to a perinuclear structure in unstimulated cells (column 2, row 1). In the basal state, both constructs colocalized in the cytoplasm, displayed by a yellow fluorescent pattern (column 3, row 1). Activation with synthetic androgen R1881 induced GFP-AR to move from the cytoplasm to the nucleus while RFP-RACK1 remained in the cytoplasm (column 3, row 2).

Ron et al. (17) showed RACK1 movement upon PKC activation from the Golgi apparatus to the cell periphery alongside βIIIPKC. Also, cross-talk has been reported between the AR and PKC signaling pathways. The PKC signaling pathway has been reported to negatively regulate AR-dependent transcription. Thus, we next sought to examine whether AR translocates to the nucleus in the presence of RACK1 and upon PKC activation. Upon treatment with PKC activator indolactam V, unliganded GFP-AR remained cytoplasmic (column 1, row 3). However, in the presence of RFP-RACK1 androgen-independent nuclear translocation of GFP-AR was observed upon exposure to indolactam V (column 3, row 3). Furthermore, this movement was reduced in the presence of PKC inhibitor GF109203X (column 3, row 4). These results suggest that RACK1 has a role in ligand-independent movement of AR induced by activation of the PKC signaling pathway.

**RACK1 and Activation of PKC Signaling Pathway Suppress AR Transactivation**—We next determined the potential influence of RACK1 on AR transactivation in reporter assays using the pPSALuc reporter. Briefly, AR was transiently transfected into the AR-negative and androgen-independent prostate cancer PC3M cell line in the absence and presence of RACK1 together with pPSALuc. As shown in Fig. 5, R1881 induces AR transactivation up to 6-fold (compare bars 1 and 2). However, the addition of RACK1 repressed androgen-induced AR transactivation to a level comparable to that observed in the absence of R1881 (compare bars 2 and 3).
Because we observed androgen-independent AR movement upon PKC activation and in the presence of RACK1, we also investigated their potential effect on androgen-dependent and -independent transcriptional activation of AR. Upon treatment with PKC activator indolactam V alone, AR activity was comparable to that observed in the absence of any stimulus (compare bars 1 and 3) whereas upon treatment with PKC inhibitor GF109203X alone, AR activity was enhanced 7.5-fold (compare bars 1 and 4), suggesting a potential role for PKC signaling pathway in ligand-independent AR transactivation. Similarly, GF109203X enhanced R1881-dependent AR transactivation further 3-fold (compare bars 2 and 3) but indolactam V slightly reduced reporter activity (compare bars 2 and 5).

In the presence of GF109203X, the enhancement of reporter activity observed in the presence of AR alone was reduced 5-fold upon cotransfection with RACK1 (compare bars 4 and 10). A reduction was also observed upon treatment with indolactam V (compare bars 3 and 9). The addition of RACK1 also reduced androgen-dependent AR transactivation upon treatment with indolactam V and GF109203X (compare bars 5 and 11, 6 and 12). These results suggest a possible inhibitory role for RACK1. However, GF109203X appears to reverse the repression of both androgen-dependent and -independent AR activity observed upon treatment with indolactam V.

PKC Activation Causes a Decrease in AR Recruitment to PSA Promoter—Because PKC activation induces AR movement to the nucleus aided by RACK1 but has a repressive effect upon AR-mediated transcription, we next sought to determine whether the AR is still recruited to target genes. To investigate this, we used chromatin immunoprecipitation assays in LNCaP cells utilizing an antibody specific for the AR to examine the recruitment of the AR to the AR-responsive PSA promoter in the presence and absence of indolactam V. It has been previously shown that the AR rapidly associated with the PSA promoter upon androgen stimulation (26). Interestingly, we detected a decrease in AR association with the PSA promoter after 1 h of treatment with indolactam V suggesting there is a decrease in AR recruitment to the promoter upon PKC activation (Fig. 6, compare lanes 1 and 3).

PKC Activation Represses PSA mRNA Expression—Since we found that PKC activation represses AR transactivation and decreases AR recruitment to the PSA promoter we next examined PSA expression levels. We analyzed the effect of treatment with indolactam V for 4, 8, 24, or 48 h on PSA mRNA expression in LNCaP cells by RT-PCR. As shown in Fig. 7, prolonged exposure to indolactam V caused a reduction in PSA expression. PSA mRNA levels were decreased following treatment for 48 h in comparison to treatment for 4 h. Therefore, activation of the PKC signaling pathway appears to suppress PSA mRNA expression.

PKC Activation Has a Repressive Effect on AR and PSA Protein Expression in Androgen-treated LNCaP Cells—To test the possibility that PKC activator indolactam V may alter the cellular levels and/or localization of AR, cytosolic and nuclear levels of AR protein were determined by Western blotting. LNCaP cells were incubated with R1881 and indolactam V or GF109203X and cytosolic and nuclear extracts were prepared from cells harvested after 48 h (Fig. 8). In androgen-treated cells, there was an increase in nuclear AR, which reflects stabilization of the protein in the presence of ligand and movement to the nucleus (Fig. 8A, lane 1). When cells were exposed to R1881 and indolactam V, there was a decrease in cytosolic and nuclear AR levels compared with cell extracts treated with R1881 only (compare lanes 1 and 2). However, nuclear levels of AR were higher than cytosolic levels (lane 2). Nuclear extracts prepared from cells exposed to R1881 and GF109203X demonstrated a similar increase in nuclear AR levels compared with R1881-only treated cells (compare lanes 1 and 3). This suggests the PKC inhibitor may reverse the repression seen upon PKC activation or the presence of ligand is stabilizing AR and inducing nuclear translocation.

The whole cell levels of PSA protein, an androgen-regulated gene, were also investigated under the same conditions (Fig. 8B). Similar to the results obtained for AR, exposure of R1881-treated cells to indolactam V prevented PSA protein expression (lane 2), whereas in the presence of GF109203X PSA protein expression is similar to that observed upon R1881 treatment only (compare lanes 1 and 3). Whole cell levels of RACK1 remained relatively constant for the 48 h duration of the experiment (Fig. 8C).
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Fig. 7. Suppression of PSA mRNA levels by treatment with indolactam V. LNCaP cells were exposed to indolactam V at the indicated time points. Total RNA was extracted and submitted to RT-PCR using PSA-specific primers. β-Actin RT-PCR served as a control for initial RNA amounts.

Fig. 8. Repressive effect of indolactam V on AR and PSA protein expression in androgen-treated LNCaP cells. LNCaP cells were incubated with R1881 (10 nM) and indolactam V (INDOV, 10 μM) or GF109203X (GFX, 10 μM) for 48 h, harvested and analyzed by Western blotting. A, AR protein levels in cytosolic and nuclear cell extracts; B, PSA protein levels in whole cell lysates; C, RACK1 protein levels in whole cell lysates.

DISCUSSION

In this study, we have identified RACK1 as an AR-interacting protein. RACK1 has been previously shown to be a PKC-anchoring protein that determines the localization of activated PKCβII and has become widely perceived as a scaffolding protein coordinating the interaction of key signaling molecules (17). These findings combined with the fact that a number of scaffolding proteins including caveolin, filamin, and APPL are adaptors of AR-mediated transactivation implicated a potential role for RACK1 in AR function (11, 28, 29). Our studies identify RACK1 as the first scaffolding protein required for ligand-independent AR nuclear translocation.

Steroid hormone receptors are known to function as ligand-dependent transcription factors. However, several steroid receptors such as the chicken progesterone and human estrogen receptor can be activated in the absence of steroid by growth factors, neurotransmitters, and agents that increase kinase activity or decrease phosphatase activity (30–34). Other members of the superfamily including the human progesterone and glucocorticoid receptors are inactive in the absence of ligand but can be activated in a synergistic fashion by the co-treatment with kinase modulators (35, 36).

It has been reported that AR can be activated in the absence of cognate ligand by growth factors, cytokines and compounds that elevate cAMP (3, 6). Studies have also revealed synergism between PKA/PKC and AR pathways to stimulate AR transcriptional activation (3, 37, 38). So far, only the potential action of IGF-I and EGF on AR nuclear transfer has been investigated (39). However, these growth factors alone were unable to initiate the nuclear translocation of AR in the absence of androgen. We find that PKC activation induces AR movement to the nucleus but only in the presence of RACK1. Ron et al. (40) demonstrated that upon PKC activation RACK1 and PKCβII moved together to the Golgi. In this report, RACK1 remains in the cytoplasm while AR becomes nuclear. Previous reports have shown RACK1 translocation to the nucleus upon PKA activation and ethanol exposure.

An interesting feature contained within the AR-interacting domain in RACK1 is the presence of the sixth WD-40 domain. This repeat contains Tyr-246 which has been shown to be phosphorylated by Src and its phosphorylation mediates in part the Src-RACK1 interaction. PKC activation was found to enhance tyrosine phosphorylation of RACK1 and the Src-RACK1 interaction (19, 41). Interestingly, this repeat also contains, at least in part, the binding site for both PKC and Src. PKC activation may induce this post-translational modification to enable RACK1 to interact with other signaling proteins such as AR. It would be interesting to investigate whether RACK1 tyrosine phosphorylation is necessary for its role in AR signaling or indeed what other factors may regulate the interaction of RACK1 and AR. Migliaccio et al. (42) demonstrated an agonist-dependent simultaneous interaction of AR and ER with Src in prostate cancer cells. It may be that RACK1 brings Src in close proximity to AR following PKC activation thereby potentially facilitating AR phosphorylation. Recently, Yaku et al. (43) identified RACK1 as an inhibitory scaffolding protein for NMDA receptor phosphorylation and function by inhibiting phosphorylation of the NR2B subunit of NMDA by Fyn tyrosine kinase. Other adaptor proteins such as protein kinase A-anchoring proteins have been shown to bring PKA, PKC, and protein phosphatase 2B together (44).

Our findings show that RACK1 represses androgen-dependent AR transactivation. Similarly, RACK1 has also been shown to interact with the transcription factors p73 and NFAT and repress their transactivation (45, 46). Furthermore, RACK1 has been found to directly interact with Epstein-Barr virus trans-activator protein BZLF1, a c-Fos-related protein (47, 48). Interestingly, nuclear RACK1 has a role in the induction of c-Fos mRNA and protein expression upon acute exposure of cells to ethanol. PAC1, the type I receptor for PACAP was identified as a putative downstream gene that is altered in response to the induction of c-Fos by ethanol via RACK1 (49). Although, in this study full-length RACK1 was not detected in the nucleus, it would be interesting to investigate whether deletion fragments of RACK1 have a nuclear role. A 100 kDa fragment of filamin has been found to colocalize with AR in the nucleus and repress AR transactivation by competing with coactivator TIF2 (50). This role of filamin as a nuclear transcription modulator was entirely unexpected because filamin is known as a cytoplasmic scaffolding protein. AR has also been shown to associate with another actin-binding protein expressed in muscle, supervillin (51). A deletion peptide of supervillin was found to colocalise in the nucleus with activated AR and enhance AR transactivation. Our studies also show that PKC activation by indolactam V and RACK1 have a repressive effect on AR transactivation. RACK1 may be acting as an adaptor bringing AR in close proximity to PKC, which in turn modifies the receptor and affects its transcriptional function. However, the formation of a trimeric complex remains to be demonstrated. Previous studies reported repression of AR-mediated gene induction by PKC activator and phorbol ester TPA, which was at least in part due to overexpression of c-Jun and
c-Fos (52). One study demonstrated an enhancement of AR activity by TPA but not by stimulation of the PKA pathway in the absence of androgen (5). In contrast, it has been shown that AR can be activated by PKA activator forskolin in a ligand-independent manner (3). The variation of observations by these studies indicates that the context of the promoter in the reporter gene governs ligand-independent activation of AR.

Our study shows that PKC activation represses the androgen induction of PSA protein expression, which is in agreement with the repression previously observed by TPA treatment (53). The inability of AR to associate with the endogenous PSA superfamily also remains to be determined. Further investigation is required to ascertain the role in the signaling of both of these PKC isoforms. Further investigation is required to ascertain the context of the promoter in the regulation of prostate cancer to androgen independence. It has been suggested that PKC activation AR translocates to the nucleus in the absence of ligand, aided by scaffolding protein RACK1 but is unable to associate with target gene promoter sequences and initiate their transcription. The implication of these findings for alternate activation of the AR may be important in understanding the progression of prostate cancer to androgen independence. It has been suggested that PKCβ may be down-regulated in early prostate cancer and PKCε has been shown to have the potential to advance the recurrence of prostate cancer (54, 55). RACK1 has been shown to have a role in the signaling of both of these PKC isoforms. Further investigation is required to ascertain whether Src and PKC modulate the AR-RACK1 interaction and elucidate the mechanism of repression of AR transcriptional ability. A role for RACK1 in the regulation of other members of the SHR superfamily also remains to be determined.

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