Short Communication

Increased Expression of the Interleukin-11 Receptor and Evidence of STAT3 Activation in Prostate Carcinoma

Cara L. Campbell,* Zhong Jiang,[†]
Diane M. F. Savarese,[‡] and Todd M. Savarese*

From the Cytokine/Cytokine Receptor Laboratory,* LINK Laboratories, University of Massachusetts Cancer Center, University of Massachusetts Medical School, Worcester; and the Department of Pathology,[†] and the Division of Hematology/Oncology,[‡] University of Massachusetts/Memorial Health Care, Worcester, Massachusetts

Previous investigations have shown that interleukin-6, a member of the JAK-STAT activating family of cytokines, plays an important role in prostate carcinoma. Here we demonstrate the co-expression of another member of this cytokine family, interleukin-11 (IL-11), and components of its receptor (interleukin-11 receptor; IL-11R), ie, IL-11R α (involved in ligand recognition), and gp130 (involved in signal transduction) in cultured normal and malignant prostate-derived epithelial cell lines. In the DU-145 prostate carcinoma cell line, rhIL-11 stimulates a transient and dose-dependent increase in the tyrosine 705-phosphorylated, active form of STAT3 (STAT3 P-Tyr705), involved in the downstream signaling of IL-11R and other members of the gp130-dependent receptors. The ability of IL-11 to activate STAT3 in prostate-derived cells may be mechanistically important, given recent data suggesting that constitutively activated STAT3 may be associated with the malignant phenotype. In 51 human primary tissues derived from normal prostate, benign prostatic hyperplasia, and prostate carcinomas, IL-11R α and gp130 were commonly expressed, with a statistically significant elevation in the expression of IL-11R α in prostate carcinoma. Also, the tyrosine-phosphorylated, activated form of STAT3 was observed more prominently in the nuclei of cells residing in malignant glands compared to those in nonmalignant samples. Thus, the IL-11 receptor system is up-regulated in prostate carcinoma, and may be one part of a cytokine network that maintains STAT3 in its activated form in these tissues. (*Am J Pathol* 2001, 158:25–32)

Prostate carcinoma is one of the leading causes of cancer deaths among men in the United States.¹ As part of an effort to better understand the biology of normal prostate tissue and its neoplastic counterpart, we have been investigating the presence of cytokine and growth factor networks in these tissues, because these cell signaling systems play a key role in the cellular events that are important in malignancy, such as growth, cell survival, and motility. Here we report that the receptor for interleukin-11 (IL-11) is present in prostate-derived tissue, and is expressed to a greater degree in prostate carcinoma compared to nonmalignant prostate tissue.

The IL-11 receptor (IL-11R) mediates the action of IL-11, a 19.1-kd pleiotropic cytokine that was initially cloned from a bone-marrow stromal cell line.² Whereas the hematopoietic effects of IL-11, which include stimulation of megakaryocyte maturation and platelet production,³ and growth stimulation of CD34⁺ hematopoietic progenitor cells,⁴ have been well studied, this cytokine has also been shown to mediate inhibition of adipogenesis,⁴ stimulation of osteoclasts,⁵ and cytoprotection of gut mucosa.^{6–9} The IL-11 receptor is a member of a family of cytokine receptors, sometimes referred to as the gp130-dependent family of receptors, which includes the receptors for IL-6, leukemia inhibitory factor, ciliary neurotrophic factor, oncostatin M, and cardiotrophin.¹⁰ The α subunit of the IL-11R, IL-11R α , is required for high affinity binding of the ligand; on ligand binding, gp130, the subunit responsible for signal transduction, is recruited to the receptor complex.¹¹ It is not known whether IL-11R-associated gp130 undergoes homodimerization, as it does in the case of the IL-6 receptor, or if an additional, as yet unidentified, subunit is involved.¹² Among the signaling systems activated by IL-11R and other members of this

Supported by an American Cancer Society institutional grant, the University of Massachusetts Cancer Center, and the Department of Pathology, University of Massachusetts/Memorial Health Care.

Accepted for publication September 25, 2000.

Address reprint requests to Todd M. Savarese, Ph.D., UMass Cancer Center, Rm HB-774, University of Massachusetts Medical School, 55 Lake Ave. North, Worcester, MA 01655. E-mail: todd.savarese@umassmed.edu.

receptor family is the JAK-STAT pathway. On ligand binding, these receptors activate members of the JAK kinase family; these kinases phosphorylate tyrosine residues in the cytoplasmic domains of the gp130 subunit, which in turn serve as docking sites for members of a family of latent transcription factors, the so-called signal transducers and activators of transcription proteins, ie, STAT proteins.^{13,14} These recruited STAT proteins undergo tyrosine phosphorylation, which permits their dimerization and translocation to the nucleus where they alter gene expression.¹⁵ Indeed, IL-11 has been shown to activate the JAK1 and JAK2 receptor-associated kinases,^{16,17} triggering the activation of STAT1 and STAT3.^{12,18} The latter action may be especially important, as constitutive or aberrant activation of STAT3 has recently been associated with the malignant phenotype. Evidence for this includes: 1) constitutively activated STAT3 has been reported in a variety of carcinomas and hematological malignancies,^{19–24} 2) cell transformation by *src* seems to be associated with STAT3 activation,^{25,26} 3) a mutant form of STAT3 that spontaneously dimerizes and self-activates can act as an oncogene,²⁷ and 4) transfection of cell lines with dominant-negative forms of STAT3 results in growth suppression and/or induction of apoptosis.^{23,24,28} Other signaling systems that may be activated by the IL-11R include MAP kinase, the ribosomal S6 protein kinase, pp90^{rsk},²⁹ *src*-family tyrosine kinases, eg, p60^{src} and p62^{yes}, and phosphatidylinositol-3 kinase.^{30,31}

Two developments prompted this study. First, there is evidence that one member of the cytokine family that activates the JAK-STAT pathway, IL-6, may be important in prostate biology. IL-6 is present in seminal fluid and elevated plasma levels of this cytokine have been associated with increased morbidity in prostate cancer patients.³² In addition, IL-6 acts as an autocrine growth factor in several prostate cancer cell lines, and this growth stimulation is accompanied by activation of STAT3.³³ These data suggest that other members of this cytokine family might also be important in both normal and malignant prostate epithelial cells. Second, using a reverse transcriptase-polymerase chain reaction (RT-PCR)-based screen for the expression of a number of prototypical cytokines and their receptors, we have discovered that one of the potential autocrine loops expressed by a normal human prostate epithelial cell line involves IL-11 and its receptor. As a result, we investigated the expression of components of the IL-11 system in prostate-derived cells and tissues.

Materials and Methods

Cell Culture

Normal human prostate epithelial cells were obtained from Clonetics (San Diego, CA); these cells were cultured for 2 to 4 passages in a defined medium as previously described.³⁴ Culturing of the DU-145, PC-3, and LNCaP prostate carcinoma cell lines (all obtained from the ATCC, Rockville, MD) were performed as outlined previ-

ously.³⁵ Recombinant human IL-11 (rhIL-11) was obtained from R&D Systems (Minneapolis, MN).

Detection of IL-11 and IL-11R α mRNA by RT-PCR

The basic RT-PCR methodology used here has been described.³⁴ For the detection of IL-11 mRNA, the following primers were used: sense: 5'-ACT GCT GCT GCT GAA GAC TCG GCT GTG A-3', antisense: 5'-ATG GGG AAG AGC CAG GGC AGA AGT CTG T-3'; for IL-11R α , sense: 5'-GCC AAG CAG CCG ACT ATG AGA A-3', antisense: 5'-AGT AGC CGA GGG TGT GGT TGG A-3'. Amplification was performed in a Stratagene Robocycler Gradient 40 thermal cycler (Stratagene, La Jolla, CA) under the following conditions: denaturation, 94°C, 45 seconds; annealing, 63°C for IL-11, 64°C for IL-11R α , 45 seconds; extension 72°C, 2 minutes; 35 cycles followed by a 10-minute polishing step at 72°C. PCR products (expected sizes: 322 bp for IL-11, 712 bp for IL-11R α) were resolved by electrophoresis using 1.5% agarose gels containing ethidium bromide. To confirm the identity of the PCR products, a Southern blotting technique was used. Gels were denatured in 0.2 N NaOH, 0.6 mol/L NaCl for 30 minutes, then washed in 25 mmol/L sodium phosphate buffer, pH 6.5; DNA fragments were transferred onto a GeneScreen nylon membrane (New England Nuclear-Dupont, Boston, MA) in the latter buffer overnight. The following antisense oligonucleotides to internal sequences within the expected PCR products, IL-11, 5'-GAT CTG GCT TTG GAA GGA CGG TGG TGG CT-3', and IL-11R α , 5'-GGA CGG TAC TGC AAA CGG AAC TTG AGC A-3', were ³²P-end-labeled with T4 polynucleotide kinase and used to probe the membrane in a hybridization buffer consisting of 2× standard saline citrate (SSC), 50% deionized formamide, 10% dextran sulfate, 0.02% bovine serum albumin, 0.02% polyvinylpyrrolidone, 0.02% Ficoll, 1% sodium dodecyl sulfate (SDS), and 500 mg/ml tRNA overnight at 42°C on an orbital shaker. The membrane was then washed in 2× SSC, 1% SDS at 55°C (20 minutes), two washes (15 minutes) in 0.1× SSC, 0.1% SDS, 55°C, and one 5-minute wash in 0.1% SSC at room temperature, then sealed and exposed to Kodak BioMax MS film (Eastman-Kodak, Rochester, NY) for 2 hours.

Secretion of IL-11 by Cell Lines as Determined by Enzyme-Linked Immunosorbent Assay

Normal and malignant cell lines (see above) were plated in their respective growth media at 1×10^5 cells/well in six-well dishes and incubated in a humidified 37°C incubator in a 95% air-5% CO₂ atmosphere for 96 hours. Culture media incubated without cells served as controls. Conditioned media was then harvested and stored at -70°C, and cells were trypsinized and counted using a hemocytometer. The conditioned media was thawed and centrifuged briefly before assay for soluble IL-11 protein

using a commercially available enzyme-linked immunosorbent assay kit (R&D Systems).

Immunoblotting of STAT3 Phosphorylated at Tyr705

DU-145 prostate carcinoma cells (0.5×10^6) were plated in minimal essential medium containing 0.5% fetal bovine serum and incubated at 37°C for 48 hours to reduce basal levels of activated STAT3. Cells were then incubated in serum-free minimal essential medium for 4 hours, then treated with serum-free medium containing various concentrations of rhIL-11 for periods of up to 60 minutes. Cells were lysed in 62.5 mmol/L Tris-HCl, pH 6.8, 2% SDS, 10% glycerol, 50 mmol/L dithiothreitol, 0.1% bromophenol blue, heated to 95°C for 5 minutes, placed on ice, and loaded onto 10 to 15% gradient SDS-polyacrylamide gel electrophoresis gels (Amersham Pharmacia, Piscataway, NJ). After electrophoresis in a PhastSystem apparatus (Amersham Pharmacia), gels were electrotransferred onto nitrocellulose membranes; these membranes were analyzed for the presence of Tyr705-phosphorylated STAT3 using an antibody from New England Biolabs (Beverly, MA), according to the manufacturer's procedures.

Immunohistochemical Analysis of Prostatic Tissues and Cell Lines

Formalin-fixed, paraffin sections (5 μ m) of normal prostate, benign prostatic hyperplasia, and prostate carcinoma were obtained either from the Cooperative Human Tissue Network (Columbus, OH) or the Department of Pathology at the University of Massachusetts/Memorial Health Care System (Worcester, MA) under a human subjects protocol approved by the University of Massachusetts Medical School human subjects institutional review board. These sections were deparaffinized by heating the slides to 60°C for 15 minutes and then subjecting them to two 5-minute changes in 100% xylene; the sections were then rehydrated by serial incubations in 100%, 90%, and 80% ethanol, followed by phosphate-buffered saline (PBS). The sections were then subjected to an antigen retrieval procedure: slides were placed in 10 mmol/L sodium citrate, pH 6.0, autoclaved at 121°C for 10 minutes, then placed in PBS for 30 minutes. In the case of cell lines, cultured cells were harvested by trypsinization and centrifuged onto poly-L-lysine-treated glass slides, or the cells were plated in their appropriate growth media on glass slides and grown for 24 hours; the cells were then fixed for 5 minutes in methanol at -20°C. After this, specimens were treated with methanol containing 0.3% H₂O₂ for 5 minutes to inactivate endogenous peroxidase activity. The samples were washed with PBS and nonspecific binding sites blocked by incubating with 5% goat serum in PBS for 90 minutes in a humidified chamber at room temperature. Samples were then incubated for 60 minutes at room temperature with rabbit anti-human IgG polyclonal antibodies to IL-11R α or

gp130 (Santa Cruz Biotechnology, Santa Cruz, CA) or rabbit polyclonal IgG controls (Santa Cruz Biotechnology) at concentrations of 3.5 μ g/ml (IL-11R α) or 1.0 μ g/ml (gp130) in PBS containing 5% goat serum. Specimens were then rinsed in wash buffer (PBS containing 0.5% bovine serum albumin, 0.1% Tween-20) and incubated for 30 minutes with biotinylated goat anti-rabbit IgG (rabbit ABC staining kit, Santa Cruz Biotechnology) diluted according to the manufacturer's protocol. Next, a solution of avidin-conjugated horseradish peroxidase (ABC staining kit) was applied for 30 minutes, according to the manufacturer's recommendations. Slides were then rinsed twice in wash buffer and treated at room temperature with a H₂O₂/3-amino-9-ethylcarbazole substrate solution prepared as recommended by the manufacturer (Sigma Chemical Co., St. Louis, MO); color development time was in the range of 4 to 8 minutes. To confirm the specificity of the anti-IL-11R α staining, the anti-IL-11R α antibody was preincubated for 2 hours at room temperature with a fivefold excess by weight of the specific IL-11R α peptide that this antibody was raised against (Santa Cruz Biotechnology) before using this antibody in the above-described immunostaining method. This preabsorption procedure was found to block immunostaining in prostate tissues, indicating that the staining observed was specific for IL-11R α . Similarly, preincubation of the anti-gp130 antibody with a 10-fold excess of the specific gp130 peptide that this antibody was raised against (Santa Cruz Biotechnology) also blocked tissue staining. None of the tissues treated with control, nonspecific antibodies demonstrated staining.

A subset of paraffin sections was also screened for the presence of STAT3 phosphorylated at Tyr705 (STAT3 P-Tyr705), the activated form of STAT3 that is generated downstream of IL-11R/gp130 activation. Sections were processed and stained using a 1:100 dilution of a rabbit polyclonal IgG anti-human STAT3 P-Tyr705 antibody (Cell Signaling Technology, Beverly, MA), or rabbit polyclonal IgG controls using the same immunohistochemical methodology described above, except that the incubation with the primary antibody was performed for 24 hours at 4°C and the secondary antibody incubation time was increased to 1 hour. No staining is observed if nonspecific rabbit polyclonal IgG is used. To further determine the specificity of the staining, the anti-STAT3 P-Tyr705 antibody was preabsorbed for 2 hours at room temperature with a 10-fold excess by weight of the phosphorylated peptide which the antibody was raised against (SAAPY*LKTK, where Y* is a phosphotyrosyl residue; a gift from Dr. Nicole Stark, Cell Signaling Technology). This absorption procedure completely eliminated the nuclear staining of the prostate tissue samples.

Results

Expression of IL-11 and Its Receptor in Normal and Malignant Cell Lines

As part of our laboratory's effort to uncover novel autocrine and paracrine axes that may play important func-

Figure 1. Evidence for the expression of IL-11 and IL-11R α in normal and malignant prostate epithelial cell lines. **A:** Expression of the mRNA for IL-11 (left) and IL-11R α (right) in the normal prostate epithelial cell line, PrEC (lane 2) and the prostate carcinoma cell lines DU-145, PC-3, and LNCaP (lanes 3–5, respectively) as determined by RT-PCR. Experimental procedures are described in Materials and Methods. Identity of the PCR bands were confirmed by Southern blotting using antisense oligonucleotide probes specific to internal sequences within the expected PCR products (see Material and Methods). **B:** Secretion of IL-11 by normal and malignant prostate epithelial cell lines as detected by enzyme-linked immunosorbent assay. Experimental procedures are described in Materials and Methods. In the case of each cell line, “control” refers to the respective culture medium incubated in the absence of cells (in the case of PrEC, PrEC growth medium,³⁴ DU-145, minimal essential medium with 10% fetal bovine serum; LNCaP, RPMI 1640 with 10% fetal bovine serum). The IL-11 levels in all control samples were less than the detection limits of the assay (<8 pg/ml), as indicated by the asterisk. Values represent the mean $\pm$ SD for three independent determinations. **C:** Immunohistochemical staining of PrECs (top left) and LNCaP cells (bottom left) for IL-11R α . Experimental methodology is described in Material and Methods. **Right:** The immunostaining was performed on these cell types using antibodies to IL-11R α that had been preincubated with a fivefold excess by weight of a IL-11R α -specific peptide to which the antibody had been raised (blocking peptide). Original magnification, $\times$ 600. **D:** Activation of phosphorylation of STAT3 at Tyr705 by rhIL-11 in DU-145 prostate carcinoma cells, as determined by immunoblotting. **Top:** Time course in minutes (“”) of STAT3 phosphorylation in the absence (left) or presence (right) of 250 ng/ml rhIL-11. Pos Cont, positive control for STAT3 P-Tyr705, SK-N-MC cells treated with ciliary neurotrophic factor. **Bottom:** Dose response relationship. Cell treatments and immunoblotting methodology are described in Materials and Methods.

tional roles in the prostate, we examined a commercially obtained normal prostate epithelial cell line (referred to as PrECs) for the expression of a variety of cytokines, growth factors, and their cognate receptors using an RT-PCR-based screen. Among the factors tested were interleukins 1 (α and β) through 15, and their receptors, excepting the receptors for IL-12, 13, and 14. One of the salient findings of this study was the co-expression of IL-11 and its receptor in the PrECs (Figure 1A, lane 2). To determine whether co-expression of IL-11 and IL-11R α occurred in malignant prostate epithelial cells as well, we also tested several frequently studied prostate carcinoma cell lines, DU-145, PC-3, and LNCaP cells, for the presence of these mRNAs using RT-PCR. As shown in Figure 1A, each of these cell lines also expressed both IL-11 and IL-11R α . The identities of these mRNAs were confirmed by Southern blotting using internal probes specific to the respective sequences. We then determined if these mRNAs were expressed as proteins in these cells. Cell lines were plated in their respective growth media and incubated for 96 hours; the conditioned media was then harvested and assayed for human IL-11 using an en-

zyme-linked immunosorbent assay. Each of the three cell lines tested (PC-3 cells were not studied) secreted IL-11 (Figure 1B); DU-145 cells expressed this cytokine at the highest level (97.4 ± 1.6 pg/10⁵ cells), followed by PrECs (57.4 ± 5.1 pg/10⁵ cells), then LNCaP cells (33.1 ± 13.2 pg/10⁵ cells). Control media, ie, media that was incubated in the absence of cells, contained less than detectable levels of human IL-11 (<8 pg/ml). By immunohistochemistry, the specific expression of IL-11R α was demonstrated in all four of these cell lines (for examples, see Figure 1C). All these cell lines stained positively for gp130, the other IL-11 receptor subunit (data not shown). That this IL-11 receptor is functional was demonstrated in studies with serum-starved DU-145 cells. rhIL-11 was able to mediate a transient activation of the phosphorylation of STAT3 on Tyr705 (STAT3 P-Tyr705) in these cells, with a peak at 10 minutes and a return to basal levels by 60 minutes; this activation was also dose-dependent (Figure 1D). Thus co-expression of IL-11 and its receptor, ie, a potential autocrine loop, is commonly observed in human cultured prostate cells.

Figure 2. Immunohistochemical staining of normal human prostate and primary prostate carcinoma for IL-11R α . Experimental procedures are described in Materials and Methods. **A:** Normal prostate tissue section showing weak epithelial cell staining, but high expression of IL-11R α in stromal cells. **B:** Expression of IL-11R α in high-grade prostatic intraepithelial neoplasia (PIN, **arrows**). **C:** High level of expression of IL-11R α in prostate carcinoma with perineural invasion (**arrow**). **D:** High level of expression of IL-11R α in prostate carcinoma. Original magnification, $\times 200$.

Expression of IL-11R Components in Normal Human Prostate, Benign Prostatic Hyperplasia (BPH), and Prostate Carcinoma

We then asked if components of the IL-11 system were present in primary prostate tissues. Twenty-three primary prostate carcinomas (with combined Gleason scores ranging from 6 to 9) and 28 nonmalignant specimens (normal prostate or BPH) were analyzed for the expression of IL-11R α by immunohistochemistry. Several examples of these immunostains are presented in Figure 2. It was noted that the epithelial cells of the nonmalignant specimens displayed weak staining for IL-11R α (Figure 2A), whereas more frequent and prominent staining occurred within the high-grade prostatic intraepithelial neoplasia (PIN) and invasive carcinoma (Figure 2, B–D). Staining was observed in the stroma of all prostate-derived specimens. To quantify these observations, epithelial cells within the glands of the prostate samples were scored for positive staining using the following scale: if no cells were positive, a score of 0 was given; if >0 but $\leq 25\%$ of the cells stained positively, a score of 1+ was given; if >25 but $\leq 50\%$ of the cells were positive, a score of 2+ was assigned; and if the number of positive cells was $>50\%$, a score of 3+ was given. Fourteen of the 23 carcinomas tested had a score of 3+ (61%) for IL-11R α

staining, representing frequent expression of this receptor, whereas only nine (39%) had scores of 2+ or 1+, representing moderate to low frequency of IL-11R α expression; none of the carcinoma samples stained negatively (Table 1). In contrast, among the 28 nonmalignant samples, six (21%) displayed no staining for IL-11R α , 22 (79%) had scores of 2+ or 1+, and none had a 3+ score. Statistically, the scoring difference between the malignant *versus* nonmalignant samples was highly significant ($P < 0.001$) using a two-sided Fisher's exact test. There was no significant association, however, between IL-11R α staining and Gleason score, nor was there was any significant difference in extent of IL-11R α staining be-

Table 1. Expression of IL-11R α in Primary Prostate Carcinomas *Versus* Normal Prostate and Benign Prostatic Hyperplasia (BPH) as Determined by Immunohistochemistry

Histopathology	IL-11R α Expression score* (No. samples)				Total
	0	1+	2+	3+	
Adenocarcinoma	0	5	4	14	23
Normal/BPH	6	20	2	0	28

*Scoring: negative for staining, 0; >0 to $\leq 25\%$ epithelial cells staining positive, 1+; >25 to $\leq 50\%$ epithelial cells staining positive, 2+; $>50\%$ epithelial cells staining positive, 3+.

tween BPH and normal prostate tissues. These results indicate that IL-11R α may become up-regulated during the malignant transformation of prostate epithelial cells, and/or that there is an expansion of an IL-11R α -expressing cell type that occurs during this process. We also performed immunohistochemical staining for gp130, the signal transducing component of the IL-11 receptor; gp130 was universally expressed in the epithelial and stromal portions of these samples (data not shown), as has been observed in many other tissues and cell types.¹⁰ We were unable to obtain an antibody to human IL-11 that was satisfactory for the immunostaining of formalin-fixed paraffin sections; as such, we were unable to determine whether potential IL-11 autocrine or paracrine axes are found in these prostate specimens.

Expression of Tyrosine-Phosphorylated STAT3 in Normal Human Prostate, BPH, and Primary Prostate Carcinoma

We then asked if the activated form of STAT3, ie, STAT3 phosphorylated at Tyr705 (STAT3 P-Tyr705), which would be produced as a consequence of activation of the IL-11R, other members of the gp130-dependent family of receptors, or certain growth factor receptors such as erbB1,³⁶ were present in these prostate-derived tissues. An immunohistochemical assay was developed that used antibodies that specifically recognize only this phosphotyrosine form of STAT3. Because STAT3 P-Tyr705 is the species that dimerizes, enters the nucleus, and acts as a transcription factor,¹⁵ it would be predicted that one would observe primarily nuclear staining in tissues where STAT3 is activated. A total of 21 prostate carcinomas and 28 specimens of normal or BPH were immunostained for STAT3 P-Tyr705. As shown in Figure 3, nuclear staining was obtained in the glandular epithelial cells of both the malignant (Figure 3A) and nonmalignant samples (Figure 3B). The immunostains were quantitated using the scoring system described above for IL-11R α staining. As in the case of IL-11R α , it was noted that the epithelial cells in many of the nonmalignant specimens stained relatively weakly for STAT3 P-Tyr705 as compared to those in the malignant samples. For example, 15 of the 21 carcinomas (71%) displayed frequent staining for STAT3 P-Tyr705 (>50% of the cells staining positively), four (19%) displayed low to moderate staining (1+ or 2+), and only two samples were negative for this form of the protein (Table 2). In contrast, seven of the 28 normal/BPH samples (25%) did not stain for STAT3 P-Tyr705, 19 (66%) exhibited low to moderate staining (1+ or 2+), and only two displayed a high percentage of positively staining cells (3+). The scores for the malignant samples were significantly different from the nonmalignant ($P = < 0.0001$) using a two-sided Fisher's exact test. A comparison of the scores for IL-11R α expression and STAT3 P-Tyr705 among the benign samples reveals that there is some discordance between the two. In at least five cases, moderate expression of activated STAT3 was observed in samples where IL-11R α was absent from the epithelial cells. This suggests that cytokines or growth

Figure 3. Immunohistochemical staining of prostate carcinoma (A) and normal prostate tissue (B) for the tyrosine-phosphorylated, activated form of STAT3 (STAT3 P-Tyr705). Note the strong nuclear staining in prostate carcinoma (A) as compared to the weak, more sporadic staining in the normal epithelial cells (B). Experimental methodology is described in Materials and Methods. Original magnification, $\times 400$.

factors other than those that activate the IL-11 receptor, or some other mechanism, are contributing to the activation of STAT3 in these cases.

Discussion

The major finding of this investigation is that components of the IL-11 receptor system are commonly expressed in normal prostate, BPH, prostate carcinomas, and cell lines, and that the expression of the IL-11R α subunit is elevated in prostate carcinoma relative to their BPH or normal prostate counterparts. Furthermore, the tyrosine-phosphorylated, activated form of STAT3, a signal transducing protein downstream of the IL-11 and other cytokine receptors, appears to be more prominent in primary prostate carcinoma cells compared to the epithelial cells of BPH or normal prostate. The presence of the IL-11

Table 2. Presence of STAT3 P-Tyr705 in Primary Prostate Carcinomas Versus Normal Prostate and BPH as Determined by Immunohistochemistry

Histopathology	STAT3 P-Tyr705 Score* (No. samples)				Total
	0	1+	2+	3+	
Adenocarcinoma	2	2	2	15	21
Normal/BPH	7	13	6	2	28

*Scoring: negative for staining, 0; >0 to $\leq 25\%$ epithelial cells staining positive, 1+; >25 to $\leq 50\%$ epithelial cells staining positive, 2+; $>50\%$ epithelial cells staining positive, 3+.

system in prostate tissue is not without precedent. Douglas and colleagues³⁷ found the mRNA for IL-11R α in 15 of 15 primary breast carcinomas; IL-11 has also been reported in some breast cancer cell lines.^{38,39} A recent immunohistochemical-based study from our laboratory revealed that the malignant epithelial cells of 41 of 44 primary human ovarian tumors co-express IL-11R α and gp130, and that co-expression of IL-11 and its receptors are frequently observed in cell lines derived from both a variety of solid tumors and hematological malignancies.⁴⁰ Thus the IL-11 system may be more commonly expressed in malignant tissues and some of their normal counterparts than originally appreciated.

The exact function of IL-11 and other JAK-STAT activating cytokines in the prostate remains unclear. IL-6, which shares many overlapping actions with IL-11 in a number of cell systems, has been shown to activate androgen receptor-mediated gene expression in a STAT3-dependent manner in LNCaP prostate cancer cells.⁴¹ Importantly, IL-6 was found to mediate androgen receptor activation synergistically with androgens, but was capable of triggering androgen receptor activation in the absence of androgen.⁴¹ Thus in the normal prostate IL-6 (and IL-11) might act to sensitize androgen receptor-expressing prostate epithelial cells to the actions of androgens, whereas in the case of malignancy, up-regulation of these STAT3 activating cytokine systems might drive androgen receptor activation in the absence of androgens, thereby playing a role in the development of androgen independence. Another potentially relevant finding is that IL-11-expressing breast cancer cells can up-regulate the expression of aromatase in breast adipose stromal cells; by this paracrine interaction, the carcinoma cells might increase the level of stromal cell-generated estrogen.³⁸ Based on these observations, the relationship between expression of JAK-STAT activating cytokines and sex steroid metabolism in steroid-response tissues such as prostate and breast deserves further study. One may also consider the potential role of the IL-11 system in aspects of the biological behavior of prostate carcinoma, such as the predilection of this malignancy to metastasize to bone. Because IL-11 is produced by bone-marrow stromal cells,³ IL-11R-bearing prostate carcinoma cells might be chemotactically attracted to this site; alternatively, if IL-11 serves as a growth or survival factor for these cells, the IL-11-rich bone marrow might be a hospitable environment for metastasizing cells.

The presence of the activated form of STAT3 and its up-regulation in prostate carcinoma, has important implications in terms of prostate cancer biology. As previously discussed, there is a growing body of evidence associating constitutive or aberrant activation of STAT3 with oncogenic transformation in human cancers.^{19–28} Constitutively activated STAT3 has recently been reported in prostate carcinoma cell lines;³³ furthermore, transfection of these cells with dominant-negative forms of STAT3 triggers a suppression of their proliferation.⁴² The data presented here provides evidence that activated forms of STAT3 occur in primary prostate carcinomas as well. Based on the findings presented here that the IL-11

receptor is commonly and prominently expressed in prostate cancers, that IL-11 can trigger STAT3 activation in prostate-derived cells, and the recent data that IL-6 may be an important inducer of STAT3 activation and prostate epithelial cell growth,^{33,42} one may postulate that there are multiple cytokine networks involving the gp130-dependent receptors and other systems operating in prostate carcinoma whose function is to maintain STAT3 in an activated state, thus helping to drive the malignant phenotype.

References

1. Landis SH, Murray T, Bolden S, Wingo PA: Cancer statistics, 1999. *CA Cancer J Clin* 1999, 49:8–13
2. Paul S, Bennet F, Calvetti J, Kelleher K, Wood C, O'Hara Jr R, Leqary A, Sibley B, Clark S, Williams D, Yang Y-C: Molecular cloning of a cDNA encoding interleukin-11, a stromal cell-derived lymphopoietic and hematopoietic cytokine. *Proc Natl Acad Sci USA* 1990, 87:7512–7516
3. Du XX, Williams DA: Interleukin-11: review of molecular, cell biology and clinical use. *Blood* 1997, 89:3897–3908
4. Keller DC, Du XX, Srouf EF, Hoffman R, Williams DA: Interleukin-11 inhibits adipogenesis and stimulates myelopoiesis in human long-term marrow cultures. *Blood* 1993, 82:1428–1435
5. Girasole G, Passeri G, Jilka RL, Manolagas SC: Interleukin-11: a new cytokine critical for osteoclast development. *J Clin Invest* 1994, 93:1516–1524
6. Keith Jr JC, Albert L, Sonis ST, Pfeiffer CJ, Schaub RG: IL-11, a pleiotrophic cytokine: exciting new effects of IL-11 on gastrointestinal mucosal biology. *Stem Cells* 1994, 12:79–90
7. Potten CS: Interleukin-11 protects the clonogenic stem cells in murine small intestinal crypts from impairment of their reproductive capacity by radiation. *Int J Cancer* 1995, 62:356–361
8. Orazi A, Du X, Yan Z, Kashai M, Williams DA: Interleukin-11 prevents apoptosis and accelerates recovery of small intestinal mucosa in mice treated with combined chemotherapy and radiation. *Lab Invest* 1996, 75:33–42
9. Du XX, Liu Q, Yang Z, Orazi A, Rescoria FJ, Grosfeld JL, Williams DA: Protective effects of interleukin-11 in a murine model of ischemic bowel necrosis. *Am J Physiol* 1997, 272:G545–G552
10. Kishimoto T, Akira S, Narazaki M, Taga T: Interleukin-6 family of cytokines and gp130. *Blood* 1995, 86:1243–1254
11. Heinrich PC, Behrmann I, Muller-Newen G, Schaper F, Graeve L: Interleukin-6 type cytokine signaling through the gp130/Jak/STAT pathway. *Biochem J* 1998, 334:297–314
12. Dahmen H, Horsten U, Kuster A, Jacques Y, Minvielle S, Kerr IM, Ciliberto G, Paonessa G, Heinrich PC, Muller-Newen G: Activation of the signal transducer gp130 by interleukin-11 and interleukin-6 is mediated by similar molecular interactions. *Biochem J* 1998, 331:695–702
13. Stahl N, Boulton TG, Farruggella T, Ip NY, Davis S, Wittuhn BA, Quelle FW, Silvennoinen O, Barbieri G, Pellegrini S, Ihle J, Yancopoulos GD: Association and activation of Jak-Tyk kinases by CNTF-LIF-OSM-IL-6 β receptor components. *Science* 1994, 263:92–95
14. Stahl N, Farruggella TJ, Boulton TG, Zhong Z, Darnell JE, Yancopoulos GD: Choice of STATs and other substrates specified by modular tyrosine-based motifs in cytokine receptors. *Science* 1995, 267:1349–1353
15. Darnell Jr JE: STATs and gene regulation. *Science* 1997, 277:1630–1635
16. Berger LC, Hawley TSD, Lust JS, Goldman SJ, Hawley RG: Tyrosine phosphorylation of JAK-TYK kinases in malignant plasma cell lines growth-stimulated by interleukins 6 and 11. *Biochem Biophys Res Commun* 1994, 202:596–605
17. Yin T, Yasukawa K, Taga T, Kishimoto T, Yang Y-C: Identification of a 130 kilodalton tyrosine-phosphorylated protein induced by interleukin-11 as JAK2 tyrosine kinase, which associates with gp130 signal transducer. *Exp Hematol* 1994, 22:467–472

18. Yang Y-C, Yin T: Interleukin (IL)-11 mediated signal transduction. *Ann NY Acad Sci* 1995, 762:31-40
19. Garcia R, Yu C-L, Hudnall A, Catlett R, Nelson KL, Smithgall T, Fujita DJ, Ethier SP, Jove R: Constitutive activation of Stat3 in fibroblasts transformed by diverse oncoproteins and in breast carcinoma cells. *Cell Growth Differ* 1997, 8:1267-1276
20. Gouilleux-Gruart V, Gouilleux F, Desaint C, Claisee JF, Capiod JC, Delobel J, Weber-Nordt R, Dusanter-Fourt I, Dreyfus F, Groner B, Prin L: STAT-related transcription factors are constitutively activated in peripheral blood cells from acute leukemia patients. *Blood* 1996, 87:1692-1697
21. Weber-Nordt RM, Egen C, Wehinger J, Ludwig W, Gouilleux-Gruart V, Mertelsmann R, Finke J: Constitutive activation of STAT proteins in primary lymphoid and myeloid leukemia cells and in Epstein-Barr virus (EBV)-related lymphoma cell lines. *Blood* 1996, 88:809-816
22. Chai SK, Nichols GL, Rothman P: Constitutive activation of JAKs and STATs in BCR-Abl-expressing cell lines and peripheral blood cells derived from leukemic patients. *J Immunol* 1997, 159:4720-4728
23. Grandis JR, Drenning SD, Chakraborty A, Zhou M-Y, Zeng Q, Pitt AS: Requirement of Stat3 but not Stat1 activation for epidermal growth factor receptor-mediated cell growth in vitro. *J Clin Invest* 1998, 102:1385-1392
24. Catlett-Falcone R, Landowski TH, Oshiro MM, Turkson J, Levitski A, Savino R, Ciliberto G, Moscinski L, Fernandez-Luna JL, Nunez G, Dalton WS, Jove R: Constitutive activation of Stat3 signaling confers resistance to apoptosis in human U266 myeloma cells. *Immunity* 1999, 10:105-115
25. Yu C-L, Meyer DJ, Campbell GS, Lerner AC, Carter-Su C, Schwartz J, Jove R: Enhanced DNA-binding activity of a Stat3-related protein in cells transformed by the Src oncoprotein. *Science* 1995, 269:81-83
26. Bromberg JF, Horvath CM, Besser D, Lathem WW, Darnell Jr JE: Stat3 activation is required for cellular transformation by v-src. *Mol Cell Biol* 1998, 18:2553-2558
27. Bromberg JF, Wrzeszczynska MH, Devgan G, Zhao Y, Pestell RG, Albanese C, Darnell Jr JE: Stat3 as an oncogene. *Cell* 1999, 98:295-303
28. Niu G, Heller R, Catlett-Falcone R, Coppola D, Jaroszeski M, Dalton W, Jove R, Yu H: Gene therapy with dominant negative Stat3 suppresses growth of the murine melanoma B16 tumor in vivo. *Cancer Res* 1999, 59:5059-5063
29. Yin TG, Yang Y-C: Involvement of MAP kinase and pp90^{ras} activation in signalling pathways shared by interleukin-11, interleukin-6, leukemia inhibitory factor and oncostatin M in mouse 3T3 L1 cells. *J Biol Chem* 1994, 269:3731-3738
30. Fuhrer DK, Yang Y-C: Activation of src-family protein tyrosine kinases and phosphatidylinositol-3-kinase in 3T3 mouse preadipocytes by interleukin-11. *Exp Hematol* 1996, 24:195-203
31. Fuhrer DK, Yang Y-C: Complex formation of JAK2 with PP2A, PI3K, and yes in response to the hematopoietic cytokine interleukin-11. *Biochem Biophys Res Commun* 1996, 224:289-296
32. Twillie DA, Eisenberger MA, Carducci MA, Hsieh W-S, Kim WY, Simons JW: Interleukin-6: a candidate mediator of human prostate cancer morbidity. *Urology* 1995, 45:542-549
33. Lou W, Ni Z, Dyer K, Tweardy DJ, Gao AC: Interleukin-6 induces prostate cancer cell growth accompanied by activation of Stat3 signaling pathway. *The Prostate* 2000, 42:239-242
34. Campbell CL, Savarese DMF, Quesenberry PJ, Savarese TM: Expression of multiple angiogenic cytokines in cultured normal human prostate epithelial cells: predominance of vascular endothelial growth factor. *Int J Cancer* 1999, 80:868-874
35. Savarese DMF, Valinski H, Quesenberry PJ, Savarese TM: Expression and function of colony stimulating factors and their receptors in human prostate carcinoma cell lines. *The Prostate* 1998, 34:80-91
36. Fu X-Y, Zhang J-J: Transcription factor p91 interacts with the epidermal growth factor receptor and mediates activation of the c-fos gene promoter. *Cell* 1993, 74:1135-1145
37. Douglas AM, Goss GA, Sutherland RL, Hilton DJ, Berndt MC, Nicola NA, Begley CG: Expression and function of members of the cytokine receptor superfamily on breast cancer cells. *Oncogene* 1997, 14:661-669
38. Crichton MB, Nichols JE, Zhao Y, Bulun SE, Simpson ER: Expression of transcripts of interleukin-6 and related cytokines by human breast tumors, breast cancer cells, and adipose stromal cells. *Mol Cell Endocrinol* 1996, 118:215-220
39. Lacroix M, Siwek B, Marie PJ, Body JJ: Production and regulation of interleukin-11 by breast cancer cells. *Cancer Lett* 1998, 127:29-35
40. Campbell CL, Nelson B, Quesenberry P, Savarese T: Coexpression of IL-11 and IL-11 receptor in primary neoplasms (ovarian) and cultured cell lines. *Proc Am Assoc Cancer Res* 1999, 40:177
41. Chen T, Wang LH, Farrar WL: Interleukin 6 activates androgen receptor-mediated gene expression through a signal transducer and activator of transcription 3-dependent pathway in LNCaP prostate cancer cells. *Cancer Res* 2000, 60:2132-2135
42. 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