Antisense RNA-mediated reduction of p53 induces malignant phenotype in nontumorigenic rat urothelial cells

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p53 mutation is commonly associated with high-grade, high-stage human urothelial carcinomas. Recent studies suggest that p53 mutation in low-grade, low-stage bladder carcinomas may be correlated with the progression of the disease. In the present study, we used antisense RNA methodology in vitro to evaluate the significance of the loss of p53 function at an early stage of urinary bladder carcinogenesis. An immortalized nontumorigenic rat urothelial cell line (MYP3) that strongly expresses wild-type (WT) p53 was transfected with a plasmid (pDL-SRα-296) containing a rat WT p53 cDNA in antisense orientation. The transfection resulted in a significant reduction in p53 mRNA expression and protein synthesis, in stimulation of anchorage-dependent growth, and in acquisition of anchorage-independent growth potential. Three such clones, when tested in athymic nude mice, all formed muscle-invasive, high-grade transitional cell carcinomas at s.c. injection sites. When cells were inoculated into an orthotopic site (urinary bladder), one of two antisense transfectants tested formed bulky tumors in the bladder in all seven nude mice and metastasizes to lungs in three of the seven mice. Analysis of these cells revealed a decrease in the expression of p21 (WAF1, sdi1, or CIP1) and retinoblastoma (RB) gene product. Phosphorylation of RB protein was not inhibited when the cells were starved. No significant difference was observed in the expression of p16 protein. In cell cycle analysis, all antisense transfectants tested escaped from G1 arrest by starvation. Furthermore, secretion of interleukin (IL)-6 into culture medium was increased significantly. Treatment with anti-IL-6 antibody suppressed anchorage-dependent growth. This study directly demonstrates that the loss of p53 function at an early stage of urothelial carcinogenesis may result in acquisition of a malignant phenotype by regulating IL-6 production as well as cell cycle related genes.

Introduction

The p53 gene was identified as a tumor-suppressor gene because of its ability to inhibit malignant transformation (1,2). Mutations or deletions of the p53 gene are the most frequent molecular event in human oncocarcinogenesis (2,3). In urinary bladder carcinogenesis, p53 gene deletion or mutation is associated with high-grade, high-stage human urothelial carcinomas (4–8), and it may be involved in the progression of superficial to deeply invasive bladder cancer (9). However, recent human and animal studies also indicate that p53 gene inactivation may occur in an early stage of bladder carcinogenesis (10,11), and that p53 gene mutation in carcinoma in situ or superficial bladder cancer is associated with a high rate of disease progression (12,13).

In the present study, to examine the significance of the early loss of p53 function in urinary bladder carcinogenesis in vitro, we used antisense RNA methodology. We introduced wild-type (WT*) p53 cDNA in an antisense orientation under the control of the SRt promoter into a rat nontumorigenic urothelial cell line, MYP3, that strongly expresses WT p53.

Materials and methods

Cells and cell culture

MYP3 is a hyperdiploid (44, XY, +1, +7) cell line which maintains the characteristics of epithelial cells in culture. The cells do not form tumors in nude mice, nor do they form colonies in soft agar (14). The cells were grown in Ham’s F12 medium (Life Technologies, Inc., Gaithersburg, MD) supplemented with 10% FCS (Life Technologies, Inc.), 10 µM non-essential amino acids (Life Technologies, Inc.), 2.7 mg/ml dextrose (Sigma, St Louis, MO), 1 µg/ml hydrocortisone (Sigma), 5 µg/ml transferrin, 10 µg/ml insulin, 10 ng/ml epidermal growth factor, 100 µg/ml streptomycin, and 100 U/ml penicillin (all from Life Technologies, Inc.) in a humidified atmosphere of 95% air and 5% CO₂ at 37°C. This medium was designated as complete medium. There is no activating mutation in the p53 gene in MYP3 when examined by polymerase chain reaction (PCR)-single-strand conformation polymorphism analysis (Okamoto and Oyasu, unpublished observations).

Preparation of rat WT p53 cDNA and plasmid construction

Rat WT p53 cDNA was prepared from mRNA of MYP3 cells by reverse transcriptase (RT)-PCR. The two nucleotide bases used were 5′-gaattcaggatgactactg-3′ as an up-stream primer and 5′-gaattcatggag-3′ as a down-stream primer (15). Additional nucleotides for EcoRI sites were linked to both primers at their 5′ end. One µg of total RNA was reverse-transcribed by Moloney murine leukemia virus reverse transcriptase (Life Technologies, Inc.) at 42°C for 60 min in a 10 µl mixture with the down-stream primer. One µl of RT mixture was subjected to PCR in a 20 µl mixture [10 mM Tris–HCl, pH 8.3, 50 mM KCl, 1.5 mM MgCl₂, 0.01% gelatin, 20 mM each dNTP (A,G,T,C), 0.5 U Taq polymerase (Cetus Perkin-Elmer, Norwalk, CT), and 0.25 pmol primer] (16). Forty cycles of reaction at 94, 55, and 72°C for 60, 90, and 150 s, respectively, were run in a DNA thermal cycler (Cetus Perkin-Elmer). The PCR products (1029 bp; exons 1–9) were digested with EcoRI and purified by preparative gel electrophoresis and the Gene clean II kit (BIO 101 Inc., La Jolla, CA). The purified fragment was ligated to the EcoRI site of pCDL-SRt296 (provided by Dr Yutaka Takebe) in sense and antisense orientation. This is a mammalian expression vector under the transcriptional control of the SRt promoter (the simian virus 40 early promoter and the R-U5 sequence from the long terminal repeat of human T-cell leukemia virus type I) (17). The insert as a rat WT p53 cDNA fragment was confirmed by nucleotide sequencing. These recombinant plasmids containing sense- or antisense-oriented rat WT p53 cDNA fragment were designated as pSRt-p53S and pSRt-p53AS, respectively (Figure 1A). The direction of the ligated fragment from the promoter was confirmed by restriction enzyme digestion with PstI (Life Technologies, Inc.).

Transfection

Cells (5×10⁵ cells/dish) were seeded in 100 mm culture dishes (Falcon, Lincoln Park, NJ) in the complete medium. Twenty-four h later, the cells were transfected with 20 µg of either pSRt-p53S or pSRt-p53AS mixed with 1 µg of a selectable vector, pSV2neo (provided by Dr Yusuke Ebina), by the polybrene method (18). The cells were incubated for 24 h in the
complete medium and then trypsinized and seeded at a 1:5 ratio in 100 mm culture dishes in the complete medium. Forty-eight h later, the cells were switched to a selective medium containing Geneticin (800 µg/ml G418; Life Technologies, Inc.). After 14 days of culture in the selective medium, G418-resistant clones were isolated and expanded in a 24-well cluster dish (Falcon).

Isolation of RNA and Northern blot analysis

Cells grown in monolayers were harvested at early confluence. RNA was prepared by lysing of cells in hypotonic buffer containing Nonidet P-40. Cells grown in monolayers were harvested at early confluency. RNA was isolated by oligo-dT cellulose (Collaborative Research, Bedford, MA). Cytoplasmic RNA (20 µg) or Poly A+ RNA (1 µg) was electrophoresed onto a formaldehyde/1.0% agarose gel, blotted onto a nylon filter (ICN Biomedicals, Irvine, CA), and hybridized with a 32P-labeled cDNA probes or RNA probes. The cDNA probes used were a 1.0 kb EcoRI-EcoR1 fragment of pSRtsp53AS for rat p53, and a 2.1 kb Xho1-Xho1 fragment of the pHEB-A1 for β-actin (American Type Culture Collection, Rockville, MD). Rat Interleukin (IL)-6 cDNA was prepared from mRNA of primary-culture stromal cells derived from rat bladder by RT-PCR as described above. The two nucleotide bases used were 5'-ATGAGTTCCTCCGAAAGAAC-3' as an up-stream primer and 5'-TTGCTCTGAATGCACCTCTGGTTG-3' as a down-stream primer (20). The PCR products (433 bp) were subcloned into the pCR™II vector with the use of a TA cloning kit (Invitrogen, San Diego, CA). The insert as a rat IL-6 cDNA fragment was confirmed by nucleotide sequencing. The 452 bp EcoRI1-EcoRI1 fragment containing rat IL-6 cDNA was excised from the vector and was used as a probe. A 1.0 kb EcoRI1-EcoRI1 fragment of pSRtsp53AS for rat p53 was ligated to the EcoRI site of pGEM-4Z (Promega, Madison, WI), and then sense- and antisense-oriented RNA probe for rat p53 was prepared with a commercial kit (Riboprobe Gemini II Core System, Promega).

Western blot analysis

Cells were starved by culturing in F-12 medium containing 0.1% FCS. Zero to 72 h later, cells were harvested by centrifugation, washed once with ice-cold phosphate-buffered saline (PBS), and lysed on ice for 30 min in 0.9% PBS containing 1% NP40, 10 µg/ml leupeptin, 10 µg/ml aprotinin, 2 mM 4-(2-aminoethyl) benzenesulfonyl fluoride, 1 mM sodium o-vanadate, 10 mM sodium fluoride, and 5 mM sodium pyrophosphate. The samples were centrifuged at 4°C for 15–20 min, and supernatants were transferred into new tubes. The samples (100 µg protein/lane) were electrophoresed on SDS-polyacrylamide gel. Proteins from gels were transferred to nitrocellulose (Bio-Rad, Hercules, CA), and p53, WAF1/p21, Rb, and p16 were detected, respectively, with mouse anti-p53 monoclonal antibody (Ab-1; Oncogene Science, Uniondale, NY), rabbit anti-WAF1 polyclonal antibody (Oncogene Science), mouse anti-retinoblastoma (Rb) gene product antibody (Trition Biosciences Inc., Alameda, CA), rabbit anti-p16 polyclonal antibody (M-156; Santa Cruz Biotechnology Inc., Santa Cruz, CA), and an ECL kit (Amersham, Arlington Heights, IL).

Cell cycle determination

Cells were starved by culturing in F-12 medium containing 0.1% FCS. Zero to 48 h later, cells were harvested by centrifugation, washed, and fixed in ice-cold 70% ethanol. Cells were then washed with PBS, treated with RNase (500 units/ml; Sigma) at 37°C for 15 min, and finally stained with propidium iodide at 50 µg/ml in PBS. Cell cycle analysis was performed with a fluorescence-activated cell analyzer (Coulter Co., Miami, FL) and fitting program, MOD-Fit (Verity Software House Inc., Topsham, MA).

Anchorage-dependent growth

Cells were grown for 24 h in a 96-well plate containing the complete medium. Then cells were cultured in F-12 medium containing 0.1% FCS in flat-bottom 96-well plates (Falcon) at 1.0×104 cells per well. After 0–4 days of culture, cell proliferation was assessed by addition of 20 µg of the vital dye 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT, 1 mg/ml; Sigma) for 4 h. The blue dye taken up by cells was dissolved in dimethylsulfoxide (100 µl/well), and its optical density at 495 nm was read on an automated microplate reader (Bio-Tec, Winooski, VT) (21). A preliminary study with the MTT assay showed that there was no quantitative difference in absorbance

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Fig. 1. Characterization of stably transfected p53AS cells. (A) The 1029 bp rat p53 cDNA (exons 1–9) was cloned into the EcoRI site of a mammalian expression vector, pcDL-SRts296, in antisense orientation under the transcriptional control of SRt2 promoter. These recombinant plasmids were designated as pSRts-p53AS. SV40, simian virus 40; ori, origin; ampR, ampicillin resistance; HTLV1 LTR, long terminal repeat of human T-cell leukemia virus type I. (B) and (C) Northern blot analysis for detecting sense (intronic) and antisense (introduced) p53 mRNA. Poly A+ RNA (1 µg/lane) was fractionated on 1.0% denaturing agarose gel, transferred to a nylon filter, and hybridized to 32P-labeled antisense-oriented (B) or sense-oriented (C) RNA probe for rat p53. (D) Western blot analysis for detection of p53 protein. Cell lysates (100 µg protein/lane) were electrophoresed on SDS-polyacrylamide gel, transferred to nitrocellulose, and stained with mouse anti-p53 monoclonal antibody. Lanes a, MYP3neo; lanes b, p53AS-1; lanes c, p53AS-3; lanes d, p53AS-4; lanes e, p53AS-C; lanes f, p53AS-E.
between MYP3 and its transfectants. Thus, we were able to use absorbance for estimating the number of cells without specific correction.

**Anchorage-independent growth**

Cells were seeded at a density 1 x 10^4 cells/35 mm dish in 2 ml of 0.3% Noble agar (Difco, Detroit, MI) in the complete medium. This suspension was layered over 2 ml of 0.6% agar in the complete medium in 35 mm dishes (Falcon). The cells were incubated in a humidified atmosphere of 95% air and 5% CO_2 at 37°C. One ml of 0.3% agar in the complete medium was added at days 9 and 18. After 28 days, colonies of >20 cells were counted.

**Tumorigenicity in athymic nude mice**

After trypsinization, cells were washed twice with Ham’s F-12 medium without serum and resuspended in 0.2 ml of the medium without serum or Matrigel. After 28 days, colonies of tumor cells were grossly recognizable and processed for routine histologic examination.

**Preparation of conditioned medium (CM) and enzyme-linked immunosorbent assay for IL-6**

Cells were grown to confluency (~2.5 x 10^6 cells/dish) in 100 mm dishes (Falcon) in the complete medium, washed 3 times with the serum-free F-12 medium, and cultured for an additional 48 h in 10 ml of the serum-free medium. The concentration of IL-6 in CM was measured by enzyme-linked immunosorbent assay with the use of polyclonal rabbit anti-human IL-6 (Genzyme) as a second antibody. Both antibodies are known to react to rat p53.

**Results**

**Isolation of antisense transfectants that express a reduced level of p53**

MYP3 cells were transfected with an expression vector containing antisense-oriented rat p53 cDNA under transcriptional control of the SRα promoter (Figure 1A). Five clones (p53AS-1, p53AS-3, p53AS-4, p53AS-C, p53AS-E) were isolated. They showed a marked reduction in both p53 mRNA and protein as compared to the control cells transfected with only pSV2neo (MYP3neo). Northern blot analysis with an antisense-oriented RNA probe for sense p53 mRNA demonstrated a marked reduction in the expression of p53 mRNA in all of these transfectants (Figure 1B). All transfectants also showed a significant decrease in p53 protein (Figure 1D) by Western blot analysis. We confirmed the expression of antisense transcripts of rat p53 in all five transfectants by Northern blot analysis with a sense-oriented RNA probe (Figure 1C). Unique integration of rat p53 cDNA was confirmed by PCR and Southern blot analysis (data not shown).

**Growth potential of antisense transfectants on plastic surface and in soft-agar culture**

All transfectants with introduced antisense RNA demonstrated higher cell counts than did MYP3neo [163%–173% of the control cells (MYP3neo; P < 0.001), counted on day 4] (Figure 2A). The cells transfected with sense-oriented rat p53 cDNA (p53S) showed a growth potential similar to that of MYP3neo (Figure 2A). In soft-agar assay, all transfectants with antisense RNA formed colonies (89–367/1 x 10^4 cells) (Figure 2B), whereas MYP3neo and p53S did not (Figure 2B). In soft agar (Figure 2), and MYP3neo as a negative control (5 x 10^5 cells) were injected s.c. at the dorsal flanks of nude mice with or without 50% Matrigel. MYP3neo did not form tumors. In contrast, all clones expressing the antisense RNA formed tumors with or without Matrigel (Table I). Microscopic grossly recognizable tumors and processed for routine histologic examination.

**Table I. Tumorigenicity of antisense-p53 transfectants in athymic nude mice**

<table>
<thead>
<tr>
<th>Cells</th>
<th>Tumors/mice injected</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>with Matrigel</td>
</tr>
<tr>
<td>MYP3neo</td>
<td>0/5</td>
</tr>
<tr>
<td>p53AS-3</td>
<td>5/5</td>
</tr>
<tr>
<td>p53AS-4</td>
<td>5/5</td>
</tr>
<tr>
<td>p53AS-C</td>
<td>5/5</td>
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Cells (5 x 10^6) were injected s.c. in dorsal flanks of nude mice with or without 50% Matrigel. The mice were monitored twice a week for appearance of tumors and were killed after 11–13 weeks.
Table II. Tumorigenicity and metastatic potential of antisense-p53 transfectants in heterotopically transplanted rat bladder (HTB) system in athymic nude mice

<table>
<thead>
<tr>
<th>Cells</th>
<th>Tumorigenicity in HTB</th>
<th>Lung metastasis</th>
</tr>
</thead>
<tbody>
<tr>
<td>MYP3neo</td>
<td>0/3</td>
<td>0/3</td>
</tr>
<tr>
<td>p53AS-3</td>
<td>7/7</td>
<td>3/7</td>
</tr>
<tr>
<td>p53AS-4</td>
<td>0/8</td>
<td>0/8</td>
</tr>
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</table>

Cells (5×10^6) suspended in 0.5 ml of serum-free medium were instilled into the nude mouse HTB which was pre-treated with 1.0% Nonidet P-40 and Matrigel to remove the preexisting normal epithelial cells as reported previously (22). The mice were killed 19–25 weeks after inoculation.

examination showed them to be high-grade transitional cell carcinomas.

The tumorigenicity of the cells was also tested at an orthotopic site, namely, in our unique nude-mouse HTB system. All seven mice receiving p53AS-3 cells formed bulky tumors (Table II and Figure 3A and B). They were high-grade, poorly differentiated transitional cell carcinomas which were deeply invasive through the muscle layer. Multiple foci of pulmonary metastasis were observed in three of the seven mice (Table II and Figure 3C). In contrast, p53AS-4 cells as well as control MYP3neo did not form tumors in either the bladder or the lungs.

Expression of IL-6 mRNA and IL-6 release into CM by antisense transfectants

Because p53 was reported to be involved as a transcriptional repressor of IL-6 gene expression during cellular differentiation and oncogenesis (23–25), we examined the effect of antisense transfection on the expression of IL-6 mRNA and protein. All antisense transfectants demonstrated IL-6 mRNA at significantly higher levels than did MYP3neo (Figure 4A). IL-6 was secreted into CM by p53AS-3, p53AS-4, and p53AS-C (289 ± 18, 216 ± 31, and 107 ± 10 pg/ml/48 h/5×10^6 cells, respectively) at much higher levels than that by MYP3neo or p53S (4.9 ± 0.6 and 3.9 ± 0.5 pg/ml/48 h/5×10^6 cells, respectively) (Figure 4B).

Effect of anti-IL-6 antibody on growth of antisense transfectants

We demonstrated in our previous report that MYP3 had IL-6 receptor and that its growth was stimulated by exogenous IL-6 (26). Therefore, we added polyclonal rabbit anti-human IL-6 antibody (50 µg/ml) to the medium of the antisense transfec-
tants. Its neutralizing activity as well as its specificity for IL-
6 have been confirmed by complete inhibition of the mitogenic effect of recombinant IL-6 (27; our own data, unpublished).

In all antisense transfectants tested, growth was suppressed significantly by the addition of anti-IL-6 antibody (P < 0.001, Figure 4C).

Expression of p53, Rb protein and the negative cell cycle regulators WAF1/p21 and p16

Both p53 and Rb proteins play a major role in the control of the cell cycle in G0–G1 phase. Expression of Rb is regulated directly at the transcriptional level (28,29) as well as indirectly (30) by p53. The underphosphorylated form of Rb, which exists as a complex with transcription factors E2F, is believed to be the functionally active form of Rb and to exert a negative effect on the cell cycle. Its phosphorylation releases E2F, and the unbound E2F stimulates transcription of cellular genes implicated in the induction of S phase (30). Rb protein is a target for the enzymatic activity of cyclin–cyclin dependent kinase (CdK) complexes. p21 is transcriptionally regulated by p53 and inactivates cyclin E-CdK2, cyclin A-CdK2, cyclin D1-, D2- and D3-CdK4 complexes, and consequently it inhibits Rb phosphorylation (30,31). p15, p16, and p21 also function as cyclin-dependent kinase inhibitors and contribute to cell cycle arrest by inhibiting phosphorylation of Rb.

We examined the cell lysates of the transfectants for the expression of p53, WAF1/p21, Rb protein and its phosphoryl-
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**Fig. 4.** Production and release of IL-6 by p53AS clones. (A) Northern blot analysis for detecting IL-6 mRNA by MYP3 and p53AS clones. Cytoplasmic RNA (20 µg/lane) was fractionated on 1.0% denaturing agarose gel, transferred to a nylon filter, and hybridized to 32P-labeled probe for rat IL-6. Lane a, MYP3neo; lane b, p53AS-3; lane c, p53AS-4; lane d, p53AS-C. (B) Secretion of IL-6 into CM. The levels of IL-6 were measured by ELISA. Bars denote SD of triplicate samples. *, P < 0.001 as compared with MYP3neo. (C) Effect of anti-IL-6 antibody on growth of p53AS clones. Polyclonal rabbit anti-IL-6 antibody (50 µg/ml) was added to cultures for assessment of its effect on the anchorage-dependent growth of p53AS clones. After 4 days of culture, cells were counted by MTT assay. Bars denote SD of triplicate samples. *, P < 0.001; #, P < 0.01 as compared with the respective untreated controls.

Fig. 5. Western blot analysis for p53 (A), and WAF1/p21 and Rb (B) protein. Cells were starved for 0–72 h in F-12 medium containing 0.1% FCS, and then cell lysates were isolated and electrophoresed on SDS-polyacrylamide gel (100 µg protein/lane). Proteins from gels were transferred to nitrocellulose, p53, WAF1/p21 and Rb were detected, respectively, with mouse anti-p53 monoclonal antibody, rabbit anti-WAF1 polyclonal antibody, mouse anti-Rb gene product antibody, and an ECL kit. (A) Arrowheads indicate p53 protein. (B) Arrowheads indicate hyperphosphorylated Rb protein (116 kDa), and arrows indicate hypophosphorylated Rb protein (110 kDa).

Fig. 6. Cell cycle analysis of p53 antisense transfectants. Cells were starved for 0–48 h in F-12 medium containing 0.1% FCS, harvested, and fixed in ice-cold 70% ethanol. Cells were then treated with RNase (500 units/ml) at 37°C for 15 min and finally stained with propidium iodide at 50 µg/ml in PBS. Cell cycle analysis was performed with a fluorescence-activated cell analyzer.
cytometric analysis. G1 arrest was observed in MYP3neo after starvation (G0-G1 fraction; 62.2% before starvation, 87.0% after starvation; Figure 6). In contrast, antisense transfectants showed an escape from G1 arrest by starvation (G0-G1 fraction; 43.5% and 46.4%, respectively, before and after starvation) in p53AS-3 or a minimal effect on cell cycle (44.9% and 60.7%, respectively, before and after starvation) in p53AS-4 (Figure 6). The findings were in keeping with the growth curves depicted in Figure 2.

Discussion

Mutations or deletions of the p53 tumor suppressor gene are the most frequent molecular event in human oncogenesis (2,3). Recent reports indicate that p53 gene mutations occur not only in the advanced stage of bladder cancer, but also in superficial (low-stage) bladder carcinoma, dysplasia, and carcinoma in situ and that superficial bladder cancer or carcinoma in situ with p53 gene mutations is associated with a high rate of disease progression (10–12). Although the above observation suggests a causal role of p53 alterations in the progression of the disease, evidence to support the suggestion is currently not available. The present study was an attempt to evaluate the significance of the loss of p53 function at an early stage of urinary bladder neoplasia. Our strategy was to utilize an immortalized non-tumorigenic rat urothelial cell line, MYP3, which strongly expresses WT p53, to inhibit p53 gene function by transfection with a plasmid containing a rat WT p53 cDNA in antisense orientation, and to evaluate the effects in vivo and in vitro. We observed a significant reduction of p53 mRNA expression and protein production, a marked increase in growth rate on a plastic surface, acquisition of anchorage-independent growth, and tumorigenicity and metastatic potential in nude mice. Thus, the marked alterations in biological behavior are impressive: our observations indicate that p53 is indeed a powerful suppressor of oncogenicity, and that its inactivation in a nontumorigenic urothelial cell line is sufficient to convert it to a fully malignant phenotype. In view of the fact that p53 is a transcription factor and mediates arrest of the cell cycle in the G1 phase after sublethal DNA damage, we examined the expression of some of the genes involved in the cell cycle. In the control cells, p53 protein level increased markedly from G1 arrest after starvation unlike control MYP3neo by cell cycle analysis.

We also examined the expression of IL-6 because p53 has been shown to be a transcriptional repressor in modulating IL6 gene expression and oncogenesis (23–25). The transfectants showed a marked increase in the secretion of IL-6 into the culture medium, and anti-IL-6 antibody inhibited their growth significantly. Thus, an antisense p53-mediated increase in IL-6 production is one of the genes altered by inactivation of p53 and may play an important role in bladder carcinogenesis. This mechanism has been suggested by us recently (26).

It is of considerable interest that one of the transformants, p53AS-3, formed invasive tumors in the orthotopic model (HTBs), whereas the other line, p53AS-4, did not, even though both were tumorigenic when tested at a heterotopic site (subcutaneous tissue). p53AS-3 formed more colonies in soft agar culture and secreted more IL-6 than did p53AS-4, and p53AS-4 expressed WAF1/p21 and Rb protein slightly more than did p53AS-3. Moreover, p53AS-3 escaped from G1 arrest more than did p53AS-4 when they were starved. It is unlikely, however, that these minor differences are responsible for the failure of p53AS-4 to form tumors at the orthotopic test site (HTBs). It appears that the gene alterations associated with p53AS-4 are sufficient to allow growth in subcutaneous tissue, but insufficient to do so in the urinary bladder. It is well known that inactivation of p53 by mutation or deletion frequently causes spontaneous transformation in a variety of cells (35,36). In our future investigations, we will attempt to identify the gene(s) that are altered by inactivation of p53 in vitro and in vivo.

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References


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