Androgen Receptor Mediates Non-genomic Activation of Phosphatidylinositol 3-OH Kinase in Androgen-sensitive Epithelial Cells

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Androgens are known to modulate many cellular processes such as cell growth and survival by binding to the androgen receptor (AR) and activating the transcription of target genes. Recent data suggested that AR can also mediate non-transcriptional actions outside the nucleus in addition to its ligand-inducible transcription factor function. Here, we describe a transcription-independent activation of the phosphatidylinositol 3-OH kinase (PI3-K) signaling pathway by androgens. Using non-transformed androgen-sensitive epithelial cells, we show that androgens enhance the PI3-K activity by promoting accumulation of phosphoinositide-3-P phospholipids in vitro. This activation is found in conjunction with an increased time-dependent phosphorylation of the downstream kinase AKT/protein kinase B on both Ser173 and Thr308 residues. Hormone-stimulated phosphorylation of AKT requires AR since incubation with the anti-androgen bicalutamide completely abolishes the androgen-stimulated AKT phosphorylation. Accordingly, we show that androgens increase AKT phosphorylation level in prostate carcinoma PC3 cells only if they have been transfected with AR. Downstream, androgens enhance phosphorylation of transcription factor FKHR (Forkhead in rhabdomyosarcoma)-L1 and proapoptotic Bad protein and promote cell survival as they can counteract an apoptotic process. We also report that non-genomic effects of androgens are based on direct interaction between AR and the p85α regulatory subunit of class I(A) PI3-K. Together, these novel findings point out an important and physiologically relevant link between androgens and the PI3-K/AKT signaling pathway in governing cell survival.

Steroid hormones exert most of their effects by binding to specific nuclear receptors, which act as transcription factors (1–3). The relationship between steroid target genes and steroid physiological actions suggest that mechanisms, different from those controlling the transcriptional activity of nuclear receptors, may be involved. In recent years, steroids have been found to control the activity of multiple signaling pathways via non-genomic mechanisms (for a review, see Ref. 4). Non-genomic activities of estrogen receptor (ER)αβ were the first described and associated to vascular protection through nitric oxide production in endothelial cells, involving the phosphatidylinositol 3-OH kinase (PI3-K) and subsequent endothelial nitric-oxide synthase activation (5–8). Estrogens and both ERα and ERβ rapid effects have been studied in a wide range of physiological or pathological conditions and cell types, including neurons (9), endothelial cells, osteoblasts, and breast cancer cells (10). Some other steroid receptors such as progesterone receptor (PR) (11–14) but also androgen receptor (AR) (15–17) have been recently highlighted to mediate non-genomic activities. Kousteni et al. (17) demonstrated that ERα, ERβ, and AR, associated in the same complex, can protect osteoblasts and osteocytes from apoptosis through the Src/She/ERK signaling pathway. Moreover, such association of either AR with ERα in LNCaP cells or AR with ERα in MCF-7 and T47-D cell lines was described to trigger cell proliferation through the same pathway (16). In PC3 cells stably expressing AR, androgens are able to increase the enzymatic activities of several cytoplasmic kinases such as the mitogen-activated protein kinases (MAPKs), the protein kinase C, and the PI3-K (15). Although non-genomic steroid receptor activities mediated by the PI3-K pathway are now well documented for ER and PR, they remain poorly investigated concerning AR.

Three classes of PI3-K have been described depending on their primary structure and substrate specificity (18, 19). Class I(A) comprises five adapter subunits currently known. Of these, three are encoded by the p85α gene, generating p85α, p55α, and p50α by alternative splicing. These regulatory subunits regulate catalytic subunits p110α, p110β, and p110δ, which enhance phosphorylation of phosphoinositides (PtdIns-P) in D3 position of the inositol ring (20). The class I(A) p85α/p110α heterodimer, the most studied PI3-K, supports a large number of insulin and growth factor cellular effects (21–24). In vivo, the primary phosphorylated lipids generated by PI3-K class I(A) are the PtdIns-3,4-P2 and PtdIns-3,4,5-P3, which in turn recruit signaling kinases such as the AKT/protein kinase B (25). Acti-

The abbreviations used are: ER, estrogen receptor; AR, androgen receptor(s); PR, progesterone receptor; PI3-K, phosphatidylinositol 3-OH kinase; ERK, extracellular signal-regulated kinase; MAPK, mitogen-activated protein kinase; ANOVA, analysis of variance; PARP, poly-ADP ribosyl polymerase; VDEC, vas deferens epithelial cells; PMFSF, phenylmethlysulfonfluoride; PBS, phosphate-buffered saline; GST, glutathione S-transferase; CHX, cycloheximide; IGF-I, insulin-like growth factor-I; PtdIns, phosphoinositides; P, phosphorylated; h, human; FKHR, Forkhead in rhabdomyosarcoma.
vation of AKT by phosphorylation of the Ser473 and Thr308 residues (26) mediates many of the downstream PI3-K effects, including protection against apoptosis. This control involves the phosphorylation of many proteins, including the transcription factor of the Forkhead family (FKHR) (27) and the pro-apoptotic factor Bad (28, 29).

In this study, we report that androgens can activate PI3-K in androgen-sensitive epithelial cells. The PI3-K activation results in the phosphorylation of the downstream AKT/protein kinase B, FKHR-L1 and Bad protein and is supported by direct interactions between AR and p85α, involving the N-terminal SH3 and C-terminal SH2 domains of the regulatory subunit. Finally, we demonstrate that androgenic activation of the PI3-K/AKT pathway by AR acts as an anti-apoptotic stimulus in epithelial cells.

EXPERIMENTAL PROCEDURES

Reagents—R1881, insulin-like growth factor-I (IGF-I), and Wortmannin were obtained from Sigma. LY294002 was purchased from Biomol, and Bicalutamide (Casodex) was purchased from AstraZeneca. Antibodies against PI3-K subunit p85α (catalog number 06-195) and AR (catalog number PG21) were from Upstate Biotechnology; AKT (catalog number 9272), phospho-AKT Ser473 (catalog number 9258), and AR (catalog number PG21) were from Upstate Biotechnology; TCTACGTAAGTTC-3 (H11032) were from Trevigen Inc.; and β-actin (catalog number A2068) was from Sigma. Cell Culture and Transfections—Mouse vas deferens epithelial cells (VDEC) were cultured as already described (30). Cells were starved for 16 h in Dulbecco’s modified Eagle’s medium/ham F12, gentamycin (50 μg/ml) and then stimulated with or without R1881 or IGF-I. When used, LY294002 or Wortmannin were added 30 min before hormone inductions and were maintained through the experimental procedure. PC3 cell lines grown in Glutamax-Dulbecco’s modified Eagle’s medium containing gentamycin (50 μg/ml) and 10% fetal calf serum. Cells were transfected using the PEI ExGen 500 procedure (Euromedex).

Plasmids and Constructions—The pSG5-hAR and pGEX4T1-p85α plasmids were kindly provided by Prof. C. Sultan (Montpellier, France) and Dr. B. Payrastre (Toulouse, France), respectively. The p85α expression vector was constructed into the Ncol and EcoRI sites of pBRK by subcloning the full-length p85α PCR fragment amplified from pGEX4T1-p85α using the upstream 5′-CATGCCATGGTTGCTGAGGG-GATCCTCGAGCATGTCCCCGTAAGGTCCGGA-3′ and the downstream 5′-CGCGGATCCGCGATGGAAGTGCAGTTAGGGC-3′ (H11032) primers. The SH3, BCR, N-SH2, and C-SH2 domains of p85α were obtained by reverse transcriptase-PCR and then subcloned into the pGEX vector (Promega). BamHI/EcoRI digestion fragments were further ligated into the pGEX-4T1 expression vector (Amerham Biosciences) in-frame with the open reading frame of p85α in the same buffer overnight at 4 °C (Tris 10 mM, NaCl 100 mM, EDTA 1 mM). Beads were resuspended in CHCl3 and analyzed by TLC using CHCl3:CH3OH:NH4:H2O (90:70:6:14). The phosphorylated lipids were visualized by autoradiography and quantified using a phosphorimaging device (Bio-Rad).

Western Blot Analysis—Cells were harvested, washed in ice-cold PBS buffer, and lysed in radioimmunoprecipitation buffer (50 mM Tris-HCl, 1% Nonidet P-40, 0.25% sodium deoxycholate, 150 mM NaCl, 1 mM EDTA, 1 mM PMSF, 1 μg/ml aprotinin, 1 μg/ml leupeptin). Total proteins (40 μg) were boiled in Laemmli sample buffer and subjected to SDS-PAGE. Proteins were then transferred to nitrocellulose membranes (Hybond ECL, Amersham Biosciences) followed by blocking in Tris-buffered saline 1×, 10% powdered milk, and incubated with the indicated primary antibodies in the same buffer overnight at 4 °C. The membranes were washed three times with 1× Tris-buffered saline, 0.05% Tween 20, and then incubated 1 h with either anti-rabbit (P.A.R.L.S) or anti-mouse (Amerham Biosciences) peroxidase-conjugated IgG. Detection was performed using ECL system (PerkinElmer Life Sciences).

GST Pull-down Experiments—Fresh overnight cultures of BL21 pLysEs Escherichia coli strain transformed with GST-fused constructs were diluted 1:10 in LB medium containing ampicillin (100 μg/ml). Isopropyl-1-thio-β-d-galactopyranoside was added in growing exponential bacterial culture to a final concentration of 1 mM and incubated for 4 h at 30 °C. Cells were resuspended in STE buffer (10 mM Tris, pH 8, 150 mM NaCl, 1 mM EDTA). After 10,000 × g centrifugation during 10 min at 4 °C, pellets were frozen during 5 min, resuspended in STE buffer containing 1 mM dithiothreitol and 10% sarcosyl. Lysates were sonicated for 1 min and clarified at 10,000 × g for 10 min at 4 °C. The bacterial supernatant was rocked overnight at 4 °C with glutathione-Sepharose 4B (Amersham Biosciences), and beads were washed three times with PBS containing Triton X-100 (0.1%) and PMSF (1 mM). 35S-labeled AR and p85α proteins were generated in vitro using the TNT T7/T7-coupled reticulocyte lysate system (Promega). 35S-labeled proteins were incubated with the beads in binding buffer (20 mM Hepes, pH 7.4, 100 mM NaCl, 0.1% Triton X-100, 0.2 mg of bovine serum albumin, 2 mM dithiothreitol, 10 μM ZnCl2, 2 mM EDTA, 1 mM PMSF, and 1 μg/ml aprotinin) by rocking 2 h at 4 °C. The glutathione-Sepharose beads were washed three times in PBS buffer containing 0.2% Triton X-100 and 150 mM NaCl, re suspended in Laemmli buffer, released from beads by boiling for 5 min, and subjected to SDS-PAGE analysis. Fractionated proteins were stained with Coomassie Blue, visualized by autoradiography, and quantified using a phosphorimaging device (Bio-Rad).

Immunoprecipitation—VDE were washed with PBS and lysed in ice-cold radioimmunoprecipitation buffer. Cell lysates were centrifuged, and 500 μg of total protein was subjected to immunoprecipitation using anti-p85α antibodies (06-195) and protein A-Sepharose CL-4B (Amersham Biosciences) overnight at 4 °C. After five washes with PBS buffer, 0.2% Triton X-100 and 150 mM NaCl, the immunocomplexes were eluted in Laemmli buffer and analyzed by Western blotting as described above.

Apoptosis—Following treatment, the VDEC monoclonal was fixed with Clarke buffer (CH3OH/CH3COOH 3:1) at 4 °C for 10 min, and nuclei were stained with Hoescht 33342 (Sigma) for 30 min in the dark at room temperature. Cells were analyzed for the appearance of pyknotic nuclei and apoptotic body formation using a Zeiss Axioplan microscope. For flow cytometry analysis, cells were recovered and washed in PBS. Pellets were resuspended in an RNase A (50 μg/ml), propidium iodide (50 μg/ml) solution and kept 1 h at 4 °C in the dark. Cell suspensions were analyzed using a Beckman Coulter fluorescence-activated cell sorter. The percentage of apoptotic cells was determined by evaluating sub-G1 nuclei accumulation. At less 15,000 events were measured for each sample.

Statistical Analysis—Statistical analyses were performed by a one-way analysis of variance (ANOVA) followed by Fisher’s test. Values of p < 0.01 were considered significant and are presented under “Results.”
RESULTS

Androgens Stimulate the PI3-K/AKT Pathway in Androgen-sensitive Epithelial Cells—The ability of androgens to enhance the PI3-K activity in non-tumoral VDEC that express endogenous AR was tested using R1881. The PI3-K p85α regulatory subunit was immunoprecipitated, and the phosphorylation status of the PI3-K substrates (PtdIns-3-P versus PtdIns) was assessed by thin layer chromatography. A 10–20-min exposure of VDEC with R1881 was associated with a 2.3-fold increase in the PI-3-P production in vitro (Fig. 1A). This lipid kinase activity is dependent on the PI3-K as it was inhibited by wortmannin, an inhibitor of the p110 catalytic subunit of the PI3-K (31). The activation of the lipid kinase activity in response to this short androgenic stimulation was unlikely to be related to modifications in the steady-state levels of p85α and AR as they remained unchanged during the treatment (data not shown).

PI3-K catalyzes the formation of PtdIns(3,4,5)P3 on the intracellular membrane surface and leads to the recruitment of pleckstrin homology-containing domain proteins within the cell. Among these is the Ser/Thr kinase AKT, which mediates a large range of the PI3-K cellular effects. To determine whether androgens can activate AKT, we monitored its phosphorylation status on the Ser473 residue, known to be regulated in a PI3-K-dependent manner by growth factors. A 20-min incubation with R1881 resulted in a 4.6-phosphorylation fold increase, similar to that obtained under IGF-I exposure (Fig. 1B). Nevertheless, full AKT activation is dependent on the phosphorylation of two specific sites, Ser473 in the COOH regulatory region but also Thr308 in the kinase domain. We thus tested the capacity of androgens to change the phosphorylation status of Thr308, in addition to that of Ser473. In the presence of R1881, Thr308 phosphorylation was up-regulated and reached the highest level within 10 min (Fig. 1C). The phosphorylation on the Ser473 residue in response to androgens was time- and dose-dependent (Fig. 1, C and D). Maximum phosphorylation is observed upon 10 min and significant for 10 nM R1881. To determine whether PI3-K is required or not for AKT phosphorylation in response to androgens, the Ser473 phosphorylation status was analyzed in the presence of increasing concentrations of LY294002, a PI3-K inhibitor (31). Co-incubation of VDEC with LY294002 and R1881 dramatically decreased AKT phosphorylation, even with an inhibitor concentration as low as 5 μM (Fig. 1E). Previously, we have demonstrated that AR level is dependent on the PI3-K/AKT pathway and that 50 μM LY294002 could abolish AR expression after long exposure (30). As shown in Fig. 1E, the use of the PI3-K inhibitor did not affect AR accumulation over a short incubation. It was then investigated whether intracellular effectors...
downstream to AKT, such as the transcription factor FKHR-L1 or the pro-apoptotic factor Bad, could be phosphorylated following androgenic stimulation. These two proteins are known to be inhibited by AKT through phosphorylation of Ser256 and Ser112, respectively. FKHR-L1 phosphorylation was significantly increased following a 20-min R1881 stimulation and reached its maximal level after 1 hour of treatment (Fig. 2A). The Bad protein was highly and rapidly phosphorylated. Unlike FKHR-L1, the high phosphorylation level of Bad in response to androgens was transient, reaching a maximum between 10 and 20 min of treatment (Fig. 2B). Present data suggest that different mechanisms could be involved in the control of FKHR-L1 and Bad phosphorylation, even if they are a substrate of the same kinase. These observations demonstrate that androgen stimulation of AKT leads to phosphorylation of cognate substrates.

**AR Is Required for the Androgen-dependent PI3-K/AKT Pathway Stimulation**—To demonstrate whether AR is involved in the androgenic activation of the PI3-K, AKT phosphorylation on Ser473 was analyzed in the presence of both R1881 and bicalutamide, a synthetic anti-androgen hormone. Bicalutamide blocked R1881-induced AKT phosphorylation (Fig. 3A), suggesting that AR is required for activation of the PI3-K/AKT pathway. This effect did not rely on changes in AR accumulation since it remained at steady-state levels in the different experimental conditions. We also analyzed AKT Ser473 phosphorylation in PC3 prostate carcinoma cells, which do not express endogenous AR. These cells were transfected with increasing amounts of either an AR-expressing construct or the corresponding empty vector. As shown in Fig. 3B, the increase in the phosphorylation level on the Ser473 residue was dependent on the presence of the AR. In this transient transfection assay, although AR was able to mediate a partial AKT phosphorylation in untreated cells (Fig. 3C), this 3.0-fold stimulation was further enhanced when AR was activated by R1881. These results clearly indicate that androgen receptor is a central mediator for androgen action on PI3-K pathway since PC3 cells devoid of endogenous AR are unable to enhance AKT phosphorylation in response of R1881.

**The Androgen Activation of the PI3-K/AKT Pathway Is Supported by a Physical Interaction between AR and p85α**—Prior
FIG. 4. Androgen receptor interacts with the p85α regulatory subunit of PI3-K. A, interaction between AR and p85α. VDEC were starved for 16 h, and then incubated with or without R1881 (10 nM) for 10, 20, and 30 min or with ethanol for 20 min as a control. Precleared cell lysates were immunoprecipitated with anti-p85α antibody, and the immune complexes were analyzed by Western blotting with anti-AR and anti-p85α antibodies. B, in vitro pull-down assay of 35S-labeled p85α against full-length, N-terminal, DNA binding domain (DBD)-hinge, C-terminal domains of hAR fused to GST. C, in vitro pull-down assay of 35S-labeled hAR against full-length, SH3, Bcr, N-SH2, C-SH2 domains of p85α fused to GST. The Input lanes were loaded with 20% of the amount of 35S-labeled proteins used in the binding reactions. The amount of GST-fused recombinant proteins was monitored by Coomassie Blue staining, and radioactive signals of radiolabeled proteins were analyzed using a phosphorimaging device.
investigations demonstrated that some steroid receptors, such as glucocorticoid and estrogen receptors, could interact with the p85α regulatory subunit of PI3-K. To determine whether such interactions can occur between p85α and AR, we performed p85α immunoprecipitation in VDEC. As suspected, AR was detected in the p85α immune complex, and interaction is enhanced by R1881 stimulation upon 10 min (Fig. 4A). The interaction features were then investigated using GST pull-down assays. We monitored the binding of 35S-p85α produced in vitro to the full-length, N-terminal domain, DNA binding domain, or ligand binding domain of AR fused to GST. All the AR truncated constructs tested bound p85α protein, but the DNA binding domain-hinge and C-terminal domain exhibits slight interactions regarding N-terminal domain (Fig. 4B). We next investigated the interaction abilities of the different p85α domains to radiolabeled 35S-AR; only the SH3 and C-SH2 domains showed consistent binding (Fig. 4C). The binding of androgen receptor to p85α in vitro was independent of the ligand (data not shown). Taken together, these data demonstrate that AR/p85α interaction occurs throughout direct interaction involving SH3 and C-SH2 motifs of the regulatory subunit.

**Androgens Protect VDEC against Cycloheximide-induced apoptosis through PI3-K pathway**—As R1881 can stimulate the PI3-K pathway, we investigated whether this androgen-dependent non-genomic mechanism could be able to control cell...
survival. To prove this hypothesis, we first determined whether the apoptotic inducer cycloheximide (CHX) triggers apoptosis in VDEC following a 24-h treatment. It resulted in monolayer injury by cells cutting off, changes in nuclei morphology with the appearance of pyknotic nuclei, and apoptotic bodies as attested by DNA staining experiments (Fig. 5A). To ensure that these observations arose from an apoptotic process, we performed Western blot analysis to monitor PARP cleavage, which is specific to the apoptotic process. As expected, CHX induced accumulation of the cleaved PARP form at 85 kDa (Fig. 5A). We then investigated androgen effects on cycloheximide-induced apoptosis and determined the percentage of apoptotic cell population by flow cytometry by measuring hypoploid nuclei accumulation. VDEC were pretreated for 48 h with R1881 (10 nM) to increase AR expression (30) and with LY294002 to prevent any non-genomic action of androgens and to ensure that the treatment could not interfere with the PI3-K cell survival pathway. Cells were then starved for 16 h in minimum medium, a treatment that did not decrease AR level but reset growth factor-dependent signaling pathways. At this point, VDEC were incubated for 1 h with R1881 in the presence or in the absence of LY294002 prior to the addition of cycloheximide to analyze the non-genomic action of androgens on apoptosis. Such an R1881 incubation was able to prevent cell death, reducing apoptotic cell population from 81 to 51% (Fig. 5B). In contrast, LY294002 co-incubation with R1881 blocked the androgen protective effects in a dose-dependent manner, when LY294002 alone had no effect. Taken together, these results indicate that R1881 supports an important role in epithelial cell survival through the non-genomic activation of PI3-K.

DISCUSSION

We show here that the androgen receptor is able to activate the PI3-K/AKT pathway through direct interaction with the p85α regulatory subunit in response to synthetic but also to natural androgens (not shown). Such androgenic activation of the PI3-K leads to the phosphorylation of AKT and the downstream targets Bad and FKHR-L1. Since androgen withdrawal has the potential to inhibit epithelial cell growth and to induce epithelial apoptosis in the epididymis, vas deferens, seminal vesicle, and prostate, many efforts have been devoted to decipher the mechanisms by which they are able to act in these organs. Although androgens were demonstrated to act on proliferation processes through the ras/MAPK signaling cascade, their effect on the PI3-K/AKT pathway has remained little investigated until now. The PI3-K pathway is a central signaling crossroad that integrates a variety of extracellular signals and controls several cellular functions, including cytoskeletal organization, cell division, and survival maintenance (32–34). Among the proteins involved in the regulation of cell death, the transcription factors of the Forkhead family (27) and the pro-apoptotic protein Bad (28, 29), a member of the Bcl-X family, are the key targets of the PI3-K/AKT pathway. Using differentiated androgen-sensitive epithelial cells (VDEC) (35, 36), we demonstrate that androgens are able to stimulate the PI3-K, leading within minutes to the phosphorylation of AKT and its downstream targets Bad and FKHR-L1. Furthermore, such an activation of the PI3-K survival pathway is associated with a clear protection against apoptosis by androgens in differentiated VDEC. The present data allow us to depict a new mechanism for androgen action on male genital epithelia, which is different from that proposed during fetal and early postnatal development where androgens are described as indirect regulators of epithelial cell physiology via mesenchymal paracrine-acting factors such as IGF-I and epidermal growth factor (37, 38). Here, we show that androgens act directly on differentiated epithelial cells and trigger survival signaling by mobilizing the PI3-K, the primary effector of the IGF-I transduction cascade. Interestingly, Li et al. (39) have also demonstrated a link between androgen and growth factor signaling pathways in DU145 cells in which AR can directly activate the FKHR transcription factor, but in an AKT-independent manner. Nonetheless, such a mechanism is likely to be specific to this tumoral cell line as we observed a rapid increase in the PI3-K-dependent phosphorylation of AKT in response to androgens in non-transformed differentiated epithelial cells. One important issue of this work is to further determine the molecular targets and subsequent mechanisms involved in the androgen-dependent repression of cell death processes in VDEC.

Studies of non-genomic mechanisms have shown that steroids could alternatively act through classical steroid receptors or through atypical membrane receptors (40). Our results clearly show that the classical androgen receptor (AR) is required for PI3-K pathway activation. Indeed, in the presence of the anti-androgen bicalutamide, which antagonizes AR, R1881 cannot stimulate AKT Ser473 phosphorylation. Accordingly, in PC3 cells that lack endogenous AR expression, AKT phosphorylation is insensitive to androgens but is enhanced when AR is transiently overexpressed in these cells. This non-genomic stimulation is supported by an interaction of AR with the PI3-K regulatory subunit p85α as demonstrated by immunoprecipitation in VDEC. Our in vitro analyses clearly indicate that two main domains of p85α are involved in the interaction with AR, the SH3 motif and the C-terminal SH2 domain. These domains are known to bind proline-rich regions and phospho-Tyr respectively, and AR has multiple tyrosine residues and a proline stretch in its N-terminal sequence (41). These data strengthened the central role of this AR region in the non-genomic transduction signal. Indeed, the AR proline stretch is known to interact with the SH3 motif of the Src kinase and allows the formation of an active AR-Src kinase complex in a ligand-dependent manner in some breast and prostate cell lines (16). Interestingly, we did not observe a ligand-dependent interaction between AR and p85α in pull-down experiments. Such a lack of hormone sensitivity can be explained by the cell-free system context, in which interacting domains are unmasked and available for binding. This hormone-independent interaction between AR and p85α can be linked with AR overexpression in PC3 cells that leads to an increase in AKT phosphorylation in the absence of ligand. We hypothesize that AR overexpression results in the titration of HSP chaperone known to block AR activity in absence of hormone. Such a mechanism could result in constitutive AR/p85α interaction leading to an increase in AKT phosphorylation level in PC3 cells. Another hypothesis is that AR overexpression increases its availability at the plasma membrane, leading to furthered PI3-K stimulation.

Taken together, these results clearly point out that the androgenic activation of PI3-K represents an alternative pathway by which androgens could protect the epithelial cells against apoptosis. Further investigations are necessary to determine the extent of the non-genomic androgen actions during genital tract development and/or early prostate carcinogenesis stages.

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