

Requirement for NF- κ B in Interleukin-4-Induced Androgen Receptor Activation in Prostate Cancer Cells

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BACKGROUND. Accumulating evidence suggest a critical role of activation of androgen receptor (AR) by nonandrogen in the development of androgen independent prostate cancer. Previous study identified that interleukin-4 (IL-4) enhances AR activation in the absence of androgen or in the very low levels of androgen in prostate cancer cells. In this study, the mechanism of IL-4-induced AR activation was investigated.

METHODS & RESULTS. The induction of AR activation by IL-4 can be suppressed by expression of the I κ B α , an inhibitor of NF- κ B. The enhanced expression of AR-mediated prostate-specific antigen (PSA) by IL-4 was blocked by the expression of I κ B α . IL-4 increases NF- κ B transcriptional activity in prostate cancer cells which can be blocked by the addition of IL-4 antibody. IL-4 can also rapidly activate NF- κ B. Furthermore, the IL-4-induced NF- κ B activation and nuclear translocation can be blocked by LY294002, a PI3K/Akt specific inhibitor, suggesting that IL-4-induced NF- κ B activation is mediated by activation of PI3K/Akt pathway.

CONCLUSION. In combination with previous study that IL-4 activates PI3K/Akt pathway, activation of PI3K/Akt > NF- κ B pathways may be responsible for IL-4-induced AR activation in prostate cancer cells. Taken together, these studies suggest that IL-4 > PI3K/Akt > NF- κ B signaling pathways, which activate AR signaling, may play an important role during the progression of androgen independent prostate cancer cells. *Prostate* 64: 160–167, 2005.

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KEY WORDS: prostate cancer; IL-4; NF- κ B; androgen receptor; PSA

INTRODUCTION

Prostate cancer cells depend on androgen on its growth. Androgen regulates the expression of androgen-regulated genes such as prostate-specific antigen (PSA) through the binding of the androgen receptor (AR) to the androgen-responsive elements (AREs) in the promoters of the PSA. When androgen is depleted, prostate cancer cells initially undergo apoptosis and die. However, most patients will relapse to hormone refractory disease due to the growth of androgen recurrent cancer cells. Accumulating evidence demonstrate abnormal AR signaling contributes to androgen-independent growth of prostate cancer. AR can be activated by growth factors and cytokines to display enhanced activity in the presence of low level of androgen or to function even in the absence of androgen [1–4]. Previous studies demonstrated that IL-4 activates AR-

mediated gene expression by activation of the AR in a ligand-independent manner or in the presence of very low levels of androgen [5].

Interleukin-4 (IL-4) is a pleiotropic type I cytokine produced by T cells, mast cells, and basophils in response to receptor-mediated activation events [6].

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IL-4 has biological effects on many immune cells, including B and T lymphocytes, mast cells, and macrophages, and plays a central role in regulating inflammatory and cell-mediated immune responses [7]. In addition to its effect on immune cells, IL-4 has a variety of other functions including effects on hematopoietic tissues, tissue adhesion, and inflammation [7].

IL-4 exerts its function through activation of the IL-4 receptor, designated IL-4R α , by tyrosine phosphorylation [8]. The receptor consists of a 140-kDa IL-4R α chain that binds IL-4 with high affinity (Kd 20–300 pM). IL-4R α activation results in tyrosine phosphorylation of multiple receptor-associated kinases including Janus-family (Jak) tyrosine kinases (Jak1, Jak2, and Jak3) [9–11], insulin receptor substrate (IRS-1/2) proteins [12,13], Shc [14], and signal transducers and activators of transcription (Stat6) [15] for the initiation of signal transduction. Among the molecules that interact with phosphorylated IRS-1/2 molecules are the regulatory subunit of phosphoinositide-3-kinase (PI3K) and the adapter molecule, Grb-2. These interactions lead to the activation of the PI3K and Ras/MAPK signaling pathways, respectively [7].

The PI3K/Akt cell signaling pathway is an important regulator of growth and survival in many cell types including prostate [16,17]. Akt can be activated by various growth factors by activating PI3K and subsequently phosphorylating Akt at Thr³⁰⁸ or Ser⁴⁷³ [18–21]. The PI3K/Akt pathway is negatively regulated by *PTEN*, a tumor suppressor gene that has been found to be deleted in many cancer cells including prostate [22].

Akt can activate NF- κ B pathway via phosphorylation and activation of molecules in the NF- κ B pathway [23,24]. Phosphorylation of Akt induces phosphorylation of IKKs. Activation of IKKs later induces the phosphorylation and degradation of I κ B and liberates NF- κ B from I κ B to initiate nuclear translocation of NF- κ B. Once the NF- κ B complexes translocate to the nucleus, it binds to NF- κ B-specific DNA-binding motif and regulate gene expression [23–25]. The Akt > NF- κ B pathway is known to be a strong cell survival pathway [26–28]. NF- κ B has been shown activated in the androgen-independent prostate cancer cell lines DU145 and PC-3, but NF- κ B has little activity in the androgen-sensitive LNCaP prostate cancer cell line [29]. NF- κ B binding activity is upregulated in the LAPC-4 xenograft model of androgen-independent prostate cancer [30], suggesting a potential role of NF- κ B activation in prostate cancer progression.

In this study, we investigated whether activation of AR signaling by IL-4 is mediated by activation of NF- κ B pathway in prostate cancer cells. We demonstrate that PSA production induced by IL-4 is mediated by activation of NF- κ B through Akt pathways. Our results

suggest that IL-4 > PI3K/Akt > NF- κ B signaling pathways, which activate AR signaling, may play an important role during the progression of androgen independent prostate cancer cells.

METHODS AND MATERIALS

Cell Culture

LNCaP cells were maintained in RPMI1640 supplemented with 10% of FBS, 100 U/ml of penicillin, and 100 μ g/ml of streptomycin at 37°C in 5% CO₂ incubator.

Transfection and Luciferase Assay

The plasmids expressing NF- κ B p65 and luciferase reporter containing a 5 \times NF- κ B consensus binding motifs were described previously [31]. The mutant form of I κ B α was generated by substituting serine residues at 32 and 36 with alanine [32]. Luciferase reporter containing AR responsive PSA promoter was described previously [5]. Twenty-four hours before transfection, 2 \times 10⁵ cells were plated in a 12-well plate. Cells were transfected using Superfect (Qiagen, Valencia, CA) according to the manufacturer's instructions. The total amount of plasmid DNA used was adjusted to 2.5 μ g/well by the addition of empty plasmid. Three hours after transfection, the DNA:liposomes mixture was removed, and cells were treated with phenol red-free medium containing 5% CS-FBS with either 0.1 nM R1881 (Sigma, St. Louis, MO) or in the absence of R1881. Cells were also treated with different concentrations of the recombinant IL-4 (R&D Systems, Minneapolis, MN) and LY294002 (Promega, Madison, WI). Cell extracts were obtained 24 or 48 hr later and luciferase activity was assayed using the Luciferase Assay System (Promega). Protein concentrations in cell extracts were determined by Coomassie Plus protein assay (Pierce, Rockford, IL) and luciferase activities obtained were normalized by protein concentrations of the samples. Transfection efficiency was also monitored by cotransfection with plasmid containing green fluorescent protein and visualized with a fluorescent microscope at 488 nm. All transfection experiments were performed in triplicate and repeated at least three times.

Nuclear Lysate Preparation

Nuclear protein extracts were prepared as described previously with modification [33]. Briefly, for nuclei preparation, cells were harvested, washed with PBS twice, and resuspended in hypotonic buffer (10 mM HEPES, pH 7.9, 10 mM KCl, 0.1 mM EDTA, 0.4% NP40, 0.5 mM PMSF, 0.5 mM DTT, 1 mM NaV, and 1 mM NaF) and incubated on ice for 10 min. Nuclei were precipitated with 10,000 \times g centrifugation at 4°C for 10 min. After washing once with hypotonic buffer, the

nuclei were lysed in the lysis buffer (10 mM HEPES, pH 7.9, 400 mM NaCl, 1 mM EDTA, 1 mM DTT, 0.5 mM PMSF, 1 mM NaV, and 1 mM NaF) and incubated on ice for 30 min. The nuclear lysates were precleared by $10,000 \times g$ centrifugation at 4°C for 15 min. Protein concentration was determined by Coomassie Plus protein assay kit.

Electromobility Shift Assay

Electromobility shift assay (EMSA) was performed as described previously [34]. For determination of the NF- κ B DNA binding activity, nuclear extracts (10 μg) were incubated in a final volume of 20 μl (10 mM HEPES, pH 7.9, 80 mM NaCl, 10 % glycerol, 1 mM DTT, 1 mM EDTA, 100 $\mu\text{g}/\text{ml}$ poly(dIdC)) with radiolabeled double stranded NF- κ B consensus binding motif 5'-AGTTGAGGGGACTTCCAGGC (Promega). For supershift assay, the cell lysis was preincubated with NF- κ B p50 antibody (Cell Signaling Technology, Beverly, MA). 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 obtained in high salt buffer as described [35] 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 either with antibodies of NF- κ B (p65) (Santa Cruz Biotechnology, Santa Cruz, CA), phosphorylated I κ B α , total Akt, or phosphorylated Akt (Thr⁴⁷³) (Cell Signaling Technology). Following secondary antibody incubation, immunoreactive proteins were visualized with an enhanced chemiluminescence detection system (Amersham Pharmacia Biotech, Buckinghamshire, England).

PSA Protein Analysis

PSA protein expression was determined by ELISA with the use of anti-PSA as primary antibody according to the manufacturer's instruction (Beckman Coulter, Fullerton, CA) as described previously [5]. PSA expression was normalized to the concentration of the protein of the samples.

RESULTS

IL-4 Induces NF- κ B Activation in LNCaP Cells

Androgen sensitive LNCaP cells express limited amounts of activated NF- κ B. We first attempted to investigate whether IL-4 activates NF- κ B in LNCaP cells. LNCaP cells were cultured in regular medium and switched into medium containing 2% charcoal-

stripped serum for 24 hr and the cells were exposed to IL-4 (20 ng/ml) for various incubation time. Cells were harvested and the nuclear fraction was separated and used for the gel shift assay using radiolabeled NF- κ B binding motif. Upon IL-4 stimulation, NF- κ B is rapidly activated within 10 min and lasts for more than 2 hr (Fig. 1A). Supershift assay using anti-NF- κ B p50 antibody revealed a band shift, indicating the specificity of the NF- κ B band (Fig. 1A).

When NF- κ B is activated, it is translocated into nucleus. Western blot was then performed to test whether IL-4 stimulates translocation of NF- κ B molecule. Cells were cultured in regular medium, switched into medium containing 2% charcoal stripped medium, and exposed to IL-4 (20 ng/ml) for various time periods ranging from 0 to 120 min. Nuclear and cytosolic fractions were separated and Western blot was performed using p65 antibody. As shown in Figure 1B, NF- κ B protein was detected in the cytosolic fraction but was not detected in the nuclear fraction of LNCaP cells in the absence of IL-4. However, NF- κ B protein can

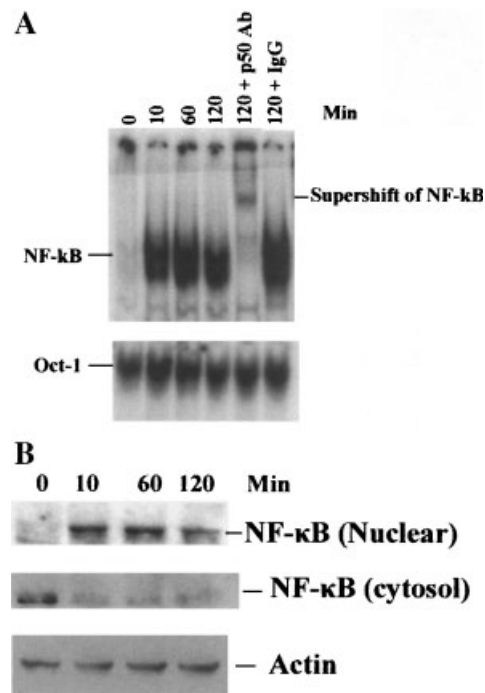


Fig. 1. IL-4 activates NF- κ B in LNCaP cells. LNCaP cells were treated with IL-4 (20 ng/ml) for different time ranging from 0 to 120 min. **A:** Nuclear extracts were prepared and NF- κ B DNA binding activity was determined by EMSA using NF- κ B consensus binding sequences. For supershift, nuclear extracts from IL-4 treatment (120 min) were preincubated with NF- κ B p50 antibody or control IgG antibody and DNA binding was analyzed. Oct-1 was used as a control for demonstrating the nuclear extracts used contain similar activities of a transcription factor. **B:** Nuclear extracts and cytosolic extracts were analyzed by Western blot using NF- κ B p65 antibody. Actin was used as a loading control.

be detected in the nucleus after 10 min upon IL-4 treatment, further suggesting that IL-4 activates NF- κ B in LNCaP cells (Fig. 1B). Collectively, these results demonstrate that IL-4 activates NF- κ B molecule in LNCaP prostate cancer cells.

IL-4 Enhances Expression of Phosphorylated I κ B α Protein

Inactivated NF- κ B proteins form dimers in the cytoplasm that are bound to I κ B proteins. After stimulation, I κ B proteins are phosphorylated and degraded via the proteasome pathway allowing the translocation of NF- κ B complexes to the nucleus. Therefore, increased expression of phosphorylated I κ B proteins is correlated with elevated NF- κ B activation. To investigate if IL-4 increases the levels of expression of phosphorylated I κ B α in prostate cancer cells, LNCaP cells were treated with IL-4 and the expression of phosphorylated I κ B α protein was determined by Western blot using antibody against phosphorylated I κ B α . As expected, IL-4 enhances the expression of phosphorylated I κ B α protein in LNCaP cells (Fig. 2). Cotransfection expression with a mutant I κ B α , an inhibitor of NF- κ B, greatly diminished IL-4-induced phosphorylated I κ B α expression. Treatment with LY294002, the Akt inhibitor, together with the expression of the mutant I κ B α , completely abolished IL-4-induced expression of phosphorylated I κ B α (Fig. 2).

IL-4 Stimulates NF- κ B-Mediated Transcription Activity

To further investigate the effect of IL-4 on NF- κ B function, we examined if IL-4 activate NF- κ B-mediated gene transcription. LNCaP cells were transiently transfected with an NF- κ B responsive luciferase reporter, and treated with different concentrations of IL-4 (from 0 to 20 ng/ml) for 48 hr. IL-4 activate NF- κ B-mediated gene transcription activity in a dose dependent manner (Fig. 3). Addition of antibody specific for IL-4 abolished IL-4-induced NF- κ B transcriptional activation, sug-

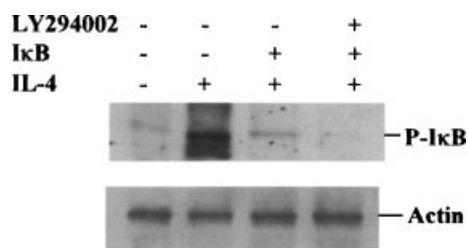


Fig. 2. IL-4 increase phosphorylated I κ B expression. LNCaP cells were transiently cotransfected with either vector control or I κ B plasmid and incubated with IL-4 (20 ng/ml) in the absence of LY294002 or in the presence of LY294002 (20 μ M). Protein extracts (20 μ g) were used for Western blot analysis using phosphorylated I κ B antibody. Actin was used as a loading control.

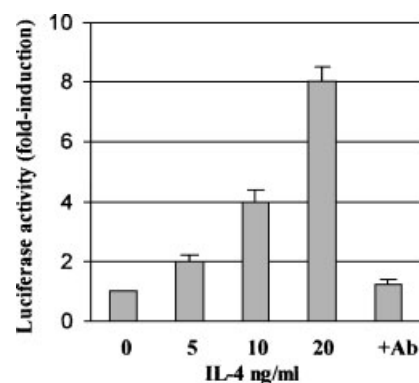


Fig. 3. IL-4 enhances NF- κ B transcription activity. LNCaP cells were transiently transfected with an NF- κ B responsive luciferase reporter, and treated with different doses of IL-4 for 48 hr. For specificity, IL-4 antibody (2 μ g) was included in an IL-4 treatment (20 ng/ml) as indicated as Ab. Luciferase activity was determined. Protein concentration in cell extracts was determined by Coomassie Plus protein assay. Luciferase activities were normalized by protein concentrations. All transfection experiments were performed in triplicate wells and repeated at least three times. Values were expressed as mean \pm SD of triplicate samples.

gesting the specificity of IL-4 on NF- κ B-mediated gene transcriptional activation.

IL-4 Activates NF- κ B via Activation of Akt in Prostate Cancer Cells

We have previously demonstrated that IL-4 activates Akt pathway in LNCaP cells [5]. Since Akt can activate NF- κ B via the IKK complex in response to TNF or PDGF stimulation [23,36], we examined whether IL-4 activation of NF- κ B requires activation of Akt in prostate cancer cells. LNCaP cells were grown in regular medium and switched into medium containing 2% charcoal stripped serum for 24 hr before IL-4 stimulation. Three hours before IL-4 stimulation, cells were either treated with LY294002, inhibitor of PI3K/Akt pathway, or ethanol as a control. The cells were then treated with IL-4 (20 ng/ml) for 1 hr and nuclear fraction was obtained at the end of incubation. As shown in Figure 4A, treatment with PI3K/Akt inhibitor decreased IL-4-induced Akt activation, accompanied by decreased NF- κ B activation. As shown in Figure 4B, IL-4-induced NF- κ B-mediated gene luciferase activity was greatly inhibited by the treatment of 5 μ M of LY294002, and almost completely abolished by addition of 25 μ M of LY294002. These results suggest that NF- κ B activation by IL-4 in LNCaP cells is mediated via activation of the Akt pathway.

Blockage of NF- κ B Activation Inhibits IL-4-Mediated AR Activation

Since IL-4 activates Akt > NF- κ B signaling in LNCaP cells, we examined whether inhibition of these

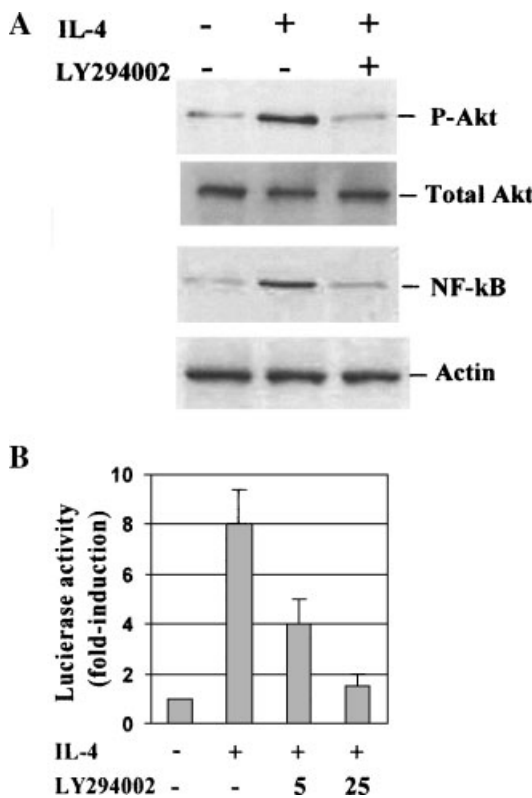


Fig. 4. Inhibition of Akt reduces IL-4-induced NF- κ B activation. **A:** Akt inhibitor inhibits IL-4-induced NF- κ B nuclear expression. LNCaP cells were treated with or without LY294002 and stimulated with IL-4 (20 ng/ml) for 1 hr. Nuclear proteins were analyzed by Western blots using Akt and NF- κ B p65 antibodies, respectively. Actin was used as a loading control. **B:** Akt inhibitor inhibits IL-4-induced NF- κ B activation. LNCaP cells were transfected with NF- κ B responsive luciferase reporter and treated with IL-4 (20 ng/ml) together with different doses of LY294002 (from 0 to 25 μ M) for 24 hr. Protein concentration in cell extracts was determined by Coomassie Plus protein assay. Luciferase activities were normalized by protein concentrations. All transfection experiments were performed in triplicate and repeated at least three times. Values were expressed as mean \pm SD of triplicate samples.

signaling pathways suppresses IL-4-mediated PSA transcription. As shown in Figure 5A, transfection expression of the mutant κ B α almost completely blocked IL-4-induced PSA-luciferase activity. LY294002, a PI3K-Akt specific inhibitor, greatly abolished IL-4-induced PSA-luciferase activity. Addition of PD98059, a MAPK inhibitor, failed to inhibit IL-4-induced PSA-luciferase activity. These results suggest that Akt > NF- κ B signaling pathways mediate IL-4-induced PSA transcription.

We next determined whether blockage of NF- κ B activation inhibit PSA protein production in LNCaP cells. Inhibition of Akt and NF- κ B suppresses PSA protein production in LNCaP cells (Fig. 5B). The IL-4-induced PSA protein expression was inhibited by the

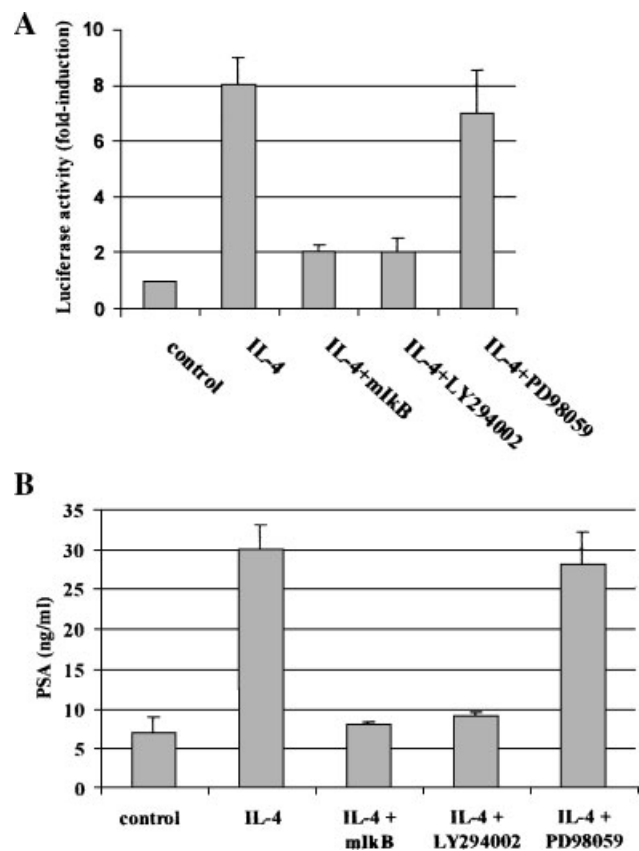


Fig. 5. Blockage of NF- κ B activation inhibits IL-4-mediated AR activation. **A:** Effect of expression of a mutant κ B α (mlkB), LY294002, and PD98059 (20 μ M) on IL-4-mediated PSA promoter activation. LNCaP cells were transfected with PSA promoter reporter, and treated with different inhibitors plus IL-4 (20 ng/ml) in the presence of 0.1 nM RI881. Protein concentration in cell extracts was determined by Coomassie Plus protein assay. Luciferase activities were normalized by protein concentrations. All transfection experiments were performed in triplicate and repeated at least three times. Values were expressed as mean \pm SD of triplicate samples. **B:** Effect of expression of a mutant κ B (mlkB), LY294002, and PD98059 (20 μ M) on IL-4-mediated PSA protein expression. LNCaP cells were treated with different inhibitors plus IL-4 (20 ng/ml) for 24 hr in the presence of 0.1 nM RI881. The level of PSA expression was determined by PSA ELISA kit.

mutant κ B α , the NF- κ B inhibitor, and by LY294002, a PI3K-Akt inhibitor, but not by PD98059, a MAPK inhibitor. This result further demonstrates that activation of AR by IL-4 is mediated at least in part by Akt > NF- κ B signaling pathway.

NF- κ B Enhances AR-Mediated Gene Transcription Activation

In order to directly assess the role of activation of NF- κ B signaling by IL-4 in prostate cancer cells, we investigated whether activation of NF- κ B signaling is sufficient to activate AR signaling in prostate cancer cells. LNCaP cells were transiently cotransfected with a

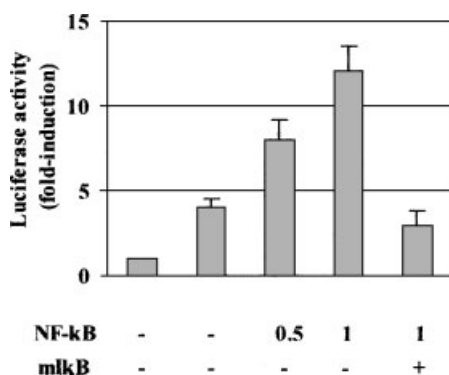


Fig. 6. NF- κ B enhances PSA promoter transcriptional activation. LNCaP cells were cotransfected with the luciferase reporter containing PSA promoter and different amount of NF- κ B plasmid (p65) in the absence of androgen. Mutant I κ B α plasmid (0.2 μ g) was also cotransfected to block NF- κ B activity. The total amount of plasmid DNA was adjusted to 2.5 μ g/well by the addition of empty plasmid. Cell extracts were isolated and luciferase activity was determined. The luciferase activity was normalized by protein concentrations of the samples. All transfection experiments were performed in triplicate and repeated for three times.

constitutively activated NF- κ B or vector control, and luciferase reporter containing androgen-responsive promoter of PSA (PSA-Luc). Total DNA content was kept constant in all experiments. Cell lysates were collected and luciferase activity measured. As shown in Figure 6, NF- κ B enhances PSA promoter activity in a dose dependent manner, which can be blocked by coexpression of the mutant I κ B α , the inhibitor of NF- κ B.

DISCUSSION

IL-4 is significantly elevated in serum of patients with hormone refractory prostate cancer compared with values in hormone-sensitive prostate cancer, and the levels of IL-4 directly correlated with elevated PSA [37]. We previously demonstrated that IL-4 enhances PSA expression by activation of AR signaling in LNCaP prostate cancer cells [5]. In this study, we investigated the mechanism of IL-4-induced AR activation and PSA expression in LNCaP cells and demonstrated that NF- κ B activation by IL-4 plays critical role in mediating IL-4-induced AR activation and enhanced PSA expression in prostate cancer cells.

IL-4 can activate many cellular signaling pathways. Zamorano et al. suggested activation of NF- κ B upon IL-4 treatment in T-cells [38]. We determined whether IL-4 can activate NF- κ B in prostate cells. IL-4 indeed activated NF- κ B in LNCaP cells in androgen depleted condition. IL-4 stimulated nuclear translocation of NF- κ B molecule and NF- κ B binding activity to its DNA binding motif. We further demonstrate that IL-4-induced NF- κ B activation is through activation of

PI3K/Akt pathway. Inhibition of Akt activation by LY294002 blocked IL-4-induced NF- κ B activation in LNCaP cells. Furthermore, blockage of Akt or NF- κ B activation inhibits IL-4-induced AR activation and PSA expression in LNCaP cells, suggesting that IL-4-induced AR activation is through activation of Akt > NF- κ B signaling pathway.

NF- κ B is implicated in the control of cell proliferation, apoptosis, and transformation in normal and malignant cells. Amplification, overexpression, or rearrangement of genes coding for Rel/NF- κ B factors have been found in cancer cells. Constitutive activation of NF- κ B is also a common characteristic of many tumors. NF- κ B has been shown activated in the androgen-independent prostate cancer cell lines Du145 and PC-3, but has little activity in the androgen-sensitive LNCaP cells [29,39]. NF- κ B binding activity is upregulated in the LAPC-4 xenograft model of androgen-independent prostate cancer [30], suggesting a potential role of NF- κ B activation in prostate cancer progression. Reports on the role of NF- κ B in AR signaling are conflict. Some showed NF- κ B negatively regulates AR function [40], and others showed NF- κ B enhances AR-mediated PSA expression [30]. Our results demonstrate that NF- κ B (p65) enhances AR-mediated PSA transcription activation and promotes PSA production in LNCaP cells in the absence of androgen. Collectively, these results suggest that NF- κ B increases AR-mediated gene activation in the absence of androgen, while decreases androgen-induced AR activation in the presence of androgen.

The AR signaling is commonly active in hormone refractory prostate cancer despite the presence of very low levels of androgen. Recent studies suggested that AR activation plays a central role for hormone refractory progression of prostate cancer [41,42]. The link of IL-4 activation of AR signaling through activation of PI3K/Akt and NF- κ B signaling in prostate cancer cells may provide an additional insight of prostate cancer progression to androgen independent state. In view of that the level of IL-4 is significantly elevated in androgen independent prostate cancer, and that Akt and NF- κ B signaling is critical in protecting cells from apoptotic cell death, the IL-4-induced activation of Akt and NF- κ B signaling may provide a survival signal for prostate cancer cells under androgen-deprived condition. Therapeutic approaches targeting IL-4 > Akt > NF- κ B signaling pathways may provide an opportunity for drug development for advanced prostate cancer.

REFERENCES

1. Connolly JM, Rose DP. Regulation of DU145 human prostate cancer cell proliferation by insulin-like growth factors and its interaction with the epidermal growth factor autocrine loop. *Prostate* 1994;24:167-175.

2. Cronauer MV, Hittmair A, Eder IE, Hobisch A, Culig Z, Ramoner R, Zhang J, Bartsch G, Reissigl A, Radmayr C, Thurnher M, Klocker H. Basic fibroblast growth factor levels in cancer cells and in sera of patients suffering from proliferative disorders of the prostate. *Prostate* 1997;31:223–233.
3. Culig Z, Hobisch A, Cronauer MV, Radmayr C, Trapman J, Hittmair A, Bartsch G, Klocker H. Androgen receptor activation in prostatic tumor cell lines by insulin-like growth factor-I, keratinocyte growth factor, and epidermal growth factor. *Cancer Res* 1994;54:5474–5478.
4. Culig Z, Hobisch A, Hittmair A, Cronauer MV, Radmayr C, Zhang J, Bartsch G, Klocker H. Synergistic activation of androgen receptor by androgen and luteinizing hormone-releasing hormone in prostatic carcinoma cells. *Prostate* 1997;32:106–114.
5. Lee SO, Lou W, Hou M, Onate SA, Gao AC. Interleukin-4 enhances prostate-specific antigen expression by activation of the androgen receptor and Akt pathway. *Oncogene* 2003;22:6037–6044.
6. Seder RA, Paul WE. Acquisition of lymphokine-producing phenotype by CD4+ T cells. *Annu Rev Immunol* 1994;12:635–673.
7. Nelms K, Keegan AD, Zamorano J, Ryan JJ, Paul WE. The IL-4 receptor: Signaling mechanisms and biologic functions. *Annu Rev Immunol* 1999;17:701–738.
8. Smerz-Bertling C, Duschl A. Both interleukin 4 and interleukin 13 induce tyrosine phosphorylation of the 140-kDa subunit of the interleukin 4 receptor. *J Biol Chem* 1995;270:966–970.
9. Witthuhn BA, Silvennoinen O, Miura O, Lai KS, Cwlc C, Liu ET, Ihle JN. Involvement of the Jak-3 Janus kinase in signalling by interleukins 2 and 4 in lymphoid and myeloid cells. *Nature* 1994;370:153–157.
10. Johnston JA, Kawamura M, Kirken RA, Chen YQ, Blake TB, Shibuya K, Ortaldo JR, McVicar DW, O'Shea JJ. Phosphorylation and activation of the Jak-3 Janus kinase in response to interleukin-2. *Nature* 1994;370:151–153.
11. Murata T, Noguchi PD, Puri RK. IL-13 induces phosphorylation and activation of JAK2 Janus kinase in human colon carcinoma cell lines: Similarities between IL-4 and IL-13 signaling. *J Immunol* 1996;156:2972–2978.
12. Wang LM, Myers MG, Jr., Sun XJ, Aaronson SA, White M, Pierce JH. IRS-1: Essential for insulin- and IL-4-stimulated mitogenesis in hematopoietic cells. *Science* 1993;261:1591–1594.
13. Sun XJ, Wang LM, Zhang Y, Yenush L, Myers MG, Jr., Glasheen E, Lane WS, Pierce JH, White MF. Role of IRS-2 in insulin and cytokine signalling. *Nature* 1995;377:173–177.
14. Zhou MM, Huang B, Olejniczak ET, Meadows RP, Shuker SB, Miyazaki M, Trub T, Shoelson SE, Fesik SW. Structural basis for IL-4 receptor phosphopeptide recognition by the IRS-1 PTB domain. *Nat Struct Biol* 1996;3:388–393.
15. Shimoda K, van Deursen J, Sangster MY, Sarawar SR, Carson RT, Tripp RA, Chu C, Quelle FW, Nosaka T, Vignali DA, Doherty PC, Grosveld G, Paul WE, Ihle JN. Lack of IL-4-induced Th2 response and IgE class switching in mice with disrupted *Stat6* gene. *Nature* 1996;380:630–633.
16. Bellacosa A, Testa JR, Staal SP, Tsichlis PN. A retroviral oncogene, akt, encoding a serine-threonine kinase containing an SH2-like region. *Science* 1991;254:274–277.
17. Nakatani K, Sakaue H, Thompson DA, Weigel RJ, Roth RA. Identification of a human Akt3 (protein kinase B gamma) which contains the regulatory serine phosphorylation site. *Biochem Biophys Res Commun* 1999;257:906–910.
18. Franke TF, Yang SI, Chan TO, Datta K, Kazlauskas A, Morrison DK, Kaplan DR, Tsichlis PN. The protein kinase encoded by the Akt proto-oncogene is a target of the PDGF-activated phosphatidylinositol 3-kinase. *Cell* 1995;81:727–736.
19. Okano J, Gaslightwala I, Birnbaum MJ, Rustgi AK, Nakagawa H. Akt/protein kinase B isoforms are differentially regulated by epidermal growth factor stimulation. *J Biol Chem* 2000;275:30934–30942.
20. Burgering BM, Coffey PJ. Protein kinase B (c-Akt) in phosphatidylinositol-3-OH kinase signal transduction. *Nature* 1995;376:599–602.
21. Alessi DR, Andjelkovic M, Caudwell B, Cron P, Morrice N, Cohen P, Hemmings BA. Mechanism of activation of protein kinase B by insulin and IGF-1. *Embo J* 1996;15:6541–6551.
22. Li J, Yen C, Liaw D, Podsypanina K, Bose S, Wang SI, Puc J, Miliaresis C, Rodgers L, McCombie R, Bigner SH, Giovanella BC, Ittmann M, Tycko B, Hibshoosh H, Wigler MH, Parsons R. PTEN, a putative protein tyrosine phosphatase gene mutated in human brain, breast, and prostate cancer. *Science* 1997;275:1943–1947.
23. Ozes ON, Mayo LD, Gustin JA, Pfeffer SR, Pfeffer LM, Donner DB. NF-kappaB activation by tumour necrosis factor requires the Akt serine-threonine kinase. *Nature* 1999;401:82–85.
24. Romashkova JA, Makarov SS. NF-kappaB is a target of AKT in anti-apoptotic PDGF signalling. *Nature* 1999;401:86–90.
25. Kane LP, Mollenauer MN, Xu Z, Turck CW, Weiss A. Akt-dependent phosphorylation specifically regulates Cot induction of NF-kappa B-dependent transcription. *Mol Cell Biol* 2002;22:5962–5974.
26. Van Antwerp DJ, Martin SJ, Kafri T, Green DR, Verma IM. Suppression of TNF-alpha-induced apoptosis by NF-kappaB. *Science* 1996;274:787–789.
27. Wang CY, Mayo MW, Korneluk RG, Goeddel DV, Baldwin AS, Jr. NF-kappaB antiapoptosis: Induction of TRAF1 and TRAF2 and c-IAP1 and c-IAP2 to suppress caspase-8 activation. *Science* 1998;281:1680–1683.
28. Wang CY, Mayo MW, Baldwin AS, Jr. TNF- and cancer therapy-induced apoptosis: Potentiation by inhibition of NF-kappaB. *Science* 1996;274:784–787.
29. Zerbini LF, Wang Y, Cho JY, Libermann TA. Constitutive activation of nuclear factor kappaB p50/p65 and Fra-1 and JunD is essential for deregulated interleukin 6 expression in prostate cancer. *Cancer Res* 2003;63:2206–2215.
30. Chen CD, Sawyers CL. NF-kappa B activates prostate-specific antigen expression and is upregulated in androgen-independent prostate cancer. *Mol Cell Biol* 2002;22:2862–2870.
31. Wang D, You Y, Case SM, McAllister-Lucas LM, Wang L, DiStefano PS, Nunez G, Bertin J, Lin X. A requirement for CARMA1 in TCR-induced NF-kappa B activation. *Nat Immunol* 2002;3:830–835.
32. Lin X, O'Mahony A, Mu Y, Gelezianas R, Greene WC. Protein kinase C-theta participates in NF-kappaB activation induced by CD3-CD28 costimulation through selective activation of Ikap-beta kinase beta. *Mol Cell Biol* 2000;20:2933–2940.
33. Lin DL, Whitney MC, Yao Z, Keller ET. Interleukin-6 induces androgen responsiveness in prostate cancer cells through up-regulation of androgen receptor expression. *Clin Cancer Res* 2001;7:1773–1781.
34. Ni Z, Lou W, Leman ES, Gao AC. Inhibition of constitutively activated Stat3 signaling pathway suppresses growth of prostate cancer cells. *Cancer Res* 2000;60:1225–1228.
35. Lee SO, Lou W, Hou M, De Miguel F, Gerber L, Gao AC. Interleukin-6 promotes androgen-independent growth in

- LNcaP human prostate cancer cells. *Clin Cancer Res* 2003;9:370–376.
36. Palombella VJ, Rando OJ, Goldberg AL, Maniatis T. The ubiquitin-proteasome pathway is required for processing the NF-kappa B1 precursor protein and the activation of NF-kappa B. *Cell* 1994;78:773–785.
 37. Wise GJ, Marella VK, Talluri G, Shirazian D. Cytokine variations in patients with hormone treated prostate cancer. *J Urol* 2000;164:722–725.
 38. Zamorano J, Mora AL, Boothby M, Keegan AD. NF-kappa B activation plays an important role in the IL-4-induced protection from apoptosis. *Int Immunol* 2001;13:1479–1487.
 39. Suh J, Payvandi F, Edelstein LC, Amenta PS, Zong WX, Gelinas C, Rabson AB. Mechanisms of constitutive NF-kappaB activation in human prostate cancer cells. *Prostate* 2002;52:183–200.
 40. Palvimo JJ, Reinikainen P, Ikonen T, Kallio PJ, Moilanen A, Janne OA. Mutual transcriptional interference between RelA and androgen receptor. *J Biol Chem* 1996;271:24151–24156.
 41. Chen CD, Welsbie DS, Tran C, Baek SH, Chen R, Vessella R, Rosenfeld MG, Sawyers CL. Molecular determinants of resistance to antiandrogen therapy. *Nat Med* 2004;10:33–39.
 42. Edwards J, Krishna NS, Grigor KM, Bartlett JM. Androgen receptor gene amplification and protein expression in hormone refractory prostate cancer. *Br J Cancer* 2003;89:552–556.