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Interleukin-6 Is Responsible for Drug Resistance and Anti-Apoptotic Effects in Prostatic Cancer Cells

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BACKGROUND. Interleukin (IL)-6-mediated anti-apoptotic effects and drug-resistance mechanisms in prostate cancer cells were investigated.
METHODS. IL-6 levels of PC-3 and LNCaP cells were studied by using ELISA. Protective effects of IL-6 on cytotoxic agent-induced apoptosis were studied by exogenous IL-6 in serum-starved PC-3 cells and by anti-sense IL-6 strategy. Western blotting and reverse transcription-polymerase chain reaction (RT-PCR) were used to determine IL-6 effects on Bcl-2 family proteins. Tetracycline-regulated Bcl-xL expression system and dominant negative STAT3 transfectants were used to study IL-6 signaling pathways and its anti-apoptosis effects.
RESULTS. Exogenous IL-6 and anti-sense IL-6 oligonucleotide treatment conferred resistance to cytotoxic agent-induced apoptosis. Among Bcl-2 family proteins, only Bcl-xL was evidently increased by IL-6 stimulation. The anti-apoptotic effect of IL-6 can be significantly attenuated by anti-sense *bcl-xL* transfection and partially abrogated in dominant negative STAT3 transfectants.
CONCLUSIONS. IL-6 is a survival factor against cytotoxic agent-induced apoptosis through both STAT3 and *bcl-xL* pathways in prostate cancer cells. *Prostate 60: 120–129, 2004.*
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KEY WORDS: **cytotoxic agents; apoptosis; prostatic neoplasms; Bcl-xL; STAT3**

INTRODUCTION

Dysregulation of apoptosis has been implicated in a variety of human diseases, including autoimmune disorders, viral infections, neurodegenerative disorders, and cancers [1]. As generally assumed, many chemotherapeutic agents destroy cancer cells through activation of apoptotic pathways. In addition, inability to activate the intrinsic apoptotic program is a newly recognized mechanism of drug resistance that leads to treatment failures. Therefore, elucidating molecular factors that counteract apoptosis mechanism may be crucial to overcome drug resistance in anti-cancer therapies.

Abbreviations: IL-6, interleukin-6; ELISA, enzyme-inked immunosorbent assay; STAT3, signal transducers and activator of transcription 3; RT-PCR, reverse transcription-polymerase chain reaction; β-lap, β-lapachone.

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Interleukin (IL)-6 is a pleiotropic cytokine that exhibits many physiological functions, including host defense, bone metabolism, and acute phase responses [2]. IL-6 can exert differentiation and also induce growth-inhibitory or growth-stimulatory activities, depending on the nature of responsive target cells [3]. A variety of malignant tumors including myeloma, renal cell carcinoma, cervical carcinoma, AIDS Kaposi's sarcoma-derived cells, and prostatic carcinoma, have been shown to express or synthesize IL-6. Most importantly, autocrine growth stimulation has been suggested as one of the action mechanisms of IL-6-related signaling pathway [4]. A recent study has shown that although epithelial cells from a benign hyperplastic prostate does not respond to IL-6, an IL-6-mediated autocrine and/or paracrine growth mechanism was found in prostatic carcinoma cells, such as LNCaP, PC-3, and DU145 cells [5]. This observation suggests that elevated levels of IL-6 are associated with neoplastic growth of the prostate carcinoma [6]. In accordance with these findings, IL-6 was found to act as a resistance factor against some chemotherapeutic agents in DU145 and PC-3 cells [6,7].

IL-6 has been shown to be a predominant survival factor for multiple myeloma cells in that it inhibits apoptosis induced by serum starvation, dexamethasone, and Fas [8–10]. Contrarily, it has also been demonstrated that IL-6 was important for differentiation and apoptosis of pre-B cell [11]. A number of studies have recently shown that the anti-apoptotic ability of IL-6 was associated with expressions of the Bcl-2 family proteins [12]. Interestingly, the IL-6-mediated anti-apoptotic effect may be associated with the STAT3-independent Bcl-2 upregulation [13]. A recent study further showed that upregulation of the Bcl-xL protein was detected in human myeloma cell lines and patients' myeloma cells at relapse and that serum IL-6 levels tended to rise upon disease progression [14]. Although the action mechanisms of IL-6 in multiple myeloma have been extensively studied, the exact pathways of IL-6-mediated anti-apoptosis, which leads to failure of cytotoxic therapies for prostate cancer, have been far less explored.

In this study, we investigated the mechanisms of IL-6 in modulating cellular sensitivity to cytotoxic agents in prostatic cancer cells. The expressions of *bcl*-2 family genes in prostatic cancer cells in response to IL-6 were also examined.

MATERIALS AND METHODS

Cell Culture

PC-3 and LNCaP cell lines were purchased from American Type Culture Collection and cultured in the RPMI1640 medium supplemented with 10% fetal calf serum (FCS), 100 U/ml penicillin, and 0.1 mg/ml streptomycin. Cultures were routinely administered a fresh medium three times a week, and confluent cultures were dispersed weekly with PBS containing 0.05% trypsin and 0.02% EDTA for subsequent passages.

IL-6 ELISA

IL-6 levels in the supernatant of cultured PC-3 cells were quantified by using an IL-6 ELISA kit (R&D Co., Minneapolis, MN). Briefly, cells (1×10^6) were incubated in a serum-free (SF) RPMI medium for 24 hr, and then cultured in the RPMI medium for various time intervals with or without FCS. The cells were pelleted and the supernatant was collected.

Quantification of Cytotoxic Agent-Induced Apoptosis by ELISA

Cells were pretreated with IL-6 (60 ng/ml) for up to 1 hr before exposure to various cytotoxic agents including 3 μM β-lapachone (Sigma Chemical Co., St. Louis, MO) for up to 8 hr, and 10 μM doxorubicin (Pharmacia & Upjon Co. Ltd., Milan, Italy), 20 μM VP-16 (etoposide, Sigma Chemical Co.), and 50 μM cisplatin (Pharmacia & Upjon Co. Ltd.) for up to 16 hr, respectively to induce apoptosis. A previous study demonstrated that β-lapachone, a topoisomerase inhibitor, was selectively toxic to prostatic cancer cells in a SF culture condition [15]. β-lapachone was prepared according to the procedures described previously [16]. An ELISA-based cell death assay kit (Boehringer Mannheim GmbH, Germany) that measures cytoplasmic DNA-histone complexes generated during apoptotic DNA fragmentation was used. Briefly, an anti-histone first antibody was coated on wells, which were then loaded with cytoplasmic lysates. Cytoplasmic extracts from control or drug-treated cells were normalized to total cell numbers. The second antibody was anti-DNA antibody conjugated to peroxidase. The ELISA was then developed by adding the peroxidase substrate. Finally, the absorbance at 405 nm was measured using a microplate autoreader.

DNA Fragmentation Assay and Morphological Features of Apoptosis

Cells were harvested and washed with PBS. DNA fragmentation assays were done and analyzed as described previously [17]. Morphological and nuclear characteristics were also used to verify the presence of apoptotic events in prostatic cancer cells treated by cytotoxic agents, which included cell shrinkage, chromatin condensation, and formation of apoptotic bodies.

Reverse Transcription Polymerase Chain Reaction RT-PCR

RNAs from PC-3 cells treated with IL-6 for various time intervals were prepared with the commercial kits (BIOTECX Lab., Inc., Houston, TX). Total RNA was subjected to first-strand cDNA synthesis using oligo-dT (Amersham Pharmacia Biotech, Inc., Hong Kong, China) and moloney murine leukemia virus reverse transcriptase (GIBCO BRL, Life Technologies, Gaithersburg, MD) at 37°C for 2 hr. The reaction was stopped by incubation at 70°C for 10 min. The primers used for *bcl-xL* amplification were synthesized according to the sequences reported previously [18].

For semi-quantitative PCR for *bcl-xL*, the 5′-primer and the 3′-primer were 5′-AGTCAGTTTAGTGATG-TC-3′ and 5′-GGATGTTAGATCACTGAA-3′, respectively. PCR (1 min at 96°C, 1 min at 55°C, 2 min at 72°C) was performed at 25 cycles. With this condition, relative quantitative analysis of PCR products can be achieved. PCR products were separated by agarose gel electrophoresis and transferred to nylon membranes, which were then vacuum dried for X-ray film exposure.

IL-6-Specific Anti-Sense Oligonucleotide

PC-3 cells were treated with 20 μM IL-6-specific anti-sense or sense oligonucleotide phosphorothioates (Genset Co., San Diego, CA) for 12 hr and then with 1 μM β-lapachone for another 8 hr. These oligonucleotides were not designed for protein expression. Apoptosis was determined by using an ELISA-based quantitative assay. Data were calculated from three independent experiments.

Establishment of Anti-Sense-bcl-xL and Dominant-Negative STAT3 Transfectants

The parent PBSTR-1 retroviral vector was generously provided by Dr. S. A. Reeves (Massachusetts General Hospital, Boston, MA). It is a modified version of the previously described tetracycline (Tet) system [19]. The Tet-controlled system has been described elsewhere [17]. Human *bcl-xL* cDNA was cloned into a *Bam*H1 site of the PRSTR-1 vector in an anti-sense orientation.

As previously reported, STAT3 are readily activated in response to IL-6 [20]. To explore whether STAT3 is involved in IL-6-mediated anti-apoptotic signaling, we utilized the dominant-negative STAT3 (DN-STAT3) gene to inhibit the activity of endogenous STAT3 function. A recent study demonstrated that the STAT3F and STAT3D mutants, in which Tyr-705 (a phosphoacceptor site of STAT3) was mutated to phenylalanine (STAT3F) and Glu-434 and Glu-435 of the DNA-binding domain were mutated to alanines (STAT3D), act specifically in a dominant-negative manner [21]. The two vectors, STAT3F and STAT3D, were generously provided by Dr. Toshio Hirano (Department of Molecular Oncology, Biomedical Research Center, Osaka University Medical School, Suita, Osaka, Japan). We transfected PC-3 cells with the expression vectors of STAT3F, STAT3D, or a *Neo* control vector. The representative stable G418-resistant PC-3 transfectants were subjected to the determination of STAT3 mutant protein expressions using an anti-hemagglutinin (HA) antibody to specifically immunoprecipitate the HA-tagged proteins and further immunoblotting with the anti-STAT3 antibody (Upstate Biotech, Lake Placid, NY).

Anti-sense *bcl-xL*, *Neo*, and DN-STAT3 vectors were transfected into exponentially growing PC-3 cells using LipofactAmine reagent (GIBCO BRL, Life Technologies, Gaithersburg, MD) according to the manufacturer's instructions. To select stable clones, G418 and puromycin were added into DN-STAT3 and anti-sense *bcl-xL* transfectants, respectively. The G418 and puromycin resistant clones were individually selected, expanded and then assayed for expressions of transfected cDNAs by Western blotting. To study whether IL-6 treatment can induce *bcl-xL* upregulation, which then contributes to the anti-apoptotic behavior, *bcl-xL* or *Neo*transfectants were treated with 60 ng/ml IL-6 for 16 hr after incubation with or without 1 μg/ml tetracycline for 24 hr. The cell lysates were subjected to Western blot analysis using an anti-Bcl-xL antibody.

Western Blot Analysis

Cells were lysed in a lysis buffer (50 mM Tris-HCl, 150 mM NaCl, 1 mM EGTA, 1% NP-40, 1 mM NaF, 1 mM Na_3VO_4, 1 mM phenylmethylsulfonyl fluoride, 1 mg/ml aprotinin, and leupeptin, pH7.4) for 20 min on ice. The lysates were centrifuged at 14,500 rpm for 20 min at 4°C. Protein concentrations were then determined using a commercial BCA kit (PIERCE Life Science Co., Rockford, IL). A sample of 50 μg of each lysate was subjected to a 12% SDS–polyacrylamide gel electrophoresis (PAGE). Finally, proteins were transferred to nitrocellulose papers and immunoblotted with anti-Bcl-2, anti-IL-6, anti-Bcl-xL, anti-Bax, anti-Bad, or anti-STAT3 antibodies (Santa Cruz Biotechnology, Santa Cruz, CA). The detection was performed by using an enhanced chemiluminescence kit (Amersham Pharmacia Biotech).

Statistical Analysis

All statistical comparisons were made by using the Student's *t*-test with an $\alpha = 0.05$. Data were presented as mean ± SD of several independent experiments.

RESULTS

Secretion of IL-6 by PC-3 But Not LNCaP Cells Is Serum-Dependent

To examine the secretion levels of IL-6, PC-3, and LNCaP cells were cultured in a medium containing 10% FCS or a SF medium for 24 hr. Figure 1A shows the kinetics of the endogenous IL-6 secretion. PC-3 cells produced a substantial level of IL-6 (878 ± 216 pg/ml) at 8 hr and reached a plateau level (1,123 ± 238 pg/ml) at 12 hr when cultured in a medium containing 10% FCS. In contrast, secretion of IL-6 by PC-3 cells was significantly reduced when they were cultured in a SF medium. As compared to PC-3 cells, LNCaP cells secreted minimal levels of IL-6 (mean: 186 ± 43 pg/ml) either in a SF medium or serum-containing medium. In line with the IL-6 ELISA data, Western blot analysis also revealed that specific anti-IL-6 antibody detected a significant amount of IL-6 protein in PC-3 cells cultured in a serum-containing medium. Only very little IL-6 protein was detected in PC-3 cells cultured in a SF medium or in LNCaP cells maintained in a medium with or without serum (Fig. 1B).

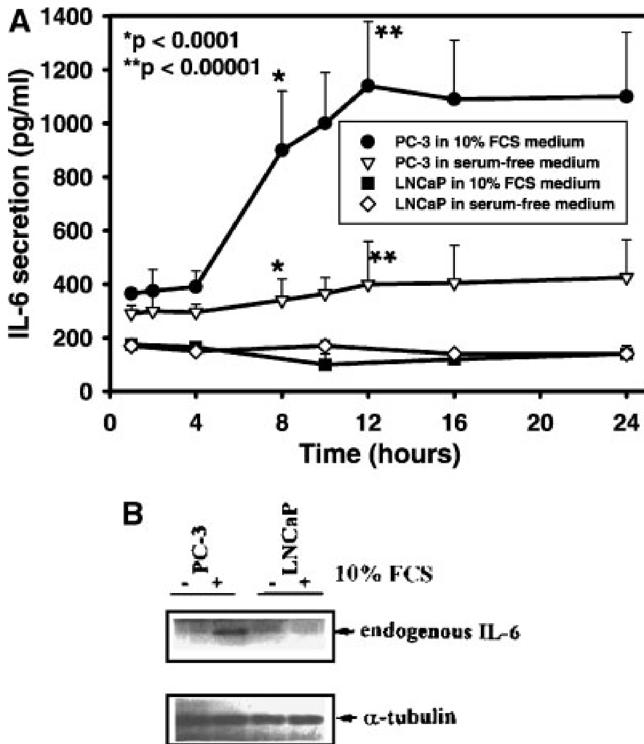

Fig. 1. Secretion of IL-6 by PC-3 and LNCaP cells. IL-6 secreted into the medium was quantified by ELISA (**A**) or Western blotting (**B**). Data (mean ± SD) were calculated from at least three independent experiments.

Inhibition of Cytotoxic Agent-Induced Apoptosis by Exogenous IL-6

We examined whether IL-6 affects cytotoxic agent-induced apoptosis in PC-3 and LNCaP cells. The extent of apoptosis was determined by using the ELISA-based quantitative assay. As shown in Figure 2A, exogenous IL-6 caused a dose-dependent inhibition of

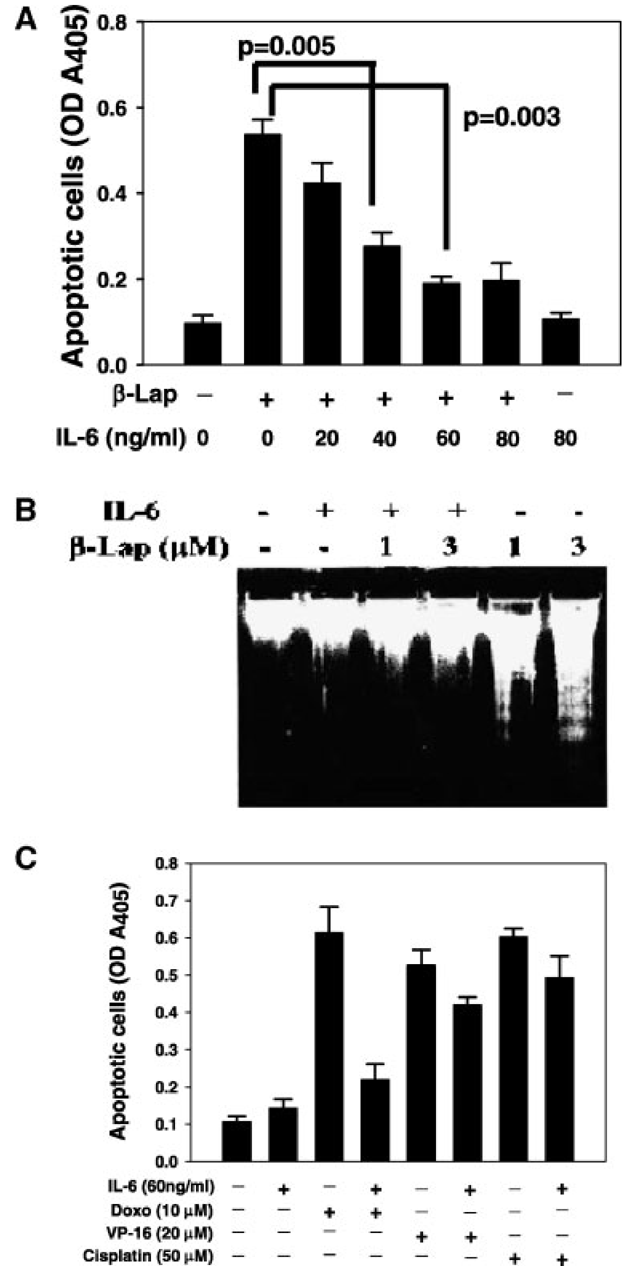

Fig. 2. Effect of exogenous IL-6 on cytotoxic agent-induced apoptosis in PC-3 cells. **A**: Inhibition of β-lapachone induced apoptosis by IL-6. **B**: IL-6 inhibits β-lapachone induced DNA fragmentation. **C**: Inhibition of cytotoxic agent induced apoptosis by IL-6. All data were calculated from three independent experiments. Doxo, doxorubicin.

β-lapachone-induced apoptosis in PC-3 cells cultured in a SF medium. Maximal cell protection from apoptosis was achieved by IL-6 at 60 ng/ml. With the same settings, the IL-6 protective effect was less prominent in LNCaP cells (data not shown) than in PC-3 cells. Morphological and nuclear characteristics further verified the anti-apoptotic effect in that typical features of apoptosis such as cell shrinkage; chromatin condensation and formation of apoptotic bodies were observed in β-lapachone-treated PC-3 cells (1 μM for 12 hr) but much less in IL-6 pretreated cells (data not shown). DNA ladder assay also confirmed that IL-6 treatment made PC-3 cells resistant to DNA fragmentation induced by 1 or 3 μM β-lapachone (Fig. 2B). Furthermore, the ELISA-based apoptosis assay showed that IL-6 at 60 ng/ml for 1 hr attenuated 68% ($P < 0.01$), 19% ($P < 0.05$), and 20% ($P = 0.06$) of apoptosis induced by doxorubicin, VP-16, and cisplatin, respectively (Fig. 2C). These results suggest that IL-6 inhibits cytotoxic agent-induced apoptosis in a dose-dependent manner and act as a survival factor for prostatic cancer cells.

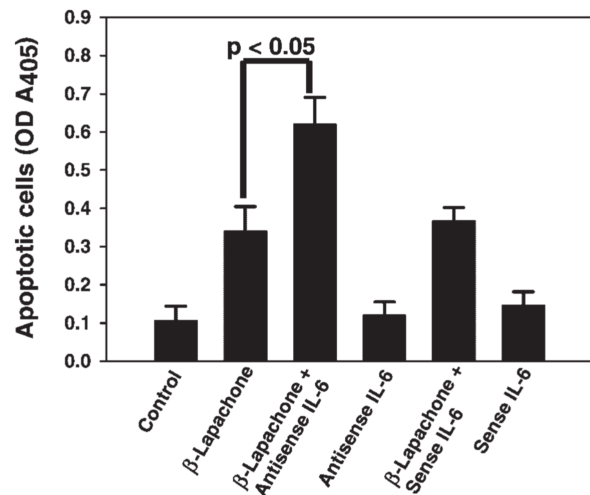

Fig. 3. Enhancement of β-lapachone-induced apoptosis by IL-6-specific anti-sense oligonucleotide. Data were calculated from three independent experiments.

Downregulation of IL-6 Secretion Sensitizes PC-3 Cells to β-Lapachone

We have demonstrated that PC-3 cells constitutively secrete substantial amounts of IL-6 in a serum-containing medium but only minimal levels in a SF medium. We hypothesized that blockage of the IL-6 autocrine circuit by inhibiting IL-6 secretion may sensitize PC-3 cells to β-lapachone and other cytotoxic agents even in the presence of a serum-containing medium. To address this issue, we treated PC-3 cells with 20 μM IL-6-specific anti-sense or sense oligonucleotide phosphorothioates for 12 hr and then with 1 μM β-lapachone for another 8 hr. In results, pretreatment with IL-6-specific anti-sense oligonucleotide obviously enhanced β-lapachone-induced apoptosis, but the sense oligonucleotide could not (Fig. 3). Flow cytometric analysis revealed that treatment with anti-sense IL-6 oligonucleotides for 12 hr significantly reduced endogenous IL-6 secretion in PC-3 cells (data not shown). These results implicate that abrogation of IL-6 secretion can sensitize PC-3 cells to cytotoxic agents like β-lapachone.

Effects of IL-6 on Bcl-2 Gene Family Expressions

The mechanism by which IL-6 protects PC-3 cells from cytotoxic agent-induced apoptosis was investigated. We examined the levels of Bcl-2 family proteins in PC-3 cells treated with IL-6. Cells were preincubated in a SF medium for 24 hr and then treated with 60 ng/ml IL-6 for various time intervals. Western blot analysis of total cellular proteins showed that Bcl-xL protein levels were increased over threefold at 8 hr of IL-6 treatment and remained elevated for up to 24 hr. There were no changes in other Bcl-2 family members such as Bcl-2, Bax, or Bad (Fig. 4A). Notably, the upregulation of Bcl-xL by IL-6 appeared to be dose-dependent in that the maximal response of Bcl-xL was achieved with IL-6 at 40 ng/ml. Only minimally increased expressions of Bcl-2 but not Bax or Bad were noted in response to escalated doses of IL-6 (Fig. 4B). The IL-6-induced elevation of Bcl-xL levels was consistently seen in four independent experiments. RT-PCR assays also showed that the maximal increase of *bcl-xL* mRNA expression was detected at 4 hr of IL-6 exposure (Fig. 4C).

Role of Bcl-xL in IL-6-Mediated Anti-Apoptosis

To determine whether the IL-6-mediated upregulation of *bcl-xL* contributed to its anti-apoptotic behavior, we established a tetracycline (Tet)-regulated anti-sense *bcl-xL* retroviral vector and transfected it into PC-3 cells. The puromycin-resistant PC-3 clones were picked up and maintained in the presence of Tet. Western blot analysis revealed that intracellular Bcl-xL protein level was significantly decreased in anti-sense *bcl-xL*-transfected PC-3 cells when Tet had been removed for 24 hr (Fig. 5A). The Bcl-xL levels in cells transfected with the control vector, *Neo*, were not affected by the presence or absence of Tet (Fig. 5A). ELISA-based apoptosis assay showed that the protective effect of IL-6 was significantly attenuated in anti-sense *bcl-xL*-transfected PC-3 cells treated with β-lapachone when cultured in the absence of Tet for 24 hr (Fig. 5B). These findings imply that Bcl-xL protein plays an important role in IL-6-mediated anti-apoptosis.

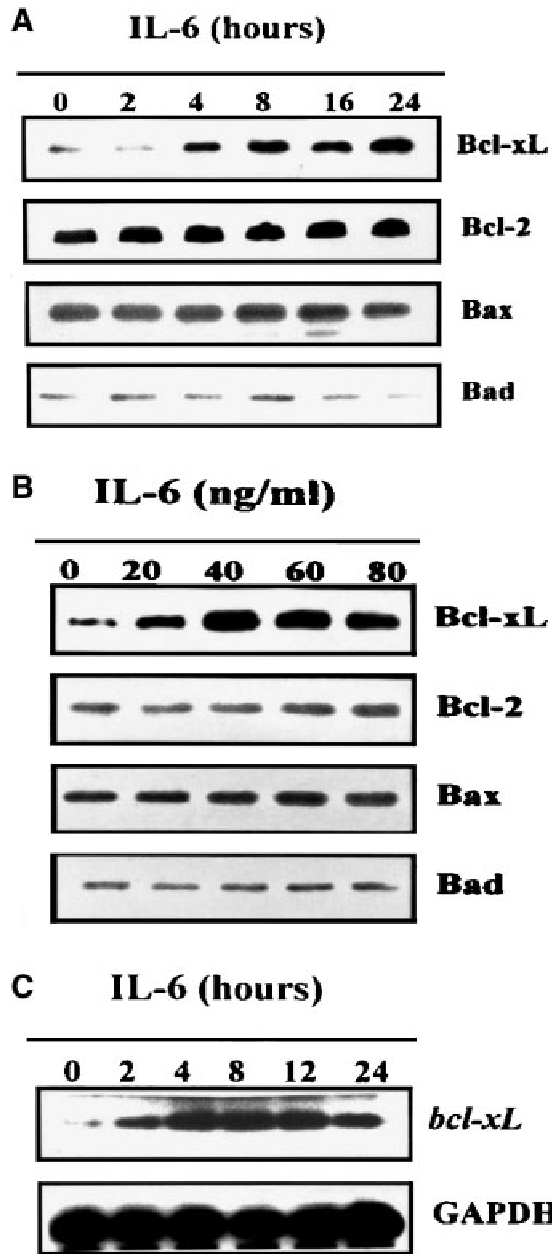

Fig. 4. Western blot analysis of the Bcl-2 family protein expressions in response to IL-6 stimulation in PC-3 cells. Expressions were examined at different time intervals (**A**) or escalated concentrations (**B**) of IL-6 stimulation. **C**: Semi-quantitative RT-PCR analysis of Bcl-xL mRNA expression. The Bcl-xL upregulation by IL-6 appeared to be time- and dose-dependent.

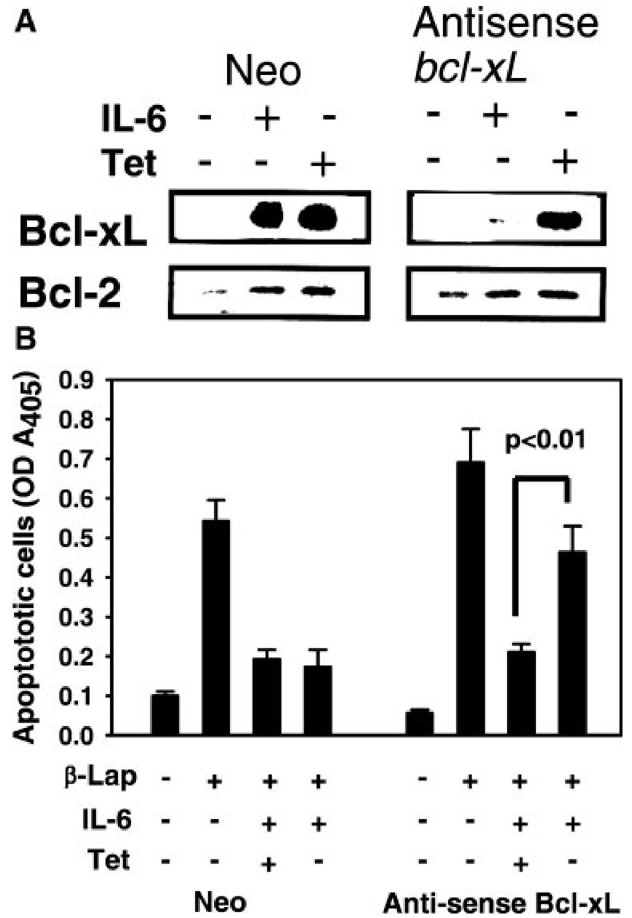

Fig. 5. Inhibitory effect of anti-sense Bcl-xL on IL-6-mediated Bcl-xL upregulation and anti-apoptosis in PC-3 cells. **A**: Determination of Bcl-xL and Bcl-2 protein levels in anti-sense bcl-xL transfected cells by Western blot analysis. Neo: the control cell clone transfected with the *Neo* vector. **B**: Anti-sense Bcl-xL transfectant cultured in the absence of Tet was more sensitive to β-lapachone even under IL-6 protection. Data were calculated from four to six independent experiments.

STAT3 Is Also Involved in IL-6-Mediated Anti-Apoptotic Effect

By Western blot analysis, substantial amounts of STAT3 proteins can be seen in the STAT3F and STAT3D transfectants but not in the *Neo* control PC-3 cells (Fig. 6A). These transfectants were then treated with IL-6, β-lapachone, or IL-6 plus β-lapachone for 8 hr and analyzed for the extent of apoptosis. In results, the anti-apoptotic effect of IL-6 was partially abrogated in the two DN-STAT3 cells but not in the *Neo* control cells (Fig. 6B). We further examined whether IL-6-mediated upregulation of Bcl-xL levels would be inhibited in STAT3F or STAT3D cells. Western blot analysis revealed that the IL-6-mediated Bcl-xL expression was not affected in the two DN-STAT3 mutants (Fig. 6C). To further clarify whether Bcl-xL acts independently of the STAT3 pathway in IL-6-mediated anti-apoptosis, the STAT3D mutant clone was transfected with tetracycline-controlled anti-sense *bcl-xL* expression vector (Fig. 6D). In STAT3D, IL-6 saved only about half of the apoptotic events induced by β-lap when anti-sense *bcl-xL* expression was suppressed by the presence of tetracycline. When anti-sense *bcl-xL* was expressed by

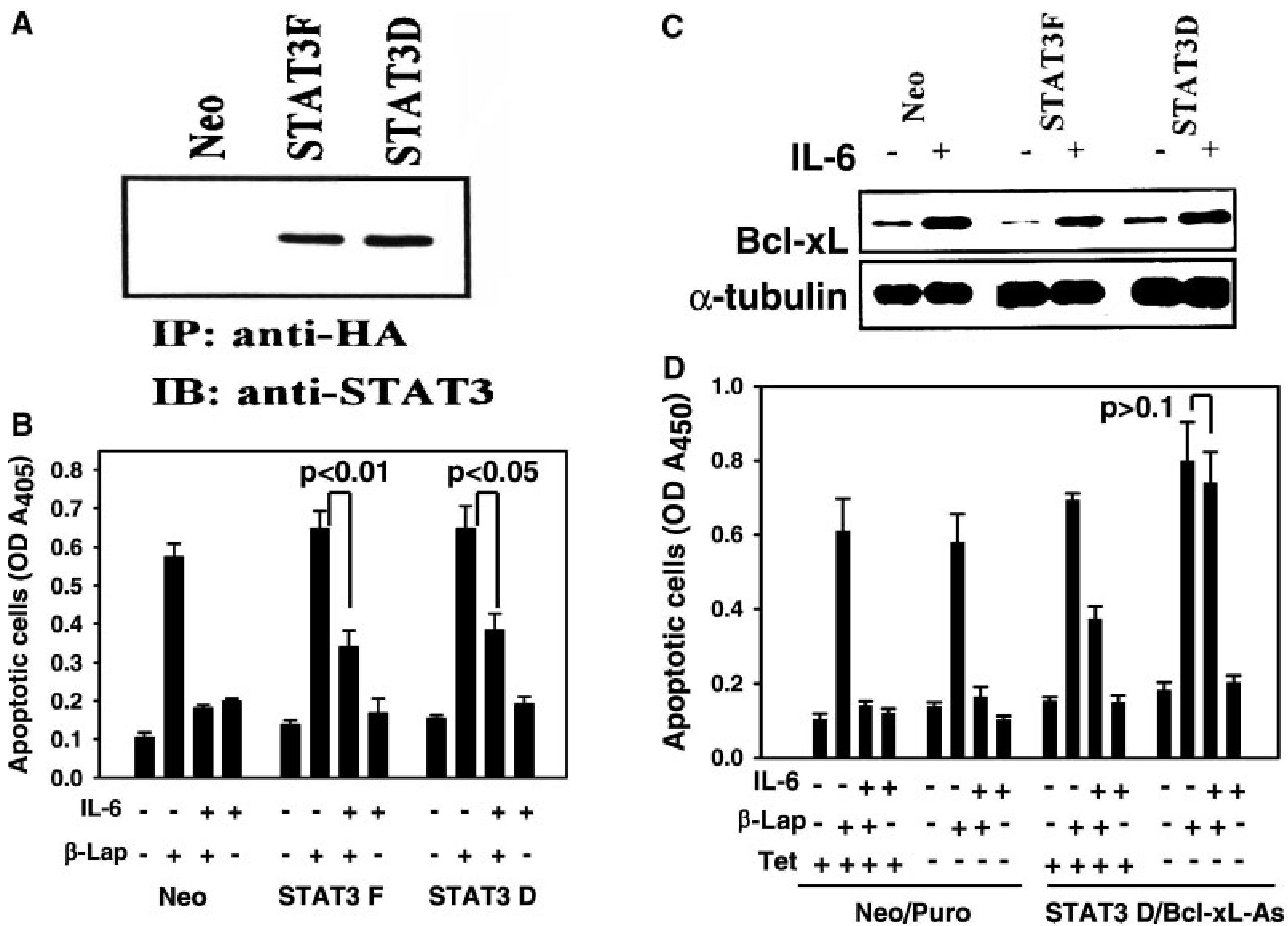

Fig. 6. Partial abrogation of IL-6-mediated anti-apoptosis in dominant-negative (DN) STAT3 transfectants. **A**: Expression of the DN STAT3 mutant proteins, STAT3D and STAT3F, in PC-3 cells. **B**: Expression of the DN STAT3 mutant proteins can attenuate the protective effect of IL-6 from β-lapachone-induced apoptosis. Data were from three independent experiments. **C**: Blockage of the intracellular STAT3 activity did not affect IL-6-mediated Bcl-xL upregulation determined by Western blot analysis. α-tubulin: internal control. **D**: Blockage of Bcl-xL by anti-sense strategy further attenuates IL-6-mediated anti-apoptosis in the DN STAT3D mutant.

removing tetracycline, the extent of apoptosis induced by β-lap was full even with the presence of IL-6, which indicates that IL-6 no longer protects cells from apoptosis when both the STAT3 and Bcl-xL pathways are defective. These experiments suggest that STAT3 is partially involved in the IL-6-mediated anti-apoptosis and that STAT3 signaling is not a prerequisite for Bcl-xL expression.

DISCUSSION

Previous studies have consistently demonstrated that PC-3 and DU145 cells secrete a large amount of IL-6, while LNCaP cells do not [6]. Our data further demonstrated that IL-6 secretion was significantly attenuated when PC-3 cells were cultured in a SF medium. This finding suggests that some serum factors may play a role in the regulation of IL-6 synthesis. In contrast to LNCaP cells, PC-3 and DU145 cells are highly aggressive with respect to their growth rate in culture and tumorigenicity in animal models [22]. This raised the possibility that prostatic carcinoma cells with elevated IL-6 levels may be associated with a more aggressive phenotype. Androgen-independence or aggressive phenotype may occur when prostatic tumor cells acquire the ability to secrete their own survival factor, such as IL-6. This concept warrants further investigations. However, results of clinical investigations appeared to support that IL-6 expressions in renal cell carcinoma and prostatic carcinoma are associated with a more aggressive tumor behavior [6,23].

Accumulating data suggest that acquiring ability to resist apoptosis is an important factor to develop hormone resistance and a more aggressive phenotype in human prostate cancer [24]. A recent study indicated that IL-6 is a survival factor for normal hematopoietic

and leukemic cells [25] and is able to block apoptosis induced by wild-type p53, transforming growth factor-β1, and several cytotoxic agents [26]. Taken together, these may imply that the IL-6 signaling pathway may contribute to the chemoresistance of hormone-independent prostate cancer. Our results demonstrated that exogenous IL-6 rendered serum-starved PC-3 cells resistance to apoptosis induced by cytotoxic agents including β-lapachone, doxorubicin, VP-16, and cisplatin. In contrast, specific blockade of IL-6 by the antisense oligonucleotide strategy sensitized PC-3 cells to these drugs. These findings suggest that IL-6 acts as a survival factor for prostatic cancer cells in our experimental setting. Nonetheless, it has also been shown that IL-6 may act to promote cell proliferation and growth. Schaeffer et al. have demonstrated that IL-6 induced the activation of the Src family kinase, Hck, which mediated proliferative signaling in murine pro-B-cell line model upon growth factor stimulation [27]. In line with our findings, Borsellino et al. have shown that anti-IL-6 antiserum moderately inhibited the growth of PC-3 and DU145 cells and sensitized both cell lines to cytotoxic agents such as VP-16 and cisplatin [7]. However, Oritani et al. have reported contradictory results where IL-6 signaling led to macrophage differentiation and apoptosis in 1A9-M cells [11]. These data suggest that different types of cells may respond to the same IL-6 stimuli in a number of different ways.

The mechanisms by which IL-6 protects prostate cancer cells from drug-induced apoptosis are largely unknown. The Bcl-2 family proteins are known to be involved in regulating the threshold beyond which cells undergo stress-induced apoptosis [28]. Bcl-2 can inhibit apoptosis induced by diverse stimuli in vitro. In addition, overexpression of *bcl-2* in LNCaP cells conferred resistance to apoptotic stimuli in vitro and androgen depletion in vivo [12]. Our hypothesis is that IL-6 signaling may confer cellular resistance to apoptosis by way of upregulating the expressions of intrinsic *bcl-2* family members. Our data revealed that exogenous recombinant IL-6 did significantly enhance the endogenous *bcl-xL* gene expression in serum-starved PC-3 cells at both the mRNA and protein levels, but did not affect the expressions of other *bcl-2* members tested such as *bcl-2*, *bax*, or *bad*. We have also demonstrated that transfection of PC-3 cells with the antisense*bcl-xL* resulted in an attenuation of IL-6-mediated anti-apoptotic effects. Similar findings were also seen in a different cell type where IL-6 induced *bcl-xL* mRNA without upregulating *bcl-2* levels in an IL-6-dependent myeloma cell line model [29]. These results implicate that IL-6-induced upregulation of *bcl-xL* may be responsible for the chemoresistance phenotype in some specific types of cancer cells. However, although the IL-6-mediated upregulation of Bcl-xL protein could be completely abrogated by anti-sense *bcl-xL* induced by removing tetracycline, only about 60% of the anti-apoptotic effects of IL-6 were abolished. It indicates that *bcl-xL* only partially contributes to the anti-apoptotic effects of IL-6 in PC-3 cells.

Although Bcl-2 appeared to be not involved in the IL-6-mediated anti-apoptosis in our experimental model, the possible role of Bcl-2 in mediating drug resistance of prostatic cancer cells can still be present. In fact, overexpression of *bcl-2* has been shown to confer drug resistance phenotype to prostatic cancer cells in vitro [30]. Bcl-xL and Bcl-2 expressions have been reported to be regulated by each other in some normal tissues and tumors [31], which indicates that these two anti-apoptotic proteins ''take turns'' in serving as the predominant regulators of apoptosis at various stages of cell differentiation and activation.

It was recently proposed that stimulation of the JAK-STAT and/or MAPK pathways by IL-6 and forskolin activated the androgen receptor (AR) in the absence of androgens in LNCaP prostate cancer cells [32]. The mechanisms possibly include phosphorylation of the AR coactivator-1 [33] and/or recruitment of the coactivator p300 [34]. While these mechanisms may account for the continued activity of the androgen-signaling axis following androgen ablation, the contribution of AR signaling to prostate cell growth in vivo is still largely unknown. It is likely that IL-6 activates AR-mediated gene expression through the STAT3 pathway [35]. Previous studies have demonstrated that the crosstalk between IL-6 and AR signaling occurs by direct physical and functional interactions between STAT3 and AR in prostate cancer cells [36]. Therefore, activated STAT3 signaling is important for IL-6 to activate AR and may play an important role in prostate cancer progression.

Previous studies confirmed that IL-6 exerts its multiple biological function or activates a set of different genes by activating the JAK-STAT3 pathway [37]. STAT3 transcriptional activity could be enhanced by interaction with other transcription factors. An interesting example was the forkhead transcription factor forkhead-related transcription factor (FKHR). FKHR specifically enhanced the activity of STAT3-dependent promoters, such as the α2M promoter. It indicated that FKHR could modulate the IL-6-induced transcriptional activity by enhancing STAT3 action [38]. Furthermore, although STAT3 activity has been found to be involved in the upregulation of Bcl-2 in pro-B cells [21], our data showed that the blockage of STAT3 activity did not alter the IL-6-mediated expression of Bcl-xL protein in PC-3 cells. This finding suggests that some unknown factors that are downstream to STAT3 but distinct from Bcl-xL are responsible for the STAT3 signaling effects and partially explain the IL-6-

mediated cytoprotection. Interestingly, many studies have demonstrated that IL-6 was able to activate PI3K pathway to regulate prostate cancer progression. Recent studies have shown IL-6-induced activation of the PI3K/Akt pathway was involved in the protection against apoptosis, as well as in enhanced proliferation of multiple myeloma cells. Also, in human cervical cancer cells, the PI3K pathway was crucially involved in the IL-6-mediated prevention of apoptosis, which coincided with the upregulation of the anti-apoptotic protein Mcl-1 [39]. In fact, our recent data also showed that the anti-apoptotic effect of IL-6 is mediated in part by Mcl-1 expression through the PI3-K/Akt-dependent pathway [40]. Putting together, we conclude that IL-6 functions as a chemoresistance factor in PC-3 cells, and its expression may be associated with an aggressive phenotype of human prostate cancer. Modulation of IL-6 expression or its related signaling pathway may be a promising strategy of treatment for hormone-refractory and drug-resistant prostate cancer.

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