Androgen Blocks Apoptosis of Hormone-dependent Prostate Cancer Cells

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ABSTRACT

Androgen plays a critical role in the promotion and growth of prostate cancer. Androgen ablation has an expanding role in prostate cancer treatment and is now used to improve the efficacy of radiation therapy in addition to its role in treatment of metastatic disease. Here we show that androgen interferes with induction of prostate cancer cell death induced by a variety of stimuli. The effect of androgen on cell death occurs predominantly by interference with caspase activation and the inhibition of caspase cleavage in both the extrinsic and intrinsic cell death pathways. Androgen inhibited apoptosis induced by both tumor necrosis factor-α (TNF-α) and by Fas activation with or without concomitant irradiation. An antiapoptotic effect was seen in the presence of R1881, dihydrotestosterone, and also 17β-estradiol within 24 h of death induction. Sustained inhibition of apoptosis at 72 h was seen only with R1881, dihydrotestosterone, cyproterone acetate, and hydroxyflutamide. Androgen treatment inhibited activation of caspases-8, -7, and -9 by TNF-α +/- irradiation. Androgen attenuated BAX expression and blocked appearance of the proapoptotic p18 fragment of BAX. Androgen also abrogated BID cleavage induced by TNF-α +/- irradiation that contributed to a decrease in cytochrome c egress from mitochondria induced by TNF-α +/- irradiation. There was also decreased mitochondrial depolarization in response to TNF-α +/- irradiation. Production of the proapoptotic lipid metabolite ceramide was not affected by androgen, but androgen acted downstream from ceramide generation because R1881 blocked cell death induction by bacterial sphingomyelinase. Inhibition of phosphoinositol-3-kinase activity by wortmannin induced apoptosis that was also blocked by androgen, but there was no effect on protein levels or phosphorylation of AKT, indicating that R1881 did not interact with survival signaling of phosphoinositol-3-kinase. Lastly, androgen inhibited activation of nuclear factor-κB during death induction, but the effect of androgen on cell death was not mediated by interference with the nuclear factor-κB pathway. The data suggest that androgen induced blockade of caspase activation in both intrinsic and extrinsic cell death pathways and thereby was able to protect prostate cancer cells from apoptosis induced by diverse stimuli.

INTRODUCTION

Since the discovery by Huggins that androgen ablation benefited patients with advanced prostate cancer, we have come to understand that androgens play a critical role in the development, progression, and treatment of prostate cancer (1, 2). Currently androgen ablation is the only proven treatment that confers unequivocal, but temporary, benefit on patients with metastatic prostate cancer (3, 4). More recently use of androgen ablation as adjunctive treatment for localized prostate cancer has been undertaken. Studies during the last decade have shown that, when combined with radiation therapy, androgen ablation improved survival of patients with locally advanced prostate cancer (5). There is also increasing evidence that early androgen ablation, despite its long-term morbidity, improves cause-specific survival compared with delayed hormonal therapy in early metastatic prostate cancer (6–8). Androgens are also believed to be important prostate cancer promoters. Interference with the synthesis of DHT3, the androgen on which prostatic epithelial cells are most dependent, is under investigation for prostate cancer prevention in the nationwide randomized Prostate Cancer Prevention Trial (9, 10).

We reported that androgens protected androgen-responsive LNCaP human prostate cancer cells from programmed cell death induced by etoposide (11). LNCaP cells undergo growth arrest but not apoptosis, in response to androgen deprivation, making these cells a good model for hormone-responsive prostate cancer. Our data suggested that cytotoxic treatments for prostate cancer would have greater efficacy if delivered concurrently with androgen ablation. We have also shown that LNCaP cells are highly resistant to radiation-induced cell death but can be sensitized to irradiation by death ligands such as TNF-α and agonistic Fas antibodies (12, 13).

Androgen can inhibit cell death by acting as a survival factor for normal prostatic epithelial cells. Prostatic epithelium undergoes apoptosis shortly after androgen deprivation. The rat prostate gland involutes within 3 weeks of castration (14, 15). The transplantable human PC-82 prostate cancer xenograft also undergoes apoptosis after castration (16). Human prostatic epithelium and the majority of prostate cancer cells respond quite rapidly to androgen ablation and undergo apoptosis (17, 18). The synthetic androgen R1881 has been shown to support LNCaP cell survival in the presence of a PI3K inhibitor that blocked AKT phosphorylation and thereby AKT activity (19). These experiments showed that androgen acted as a survival factor through pathway(s) independent of PI3K-PTEN-AKT.

We now show that androgen blocks LNCaP cell death in large part by interfering with caspase activation in both intrinsic and extrinsic cell death pathways. These experiments provide further support for the notion that cytotoxic therapies may have greater effect on prostate cancer cells in the androgen-deprived milieu.

MATERIALS AND METHODS

Cell Culture and Apoptosis Assays. Cell culture conditions and death induction have been described previously (12, 13). Modified IMEM (Life Technology Inc., Gaithersburg, MD) containing 5% FCS was usually used for culturing LNCaP cells. For the experiments, the medium was replaced by the modified IMEM containing 5% charcoal-treated calf serum without phenol red 24 h before treatment with okadaic acid or death ligands. Synthetic androgen R1881 (10−9 M, unless stated otherwise) was added to the culture when the culture medium was replaced by the medium with charcoal-treated calf serum. Other steroid hormones were used at the concentrations indicated. Cyproterone acetate was obtained from Sigma Chemical Co. Hydroxyflutamide was obtained from the Schering Corporation (20). Cell death was measured by in situ end labeling that we had previously shown correlates well with appearance of the morphological hallmarks of cell death (21). We routinely used 8 Gy for experiments in which irradiation was combined with TNF-α (12). For some experiments with other agents, 20 Gy were used. Bacterial sphingomyelinase was used at a concentration of 300 mU/ml.

Western Blotting. Western blotting was carried out as described previously (12).

Ceramide. Ceramide assays were done as described previously (12).

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3 The abbreviations used are: DHT, dihydrotestosterone; PARP, poly(ADP-ribose) polymerase; TNF, tumor necrosis factor; PI3K, phosphatidylinositol trisphosphate kinase; TNFR1, TNF receptor 1; IMEM, improved minimal essential medium; NF, nuclear factor; FACS, fluorescence-activated cell sorter/sorting.
NF-kB Transcriptional Activity Assay. The NF-kB transcription reporter vector, pNF-kB-Reporter, was purchased from Clontech Laboratories Inc. (Palo Alto, CA). After the vector was transiently transfected into cells with effectene (QIAGEN Inc., Valencia, CA), the cells were cultured in IMEM without phenol red with 5% charcoal-treated calf serum overnight. The cells were treated with TNF-α and/or irradiation. Six h after the treatment, the cells were washed and dissolved into the solubilization buffer that was a part of the luciferase assay kit (Promega Corp., Madison, WI). The luciferase assay of transcriptional activity was performed according to the manufacturer’s protocol (Promega) using a Lumat LB9501 luminometer (Berthold Pty Ltd., Bundoora, Australia).

Cytochrome c Egress. Cytochrome c egress was assayed by confocal microscopy. The cells were cultured on glass-bottomed microwell dishes (MatTek Co., Ashland, MA). After treatment with TNF-α and/or irradiation, the cells were incubated for 24 or 48 h and fixed with 10% formaldehyde. The cells were permeabilized with 0.1% Triton X-100, and stained with mouse anti-cytochrome c oxidase I monoclonal antibody (Molecular Probes Inc., Eugene, OR) and with antimouse IgG conjugated with Texas Red (Jackson ImmunoResearch Laboratories Inc., West Grove, PA). After this, the cells were further stained with rabbit anti-cytochrome c polyclonal antibody (Santa Cruz Biotechnology Inc., Santa Cruz, CA) and with antirabbit IgG conjugated with FITC (Jackson ImmunoResearch Laboratories Inc., West Grove, PA). After this, the cells were further stained with rabbit anti-cytochrome c polyclonal antibody (Santa Cruz Biotechnology Inc., Santa Cruz, CA) and with antirabbit IgG conjugated with FITC (Jackson ImmunoResearch Laboratories Inc., West Grove, PA). After this, the cells were further stained with rabbit anti-cytochrome c polyclonal antibody (Santa Cruz Biotechnology Inc., Santa Cruz, CA) and with antirabbit IgG conjugated with FITC (Jackson ImmunoResearch Laboratories Inc., West Grove, PA). After this, the cells were further stained with rabbit anti-cytochrome c polyclonal antibody (Santa Cruz Biotechnology Inc., Santa Cruz, CA) and with antirabbit IgG conjugated with FITC (Jackson ImmunoResearch Laboratories Inc., West Grove, PA). After this, the cells were further stained with rabbit anti-cytochrome c polyclonal antibody (Santa Cruz Biotechnology Inc., Santa Cruz, CA) and with antirabbit IgG conjugated with FITC (Jackson ImmunoResearch Laboratories Inc., West Grove, PA). After this, the cells were further stained with rabbit anti-cytochrome c polyclonal antibody (Santa Cruz Biotechnology Inc., Santa Cruz, CA) and with antirabbit IgG conjugated with FITC (Jackson ImmunoResearch Laboratories Inc., West Grove, PA). After this, the cells were further stained with rabbit anti-cytochrome c polyclonal antibody (Santa Cruz Biotechnology Inc., Santa Cruz, CA) and with antirabbit IgG conjugated with FITC (Jackson ImmunoResearch Laboratories Inc., West Grove, PA). After this, the cells were further stained with rabbit anti-cytochrome c polyclonal antibody (Santa Cruz Biotechnology Inc., Santa Cruz, CA) and with antirabbit IgG conjugated with FITC (Jackson ImmunoResearch Laboratories Inc., West Grove, PA).

Mitochondrial Depolarization. The assay was performed by using the DePsipher kit (Trevigen, Gaithersburg, MD; Ref. 22). Briefly the cells were collected and washed with PBS(−), and suspended in DePsipher (JC-1, 5,5′,6,6′-tetrachloro-1,1′,3,3′-tetraethylbenzimidoxyl carbocyanine iodide) solution (final concentration, 5 µg/ml). After incubation at 37°C for 20 min, the samples were washed with PBS(−) twice and analyzed at 488 nm argon laser by flow cytometry (FACStar plus, Becton Dickinson, San Diego, CA).

RESULTS

Effect of Androgen on Caspase-dependent Cell Death. Okadaic acid causes rapid and complete LNCaP cell death within 48 h of exposure via activation of the caspase-dependent intrinsic death pathway (12). The synthetic androgen R1881 diminished cell death by causing a delay in caspase activation as suggested by Western blot analysis of PARP cleavage (Fig. 1, A and B). To examine the effect of androgen on more clinically relevant models of LNCaP apoptosis, we used death ligands and irradiation. LNCaP cell death is induced minimally by the agonistic Fas antibody (clone CH-11), but to a much greater degree by CH-11 + irradiation (13). Androgen completely abrogated cell death induction by CH-11 + irradiation (Fig. 1C).

Rapid caspase-dependent LNCaP cell death is also induced by exposure to the combination of TNF-α and CH-11 (13). Combined treatment with CH-11 and TNF-α is synergistic and causes apoptosis within 24 h at concentrations of death ligands that alone have minimal or no effect on LNCaP cells. The combined effect is enhanced further by concurrent exposure to irradiation. Androgen effectively blocked apoptosis under these conditions (Fig. 1D). TNF-α sensitization of LNCaP cells to CH-11 is attributable in part to increased expression...
of Fas on the cell surface. This increase is mediated by NF-κB and blocked by IkB super repressor. R1881 reduced Fas expression on the cell surface only slightly and, therefore, probably affected apoptosis downstream from Fas activation (Fig. 1E). The reduction in cell surface Fas was consistent with reduced levels of total cellular Fas seen in androgen-treated cells (Fig. 1F). However, there was no effect of androgen on levels of Fas ligand (Fig. 1F).

TNF-α at concentrations of 10–40 ng/ml induces a moderate degree of LNCaP cell death over 72 h. TNF-α sensitizes LNCaP cells to γ-irradiation resulting in an ~2- to 3-fold increase in cell death at 72 h (12). We treated cells with different concentrations of TNF-α in the presence of serum-free medium +/- 1 nM R1881. After a 72-h incubation, we observed up to 67% less cell death in the cultures that included R1881 that appeared to limit the degree of cell death induced by TNF-α (Fig. 2A). The data at 72 h after exposure to TNF-α and irradiation are shown in Fig. 2B. Note that in contrast to a near complete block of cell death by CH-11 + irradiation, R1881 caused only a partial block of cell death attributable to TNF-α + irradiation. Androgen inhibition of TNF-α-induced cell death was not attributable to a change in TNFR1 expression, because levels of TNFR1 were unchanged in the presence of androgen treatment (Fig. 2C).

To examine the ligand specificity of the antiapoptotic effects of the androgen receptor, we treated cells with different steroid hormones. We used these hormones at concentrations that we had previously shown activated androgen-responsive reporter gene activity in prostate cancer cell lines (23). R1881 and DHT inhibited cell death 72 h after treatment with TNF-α and irradiation. Testosterone, 17β-estradiol, and androstenedione, an adrenal androgen, did not inhibit cell

4 K. Kimura and E. P. Gelmann, unpublished observations.
which has no effect on LNCaP cell growth, also had no effect on hydroxyflutamide as agonists (20, 26). On the other hand, flutamide, mutant androgen receptor that recognizes cyproterone acetate and nonsteroidal antihormones is shown in Table 1. LNCaP cells have a (24). A summary of apoptosis inhibition by steroid hormones and of heterologous steroid hormones and receptors as suggested recently (24). Because LNCaP cells do not express estrogen receptor (25), we

**Androgen Effect on Caspase Activation.** TNF-α + irradiation activates both intrinsic and extrinsic death pathways in LNCaP cells (13). Extrinsic pathways are activated by the activation of caspase-8 and its downstream effector caspase-7. The intrinsic pathway is activated via BID cleavage by caspase-8 and subsequent activation of caspase-9. Androgen blocked caspase-8 and caspase-7 activation substantially and had a lesser effect on caspase-9 activation (Fig. 3). We have observed that, in prostate cancer cells, increased procaspase expression is required for cell death execution (27). We note that R1881 did not block procaspase synthesis and, if anything, increased levels of procaspases (compare, for example, Fig. 3, caspase-7, Lanes 1 and 5 or 3 and 7). Therefore, the cells initiated a death response, as seen by increased procaspase levels, but were unable to carry out caspase cleavage.

Because caspase-9 cleavage was inhibited to a lesser degree than caspase-8 and -7, we wanted to see whether androgen affected cytochrome c efflux and mitochondrial depolarization. Using antibodies to cytochrome c and cytochrome c oxidase, we observed with confocal microscopy that androgen caused a block in cytochrome c egress from mitochondria (Fig. 4A). We also examined the effect of androgen on mitochondrial depolarization as measured by the efflux of the fluorescent dye JC-1. In the presence of R1881, there was a low level of mitochondrial depolarization after treatment with either TNF-α or irradiation. However, androgen reduced the mitochondrial depolarization that was induced by combined treatment with TNF-α + irradiation (Fig. 4B).

Because we found an inhibitory effect of androgen on cytochrome
c release from mitochondria and mitochondrial membrane depolarization, we asked whether the effect on mitochondrial activation was associated with changes in the activation of members of the BCL-2 family of proteins. BAX expression was increased by TNF-α +/--irradiation (Fig. 4C). Androgen inhibited the induction of BAX expression by TNF-α. Of note was that calpain-dependent cleavage of BAX to an active p18 subunit was inhibited in the presence of androgen (28–30). We also observed that BID cleavage, seen prominently at 72 h after treatment with TNF-α + irradiation, was also inhibited by androgen. Because BID is cleaved by caspase-8, we expected to see an attenuation of BID cleavage because caspase-8 activation was inhibited by androgen (Fig. 3). We examined the BCL-X_L expression in the same samples and, in contrast to the effects on BAX and BID, found no effect of R1881 on BCL-X_L expression.

**Androgen and Ceramide Production.** Ceramide production accompanies the induction of LNCaP cell death after exposure to TNF-α + irradiation. This is demonstrated by the increase in ceramide production seen after treatment with both agents compared with either alone (12). The presence of androgen had no discernable effect on ceramide production in LNCaP cells (Fig. 5A). However, when cell death was induced by bacterial sphingomyelinase, which acts by increasing production of endogenous ceramide, death was blocked by androgen (Fig. 5B). Bacterial sphingomyelinase causes caspase-dependent LNCaP cell death in a manner that also sensitizes the cells to irradiation, similarly to TNF-α (31). Therefore, the data suggest that interference of ceramide-induced cell death by androgen occurred downstream from ceramide generation.

**Androgen Acts Downstream from the PI3K Survival Pathway.** Disruption of the PTEN suppressor oncogene in LNCaP cells and many advanced prostate cancers leads to activation of the PI3K survival pathway (32–36). It has been shown that the PI3K inhibitor LY294002 can block AKT phosphorylation and induce caspase-dependent LNCaP cell death that can be blocked by R1881 (19). However, R1881 does not prevent the blockade of AKT phosphorylation by PI3K inhibitors, although it does inhibit apoptosis, which suggests that there is an effect of R1881 on apoptosis downstream from AKT. We used wortmannin to inhibit PI3K and induce LNCaP cell death. Because wortmannin is a PI3K inhibitor, it works upstream from PTEN and abrogates the effect of PTEN inactivation in LNCaP cells. When combined with irradiation, wortmannin caused very rapid

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**Table 1** Effect of steroid hormones and nonsteroidal antagonists on cell death at 72 h

<table>
<thead>
<tr>
<th>Hormone</th>
<th>Concentration</th>
<th>TNF-α</th>
<th>TNF-α + 8 Gy</th>
</tr>
</thead>
<tbody>
<tr>
<td>R1881</td>
<td>1 nM</td>
<td>90.8 ± 12.8</td>
<td>39.3 ± 4.0</td>
</tr>
<tr>
<td>DHT</td>
<td>10 nM</td>
<td>97.1 ± 4.2</td>
<td>72.0 ± 2.8</td>
</tr>
<tr>
<td>Testosterone</td>
<td>100 nM</td>
<td>101.3 ± 5.1</td>
<td>110.4 ± 5.3</td>
</tr>
<tr>
<td>17β-estradiol</td>
<td>100 nM</td>
<td>118.3 ± 6.6</td>
<td>121.3 ± 1.9</td>
</tr>
<tr>
<td>Androstenedione</td>
<td>10 nM</td>
<td>118.5 ± 4.1</td>
<td>102.0 ± 6.5</td>
</tr>
<tr>
<td>Flutamide</td>
<td>10 nM</td>
<td>107.3 ± 6.2</td>
<td>117.7 ± 3.8</td>
</tr>
<tr>
<td>Cyproterone acetate</td>
<td>100 nM</td>
<td>120.5 ± 4.7</td>
<td>122.5 ± 1.9</td>
</tr>
<tr>
<td>Hydroxyflutamide</td>
<td>100 nM</td>
<td>85 ± 19.6</td>
<td>71.5 ± 3.4</td>
</tr>
</tbody>
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![Fig. 3](image-url) The effect of 1 nM R1881 on the cleavage of caspase-8, -7, and -9 induced by 40 ng/ml TNF-α and 8 Gy irradiation at 72 h after treatment. The cleavage products of caspases are p44, p42, and p18 for caspase-8, p17 for caspase-7, and p37 for caspase-9.
cell death within 24 h of treatment (Fig. 6). Note that death induction by wortmannin and irradiation occurred more rapidly than death induction by TNF-α and irradiation. R1881 blocked the induction of cell death induced by wortmannin and irradiation. This confirms and expands the observations by Carson et al. that androgen acts downstream to block cell death induced by PI3K inhibitors (19).

Androgen Effects on TNF-α-induced NF-κB Activity. Although in many cell types activation of the transcription factor NF-κB has antiapoptotic effects, in LNCaP cells induction of NF-κB activity by TNF-α is proapoptotic (37, 38). To ask whether the antiapoptotic effects of R1881 were to some degree mediated by affecting NF-κB activity, we assayed NF-κB reporter activity in LNCaP cells in the presence and absence of androgen. Fig. 7A shows that the presence of 1 nM R1881 caused a 50% reduction in NF-κB activity induced by TNF-α. Despite this observation, we do not believe that R1881 exerts a major antiapoptotic effect by 50% inhibition of NF-κB activity because blockade of NF-κB with IκB super repressor resulted in complete inhibition of procaspase-8 and -7 production in response to TNF-α. In contrast, R1881 slightly diminished procaspase-8 expression and increased baseline procaspase-7 expression (Fig. 3). The inhibition of NF-κB activity was independent of any effect R1881 might have had on the PI3K survival pathway or the ability of R1881 to affect AKT phosphorylation. As shown in Fig. 7B, wortmannin had no effect on NF-κB activity induced by TNF-α. In other systems, PI3K has been shown to affect NF-κB activation (39, 40).

**DISCUSSION**

Androgen is a critical factor that sustains the survival of normal prostate epithelial cells. For example, rat prostatic epithelium undergoes apoptosis within 3 weeks after castration (14, 15). Androgen withdrawal also induces death in vivo in human prostate adenocarcinoma (17, 18). Androgens also have antiapoptotic effects in prostate...
androgen on procaspase levels after exposure to TNF-α in intrinsic and extrinsic pathways. In addition, there was an effect of androgen blocks apoptosis after activation of death receptors in both intrinsic and extrinsic cell death pathways in LNCaP cells (12, 13).

Glands alone or in combination with irradiation induce activation of initiation and interfere primarily with caspase activation. Death antiapoptotic effects of androgen act downstream from cell death prostate epithelial cells. Here we have shown that, in LNCaP cells, the survival factor, preventing the initiation of the cell death cascade in irradiation alone in patients with locally advanced prostate cancer (5, 41).

Androgen withdrawal and irradiation produce longer disease-free and overall survival than irradiation alone in patients with locally advanced prostate cancer (5, 41).

It has been assumed that androgen plays an important role as a survival factor, preventing the initiation of the cell death cascade in prostate epithelial cells. Here we have shown that, in LNCaP cells, the antiapoptotic effects of androgen act downstream from cell death initiation and interfere primarily with caspase activation. Death ligands alone or in combination with irradiation induce activation of intrinsic and extrinsic cell death pathways in LNCaP cells (12, 13). Androgen blocks apoptosis after activation of death receptors in both intrinsic and extrinsic pathways. In addition, there was an effect of androgen on procaspase levels after exposure to TNF-α. Increases in caspase-8 expression were attenuated in the presence of R1881. In contrast, there appeared to be higher basal levels of procaspase-7 in the presence of R1881 (Fig. 3). Therefore, there were variable effects of androgen on procaspase levels.

The spectrum of possible mechanisms for steroid hormone action have recently been expanded by evidence that cytoplasmic steroid hormone receptors bound to homologous or heterologous ligands can interfere with SRC-SHC signaling and cell death (24). The antiapoptotic effects reported by Kousteni et al. (24) were of relatively low magnitude, occurred early after induction of cell death, and were not examined for their impact on DNA fragmentation. Although we were able to detect antiapoptotic effects of estrogen on early phases of LNCaP cell death, later phases of apoptosis were affected significantly only by R1881 and DHT. Kousteni et al. showed that steroid hormones blocked apoptosis in HeLa cells expressing exogenous androgen or estrogen receptor genes. We saw no effect of R1881 on induction of apoptosis in TSU-Pr1 prostate cancer cells that were transfected with a human androgen receptor expression plasmid despite the fact that androgen-responsive reporter constructs were activated by androgen exposure of these transfected cells (data not shown).

One consequence of androgen inhibition of caspase-8 activation was diminished BID cleavage. Cleaved BID has a proapoptotic effect on mitochondria to activate cytochrome c egress by forming a complex with BAX (42–44). We also observed that in the presence of androgen there was less BAX p18, a calpain cleavage product that is proapoptotic (29, 30). In contrast, we observed no change in levels of the antiapoptotic protein BCL-XL. Therefore, the effect of androgen on BID and BAX cleavage likely diminished mitochondrial cytochrome c release. BAX, but not BID, is known to cause a change in mitochondrial membrane potential (42, 45). Therefore, the inhibition of membrane depolarization by androgen most likely resulted from the effect of androgen on BAX.

Ceramide production is induced by TNF-α in LNCaP cells, and ceramide production correlates with death induction (12). Although androgen inhibited caspase-8 activation, there was no significant effect of androgen on ceramide production. Moreover, death induction by bacterial sphingomyelinase was blocked by androgen. It appears, therefore, that androgen inhibits cell death downstream from ceramide production induced by either TNF-α or bacterial sphingomyelinase. Ceramide production induced by death receptor activation was not significantly affected by androgen treatment.

An important survival signal in advanced prostate cancer is inactivation of the PTEN phosphatase. LNCaP cells have inactivated PTEN and, therefore, are under a continuous survival stimulus via the PI3K/AKT pathway (46). Restoration of PTEN function to LNCaP cells results in growth inhibition and cell death (47). However, it has been shown that androgen interferes with cell death induced by the pharmacological inhibition of the PI3K survival signal (19). We have confirmed these observations with the PI3K inhibitor wortmannin and have also shown that although wortmannin sensitzes LNCaP cells to irradiation, androgen blocks LNCaP cell death after exposure to wortmannin + irradiation, in part by inhibiting caspase activation.

Androgen may act upstream from AKT to enhance the PI3K survival signal. Some time ago, we demonstrated that DHT increased

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**Figure 6.** A, LNCaP cell death after treatment with 100 nM wortmannin, and 8 Gy irradiation at 24 h after treatment. B, Western blotting showing expression of AKT and phospho-AKT in LNCaP cells treated with 100 nM wortmannin, 8 Gy, and 8 Gy + 1 nM R1881 at 24 h after treatment.

**Figure 7.** A, the effect of 1 nM R1881 on the NF-κB activity induced by TNF-α + 8 Gy irradiation at 6 h. B, the effect of wortmannin on the NF-κB activity induced by TNF-α plus irradiation at 6 h after treatment.
intracellular phosphatidylinositol trisphosphate (48). Androgen treatment of LNCaP cells did not increase AKT phosphorylation (Fig. 6B). This may be attributable to the absence of PTEN expression that confers constitutive activation of PI3K (46). However, androgen may act as a survival factor for prostate cells with intact PTEN by enhancing the synthesis of phosphatidylinositol trisphosphate and AKT phosphorylation. It is noteworthy that, whereas neither wortmannin alone nor 8 Gy irradiation alone had any effect on AKT phosphorylation, 8 Gy plus wortmannin decreased AKT phosphorylation (Fig. 6B). This implies that in the presence of PI3K inhibition, irradiation affects the AKT-mediated survival pathway that results in BAD activation (49).

Exposure of cells to TNF-α results in activation of caspases and also of NF-κB. In some cell types, including most nontransformed cells, NF-κB has an antiapoptotic effect that contributes to the modulation of TNF-α signaling and lays a role in determining whether TNF-α induces cell death or an inflammatory response (50, 51). In prostate cancer cells, we have found that constitutive expression of 1κB results in relative resistance to apoptosis induction by death ligands +/- irradiation. Therefore, we thought that it was important to examine the effect of androgen on NF-κB activity in LNCaP cells. We did not find a relationship between the small effect of androgen on NF-κB activation after exposure to TNF-α and the antiapoptotic effect of the hormone. The effect of androgen on NF-κB activation by TNF-α was independent of any effect on the PI3K pathway because wortmannin had no effect on NF-κB activation induced by TNF-α +/- irradiation.

The interaction of androgen with the cell survival and death is complex. For example, androgen represses the expression of clusterin/TRPM-2, the expression of which is up-regulated during cell death (52, 53). Clusterin/TRPM-2 is antiapoptotic and can interfere with LNCaP cell death after exposure to TNF-α (54). Therefore, androgen can suppress as well as stimulate the expression of antiapoptotic proteins.

REFERENCES


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