Chemotherapeutic agents induce the expression and activity of their clearing enzyme CYP3A4 by activating p53

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Cytochrome P450 (P450) enzymes are abundantly expressed in the human liver where they hydroxylate organic substrates. In a microarray screen performed in human liver cells, we found a group of eleven P450 genes whose expression was induced by p53 (CYP3A4, CYP3A43, CYP3A5, CYP3A7, CYP4F2, CYP4F3, CYP4F11, CYP4F12, CYP19A1, CYP21A2 and CYP24A1). The mode of regulation of four representative genes (CYP3A4, CYP3A7, CYP4F2 and CYP4F3) was further characterized. The genes were induced in a p53-dependent manner in HepG2 and HuH6 cells (both are cancer-derived human liver cells) and in primary liver cells isolated from human donors. Furthermore, p53 was found to bind to p53-responsive elements in the genes' DNA-regulatory regions and to enhance their transcription in a reporter gene assay. Importantly, when p53 was activated following the administration of either of three different anticancer chemotherapeutic agents (cisplatin, etoposide or doxorubicin), it was able to induce CYP3A genes, which are the main factors in systemic clearance of these agents. Finally, the p53-dependent induction of P450 genes following either Nutlin or chemotheraphy treatment led to enhanced P450 enzymatic activity. Thus, in addition to the well-established role of p53 at the tumor site, our data unravels a novel function of hepatic p53 in inducing P450 enzymes and position p53 as a major factor in the hepatic response to xenobiotic and metabolic signals. Importantly, this study reveals a novel pathway for the induction of CYP3As by their substrates through p53, warranting the need for careful consideration when designing systematically administered chemotherapeutic regimens.

Introduction

Genes from the cytochrome P450 (P450) superfamily encode enzymes that catalyze hydroxylation reactions. Each P450 enzyme has different substrate specificities and, accordingly, different tissue distributions. Many P450 enzymes are abundantly expressed in the liver where they hydroxylate a wide range of substrates wired to many metabolic circuits and are involved in the metabolism of both endogenous (e.g. lipids, steroids) and exogenous (e.g. drugs, toxins) compounds. The P450 genes are divided into families and subfamilies according to their homology, activity and substrate specificity (1,2). The four members of the CYP3A subfamily (CYP3A4, CYP3A43, CYP3A5 and CYP3A7) are liver-resident enzymes paramount to drug metabolism. Particularly, CYP3A4, the most abundant P450 enzyme in adult liver, is responsible for metabolizing more than 50% of clinically used drugs. An important group of drugs heavily affected by CYP3A4 is anticancer chemotherapeutics, a great number of clinically used chemotherapeutic agents serve as substrates for CYP3A4 (3). More often than not, CYP3A4 facilitates the systemic clearance of these compounds, sometimes leading to reduced efficiency or even treatment failure. In some cases, substrates of CYP3A4 also serve as transcriptional inducers of its gene resulting in increased activity and thus enhanced clearance of the inducing drug (4). For example, tamoxifen, an estrogen-receptor modulator used for treatment of breast cancer, was shown to induce CYP3A4 leading to its own clearance (5). In addition to inducing its own clearance, a drug that induces CYP3A4 may as a result induce the clearance of concomitantly administered drugs. This option is particularly relevant to anticancer treatment regimens that often include a combination of drugs. Indeed, administration of tamoxifen combined with aromatase inhibitors (which are also CYP3A substrates) to breast cancer patients led to a reduction in their plasma levels (6). Therefore, the efficiency of a given drug is highly dependent upon CYP3A activity levels and the transcriptional regulation of CYP3A4 genes is a crucial determinant in drug administration regimens (4,7).

The CYP4F subfamily is also abundant in the liver where it is involved in lipid metabolism. CYP4F enzymes catalyze ω-hydroxylation of long fatty acids that, following two more reactions, can enter the more utilized pathway of fatty acid catabolism, ω-oxidation. Consequently, these fatty acids can be either completely oxidized to produce available energy, metabolized to succinate and acetyl-CoA, or alternatively be excreted in the urine (8). In addition to fatty acid metabolism, CYP4F enzymes take part in several metabolic pathways that are involved in both physiological and pathological aspects such as hypertension and inflammation (9). Similarly to CYP3As, the CYP4F subfamily is subjected to transcriptional induction in response to stimuli. For instance, activation of adenosine monophosphate-activated protein kinase (AMPK), a master regulator of metabolism, leads to induction of CYP4F2 expression (10).

The p53 protein is a sequence-specific transcription factor with established roles in many biological processes. The classic property of p53 is tumor suppression accomplished by regulation of various gene networks leading to cell-cycle arrest, apoptosis etc (11,12). In recent years, p53 was also found to regulate metabolic pathways such as glycolysis and oxidative phosphorylation (13,14). Accordingly, p53 was shown to be phosphorylated and activated by the metabolic sensor AMPK (15,16). AMPK accelerates adenosine triphosphate production by modulating metabolic pathways such as enhancing oxidative phosphorylation and fatty acid oxidation (17). The activation of p53 by AMPK leads to activation of metabolism-related genes such as GAMA, resulting in increased fatty acid oxidation (18). Also, p53 was found to regulate two mouse P450 genes, Cyp7a1 and Cyp8b1, thus affecting hepatic lipid metabolism (19).

In this study, we show that p53 is a regulator of a variety of P450 genes in cells of hepatic origin—11 members from five different P450 subfamilies were induced by p53. Focusing on genes from two P450 subfamilies—CYP3A and CYP4F, we demonstrate the ability of p53 to bind to DNA-regulatory elements in the genes and to promote their transcription. Interestingly, we show that following a chemotherapeutic stimulus, p53 induces the expression and enzymatic activity of CYP3As, the main enzymes responsible for clearing chemotherapeutics. The presented data points to a scenario in which p53 plays a systemic role in response to chemotherapy in addition to its established role at the tumor site. Considering these findings may help in improving the efficacy of chemotherapeutic treatment regimens.

Materials and methods

Reagents, vectors and antibodies
Nutlin-3a was purchased from Alexis biochemicals (San Diego, CA), doxorubicin, etoposide and AICAR were purchased from Sigma. pRetroSuper vectors harboring sh-RNA against human p53 (sh-p53) or mouse noxa (sh-con) were kindly provided by Dr Doron Ginsburg (Bar-Ilan University, Israel). The p53 construct pC53-SN3 was kindly provided by Prof. Bert Vogelstein (Johns Hopkins University School of Medicine, Baltimore, MD). Reporter

Abbreviations: AMPK, adenosine monophosphate–activated protein kinase; P450, cytochrome P450; p53REs, p53-responsive elements; mRNA, messenger RNA.

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gene vectors were purchased from New England Biolabs (Ipswich, MA). The following antibodies were used: α-p53 (DO-1), α-p53 (CM1) (Novoceastra Laboratories, UK) α-p21 (Santa Cruz Biotechnology, sc-397) α-gapdh (Chemicon, mab374), goat antiamouse or goat antirabbit horseradish peroxidase-conjugated antibodies (Jackson ImmunoResearch Laboratories).

**Cell culture**

All cell lines were cultured in a humidified incubator at 37°C and 5% CO2 in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal calf serum and Pen/Strep solution (Biological industries, Beit-Haemek, Israel). HepG2 cells were kindly provided by Prof. Yehiel Zick; Hep3B and Huh6 cells were kindly provided by Prof. Yosef Shaul (both from the Weizmann Institute of Science, Israel).

**Retroviral infection**

Amphotropic and ecotropic Phoenix-packaging cells were transfected with 10 µg of DNA of the appropriate retroviral construct by a standard calcium phosphate procedure. Culture supernatants were collected 48 h post transfection and filtered. Cells were infected with the filtered viral supernatants in the presence of 4 µg/ml polybrene (Sigma) for 12 h, after which the medium was changed. Fresh viral suspensions were added after a 24 h interval for an additional 12h. Infected cells were selected using Blasticidin (10 µg/ml) for 20 days.

**Microarray**

RNA from HepG2 cells treated with Nutlin (10 µM) or its solvent for 24 h was collected (two separate biological replicates were used for each cell type and each treatment). Complementary DNA samples were generated and labeled using Affymetrix 1-cycle expression kit (Affymetrix, Santa Clara, CA) and hybridized to Human Gene 1.0 ST. Retroviral microarray using Affymetrix hybridization kit materials according to manufacturer’s instruction. Data were processed by applying the RNA algorithm and using the Partek software. The entirety of the microarray results was deposited in GEO (accession #GSE30137).

**RNA isolation and quantitative real-time PCR**

Total RNA was isolated using Nucleospin kit (Macherey-Nagel) according to the manufacturer’s protocol. A 2 µg aliquot of total RNA was reverse transcribed using Bio-RT (Bio Labs) and random hexamer primers. Quantitative real-time PCR (QRT-PCR) was performed with an ABI-7300 instrument (Applied Biosystems, Foster City, CA) using Platinum SYBR Green qPCR SuperMix (Invogen, Carlsbad, CA). Gene values were normalized to 18S values. QRT-PCR data are described in arbitrary units. The details of the primers used in this study are presented in Supplementary Table 1, available at Carcinogenesis Online.

**Isolation and culturing of primary human liver cells**

Human liver cells were isolated as described previously (20). Informed consent was obtained from each donor and the study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki as reflected in a priori approval by the Committee on Clinical Investigations (Institutional Review Boards of Sheba Medical Center and Rabin Medical Center).

**Chromatin immunoprecipitation**

Cells underwent cross-linking (1% formaldehyde, room temperature, 10 min) followed by quenching (glycine 125 mM). Cells were rinsed using cold phosphate-buffered saline, incubated with 20% trypsin (Gibco), again washed using phosphate-buffered saline, scraped and centrifuged. Cells were lysed (5 mM 1,4-piperazinediethane-sulfonic acid, pH 8.0, 85 mM KC1, 0.5% NP-40, 1% protease inhibitors) on ice for 20 min. Nuclei were collected by centrifugation 4000rpm, resuspended in nuclear lysis buffer (50 mM Tris–Cl, pH 8.1, 10 mM ethylenediaminetetraacetic acid , 1% sodium dodecyl sulfate, 1% protease inhibitors) and incubated on ice for 10 min. Samples were sonicated to an average DNA fragment length of 500 bp and then centrifuged (20,000g). The chromatin solution was precleared by adding protein A beads (2h, 4°C; Santa Cruz Biotechnology). Immunoprecipitation of chromatin—rotation, 12h, 4°C, 1 µg polyclonal α-p53 antibody—either CM1 or H47 was followed by incubation with 30 µl protein A beads (2h). Immunoprecipitates were consecutively washed with dilution buffer (100 mM Tris–Cl, pH 9.0, 500 mM LiCl, 1% NP-40, 1% deoxycholic acid, 1% protease inhibitors), TSE 150, TSE 500 and TE pH 8. Samples were treated with 10 µg RNase A (30 min), followed by 30 µg of proteinase K treatment (2h, 50°C) and incubation at 65°C overnight. DNA samples were extracted using QIAquick PCR Purification Kit (Qiagen). QRT–PCR was performed as described above with each sample containing 2 µl of immunoprecipitated DNA; values were normalized for 1% input values.

**Reportor gene assay**

Cells were plated in 48-well plates in triplicates (5 × 104 cells/well) and 24 h later were transfected (lipofectamine 2000, Invitrogen, Carlsbad, CA) with 450 ng/well of pCluc-Basic2 harboring the p53-responsive elements (p53REs) specified in Figure 3A in addition to 10 ng/well of pCMV-GLuc and 10 ng/well of p533-N3. Luminescence values were estimated 48 h later from 20 µl of culture medium. Values of the Cypridinina enzyme (encoded from pCluc-Basic2) were normalized to values of Gaussia luciferase (encoded from pCMV-GLuc).

**Cytochrome P450 activity assay**

P450 3A0 activity was measured using a commercial kit according to the manufacturer’s instructions (Promega, Cat# V9001 (21)). Briefly, cells were plated on 96-well plates (3 × 104 cells per well) and treated with P450-inducing compounds the next day. After 48 h, medium containing a P450-activated procluciferin was added to the cells for 1 h followed by quantification of luminescence.

**Statistical analysis**

All presented data are representative experiments from at least three repeats. Columns marked with asterisks denote a significant (P < 0.05) elevation, measured by a student t-test compared with unmarked columns.

**Results**

**p53 induces the expression of P450 genes in human liver cells**

In order to explore hepatic-related p53-dependent gene networks, we analyzed the transcriptome of hepatocyte-derived cells—HepG2. These cells retain most of its original hepatic features and serve as an excellent and extensively used in vitro model for both hepatocyte research and p53 pathway analysis (10,15,22,23). We treated HepG2 cells with Nutlin, a compound that disrupts the interaction between p53 and its negative regulator MDM2, thus stabilizing p53 protein level, leading to its accumulation and to the activation of p53-dependent transcription (24). To avoid any p53-independent effects of Nutlin, we used a control of HepG2 cells stably expressing short-hairpin RNA targeting p53 (termed HepG2sh-p53), whereas the cells with unaffected p53 levels stably expressed a non-relevant short-hairpin RNA (termed HepG2sh-con) as described previously (25). HepG2sh-p53 cells treated with Nutlin showed a prominent increase in p53 protein levels and in its downstream target gene—p21 as compared with untreated cells. In contrast, HepG2sh-con cells exhibited lower p53 and p21 levels compared with HepG2sh-p53 cells in the presence or absence of Nutlin (Figure 1A). Therefore, this experimental setup is suitable for analyzing p53-dependent transcriptional patterns.

Next, we analyzed the transcriptome of HepG2 cells under these conditions using a gene expression microarray. Following an analysis of variance and exclusion of genes with a fold change lower than 2 or a P value higher than 0.05, we found a total of 341 annotated genes whose expression was induced by p53 (25). Strikingly, out of this group, 11 genes were members of the cytochrome P450 superfamily: CYP3A4, CYP3A43, CYP3A5, CYP1A2, CYP4F2, CYP4F3, CYP4F11, CYP4F12, CYP19A1, CYP2A12 and CYP2A4. In order to corroborate our findings, we repeated the above-mentioned experimental setup and performed a QRT–PCR analysis that completely reproduced the pattern observed in the microarray analysis (Figure 1B).

In order to evaluate whether this pattern of expression is a more general phenomenon, we evaluated the effect of p53 activation on four representative genes from two different P450 subfamilies (CYP3A4, CYP3A7, CYP4F2 and CYP4F3) in other hepatoc-derived cells. First, we treated Huh7sh-p53 cells (also from a hepatic origin and harboring wild-type p53) with Nutlin, which resulted in both the stabilization of p53 protein levels and the induction of messenger...
RNA (mRNA) levels of all four genes in comparison with the controls (Figures 2A and 2B). In addition, we treated normal primary liver cells isolated from human donors with Nutlin and observed both p53 stabilization and induction of CYP3A4, CYP3A7 and CYP4F2 (Figures 2C and 2D). We were not able to detect CYP4F3 mRNA in these cells.
Taken together, the findings presented in Figures 1 and 2 show a role for p53 in regulating the expression of P450 genes from a number of gene families in several liver-derived cells. Due to lack of validated commercial antibodies, we were unable to evaluate the protein levels of P450 enzymes. However, the QRT–PCR analysis was performed with TaqMan probes for higher precision and specificity of detection (Supplementary Table 1, available at Carcinogenesis Online).

Fig. 2. The expression of P450 genes is induced by p53 in liver-derived cells. (A) Huh6 cells were treated with the p53-activating agent Nutlin (25 µM) for 48h, total protein was extracted and the protein level of p53 was analyzed in a western blot analysis. (B) Huh6 cells were treated with the p53-activating agent Nutlin (25 µM) for 48h, total RNA was extracted and the mRNA levels of the indicated genes were analyzed in a QRT–PCR analysis. (C) Primary human liver cells were treated with the p53-activating agent Nutlin (10 µM) for 24h, total protein was extracted and the protein level of p53 was analyzed in a western blot analysis. (D) Primary human liver cells were treated with the p53-activating agent Nutlin (10 µM) for 24h, total RNA was extracted and the mRNA levels of the indicated genes were analyzed in a QRT–PCR analysis. Results are of a representative experiment from three experiments.
Fig. 3. p53 binds to the DNA-regulatory elements of P450 genes and through them enhances their transcription. (A) The promoters of CYP3A4 and CYP3A7 and the introns of CYP4F2 and CYP4F3 contain p53-responsive elements. The sequence of the indicated p53REs is detailed in the left-hand side of panel, the core elements are underlined. p53REs are schematically depicted on the right-hand side of the panel; gray rectangles represent the p53RE with its location indicated in base pair numbers relative to the transcription start site (the number indicated is of the first base in the p53RE). (B) p53 binds to the genes’ p53REs. HepG2 cells were treated with the p53-activating agent Nutlin (10 µM) for 24 h and then subjected to chromatin immunoprecipitation with two separate polyclonal α-p53 antibodies. The immunoprecipitated DNA was then subjected to QRT–PCR analysis with primers amplifying the indicated p53RE regions. Samples are normalized to 1% of total DNA. (C) p53 elevates gene expression through the p53REs of P450 genes. p53 was ectopically expressed in the p53-null cell line Hep3B along with constructs harboring the indicated p53REs upstream to a minimal promoter and the luciferase gene (Cypridina). Luminescence values were measured as indication for transcription levels and normalized to a constitutively expressed luciferase gene (Gaussia). Results are of a representative experiment from three experiments.
It is well established that p53 induces gene expression by binding to DNA-regulatory elements termed p53REs in its target genes (26). For that reason, we scanned the sequence of the genes’ introns, exons and promoters (10 kb upstream of the genes’ transcription start site—TSS) for p53REs using the p53MH algorithm (27). Indeed, the distal promoters of CYP3A4 and CYP3A7 each contained a putative p53RE and so did the third intron of CYP4F2 and CYP4F3 (Figure 3A). The p53REs of CYP4F2 and CYP4F3 are completely identical, agreeing with the notion that the two genes have originated from a gene-duplication event (2). Likewise, the p53REs of CYP3A4 and CYP3A7 are strikingly similar with only one nucleotide differing between them.

To determine whether these putative p53REs are indeed occupied by p53 in a cellular context, we performed a chromatin immunoprecipitation experiment in which HepG2sh-con cells were treated with either Nutlin or its solvent as a control and their chromatin was immunoprecipitated with two different α-p53 polyclonal antibodies (H47 and CM1). p53 was able to bind all p53REs following Nutlin treatment as deduced by the enrichment of these DNA segments in p53-precipitated DNA (Figure 3B). The binding of p53 to the CYP4F2 p53REs was similar in its extent to the binding of p53 to the known p53RE of p21 (Figure 3B). Furthermore, no binding was observed in the negative control in which primers amplifying a non-relevant genomic area were used (data not shown).

In order to show that the aforementioned p53REs are functional (i.e. able to induce transcription in a p53-dependent manner), we performed a reporter gene assay in which the p53REs were cloned upstream to a minimal promoter followed by a luciferase reporter gene. This construct was co-transfected with either a p53-harboring vector or a control vector to the hepatic-derived, p53-null cell line, Hep3B. All three p53REs significantly enhanced the expression of luciferase in the presence of exogenous p53, similarly to the positive control (a construct harboring the consensus sequence of p53RE) (Figure 3C). Collectively, the findings presented in Figure 3 uncover the mode of regulation of the p53-dependent induction of P450 genes, namely, binding to p53REs in DNA-regulatory elements and the subsequent promotion of transcription.

p53 binds to p53REs in the DNA-regulatory elements of P450 genes, thereby inducing their transcription

Anticancer chemotherapeutic agents activate p53 to induce their own clearing enzymes.

DNA damage is another kind of p53-activating signal. Due to their DNA-damaging attributes, many of the clinically used anticancer chemotherapeutic compounds lead to activation of p53 and the subsequent transcriptional activation of p53 target genes (29). In addition, many anticancer chemotherapeutic compounds are metabolized and thus inactivated by the CYP3A subfamily of enzymes. In order to assess the p53-dependent induction of CYP3A genes following chemotherapeutics, we treated HepG2 cells with either of three widely used genotoxic chemotherapeutic drugs—doxorubicin (Dox), etoposide (Eto) and cisplatin (Cis). Cells were treated with concentrations reflecting the peak plasma concentrations of each agent as reported in clinical measurements (30–33). These compounds are known to activate p53 (29) and to be metabolized by CYP3A4 (3). As expected, all three chemotherapeutics led to phosphorylation of p53 at serine 15 (an indicator of its activation), Dox or Cis treatment also resulted in marked elevation in p53 protein levels (Figure 5A). These drugs induced the expression of the CYP3A4 and CYP3A7 genes in a p53-dependent manner (Figure 5B). In order to assess the functional effect of this induction, we examined the activity of the CYP3A4 enzyme following treatment with either rifampicin (Rif, a well-known inducer of CYP3A4), Nutlin, Dox or Eto (we did not examine the effect of Cis due to its relatively low effect on CYP3A4 mRNA expression). Remarkably, Nutlin-treated HepG2sh-con cells showed a
significant increase in CYP3A4 activity, whereas HepG2sh-p53 cells did not. Similarly, HepG2sh-p53 treated with either Dox or Eto showed a significant increase in CYP3A4 activity, this increase was milder in HepG2sh-p53 cells (Figure 5C). Thus, following its activation, p53 is able to significantly induce CYP3A enzymatic activity in liver cells activity.

**Discussion**

In this report, we show that p53 regulates several P450 genes involved in various metabolic pathways. p53 induces the activity of CYP3A4, which is the main drug-metabolizing enzyme in adult liver (3). p53 regulates *CYP4Fs* that metabolize lipids and affects...
physiological pathways such as inflammation and hypertension (8). p53 regulates the expression of CYP19A1, an enzyme that converts androgen to estrogen. CYP21A2, another enzyme induced by p53, catalyses a crucial step in glucocorticoid synthesis. Finally, the p53-induced CYP21A2 enzyme hydroxylates and thus inactivates vitamin D₃ and is considered the main catabolic enzyme of vitamin D (1). In addition to our findings, p53 was shown previously to reduce the expression of two mouse P450 genes—Cyp7a1 and Cyp8b1 (19). The regulation of a variety of P450 genes by p53 establishes a novel and direct role for p53 in aspects of lipid, steroid, vitamin and drug metabolism and may help in promoting our understanding of their associated pathways.

Until now, delineation of molecular mechanisms for the induction of CYP3A genes was concentrated on nuclear receptors (3). The results described above unravel another route for the induction of CYP3As, through activation of p53, its binding to p53REs and the subsequent transcriptional enhancement of CYP3As. Of note, the functional p53REs in CYP3A4 is located upstream of its conventional promoter (Figure 3A). This finding should be considered when studying CYP3A4 induction because many of the studies exploring CYP3A4 induction patterns use a cloned promoter with an approximate length of 1 kb, which lack the p53RE (e.g. (34,35)). Therefore, some drugs that may not induce CYP3A4 in these assays would be a ‘false negative’ and would actually induce CYP3A4 through its p53RE in an endogenous context.

Our findings show that p53 responds to a chemotherapeutic signal or to Nutlin to transcriptionally induce CYP3A genes in liver cells. This molecular observation may bear clinical relevance considering the widespread use of anticancer chemotherapeutic drugs and the optional future use of Nutlin as an anticancer compound (24,36). Both Nutlin and genotoxic chemotherapy are administered systemically and thus reach the liver and may trigger hepatic p53 to induce CYP3A genes. Consequently, the elevated activity level of CYP3A enzymes may lead to reduced efficacy of the drugs. Similar cases of a drug inducing its own clearance leading to undertreatment have been reported in other cases of CYP3A induction (4,5). Moreover, cases of drug–drug interactions in which a drug induces CYP3A4, leading to the clearance of co-administered drugs were reported (6). Because most anticancer treatment regimens are composed of several concomitantly administered drugs (in most cases, at least one of which is a p53-activating drug), such drug–drug interactions should be carefully considered. A combined administration of a p53-inducing drug with a second drug that is catalyzed by CYP3A enzymes could prove to be counterproductive—not only would the first drug’s efficacy be curtailed by induced CYP3A activity but so would the efficacy of the second drug.

It is worth mentioning that the examination of P450 induction in rodent models is problematic because the transcriptional response of P450 genes varies greatly between species (3) and therefore studies regarding P450 induction were traditionally done in human cell lines. Accordingly, treating mice with etoposide or Nutlin did not lead to CYP3A induction (data not shown).

Our finding linking between chemotherapy treatment and p53 in the liver rather than in a specific tumor site sheds light on the systemic roles of p53 in response to chemotherapy. p53 may initiate a hepatic process leading to chemotherapy clearance potentially resulting in reduced efficacy. However, this option warrants further investigation. In conclusion, in addition to the well-established role of p53 in preventing tumorigenesis, we describe here a novel function of p53 in liver-specific molecular pathways relating to chemotherapy clearance.

The regulation of chemotherapy-clearing enzymes by p53 may influence drug administration regimens and carefully considering it could prove beneﬁciary and improve drug efﬁcacy.

Supplementary material
Supplementary Table 1 can be found at http://carcin.oxfordjournals.org/

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