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ORIGINAL ARTICLE

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Modulation of multidrug resistance-associated protein 1 (MRP1) by p53 mutant in Saos-2 cells

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Abstract The p53 tumor suppressor gene is one of the most frequently mutated genes in human cancer and the mutation is correlated with a poor prognosis in cancer therapy. Upregulation of multidrug resistance-associated protein 1 (MRP1) and increase in drug resistance have been found to be induced by p53 mutation. Human osteosarcoma Saos-2 cells, a p53-null cell line, was transfected with p53 with mutations at codon 143 (V to A), 175 (R to H), 248 (R to W), 273 (R to H) and 281 (D to G). Among the different transfectants, overexpression (about 42-fold) of MRP1 was detected in p53-R175H cells. Furthermore, the p53-R175H cells were 2.5-fold more resistant to doxorubicin (DOX) and had a 4-fold greater DOX efflux rate than the control cells 1h after DOX treatment. Transfection with antisense MRP1 oligonucleotides demonstrated a DOX sensitization effect (about 2-fold) in p53-R175H transfectants but not in control cells. In addition, transfection with antisense p53 oligonucleotides greatly suppressed MRP1 expression and reversed DOX resistance in p53-R175H cells but had no effect in control cells. The results suggested that p53-R175H might induce MRP1 expression and DOX resistance in cells.

Keywords MRP1 · Drug resistance · p53 mutation · Doxorubicin

Introduction

The occurrence of drug resistance is one of the main obstacles in cancer therapy. The resistance is mediated by several mechanisms. For example, inactivation or downregulation of topoisomerase II, upregulation of multidrug resistance-related proteins, e.g. P-glycoprotein (Pgp) and multidrug resistance-associated protein 1 (MRP1) [1, 2, 3], detoxification enzymes, e.g. glutathione-S-transferase π (GSTπ) [4, 5], and DNA damage-repair enzymes, e.g. ERCC1 and ERCC2 [6].

MRP1 was discovered in 1992. It is a 190-kDa transmembrane protein which belongs to the ABC transporter superfamily and is classified as ABCC1 [1, 7, 8, 9]. MRP1 has three multitransmembrane domains and shares 15% amino acid identity with Pgp. The protein acts as an ATP-dependent drug efflux pump mediated by glutathione (GSH). Transfection of cells with MRP1 cDNA increases their resistance to a wide variety of drugs such as anthracyclines, vinca alkaloids and epipodophyllotoxins [10]. Cells with MRP1 overexpression demonstrate a decrease in drug accumulation and marked changes in intracellular drug distribution [10]. This suggests that drug resistance as a result of MRP1 overexpression may correlate with a greater drug efflux in cells.

The expression of MRP1 is known to be regulated by different mechanisms. Induction of MRP1 has been detected in cells exposed to prooxidants such as *tert*-butylhydroquinone or cytokines such as interleukin-6 [11, 12, 13]. Antisense suppression of N-myc, however, results in downregulation of MRP1 in neuroblastoma cells. Involvement of N-myc in the MRP1 regulatory pathway is therefore suggested [14]. Recent studies have revealed that wild-type p53 demonstrates a transcription suppression effect on *mrp1* promoter activity [15]. However, the temperature-sensitive p53 mutant (Val138) has been found to induce MRP1, and to decrease drug accumulation and drug sensitivity in human LNCaP prostate cancer cells [16]. The tumor suppressor gene p53 is frequently mutated in human cancers. Loss of wild-type p53 protein function by frequent mutation is related to tumor progression and acquired drug resistance in cancer therapy [17, 18, 19, 20, 21, 22].

In contrast to the temperature-sensitive p53 mutant (Val138), the relationship between MRP1 expression and

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various p53 mutants common in human cancers had previously not been investigated and was therefore the subject of the present study. Human osteosarcoma Saos-2 cells, a p53-null cell line, were transfected with five different p53 mutants (V143A, R175H, D248W, R273H and D281G). Among the transfectants, MRP1 was found to be overexpressed in p53-R175H cells. The p53-R17H transfectants were also found to be more resistant to doxorubicin (DOX) and showed a higher rate of DOX efflux than cells transfected with the control vector. Subsequent experiments including MRP1 and p53 antisense oligonucleotides indicated that the DOX resistance in Saos-2 cells might be mediated through the p53-R175H and MRP1 pathway.

Materials and methods

Cell lines

The human osteosarcoma Saos-2 mutant p53 transfectants were a gift from Dr. A.J. Levine, Rockefeller University. The Saos-2 cells were transfected with pCMV-Bam-Neo vector alone as the control or vectors carrying the p53 gene with mutations at codon 143 (V to A), 175 (R to H), 248 (R to W), 273 (R to H) or 281 (D to G) [23]. All transfected cell lines were grown in Dulbecco's modified Eagle's medium (DMEM) with 10% fetal bovine serum (Invitrogen, Carlsbad, Calif.) and 200μg/ml G418 (Calbiochem, La Jolla, Calif.) at 37°C in a humidified incubator under an atmosphere containing 10% CO_2. The doubling time of all these cells was about 28h.

MTT cell viability assay

Saos-2 cells were seeded in a 96-well plate for 24h and then exposed to various concentrations of DOX for 48h. At the end of the incubation with drug, the cells were incubated in 50μl of a 0.1mg/ml solution of 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) at 37°C for 3h and lysed in 150μl dimethyl sulfoxide at room temperature for 30min. The absorbance of each well was measured at 580nm in a microplate reader.

RNA preparation and reverse transcription-polymerase chain reaction (RT-PCR)

Cells were lysed with TRI reagent (Molecular Research Center, Cincinnati, Ohio). Total RNA was extracted with 1-bromo-3-chloropropane reagent (Molecular Research Center), precipitated with isopropanol and solubilized in RNase-free water. The first cDNA was synthesized by 5μg total RNA in the presence of oligo-$dT_{12–18}$ primer (Invitrogen) and AMV reverse transcriptase (Promega, Madison, Wis.). Amplification of a 515-bp MRP1 cDNA fragment was performed with the MRP1-specific forward primer 5′-AGGAGGCGCCCTGGCAAATC-3′ (residues 4011–4030) and the MRP1-specific backward primer 5′-GGCCCGGGCTAGGCACACAA-3′ (residues 4506–4525) [24]. Thermocycling was carried out as follows: 94°C for 1min and 35 amplification cycles of 94°C for 30s, 55°C for 40s and 72°C for 1min, followed by 72°C for 5min. The amplification products were resolved on 1.2% agarose gel and purified as a probe for Northern blot analysis.

Northern blot analysis of MRP1 mRNA levels

Total RNA (5μg) was run in 1% agarose/7*M* formaldehyde denaturing gel and transferred to a nylon membrane (Amersham Pharmacia Biotech, Little Chalfont, UK). The cDNA probes amplified by the RT-PCR reaction were labeled with a Redprime II random prime labeling system (Amersham Pharmacia Biotech) using [^{32}P]dCTP. Hybridization was performed at 65°C for 2h using Rapid-hyb buffer (Amersham Pharmacia Biotech). After stringent washes, the pattern of mRNA expression was visualized by exposure of membranes to X-ray film with an intensifying screen at −80°C. The signal of each band was scanned and quantitated by Kodak Digital Science 1D image analysis software version 3.0 (Eastman Kodak, New York, N.Y.). The expressions of the bands in each experiment were first normalized by the levels of the corresponding 28S rRNA. The relative expression of each band was then calculated with respect to the expression of the control cells designated as 1.

Western blot analysis of MRP1 and p53 protein levels

Cells were lysed in Laemmli's lysis buffer containing 1% Triton-X 100 and scraped using a cell lifter. Protein (30μg) was resolved by denaturing 8% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to an Immobilon-P membrane (Millipore, Bedford, Mass.). Membranes were blocked with 5% non-fat dried milk at 4°C overnight and incubated with goat polyclonal anti-MRP1 antibody (Santa Cruz Biotechnology, Santa Cruz, Calif.) or mouse monoclonal anti-p53 antibody (Calbiochem) at room temperature for 2h. Membranes were then washed three times with 1X phosphate-buffered saline (PBS) in 0.1% Tween-20 and incubated with the respective secondary antibody conjugated with horseradish peroxidase at 1:10,000 dilution (Santa Cruz Biotechnology). The signals were visualized by enhanced chemiluminescence (ECL, Amersham Life Science, Little Chalfont, UK), scanned and quantitated by Kodak Digital Science 1D image analysis software version 3.0. The relative expression of each band in each experiment was calculated by designating the expression of the control cells as 1.

DOX efflux analysis

After incubation with 8.62μM DOX for 1h, cells were incubated in drug-free medium for up to 8h. At the indicated times, the cells were trypsinized, washed once with 1XPBS, and resuspended in serum-free phenol-red-free DMEM. The cellular levels of DOX were assessed in terms of fluorescent intensity with excitation at 488nm and emission at 570nm on a FACSort flow cytometer (Becton Dickinson, Mountain View, Calif.). A total of 10,000 events were collected in each analysis. Data acquired were analyzed by WinMDI version 2.7 software and the median channel value for the fluorescent intensity profile of each treatment was recorded. DOX efflux (as a percentage) was calculated as: (D0−Dt)/D0, where Dt represents the median channel values of cells at various times after DOX treatment and D0 represents the median channel value of cells immediately after DOX treatment.

Transient transfection with antisense MRP1 and p53 oligonucleotides

The 20-base antisense oligonucleotides complementary to the coding region of MRP1 (5′-TGCTGTTCGTGCCCCCGCCG-3′) or p53 (5′-CCCTGCTCCCCCTGGCTCC-3′) mRNA were used [14, 25]. Sense oligonucleotides with sequences complementary to the antisense oligonucleotides were used as the control. All bases were phosphorothioated to increase the stability of the oligonucleotides (Biobasic, Canada). The cells were washed twice with serum-free DMEM before transfection with oligonucleotides. Oligonucleotides at a concentration of 200n *M* were allowed to form complexes with oligofectamine (Invitrogen) in serum-free DMEM for 20min at room temperature. The cells were then incubated with oligofectamine/oligonucleotide complex for 4h at 37°C. After transfection, the cells were incubated in complete medium for drug treatment.

Results

Overexpression of MRP1 in Saos-2 p53-R175H transfectants

To determine the differences in the expression of MRP1 between the p53 mutants, the mRNA and protein steady-state levels of MRP1 in Saos-2 cells were determined by Northern and Western blot analyses, respectively. Transfection of Saos-2 cells with p53-R175H mutant led to a 6-fold increase in MRP1 mRNA levels and a 42-fold increase in MRP1 protein levels, while there were no changes with the other transfectants (Fig. 1).

MRP1 overexpression correlated with DOX resistance and enhanced DOX efflux in p53-R175H cells

DOX is a common antitumor drug that intercalates into nucleic acids and inhibits DNA synthesis. Since overexpression of MRP1 is known to be associated with DOX resistance and increased DOX efflux in cells, the DOX sensitivity and efflux in p53-R175H cells were examined in parallel with control cells. Saos-2 cells transfected with p53-R175H appeared to be about 2.5-fold more resistant to DOX than cells transfected with the control vector (Fig. 2A). The IC_{50} of DOX for Saos-2 control cells was 0.2μM, whereas it was 0.49μM for p53-R175H transfectants. One of the features of cells showing MRP1-associated drug resistance is an increase in drug efflux rate. Flow cytometric analysis of Saos-2 cells which had been exposed to DOX for 1h and then incubated in drug-free condition revealed that the intracellular DOX content dropped more rapidly in p53-R175H transfectants than in control cells: for the first hour after drug exposure, the efflux rate was fourfold faster from p53-R175H cells than from control cells (Fig. 2B). These results suggest that MRP1 overexpression induced DOX resistance in p53-R175H cells. To confirm these findings, the effect of antisense MRP1 oligonucleotides on the DOX sensitivity of p53-R175H and control cells was investigated. As shown in Fig. 3A, antisense MRP1 oligonucleotides suppressed the expression of MRP1 by sixfold and also increased DOX sensitivity by about twofold in p53-R175H cells. On the other hand, the sense MRP1 oligonucleotides had no effect on MRP1 expression or DOX sensitivity of p53-R175H cells (Fig.3A). The IC_{50} values of DOX in p53-R175H cells pretreated with antisense and sense MRP1 oligonucleotides, or with no pretreatment were 0.25μM, 0.45μM and 0.48μM,

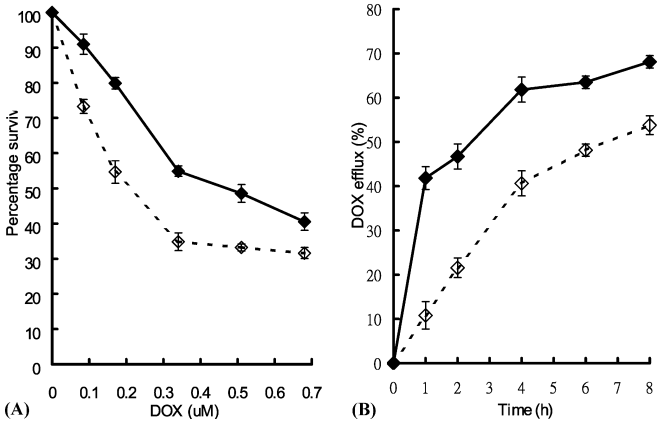

Fig. 2 **A** Sensitivity of Saos-2 cells to DOX treatment. Cells were incubated with various concentrations of DOX for 2days and drug toxicity was assessed by the MTT assay. The percentage cell survival was calculated as the ratio between cells with drug treatment and cells without drug treatment. **B** Flow cytometric analysis of DOX accumulation in Saos-2 control cells and p53-R175H cells. Cells were treated with 8.62 μM DOX for 1h and then incubated in drug-free medium for various times before collection for flow cytometric analysis. DOX efflux was calculated as described in Materials and methods (◇ control cells without p53 transfection, ◆ cells transfected with p53-R175H). The results shown are the average from four separate experiments

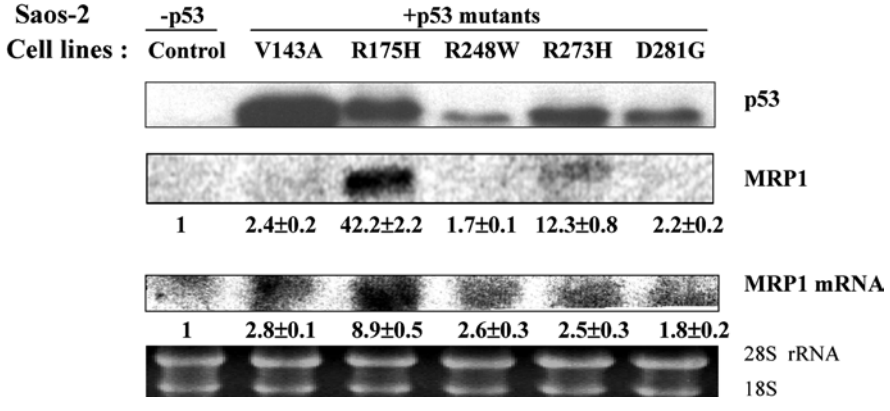

Fig. 1 Steady-state levels of p53, MRP1 proteins and MRP1 mRNA in Saos-2 cells transfected with different p53 mutants. Protein expression was verified by Western blot analysis using anti-p53 or anti-MRP1 antibodies. MRP1 mRNA expression was assessed by Northern blot analysis. The cDNA probe for *mrp1* was amplified by RT-PCR, radiolabeled with ^{32}P-dCTP and then hybridized with 5μg total RNA on nylon membrane. Equal RNA loading was demonstrated by the levels of 18S and 28S rRNA. The results shown are from a representative of four repeated experiments. The relative expression of each band (mean ± SD of four sets of experiments) is shown below each band. The procedure by which the relative expression of the band was calculated is described in Materials and methods. (*Control* cells transfected with vector only)

respectively. MRP1 in p53-null control cells was barely detectable and there were no apparent changes in DOX sensitivity or MRP1 expression in the control cells following treatment with sense and antisense MRP1 oligonucleotides. Overall, the results shown in Fig. 3A provide further evidence that the DOX resistance observed in p53-R175H cells is associated with MRP1 overexpression in the cells.

p53-R175H induced MRP1 expression and associated DOX resistance in Saos-2 cells

To illustrate that the overexpression of MRP1 and associated DOX resistance in Saos-2 p53-R175H cells was due to transfection with the p53-R175H mutant, the protein levels of p53 and MRP1, and DOX sensitivity in p53-R175H and control Saos-2 cells were studied upon incubation with antisense p53 oligonucleotides. The antisense p53 oligonucleotides not only suppressed the levels of p53 protein (17-fold decrease) but also the levels of MRP1 protein (10-fold decrease) in p53-R175H cells (Fig. 3B). Furthermore, the antisense treatment also increased DOX sensitivity by 2-fold in p53-R175H cells. In contrast, sense p53 oligonucleotides appeared to have no effect on p53 levels, MRP1 levels or DOX sensitivity in p53-R175H cells (Fig. 3B). Neither sense nor antisense p53 oligonucleotides altered the expression of MRP1 or DOX sensitivity in p53-null Saos-2 control cells. The results shown in Fig. 3B confirmed the role of p53-R175H in inducing MRP1 expression and associated DOX resistance in Saos-2 cells.

Discussion

p53-R175H induces MRP1 expression and associated DOX resistance in Saos-2 cells. Compared to the level

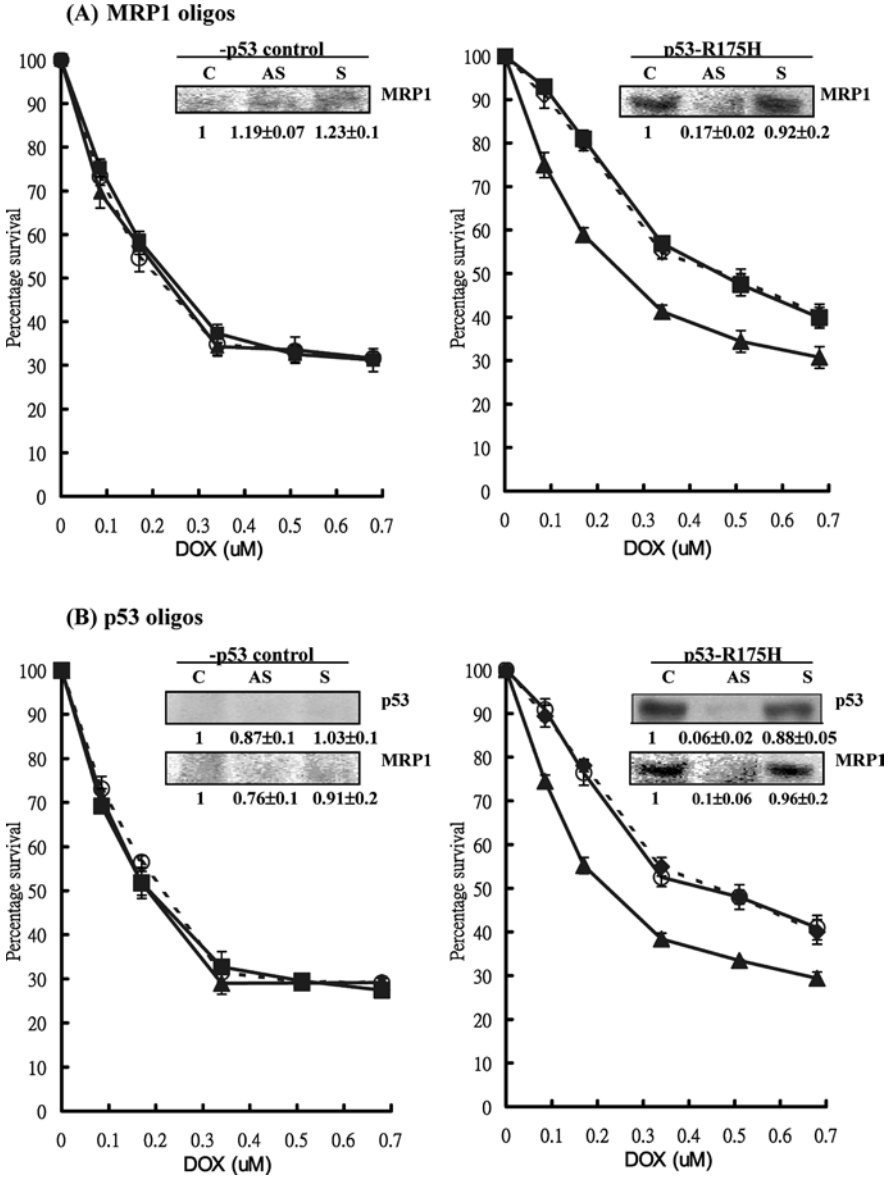

Fig. 3 A The effect of MRP1 oligonucleotides on DOX sensitivity and MRP1 protein levels in Saos-2 control cells (*left*) and p53-R175H transfectants (*right*). **B** The effect of p53 oligonucleotides on DOX sensitivity, p53 and MRP1 protein levels in Saos-2 control cells (*left*) and p53-R175H transfectants (*right*). Cells were transfected with 200n*M* of sense or antisense oligonucleotides for 4h in serum-free medium and then used for Western blot analysis or incubated with various concentrations of DOX in complete medium for 48h for the MTT assay. The relative expressions of each band (mean ± SD of four sets of experiments) are shown below each individual protein band. The relative expressions of the protein were calculated as described in Materials and methods (*C* cells without oligonucleotide transfection, *AS* cells transfected with (**A**) MRP1 or (**B**) p53 antisense oligonucleotides, *S* cells transfected with (**A**) MRP1 or (**B**) p53 sense oligonucleotides; ○ cells without oligonucleotide transfection ▲ cells transfected with (**A**) MRP1 or (**B**) p53 antisense oligonucleotides, ◆ cells transfected with (**A**) MRP1 or (**B**) p53sense oligonucleotides). The results shown are the average of four separate experiments

of MRP1 in Saos-2 control cells transfected with the empty vector only, MRP1 was highly expressed in p53-R175H cells. As MRP1 protein acts as an ATP-dependent drug efflux pump, its overexpression may increase drug efflux in cells and consequently lead to drug resistance. Compared to the p53-null control cells, increases in both drug efflux, as measured by flow cytometric analysis, and drug resistance, as measured by the MTT assay, were detected in MRP1-overexpressing p53-R175H cells (Fig. 2). The role of MRP1 expression in DOX resistance in p53-R175H cells was further confirmed as transient transfection with antisense MRP1 oligonucleotides, but not with the sense oligonucleotides, suppressed MRP1 protein expression and increased DOX sensitivity in these cells. The DOX resistance detected in p53-R175H cells would be unlikely to be related to the expression of other drug resistance proteins such as Pgp, topoisomerase II, and GSTπ, as their expression levels were similar between the control and p53-175H transfectants (results not shown). The role of p53-R175H in MRP1 induction and related DOX resistance in Saos-2 cells was confirmed as both the MRP1 level and drug sensitivity were suppressed when the p53-R175H level in Saos-2 p53-R175H transfectants was reduced by p53 antisense oligonucleotides (Fig. 3). The results relating to p53-R175H-induced MRP1 expression may be compared with those of the studies using a temperature-sensitive p53 mutant (Val138) by which MRP1 and drug resistance are induced in LNCaP cells [16].

The mechanisms by which p53-R175H regulates MRP1 expression have yet to be fully elucidated. Following cotransfection with *mrp1* promoter, wild-type p53 significantly suppresses *mrp1* promoter activity in p53-null human H1299 cells [15]. Although a p53 consensus binding sequence has not been detected in the *mrp1* promoter region, transcription suppression of *mrp1* promoter by wild-type p53 is believed to occur through direct interaction of the p53 protein with transcription factor, e.g. SP1 [15]. The SP1 binding site in the promoter sequence of MRP1 is thought to be essential for optimal transcriptional activity of the gene [3]. In another study, conversion of p53 mutant (val138) from its wild-type conformation to a mutant conformation by a temperature shift has been shown to induce MRP1 expression and also to decrease drug accumulation and drug sensitivity in human prostate cancer LNCaP cells [16]. In addition to MRP1, wild-type and mutant p53 have demonstrated differences in transcriptional activity for various promoters, e.g. *mdr1*, *pcna* and *egfr* [26, 27, 28]. Wild-type p53 protein suppresses the transcription of *mdr1* promoter activity while different p53 mutants have been found to activate the *mdr1* promoter in cells [29]. Although MRP1 can be induced by many different agents, including p53-R175H, as shown in the present study, information regarding the induction mechanism is still lacking. p53-R175H may transcriptionally activate the MRP1 gene or interfere with its downstream metabolic pathways, e.g. mRNA stability and protein stability. The details of this pathway require further investigation. Nevertheless, the findings of the present study agree with previous reports that the effect of p53 on the expression of MRP1 depends on the nature of the p53 mutations.

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References

1. Cole SPC, Bhardwaj G, Gerlach JH, Mackie JE, Grant CE, Almquist KC, Stewart AJ, Kurz EU, Duncan AMV, Deeley RG (1992) Overexpression of a transporter gene in multidrug resistant human lung cancer cell line. Science 258:1650–1654
2. Labroille G, Belloc F, Bilhou-Nabera C, Bonnefille S, Bascans E, Boisseau MR, Bernard P, Lacombe F (1998) Cytometric study of intracellular P-gp expression and reversal of drug resistance. Cytometry 32:86–94
3. Zhu Q, Center MS (1996) Evidence that SP1 modulates transcriptional activity of the multidrug resistance-associated protein gene. DNA Cell Biol 15:105–111
4. Borst R, Kool M, Evers R (1997) Do cMOAT (MRP2), other MRP homologues and LRP play a role in MDR? Semin Cancer Biol 8:205–213
5. Deeley R, Cole SPC (1997) Function, evolution and structure of multidrug resistance protein (MRP). Semin Cancer Biol 8:193–204
6. Volm M, Mattern J (1996) Resistance mechanisms and their regulation in lung cancer. Crit Rev Oncog 7:227–244
7. Grant CE, Valdimarsson G, Hipfner DR, Almquist KC, Cole SPC, Deeley RG (1994) Overexpression of multidrug resistance-associated protein (MRP) increases resistance to natural product drugs. Cancer Res 4:357–361
8. Higgins CF (1992) ABC transporters: from microorganisms to man. Annu Rev Cell Biol 8:67–113
9. Loe DW, Deeley RG, Cole SPC (1996) Biology of the multidrug or resistance-associated protein in MRP. Eur J Cancer 32A:945–957
10. Cole SP, Deeley RG (1998) Multidrug resistance mediated by the ATP-binding cassette transporter protein MRP. Bioessays 20:931–940
11. Lee G, Piquette-Miller M (2001) Influence of IL-6 on MDR and MRP-mediated multidrug resistance in human hepatoma cells. Can J Physiol Pharmacol 79:876–884
12. Schrenk D, Baus PR, Ermel N, Klein C, Vorderstmann B, Kauffmann HM (2001) Up-regulation of transporters of the MRP family by drugs and toxins. Toxicol Lett 120:51–57
13. Yamane Y, Furuichi M, Song R, Van NT, Mulcahy RT, Ishikawa T, Kuo MT (1998) Expression of multidrug resistance protein/GS-X pump and γ-glutamylcysteine synthetase genes is regulated by oxidative stress. J Biol Chem 273:31075–31085
14. Kuss BJ, Corbo M, Lau WM, Fennell DA, Dean NM, Cotter FE (2002) In vitro and in vivo downregulation of MRP1 by antisense oligonucleotides: a potential role in neuroblastoma therapy. Int J Cancer 98:128–133
15. Wang Q, Beck WT (1998) Transcriptional suppression of multidrug resistance-associated protein (MRP) gene expression by wild-type p53. Cancer Res 58:14517–14522
16. Sullivan GF, Yang JM, Vassil A, Yang J, Bash-Babula J, Hait WN (2000) Regulation of expression of the multidrug resistance protein MRP1 by p53 in human prostate cancer cells. J Clin Invest 105:1261–1267
17. Aas T, Borresen AL, Geisler S, Smith-Sorensen B, Johnsen H, Varhaug JE, Akslen LA, Lonning PE (1996) Specific p53 mutations are associated with denovo resistance to doxorubicin in breast cancer patients. Nat Med 2:804–810

18. Chang FL, Lai MD (2001) Various forms of mutant p53 confer sensitivity to cisplatin and doxorubicin in bladder cancer cells. J Urol 166:304–310
19. Hawkins DS, Demers GW, Galloway DA (1996) Inactivation of p53 enhances sensitivity to multiple chemotherapeutic agents. Cancer Res 56:892–898
20. Keshelava N, Zuo JJ, Chen P, Waidyaratne SN, Luna MC, Gomer CJ, Triche TJ, Reynolds CP (2001) Loss of p53 function confers high-level multidrug resistance in neuroblastoma cell lines. Cancer Res 61:6185–6193
21. Levine AJ, Perry ME, Chang A (1994) The 1993 Walter Hubert Lecture – the role of p53 tumor suppressor gene in tumorigenesis. Br J Cancer 69:409–416
22. Wahl AF, Donaldson KL, Fairchild C, Lee FY, Foster SA, Demers GW, Galloway DA (1996) Loss of normal p53 function confers sensitization to taxol by increasing G2/M arrest and apoptosis. Nat Med 2:72–79
23. Dittmer D, Pati S, Zambetti G, Chu S, Teresky AK, Moore M, Finlay C, Levine AJ (1993) Gain of function mutations in p53. Nat Genet 4:42–46
24. Esser J, Esser P, Mietz H, Hueber A, Kociok N, Schraermeyer U, Krieglstein GK (2000) Multidrug resistance-associated proteins in glaucoma surgery. Graefes Arch Clin Exp Ophthalmol 238:727–732
25. Stewart AJ, Canitrot Y, Baracchini E, Dean NM, Deeley RG, Cole SPC (1996) Reduction of expression of the multidrug resistance protein (MRP) in human tumor cells by antisense phosphorothioate oligonucleotides. Biochem Pharmacol 51:461–469
26. Deb S, Jackson CT, Subler MA, Martin DW (1992) Modulation of cellular and viral promoters by mutant human p53 proteins found in tumor cells. J Virol 66:6164–6170
27. Ludes-Meyers JH, Subler MA, Shivakumar CV, Munoz RM, Jiang P, Bigger JE, Brown DR, Deb SP, Deb S (1996) Transcriptional activation of the human epidermal growth factor receptor promotor by human p53. Mol Cell Biol 16:6009–6019
28. Zastawny RL, Salvino R, Chen J, Benchimol S, Ling V (1993) The core promoter region of the P-glycoprotein gene is sufficient to confer differential responsiveness to wild-type and mutant p53. Oncogene 8:1529–1535
29. Lin-Lee YC, Tatebe S, Savaraj N, Ishikawa T, Kuo MT (2001) Differential sensitivities of the MRP gene family and gamma-glutamylcysteine synthetase to prooxidants in human colorectal carcinoma cell lines with different p53 status. Biochem Pharmacol 61:555–563