

Interleukin-6 Protects LNCaP Cells From Apoptosis Induced by Androgen Deprivation Through the Stat3 Pathway

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BACKGROUND. Elevated expression of interleukin-6 (IL-6) is implicated in the progression of hormone refractory prostate cancer. Previous studies demonstrated that IL-6 promotes androgen-independent growth of prostate cancer cells. In this study, the effect of IL-6 on apoptosis induced by androgen deprivation was investigated.

METHODS. The effect of IL-6 on apoptosis induced by androgen deprivation in LNCaP cells was examined by cell death ELISA and Western blot using cleaved poly (ADP-ribose) polymerase (PARP) and caspase-9, as well as Bcl-x_L and phosphorylated Bad. The Stat3 in IL-6-mediated anti-apoptosis in prostate cancer cells was examined using either dominant-negative or constitutively activated Stat3 mutants.

RESULTS. Overexpression of IL-6 renders androgen sensitive LNCaP human prostate cancer cells more resistant to apoptosis induced by androgen deprivation. LNCaP cells undergo apoptosis after 72 hr of androgen deprivation, an outcome is largely absent in clones overexpressing IL-6 as measured by cell death ELISA and chromatin degradation assays. IL-6 over-expressing cells resulted in a significant decrease in the expression of cleaved PARP and cleaved caspase-9 as well as an increase in the expression of Bcl-x_L and phosphorylated Bad. Addition of IL-6 antibody completely abolished the anti-apoptotic activity of IL-6. This protective effect of IL-6 was reversed by the expression of a dominant-negative Stat3 mutant, Stat3F. Furthermore, ectopic expression of a constitutively active Stat3 antagonized androgen deprivation-induced cell death of LNCaP cells.

CONCLUSION. These results indicate that IL-6 protects androgen sensitive LNCaP cells from apoptosis induced by androgen deprivation, and Stat3 activation play an important role in IL-6-mediated anti-apoptosis in prostate cancer cells. *Prostate* 60: 178–186, 2004.

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KEY WORDS: IL-6; prostate cancer; apoptosis; Stat3; androgen-independence

INTRODUCTION

Prostate cancer is the most commonly diagnosed cancer and second leading cause of cancer death in American men. Initially, the growth of prostate epithelial cells is dependent on androgen. When androgen is depleted, these cells undergo apoptosis and die, the basis for androgen ablation therapy, a common treatment for prostate cancer [1,2]. However, most patients will relapse to hormone refractory disease due to the growth of androgen-independent cancer cells. In this stage, cells are more resistant to apoptotic cell death and thus androgen ablation is ineffective [2].

Interleukin-6 (IL-6) is a 21–28 Kd multifunctional cytokine involved in many cellular processes such as

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inflammation, cell differentiation, and proliferation [3]. IL-6 production correlates with tumor progression in human cancer such as pleural mesothelioma, glioblastoma, and ovarian and prostate cancer [4–7]. The expression of IL-6 and its receptor is consistently demonstrated in human prostate cancer cell lines and in freshly isolated human prostate carcinoma and benign prostate hyperplasia [8–10]. Clinically, the levels of IL-6 in serum are significantly elevated in many men with advanced, hormone-refractory prostate cancer [4,11]. IL-6 has been suggested to have both growth-promoting and inhibiting activities in androgen-sensitive LNCaP human prostate cancer cells in vitro [12–15]. IL-6 activates AR-mediated gene expression in LNCaP cells in vitro [14,16–18], suggesting that IL-6 may play a critical role during the progression of prostate cancer. In addition, overexpression of IL-6 in androgen sensitive LNCaP human prostate cancer cells promotes LNCaP cell androgen-independent growth in vitro and in vivo [19].

The biological activities of IL-6 are mediated by the IL-6 receptor. IL-6 receptor is composed of two components, a 80 Kd transmembrane protein that has ligand-binding capacity and a 130 Kd membrane glycoprotein (gp130) that mediates signal transduction following IL-6 binding [20]. The IL-6 receptor is abundant in many types of prostate cells including both androgen-dependent and androgen-independent cells [10]. In addition to the transmembrane IL-6R, a soluble IL-6 form of IL-6R (sIL-6R) can be generated either from truncated membrane protein or translation from an alternatively spliced mRNA [21]. IL-6 can bind to this soluble form of the receptor, an alternate target for the biologic activity [21]. IL-6 signaling through gp130 transduces signals into the interior of the cell through several major signaling pathways including the Janus kinase (JAK)-Signal Transducers and Activators of Transcription (STAT) pathway [22], mitogen-activated protein (MAP) kinase pathways [23], and the phosphatidylinositol (PI) 3-kinase-AKT pathways [24].

Apoptosis is a physiological cellular suicide program that maintains tissue homeostasis with pro-apoptotic and anti-apoptotic protein family members implicated in cell survival/death decisions. The role of IL-6 in the regulation of apoptosis is demonstrated in many cancer cells. Overexpression of IL-6 increases anti-apoptotic activity and thereby tumorigenic potency in basal cell carcinoma [25]. IL-6 can regulate the anti-apoptotic Bcl-2 family proteins, and the expression of Mcl-1, a Bcl-2 family member, was significantly induced by IL-6 [26].

While IL-6 plays a critical role in the development of androgen independent prostate cancer, the molecular mechanisms of IL-6 mediated androgen independence are largely unknown. In this study, we demonstrated

that IL-6 can protect androgen-sensitive LNCaP human prostate cancer cells from apoptotic death induced by androgen depletion. Furthermore, the anti-apoptotic activity of IL-6 is mediated by Stat3 signaling pathway.

MATERIALS AND METHODS

Tissue Culture

The LNCaP cells were maintained in RPMI 1640 supplemented with 10% of FBS, 100 U/ml of penicillin, and 100 µg/ml of streptomycin at 37°C in 5% CO₂ incubator. Transfection and selection of LNCaP cells stably expressing IL-6 were described previously [13]. The clones overexpressing IL-6 (LN-S15, LN-S17) and its neo control (transfected with vector alone) cells were cultured in the same medium but containing 0.3 mg/ml of G418. In order to investigate the effect of androgen deprivation, cells were cultured in a medium containing 10% charcoal-stripped FBS (CS-FBS) instead of regular 10% FBS.

In Vitro Growth Assay

LNCaP and IL-6 over-expressing cells (LN-S15 and LN-S17) (10⁴/well) were plated in 12-well plates in RPMI 1640 containing 10% FBS. After 2 days in regular culture medium with 10% FBS, cells were switched into RPMI 1640 medium containing either 10% FBS or 10% CS-FBS (Hyclone, UT). Three days later, cells were trypsinized and counted with a Coulter counter. For the IL-6 antibody test, 20 µg of IL-6 antibody (Sigma Chemicals Co.) was added per ml of culture medium.

Apoptosis ELISA Test

The ELISA kit was obtained from Roche Molecular Biochemicals (Indianapolis, IN) and the protocol was followed according to the manufacturer's instructions. For the preparation of samples, LNCaP and IL-6 over-expressing cells (LN-S15 and LN-S17) (10⁴/well) were plated in 12-well plates in RPMI 1640 containing 10% FBS. After 2 days in regular culture medium with 10% FBS, cells were switched into a medium containing either 10% FBS or 10% CS-FBS (Hyclone). Three days later, cell lysates were obtained and cell death was analyzed and the absorbance values were normalized by the cell number.

Fluorescent Microscopic Studies

Cells (5 × 10³) were plated in microslides in normal medium for 2 days and switched into a medium containing CS-FBS after washing. Three days later, cells were fixed in 8% paraformaldehyde for 15 min, washed and stained with Hoechst 33258 solution (5 µg/ml in PBS, 5 min). Cells were then washed with PBS, mounted and examined under fluorescent microscope.

Transfection

LNCaP, LN-S15, and LN-S17 cells were transiently transfected with 2 μ g of either a dominant negative Stat3 (Stat3F) or a constitutively activated Stat3 mutant (Stat3c) using SuperFect Transfection Reagent (QIAGEN, Inc., Valencia, CA). Briefly, cells (1×10^6) were plated into 6-well plates and allowed to adhere for 24 hr. Cells were transfected with either Stat3F or Stat3c in serum-free medium for 4 hr, incubated with complete medium for 24 hr, and then were switched into a medium containing CS-FBS. Three days later, the cell lysates were prepared and used for the quantitation of apoptosis by the ELISA kit, growth assay, Western blot analysis, and electromobility shift assay (EMSA). For controls, the same amount of empty vector was used for transfection.

EMSA

Whole cell extracts were prepared and EMSA was performed as described previously [27]. For determination of the Stat3 DNA binding activity, whole cell extracts (20 μ g) were incubated in a final volume of 20 μ l (10 mM HEPES, pH 7.9, 80 mM NaCl, 10% glycerol, 1 mM DTT, 1 mM EDTA, 100 μ g/ml poly(dIdC) with radiolabeled double stranded Stat3 consensus binding motif 5'-GATCCTTCTGGGAATTCCTAGATC (Santa Cruz Biotechnologies, CA). The protein-DNA complexes were resolved on a 4.5% non-denaturing polyacrylamide gel at room temperature, and the results were autoradiographed using Molecular Imager FX System (Bio Rad, Hercules, CA).

Western Blot

Whole cell extracts were obtained, as described previously [19], and resolved in 8–12% SDS-PAGE depending on the molecular weight of the protein to be detected. After blocking overnight 4°C in 5% milk in PBS-0.1% Tween 20, membranes were incubated overnight with antibodies of cleaved caspase-9 (Cell Signaling Technology, MA), cleaved poly (ADP-ribose) polymerase (PARP) (Cell Signaling Technology), Bcl-x_L (Santa Cruz Biotechnologies), or phosphorylated Bad (Cell Signaling Technology). Following secondary antibody incubation, immunoreactive proteins were visualized with an enhanced chemiluminescence detection system (Amersham Pharmacia Biotech, Buckinghamshire, England).

Statistical Analysis

Values were expressed as the mean \pm SE. Statistical analyses were performed by one-way ANOVA, followed by the Student–Newman–Keuls test for multiple comparisons, with a $P < 0.05$ significant.

RESULTS

Overexpression of IL-6 in LNCaP Cells Confers Resistance to Androgen Deprivation-Induced Apoptosis

Previous studies have demonstrated that the androgen sensitive LNCaP cells express the IL-6 receptor, but express no detectable IL-6 protein [12–15,28]. To test the effect of IL-6 on LNCaP cell function, we ectopically expressed IL-6 by introduction of a full-length IL-6 cDNA into IL-6-negative LNCaP cells as described previously [13]. Two stable IL-6 independent transfectants (LN-S15 and LN-S17) were isolated that express high levels of IL-6 (2,465 and 2,743 pg/ml/ 10^6 cells, respectively) with a vector-alone control (neo). We previously demonstrated that the growth of androgen sensitive LNCaP cells in culture was reduced by about 50% after 48 hr in androgen-deprived charcoal-stripped serum condition compared with that in the normal serum condition [19]. However, in the IL-6 overexpression cells, there was only a 5–10% decrease in growth under these androgen-deprived conditions compared with growth in normal serum, suggesting that overexpression of IL-6 can enhance the growth of LNCaP cells in the androgen deprived condition in vitro.

Androgen deprivation triggers apoptosis in both normal and malignant androgen-dependent prostate epithelial cells. LNCaP cells are androgen sensitive human prostate cancer cells. To test whether LNCaP cells undergo programmed cell death in androgen deprived condition in vitro, and whether IL-6 can prevent apoptosis, LNCaP cells, LNCaP-neo, and IL-6 overexpression cells (LN-S15 and LN-S17) were cultured in RPMI 1640 with 10% FBS and then switched to RPMI 1640 with 10% CS-FBS in which physiological levels of androgen were deprived. Death was assessed 72 hr later through different techniques. The characteristic morphology of apoptosis was determined by staining with Hoechst 33258. Figure 1 shows apoptotic morphologies of the representative parental LNCaP and IL-6 overexpressing LN-S17 cells in androgen deprived conditions. A typical apoptotic morphology including chromatin condensation and nuclear fragmentation was clearly observed in the parental LNCaP and neo control cells cultured in the CS-FBS conditions, but not in IL-6-over-expressing cells (LN-S15 and LN-S17).

Apoptotic cell death was determined using the apoptosis specific ELISA assay to evaluate DNA fragmentation. As shown in Figure 2, parental LNCaP and neo control cells showed significant levels of apoptotic death in CS-FBS as compared to normal FBS ($P < 0.01$), whereas IL-6 over-expressing cells showed a significant lower level of apoptotic death as compared to parental LNCaP and neo control cells ($P < 0.01$).

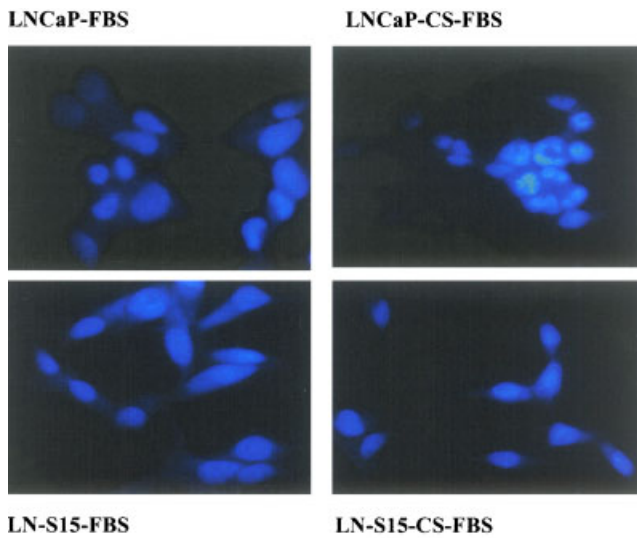


Fig. 1. Expression of interleukin-6 (IL-6) protects LNCaP cells against apoptosis induced by androgen deprivation. Parental LNCaP and IL-6 overexpressing LN-15 cells (LN-S15) were cultured in either normal FBS or androgen deprived CS-FBS conditions for 3 days. The cells were stained with Hoechst 33258 fluorescent dye and examined by fluorescent microscope. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

Addition of IL-6 antibody to the cell culture media almost completely blocked the anti-apoptotic activity of IL-6 ($P < 0.01$), indicating that the anti-apoptotic effect was mediated specifically by IL-6.

Immunoblots using antibodies against several pro-apoptotic and anti-apoptotic proteins were performed to determine the effect of IL-6 on apoptosis. When apoptosis occurs, caspases are cleaved into active

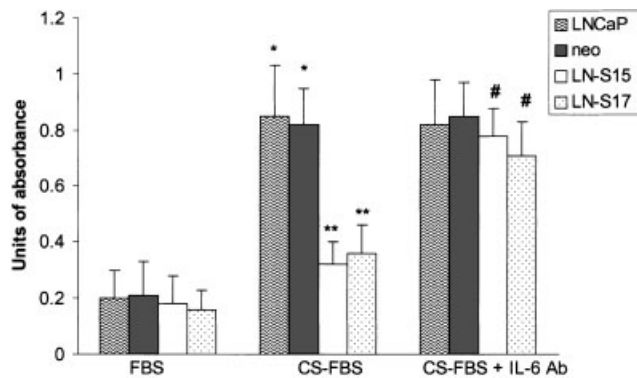


Fig. 2. Cell death analysis by a specific ELISA kit. Parental LNCaP, neo, and IL-6 overexpressing (LN-S15 and LN-S17) cells were cultured in either normal FBS, androgen deprived CS-FBS, or CS-FBS plus 20 $\mu\text{g}/\text{ml}$ of IL-6 antibody conditions. Data are expressed as mean \pm SE of four independent experiments. *, $P < 0.01$ compared with neo control cells cultured in normal FBS conditions; **, $P < 0.01$ compared with neo controls in CS-FBS conditions; #, $P < 0.01$ compared with LN-S15 and LN-S17 in the CS-FBS condition in the absence of the IL-6 antibody.

enzymes from inactive precursors and PARP is also cleaved. When parental LNCaP cells or neo control LNCaP cells were androgen deprived, elevated levels of the cleaved PARP and cleaved caspase-9 were detected (Fig. 3A). However, in IL-6 overexpressing LN-S15 and LN-S17 cells significantly lower levels of cleaved PARP and cleaved caspase-9 were observed as compared to the parental LNCaP cells or to the neo control cells when grown in androgen deficient

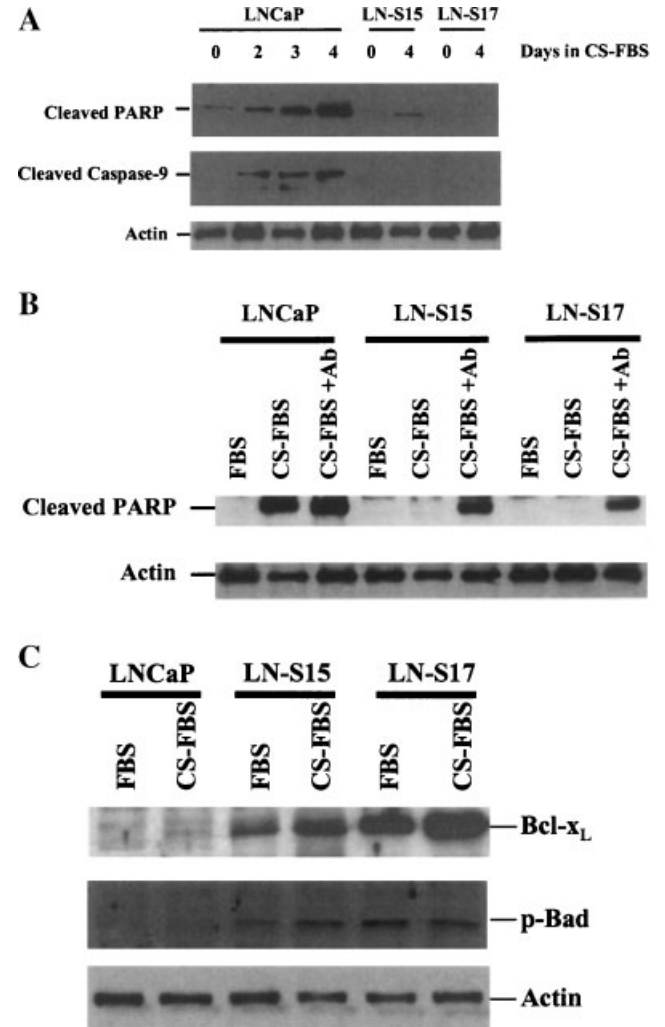


Fig. 3. Effect of IL-6 on the expression of pro-apoptotic and anti-apoptotic proteins in LNCaP cells in androgen deprivation conditions. Immunoblots were prepared from 40 μg of whole cell lysate from parental LNCaP, neo, and IL-6 overexpressing LNCaP clone LN-S15 and LN-S17 cells cultured either in normal FBS or androgen deprived charcoal-stripped FBS (CS-FBS) conditions for 3 days as indicated. **A:** Immunoblots were analyzed with cleaved poly (ADP-ribose) polymerase (PARP) and cleaved caspase-9 antibodies. **B:** Twenty microgram per milliliter of IL-6 antibody were added to the androgen deprived charcoal stripped culture medium (CS-FBS-Ab), and whole cell lysate were immunoblotted with cleaved PARP antibody. **C:** Immunoblots were analyzed with Bcl- x_L and phospho-Bad antibodies.

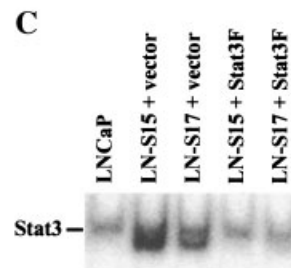
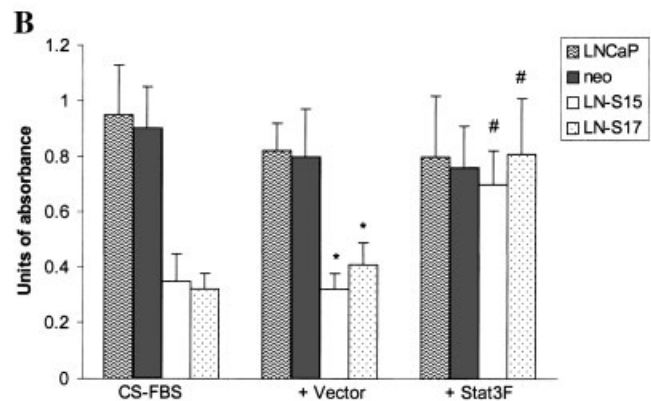
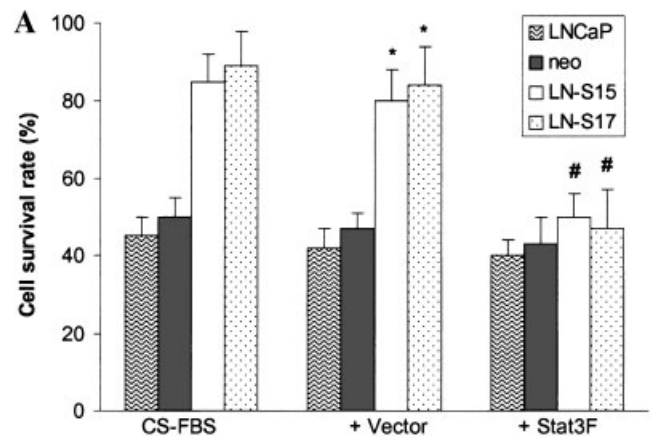
conditions (Fig. 3A). Addition of IL-6 antibody to the IL-6 overexpressing LN-S15 and LN-S17 cell culture media significantly elevated the levels of cleaved PARP expression (Fig. 3B), further suggesting a role for IL-6 specifically. Concomitantly, the increase in anti-apoptotic protein was observed in LN-S15 and LN-S17 cells. When cells were grown in androgen-deficient media, the expression of Bcl-x_L and phosphorylated Bad proteins was significantly increased in the IL-6 overexpressing LN-S15 and LN-S17 cells compared to parental LNCaP cells (Fig. 3C). Collectively, these data demonstrate that LNCaP cells undergo apoptotic cell death upon androgen withdrawal, and that IL-6 protects these cells from androgen deprivation induced apoptosis.

Stat3 Activation Mediates Anti-Apoptotic Activity of IL-6

We have previously demonstrated that IL-6 significantly activates Stat3 in LNCaP cells both with androgen (normal FBS) and without androgen (CS-FBS) [19]. To elucidate the mechanism underlying this anti-apoptotic effect of IL-6, we investigated whether activation of Stat3 by IL-6 is responsible for this effect. We transfected a dominant-negative Stat3 mutant, Stat3F, into the LNCaP parental, neo, and IL-6 overexpressing cells. The dominant-negative Stat3 construct carries a phenylalanine substitution of the tyrosine residue at 705 that causes a reduction of the tyrosine phosphorylation of wild type Stat3 and inhibits the action of endogenous Stat3 [27,29,30]. As

Fig. 4. Stat3F inhibits anti-apoptotic activity of IL-6. **A:** Stat3F inhibits IL-6 induced LNCaP cell survival in androgen deprived media. Parental LNCaP, neo, IL-6 overexpressing LN-S15 and LN-S17 cells were cultured in RPMI 1640 supplemented with 10% CS-FBS. The cells were then transiently transfected with equal amounts (2 μ g) of either vector controls or Stat3F. The cell numbers were counted after 3 days in CS-FBS condition and cell survival values were expressed as % relative to the complete FBS. **B:** Stat3F blocks anti-apoptotic activity of IL-6 in androgen deprived conditions. Parental LNCaP, neo, IL-6 overexpressing LN-S15 and LN-S17 cells were cultured in RPMI 1640 supplemented with 10% CS-FBS. The cells were then transiently transfected with equal amounts (2 μ g) of either vector controls or Stat3F. Quantitation of apoptosis by a special ELISA kit in these cells after cultured in androgen deprived CS-FBS for 3 days. Data are expressed as mean \pm SE of four independent experiments. *, $P < 0.05$ compared with neo control in CS-FBS conditions; #, $P < 0.05$ compared with LN-S15 and LN-S17 transfected with vector control in the CS-FBS condition. **C:** Effect of Stat3F on Stat3 activity. IL-6 overexpressing LN-S15 and LN-S17 cells were transfected with either vector alone or Stat3F. After transfection, the cells were continuously cultured in androgen deprived condition for 3 days and the cell extracts were prepared. The Stat3 activity was analyzed using electromobility shift assay (EMSA) as described in "Materials and Methods."

seen in Figure 4A, when Stat3F was transfected, the anti-apoptotic activity of IL-6 was abolished in IL-6 overexpressing LN-S15 and LN-S17 cells. When these cells were put into media without androgen, the survival rates were greater than 85%. In cells transfected with Stat3F, survival rates were less than 50%, a level observed in the parental LNCaP and neo control cells grown in the absence of androgen. The effect of transfecting expression of Stat3F on apoptosis was also examined by ELISA. As shown in Figure 4B, transient transfection of Stat3F into IL-6 overexpressing LN-S15 and LN-S17 cells resulted in a significant increase in apoptotic death ($P < 0.01$). Figure 4C demonstrated that the levels of Stat3 activity in these IL-6 overexpressing LN-S15 and LN-S17 clones were significantly decreased after the transient transfection of Stat3F. These studies confirm that blocking Stat3 activation reverses the anti-apoptotic activity of IL-6.



Stat3 Activity Antagonizes Androgen-Deprivation Induced Death in LNCaP Cells

The anti-apoptotic activity of IL-6 from androgen deprivation appears to be mediated by Stat3 activation in LNCaP cells. We further investigated whether increased Stat3 activity might be sufficient to protect cells from androgen deprivation-induced apoptosis. To demonstrate a direct effect of enhanced Stat3 activity on LNCaP cell apoptosis induced by androgen deprivation, LNCaP cells were transfected with a constitutively activated Stat3 mutant, Stat3c, and vector control, respectively. Stat3c is a constitutively activated Stat3 (a mutant produced by substitution of the cysteine residues within the COOH-terminal loop of the SH2 domain of Stat3) that induces cellular transformation and tumor formation in nude mice [31]. Previous studies have demonstrated that ectopic expression of Stat3c into LNCaP cells enhanced Stat3 activation and promoted cell androgen independent growth [32]. After transfection with Stat3c, the cells were deprived of androgen for 72 hr, starting 12 hr after transfection, and the cell lysis was collected. Cell death was determined by the ELISA assay. As shown in Figure 5, overexpression of constitutively activated Stat3 in LNCaP cells resulted in significantly less cell death as compared to the vector control cells ($P < 0.01$) without androgen, and addition of the dominant-negative Stat3 (Stat3F) restored the level of cell death to that of the vector control, suggesting that the constitutive activation of Stat3 is sufficient to protect LNCaP undergoing apoptosis induced by androgen deprivation.

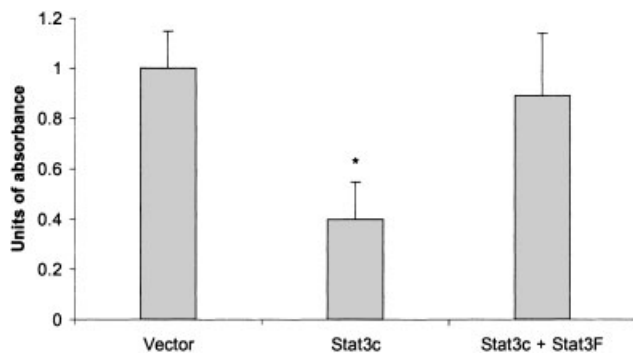


Fig. 5. Constitutively activated Stat3 protects LNCaP cells apoptosis induced by androgen deprivation. LNCaP cells were cultured in RPMI 1640 supplemented with 10% CS-FBS. The cells were then transiently transfected with equal amounts (2 μ g) of either vector controls or constitutively activated Stat3c or Stat3c plus Stat3F. Quantitation of apoptosis by a special ELISA kit was performed after cultured in androgen deprived CS-FBS for another 3 days. Data are expressed as mean \pm SE of four independent experiments. *, $P < 0.05$ compared with vector control in CS-FBS conditions.

DISCUSSION

In this study, we have investigated the molecular mechanisms by which IL-6 promotes androgen independent progression of prostate cancer cells. We demonstrated that IL-6 protects androgen sensitive LNCaP cells from androgen deprivation induced apoptosis through activation of Stat3 signaling pathway.

We previously demonstrated that overexpression of IL-6 in androgen sensitive LNCaP cells enhances androgen independent growth in vitro and in vivo [19]. To understand the mechanism of IL-6 induced androgen independent growth of LNCaP cells, we analyzed the effect of IL-6 on apoptosis induced by androgen deprivation. Several lines of evidence from this study demonstrate that overexpression of IL-6 can protect androgen deprivation-induced apoptosis in LNCaP cells. First, overexpression of IL-6 rescued LNCaP cells from cell death induced by androgen withdrawal. Second, LNCaP cells underwent apoptosis 72 hr androgen was removed, an outcome is largely absent in clones overexpressing IL-6. Third, IL-6 overexpressing cells resulted in a significant decrease in the expression of pro-apoptotic proteins such as cleaved PARP and cleaved caspase-9, and an increase in the expression of the anti-apoptotic proteins Bcl-x_L and phosphorylated Bad compared to the parental LNCaP cells. Forth, addition of IL-6 antibody completely abolished the anti-apoptotic activity of IL-6, suggesting that anti-apoptosis is specifically mediated by IL-6.

IL-6 has been implicated in the modulation of growth and differentiation in many malignant tumors and is associated with poor prognosis in several solid and hematopoietic neoplasms such as renal cell carcinoma, ovarian cancer, lymphoma, and melanoma [33]. The role of IL-6 in prostate cancer development and progression has been a subject of intensive investigation. The expression of IL-6 and its receptor has been consistently demonstrated not only in human prostate cancer cell lines but more importantly in human prostate carcinoma and benign prostate hyperplasia obtained directly from patients [8–10]. Clinically, the levels of IL-6 in the sera are significantly elevated in the patients with hormone refractory and metastatic prostate cancer compared to hormone sensitive prostate cancer [4,11]. Multiple studies have demonstrated that IL-6 is elevated in the sera of patients with metastatic prostate cancer and that the levels of IL-6 correlate with tumor burden as well as serum PSA or clinically evident metastases [4,11]. Collectively, these clinical data suggest that elevated IL-6 levels are associated with prostate cancer progression to an androgen-independent phenotype.

In addition to the clinical data on the role of IL-6 in androgen independent prostate cancer, experimental

studies demonstrate that IL-6 plays a critical role in prostate cancer cell growth and differentiation. IL-6 can function as a paracrine growth factor for the human LNCaP androgen-sensitive prostate cancer cells and an autocrine growth factor for the human DU145 and PC-3 androgen-insensitive prostate cancer cells [12]. IL-6 can activate AR-mediated gene expression by activation of the AR through a Stat3 pathway in LNCaP cells [13,16,17]. Our previous studies demonstrated that overexpression of IL-6 increases PSA mRNA expression and enhances AR activation in LNCaP cells [19]. In addition, overexpression of IL-6 promotes androgen independent growth of androgen sensitive LNCaP cells in vitro and in vivo [19]. These studies demonstrate that IL-6 activates AR signaling in a ligand-independent manner and induces a synergistic AR response with very low concentrations of androgen.

Accumulating evidence has demonstrated that abnormal AR signaling has contributed to prostate cancer androgen independent growth. Several reports suggested that the AR can be activated by growth factors and cytokines to display enhanced activity in the presence of androgen or to function even in the absence of androgen [34–37]. Recently, results from a number of groups demonstrated that IL-6 activates AR-mediated gene expression by activation of the AR through a Stat3 pathway in LNCaP cells [13,14,16,17]. Induction of neuroendocrine differentiation has also been suggested in the androgen independent prostate cancer cells [38,39]. IL-6 can induce LNCaP cell neuroendocrine differentiation mediated by the activation of Stat3 and MAPK signalings [40–42]. Androgen withdrawal triggers apoptosis in both normal and malignant androgen-dependent prostate epithelial cells. However, androgen-refractory prostate cancer cells do not undergo apoptosis [1]. Thus alteration of apoptotic signaling pathways should be critical for the survival of androgen-refractory prostate cancer cells. In this study, we demonstrate that IL-6 protects LNCaP cells from undergoing apoptotic death induced by androgen deprivation.

The effect of IL-6 on cell proliferation, differentiation, and survival is mediated by differential activation of several major signaling pathways including JAK-STAT, MAPK, and PI3K-Akt. The question arises as to what is the mechanism of anti-apoptosis induced by IL-6 in LNCaP cells. Apoptosis can be induced in response to various cytotoxic stimuli including cytokines and growth factors. These stimuli activate a series of tightly controlled intracellular signals including Stat3. The role of Stat3 in the protection of apoptosis has been already suggested in many cancer cells including prostate [43]. In U266 myeloma cells, constitutive activation of Stat3 signaling confers resistance to apoptosis [44]. In addition, Stat5 and Stat3 have also been demonstrated

to activate the Bcl-x_L expression directly [44,45]. In this study, we have clearly shown that the anti-apoptotic activity of IL-6 is mediated mainly by Stat3 signaling. We demonstrated that IL-6 overexpressing cell clones are protected from androgen-deprivation induced apoptosis through activation of Stat3 signaling. This protective effect of IL-6 was reversed by the expression of a dominant-negative Stat3 mutant, Stat3F. Furthermore, ectopic expression of a constitutively active Stat3 antagonized androgen deprivation-induced cell death of LNCaP cells.

In conclusion, we demonstrate that overexpression of IL-6 protects androgen sensitive LNCaP prostate cancer cells from apoptotic death induced by androgen deprivation through activation of Stat3 signaling pathway. Since both IL-6 levels are significantly elevated in hormone refractory prostate cancer and Stat3 activity is elevated in prostate cancer [46], targeting IL-6/Stat3 signaling may be of therapeutic value in the treatment of androgen independent prostate cancer.

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