The expression of P-glycoproteins encoded by the mdr gene family is associated with the emergence of the multidrug resistance phenotype in animal cells. mdr expression can be induced by many extracellular stimuli including cytotoxic drugs and chemical carcinogens. However, little is known about the mechanisms involved. Here, we report that the expression of the rat mdr1b can be induced by anticancer drug daunorubicin. Further analysis identified a bona fide p53-binding site spanning from base pairs −199 to −180 (5′-GAACATGTA-GAGACATGCT-3′) in the rat mdr1b promoter that is essential for basal and daunorubicin-inducible promoter activities. In addition, our results show that wild-type p53 can up-regulate not only the promoter function but also endogenous expression of the rat mdr1b. To the best of our knowledge, this is the first report showing that a specific p53-binding site is involved in the transcriptional regulation of mdr gene by wild-type p53. Since p53 is a sensor for a wide variety of genotoxic stresses, our finding has broad implications for understanding the mechanisms involved in the inducible expression of mdr gene by anticancer drugs, chemical carcinogens, UV light, and other DNA-damaging agents.

Multidrug resistance (MDR), 1 a major obstacle to the effective chemotherapy of many human malignancies, is characterized by the increased survival of cells in the presence of cytotoxic drugs with unrelated structures. A major mechanism for the development of MDR phenotype is overexpression of P-glycoproteins which are encoded by the MDR gene family (for reviews, see Refs. 1 and 2). The MDR gene family contains two members in humans and three in rodents. However, only one human (MDR1) and two rodent (mdr1a and mdr1b) mdr genes are functionally related to the MDR phenotype. High mdr mRNA levels are seen in certain tumor types before chemotherapy and, in some cases, are associated with relapse following chemotherapy (for reviews, see Refs. 1 and 3).

Increased mdr gene expression occurs in cultured cells selected by continuous exposure to both anticancer drugs and other cytotoxic agents, in which gene amplification is believed to be often associated with the overexpression of mdr genes (4, 5). However, increased mdr gene expression preceding gene amplification has been observed in early passages of drug-selected cells (6). Transient exposure of cells to different cytotoxic agents such as antitumor drugs (7–10), chemical carcinogens (11–19), and UV irradiation (20), etc. is also able to activate mdr expression, indicating that increased mdr expression is mediated by complex mechanisms.

The precise mechanisms of the induction of mdr gene expression by anticancer drugs, chemical carcinogens, UV, and other DNA-damaging agents remain unknown. It has been suggested that both post-transcriptional and transcriptional mechanisms are involved (7). A possible role for the cytoskeleton in post-transcriptional stabilization of mdr1 mRNA in rat hepatocytes treated with certain agents was suggested (21). On the other hand, in rat liver cells, it was found that doxorubicin-mediated mdr1 mRNA induction was fully inhibited by actinomycin D, suggesting that transcriptional regulation is involved (10). Nuclear run-off and transfection analyses showed that AAF-, methylcholanthrene-, aflatoxin B1-, methyl methanesulfonate-, or mitoxantrone-induced mdr1 expression is also associated with increased rates of transcription (9, 11, 15).

Here, we show that the expression of the rat mdr1b can be induced by anticancer drug daunorubicin. Further analysis demonstrates that a bona fide p53-binding site (5′-GAACATGTA-GAGACATGCT-3′) located within bp −199 to −180 of rat mdr1b promoter is essential for not only basal but also daunorubicin-inducible promoter functions. We also provide evidence indicating that both the promoter activity and endogenous expression of the rat mdr1b could be modulated by wild-type p53. Although the modulation of mdr expression by either mutant or wild-type p53 has been noted, no p53-binding sites have been identified previously (22–27). The present report represents the first evidence that a specific p53-binding site is involved in the transcriptional regulation of the mdr gene. Since p53 is responsive to a variety of genotoxic stresses (for reviews, see Refs. 28 and 29), which also induce mdr gene expression, our finding has important implications for understanding mechanisms involved in the inducible expression of drug-resistant genes by DNA-damaging agents.

**MATERIALS AND METHODS**

**Reagents**—Reagents were purchased from the following companies: [α-32P]dNTPs, [α-32P]UTP, and [14C]chloramphenicol from ICN Biomedicals (Costa Mesa, CA); poly(dI-dC)-poly(dI-dC) and acetyl-coenzyme A from Pharmacia/LKB (Upsala, Sweden); oligonucleotides from Genosys Inc. (Houston, TX); and rabbit polyclonal antibodies against c-Jun and p65 subunit of NF-κB from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA) and p53 antibody PAb421 from Calbiochem (Cambridge, MA). All other reagents were purchased from Sigma.

**Plasmids**—Wild-type (pCMVp53) and mutant (pCMVp53<sub>mut</sub>) p53 expression vectors were generously provided by Dr. G. Lozano of M. D. Anderson Cancer Center. Rat mdr1b-CAT reporter constructs (∼1288 RMI CAT, ∼243 RMI CAT, ∼163 RMI CAT, and ∼243 RMI CAT-xm)
were constructed as described previously (30, 31). –214 RMICAT, –214 RMICAT-m1, and –214 RMICAT-m2 were constructed by a PCR method using –1288 RMICAT as the template and 5′-TCCATTCTACTGACACGTGATGGGCTCTAG-3′ as the 3′ primer in combination with each of the following 5′ primers: 1) 5′-GGGGGATCCATATGGAGAGTTACCTGA-3′; 2) 5′-GAGTCTTCCTTAG-3′; 3) 5′-GGGGGTACCATATGGAGAGTTACCTGA-3′; 2) 5′-GGGGGTACCATATGGAGAGTTACCTGA-3′; 3) 5′-GGGGGTACCATATGGAGAGTTACCTGA-3′; 3) 5′-GGGGGTACCATATGGAGAGTTACCTGA-3′.

The –214/–1288 thymidine kinase (tk)-CAT recombinant was constructed by a PCR method using pGEM (30) as the template, 5′-CCAGGTTAGGGAATTGCAAGTGAACATGTAGAGACATGTCTGTG as the 5′-primer, and 5′-TGGGATCCATCTGACAGGAAGGTGAGAGTGTTAATG. All three 5′ primers contained a KpnI site (underlined). The PCR products were digested with KpnI and XbaI and inserted into the KpnIXbaI sites of a CAT vector (15).

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Two micrograms of total RNA isolated from H-4-II-E cells was used for reverse transcriptase reaction. On completion of the reverse transcriptase reaction, the enzyme was inactivated by heating to 94 °C for 5 min. Ten picomoles of each 5′ primer (5′-CTGGAGAAGCTGATACGCTTCAACAAGGTGAGAGGCTCTATGACAGGAAAGGTGAGAGGTGTTAATG) and 3′ primer (5′-AGCGGAGGCTGAGTAATGGAGAGGCTCTATGACAGGAAAGGTGAGAGGTGTTAATG) were used as a reference. The autoradiograph is representative of results from three independent experiments.

RESULTS

Daunorubicin Induces mdr1b Expression in Rat Hepatoma Cells—To investigate whether the expression of the rat mdr1b gene expression is regulated by the anticancer drug daunorubicin, we treated rat hepatoma H-4-II-E cells with daunorubicin (7 μg/ml). At various time intervals, cells were harvested and mdr1b mRNA levels were measured by the RNase protection assay. As shown in Fig. 1, the steady-state mdr1b mRNA levels in these cells were elevated after exposure to daunorubicin for 12 and 24 h. Increases of about 3–5-fold were seen in three independent experiments. Similar results were obtained in cells treated with adriamycin and chemical carcinogen 2-AAAF (data not shown). These results demonstrated that rat mdr1b expression can be induced by these cytotoxic agents in rodent cells.

Rat mdr1b Promoter Responds to Daunorubicin Treatment—To investigate the possible involvement of transcriptional regulation in the induction of the rat mdr1b gene expression by daunorubicin and, if so, to identify DNA sequences responsible for the daunorubicin induction of mdr1b expression, we generated a set of 5′ deletion mutant CAT constructs and transfected them into H-4-II-E cells following treatment with or without daunorubicin. When –1288 RMICAT, –243 RMICAT, and –214 RMICAT promoter reporter constructs containing –1288, 243, and 214 bp of the rat mdr1b upstream sequences, respectively, plus 125 bp downstream from the transcription start site were transiently transfected into H-4-II-E cells, CAT activities increased an approximately 1.6–1.9-fold in daunorubicin treated versus untreated cells (p < 0.05) (Fig. 2). However, when –163 RMICAT, which contains additional deletion to –163 bp was transfected, basal transcriptional activities were reduced more than 80%. More importantly, the deletion also abolished daunorubicin inducibility (Fig. 2). Together,
these results indicated that the rat mdr1b promoter can respond to daunorubicin treatment and that the sequence from bp −214 to −163 is essential for the promoter’s daunorubicin responsiveness.

We previously identified a NF-κB-binding site (bp −167 to −158) involved in basal and insulin-induced promoter function (31). Since it was reported that daunorubicin can induce the NF-κB activity in human fibrosarcoma HT1080 cells and HL-60 promyelocytes (36, 37), it is possible that NF-κB was also responsible for the inducible promoter activity of the rat mdr1b by daunorubicin in the H-4-II-E cells. To test this possibility, we transfected −243 RMICAT-κm, in which the NF-κB-binding site was mutated (31), into H-4-II-E cells following daunorubicin treatment. As shown in Fig. 2, although basal activity was reduced when compared with the wild-type −243 RMICAT, −243 RMICAT-κm still retained daunorubicin responsiveness. Besides, we did not observe an obvious increase of NF-κB binding activity after daunorubicin treatment using GMSAs (data not shown). These results suggested that NF-κB may not be directly involved in the daunorubicin-inducible promoter function of the rat mdr1b in H-4-II-E cells.

−214 to −177 bp Is Sufficient to Confer mdr1b Promoter Inducibility by Daunorubicin—To further substantiate the above observations, we generated two additional constructs by inserting sequences from bp −214 to −127 (containing NF-κB site) or −214 to −177 (containing no NF-κB site), respectively, into a pBLCAT2 vector containing the tk basal promoter and a CAT reporter gene. These constructs were then transiently transfected into H-4-II-E cells, and CAT expression was measured. As shown in Fig. 3, both constructs are capable of responding to daunorubicin treatment, giving rise to comparable levels of induction, whereas daunorubicin did not have effects on the tk promoter. These results suggested that NF-κB site is dispensable for the inducibility of mdr1b promoter, and that the sequence from bp −214 to −177 may contain important cis-acting elements responsible for the induction of mdr1b promoter activity by daunorubicin.

Daunorubicin Induces Formation of a Specific Protein-DNA Complex Within bp −201 to −177—To test whether daunorubicin treatment could induce protein DNA binding at sequences within bp −214 to −177, we prepared nuclear extracts from H-4-II-E cells treated with or without daunorubicin and performed GMSAs. As shown in Fig. 4A, a major DNA-protein complex was formed in the daunorubicin-untreated nuclear extracts when a double-stranded oligonucleotide spanning bp −214 to −177 was used as the probe (lane 1, C1). The binding activity of this complex remained largely unchanged after daunorubicin treatment (lanes 2−5 versus lane 1). However, a slow migrating protein-DNA complex was induced 1 h after treatment (C2, lane 2). The binding activity of this induced complex remained elevated but gradually reduced throughout the 12-h induction period (lanes 2−5).

To further characterize the sequence specificity of this daunorubicin-induced DNA binding activity, double-stranded oligonucleotides covering the left and right regions of bp −214 to −177 (fragments A and B, Fig. 4C) were used in competition GMSA. Fig. 4B shows that both the unlabeled probe (lane 2) and fragment B (bp −201 to −177) (lane 4) could compete for daunorubicin-induced DNA-protein complex, indicating that the induced protein binding required the sequence residing within fragment B.

To further define the binding sequence of the induced protein complex, two site-directed mutant oligonucleotides (M1 and M2) containing mutations on 5′- or 3′-end of fragment B, respectively (Fig. 4C), were used as competitors. However, neither mutated oligonucleotides could compete for daunorubicin-induced DNA-protein complex (Fig. 4B, lanes 5−6), suggesting that the daunorubicin-induced protein binding required both the 5′- and 3′-sequences of fragment B (bp −201 to −177).

Daunorubicin-induced DNA-binding Protein Is p53—In examining the DNA sequence of bp −201 to −177 (fragment B), we found within it a sequence (bp −199 to −180) strikingly similar to the p53-binding consensus sequence 5′-PuPuPuCA/TA(T/A)GP(PyPyPy)-3′ (38), with only 2 base pair mismatches. A comparison of the putative mdr16 p53-binding site with the p53 consensus sequence and the p53-binding site from the gadd45 third intron (39) is shown in Fig. 5B. To determine whether the sequence located between bp −199 and −180 was indeed a p53-binding site, we carried out GMSAs using a double-stranded oligonucleotide spanning bp −214 to −177 as the probe and nuclear extracts prepared from daunorubicin-treated H-4-II-E cells in the presence of various unlabeled oligonucleotides as competitors. As shown in Fig. 5A, daunorubicin-induced DNA-protein complex was efficiently competed only by the gadd45 p53-binding sequences (lane 2), but not by the mutated gadd45 p53-binding sequence (lane 3) and other unrelated sequences, i.e. AP-1 (40) and NF-κB (41) (lanes 4 and 5). Instead, the protein binding activity was actually enhanced by these unrelated oligonucleotides (compare lanes 3−5 to lane 1). The exact reasons for the enhanced binding activities are
not clear at the present.

To further strengthen this observation, antibodies were used in GMSAs. As shown in Fig. 5A, the daunorubicin-induced protein-DNA complex was supershifted by p53 antibody PAb421 (lane 6), whereas c-Jun and NF-κB p65 antibodies did not affect the formation of induced DNA-protein complexes (lanes 7–8). Extracts were assayed for binding to the labeled double-stranded oligonucleotide (bp –214 to −177) used in competition experiments. C, the mdr1b promoter sequence located between bp −199 and −180 (5′-GAACATGTAGACATGCTT-3′) is a p53-binding site.

To determine whether the rat p53 in H-4-II-E cells is a wild-type or mutant form, we amplified cDNA copies of the rat p53 by reverse transcriptase-PCR and sequenced it directly (see "Materials and Methods"). The result showed that H-4-II-E cells has a wild-type rat p53 mRNA (data not shown) with a sequence consistent with that published previously (42).

p53-binding Site Is Required for the Daunorubicin-inducible promoter Activities—To characterize the functional role of the
identified p53-binding site, the same mutations in Fig. 4C were introduced within the context of the wild-type −214 RMICAT construct, and resultant recombinants were designated −214 RMICAT-m1 and −214 RMICAT-m2. These mutant constructs were then transfected into H-4-II-E cells following treatment with or without daunorubicin. As shown in Fig. 6A, both mutations abolished the daunorubicin responsiveness. Similar results were obtained when the same mutations were introduced into heterologous (tk) promoter constructs (Fig. 6B). These results suggested that the integrity of p53 binding is essential for the daunorubicin inducible-promoter function of the rat mdr1b.

It has been reported that promoters containing p53-binding sites, in some cases, essentially showed no obvious DNA damage responsiveness in transient transfection assays after the treatment of UV or other DNA-damaging agents, whereas higher levels of the induction of the same reporters were seen in stable transfectants (43). Consistent with these observed only low levels of inductions of mdr1b CAT activities by daunorubicin were observed in our transient transfection assays (Figs. 2, 5, 6, A and B). To test whether the rat mdr1b promoter can respond to daunorubicin more dramatically in stable transfectants than in transiently transfected cells, we stably transfected both −214 RMICAT and −214 RMICAT-m1 into H-4-II-E cells. As expected, wild-type CAT reporter (−214 RMICAT) exhibited more significant responsiveness to daunorubicin (4-fold, Fig. 6C), which is comparable with the induction levels of mdr1b mRNA by daunorubicin (Fig. 1). As a control, −214 RMICAT-m1 in stably transfected H-4-II-E cells failed to respond to daunorubicin (Fig. 6C). These results further strengthened the notion that the p53-binding site is required for the promoter’s daunorubicin responsiveness. Why the fold induction is different between transient and stable transfectants is unclear but could be due to the participation of chromatin proteins or structure in p53-mediated gene expression, since studies have indicated that transiently transfected DNA, unlike stably transfected templates, are not efficiently packed into chromatin (44). Consistent with this finding, a recent report showed that high mobility group protein-1, an important component of chromatin, is a coactivator of p53 (45).

Wild-type but Not Mutant p53 Transactivates mdr1b Promoter Activity—To investigate whether p53 is able to regulate rat mdr1b promoter function, A1–5 cells, which were derived from primary REFs transformed by a p53 temperature-sensitive mutant p53Val135 (46), were stably transfected with reporter constructs containing either a wild-type p53-binding site (−214 RMICAT) or a mutated p53 site (−214 RMICAT-m1). As expected, when stably transfected A1–5 cells were shifted from the restrictive (37 °C, cells contain mutant p53) to permissive (32.5 °C, cells contain wild-type p53) temperature (46), CAT activity was clearly induced in cells stably transfected with wild-type −214 RMICAT but not those stably transfected with mutant −214 RMICAT-m1 (Fig. 7A).

In another set of experiments, wild-type p53 (pCMVp53) or mutant p53 (pCMVp53Val135) expression vectors were co-transfected with reporter constructs into SAOS-2 cells, which contain a homozygous deletion at the p53 gene locus and do not produce a p53 protein (47). As shown in Fig. 7B, co-transfection
mutant p53 line. Fold induction refers to that in cells cultured at 37 °C. 

Representative of the results from one of three independent pools for each cell line. Fold induction refers to that in cells cultured at 37 °C. B and C; p53-null SAOS-2 cells were transfected with 2 μg wild-type (−214 RMICAT or −214/−177 tk-CAT), p53-binding site-mutated (−214 RMICAT-m1, −m2, or −214/−177 tk-CAT-m2) mdr1b promoter reporter alone or in combination with 1 μg of wild-type p53 (pCMVp53) or mutant p53 expression vector (pCMVp53mut) as indicated. Empty control vector (pCMV) was used to normalize the amounts of the transfected DNA to a total 3 μg of DNA in each transfection reaction. Each column represents the mean of relative CAT activities from three independent experiments after normalization to the protein concentration of the cellular extracts. S.D. values are represented by the bars.

of pCMVp53 trans-activated CAT activity in cells transfected with the wild-type reporter (−214 RMICAT) but not in cells co-transfected with reporters containing mutated p53 site (−214 RMICAT-m1 or −214 RMICAT-m2). Moreover, co-transfection of mutant p53 expression vector also failed to activate wild-type as well as mutant reporters (Fig. 7B). Similar results were obtained when heterologous reporter constructs (−214/−177 tk-CAT and mutant −214/−177 tk-CAT-m2) were used in co-transfection assays (Fig. 7C). These results, collectively, demonstrated that wild-type p53 can trans-activate rat mdr1b promoter activity specifically via the identified p53-binding site.

Endogenous mdr1b Expression Is Modulated by Wild-Type p53—To assess the regulation of endogenous mdr1b expression by p53, we examined mdr1b mRNA levels following temperature shift in A1–5 cells. Cells transfected with the wild-type reporter (−214 RMICAT) but not in cells co-transfected with reporters containing mutated p53 site (−214 RMICAT-m1 or −214 RMICAT-m2). Moreover, co-transfection of mutant p53 expression vector also failed to activate wild-type as well as mutant reporters (Fig. 7B). Similar results were obtained when heterologous reporter constructs (−214/−177 tk-CAT and mutant −214/−177 tk-CAT-m2) were used in co-transfection assays (Fig. 7C). These results, collectively, demonstrated that wild-type p53 can trans-activate rat mdr1b promoter activity specifically via the identified p53-binding site.

![Fig. 7. Activation of mdr1b promoter by wild-type p53 but not mutant p53. A, CAT assay of −214 RMICAT and −214 RMICAT-m1 after stable transfection into A1–5 cells. Cells cultured at 37 °C were either shifted to 32.5 °C or continuously cultured at 37 °C for 24 h and then harvested for CAT assays. The autoradiograph shown is representative of the results from one of three independent pools for each cell line. Fold induction refers to that in cells cultured at 37 °C. B and C; p53-null SAOS-2 cells were transfected with 2 μg wild-type (−214 RMICAT or −214/−177 tk-CAT), p53-binding site-mutated (−214 RMICAT-m1, −m2, or −214/−177 tk-CAT-m2) mdr1b promoter reporter alone or in combination with 1 μg of wild-type p53 (pCMVp53) or mutant p53 expression vector (pCMVp53mut) as indicated. Empty control vector (pCMV) was used to normalize the amounts of the transfected DNA to a total 3 μg of DNA in each transfection reaction. Each column represents the mean of relative CAT activities from three independent experiments after normalization to the protein concentration of the cellular extracts. S.D. values are represented by the bars.](image)

![Fig. 8. Induction of endogenous mdr1b by wild-type p53. RNase protection analyses of rat mdr1b transcripts in A1–5, REF, and T101–4 cells. Cells maintained at 37 °C were either shifted to 32.5 °C or continuously cultured at 37 °C for different times as indicated. RNA was harvested at each time point and then subjected to RNase protection assays as described under "Materials and Methods." An 18S rRNA probe was used as a reference. The autoradiograph is representative of results from three independent experiments.](image)
known (26). Similarly, it is also unclear how the p53 mutants gain the functions to activate the human MDR1 promoter (24). In addition to repressing it, wild-type p53 was also shown to stimulate the MDR1 promoter in mdr-null cell line in a transfection assay (27). The reasons for the discrepancies among these studies are still unknown but there are many plausible explanations: (i) p53 is a multiple functional protein whose functions are regulated by a complex network (48), its regulation of gene expression may differ not only among cell types but also among physiological conditions under which assays are performed; (ii) p53 can also bind transcriptional coactivators such as CBP/p300 (49–51), which interacts with a battery of other transcriptional regulators such as NF-κB, Jun/Fos, nuclear receptors, and their coactivators (for review, see Ref. 52). The abundance of these transcriptional regulators may differ among different cell settings and thereby influence the overall expression of transfected genes; (iii) different p53 expression vectors, mdr reporter constructs, and time of analysis, may affect the overall results. It should also be noted that even the transfection procedures themselves may perturb endogenous p53 levels (53), affecting results of transient transfection as well as the transfection procedures themselves may perturb endogenous p53 levels (53), affecting results of transient transfection assays. These considerations, taken together, may explain the discrepancy of the transfection results described above. In this regard, the identification of an authentic p53-binding site in the mdr1b promoter region as described herein is of particular importance, since it is the first time a specific p53-binding site was elucidated to be implicated in the transcriptional regulation of mdr gene expression.

Our observation of the involvement of wild-type p53 but not mutant p53 in the regulation of the rat mdr1b expression may be relevant to the increased expression of the mdr1b during hepatocarcinogenesis. Although the expression of mdr1 is highly activated, mutation of p53 does not always occur during hepatocarcinogenesis, at least in its early stage of liver tumor development (54). In addition, it has been known that the mdr1b expression in rat liver can be rapidly activated by chemical carcinogens such as 2-AAF and aflatoxin B1 (12, 13), however, in rat hepatocellular carcinomas induced by these carcinogens, p53 mutations do not always occur (55, 56). More importantly, van Gijsell et al. (57) recently reported that p53 activity can be also induced by 2-AAF and aflatoxin B1 in rat liver. When rat hepatoma H-4-II-E cells (contain wild-type p53) were treated with 2-AAF, p53 activity was also induced. These results, taken together, suggested that the activation of the rat mdr1b during chemical hepatocarcinogenesis may be due to the elevated wild-type p53 activities.

In broader prospects, it has been known that p53 is a universal sensor of genotoxic stress (58), and can be induced by a wide variety of DNA-damaging agents such as UV, γ-irradiation, carcinogens, and cytotoxic drugs (for reviews, see Refs. 28 and 29). Strikingly, many of these agents are also known inducers of mdr gene expression, suggesting that p53 may contribute to the induction.

The p53-binding site identified in this study lies in the previously identified murine mdr1b enhancer region (59). It overlaps a palindromic sequence recognized by two peptides (41 and 49 kDa) (30), and adjoins a downstream NF-kB-binding site which is also important for the promoter function (31). It is believed that most inducible cis-acting elements contain multiple, distinct transcription factor-binding sites that are part of a combinatorial mechanism that relies on cooperative binding, interaction of transcriptional activator proteins, and transcriptional synergy (60). Our study of site-directed mutations demonstrated that the full promoter activity of the rat mdr1b requires the integrity of both the p53-binding site (bp –199 to –180) and NF-kB-binding site (bp –167 to –158) (Fig. 2) (31), suggesting that cooperative mechanisms between these two cis-acting elements are implicated in the regulation of the rat mdr1b expression. More recently, coactivator CBP/p300 was shown to interact with both p53 (49–51) and NF-kB (61, 62), and enhance p53- and NF-kB-dependent transactivation, respectively. The activity of the rat mdr1b promoter was also found to be enhanced by CBP/p300. Taken together, these may suggest that the binding of p53 and NF-kB to the mdr1b promoter may recruit CBP/p300 and basal transcriptional machinery to form a higher order transcription enhancer complex, similar to that proposed in interferon-β and E-selectin promoters (63, 64), which modulates inducible expression of the rat mdr1b. However, since our knowledge is rather limited at this moment, the validity of this model still needs to be further tested.

Finally, we would like to stress that, although our present results clearly demonstrated the direct involvement of p53 in the rat mdr1b gene regulation, the roles of p53 in the evolution of drug resistance in cancers remain to be critically evaluated. In clinical setting, the loss of functional p53 has been reported to be well correlated with de novo resistance to radiation and anticancer drugs, and some tumors with wild-type p53 respond well to chemotherapeutic drugs (Refs. 64–66, for review, see Ref. 29). However, it is unknown whether the correlation of drug resistance and p53 mutations is directly due to the activation of mdr by mutant p53, or other mechanisms such as alterations in drug targets, transporters, metabolisms, or the expression of genes regulating cell death and/or survival. Further studies are required to elucidate the molecular insights into how p53 regulates clinical drug sensitivity in cancer chemotherapy.

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