Activation of Phosphatidylinositol 3-Kinase/Akt Pathway by Androgen through Interaction of p85α, Androgen Receptor, and Src*

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Mei Sun‡‡, Lin Yang‡, Richard I. Feldman‡, Xia-meng Sun‡, Kapil N. Bhalla‡, Richard Jove‡, and Jin Q. Cheng‡‡

From the ‡Departments of Pathology and Interdisciplinary Oncology, University of South Florida College of Medicine and H. Lee Moffitt Cancer Center, Tampa, Florida 33612 and §Cancer Research Department, Berlex Biosciences, Richmond, California 94804

Recent studies have demonstrated that the cell growth and antiapoptotic actions of androgen could be dissociated from the transcriptional activity of the receptor and were, instead, mediated by activation of a mitogen-activated protein kinase pathway. This finding suggests an important cellular function of androgen receptor (AR) outside the nucleus. In this report, we demonstrate that androgen activates phosphatidylinositol 3-kinase (PI3K) and Akt, including AKT1 and AKT2, in receptors (1, 3, 4). For instance, recent studies have demonstrated that both AR and estrogen receptor (ER)α bind to Src oncoprotein to activate MAPK pathway leading to cell survival and proliferation (4–6).

This article has been withdrawn by the authors. The same data were used to represent different experimental conditions. Specifically, lanes 1–3 of the H2B panel of Fig. 4B were reused in lanes 1–3 of the H2B panel from Fig. 6B. Lanes 1 and 6 of the phospho-Akt panel from Fig. 4C were duplicated. Lanes 3 and 4 of the lower FLAG panel in Fig. 4C were reused in lanes 7 and 8 of the lower FLAG panel in Fig. 5A. The PI(3,4)P2 spots from lanes 1 and 2 from Fig. 7 were reused in lanes 7 and 8 of the same panel. Additionally, some PI(4)P1 spots were pasted in. The authors state that the overall conclusions of this work are not affected.

In the classical model of steroid action, steroid hormones bind to intracellular receptors and modulate nuclear transcription after translocation of steroid-receptor complexes into the nucleus. Their transcriptional activity can even be activated in the absence of steroids through cross-talk with agonist-occupied membrane receptors. However, this classical model of steroid transcriptional action has not yet given exhaustive insight into steroid action on different events, including regulation of cell survival and cell proliferation. Accumulated evidence showed that steroids are able to cause nongenomic responses of cells, i.e. responses not mediated through nuclear receptors but rather responses initiated at the plasma membrane, presumably through unconventional surface receptors (1, 3, 4). For instance, recent studies have demonstrated that both AR and estrogen receptor (ER)α bind to Src oncoprotein to activate MAPK pathway leading to cell survival and proliferation (4–6).

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To whom correspondence should be addressed: Dept. of Pathology, Univ. of South Florida, College of Medicine and H. Lee Moffitt Cancer Center, 12901 Bruce B. Downs Blvd., MDC Box 11, Tampa, FL 33612. Tel.: 813-974-8595; Fax: 813-974-5536; E-mail: jcheng@hsc.usf.edu.

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The abbreviations used are: AR, androgen receptor; ERα, estrogen receptor α; MAPK, mitogen-activated protein kinase; PI3K, phosphatidylinositol 3-kinase; PI(3,4)P2, phosphatidylinositol-3,4-biphosphate; HA, hemagglutinin; GST, glutathione S-transferase; HEK, human embryonic kidney; EGFR, epidermal growth factor receptor; DN, dominant-negative; GRD, GTPase-responsive domain;,
Inhibition of PI3K/Akt pathway attenuates androgen-induced cell proliferation and survival. A, [3H]thymidine incorporation assay. LNCaP and MCF7 cells were incubated for 2 days in phenol red-free medium containing 10% charcoal-treated serum in which hormones were depleted and then treated with or without R1881 and vehicle (0.1% Me2SO). LY294002 (10 μM), or PP1 (10 μM) for 24 h. During the last 6 h of treatment, [3H]thymidine incorporation was measured as described under “Experimental Procedures.” B, MTS assay. LNCaP and MCF7 cells were plated in 96-well plates and treated with or without R1881, and assayed with [3H]thymidine incorporation with or without LY294002 (20 μM) or PP1 (10 μM) for 1 h followed by a 6-h treatment with etoposide (25 μM). The percentage of apoptosis was determined by counting 300–500 cells. All the results were obtained from triple experiments.

We and others (16–18) have shown previously that Akt expression is frequently elevated in human prostate, ovarian, and breast cancers and that ectopic expression of constitutively active Akt or wild type Akt2 results in malignant phenotype. Active Src and Ras induce Akt activation in a PI3K-dependent manner (19, 20). Moreover, we have recently demonstrated that the ERα binds to and activates the PI3K leading to elevated Akt activity in breast cancer cells (21). In this study, we report that androgen stimulates PI3K and Akt activities, leading to cell growth and survival. The activation of PI3K/Akt is mediated by interactions of AR and p85α as well as AR and Src.

EXPERIMENTAL PROCEDURES

Plasmids—The cytomegalovirus-based expression constructs encoding wild-type, constitutively active, and dominant-negative Akt and Src have been described (12, 20). HA-p85α and p110α were kindly provided by Julian Downward (Imperial Cancer Research Fund, London, UK). Expression plasmid pSG-AR was a gift from Albert Brinkman (Erasmus University, Rotterdam, The Netherlands). FLAG-tagged full-length and truncated AR mutants and glutathione S-transferase (GST)-p85α constructs (p85α-SH3, p85α-N-SH2, and p85α-C-SH2) were created by PCR and sub-cloned into FLAG-pCMV2, pcDNA3, and pGEM-4T-1 vectors, respectively, and were confirmed by sequence analysis.

Cell Culture and Transfection—AR-negative human embryonic kidney (HEK)-293 and AR-positive human MCF7 breast cancer and LNCaP prostate cancer cells were obtained from the American Type Culture Collection and were cultured at 37 °C and 5% CO2 in DMEM (for HEK293 and MCF7) or RPMI 1640 medium (for LNCaP) supplemented with 10% fetal calf serum. Cell transfections were performed using calcium phosphate DNA precipitation or LipofectAMINE Plus (Invitrogen).

DNA Synthesis, Cell Growth, and Detection of Apoptotic Cells—Quiescent LNCaP and MCF7 cells were prepared by seeding cells in 12-well tissue culture plates and maintained for 2 days in phenol red-free DMEM medium containing 5% charcoal-stripped calf serum, in which hormones were depleted. The cells were then left unstimulated or stimulated for 24 h with 10 nm R1881 (a synthetic androgen methyltrienolone, PerkinElmer Life Sciences) and treated with or without 10 μM LY294002, a PI3K inhibitor (Sigma), or 10 μM PP1, an Src kinase inhibitor (Biomol Research Laboratories). During the last 6 h of stimulation, 5 μCi/ml [3H]thymidine (PerkinElmer Life Sciences) was added into the culture. The [3H]thymidine incorporation and cell growth assay were performed as described previously (12). For the programmed cell death, LNCaP cells were treated with or without 10 nm R1881 and 20 μM LY294002 or 10 μM PP1 for 1 h, followed by a 6-h treatment with 25 μM etoposide (Sigma). Cell death was analyzed by trypan blue staining and terminal deoxynucleotidyltransferase-mediated dUTP nick end labeling assay. The percentage of apoptosis was determined by counting 300–500 cells. The results reflected triple experiments.

In Vitro Kinase Assay—Akt kinase and PI3K assays were performed as described previously (22). For Src kinase assay, HEK293 cells were
Fig. 2. Androgen induces PI3K and Akt activation in AR-dependent manner. A. GST Pull-down Assay. The GST-p85 immunoprecipitates prepared from MCF7 cells transfected with c-Src together with or without different AR mutant plasmids. After treatment with androgen, the cells were lysed in a lysis buffer containing 50 mM HEPES (pH 7.5), 100 mM NaCl, 2 mM EDTA, 0.5% Nonidet P-40, 10% glycerol (v/v), 0.1 mM phenylmethylsulfonyl fluoride, 0.1 mM sodium orthovanadate, 1 mM phenylmethylsulfonyl fluoride, 10 μg/ml aprotinin, and 10 μg/ml leupeptin in the absence or presence of R1881. Beads were washed three times in the same buffer. Proteins were eluted from the beads with Laemmli sample buffer and separated in SDS-PAGE; protein bands were revealed by autoradiography.

Western Blotting Analysis and Co-immunoprecipitation—Western blotting and co-immunoprecipitation analyses were performed as described previously (12, 16). Briefly, cell lysates were incubated with the appropriate antibody in the presence of protein A agarose beads (Invitrogen). The immunoprecipitates were subjected to Western blotting analysis. Detection of antigen-bound antibody was carried out with the ECL system (Amersham Biosciences). The antibodies used in this study include anti-HA (Roche Applied Science), anti-FLAG (Sigma), anti-AR (Upstate Biotechnology), anti-c-Src (Santa Cruz Biotechnology, Inc.), anti-Akt1 (Upstate Biotechnology), anti-Akt2 (Upstate Biotechnology), and anti-phospho-Akt-Thr308 (Cell Signaling Technology).

GST Pull-down Assay—The GST-p85 fusion proteins and in vitro transcription and translation of 35S-methionine-labeled AR proteins were prepared as described previously (18, 24). The GST-p85a-SH3, GST-p85a-N-SH2, GST-p85a-C-SH2, or GST agarose was incubated with 35S-labeled AR proteins for 1 h at room temperature by gentle shaking in a binding buffer containing 50 mM HEPES, pH 7.4, 150 mM NaCl, 10% glycerol, 1% Triton X-100, 1 mM EDTA, 10 mM NaF, 1 mM sodium orthovanadate, 1 mM phenylmethylsulfonyl fluoride, 10 μg/ml aprotinin, and 10 μg/ml leupeptin.

RESULTS

PI3K/Akt Pathway Mediates Androgen-induced Cell Growth and Anti-apoptosis Action—Recent studies have shown that androgen treatment influences the association of AR with Src. This activates the Src/Raf-1/Erk pathway through a nongenotropic mechanism leading to cell survival and growth in both osteoblasts and epithelial cells (5, 6). Because PI3K/Akt is a major pathway promoting cell survival and growth, we examined whether PI3K/Akt mediates androgen nongenotropic action. To minimize estrogenic activity and deplete hormones in regular medium, AR-positive LNCaP cells were incubated in regular medium for 2 days, and then treated with or without synthetic androgen R1881 (10 nM) and LY294002 for 24 h. Because LNCaP cells have elevated basal levels of PI3K and Akt activity which resulted from mutation of the PTEN (25), parallel experiments were performed in AR-positive MCF7 cells to assess the role of PI3K/Akt pathway in androgen nongenomic action. After the treatment, cell growth was assayed by 3H-thymidine incorporation as shown in Fig. 1A, R1881 stimulated 3H-thymidine incorporation into newly synthesized DNA, and anti-AR (Upstate Biotechnology), anti-c-Src (Santa Cruz Biotechnology, Inc.). After washing three times with lysis buffer and one time with kinase buffer (0.1 mM HEPES, 0.02 mM MgCl2, 0.01 mM MnCl2, and 0.02 mM dithiothreitol), the immunoprecipitates were incubated with kinase reaction mixture (10 mM HEPES (pH 7.5), 100 mM NaCl, 2 mM EDTA, 0.5% Nonidet P-40, 10% glycerol (v/v), 0.1 mM phenylmethylsulfonyl fluoride, 0.1 mM sodium orthovanadate, 1 mM phenylmethylsulfonyl fluoride, 10 μg/ml aprotinin, and 10 μg/ml leupeptin), the immunoprecipitates were subjected to kinase reaction mixture (10 μM cold ATP, 2 μCi [γ-32P]ATP, and 2 μg of acid-denatured enolase) at 30 °C for 30 min (23). The reaction mixture was separated on a 10% SDS-PAGE gel, determined by autoradiography, and quantified with a PhosphorImager.

 Autoradiography.
DNA by 6–7-fold. PI3K inhibitor, LY294002, significantly inhibited the androgen-induced [3H]thymidine incorporation in both LNCaP and MCF7 cells. These data indicate that androgen-induced DNA synthesis is mediated by PI3K.

We next examined the involvement of Akt in androgen-stimulated DNA synthesis. Because MCF7 cells possess intact PI3K/PTEN/Akt pathway and are AR positive, we stably transfected MCF7 cells with dominant-negative Akt1. As illustrated in Fig. 1B, ectopic expression of dominant-negative (DN)-Akt1 dramatically inhibited androgen-stimulated thymidine incorporation. Further, MTS cell proliferation analysis revealed that cell growth stimulated by androgen was inhibited by PI3K inhibitor LY294002 in MCF7 and LNCaP cells (Fig. 1C and data not shown). Moreover, androgen treatment significantly inhibited programmed cell death induced by etoposide, whereas the PI3K inhibitor LY294002 attenuated androgen-anti-apoptotic activity by approximately 60% (Fig. 1D). In addition, inhibition of Src by either Src inhibitor PP1 or stably expressed dominant-negative Src abrogated androgen actions (Fig. 1, A–D).

These data suggest that PI3K/Akt and Src mediate the non-genomic function of androgen.

**Androgen Activates PI3K/Akt Pathway in an AR-dependent Manner**—To determine whether androgen induces PI3K/Akt kinase activity, MCF7 cells were hormone-depleted as described above, pretreated with or without the PI3K inhibitor wortmannin, and then stimulated with 10 nM R1881 for 10 min. In vitro AKT kinase assay showed an increase of endogenous AKT1 and AKT2 kinase activities upon R1881 stimulation, which was inhibited by wortmannin (Fig. 2A). To examine whether the activation is mediated by AR, AR-negative HEK293 cells were transfected with AR and then stimulated with or without R1881. As shown in Fig. 2B, both Akt1 and Akt2 activities were significantly induced by androgen stimulation in AR-transfected cells. Similarly, the PI3K inhibitor wortmannin abrogated R1881-induced Akt activation. To further determine whether androgen can activate PI3K, in vitro PI3K assay was performed with HEK293 cells transfected with AR and src.
Androgen Activation of PI3K/Akt Pathway

Fig. 4. Interaction between AR and p85α and their Interaction Is Enhanced by Androgen Stimulation. A and B, in vitro kinase assays using PI-4-P and histone H2B as substrates, respectively. C, immunoprecipitation was carried out in LNCaP cells after transient transfection with either HA-p85α or histone H2B expression plasmids. After stimulation with or without R1881, the cells were lysed and immunoprecipitated with anti-p110α or anti-HA antibody, respectively. In R1881-treated and -untreated cells, the activity was analyzed by immunoblots probed with anti-HA and anti-myc (bottom two panels), and anti-Akt1 (middle two panels) antibodies. The blots were detected with anti-phospho-Akt-T308 (top panel), anti-FLAG (middle two panels), and anti-Akt1 antibodies. The immunoprecipitates were subjected to in vitro kinase assay using PI-4-P and histone H2B as substrates, respectively.

AR Binds to P85α and their Interaction Is Enhanced by Androgen Stimulation—We and others (21, 26) have demonstrated previously that ERα binds to the p85α regulatory subunit of PI3K and induces PI3K/Akt activation. To determine whether AR is able to bind to PI3K, LNCaP cells were transiently transfected with either HA-p85α or Myc-p110α expression plasmid, and cells were incubated with or without R1881. Association of p85α or p110α with immunoprecipitated AR was analyzed by immunoblots probed with anti-HA and anti-myc antibody, respectively. In R1881-treated and -untreated cells, AR associated with p85α but not p110α (Fig. 3A and data not shown). The binding affinity between AR and p85α was increased by androgen stimulation (Fig. 3A). To examine whether p85α interacts with AR in physiological protein concentration, co-immunoprecipitation was carried out in LNCaP cells after treatment with or without R1881 for 12 h. As shown in Fig. 3B, p85α and AR associated with each other, and the association was also enhanced by R1881 treatment. As shown in Fig. 3E, full-length AR protein (Fig. 3D) was incubated with different GST-fused domains of p85α. As shown in Fig. 3E, full-length AR also only bound to the SH3 domain of p85α.

Definition of Domains Involved in p85α-AR Interaction—Previous studies have demonstrated that a number of proteins interact with different domains of p85α, especially SH3 or SH2 domains, to regulate PI3K (27, 28). Furthermore, recent reports have shown that the proline-rich region of AR binds to the SH3 domain of Src to mediate androgen-induced Src/Raf/Erk activation (5, 6). Therefore, we reasoned that the proline-rich motif of AR may interact with the SH3 domain of p85α. To test this hypothesis, a GST pull-down assay was performed with the GST-SH3, GST-N-SH2, and GST-C-SH2 domain of p85α fusion proteins (Fig. 3, C and E). The [35S]methionine-labeled AR proteins were obtained with in vitro transcription/translation using N-terminal-truncated AR-ΔN and AR-ΔNP, which include DNA binding domain, hinge region, ligand binding domain, and two C-terminal deletions of AR fragments as templates, one of which contains the proline-rich region (AR-ΔC) and the other does not (AR-ΔCP; Fig 3D). The translated AR-ΔN, AR-ΔNP, AR-ΔC, and AR-ΔCP proteins were incubated with GST-p85α-SH3, GST-p85α-N-SH2, GST-p85α-C-SH2, or GST-agarose. GST-agarose was used as a control in the presence or absence of androgen. After extensive washing, the complexes were separated in SDS-PAGE and exposed to x-ray film. Three different experiments showed that both AR-ΔC and AR-ΔCP bound to C-SH2 but not the C-SH3 domain of p85α in the presence or absence of R1881, indicating that the proline-rich region of AR does not interact with the SH3 motif of p85α. Further, AR-ΔN and AR-ΔNP failed to interact with p85α, even though they contain ligand binding domain (Fig. 3E). As a positive control, in vitro translated full-length AR protein (Fig. 3D) was incubated with different GST-fused domains of p85α.

As shown in Fig. 3E, full-length AR also only bound to the C-SH2 domain of p85α. These data indicate that the C-SH2 domain of p85α associates with the N terminus, upstream from the proline-rich region, of AR.
Interactions of AR and p85α as well as AR and Src Are Required for Androgen-induced PI3K/Akt Activation—Next, we examined the importance of interaction between AR and p85α in androgen-induced PI3K and Akt activation. HEK293 cells were transiently transfected with AR-ΔC, AR-ΔCP, AR-ΔN, AR-ΔNP, and AR and stimulated with or without R1881. In vitro kinase assay revealed that elevated PI3K and Akt kinase activities were detected in the cells transfected with AR-ΔC and AR but not the cells transfected with AR-ΔN, AR-ΔNP, and even AR-ΔCP, which binds to p85α (Figs. 3E and 4A and B). Accordingly, the elevated phosphorylation levels of Akt at Thr-308, a key residue for activation of Akt, were only observed in AR-ΔC- and AR-transfected cells (Fig. 4C), suggesting that androgen-induced PI3K/Akt activation depends upon the interaction between p85α and the N-terminal region of AR, which has to contain the proline-rich region to recruit Src (see below) but is independent of ligand binding domain of AR. These results support the notion that sex steroid hormones, including androgen and estrogen, function as growth factor (29, 30, 31) and that their nongenomic action could be mediated by cell membrane receptors such as G protein-coupled receptor (4, 32) and epidermal growth factor receptor (EGFR) (33), as well as...
sex hormone binding globulin (1). It has been shown that the interaction between the proline-rich region of AR and the SH3 domain of Src is required for androgen-induced Src-Raf-Mek-Erk activation (5, 6); thus, our results also suggest that androgen-stimulated PI3K/Akt activation, unlike MAPK, may require the interaction between AR and p85α as well as an association of AR with Src.

To test this hypothesis, we carried out in vitro Src and PI3K kinase assays in HEK293 cells transfected with different truncation mutants of AR. As illustrated in Fig. 5, androgen activates Src and PI3K kinase in AR-ΔC and AR transfected cells as expected but not in the cells transfected with ARΔCP and ARΔNP, which lack the proline-rich region and are incapable of interaction with Src (Fig. 5C). Notably, ARΔN was able to induce kinase activity of Src but not PI3K and Akt (Figs. 4 and 5), as it contains the proline-rich region and binds to Src (Fig. 5C) but not to p85α (Fig. 3E). These results indicate that the interaction of AR and Src also mediates androgen-activated PI3K and that androgen-induced Src activation is independent of the hormone binding domain of AR as well.

To demonstrate further the involvement of Src in androgen activation of PI3K/Akt, HEK293 cells were transfected with dominant-negative Src and AR and stimulated by R1881. In vitro kinase assays revealed that ectopic expression of DN-Src efficiently inhibited androgen-stimulated PI3K and Akt kinase activities (Fig. 6, A and B), suggesting that Src is required for androgen-induced PI3K/Akt activation. Next, we examined whether Src regulates the association between p85α and AR. HEK293 cells were transfected with AR, p85α, and DN-Src. After stimulation with or without androgen, coimmunoprecipitation was performed. As illustrated in Fig. 6C, expression of DN-Src decreases the binding affinity between AR and p85α in the cells treated with or without androgen. Taken together, we conclude that the triple complex between AR, p85α, and Src is required for androgen activation of PI3K/Akt pathway in a hormone binding domain-independent manner and that androgen-stimulated Src is a critical mediator for the triple complex formation and the PI3K/Akt activation.

As androgen induces PI3K and Akt activity independently of the ligand domain of AR, we reasoned that androgen could stimulate cell membrane receptor(s) and then enhance Src activity and the triple complex formation leading to the activation of the PI3K/Akt pathway. To test this hypothesis, three tyrosine receptor kinase inhibitors, AG1478, STI-571, and

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**Fig. 6. Androgen activation of PI3K/Akt and association between p85α and AR are regulated by Src.**

**A**. *In vitro* PI3K assay of the p110α immunoprecipitates prepared from HEK293 cells transfected with indicated plasmids and treated with or without R1881 (upper panel). Expression of transfected FLAG-AR and DN-sr is shown in middle and lower panels.

**B**. *In vitro* Akt kinase assay. HEK293 cells were transfected with indicated plasmids. After the stimulation with or without R1881, the cells were lysed and immunoprecipitated with anti-HA antibody. HA-Akt1 immunoprecipitates were subjected to in vitro kinase assay (upper panel). Middle and lower panels show expression of transfected Akt1, AR, and DN-Src.

**C**. DN-Src decreases the binding affinity between p85α and AR. HEK293 cells were transfected with indicated expression constructs and immunoprecipitated with anti-AR antibody. The immunoprecipitates were immunoblotted with anti-HA (upper panel) and anti-AR (lower-middle panel) antibody. The binding affinity between p85α and AR was quantified (upper-middle panel). Lower panel shows expression of transfected DN-Src.

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**WITHDRAWN**

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AG1296 were used to treat the cells prior to R1881 stimulation. In vitro PI3K kinase assay and Western blot analysis revealed that EGFR inhibitor AG1478, but not c-kit and/or platelet-derived growth factor receptor inhibitors STI-571 and AG1296 (35), attenuated androgen-induced PI3K and Akt activation. The inhibitory effects of AG1478 on R1881-stimulated PI3K/Akt activity are very similar to those of Src inhibitor PP1 (Fig. 7 and data not shown). AG1478 has also been shown to inhibit Her2/Neu (36). Therefore, these data indicate that a member(s) of ErbB family mediates androgen action on PI3K/Akt pathway.

**DISCUSSION**

Recent studies have identified a nongenomic action of androgen depending on the ability of the AR to interact with Src, which activates the MAPK pathway (5, 6). In addition to androgen, estrogen also activates the same pathway even though with some differences in the initial steps leading to the hormonal Src activation (5, 6). Inhibition of the MAPK pathway abrogates the hormone-dependent growth (30). Moreover, hormone activation of the same pathway protects osteoblasts from apoptotic stimuli (5). In this report, we have demonstrated that androgen activation of the PI3K/Akt cascade depends upon AR interaction with p85α as well as with Src and that interference with the PI3K/Akt activation inhibits androgen-stimulated cell growth and survival. In addition, we present evidence that AR mediates androgen nongenomic function and that androgen activates PI3K/Akt in a ligand binding domain-independent manner, which supports the notion that the nongenomic function of androgen and estrogen is mediated by its interaction with membrane receptors (1, 3, 4) such as G protein-coupled receptor (4, 32) and EGFR (33), as well as sex hormone binding globulin (1). Moreover, we showed that EGFR/Her2 inhibitor, but not platelet-derived growth factor receptor and c-kit inhibitors, abrogated androgen-induced PI3K and Akt activity (Fig. 7) and that dominant-negative Src deceases the association of AR and p85α (Fig. 6C). Taken collectively, these data suggest that a member(s) of the ErbB family, rather than AR, is a direct transducer(s) of androgen nongenomic action to induce Src

![Fig. 7.](image)

**Fig. 7.** EGFR inhibitor, AG1478, inhibits androgen-induced PI3K and Akt activation. HEK293 cells were transfected with indicated expression plasmids. After 36 h of transfection, cells were serum-starved overnight and treated with indicated compounds for 30 min prior to R1881 stimulation. In vitro PI3K assay was performed with anti-p110α antibody (upper panel). Phospho-Akt was detected by Western blot analysis using anti-phospho-S473 antibody (middle panel). Expression of total Akt and transfected AR is shown in the three lower panels.

| AR: | - | + | + | + | + | + | - | - | - | - | - |
| Flag-ARΔC: | - | - | - | - | - | - | + | + | + | + | - |
| R1881: | - | + | + | + | + | + | + | + | + | + | + |
| LY294002: | - | - | + | - | - | - | - | - | + | - | - |
| AG1478: | - | - | - | + | + | + | - | - | - | + | - |
| PP1: | - | - | - | - | - | - | - | - | - | - | - |

![Fig. 8.](image)

**Fig. 8.** Schematic illustration of androgen activation of PI3K/Akt pathway to promote cell growth and survival.
kinase (Fig. 5). The activated Src promotes the complex formation of AR and p85α leading to the activation of PI3K and Akt (Figs. 6C and 8).

P85α regulatory subunit of class Ia PI3K is composed of SH3, BCR, N-SH2, iSH2, and C-SH2 domains that mediate interactions between PI3K and other signal molecules. Class Ia PI3K can be activated by tyrosine kinase receptors through the binding of the SH2 domain of p85α and by GTP-bound Ras, which interacts with the catalytic subunit p110 (37). Activation of PI3K by Src family kinases through binding of the proline-rich motif interacts with the catalytic subunit p110 (37). Activation of PI3K by Src family kinases through binding of the proline-rich domain function of p85α with Src (Fig. 5). The activated Src promotes the complex formation of AR-p85α and p110 plasmids. We also thank the people at the DNA Sequence and Flow Cytometry Facilities at the H. Lee Moffitt Cancer Center.

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