

# Down-Regulation of neu/HER-2 by Interferon- $\gamma$ in Prostate Cancer Cells<sup>1</sup>

Scott L. Kominsky, Amy C. Hobeika, Faith A. Lake, Barbara A. Torres, and Howard M. Johnson<sup>2</sup>

Department of Microbiology and Cell Science, University of Florida, Gainesville, Florida 32611

## ABSTRACT

Interferons (IFNs) are known to possess potent antitumor properties. Previous studies have indicated that IFNs are capable of modulating the expression of various tumor suppressor genes and oncogenes. In this study, we looked at the effect of IFN- $\gamma$  on the neu/HER-2 proto-oncogene in the DU145, LNCaP, and PC-3 prostate cancer cell lines. IFN- $\gamma$  inhibited cell proliferation in both DU145 and PC-3 cells in a dose-dependent manner, whereas no inhibition of proliferation was seen in LNCaP cells. Correspondingly, IFN- $\gamma$  treatment of DU145 and PC-3 cells resulted in an increased production of the cyclin-dependent kinase inhibitor p21<sup>WAF1</sup>, whereas no increase in p21<sup>WAF1</sup> was seen in LNCaP cells. In addition, IFN- $\gamma$  induced phosphorylation of signal transducer and activator of transcription (STAT) 1 in DU145 and PC-3 cells, but not in LNCaP cells. Consistent with these findings, we found that IFN- $\gamma$  treatment of DU145 and PC-3 cells caused a reduction in neu/HER-2 expression, with no change seen in the LNCaP cell line. Transfection and overexpression of the transcriptional coactivator p300 in PC-3 cells suppressed the reduction in neu/HER-2 expression after IFN- $\gamma$  treatment, suggesting a role for p300 in neu/HER-2 expression. The antiproliferative activity and p21<sup>WAF1</sup> production of these cells after IFN- $\gamma$  treatment were found to be reduced as well. We propose that the down-regulation of neu/HER-2 by IFN- $\gamma$  occurs via the interaction of phosphorylated STAT1 with p300 because IFN- $\gamma$  activities requiring phosphorylated STAT1 are reduced in cells overexpressing p300. These findings suggest that neu/HER-2 may play a role in the growth of some prostate cancers and that IFN- $\gamma$  may suppress such cancers by down-regulation of neu/HER-2.

## INTRODUCTION

IFNs are a group of cytokines that produce a number of cellular and immunological effects including inhibition of proliferation and regulation of oncogenes and tumor suppressor genes in a variety of cell types. Although the antitumor properties of IFNs have been established, many of the underlying mechanisms of these activities remain unknown. We have previously found that IFN- $\gamma$  inhibition of cellular proliferation occurs via the induction of the CKI<sup>3</sup> p21<sup>WAF1</sup> (1, 2).

Like other cytokines, IFNs use the JAK/STAT signal transduction pathway. IFN- $\gamma$  binding of its cell surface receptor specifically induces STAT1 phosphorylation, resulting in translocation to the nucleus, where it binds specific  $\gamma$ -activated sites in target genes (3). Recent studies have shown that both dimerized phosphorylated STAT1 and unphosphorylated STAT1 monomer can bind the CBP p300 (4). The CBP p300 family of proteins is a group of transcriptional coactivators that can interact with a variety of DNA-binding factors (5). p300 was originally isolated and cloned because of its interaction with the E1A protein of adenovirus (6). E1A does not directly bind DNA but regulates gene expression via interactions with

multiple transcription factors (7). As a result, E1A is responsible for reducing p300 coactivation of several genes controlling activities such as cellular differentiation, cell cycle progression, and transformation (8).

The neu/HER-2 proto-oncogene has been linked to several human malignancies including breast, ovarian, and prostate cancer (9–12). Recently, it has been shown that neu/HER-2 promoter activity can be repressed by E1A (13). Furthermore, this repression was reversed both by increasing amounts of p300 as well as by normal amounts of p300 defective in E1A binding (14). These findings suggest that p300 may play a role in the regulation of the neu/HER-2 gene. Interestingly, phosphorylated STAT1 homodimers from IFN- $\gamma$ -treated extracts have also been shown to bind the E1A binding site of p300, indicating the potential for IFN- $\gamma$  to repress p300-regulated genes via phosphorylated STAT1 (4). The experiments described in this report are directed at IFN- $\gamma$ -induced down-regulation of the neu-HER-2 receptor and the possible role of phosphorylated STAT1 in this down-regulation via interaction with p300, analogous to E1A down-regulation of neu/HER-2.

## MATERIALS AND METHODS

**Reagents, Cell Lines, and Plasmids.** Purified human IFN- $\gamma$  (specific activity,  $1-4 \times 10^7$  units/ml) was obtained from Genzyme Diagnostics (Cambridge, MA). The DU145, LNCaP, and PC-3 cell lines were obtained from American Type Culture Collection (Manassas, VA). Complete media for the DU145, LNCaP, and PC-3 cell lines consisted of Eagle's MEM, Eagle's MEM + 1 mM sodium pyruvate and 2 mM L-glutamate, and F12K medium, respectively, supplemented with 10% fetal bovine serum, 200 units/ml penicillin, and 200  $\mu$ g of streptomycin. Antibodies to p21<sup>WAF1</sup>, STAT1, and p300 were obtained from Santa Cruz Biotechnology (Santa Cruz, CA). Antibodies to phosphorylated STAT1 were obtained from New England BioLabs, Inc. (Beverly, MA). Antibodies to neu/HER-2 were obtained from Upstate Biotechnology (Lake Placid, NY). The p300 plasmid pRc/RSV-p300 containing a neomycin resistance gene allowing for selection of colonies in Geneticin was generously provided by Dr. Richard H. Goodman (Vollum Institute, Oregon Health Sciences University, Portland, OR). The pRc/RSV plasmid was purchased from Invitrogen (Carlsbad, CA). Transfectam reagent was obtained from Promega (Madison, WI).

**Transfection.** PC-3 cells were transfected with the plasmids pRc/RSV and pRc/RSV-p300 after the Transfectam reagent transfection protocol. PC-3 cells ( $1.3 \times 10^5$ ) were plated in a 100-mm<sup>2</sup> plate. A mixture of either pRc/RSV or pRc/RSV-p300 DNA and Transfectam reagent was added to the PC-3 cells and left in contact with the cells for 2 h. Transfected PC-3 cells were then selected via incubation with complete media + 600  $\mu$ g/ml Geneticin (Sigma Chemical Co., St. Louis, MO).

**Antiproliferation Assay.** IFN- $\gamma$  inhibition of cell number was determined by plating DU145, LNCaP, PC-3, and PC-3(pRc/RSV-p300) cells ( $4-10 \times 10^4$  cells/well) into 6-well plates with or without 500–20,000 units/ml IFN- $\gamma$ . At 72 h, cells were trypsinized, washed, and counted. Cell counts were performed using a hemocytometer, and cell viability was assessed by trypan blue dye (0.4%) exclusion.

**Immunoprecipitation and Immunoblotting.** For preparation of cell lysates, DU145, LNCaP, PC-3, and PC-3(pRc/RSV-p300) cells ( $4-6 \times 10^6$  cells/sample) were grown in the presence or absence of 500–20,000 units/ml IFN- $\gamma$  for various lengths of time and lysed at 4°C for 20 min as described previously (1). Equal amounts of protein from cell lysates were either used directly for Western transfer (100–200  $\mu$ g/sample) or subsequently immunoprecipitated (375–500  $\mu$ g/500  $\mu$ l) with 1  $\mu$ g of an antibody specific for the protein of interest. After Western transfer, membranes were immunoblotted

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<sup>2</sup> To whom requests for reprints should be addressed, at Room 1052, Building 981, P. O. Box 110700, Department of Microbiology and Cell Science, University of Florida, Gainesville, FL 32611. Phone: (352) 846-0968; Fax: (352) 392-5922; E-mail: johnsonh@ufl.edu.

<sup>3</sup> The abbreviations used are: IFN, interferon; CKI, cyclin-dependent kinase inhibitor; STAT, signal transducer and activator of transcription; JAK, Janus kinase; CBP, cAMP-responsive element binding protein-binding protein.

with antibodies specific for the protein of interest and developed using enhanced chemiluminescence (Amersham, Arlington Heights, IL). Densitometric analysis of radiographic film using IA-200 Image Analysis Software was used to determine the percentage decrease or fold-increase between band intensities based on total pixel values.

## RESULTS

We determined the antiproliferative effects of IFN- $\gamma$  on the DU145, LNCaP, and PC-3 prostate cancer cell lines by determining the total number of cells grown in the presence or absence of 0, 500, 1000, and 5000 units/ml IFN- $\gamma$  for 72 h. IFN- $\gamma$  inhibited DU145 and PC-3 cell growth by up to 59.1% and 50.8%, respectively, whereas no inhibition of LNCaP cells was seen (Table 1). We also found that IFN- $\gamma$  inhibited DU145 and PC-3 cells in a dose-dependent manner. Thus, the proliferation of the DU145 and PC-3 cell lines is inhibited by IFN- $\gamma$  treatment, whereas LNCaP cells do not appear to be affected.

We have found previously that growth inhibition of the DU145 cells by IFN- $\gamma$  was due to up-regulation of the CKI p21<sup>WAF1</sup> (2). p21<sup>WAF1</sup> regulates the mammalian cell cycle by directly binding cyclin-dependent kinases, which are necessary for cell cycle progression, and blocking their kinase activity (15). IFN- $\gamma$ -induced up-regulation of p21<sup>WAF1</sup> describes a mechanism by which IFNs inhibit the cell cycle and subsequent cell growth. Asynchronous DU145, LNCaP, and PC-3 cells were treated with 5000 units/ml IFN- $\gamma$  for 16 and 24 h and subsequently lysed and immunoblotted. IFN- $\gamma$  induced p21<sup>WAF1</sup> production in both DU145 and PC-3 cells, but not in LNCaP cells (Fig. 1). Consistent with our previous reports, IFN- $\gamma$  up-regulation of p21<sup>WAF1</sup> correlates with inhibition of cell proliferation.

We next determined whether the signal transduction pathway of IFN- $\gamma$  was intact in the DU145, LNCaP, and PC-3 cell lines. IFNs initiate their cellular effects by binding to specific cell surface receptors. Like other cytokines, this ligand-receptor interaction induces the phosphorylation of a group of proteins known as STATs, which, in the absence of ligand, reside unphosphorylated in the cytoplasm (3). IFN- $\gamma$ -mediated signal transduction specifically phosphorylates STAT1, which then acts directly to turn on IFN- $\gamma$ -inducible genes. DU145, LNCaP, and PC-3 cells were treated with 5000 units/ml IFN- $\gamma$  for 10 min. Cell lysates were immunoprecipitated using antibodies specific for STAT1, and precipitates were subsequently immunoblotted with antibodies for both STAT1 and phosphorylated STAT1. Both DU145 and PC-3 cells had strong phosphorylation of STAT1 with IFN- $\gamma$  treatment, whereas no STAT1 phosphorylation was seen in LNCaP cells (Fig. 2). These results explain the lack of response by the LNCaP cell line to IFN- $\gamma$  because the JAK/STAT

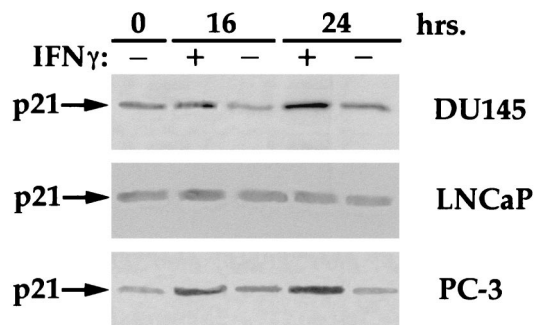


Fig. 1. IFN- $\gamma$  up-regulates p21<sup>WAF1</sup> expression in DU145 and PC-3 but not LNCaP cells. DU145, LNCaP, and PC-3 cells were treated with 5000 units/ml IFN- $\gamma$  or media alone for 0, 16, and 24 h. Cell lysates were then immunoblotted with antibodies specific for p21<sup>WAF1</sup>. Densitometric analysis of radiographic film showed a 1.5- and 1.2-fold increase in p21<sup>WAF1</sup> expression for DU145 cells and a 1.2- and 2.5-fold increase in p21<sup>WAF1</sup> expression for PC-3 cells at 16 and 24 h, respectively, in the presence of IFN- $\gamma$ . No increase in p21<sup>WAF1</sup> expression was seen in LNCaP cells. Data are representative of three experiments, each performed in triplicate.

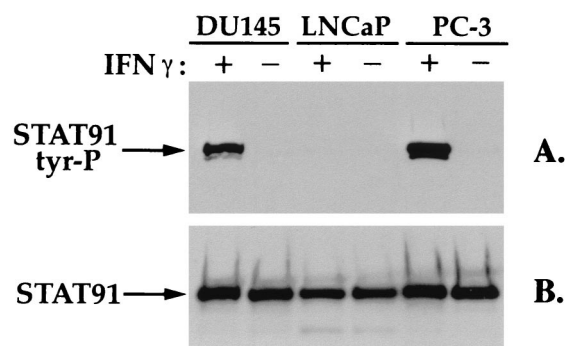


Fig. 2. IFN- $\gamma$  induces STAT1 phosphorylation in DU145 and PC-3 but not LNCaP cells. Total cell lysates from DU145, PC-3, and LNCaP cells treated with or without 5000 units/ml IFN- $\gamma$  for 10 min were immunoprecipitated using anti-STAT1 antibodies. The immunoprecipitates were subjected to immunoblotting using antibodies for phosphorylated STAT1 (A). The immunoblot was then stripped of anti-phosphorylated STAT1 and reprobed using anti-STAT1 antibodies (B). The concentrations of STAT were similar for IFN- $\gamma$ -treated and untreated cells for all cell lines. Data are representative of three experiments, each performed in triplicate.

transduction pathway is not triggered in these cells. However, the transduction pathway is active in both DU145 and PC-3 cells, consistent with up-regulation of p21<sup>WAF1</sup> and the associated antiproliferative effects seen in these cells.

Many of the mechanisms underlying the antiproliferative and cytotoxic effects of IFNs on tumor cells remain to be elucidated. We have previously shown p21<sup>WAF1</sup> to be responsible for inhibiting the cell cycle of various transformed cell lines (1, 2), but how IFN regulates proto-oncogenes is still unclear. The neu/HER-2 proto-oncogene has been implicated as a prognostic factor in several human malignancies including breast, ovarian, and prostate cancer. We looked at the effect of IFN- $\gamma$  on down-regulation of neu/HER-2 expression in the DU145, LNCaP, and PC-3 cell lines as a possible mechanism for inhibition of cell proliferation. Lysates from cells treated in the presence or absence of 5000 units/ml IFN- $\gamma$  for 96 h were immunoblotted using antibodies specific for neu/HER-2. In DU145 and PC-3 cells, we saw a 61.6% and 78.7% reduction, respectively, in neu/HER-2 expression, whereas no reduction in neu/HER-2 was seen in LNCaP cells (Fig. 3A). In addition, we saw a dose-dependent decrease in neu/HER-2 expression in DU145 and PC-3 cells (Fig. 3B). Cells were treated with 500, 1000, and 5000 units/ml IFN- $\gamma$  for 96 h, and lysates were immunoblotted. Thus, IFN- $\gamma$  reduces neu/HER-2 expression in both the DU145 and PC-3 cells, whereas no reduction is seen in the LNCaP cell line,

Table 1 Inhibition of prostate cancer cell growth by IFN- $\gamma$

Cell Line	IFN- $\gamma$ (units/ml)	Total no. of Cells ( $\times 10^5$ )	Inhibition <sup>a</sup> (%)
DU145	0	13.2 $\pm$ 0.41	
	500	8.54 $\pm$ 0.33	35.3 $\pm$ 1.3
	1000	6.72 $\pm$ 0.20	49.1 $\pm$ 1.6
	5000	5.40 $\pm$ 0.11	59.1 $\pm$ 2.1
LNCaP	0	1.50 $\pm$ 0.11	
	500	1.58 $\pm$ 0.13	
	1000	1.62 $\pm$ 0.12	
	5000	1.80 $\pm$ 0.18	
PC-3	0	2.36 $\pm$ 0.21	
	500	1.88 $\pm$ 0.11	20.3 $\pm$ 0.9
	1000	1.37 $\pm$ 0.08	42.0 $\pm$ 1.6
	5000	1.16 $\pm$ 0.06	50.8 $\pm$ 1.9

<sup>a</sup> Cells were plated into 6-well plates ( $5 \times 10^4$  cells/well) and grown in the presence or absence of IFN- $\gamma$  for 72 h, and the total number of live cells was determined. The percentage of viability for all cell lines was approximately 92%. Data are representative of three experiments, each performed in triplicate. Statistical significance was shown by Student's *t* test for the difference in cell number grown in the presence and absence of 500 ( $P < 0.05$ ), 1000 ( $P < 0.04$ ), and 5000 units/ml ( $P < 0.05$ ) IFN- $\gamma$ .

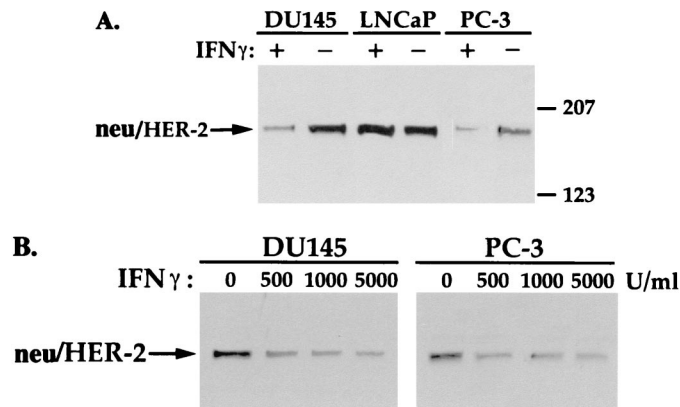


Fig. 3. A, IFN- $\gamma$  down-regulates neu/HER-2 expression in DU145 and PC-3 cells. Cell lysates from DU145, PC-3, and LNCaP cells grown in the presence or absence of 5000 units/ml IFN- $\gamma$  for 4 days were immunoblotted with antibodies specific for neu/HER-2. Densitometric analysis of radiographic film showed a 61.6% and 78.7% decrease in neu/HER-2 expression for DU145 and PC-3 cells, respectively, whereas no change in neu/HER-2 expression was seen in LNCaP cells. B, down-regulation of neu/HER-2 expression by IFN- $\gamma$  in DU145 and PC-3 cells is dose dependent. Cell lysates from DU145 and PC-3 cells treated with 0, 500, 1000, or 5000 units/ml IFN- $\gamma$  for 4 days were immunoblotted with antibodies specific for neu/HER-2. Data are representative of three experiments, each performed in triplicate.

consistent with the failure to phosphorylate STAT1 and induce p21<sup>WAF1</sup> in the latter cell line.

Although neu/HER-2 expression has been linked to tumor growth and metastasis, little is known about the transcriptional regulation of this oncogene. Previous findings have implicated the CBP p300 as a transcriptional regulator of neu/HER-2 (14). The same studies have suggested that adenovirus E1A protein binding to p300 represses neu/HER-2 promoter activity. Interestingly, there have also been reports that STAT1 from IFN- $\gamma$ -treated lysates binds p300 at the E1A binding site (4). We therefore wanted to determine whether p300 was involved in IFN- $\gamma$ -induced down-regulation of neu/HER-2 expression. PC-3 cells were transfected with a plasmid containing the p300 gene (pRc/RSV-p300). Transfected and wild-type PC-3 cells were then grown in the presence or absence of 5000 units/ml IFN- $\gamma$  for 96 h. Lysates were subsequently immunoblotted using antibodies specific for both p300 and neu/HER-2. Transfected cells showed a 30.7% increase in p300 (determined by densitometric analysis) as compared with wild-type cells (Fig. 4A). Furthermore, this increase in p300 production appeared to affect neu/HER-2 expression after IFN- $\gamma$  treatment because transfected cells showed only a 27.7% reduction in neu/HER-2 expression as compared with a 78.7% reduction in wild-type cells (Fig. 4B). This suggests that p300 may play a role in IFN- $\gamma$ -induced down-regulation of neu/HER-2.

Considering that the overexpression of p300 in PC-3 cells abrogated IFN- $\gamma$ -induced down-regulation of neu/HER-2 after IFN- $\gamma$  treatment, we wanted to determine whether the overexpression of p300 interfered with the antiproliferative effects of IFN- $\gamma$ . Transfected PC-3 cells were treated with or without IFN- $\gamma$  at concentrations of 5,000 and 20,000 units/ml for a period of 72 h. Cells were then harvested, and antiproliferative activity was determined by direct cell count. Transfected cell growth was much less inhibited after treatment with 5,000 units/ml IFN- $\gamma$  as compared with wild-type PC-3 cells, with inhibition values of 7.1% versus 50.8%, respectively (Table 2). Interestingly, treatment of transfected cells with 20,000 units/ml IFN- $\gamma$  inhibited cell growth by 44.6%, which is comparable to maximum inhibition levels seen in wild-type PC-3 cells. Thus, the antiproliferative effect of IFN- $\gamma$  is significantly reduced in PC-3 cells overexpressing p300 but is restored at higher concentrations of IFN- $\gamma$ . This suggests a dynamic and reversible interaction between IFN- $\gamma$ -induced phosphorylated STAT1 and p300.

We have previously shown that the CKI p21<sup>WAF1</sup> appears to play a role in mediating the antiproliferative effects of IFN- $\gamma$ . Because IFN- $\gamma$  has reduced antiproliferative activity on PC-3 cells overexpressing p300, we determined the ability of IFN- $\gamma$  to up-regulate p21<sup>WAF1</sup> in transfected PC-3 cells. p300-transfected PC-3 cells were treated with or without IFN- $\gamma$  at concentrations of 5,000 and 20,000 units/ml over a time period of 16 and 24 h. Cells treated for 24 h with 5,000 and 20,000 units/ml IFN- $\gamma$  showed a 1.5- and 3.7-fold increase in p21<sup>WAF1</sup> production, respectively, as determined by densitometric analysis (Fig. 5). No p21<sup>WAF1</sup> was detected at 16 h. Wild-type PC-3 cells treated with 5,000 units/ml IFN- $\gamma$  over a 24-h period showed a 2.5-fold increase in p21<sup>WAF1</sup> production. Thus, treatment of transfected PC-3 cells with 5,000 units/ml IFN- $\gamma$  led to decreased p21<sup>WAF1</sup> production as compared with wild-type PC-3 cells, correlating with the reduced antiproliferative effects of IFN- $\gamma$  on transfected PC-3 cells. Consistent with the antiproliferative effects of IFN- $\gamma$  on transfected PC-3 cells, treatment with an increased concentration of IFN- $\gamma$  (20,000 unit/ml) was able to restore p21<sup>WAF1</sup> levels to those seen after IFN- $\gamma$  treatment of wild-type PC-3 cells.

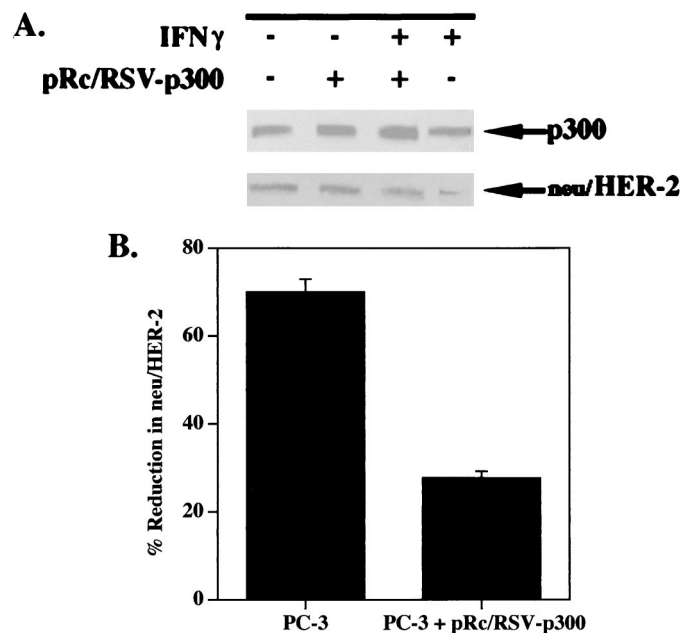


Fig. 4. A, transfection of PC-3 cells with pRc/RSV-p300 increases expression of p300 and decreases down-regulation of neu/HER-2 in response to IFN- $\gamma$  treatment. Cell lysates from PC-3 and PC-3(pRc/RSV-p300) cells grown in the presence or absence of 5000 units/ml IFN- $\gamma$  for 4 days were immunoblotted with antibodies specific for p300 and neu/HER-2. B, the percentage of reduction in neu/HER-2 expression after treatment with 5000 units/ml IFN- $\gamma$  for 4 h in PC-3 and PC-3(pRc/RSV-p300) cells was determined by densitometric scanning of radiographic film. Data are representative of three experiments, each performed in triplicate.

Table 2. Inhibition of PC-3(pRc/RSV-p300) cell growth by IFN- $\gamma$

IFN- $\gamma$ (units/ml)	Inhibition <sup>a</sup> (%)
0	
5000	7.1 $\pm$ 0.4
20000	44.6 $\pm$ 1.9

<sup>a</sup> Cells were plated into 6-well plates ( $1 \times 10^5$  cells/well) and grown in the presence or absence of IFN- $\gamma$  for 72 h, and the total number of live cells was determined. The percentage of viability was approximately 91%. Data are representative of three experiments, each performed in triplicate. Statistical significance was shown by Student's *t* test for the difference in cell number grown in the presence and absence of 5,000 and 20,000 units/ml IFN- $\gamma$  ( $P < 0.05$ ). Wild-type PC-3 cells were inhibited by 50.8% after treatment with 5,000 units/ml IFN- $\gamma$ .

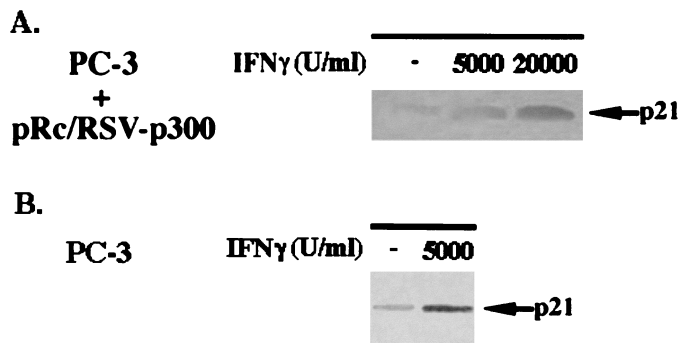


Fig. 5. IFN- $\gamma$  up-regulates p21<sup>WAF1</sup> production in wild-type PC-3 and PC-3 (pRc/RSV-p300) cells. Wild-type PC-3 and PC-3(pRc/RSV-p300) cells were treated with 0, 5,000, or 20,000 units/ml IFN- $\gamma$  for 16 and 24 h. Cell lysates were then immunoblotted with antibodies specific for p21<sup>WAF1</sup>. A, densitometric analysis of radiographic film showed a 1.5- and 3.7-fold increase in p21<sup>WAF1</sup> production after treatment of PC-3 (pRc/RSV-p300) cells with 5,000 and 20,000 units/ml IFN- $\gamma$  for 24 h, respectively. No increase in p21<sup>WAF1</sup> production was seen at 16 h. B, wild-type PC-3 cells showed a 1.2- and 2.5-fold increase in p21<sup>WAF1</sup> production after IFN- $\gamma$  treatment for 16 (data not shown) and 24 h, respectively, as determined by densitometric analysis. Data are representative of three experiments, each performed in triplicate.

## DISCUSSION

Type II IFNs have been shown to possess antiproliferative effects on various tumor cell types. Furthermore, these antiproliferative effects have been found to correlate with changes in tumor suppressor gene and oncogene expression (1, 2, 16, 17). IFN- $\gamma$  exerts its effects through the JAK/STAT signal transduction pathway, in which STAT1 molecules are phosphorylated and translocated to the nucleus, where they bind to  $\gamma$ -activated sites and activate transcription of target genes. Here we have shown that IFN- $\gamma$  exhibits strong antiproliferative effects on the prostate cancer cell lines DU145 and PC-3. Correspondingly, treatment of these cell lines with IFN- $\gamma$  resulted in an increased production of the CKI p21<sup>WAF1</sup> as compared with media control. Interestingly, IFN- $\gamma$  treatment of the prostate cancer cell line LNCaP did not result in any growth inhibition or increase in p21<sup>WAF1</sup> production. Examination of the JAK/STAT signal transduction pathway showed equal amounts of constitutive STAT1 produced in all cell lines after IFN- $\gamma$  treatment. However, IFN- $\gamma$ -treated LNCaP cells failed to produce any phosphorylated STAT1, whereas significant amounts were produced in both DU145 and PC-3 cells. Independent studies have shown that phosphorylated STAT1 molecules specifically recognize STAT-responsive elements in the p21<sup>WAF1</sup> promoter and are required for cell growth inhibition in response to IFN- $\gamma$  in various cell lines (18). This correlates with the lack of antiproliferative activity and failure to induce p21<sup>WAF1</sup> in LNCaP cells after IFN- $\gamma$  treatment.

In addition to increasing p21<sup>WAF1</sup> production, we have found that IFN- $\gamma$  treatment of DU145 and PC-3 cells results in a dose-dependent down-regulation of the neu/HER-2 proto-oncogene, although this was not seen in LNCaP cells. Recent studies have suggested that the transcriptional coactivator p300 may play a role in the regulation of neu/HER-2 (13). The adenovirus E1A protein was found to transcriptionally repress the neu/HER-2 promoter. Additional studies have shown that overexpression of p300 was able to overcome the repression of neu/HER-2 promoter activity by E1A (14). This repression was also overcome by a p300 mutant deficient in E1A binding. These studies suggest that E1A binding to p300 results in repression of neu/HER-2 promoter activity. In a separate study, phosphorylated STAT1 from IFN- $\gamma$ -treated cell extracts was found to bind to the E1A-binding domain of p300 (4). In light of this evidence, we looked at the effect of p300 overexpression on IFN- $\gamma$ -induced down-regulation of neu/HER-2 expression in PC-3(pRc/RSV-p300) cells. Inter-

estingly, the overexpression of p300 was sufficient to significantly overcome the repression of neu/HER-2 expression after IFN- $\gamma$  treatment, as seen in the case of neu/HER-2 repression by E1A. Thus, p300 appears to play a role in the expression of neu/HER-2. Furthermore, IFN- $\gamma$ -mediated repression of neu/HER-2 expression may occur via the interaction of phosphorylated STAT1 with p300 because phosphorylated STAT1 has been shown to bind to the E1A-binding domain of p300. This provides a mechanism by which IFN- $\gamma$  regulates neu/HER-2 expression and cell growth via phosphorylated STAT1 in addition to p21<sup>WAF1</sup> inhibition of cyclin-dependent kinases.

We subsequently found that the overexpression of p300 greatly reduced the antiproliferative activity of IFN- $\gamma$  on PC-3 cells, correlating with the derepression of neu/HER-2. However, this antiproliferative activity was restored after treatment with an increased concentration of IFN- $\gamma$ , thereby increasing the amount of phosphorylated STAT1 available for transcription of target genes. The overexpression of p300 in PC-3 cells also significantly inhibited the ability of IFN- $\gamma$  to increase p21<sup>WAF1</sup> production. Consistent with antiproliferation studies, IFN- $\gamma$ -induced increases in p21<sup>WAF1</sup> production were restored to levels seen in wild-type PC-3 cells after treatment with an increased concentration of IFN- $\gamma$ . Therefore, increased concentrations of IFN- $\gamma$  (and thus increased levels of phosphorylated STAT1) reversed the repressed IFN- $\gamma$ -induced activities requiring phosphorylated STAT1 in cells overexpressing p300. These studies correlate with the proposal of p300-phosphorylated STAT1 binding in response to IFN- $\gamma$  and inhibiting the function of both factors. All studies involving PC-3(pRc/RSV-p300) cells were repeated in PC-3 cells transfected with the empty vector, pRc/RSV. The results of these studies showed that PC-3(pRc/RSV) cells behaved in a manner similar to that of wild-type cells (data not shown).

In summary, we have shown that IFN- $\gamma$ -induced inhibition of prostate cancer cell line growth is accompanied by an increased production of the CKI p21<sup>WAF1</sup> and decreased expression of the proto-oncogene neu/HER-2. Our findings also implicate the transcriptional coactivator p300 in the positive regulation of neu/HER-2 gene expression. Furthermore, we propose that IFN- $\gamma$ -induced down-regulation of neu/HER-2 may involve the binding of phosphorylated STAT1 to p300, thereby inhibiting neu/HER-2 expression. Future studies will be directed at elucidating the interactions between this and other IFN- $\gamma$ -induced transcription factors and this coactivator.

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