Regulation of expression of the multidrug resistance protein MRP1 by p53 in human prostate cancer cells

Gregory F. Sullivan,[1,2] Jin-Ming Yang,[1,2] Andrew Vassil,[2] Jun Yang,[2] Judy Bash-Babula,[2] and William N. Hait[1,2,3]

[1]Department of Pharmacology,
[2]The Cancer Institute of New Jersey, and
[3]Department of Medicine, University of Medicine and Dentistry of New Jersey/Robert Wood Johnson Medical School, New Brunswick, New Jersey, USA

Address correspondence to: William N. Hait, The Cancer Institute of New Jersey, University of Medicine and Dentistry of New Jersey/Robert Wood Johnson Medical School, 195 Little Albany Street, New Brunswick, New Jersey 08901, USA. Phone: (732) 235-8064; Fax: (732) 235-8094; E-mail: haitwn@umdnj.edu.

Gregory F. Sullivan and Jin-Ming Yang contributed equally to this work.

The expression of several drug-resistance genes, including *MRP* and *p53*, increases with advancing stage of human prostate cancer. Altered transcription could account for the genotypic alterations associated with prostate cancer progression, and it was recently reported that the promoter of *MRP*1 is activated in the presence of mutant p53. To determine whether there is a relationship between p53 status and the expression of MRP1, a human, temperature-sensitive p53 mutant (tsp Val[138]) was transfected into LNCaP human prostate cancer cells. In the transfected cell line (LVCaP), the wild-type p53 produced growth arrest at the G1/S interface of the cell cycle, inhibited colony formation, and induced p21[waf1/cip1]. Temperature shifting to 38°C (p53 mutant) produced a time-dependent increase in expression of MRP1. This change in MRP1 expression was also seen in isogenic cell lines in which p53 was inactivated by human papilloma virus (HPV)16E6 protein or by a dominant-negative mutant. Functional assays revealed a decrease in drug accumulation and drug sensitivity associated with mutant p53 and increased MRP1 expression. These results provide the first mechanistic link between expression of MRP1 and mutation of p53 in human prostate cancer and support recent clinical associations. Furthermore, these data suggest a mechanism tying accumulation of p53 mutations to the multidrug resistance phenotype seen in this disease.

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Introduction

We previously explored the relationship between prostate cancer progression and the expression of drug-resistance proteins (1). Immunostaining of prostate cancer specimens from 95 patients obtained at the time of surgical resection revealed an ordered change in expression of several proteins, including the multidrug-resistance protein (MRP1), p53, topoisomerase IIα, and Bcl-2 (1). We were struck by the almost parallel increase in p53 and MRP1 staining. This suggested the possibility that MRP1 and p53 might be coregulated. This hypothesis gained further support when MRP1 promoter activity was shown to be suppressed by wild-type p53 (2).

The effect of p53 status on drug resistance has been under intense investigation. For example, Lowe et al. found that mutant p53 favored resistance to a variety of anticancer agents through interference with apoptosis (3). In contrast, our laboratory demonstrated that mutant p53 favored sensitivity to taxanes and that this was mediated by increased expression of microtubule-associated protein 4 (MAP4) (4). p53 can also regulate the expression of Bcl-2, a likely contributor to drug resistance (5) and may also regulate transport proteins such as P-glycoprotein (P-gp). For example, Chin et al. demonstrated that mutant p53 activated the *MDR*1 promoter (6). However, derepression of the endogenous *mdr*1 gene (i.e., in vivo) by mutant p53 has not been consistently demonstrated (7). The *MDR*1 gene encodes for P-gp, a member of the ATP binding cassette family of transport proteins.

MRP1, a 190-kDa membrane-spanning protein that shares 15% amino acid homology with P-gp, is also a member of the ATP binding cassette family (8). MRP is an energy-dependent transporter of amphiphilic anions, particularly of glutathione, glucuronate, or sulfate conjugates of lipophilic substances (9, 10). Four members of the MRP family have been defined and some regulate drug sensitivity (8). MRP renders cancer cells resistant to natural-product anticancer drugs such as anthracyclines, vinca alkaloids, and epipodophyllotoxins (8) and is associated with therapeutic failure in the clinic.

To study the effect of p53 on MRP1 in vivo, we transfected human prostate cancer cells (LNCaP) with a human, temperature-sensitive p53 vector, tsp Val[138]. At 38°C, the transfectants express mutant p53, whereas
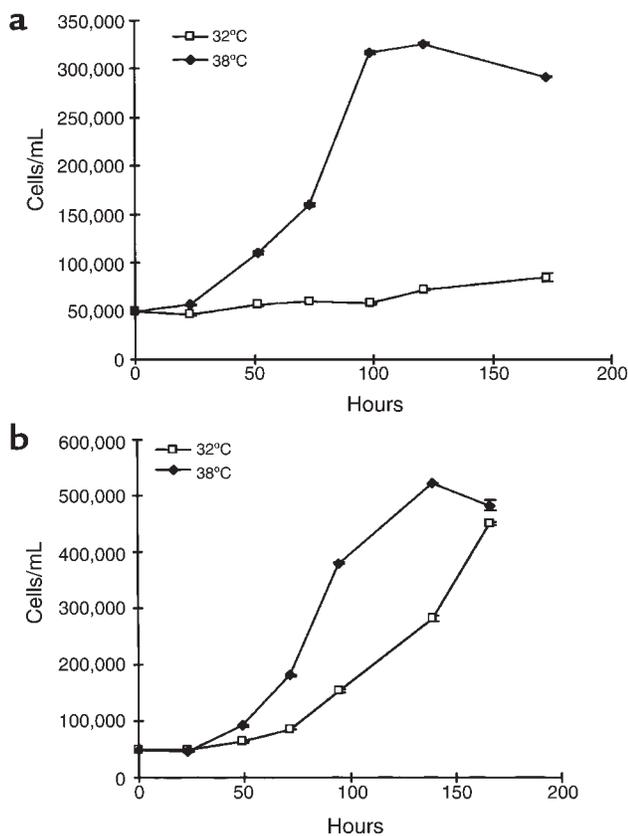
Figure 1

Effect of temperature on growth of LVCaP and LNCaP cells. Cells were plated at 3.5×10^4 cells/dish, allowed to grow for various periods, and then harvested and counted with an electronic counter. Each point represents the mean ± SD of triplicate cultures from a representative of three experiments. (**a**) LVCaP cells cultured at the permissive temperature (32°C; wild-type p53) and the restrictive temperature (38°C; mutant p53). (**b**) LNCaP cells cultured at 32°C and 38°C.

at 32°C these cells express a functionally wild-type protein (11, 12). We used the transfected cell line, LVCaP, to analyze the regulation of MRP1 by p53 in human prostate cancer.

Methods

Cell Culture. LNCaP.FGC 1740 cells frozen at passage 18 (American Type Culture Collection, Rockville, Maryland, USA) were routinely cultured in RPMI 1640 supplemented with 10% FBS, 15 mM HEPES buffer (pH 7.4), 2 mM L-glutamine and 100 µM nonessential amino acids (GIBCO BRL, Gaithersburg, Maryland, USA). LVCaP cells were routinely cultured in the LNCaP media supplemented with 50 µg/mL of Geneticin (GIBCO BRL).

The human melanoma cell lines, A875 and A875/E6, were kindly provided by M. Murphy (Fox Chase Cancer Center, Philadelphia, Pennsylvania, USA). The A875/E6 line harbors human papilloma virus (HPV)16E6. The human colon cancer cell lines RKO and RKO.mp53.13 were generous gifts from M. Kastan (St. Jude Children's Research Hospital, Memphis, Tennessee, USA).

RKO.mp53.13 was generated by transfecting parental RKO cells with a dominant-negative p53 mutant. Cells were maintained in a humidified atmosphere containing 5% CO_2/95% air and were free of contamination with mycoplasma or fungi.

Transfections. The pCMV p53 Val[138] vector was a generous gift from U. Moll (State University of New York at Stony Brook, New York, USA) and has been described previously (11). Amino acid 138 of the human p53 gene was mutated from alanine to valine (GCC → GTC). This mutation creates a temperature-dependent conformational change in the p53 protein so that it is functionally active at 32°C but not at 38°C. Transfections were carried out with 2 µg of the pCMV p53 Val[138] vector using Lipofectamine (GIBCO BRL) according to the manufacturer's instructions. After transfection, cells were allowed to recover for 48 hours before selection with 500 µg/ml of Geneticin. Colonies were selected and expanded in LVCaP media containing 15% FBS.

Cell growth. Four milliliters of LVCaP and LNCaP cells were plated at 3.5×10^4 cells/dish in 60×15 mm tissue culture dishes. After allowing the cells to attach to the plastic and grow for varying periods, cells were harvested by trypsinization and counted with an electronic counter (Coulter Corp., Miami, Florida, USA). Three determinations of cell number were made for each time point.

Clonogenic assay. LVCaP and LNCaP cells were plated at 5×10^2 cells/mL in 60×15 mm tissue culture dishes. After 2–4 weeks, media was aspirated and plates were incubated for 3 minutes in methylene blue (2.5 g of methylene blue trihydrate, 250 mL water, and 250 mL 95% ethanol). The stain was removed and plates rinsed in tepid running water. When dry, colonies were counted using a colony counting pen.
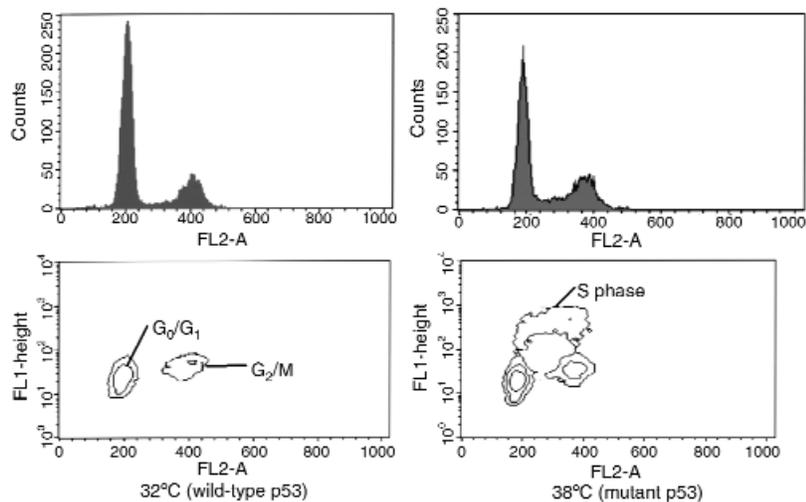
Cell-cycle analysis. Cell-cycle analysis was performed by measuring uptake of bromodeoxyuridine (BrdU) and staining of DNA with propidium iodide. Briefly, LVCaP and LNCaP were incubated with 10 µM BrdU for 1 hour at 37°C and harvested by trypsinization. Cells were washed in ice-cold PBS (137 mM NaCl [pH 7.4], 2.3 mM KCl, 10 mM Na_2HPO_4, and 1.8 mM KH_2PO_4), resuspended in 200 µL of PBS, and fixed by drop-wise addition of ice-cold 70% ethanol while vortexing. The cells were resuspended and incubated for 30 minutes in 2 N hydrochloric acid in PBS supplemented with 0.5% Triton-X100 and neutralized by rinsing once in 0.1 M sodium tetraborate (pH 8.5). Next, cells were resus-

Table 1

Effect of temperature on colony formation in LVCaP and LNCaP prostate cancer cell lines

Cell line	Temperature	Colonies (*n*)	Cloning efficiency (%)
LVCaP	32°C	0	0
LVCaP	38°C	240 ± 7	48
LNCaP	32°C	160 ± 5	32
LNCaP	38°C	190 ± 10	39

Each value represents the mean ± SD of six separate experiments.

Figure 2
Effect of temperature on cell-cycle distribution in LVCaP cells. Two-color cell cycle analysis was performed by measuring uptake of bromodeoxyuridine and staining with propidium iodide as described in Methods. DNA histograms and contour blots were generated on a Becton Dickinson FACScan analyzer.

pended with 50 μL of FITC-conjugated anti-BrdU antibody (Becton Dickinson Immunocytometry Systems, San Jose, California, USA) diluted 1:5 in PBS supplemented with 1% BSA and 0.5% Tween-20 and incubated for 30 minutes. Cells were rinsed and resuspended in 1 mL of PBS containing 5 μg/mL of propidium iodide. Fluorescence intensities were determined by quantitative flow cytometry and profiles were generated on a Becton Dickinson FACScan analyzer.

Immunoblotting. Cell pellets were lysed on ice for 30 minutes with RIPA buffer (10 mM sodium phosphate [pH 7.2], 1% Nonidet P-40, 1% sodium deoxycholate, 0.1% SDS, 150 mM NaCl, and 2 mM EDTA) supplemented with fresh 1% aprotinin, 1 mM phenylmethylsulfonyl fluoride, and 50 μg/mL leupeptin. After trituration through a 25-gauge needle, lysates were centrifuged at 14,000 g at 4°C for 10 minutes. Protein concentration was determined using the Bio-Rad Protein Assay Reagent (Bio-Rad Laboratories Inc., Hercules, California, USA). Proteins (50–100 μg) were resolved by SDS-PAGE and transferred to nitrocellulose membranes for detection of p53 and p21[waf1/cip1] or polyvinylidene difluoride membranes for detection of MRP1. The blots were incubated in blocking solution consisting of 5% milk and 3% BSA in PBS-T for 2 hours at 25°C and were then immunoblotted with monoclonal anti-human p53 protein clone DO7 (DAKO Corp., Carpinteria, California, USA), or WAF1 (Ab-1) (Calbiochem-Novachem, San Diego, California, USA), or anti-MRP1 antibody (QCRL-1; Signet, Dedham, Massachusetts, USA), or anti–β-actin antibody (Sigma Chemical Co., St. Louis, Missouri, USA). Detection by enzyme-linked chemiluminescence was performed according to the manufacturer's protocol (Amersham Life Sciences Inc., Arlington Heights, Illinois, USA). Relative expression of proteins was deter-

mined by densitometric analysis of autoradiographs using Molecular Analyst Software on a Molecular Imager FX system (Bio-Rad Laboratories, Inc.).

Northern blot analysis. RNA was prepared using Trizol Reagent according to the manufacturer's protocol (GIBCO BRL). Twenty micrograms of total RNA from each sample were electrophoresed, blotted onto nitrocellulose, and probed for *MRP*1. An α-^{32}P-labeled β-actin probe was used to measure RNA loading. Relative expression of mRNA was determined by densitometric analysis of autoradiographs using Molecular Analyst Software on a Molecular Imager FX system.

Leukotriene C_4 accumulation. Cells were seeded in 24-well plates and grown at 37°C (LNCaP) or 32°C (LVCaP). When cells were 80% confluent, LVCaP cells were shifted to 38°C and incubated for 60 hours. The growth medium was aspirated and replaced with 0.5 mL of RPMI 1640 containing 25 mM HEPES (pH 7.4) and 50 nM of [3H]leukotriene C_4 (NEN Life Science Products Inc., Boston, Massachusetts, USA). Cells were incubated with [3H]leukotriene C_4 for 2 hours and were then cooled on ice, washed three times with ice-cold PBS, and solubilized with 0.25 mL of 1% SDS. The radioactivity in each sample was determined by scintillation counting.

Table 2
Sensitivity of LNCaP and LVCaP cells to vincristine and doxorubicin at 38°C

	IC_{50} (nM)	
Drug	LNCaP	LVCaP
vincristine	8.3 ± 0.9	240 ± 43[A]
doxorubicin	180 ± 0.5	360 ± 28[A]

Each value represents the mean ± SD of three separate experiments. [A]P < 0.05 versus LNCaP cells.
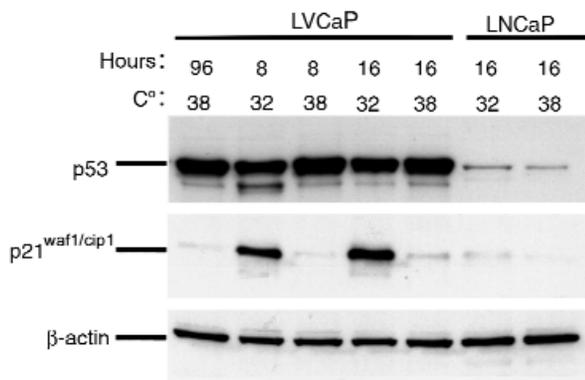
Figure 3

Effect of temperature on the expression of p53 and p21[waf1/cip1] in LVCaP and LNCaP cells. Identical amounts (50 μg) of total protein were resolved using a 15% SDS-polyacrylamide gel, transferred to nitrocellulose, and probed with monoclonal anti-human p53, monoclonal anti-human β-actin, and monoclonal anti-human p21[waf1/cip1] antibodies as described in Methods. Results are representative of three similar experiments.

Drug sensitivity. Exponential growing cells were plated into 96-well tissue culture plates at a density of 1×10^4 cells per well. Various concentrations of drugs were added to each well, and the plates were incubated at 38.5°C for 72 hours. Cell viability was measured by the MTT assay (4).

Results

Growth characteristics of cells expressing temperature-sensitive p53. To determine whether or not p53 status affected expression of MRP, we generated a stable clone expressing a temperature-sensitive p53 mutant (tsp Val[138]). Figure 1a demonstrates that at the restrictive temperature (38°C; mutant p53), the LVCaP cells proliferated with a log-phase doubling time of 28 hours. In contrast, proliferation was inhibited at the permissive temperature (32°C; wild-type p53) (Figure 1a). The parental cell line, LNCaP, expresses wild-type p53 (13) and proliferated at both the permissive and the restrictive temperatures; log-phase doubling times were 48 and 32 hours, respectively (Figure 1b).

Temperature shift also affected clonogenicity. As shown in Table 1, at the restrictive temperature (38°C; mutant p53) LVCaP cells had a 48% cloning efficiency. In contrast, shifting to the permissive temperature (32°C; wild-type p53) led to a complete loss of clonogenic capacity. Temperature shifting had no significant effect on the clonogenicity of LNCaP cells (Table 1).

Effect of p53 on cell-cycle distribution and related gene expression. We determined the effect of temperature shifting on cell-cycle distribution by BrdU uptake and propidium iodide staining. As seen in Figure 2, incubation of LVCaP cells at the permissive temperature (32°C; wild-type p53) produced G1/S arrest; the cells returned to a normal cell-cycle distribution when shifted to the restrictive temperature (38°C; mutant p53).

In contrast, temperature shifting produced no significant differences in cell-cycle distribution in the parental LNCaP cell line (data not shown).

To confirm that the G1/S arrest seen in LVCaP cells was p53-mediated, we studied the effect of temperature shifting on p21[waf1/cip1]. As seen in Figure 3, the expression of p21[waf1/cip1] was markedly increased when LVCaP cells were shifted from the restrictive temperature (38°C; mutant p53) to the permissive temperature (32°C; wild-type p53). p21[waf1/cip1] was barely detectable at 38°C in LVCaP cells, increased greatly at 8 hours, and increased further after 16 hours of culture at 32°C (Figure 3). Temperature shifting had no effect on the expression of p21[waf1/cip1] in the parental cell line (Figure 3).

Wild-type p53 represses the expression of genes such as *Bcl-2* (14) and *MAP4* (15). As shown in Figure 4, the expression of Bcl-2 protein was low in LVCaP cells cultured at the permissive temperature (32°C; wild-type p53) and increased after shift to the restrictive temperature (38°C; mutant p53).

Effect of functional status of p53 on expression of MRP1. LVCaP cells cultured at the permissive temperature (32°C; wild-type p53) expressed low levels of MRP1 protein (Figure 5). Shifting LVCaP cells to the restrictive temperature (38°C; mutant p53) increased the expression of MRP1 9-fold over a 60-hour period (Figure 5). In contrast, the parental cells (LNCaP) did not express MRP1 at either the restrictive or the permissive temperatures (Figure 5). To assess whether p53 affected MRP1 expression at the RNA level, we isolated total cellular RNA from LVCaP and LNCaP cells incubated at 38°C or 32°C for varying periods and measured MRP1 mRNA by Northern analysis. Figure 6 shows that growth of LVCaP cells at 38°C increased the expression of MRP1 mRNA; from 12 hours to 48 hours, there was a 2.4-fold increase in the expression of MRP1 mRNA. MRP1 mRNA was low in LNCaP cells and did not change with temperature shift.

To substantiate the relationship between p53 function and MRP1 expression, we measured the expression of MRP1 in additional paired human cancer cell lines in
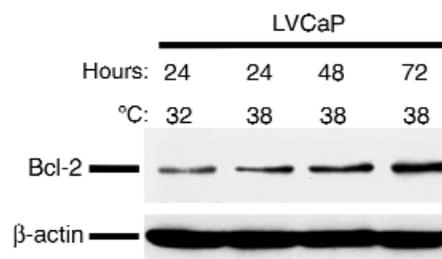
Figure 4

Effect of temperature on the expression of Bcl-2 in LVCaP cells. Identical amounts (100 μg) of total protein were resolved using a 15% SDS-polyacrylamide gel, transferred to nitrocellulose, and probed with monoclonal anti-human Bcl-2 and monoclonal anti-human β-actin antibodies as described in Methods. Results are representative of three similar experiments.
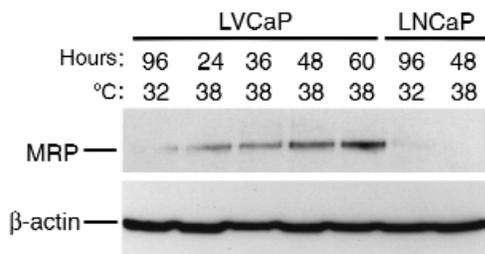
Figure 5

Effect of temperature on the expression of MRP1 in LVCaP and LNCaP cells. Identical amounts (100 μg) of total protein were resolved using a 6% SDS-polyacrylamide gel, transferred onto a polyvinylidene difluoride membrane, and probed with monoclonal anti-human MRP1 and monoclonal anti-human β-actin antibodies as described in Methods. Results are representative of three similar experiments.

which one member of the pair harbors a transcriptionally inactive p53. In the A875/E6 melanoma cell line, p53 is degraded by HPV16E6. In the RKO.mp.53.13 colon cancer cell line, p53 is inactivated through transfection with a dominant-negative mutant (16). Figure 7 demonstrates that inactivation of p53 resulted in a doubling of MRP1 protein level in A875/E6 cells and a 14-fold increase of the protein in RKO.mp.53.13 cells.

Effect of MRP1 expression on drug accumulation and sensitivity. To determine whether the increased expression of MRP1 seen with mutant p53 had functional consequences, we studied the accumulation of an MRP1-specific substrate (leukotriene C$_4$) using the LVCaP model. As shown in Figure 8, the cellular accumulation of leukotriene C$_4$ was significantly reduced in LVCaP cells grown at 38°C, compared with that in LNCaP cells ($P < 0.01$; Figure 8a) or compared with that in LVCaP cells growing at 32°C ($P < 0.01$; Figure 8b). Temperature shifting had no effect on drug accumulation in LNCaP cells (data not shown). Similar results were obtained with doxorubicin, another MRP1 substrate (data not shown).

Increased expression of MRP1 also changed the sensitivity to chemotherapeutic drugs that are transported by this protein. At 38°C (restrictive temperature; mutant p53), LVCaP cells were 29-fold more resistant to vincristine compared with LNCaP cells (IC$_{50}$ 240 ± 43 vs. 8.3 ± 0.9 nM) and were twofold more resistant to doxorubicin (IC$_{50}$ 360 ± 28 vs. 180 ± 0.5 nM) (Table 2). LVCaP cells did not proliferate at 32°C (permissive temperature, wild-type p53), making comparisons of drug sensitivity inaccurate.

Discussion

The hypothesis that mutation of p53 might contribute to drug resistance in prostate cancer was suggested by our analysis of drug-resistance proteins during the progression of human prostate cancer (1). To test this hypothesis, we created the LVCaP cell line by stable transfection of LNCaP cells with a vector containing a temperature-sensitive p53 mutant. The Val[138] mutation is in the DNA

binding region of p53 and abrogates DNA binding at the restrictive temperature by altering the conformation of the tertiary structure of the protein (11).

LVCaP cells display the anticipated phenotype after temperature shifting. At the permissive temperature, p53 adopts a wild-type conformation and is functionally active (11, 12). This was confirmed in the LVCaP cell line as measured by induction of p21[waf1/cip1] (Figure 3). p21[waf1/cip1] is part of the G1/S cell-cycle checkpoint and overexpression of this protein in LVCaP cells produces G1/S arrest (Figure 2) and inhibition of cell proliferation (Figure 1). Induction of p21[waf1/cip1] also markedly inhibited the ability of LVCaP cells to form colonies (Table 1), but did not produce apoptosis (Figure 2). This pattern of growth arrest, failure to form colonies, yet lack of initiation of apoptosis, can be explained by the expression of the antiapoptotic protein, Bcl-2, in this cell line (Figure 4). In addition, these properties make the LVCaP model a particularly useful one for the study of both biologic and pharmacological consequences of p53 alterations.

We have previously found that both the expression of MRP1 and p53 increased with advancing stage in human prostate cancer specimens (1). However, our previous measurements of p53 in human prostate cancer specimens were done by immunohistochemistry, which does not definitively discriminate between the mutant and wild-type protein. Rather, the detection of p53 by immunohistochemistry correlates well with the presence of the mutant protein because of its increased half-life. Using the LVCaP model, we find that MRP1 expression is regulated by the transcriptional activity of p53. Thus, when p53 is wild-type, the expression of MRP1 is repressed (Figure 5), and when p53 is mutant, the expression of MRP1 increases (Figure 5). Similar observations were made in paired, isogenic, human melanoma and colon cancer cells in which p53 was inactivated either by HPVE6-induced degradation or by transfection with a dominant-negative p53 mutant (Figure 7). The level
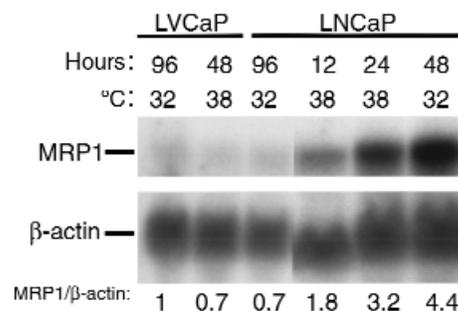
Figure 6

Analysis of MRP1 mRNA expression by Northern blot. LVCaP and LNCaP cells were cultured at 32°C or 38°C for varying periods. Twenty micrograms of total RNA from each sample were electrophoresed, blotted onto nitrocellulose, and probed for MRP1. An α-^{32}P-labeled β-actin was used to determine RNA loading.
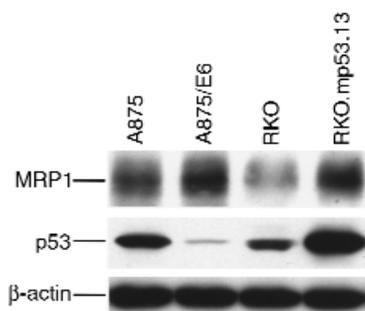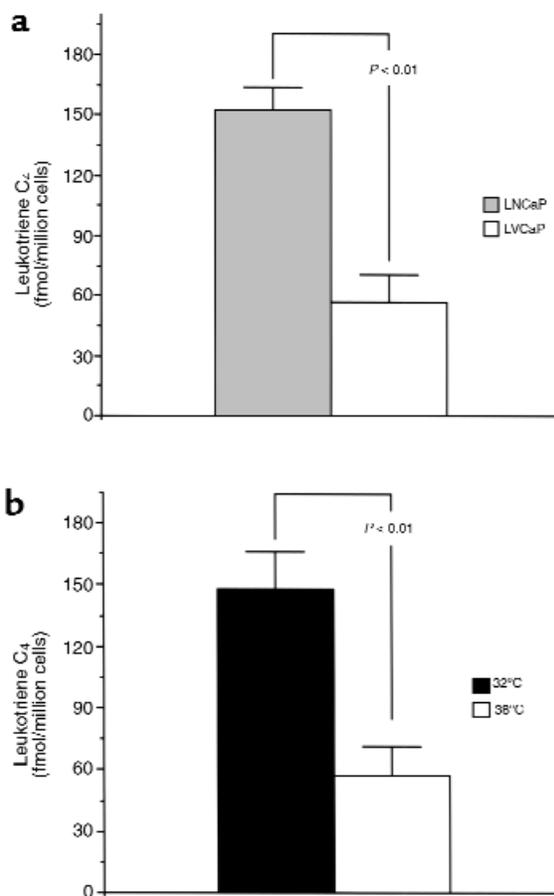
Figure 7

Effect of inactivation of p53 on the expression of MRP1 in human melanoma A875 cells and colon carcinoma RKO cells. Identical amounts (50 μg) of total protein were resolved using a 6% SDS-polyacrylamide gel, transferred onto a polyvinylidene difluoride membrane, and probed with monoclonal anti-human MRP1, monoclonal anti-human p53, and monoclonal anti-human β-actin antibodies as described in Methods.

of control of MRP1 by p53 appears to occur at least in part at the mRNA level as a shift from transcriptionally active to inactive p53 produced increases of MRP1 mRNA (Figure 6). These data are consistent with the work by Wang and Beck, who demonstrated the ability of wild-type p53 to suppress the promoter activity of MRP1 (2). Finally, p53 mutation and overexpression of MRP1 protein leads to increased function of the transporter as measured by the accumulation of leukotriene C_4 (Figure 8), an MRP1-specific substrate and the decreased sensitivity to vincristine and doxorubicin (Table 2). The decreased sensitivity to drugs was not due to changes in *MDR*1 gene expression, as P-gp was not detectable in either cell line (G.F. Sullivan and W.N. Hait, unpublished observations).

The significance of these results is strengthened by several factors. First, the relationship between p53 and MRP1 expression was originally suggested by studies done in human cancer specimens including prostate (1), colorectal (17) and non-small cell lung cancer (18). Second, unlike previous reports on the regulation of MRP1 and MDR1 that focused on activation of promoter/reporter constructs (2, 6), our results demonstrate that p53 can regulate the expression of the endogenous *MRP*1 gene, producing changes in both mRNA and protein (Figures 5, 6, and 7). The promoter regions of ABC transporters are complex, and no obvious p53-binding motif has been reported. However, repression of transcription by wild-type p53 may occur through indirect means rather than direct binding to promoter or enhancer elements (19). For example, Murphy et al. demonstrated that a microtubule-associated protein, MAP4, was transcriptionally repressed by wild-type p53, yet MAP4 does not contain an obvious p53 binding motif (15). They went on to show that a complex of wild-type p53, mSin3a, and histone deacetylase (20) mediated repression of MAP4 transcription. Third, inactivation of p53 by HPVE6 protein or by expression of a dominant-negative mutant in iso-

genic melanoma and colon carcinoma cell lines produced similar effects on MRP1 (Figure 7).

Because p53 mutations are found in approximately 50% of human cancers, this suggests that upregulation of MRP1 might be part of a cellular response to conditions of oxidative stress in which the likelihood of fixing mutations into the genome is increased. The fact that MRP1 participates in the efflux of glutathione-, glucuronate-, and sulfate- conjugated lipophilic xenobiotics and endotoxins has implicated it in a redox-controlled detoxification pathway (21–23). We also observed that the expression of GSTπ is rapidly lost as prostate epithelium undergoes malignant transformation (1). This is believed to be a consequence of hypermethylation of cytidine residues in the regulatory

Figure 8

Leukotriene C_4 accumulation in LNCaP and LVCaP cells. Cells seeded in 24-well plates were grown at 37°C (LNCaP) or 32°C (LVCaP). When cells were 80% confluent, LVCaP cells were shifted to 38°C and incubated for 60 hours. Cells were then incubated with [³H]leukotriene C_4 for 2 hours at 37°C (LNCaP) or 38°C (LVCaP). At the end of incubation, cells were cooled on ice, washed three times with ice-cold PBS, and solubilized with 0.25 mL of 1% SDS. The radioactivity in each sample was determined by scintillation counting. (**a**) Comparison of leukotriene C_4 accumulation in LVCaP to that in LNCaP cells grown at 38°C. (**b**) Comparison of leukotriene C_4 accumulation in LVCaP cells grown at 32°C to that in the same cells grown at 38°C.

sequences of the GSTπ promoter (24, 25). The loss of GSTπ can create a pro-oxidant cellular environment by shutting down a major phase II detoxification enzyme in the cancer cells (26). Because of this, many xenotoxic and endotoxic compounds may remain in a mutagenic and electrophilic state ready to interact with nucleophilic substrates, such as DNA and protein (27). In this biotransformed state, these toxins cannot be transported out of the cell by MRP1 and pose an increased threat to the stability of the genome. Therefore, the upregulation of MRP1 might represent a required compensation by cancer cells.

In summary, our results indicate that the functional status of p53 can regulate the expression of MRP1 in human prostate cancer cells. Because p53 mutations become increasingly frequent as prostate cancer advances in stage, this observation may explain one component of the drug-resistance phenotype of this common malignancy.

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