Androgen via p21 Inhibits Tumor Necrosis Factor α-induced JNK Activation and Apoptosis*

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The male hormone androgen is a growth/survival factor for its target tissues or organs. Yet, the underlying mechanism is incompletely understood. Here, we report that androgen via p21 inhibits tumor necrosis factor α-induced JNK activation and apoptosis. Inhibition by androgen requires the transcription activity of androgen receptor (AR) and de novo protein synthesis. Androgen-AR induces expression of p21 that in turn inhibits tumor necrosis factor α-induced JNK and apoptosis. Furthermore, genetic interruption of p21 alleles abolishes the inhibition by androgen. Our results reveal a novel cross-talk between androgen-AR and JNK, thereby providing a molecular mechanism underlying the survival function of androgen.

Androgens are steroid hormones that have pleiotropic functions in androgen-responsive tissues or organs and play a critical role in the development of prostate cancer (1–4). The cytoplasmic receptor of androgen (AR) is a member of the steroid hormone receptor superfamily. AR can function as a latent transcription factor in response to androgen (1, 3). After binding to androgen, the androgen-AR complex translocates into the nucleus to induce expression of androgen target genes, whose protein products are involved in many cellular activities, from proliferation to apoptosis (2, 5–8).

Although the involvement of androgen-AR in androgen-dependent prostate cancer has been well documented, the role of androgen-AR in the progression of androgen-independent prostate cancer remains elusive. It has been shown that AR can regulate stress-induced apoptosis through promotion of the proapoptotic Bcl-2 family protein Bax activity independently of its transcription activity (9).

c-Jun N-terminal kinase (JNK; also known as stress-activated protein kinase, SAPK) is a subfamily of the mitogen-activated protein kinase (MAPK) superfamily (10–12). The JNK family has three isoforms: JNK1 and JNK2, which are ubiquitously expressed, and a tissue-specific isoform JNK3, with different splicing forms (13–15). JNK can be activated by various extracellular stimuli, from proinflammatory cytokines such as tumor necrosis factor (TNF-α) to environmental stress like UV light (16). Two MAP2Ks (JNKK1/MKK4/SEK1 and JNKK2/MKK7) (17–19) and several MAP3Ks, such as MEKK1, ASK1, MLK, TAK1, and TPL-2, have been reported to be involved in mediating JNK activation through sequential phosphorylation (13).

Activated JNK can regulate the activity of several transcription factors, such as c-Jun, ATF-2, Elk-1, p53, and c-Myc, or non-transcription factors, including members of the Bcl-2 family (13, 15, 16). The activity of JNK can be regulated by protein phosphatases, NF-κB, and scaffold proteins such as IJP, β-arrestin, and JSAP1 (14, 15). Accumulating evidence shows that JNK plays a critical role in regulation of many fundamental cellular activities, including apoptosis (13, 15).

JNK has proapoptotic or antiapoptotic or no role in cell death depending on the cell context and the death stimulus (13, 20–23). Genetic evidence reveals that JNK1 and JNK2 are involved in survival of neuronal cells in mouse hindbrain and forebrain regions during development (24, 25). Recent studies show that JNK1 activation is required for interleukin 3-mediated survival of hematopoietic cells through phosphorylation and inactivation of the proapoptotic Bcl-2 family protein Bad (20, 26). However, JNK can contribute to apoptosis as well. JNK activation is required for UV-induced apoptosis (23, 27), and prolonged JNK activation contributes to TNF-α-induced apoptosis when NF-κB activation is impaired (21, 22, 28–31). It is incompletely understood how the proapoptotic activity of JNK is regulated.

It has been reported that JNK activation is involved in apoptosis induced by thapsigargin and 12-O-tetradecanoylphorbol-13-acetate in androgen-deprived prostate cancer LNCaP cells, and the activation of JNK can be inhibited by androgen with no known mechanisms (32). Inhibition of JNK by the pharmacological JNK inhibitor SP600125 also partially protected prostate cancer cells from guggulsterone-induced apoptosis (33). However, the role of JNK in apoptosis of androgen-independent prostate cancer cells is poorly understood; so is the relationship between AR and JNK upon stimulation by extracellular signals. Here, we report that androgen-AR signaling via p21Waf1/Cip1 (p21 hereafter) inhibits TNF-α-induced JNK activation and thereby suppresses apoptosis in androgen-independent, AR-positive prostate cancer cells.
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EXPERIMENTAL PROCEDURES

Reagents and Antibodies—Antibodies against JNK and hemagglutinin (HA) and the general caspase inhibitor Z-VAD-fmk were purchased from Calbiochem. Antibody against phospho-JNK was from Cell Signaling. The JNK inhibitor SP600125 was synthesized and purified (34). The synthetic fluorogenic caspase-3 substrate AC-Asp-Glu-Val-Asp-7-amino-4-trifluoromethylcoumarin (AC-DEVD-AFC) was from Clontech. The antibody against p21 was purchased from Santa Cruz Biotechnology (Santa Cruz, CA). AN-21, a polyclonal rabbit antibody raised against a 21-residue peptide corresponding to the first 21 amino acids of human and rat AR was described previously (35). Cycloheximide (CHX), Hoechst (H33258), 5-α-dihydrotestosterone, and antibodies against M2-tag and β-actin were from Sigma. The synthetic androgen R1881 was from PerkinElmer Life Sciences. The specific androgen antagonist Casodex (Cdx; bicalutamide) was from Zeneca Pharmaceuticals (Wilmington, DE). Human TNF-α was from R&D Systems (Minneapolis, MN). [γ-32P]ATP (3000 mCi/nmol) was from PerkinElmer Life Sciences.

Plasmids and Recombinant Adenoviruses—Expression vectors encoding M2-JNK1, enhanced green fluorescent protein (EGFP), glutathione S-transferase (GST)-IxBα(1–54), and GST-c-Jun(1–79) have been described previously (18, 22, 23, 34, 36). Adenoviral vectors encoding the superrepressor of NF-κB, IxBα(AA) in which Ser32 and Ser36 have been replaced by alanines (34) or LacZ have been described previously (23, 34). To generate the pcDNA3-hAR vector, the 2.8-kb hAR open reading frame was inserted into the BamHI site of the pcDNA3 vector (Invitrogen). The Kozak start sequence was inserted for higher expression. To generate the pGL3 5-ARE-LUC reporter gene that is controlled by androgen, a 27-base duplex oligomer (5′-TCGAGTCTGTTACAGGGTGTTCTTTTG-3′) was trimerized and inserted into the Sall site of the pGL3-Basic vector (Promega, Madison, WI).

Cell Culture, Transfection, Infection, and Transcription Assays—Androgen-independent LNCaP 104-R1 cells were maintained and cultured in Dulbecco’s modified Eagle’s medium supplemented with 10% dextran-coated charcoal-stripped fetal bovine serum (8). HCT116 human colon carcinoma cells (generously provided by Dr. Bert Vogelstein) were maintained on McCoy’s 5A medium supplemented with 10% fetal bovine serum. Transfections were performed by ExGen 500 (MBI Fermentas) in vitro transfection reagent, according to the manufacturer’s protocol. For typical transfection experiments, cells were transfected with various plasmids for 36 h unless indicated otherwise. For typical infection experiments, cells were usually infected with various recombinant adenoviruses for 24 h. For transfection in combination with infection experiments, cells were usually first transfected with various plasmids for 16 h followed by infection with various recombinant adenoviruses for another 24 h, unless indicated otherwise. ARE-LUC reporter gene activity and GAL4-LUC activity were determined as described previously (9, 17).

Protein Kinase Assays and Immunoblotting—Immune complex kinase assays were performed as described (17). Kinase activity was quantitated using a PhosphorImager. Immunoblot analysis was performed as described (17). The antibody-antigen complexes were visualized by the enhanced chemiluminescence (ECL) detection system (Amersham Biosciences).

Apoptosis Assays—Cells were cotransfected with various constructs in the presence of an EGFP plasmid at a ratio of 4:1. Under these conditions, cells expressing GFP also expressed cotransfected plasmid (21). Cells were subsequently infected with Ad/LacZ or Ad/IxBα(AA) at a 500 multiplicity of infection (m.o.i.), or they were left uninfected. At the time points indicated, cells were treated with stimuli and stained with Hoechst. Cell nucleus condensation was detected by fluorescence microscopy. Caspase 3 activity was measured by using the synthetic fluorogenic substrate AC-DEVD-AFC, according to the manufacturer’s manual. The liberation of 7-amino-4-trifluoromethylcoumarin from DEVD-AFC was read by a cytometer at 400 nm excitation and 505 nm emission wavelengths (34).

RESULTS

Androgen Inhibits TNF-α-induced Apoptosis through Suppression of Prolonged JNK1 Activation—The male hormone androgen is a growth/survival factor for its target tissues or organs (2, 3). However, the underlying mechanism is incompletely understood. To address this issue, we tested the effect of androgen on TNF-α-induced cell death in LNCaP 104-R1 prostate cancer cells, which can grow in the absence of androgen but still respond to it (8, 35). 104-R1 cells were infected with adenoviral vector encoding IxBα(AA) (Ad/IxBα(AA)), which is a superrepressor of NF-κB (34), and then stimulated with or without TNF-α. As expected, TNF-α induced significant cell death, as measured by apoptotic cell death assays and casp-3 assays (Fig. 1, A and B). By contrast, TNF-α-induced apoptosis was significantly suppressed when cells were incubated with a synthetic androgen, R1881 (Fig. 1, A and B). These results suggest that androgen may suppress TNF-α-induced cell death.

Because prolonged JNK1 activation is essential for TNF-α to induce apoptosis in the absence of NF-κB activation (13, 30), we were curious whether androgen inhibits prolonged JNK1 activation. To test this idea, 104-R1 cells infected with Ad/IxBα(AA) were pretreated with the control ethanol or a synthetic androgen R1881 for 30 min followed by stimulation with TNF-α for various periods of time or left alone. Immune complex kinase assays showed that TNF-α-induced JNK activation was prolonged in cells pretreated with ethanol but was transient in cells pretreated with R1881 (Fig. 1C). The conversion of JNK activation from prolonged to transient was not the result of changes in JNK expression, as measured by immunoblotting analysis using anti-JNK antibody (Fig. 1C). Under these conditions, TNF-α-induced, JNK-dependent c-Jun transcription activity was also inhibited by R1881, as measured by GAL4-c-Jun activation using a luciferase reporter gene driven by a GAL4-responsive promoter (GAL-4-LUC) (Fig. 1D).

The conversion of TNF-α-induced JNK activation from prolonged to transient by the pretreatment with R1881 prompted us to test whether androgen only prevents prolonged JNK acti-
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To determine whether the transcription activity of androgen requires AR transcription activity, Androgen exerts its biological functions via its receptor AR, which binds to androgen and translocates into the nucleus to regulate expression of genes that are involved in many cellular functions (3). To determine whether the inhibitory effect of androgen on JNK activity, expression of genes that are involved in many cellular functions (37). As expected, Cdx significantly blocked AR transcription activity, as measured by its inhibition on androgen-stimulated ARE-LUC activity in 104-R1 cells (data not shown). Immune complex kinase assays showed that pretreatment with Cdx significantly reduced the inhibitory effect of androgen on TNF-α-induced apoptosis in 104-R1/AR RNAi cells (Fig. 2C). Ectopic expression of AR in AR-negative PC-3 cells also allowed androgen to inhibit JNK activation and TNF-α-induced apoptosis (data not shown). Taken together, inhibition of JNK activation and apoptosis by androgen depends on its receptor AR.

To determine whether the transcription activity of androgen-AR is required for the suppression by androgen on TNF-α-induced JNK activity and apoptosis, we used the specific androgen antagonist Cdx to block AR transcription activity. As expected, Cdx significantly blocked AR transcription activity, as measured by its inhibition on androgen-stimulated ARE-LUC activity in 104-R1 cells (data not shown). Immune complex kinase assays showed that pretreatment with Cdx significantly reduced the inhibitory effect of androgen on JNK activation by TNF-α (Fig. 3A). Consistently, treatment of cells with a protein synthesis inhibitor CHX also abrogated the inhibition by androgen on TNF-α-induced JNK activation (Fig. 3B). CHX alone had no detectable effects on JNK activation in conditions AR protein levels were not affected by control RNAi (Fig. 2A). Immune complex kinase assays showed that although pretreatment with R1881 for 90 min inhibited JNK activation by TNF-α in 104-R1/control RNAi cells, the inhibition by R1881 was impaired in 104-R1/AR RNAi cells (Fig. 2B). Under the same conditions, R1881 significantly suppressed TNF-α-induced apoptosis in 104-R1/control RNAi cells but had no detectable inhibition on TNF-α-induced apoptosis in 104-R1/AR RNAi cells (Fig. 2C). Ectopic expression of AR in AR-negative PC-3 cells also allowed androgen to inhibit JNK activation and TNF-α-induced apoptosis (data not shown). Taken together, inhibition of JNK activation and apoptosis by androgen depends on its receptor AR.

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104-R1 cells under the conditions used (Fig. 3B), although it stimulated JNK activation in several other cell lines (22). In parallel experiments, we found that Cdx also abolished the inhibitory effect of androgen on TNF-α-induced apoptosis (Fig. 3C). Thus, the inhibitory effect of androgen on TNF-α-induced JNK1 activation and apoptosis depends on AR transcription activity and de novo protein synthesis.

p21 Mediates the Inhibition by Androgen on TNF-α-induced JNK1 Activation—The observations that the inhibition by androgen on TNF-α-induced JNK1 activation and apoptosis requires the transcription activity of AR and de novo protein synthesis prompted us to search for androgen-induced JNK inhibitors. One of the candidates was p21. It has been reported that p21 is a potential target gene of androgen (38) and that p21 expression of p21 was analyzed by immunoblotting using anti-p21 antibody. Indeed, treatment with R1881 induced expression of p21 (20 ng/ml) or left alone for 32 h. Apoptotic cell death was measured and calculated as described for Fig. 1A.

To determine whether inhibition by androgen is mediated by p21, we took a genetic approach using WT and p21−/− colon carcinoma HCT116 cells (39), in which endogenous AR was undetectable (Fig. 4C). WT and p21−/− HCT116 cells were transfected with mammalian expression vector encoding AR or empty vector. Treatment of the cells with R1881 stimulated the activity of ARE-LUC in both WT and p21−/− HCT116 cells transfected with AR but not with empty vector (Fig. 4D), demonstrating that expression of AR in both WT or p21−/− HCT116 cells rendered the cells responsive to androgen. Immune complex kinase assays showed that R1881 inhibited JNK1 activation by TNF-α in an AR-dependent manner in WT HCT116 cells (Fig. 4E, compare lane 3 with lane 2 versus lane 7 with lane 6). Under the same conditions, R1881 was unable to inhibit JNK1 activation by TNF-α in p21−/− HCT116 cells (Fig. 4F, compare lane 7 with lane 6). The inability of the R1881-AR complex to inhibit JNK activation in p21−/− HCT116 cells was not due to potential global changes in these cells because ectopic expression of p21 still inhibited JNK activation by TNF-α (data not shown).

p21 Mediates the Inhibition by Androgen on TNF-α-induced Cell Death—Next, we determined whether androgen via p21 suppresses TNF-α-induced apoptosis. To test this hypothesis, HCT116 cells were transfected with mammalian expression vectors encoding AR, EGFP (the ratio of AR to EGFP is 4:1), or empty vector, followed by infection with

FIGURE 3. AR-dependent transcription is required for androgen to inhibit JNK activation and TNF-α-induced apoptosis. A and B, 104-R1 cells infected with Ad/kbα(AA) were pretreated with either Cdx (5 μM, 30 min) (A) or CHX (1 μg/ml, 30 min) (B) followed by treatment with or without R1881 (1 nM, 90 min). Cells were stimulated with TNF-α (20 ng/ml) for 15 min. JNK activity (KA) and content (IB) were measured as described for Fig. 1C. C, 104-R1 cells infected with Ad/kbα(AA) (500 m.o.i.) were pretreated with or without Cdx (5 μM) for 30 min and R1881 (1 nM) for 60 min as indicated and then stimulated by TNF-α (20 ng/ml) or left alone for 32 h. Apoptotic cell death was measured and calculated as described for Fig. 1A.

FIGURE 4. Inhibition of TNF-α-induced JNK activation by androgen depends on p21. A, 104-R1 cells were stimulated by R1881 (1 nM) or left alone for the times indicated. Expression levels of p21 proteins were analyzed by immunoblotting using anti-p21 antibody. B, 104-R1 cells were transfected with expression vectors encoding HA-JNK1 (0.5 μg), along with Xpress-tagged p21 (1 and 2 μg, as indicated), or empty vector (2 and 1 μg). After 40 h, cells were treated with TNF-α (20 ng/ml) or left alone for 15 min. The activity of HA-JNK1 was measured by immune complex kinase assays (KA). Expression levels of HA-JNK1 and Xpress-tagged p21 were analyzed by immunoblotting (IB) using anti-HA and anti-Xpress antibodies, respectively. C, immunoblot analysis of AR levels in 104-R1 and HCT116 cells using anti-AR antibody AN21. ns, nonspecific. D, wild-type (WT) and p21−/− HCT116 cells were transfected with expression vectors encoding 3X-ARE-LUC (0.1 μg), along with AR or empty vector (0.5 μg each) for 36 h and then stimulated by R1881 (1 nM) or left alone for 8 h. Relative ARE luciferase activity was determined as described previously (8). E and F, wild-type (E) and p21−/− (F) HCT116 cells were cotransfected with expression vectors encoding HA-JNK1 (0.8 μg), along with AR or empty vector (0.4 μg each) followed by infection with Ad/kbα(AA). Cells were pretreated with R1881 (1.0 nM) for 90 min and then stimulated by TNF-α (20 ng/ml) or left alone for 15 min. The activity of HA-JNK1 was measured by immune complex kinase assays. Expression of HA-JNK1 and AR was analyzed by immunoblotting using anti-HA and anti-AR antibody, respectively.
Ad/IxBo(AA) or Ad/LacZ. Apoptotic cell death assays revealed that TNF-α-induced cell death was similar between WT and p21−/− HCT116 cells infected with HA-IxBo(AA) but not LacZ (Fig. 5A). The cell death induced by TNF-α in HCT116 cells was mainly apoptosis, rather than necrosis, because it was almost completely inhibited by the general caspase inhibition Z-VAD (Fig. 5B) and was dependent on JNK because it was inhibited by the JNK inhibitor SP600125 (Fig. 5C). C and D, wild-type and p21−/− HCT116 cells were transfected with expression vectors encoding EGFP (0.5 μg), alone with AR (2.0 μg) (C), or p21 (2.0 μg each) (D), followed by infection with Ad/IxBo(AA) (500 m.o.i.). Cells were treated with or without R1881 (1 μM) for 90 min and then stimulated with TNF-α (20 ng/ml) or left alone for 8 h. Apoptotic cell death was measured as described in Fig. 1A.

**FIGURE 5.** p21 is required for androgen-mediated suppression of TNF-α-induced apoptosis. A and B, wild-type (WT) and p21−/− HCT116 cells were infected with Ad/IxBo(AA) or Ad/LacZ (500 m.o.i.). Cells were treated with or without TNF-α (20 ng/ml) for 8 h (A) or pretreated with Z-VAD-fmk (10 μM) for 1 h or SP600125 (5 μM) for 30 min (B) and then stimulated with TNF-α (20 ng/ml) or left alone for 8 h. Apoptotic cell death was measured as described in the legend to Fig. 1B. C and D, wild-type and p21−/− HCT116 cells were transfected with expression vectors encoding EGFP (0.5 μg), alone with AR (2.0 μg) (C), or p21 (2.0 μg each) (D), followed by infection with Ad/IxBo(AA) (500 m.o.i.). Cells were treated with or without R1881 (1 μM) for 90 min and then stimulated with TNF-α (20 ng/ml) or left alone for 8 h. Apoptotic cell death was measured as described for Fig. 1A.

Ad/IxBo(AA) or Ad/LacZ. Apoptotic cell death assays revealed that TNF-α-induced cell death was similar between WT and p21−/− HCT116 cells infected with HA-IxBo(AA) but not LacZ (Fig. 5A). The cell death induced by TNF-α in HCT116 cells was mainly apoptosis, rather than necrosis, because it was almost completely inhibited by the general caspase inhibition Z-VAD (Fig. 5B) and was dependent on JNK because it was inhibited by the JNK inhibitor SP600125 (Fig. 5C). Pretreatment with R1881 inhibited TNF-α-induced apoptosis in WT HCT116 cells transfected with AR but not with empty vector (Fig. 5C), demonstrating that androgen-AR was able to inhibit TNF-α-induced apoptosis in WT HCT116 cells. By contrast, R1881 was unable to inhibit TNF-α-induced apoptosis in p21−/− HCT116 cells transfected with AR (Fig. 5D), demonstrating that the inhibition by androgen-AR on TNF-α-induced apoptosis requires p21. Taken together, these results demonstrate that p21 mediates the inhibition by androgen-AR on TNF-α-induced, JNK-dependent apoptosis.

**DISCUSSION**

Androgen is a growth/survival factor that has pleiotrophic roles in androgen-responsive tissues or organs (2, 3). Androgen also plays a critical role in the development of prostate cancer, via regulating proliferation and apoptosis (2, 3, 5). However, the relation between androgen and the intracellular signaling network is incompletely understood. Here, we report that androgen via p21 inhibits TNF-α-induced JNK1 activation, thereby suppressing apoptosis. This conclusion is based on the following evidence.

First, androgen inhibits TNF-α-induced apoptosis in androgen-independent LNCaP 104-R1 prostate cancer cells through inhibition of JNK1 activation (Fig. 1). Second, the inhibition by androgen depends on androgen-AR transcription activity and de novo protein synthesis (Figs. 2 and 3). Third, the androgen target gene p21 mediates the inhibition by androgen-AR on TNF-α-induced JNK activation and apoptosis (Figs. 4 and 5).

Our results show that androgen inhibited TNF-α-induced JNK activation and apoptosis in androgen-independent, AR-positive 104-R1 cells (Fig. 1). Furthermore, in androgen-independent AR-negative PC-3 cells that stably express AR, androgen also inhibited TNF-α-induced JNK activation and apoptosis (data not shown). Thus, in androgen-independent prostate cancer cells, androgen suppresses apoptosis induced by TNF-α (Fig. 1). It is important to point out that the inhibition by androgen on TNF-α-induced JNK and apoptosis is not limited in prostate cancer cells. In AR-expressing colon carcinoma HCT116 cells, androgen also inhibited JNK activation, thereby suppressing TNF-α killing (Fig. 5). Thus, the inhibition by androgen on TNF-α-induced JNK activation and apoptosis could likely be a general regulatory mechanism in androgen-responsive cells. Future studies are needed to test this hypothesis.

Androgen inhibits TNF-α-induced JNK activation and cell death via AR-mediated up-regulation of p21. Our results show that the inhibitory effect by androgen was abrogated by treatment with AR siRNA or the specific AR antagonist Cdx, which blocks AR transcription activity, or the protein synthesis inhibitor CHX (Figs. 2 and 3). Thus, the inhibition by androgen depends on AR-mediated transcription and de novo protein synthesis. Furthermore, our results show that p21, which is one of the androgen target genes (18), mediates the inhibition by androgen on TNF-α-induced JNK activation and apoptosis. Thus, p21 mediates the inhibition by androgen-AR on TNF-α-induced JNK activation and apoptosis.

How does p21 inhibit JNK activation? Previously, it has been reported that p21 was able to inhibit JNK itself directly (39) or inhibited mTOR-stimulated ASK1, which is one of the MAP3Ks for JNK (40). We found that androgen not only inhibited JNK activity, but it also inhibited activation of JNK. Phosphorylation of JNK at Thr183 and Tyr185, which are required for active JNKK2-JNK1 (20, 22, 36) (data not shown). These data suggest that androgen-induced p21 may inhibit upstream activators of JNK. However, knock out of ASK1 does not affect JNK activation by TNF-α (data not shown). Thus, it is unlikely that p21 inhibits TNF-α-stimulated JNK by inhibiting ASK1. Future studies are needed to determine which JNK upstream activator(s) in TNF-α signaling are inhibited by androgen-induced p21.

Our findings that androgen-AR via p21 inhibits JNK activation and thereby suppresses apoptosis induced by TNF-α may have important clinical implications. Androgen plays a critical role in the development and treatment of prostate cancer (1, 2, 41). The initial growth of prostate cancer is androgen-dependent, and therefore androgen ablation, mostly via induction of apoptosis and cell cycle arrest (2, 7, 8), has been a leading ther-
I n h i b i t i o n  o f  J N K  b y  A n d r o g e n  v i a  p 2 1

chemical choice (42). However, malignant prostate cancer often recurs. In contrast to androgen-dependent prostate cancer cells, androgen-independent prostate cancer cells are less sensitive to conventional therapies that usually induce apoptosis (1, 2, 7, 8, 43). It has been hypothesized that androgen ablation, which causes regression of androgen-dependent prostate cancer, may inadvertently select those cancerous cells that can grow independently of androgen through various mechanisms (2). To overcome this, it has been proposed that intermittent androgen ablation may delay the development of prostate tumors that are refractory to androgen ablation (44, 45). Indeed, it has been reported that intermittent androgen therapy reverts androgen-independent prostate cancer to androgen-stimulated phenotype in mice (46) and has been found to improve patient survival in clinic (45). However, cautions should be taken during the intermittent androgen therapy. Our results show that androgen can inhibit apoptosis of androgen-independent prostate cancer cells via p21-mediated inhibition on JNK activation. This suggests that androgen may still play a prosurvival role in androgen-independent prostate cancer cells under certain circumstance even though these cells can grow in an androgen-independent manner. Another implication is that depending on the type of death stimuli, androgen may play different roles in apoptosis of prostate cancer cells. In androgen-independent AR-positive prostate cancer cells, androgen may have an antiapoptotic role in TNF-α-induced apoptosis, where prolonged JNK activation is critical for TNF-α killing. However, in apoptosis elicited by other death stimuli that do not depend on prolonged JNK activation to kill, the role of androgen may be different. Future studies are needed to address these questions.

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