

IFN Unresponsiveness in LNCaP Cells Due to the Lack of *JAK1* Gene Expression

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Abstract

We reported previously that 23% of human lung adenocarcinoma cell lines were unresponsive to IFN- γ . To extend this finding to cancer cells derived from distinct tissues of origin, we assessed IFN- γ receptor signaling in the LNCaP human prostate adenocarcinoma cell line, which in previous experiments by others failed to induce a range of IFN-dependent biological responses. In this report, we show that LNCaP cells fail to respond to either IFN- γ or IFN- α because of an impairment in the proximal signaling events downstream of both IFN- γ and IFN- α/β receptors that lead to the activation of STAT1. Furthermore, we show that LNCaP insensitivity to the IFNs is a result of the absence of expression of the *JAK1* kinase, an obligate component shared by both IFN- γ and IFN- α/β receptors. *JAK1* was undetectable in LNCaP cells at both protein and message levels. Treatment of LNCaP cells with a combination of inhibitors of DNA methyltransferases and histone deacetylases induced expression of *JAK1* message. These results identify the molecular basis for IFN insensitivity in the LNCaP cell line and suggest that epigenetic silencing of key immunologic signaling components may be one mechanism by which tumor cells evade immune detection and elimination. (Cancer Res 2005; 65(8): 3447-53)

Introduction

IFN- γ is a cytokine produced mainly by natural killer cells and specific T-cell subsets which exerts pleiotropic immunoregulatory effects, including the up-regulation of MHC molecules and inhibition of cellular proliferation (1, 2), on its cellular targets. The biological actions of IFN- γ are particularly broad because almost all normal cells express functionally active IFN- γ receptors on their surfaces. Work in murine models over the last decade has provided strong evidence to suggest that IFN- γ plays a critical role in the antitumor immune response. Specifically, mice insensitive to IFN- γ [i.e., lacking expression of either the ligand-binding subunit of the IFN- γ receptor (IFNGR1^{-/-}) or the transcription factor mediating most of the downstream biological effects of IFN- γ (STAT1^{-/-})] developed higher incidences of chemically induced fibrosarcomas than their wild-type counterparts (3). In addition, mice lacking p53 and either IFNGR1 or STAT1 formed a wider spectrum of spontaneous tumors more rapidly than did mice lacking p53 alone (3). Furthermore, mice lacking the IFN- γ ligand itself were more susceptible to chemically induced carcinogenesis than wild-type mice and also developed spontaneous disseminated

lymphomas (4, 5). Together, this work showed that IFN- γ is a critical molecule mediating natural protection against primary tumor development.

Two studies showed that the tumor cell itself is a physiologically relevant target of the antitumor actions of IFN- γ . First, lipopolysaccharide-mediated rejection of the MethA fibrosarcoma was shown to require IFN- γ sensitivity at the level of the tumor (6). Specifically, lipopolysaccharide-induced MethA rejection was abrogated in wild-type recipients that were transplanted with MethA tumor cells engineered to overexpress a mutant dominant-negative IFNGR1 chain rendering only the tumor cells unresponsive to IFN- γ . Second, tumors derived from IFNGR1^{-/-} mice grew aggressively when transplanted into wild-type syngeneic hosts. However, following complementation with IFNGR1, these tumors were avidly rejected when transplanted into wild-type mice, demonstrating that IFN- γ responsiveness enhances tumor cell immunogenicity *in vivo* (3).

One prediction arising from these findings is that a subset of tumors may adaptively develop IFN- γ insensitivity to evade immune detection and/or elimination. In an initial screen of 17 human lung adenocarcinoma cell lines, 4 were found to be completely unresponsive to IFN- γ and were not able to activate STAT1 or up-regulate MHC class I expression in response to IFN- γ (3). In each case, IFN- γ unresponsiveness was shown to be the result of a lesion in the proximal IFN- γ receptor signaling pathway: one cell line lacked expression of IFNGR1, one cell line did not express *JAK1* (the Janus kinase that binds to the IFNGR1 intracellular domain), and two cell lines produced abnormal forms of *JAK2* (the Janus kinase that associates with the IFNGR2 subunit of the IFN- γ receptor).

To extend these initial observations, we assessed IFN responsiveness in prostate cancer cell lines because prostate cancers have been shown to exhibit high immunoevasive potential. In one study, 34% of primary tumors and 80% of metastases examined lacked surface expression of HLA class I molecules (7). One of the cell lines we studied, the LNCaP prostate adenocarcinoma cell line, was originally derived from a supraclavicular lymph node metastasis (8) and is heavily employed as a model for both androgen-dependent prostate cancer (9) and neuroendocrine differentiation in prostate cancer progression (10). Importantly, previous studies by others reported that LNCaP cells do not respond to IFN treatment. For example, type I IFN did not inhibit the growth of LNCaP cells (11), and LNCaP cells did not up-regulate MHC class I (12) or down-regulate *HER-2/neu* in response to IFN- γ (13). However, the molecular basis for these observations was not defined. Herein, we show that LNCaP cells fail to initiate signaling in response to either IFN- γ or IFN- α and show that IFN unresponsiveness in these cells is due to the lack of expression of *JAK1*, the Janus family kinase used in common by both IFN- γ and IFN- α/β receptors. We further document that the lack of *JAK1* expression in LNCaP cells is caused by epigenetic silencing. This study therefore not only provides additional support to the

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hypothesis that the development of IFN- γ insensitivity may be one strategy that tumors use to evade host-protective antitumor responses but also highlights a previously unrecognized variable that is relevant to LNCaP cells as a model of prostate cancer.

Materials and Methods

Electrophoretic mobility shift assay. Cells (1×10^6) were resuspended in 0.5 mL PBS containing 10% FCS and treated with either PBS or varying concentrations of recombinant human IFN- γ (Genentech, South San Francisco, CA; 10 minutes), human IFN- α /D (Schering-Plough, Kenilworth, NJ; 20 minutes), or human tumor necrosis factor- α (TNF- α ; Genentech; 10 minutes). Nuclear extracts were prepared as described previously (14), and 5 μ g were analyzed for DNA-binding activity using the 32 P-labeled m67 probe for activated STAT1 or the κ B probe for nuclear factor- κ B (NF- κ B) as described (15).

Immunoprecipitation and Western blotting. Cell lysates (10 mg) derived from LNCaP and DU-145 cells ($\sim 40 \times 10^6$ - 50×10^6) were used for immunoprecipitation and Western blotting of STAT1 as described previously (16). For JAK1, rabbit polyclonal antiserum (sc-277, Santa Cruz Biotechnology, Santa Cruz, CA) was used in the immunoprecipitation step and JAK1 antiserum (Upstate Biotechnology, Lake Placid, NY) was used in the Western blotting step. JAK2 was immunoprecipitated using rabbit antiserum from Santa Cruz Biotechnology (sc-278) and blotted with sc-294 anti-JAK2 serum from the same vendor. Proteins were separated by 8% SDS-PAGE and transferred to nitrocellulose membranes (Bio-Rad Laboratories, Hercules, CA). Blots were probed with the appropriate antibodies and developed with streptavidin-horseradish peroxidase before visualization using enhanced chemiluminescence (Amersham, Arlington Heights, IL).

Cell culture and transfection. LNCaP and DU-145 human prostate carcinoma cells were obtained from either the American Type Culture Collection (Manassas, VA) or the Memorial Sloan-Kettering Cancer Center (New York, NY) tumor bank and grown in RPMI 1640 supplemented with 10% FCS, 1% L-glutamine, 1 mmol/L sodium pyruvate, 10 mmol/L nonessential amino acids, 50 units/mL penicillin, 50 μ g/mL streptomycin, and 50 μ mol/L β -mercaptoethanol (R-10 medium). Plasmids were constructed by subcloning murine *STAT1* (kindly provided by C. Schindler, Columbia University, New York, NY) and murine *JAK1* (kindly provided by S. Nagata, Osaka University, Osaka, Japan) into the pEGFP-N1 plasmid (Clontech, Palo Alto, CA). To express green fluorescent protein (GFP) or JAK1 in LNCaP cells, tumor cells were transfected with the appropriate pEGFP-N1 construct using LipofectAMINE 2000 (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions and used for luciferase reporter assays or electrophoretic mobility shift assay (EMSA) 24 or 48 hours after transfection, respectively.

Northern blot analysis. Total RNA was isolated from LNCaP and DU-145 cells using RNazol B (Tel-Test, Friendswood, TX). Total RNA (15 μ g) was resolved on a 1.2% agarose gel and transferred to Zeta-Probe membrane (Bio-Rad Laboratories). Hybridization was done in PerfectHyb hybridization buffer (Sigma-Aldrich, St. Louis, MO) and washes were done according to the manufacturer's protocol. The probe for human JAK1 was obtained by reverse transcription-PCR (RT-PCR) from DU-145 RNA using primers 5'-GCTCC-AAGAAGACTGAGGTGAACC-3' (forward) and 5'-AACGGTGATGGTGCGATTG-3' (reverse). The probe for IRF-1 was obtained by RT-PCR from IFN- γ -stimulated DU-145 RNA using primers as described (17).

Intracellular staining. Seventy thousand cells were added to poly-L-lysine-coated coverslips overnight. Cells were then treated with 10,000 units/mL recombinant human IFN- γ for 15 minutes, 10,000 units/mL recombinant human IFN- α /D for 35 minutes, or PBS and subsequently fixed with 100% methanol. Cells were permeabilized with 0.1% Triton X-100/TBS and blocked with 5.5% normal goat serum in TBS. Phosphorylated STAT1 was detected using a primary antibody against STAT1 phospho-Tyr⁷⁰¹ (Cell Signaling, Beverly, MA) and a goat Alexa-conjugated anti-rabbit antibody (Molecular Probes, Eugene, OR). Cell nuclei were counterstained with a TOPRO-3 iodide (Molecular Probes). Samples were mounted using the Prolong Antifade Kit (Molecular Probes) and analyzed on a Zeiss laser scanning confocal microscope with a 40 \times water objective.

Luciferase reporter assays. Two hundred thousand cells were transiently transfected in triplicate with 1 μ g STAT1-dependent reporter pGAS-TA-Luc plasmid (Clontech) and 1 μ g pTK-RL reporter plasmid (Invitrogen, Carlsbad, CA). LNCaP cells were also transfected with 1 μ g of either empty pEGFP-N1 vector or pEGFP-N1/JAK1. All transfections were done using LipofectAMINE 2000. Twenty-four hours after transfection, cells were stimulated with 1,000 units/mL human IFN- γ for 6 hours and cells were subsequently lysed using Passive Lysis Buffer (Promega, Madison, WI). Luciferase reporter activity was measured in the MGM Optocomp II luminometer (MGM Instruments, Inc., Hamden, CT) and normalized to *Renilla* luciferase activity.

Inhibitor treatment. 5-Aza-2'-deoxycytidine (5-AC) and trichostatin A (TSA) were obtained from Sigma (St. Louis, MO). LNCaP cells were grown in the presence of 10 μ mol/L 5-AC and 0.3 μ mol/L TSA or DMSO as a vehicle control for 5 days. 5-AC was added with fresh medium daily, whereas TSA was added on days 1, 3, and 5. After the last drug treatment, RNA was isolated using RNazol B and reverse transcribed using Taqman reverse transcription reagents (Applied Biosystems, Foster City, CA). JAK1 transcripts were amplified using primers listed above to a 5' region of the coding sequence and the following primers to a 3' region of the coding sequence: 5'-GGAAATCTGCTACAATGGCGAG-3' (forward) and 5'-CCCCTTACAAATCTGAACGGC-3' (reverse). 18S was amplified using the following primers: 5'-CGCCGCTAGAGGTGAAATTCT-3' (forward) and 5'-CGAACCTCCGACTTTCGTCT-3' (reverse).

Results

LNCaP cells fail to initiate signaling through the IFN- γ and IFN- α / β receptors. To explain the previously reported inability of LNCaP cells to respond to either IFN- γ or IFN- α / β , we tested the hypothesis that the proximal IFN- γ and IFN- α / β receptor signaling pathways were defective in these cells. For this purpose, LNCaP and DU-145 cells (another human prostate adenocarcinoma cell line) were exposed to IFN- γ and the expression of the immediate-early gene *IRF-1* was assessed by Northern blot analysis. IFN- γ induced significant up-regulation of IRF-1 in DU-145 cells (Fig. 1). In contrast, LNCaP cells treated in the same manner with high doses of IFN- γ did not up-regulate IRF-1 mRNA expression.

If the impaired response to IFN- γ in LNCaP cells was due to a proximal signaling deficit, then IFN- γ -dependent activation of the transcription factor STAT1, which mediates not only *IRF-1* gene induction but also many of the biological functions of IFN- γ (1), should also be diminished. We therefore assessed the ability of LNCaP and DU-145 cells to activate STAT1 following treatment with IFN- γ using an EMSA that employed a radiolabeled oligonucleotide containing consensus STAT1-binding sites.

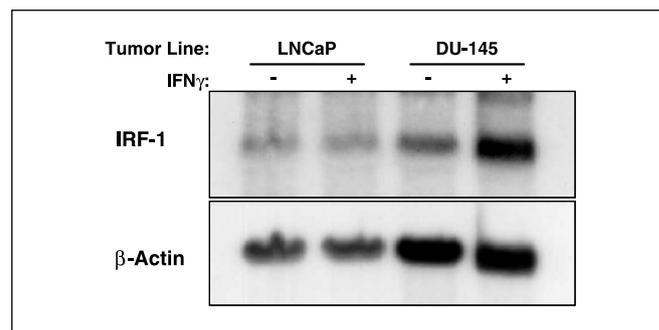


Figure 1. LNCaP cells fail to respond to IFN- γ . RNA was isolated from LNCaP and DU-145 cells stimulated with PBS or IFN- γ for 3 hours as described in Materials and Methods and probed for IRF-1 (top) and β -actin (bottom) expression by Northern blot analysis.

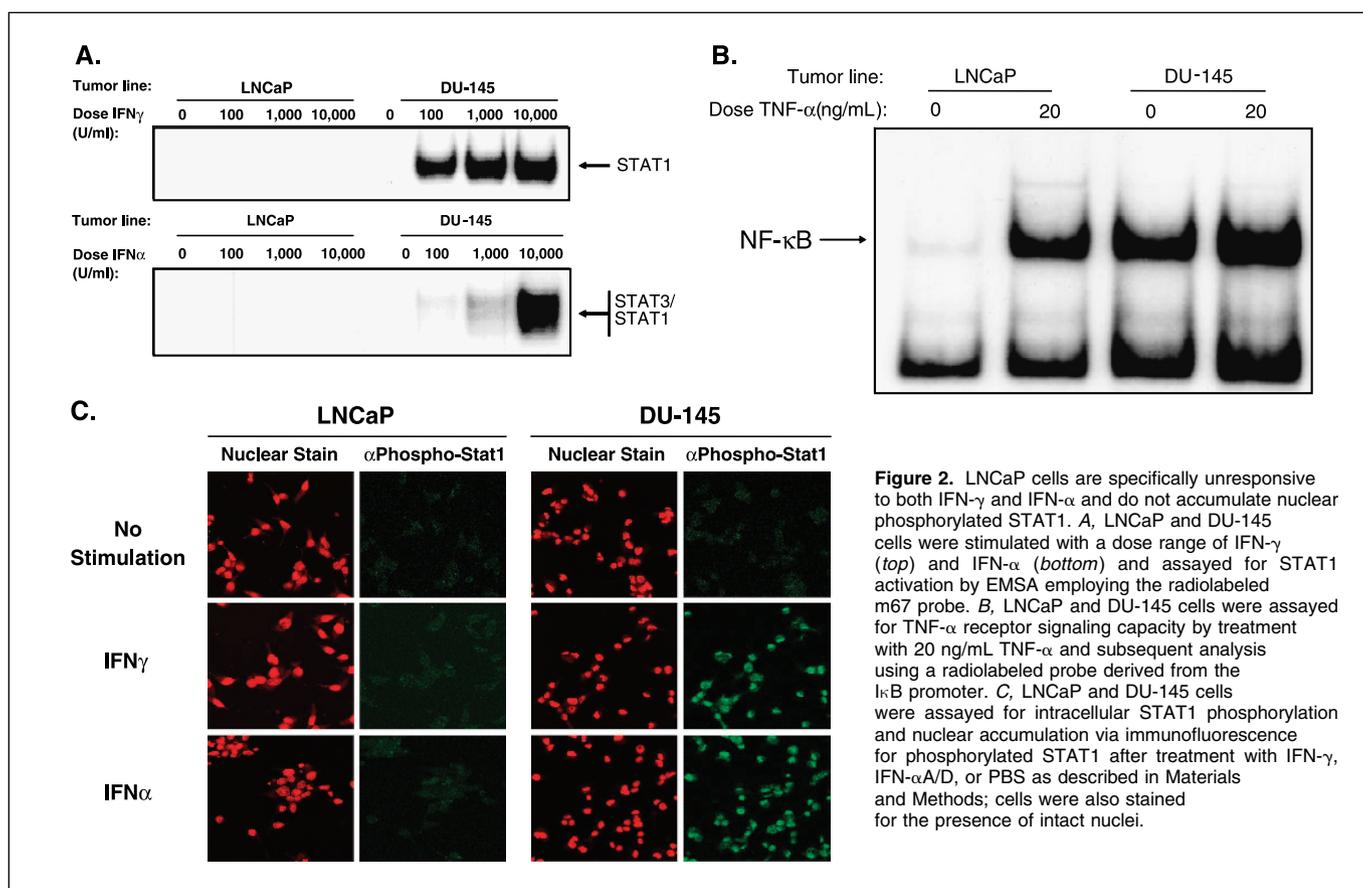


Figure 2. LNCaP cells are specifically unresponsive to both IFN- γ and IFN- α and do not accumulate nuclear phosphorylated STAT1. *A*, LNCaP and DU-145 cells were stimulated with a dose range of IFN- γ (top) and IFN- α (bottom) and assayed for STAT1 activation by EMSA employing the radiolabeled m67 probe. *B*, LNCaP and DU-145 cells were assayed for TNF- α receptor signaling capacity by treatment with 20 ng/mL TNF- α and subsequent analysis using a radiolabeled probe derived from the κ B promoter. *C*, LNCaP and DU-145 cells were assayed for intracellular STAT1 phosphorylation and nuclear accumulation via immunofluorescence for phosphorylated STAT1 after treatment with IFN- γ , IFN- α /D, or PBS as described in Materials and Methods; cells were also stained for the presence of intact nuclei.

DU-145 cells treated with IFN- γ developed dose-dependent STAT1 DNA-binding activity (Fig. 2A, top). In contrast, LNCaP cells did not, even at an input of 10,000 units/mL IFN- γ . Moreover, whereas DU-145 cells were also able to activate STAT1 and STAT3 after treatment with IFN- α , LNCaP cells did not signal through the IFN- α/β receptor even at the highest doses of IFN- α (Fig. 2A, bottom).

Because LNCaP cells failed to respond to either type of IFN, we considered the possibility that the cells were globally unresponsive to other proinflammatory/immune signaling cytokines and not selectively insensitive to ligands of the IFN- γ and IFN- α/β receptors. This possibility was explored by treating LNCaP and DU-145 cells with TNF- α and monitoring their ability to activate NF- κ B DNA-binding activity. DU-145 cells exhibited constitutively active NF- κ B, which increased following treatment with 20 ng/mL TNF- α (Fig. 2B, right lanes). LNCaP cells treated with the same dose of TNF- α exhibited a more significant increase in NF- κ B DNA-binding activity (Fig. 2B, left lanes). These data indicate that the IFN signaling deficit in LNCaP cells is due to a restricted lesion that is common to the IFN- γ and IFN- α/β receptors.

We next asked whether the inability of the IFNs to activate STAT1 DNA-binding activity or to mediate STAT1-dependent gene expression was due to impaired phosphorylation or nuclear translocation of STAT1 in response to IFN treatment. In cells capable of initiating signaling through the IFN- γ or IFN- α/β receptor, serine- and tyrosine-phosphorylated STAT1 homodimers rapidly accumulate in the nucleus after ligand binding (1). Therefore, we assessed at the single-cell level the nuclear

accumulation of phosphorylated STAT1 in LNCaP and DU-145 cells treated with either IFN- γ or IFN- α . DU-145 cells exposed to either IFN- γ or IFN- α showed clear nuclear accumulation of phosphorylated STAT1 (Fig. 2C, right). In contrast, no nuclear phosphorylated STAT1 was detected in LNCaP cells treated with either IFN- γ or IFN- α (Fig. 2C, left). Similar results were obtained with LNCaP cell lines obtained independently from American Type Culture Collection and a tumor bank maintained at the Memorial Sloan-Kettering Cancer Center. These results show that LNCaP prostate adenocarcinoma cells are uniformly unresponsive to IFN- γ and IFN- α because they fail to initiate IFN receptor signaling and thus are unable to activate STAT1.

LNCaP cells do not express JAK1 protein or transcripts. The dual unresponsiveness to both IFN- γ and IFN- α in LNCaP cells suggested that the defect mapped to a signaling component shared by both IFN- γ and IFN- α/β receptor systems. Only two signaling proteins are shared by the IFN- γ and IFN- α/β receptors: JAK1 and STAT1. We therefore monitored the expression levels of the JAK1 and STAT1 proteins and, as a control, JAK2 protein in both LNCaP and DU-145 cells by immunoprecipitation and Western blot analysis (Fig. 3A). LNCaP and DU-145 cells express similar levels of STAT1 and JAK2. However, whereas JAK1 is clearly detectable in DU-145 cells, no JAK1 protein was detected in LNCaP cells. To explore the molecular basis for the JAK1 deficiency in LNCaP cells, Northern blot analysis was employed. Using a specific JAK1 probe, no full-length JAK1 mRNA was detected in LNCaP cells, whereas mature JAK1 transcripts were abundantly expressed in DU-145 cells (Fig. 3B). Thus, LNCaP cells do not express either JAK1 message or protein.

Enforced expression of JAK1 reconstitutes IFN signaling in LNCaP cells. To formally prove that the IFN signaling defect in LNCaP cells was due to the lack of JAK1, LNCaP cells were transiently transfected with a plasmid encoding JAK1 and tested for IFN responsiveness. Transfected cells were first analyzed for the ability to activate STAT1 following exposure to IFN- γ . As determined by EMSA, control LNCaP cells engineered for expression of either GFP or STAT1 failed to activate STAT1 when incubated with 10,000 units/mL IFN- γ (Fig. 4A). In contrast, LNCaP cells engineered for enforced expression of JAK1 exhibited low levels of STAT1 DNA-binding activity without stimulation but developed significant DNA-binding activity in response to IFN- γ . These data show that enforced expression of JAK1 in LNCaP prostate cancer cells reconstitutes their ability to activate STAT1 in response to IFN- γ .

Reconstituted LNCaP cells were also monitored at the single-cell level by intracellular staining and immunofluorescence analysis for the ability to accumulate nuclear phosphorylated STAT1 in response to either IFN- γ or IFN- α (Fig. 4B). As shown previously in Fig. 2, DU-145 cells accumulated nuclear phosphorylated STAT1 after treatment with either IFN- γ or IFN- α but unmanipulated LNCaP cells or control-transfected LNCaP cells did not. However, LNCaP cells transiently expressing JAK1 displayed clear accumulation of phosphorylated STAT1 in response to treatment with either IFN- γ or IFN- α . To confirm the physiologic relevance of the signaling reconstitution, LNCaP

cells expressing GFP or JAK1 were treated with IFN- γ and IFN- γ -dependent gene induction was monitored using a luciferase reporter gene assay (Fig. 4C). Control DU-145 cells showed a 6-fold increase in luciferase activity when treated with IFN- γ . Whereas LNCaP cells transfected with GFP showed no IFN- γ -induced increase in luciferase activity, JAK1-expressing LNCaP cells showed over a 12-fold up-regulation of the luciferase reporter. Taken together, these data show that enforced expression of JAK1 in LNCaP cells reconstitutes the ability of these cells to initiate signaling through both IFN- γ and IFN- α/β receptors and thus demonstrates that the signaling defect in LNCaP cells is due to the absence of JAK1.

Recovery of JAK1 expression in LNCaP cells following treatment with inhibitors of DNA methyltransferases and histone deacetylases. To define the mechanism underlying the failure of LNCaP cells to express JAK1, we tested the hypothesis that JAK1 transcription was repressed by epigenetic mechanisms. For this purpose, LNCaP cells were treated with a combination of a DNA methyltransferase inhibitor (5-AC) and a histone deacetylase inhibitor (TSA) using a treatment regimen that was shown previously to reverse the epigenetic silencing of other genes (18). Expression of glutathione *S*-transferase π (18) and insulin-like growth factor binding protein-3 (19) was monitored by RT-PCR as endogenous controls for effective drug treatment (data not shown). As shown in Fig. 5 (*top*), a 600-bp region of the 3' coding sequence of JAK1 was detectable in DU-145 cells but was absent in control LNCaP cells treated with DMSO. In contrast, this region of JAK1 became detectable in LNCaP cells treated with the combination of 5-AC and TSA. Similar results were obtained when a different region of the JAK1 coding sequence was amplified in a RT-PCR reaction that targeted the proximal 5' region of the JAK1 coding sequence (data not shown). This result showed that the 3' region detected in 5-AC/TSA-treated LNCaP cells was not simply due to transcription of this region from a cryptic promoter in the distal half of the *JAK1* gene. The absence of JAK1 in DMSO-treated LNCaP cells was also not due to poor reverse transcription because 18S RNA was efficiently amplified from all cDNA preparations (Fig. 5, *bottom*). These data thus indicate that the absence of JAK1 is attributable to epigenetic gene silencing.

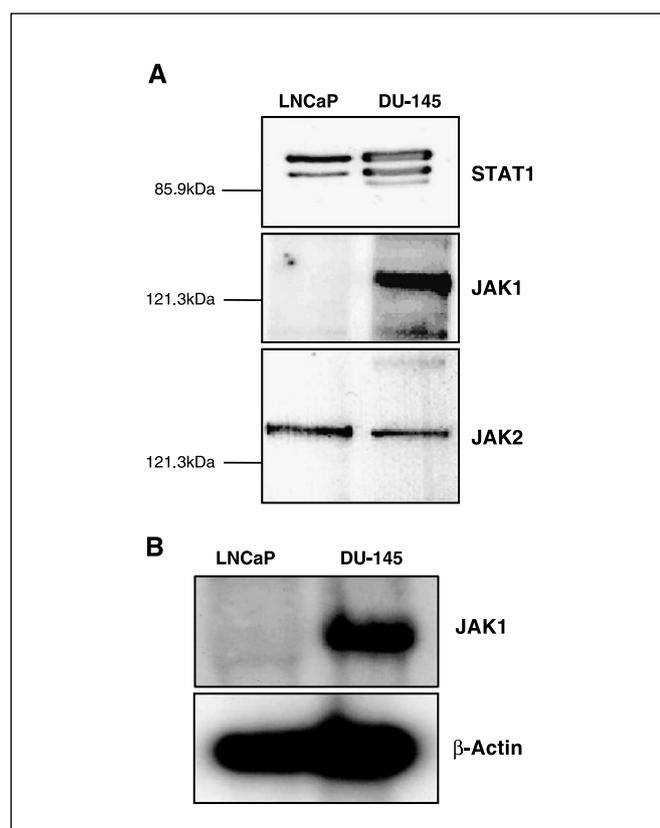


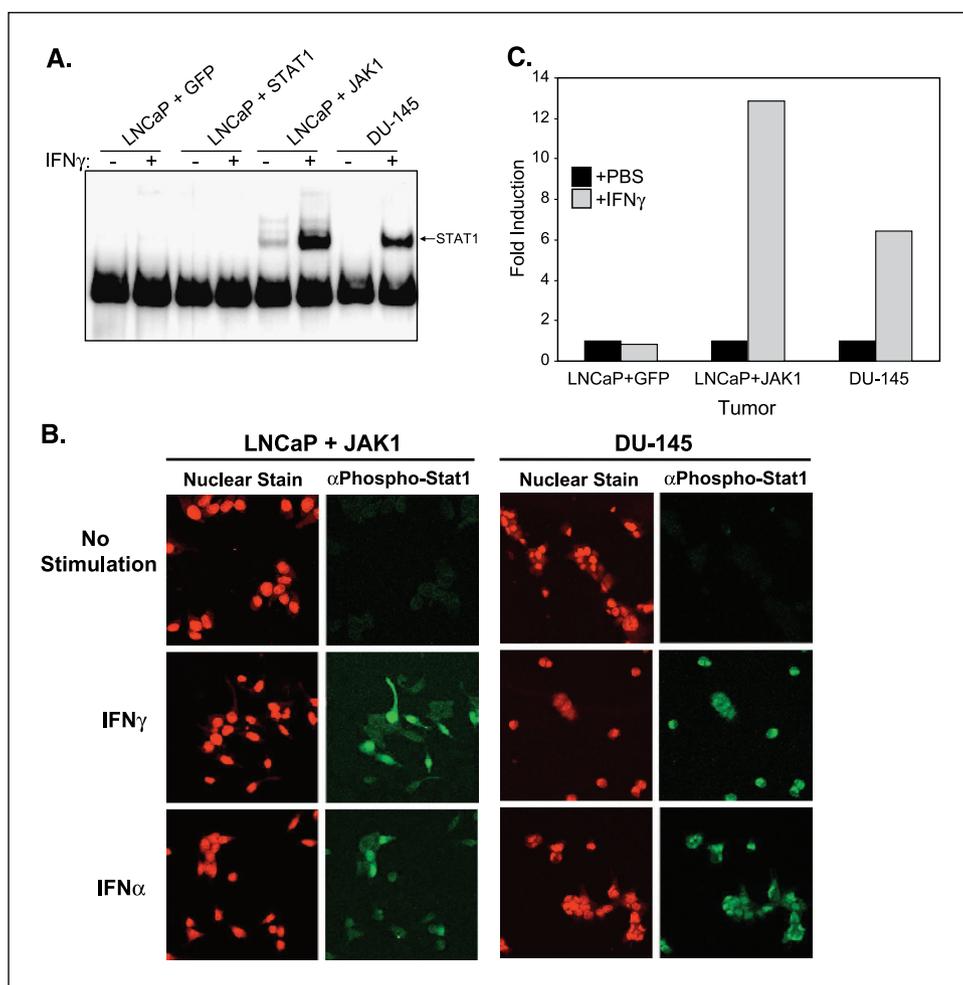
Figure 3. LNCaP cells lack expression of JAK1 protein and mRNA. A, LNCaP and DU-145 cells were monitored for the expression of STAT1, JAK1, and JAK2 proteins by immunoprecipitation/Western blot analysis on 10 mg whole cell lysate. B, RNA (15 μ g) isolated from LNCaP and DU-145 cells was probed for JAK1 (*top*) and β -actin (*bottom*) expression by Northern blot analysis as described in Materials and Methods.

Discussion

In this study, we show that the LNCaP prostate adenocarcinoma cell line is unable to initiate signaling through either IFN- γ or IFN- α/β receptor systems due to the lack of expression in these cells of the shared proximal signaling component JAK1. The dual unresponsiveness in the absence of JAK1 is consistent with published data showing that murine or human cells lacking functional JAK1 are insensitive to both classes of IFNs (20, 21). To formally show that the IFN unresponsiveness in LNCaP cells was indeed due to the lack of JAK1, we reconstituted IFN-induced STAT1 activation and STAT1-dependent gene expression by transiently expressing JAK1 in these cells. Finally, we provide evidence suggesting that the absence of JAK1 mRNA is due to epigenetic repression. These data therefore explain previous anecdotal observations of IFN resistance in LNCaP and extend the initial identification of IFN- γ unresponsiveness in human lung adenocarcinoma cell lines to tumor cells derived from the prostate.

These results and others (3, 22) provide support for the prediction that, given the critical role played by IFN- γ acting on

Figure 4. Complementation of LNCaP cells with JAK1 restores IFN signaling. **A**, enforced expression of JAK1 in LNCaP cells reconstitutes IFN- γ -induced STAT1 activation as detected by EMSA. LNCaP cells were transiently transfected with plasmids encoding either GFP (*lanes 1 and 2*), STAT1 (*lanes 3 and 4*), or JAK1 (*lanes 5 and 6*) as described in Materials and Methods. Forty-eight hours later, 1×10^6 cells from each transfection were exposed to PBS or 1,000 units/mL IFN- γ for 10 minutes, lysed, and assayed for the presence of STAT1 DNA-binding activity by EMSA as described previously. As a control, untransfected DU-145 was subjected to the same stimulation conditions and analyzed for STAT1 activation. **B**, IFN- γ and IFN- α induced nuclear accumulation of phosphorylated STAT1 assayed by intracellular staining for phosphorylated STAT1 in DU-145 cells (*right*) and LNCaP cells complemented with JAK1 (*left*); cells were also stained for the presence of nuclei. Cells were treated with PBS, 10,000 units/mL IFN- γ for 15 minutes or 10,000 units/mL IFN- α /D for 35 minutes. **C**, IFN- γ -induced, STAT1-dependent luciferase activity was monitored in DU-145 and LNCaP cells transfected with GFP or JAK1.



the tumor cell itself in host antitumor immune responses, a subset of tumors will adaptively acquire insensitivity to IFN- γ to evade immune destruction. Because the biology of IFN- γ and its receptor is now well understood (1, 2, 23), it is possible to consider the growth advantages conferred to a tumor cell, which has acquired insensitivity to IFN- γ *in vivo*. For example, diminished responses to IFN- γ would impair full processing and presentation of tumor antigens on MHC class I molecules to tumor-infiltrating lymphocytes and also impair the production of chemokines (such as IP-10, Mig, and I-TAC) that would initially attract immune effector cells into the tumor bed. In addition, tumors deficient in IFN- γ responses would also be rendered resistant to the proapoptotic (24) and antiproliferative (25) actions of IFN- γ .

The LNCaP cell line is one of the most heavily used cell lines in prostate cancer research (9). It serves as a model to study androgen-dependent prostate cancer (9), neuroendocrine differentiation (10), and development of skeletal metastases (26). However, the JAK1 deficiency identified herein has significant biological consequences and must now be considered in interpreting the data obtained in the previous studies. Importantly, LNCaP cells also represent a model to study IFN insensitivity in prostate cancer, a line of investigation that may be especially germane to the development of immunotherapeutic approaches that could be influenced by IFN-dependent up-regulation in tumor cells of antigen processing and presentation

components. For example, vaccination of cancer patients with whole autologous or allogeneic prostate cancer cells has emerged as a promising immunotherapeutic approach (27, 28). In a phase I clinical trial, seven of eight patients vaccinated with autologous irradiated, granulocyte macrophage colony-stimulating factor (GM-CSF)-secreting tumor cells mounted T-cell responses against the untransduced tumor cells (29). Due to the technical challenges of establishing autologous cell lines from surgically resected prostate cancer specimens, other clinical trials are now exploring the use of cytokine-secreting allogeneic tumor cell lines, including the LNCaP cell line, as cancer vaccines (30, 31). Preliminary data from a phase I/II trial using the GVAX vaccine, a combination of GM-CSF-secreting LNCaP and PC-3 prostate lines, suggest a therapeutic benefit from this approach (31). In this mode of vaccination, the IFN responsiveness of the tumor cell population used for vaccination may be critically important, as maximal tumor cell antigen presentation induced by exposure to the IFNs may enhance the generation of efficacious antitumor immune responses. Thus, GM-CSF-secreting LNCaP cells transduced with JAK1 to restore IFN responsiveness may be more immunogenic than the IFN-insensitive parental LNCaP cells. This consideration is also relevant to studies of tumor cells transduced with the gene encoding IFN- γ itself, especially considering that clinical trials using vaccines composed of allogeneic prostate cancer cell lines secreting interleukin-2 and IFN- γ are currently ongoing (27). A substantial number of studies have shown that

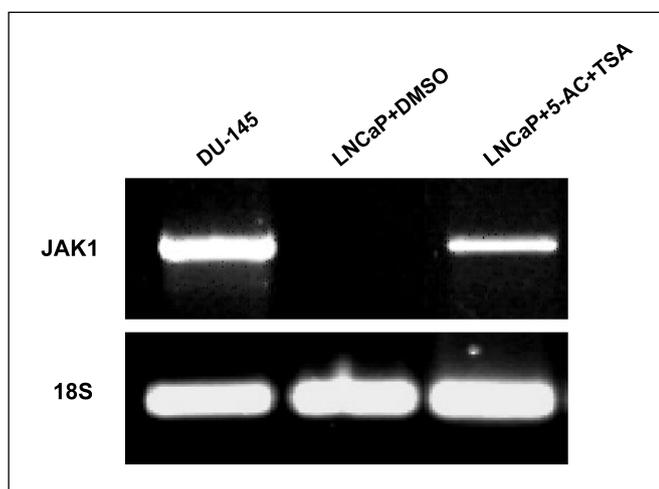


Figure 5. JAK1 coding sequence is detectable by RT-PCR after treatment with inhibitors of DNA methyltransferases and histone deacetylases. JAK1 (*top*) and 18S (*bottom*) were amplified from the cDNA from DU-145 and LNCaP cells stimulated with PBS after treatment with DMSO or the combination of 5-AC and TSA.

cells from tumors, such as melanomas (32), renal carcinomas (33), and neuroblastomas (34), transduced with IFN- γ exhibit higher levels of cell surface MHC class I than parental tumor cells. Thus, autologous or allogeneic tumor cell cytokine responsiveness may represent a critical variable in the effective use of cytokine-based gene transfer vaccination approaches. Finally, the IFN sensitivity of the tumor may influence the effectiveness of systemically given cytokines, such as IFN- α or interleukin-12 (35), which have been shown to promote host antitumor responses.

The current study and others together raise the possibility that immunologically important signaling components may be common targets of epigenetic silencing. Many studies have shown that classic tumor suppressors, including BRCA1, APC, Rb, VHL, and TP53, are often repressed in cancers through methylation or through mechanisms involving histone deacetylation (36, 37). Additionally, recent studies have shown that immunologically

relevant genes, such as MHC class I (38), MHC class II (39), and CIITA (40), are also repressed by epigenetic mechanisms in some cancers. Based on the results herein, an analysis of JAK1 in primary human prostate cancer tissues is thus clearly warranted but awaits the identification of the specific upstream promoter regulatory region of the *JAK1* gene. Nevertheless, pharmacologic de-repression not only of genes encoding immunologically important proteins but also of genes encoding the cytokine response pathways that lead to their induction might unmask the immunogenicity of a tumor and thereby condition cancer cells to be more susceptible to immunopotentiating therapeutic approaches.

The recently proposed cancer immunoeediting hypothesis emphasizes the complexity of immune system-tumor interactions (41–44). Specifically, although the immune system plays a host-protective role to block tumor development, it may also select for the eventual outgrowth of tumor variants conditioned to escape immune detection and/or elimination. A spectrum of immunoevasive strategies employed by tumors has been described, including alterations in the antigen processing and presentation pathway and also active immune suppression by tumors (45, 46). In the current report, we provide data supporting the concept that the cancer immunoeediting process may induce IFN- γ unresponsiveness in certain tumor cells and thus provide these cells with an adaptive mechanism to survive in hosts with competent immune systems. Current work is directed at elucidating the molecular basis of acquired somatic IFN- γ insensitivity in both murine tumors and human tumor isolates. In this manner, we hope to gain a better understanding of the mechanisms by which spontaneously arising IFN- γ -insensitive tumors are generated during the cancer immunoeediting process.

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