p53 is a transcription factor that is activated by genotoxic stress and mediates cell cycle arrest and apoptosis. Here we demonstrate that infection of mouse liver with recombinant E1E3-deleted adenovirus leads to p53 activation and simultaneously to the down-regulation of albumin gene expression. In vitro transcription assays indicate that transcriptional mechanisms mediated through the albumin promoter are responsible for reduced albumin mRNA levels during viral infection. Albumin expression is maintained in the liver by a combination of liver-enriched transcription factors such as CAAT enhancer-binding protein (C/EBP)α and C/EBPβ. We show that p53 wild type and tumor-derived p53 mutations repress C/EBP-mediated transcriptional activation of the albumin promoter. The binding of C/EBPα or -β to its cognate sequence in the albumin promoter is not inhibited by p53 expression. Deletion analysis and domain swapping experiments show that repression of C/EBPβ-mediated transactivation is dependent on the N-terminal domain of p53 and the transactivation domain, leucine zipper domain, and the inhibitory domain II (amino acids 163–191) of C/EBPβ. Our results provide a molecular explanation for the p53-mediated down-regulation of liver-specific gene expression after viral infection. Additionally, overexpression of p53 mutants is frequently found in undifferentiated hepatocellular carcinomas, the same mechanisms may contribute to the lack of liver-specific gene transcription in these tumors.

As a “guardian of the genome,” p53 mediates cell cycle arrest and subsequently DNA repair or apoptosis, depending on the cellular environment and the degree of cellular damage (for review, see Refs. 1 and 2). Compared with other tumor suppressor genes, p53 has an unusual spectrum of mutations in human tumors. Most of the p53 gene alterations are missense mutations, which occur in the DNA-binding domain of one p53 allele associated with a deletion of the second allele (3, 4). The spectrum of p53 gene mutations and the observation that most of the p53 missense mutations are usually strongly overexpressed in human tumors indicate a positive selection rather than a loss during carcinogenesis. Several studies show that the introduction of specific p53 mutations into tumor cells with p53 deletions in both alleles confer increased growth rate, tumorigenicity, metastatic potential, and resistance to chemotherapy (5–10).

The p53 gene appears to be a frequent target for mutations in hepatocellular carcinomas from high risk areas, whereas the average frequency of p53 missense mutations in hepatocellular carcinomas from low risk areas is only 10–30% (11, 12). The p53 alterations in hepatocellular carcinomas from high incidence areas are preferentially G to T substitutions at the third nucleotide pair of codon 249 (13). Experimental and epidemiological data indicate that aflatoxin B1 exposure results in mutations at p53 codon 249 (14, 15). Another mechanism for inactivating the function of p53 in hepatitis B associated hepatocellular carcinomas is the binding of p53 wild type by the viral protein HBx and the blockage of p53 entry into the nucleus. As a consequence liver tumors developed without evidence of p53 mutations in a HBx transgenic mouse model (16). Although p53 wild type function was impaired by HBx binding, in some animals a small proportion of cells in advanced tumors acquired p53 mutations, which suggests that p53 missense mutations harbor a selection advantage in hepatocarcinogenesis and may contribute to tumor progression.

Actually, it has been shown in several studies that in human hepatocellular carcinomas p53 mutations are closely related to the progression and the dedifferentiation of the tumors (17–20). Furthermore, it has been demonstrated that ectopic expression of the hepatocellular carcinoma hot spot mutation 249S leads to increased survival and mitotic activity of p53 −/− human hepatoma cells (8) and transgene expression in a p53 knock-out mouse results in an enhanced number of hepatocytes in the G1 phase of the cell cycle (9).

Liver-specific gene transcription in differentiated hepatocytes is controlled by a combination of cell type-specific transcription factors. One group is the C/EBP family of transcription factors, which are considered as proteins maintaining cell differentiation (for review, see Ref. 21). They are expressed at late stage during embryonic liver development and in contrast to other liver-specific transcription factors, the amount of C/EBP proteins in hepatoma cell lines is reduced to approximately 10% compared with untransformed hepatocytes (22, 23).

The tumor suppressor Rb positively controls terminal adipo-
p53 Mutations

C/EBPβ Mutations

Fig. 1. Schematic representation of the p53- and C/EBPβ-derived proteins encoded by the expression vectors used in this study. The number indicate the amino acid position of wild type proteins, assuming that the proteins are initiated at the first in frame AUG. The p53 mutations N315 and DASS are derived from the mouse p53 cDNA, whereas all other p53- and C/EBPβ-mutations are derivates from the corresponding human wild type proteins. Dotted white box, deletions; shaded gray box, evolutionary conserved regions of p53. AD, transactivation domain of C/EBPβ; B, basic DNA binding region of C/EBPβ; LZ, leucine zipper domain of C/EBPβ.

cyte differentiation by binding and activating C/EBP proteins (24). In contrast, wild type p53 inhibits the C/EBPβ-mediated gene transcription (25, 26). It was recently suspected that p53 wild type decreases sequence-specific DNA binding of C/EBP transcription factors due to protein-protein interaction (27).

Experimental Procedures

Cell Lines and Plasmids—The human hepatoma cell lines HepG2, Huh7 and Hep3B and 293T cells were obtained from the American Type Culture Collection. The Hep3B cell line was maintained in minimal essential medium (Life Technologies, Inc.) supplemented with antibiotics, L-glutamine, sodium pyruvate, and 10% heat-inactivated fetal calf serum (Life Technologies, Inc.). The 293T, HepG2, and Huh7 cell lines were grown in Dulbecco’s modified Eagle’s medium supplemented with antibiotics, L-glutamine, sodium pyruvate, and 10% heat-inactivated fetal calf serum (Life Technologies, Inc.). The cells were grown in 5% CO2 at 37 °C.

The plasmids used for transfection were CMV promoter-driven expression vectors and a CAT-reporter plasmid. The reporter plasmid contained 400 bp of the human albumin promoter (pAlbumin-CAT) upstream of the chloramphenicol acetyltransferase gene as described before (28). Northern Blot Analysis—Northern blot analysis was performed according to standard procedures. Total RNA was isolated with the Qiagen RNAeasy kit (Qiagen) according to the manufacturer’s instructions. 15 μg of total RNA was analyzed through a 1% agarose formaldehyde gel, followed by transfer to Hybond N membranes (Amersham Pharma Biotech). The Northern blot analysis was performed according to standard procedures. Total RNA was isolated with the Qiagen RNAeasy kit (Qiagen) according to the manufacturer’s instructions. 15 μg of total RNA was analyzed through a 1% agarose formaldehyde gel, followed by transfer to Hybond N membranes (Amersham Pharma Biotech).
Repression of Albumin Transcription through p53

Viral Infection of the Liver Is Associated with an Increase in p53 Expression and Repression of Albumin Gene Transcription—Viral infection of the liver triggers mechanisms in hepatocytes that are either directly related to the virus or a consequence of an immune response directed against viral epitopes. To exclude mechanisms related to the immune system, we used nude mice and nude rats for our experiments. In vivo infection of mouse and rat liver with recombinant adenovirus was performed by administration of 0.3 ml of a solution containing 1 × 10⁹ plaque-forming units/ml into the tail vein. X-gal staining of a specimen of the liver demonstrated that approximately 80–100% of the hepatocytes are infected with viral particles (Fig. 2A).

Mice were sacrificed 8 and 24 h after viral infection, and the livers were harvested for isolation of nuclear extracts and total RNA. Northern blot analysis demonstrated a significant down-regulation of albumin mRNA expression 8 and 24 h after viral infection (Fig. 2B). In contrast, the mRNA and protein levels of the liver-enriched transcription factors C/EBPα and C/EBPβ remained constant. As virus infection may induce genotoxic stress, p53 expression was studied. An increase in nuclear p53 expression was found, which was associated with down-regulation of albumin mRNA levels (Fig. 2B). This was evident 8 and 24 h after virus infection (Fig. 2B).

To assess whether the decrease of albumin mRNA expression level was transcriptionally mediated through the albumin promoter, in vitro transcription analysis was performed. Albumin-specific transcription was measured using a construct where the albumin promoter was combined with a G-less cassette of 400 bp.

In vitro transcription assays were performed with nuclear extracts, isolated at the time point 48 h after tail vein injection of rats. The construct containing adenovirus major late promoter (AdML 200) linked to a G-less cassette of 200 bp was used as a control (C) and added in all incubation mixes with the construct containing the albumin promoter (Alb 400) linked to a G-less cassette of 400 bp.

RESULTS

were labeled with [α-³²P]ATP according to random priming (Roche Molecular Biochemicals, Mannheim, Germany).

In Vitro Transcription Assay—In vitro transcription assays were performed as described by Gorski et al. (42). As DNA templates we used the G-less cassette-containing constructs Alb 400 and AdML 200 G-free, which were a generous gift from U. Schibler. Nuclear extracts were isolated from rat liver by the method described by Lichtsteiner et al. (39). Nuclear extracts and template DNA were preincubated on ice for 15 min in a total volume of 17 μl. 3 μl of the reaction mix containing 20× NTP, RNasin, and 10 μCi of [³²P]UTP were added. The transcription was performed for 45 min at 30 °C. The reaction was terminated by adding the stop buffer (250 mM NaCl, 20 mM Tris/HCl, pH 7, 5, 5 mM EDTA, 1% SDS), 2 μl of 10 mg/ml tRNA, and 4 μl of 10 mg/ml proteinase K solution. After phenol/chloroform extraction and ethanol precipitation, the RNAs were separated on a 8% polyacrylamide/urea gel, visualized by autoradiography, and quantified by Fuji phosphoimager.

Adenoviral Vector, in Vivo Infection of Mouse and Rat Liver—To exclude immunological elimination of infected hepatocytes, we used nude mice and nude rats for our experiments. Female NMRI-nu/nu mice and female NZNU-nu/nu rats were obtained from the Zentrales Tierlaboratorium (Hannover Medical School, Hannover, Germany).

The recombinant, replication-deficient adenoviral vector Ad5-CMV-lacZ was prepared, purified, and titered as described previously (37). Ad5-CMV-lacZ is an adenovirus type 5-based, E1/E3-deleted vector, containing the CMV promoter-driven E. coli lacZ gene (coding for the β-gal protein) (37). The recombinant adenovirus was stored in a buffer solution of 10% glycerol, 10 mM Tris/HCl, pH 7.4, and 1 mM MgCl₂. In vivo adenoviral infection was carried out by the administration of Ad vector into the tail vein of nude mice or rats at a concentration of 1 × 10⁹ plaque-forming units/ml. Mice and rats were injected with 0.3 ml of a solution obtained by dialysis against 10 mM Tris/HCl, pH 8.0, 1 mM MgCl₂, 140 mM NaCl at 4 °C. To evaluate the efficacy of Ad vectors infecting the hepatocytes in vivo, liver specimen of mice and rats were frozen on dry ice and subsequently frozen sections were stained with β-gal substrate X-gal (5-bromo-4-chloro-3-indolyl β-D-galactopyranoside).

were labeled with [α-³²P]ATP according to random priming (Roche Molecular Biochemicals, Mannheim, Germany).

In Vitro Transcription Assay—In vitro transcription assays were performed as described by Gorski et al. (42). As DNA templates we used the G-less cassette-containing constructs Alb 400 and AdML 200 G-free, which were a generous gift from U. Schibler. Nuclear extracts were isolated from rat liver by the method described by Lichtsteiner et al. (39). Nuclear extracts and template DNA were preincubated on ice for 15 min in a total volume of 17 μl. 3 μl of the reaction mix containing 20× NTP, RNasin, and 10 μCi of [³²P]UTP were added. The transcription was performed for 45 min at 30 °C. The reaction was terminated by adding the stop buffer (250 mM NaCl, 20 mM Tris/HCl, pH 7, 5, 5 mM EDTA, 1% SDS), 2 μl of 10 mg/ml tRNA, and 4 μl of 10 mg/ml proteinase K solution. After phenol/chloroform extraction and ethanol precipitation, the RNAs were separated on a 8% polyacrylamide/urea gel, visualized by autoradiography, and quantified by Fuji phosphoimager.

Adenoviral Vector, in Vivo Infection of Mouse and Rat Liver—To exclude immunological elimination of infected hepatocytes, we used nude mice and nude rats for our experiments. Female NMRI-nu/nu mice and female NZNU-nu/nu rats were obtained from the Zentrales Tierlaboratorium (Hannover Medical School, Hannover, Germany).

The recombinant, replication-deficient adenoviral vector Ad5-CMV-lacZ was prepared, purified, and titered as described previously (37). Ad5-CMV-lacZ is an adenovirus type 5-based, E1/E3-deleted vector, containing the CMV promoter-driven E. coli lacZ gene (coding for the β-gal protein) (37). The recombinant adenovirus was stored in a buffer solution of 10% glycerol, 10 mM Tris/HCl, pH 7.4, and 1 mM MgCl₂. In vivo adenoviral infection was carried out by the administration of Ad vector into the tail vein of nude mice or rats at a concentration of 1 × 10⁹ plaque-forming units/ml. Mice and rats were injected with 0.3 ml of a solution obtained by dialysis against 10 mM Tris/HCl, pH 8.0, 1 mM MgCl₂, 140 mM NaCl at 4 °C. To evaluate the efficacy of Ad vectors infecting the hepatocytes in vivo, liver specimen of mice and rats were frozen on dry ice and subsequently frozen sections were stained with β-gal substrate X-gal (5-bromo-4-chloro-3-indolyl β-D-galactopyranoside).
400 bp (Fig. 2C). As an internal control, the adenovirus major late promoter was included. Fig. 2C shows that 48 h after adenoviral infection of rat liver the transcriptional activity of the albumin promoter was reduced to approximately 17%, while transcription of the AdML promoter was unaffected. Therefore, these experiments indicate that transcriptional mechanisms mediated through the promoter reduce albumin mRNA levels after adenoviral transduction.

*p53 Modulates C/EBPβ-dependent Transactivation of the Albumin Promoter*—p53 wild type represses the activity of a variety of promoters by complex formation with TBP and with other transcription factors. Furthermore, p53 wild type specifically inhibits C/EBPβ-mediated transactivation (25, 26). Our in vivo results show that down-regulation of albumin gene transcription was associated with p53 overexpression. Therefore, we were interested to study the role of wild type and mutant p53 for the transcriptional control of the albumin promoter.

Binding of liver-specific transcription factors to the B- and D-site in the albumin promoter confers high transcription of the gene in differentiated hepatocytes, while this regulation is impaired in hepatoma cells. By cotransfecting expression vectors for either C/EBPα or β with an albumin promoter construct, higher transcription can be reconstituted in hepatoma cells, as shown in Figs. 3 and 4. Three hepatoma cell lines with different p53 status were included in this study. HepG2 cells express wild type p53 and Huh7 cells overexpress the p53-220C mutation, while in Hep3B cells the p53 gene is deleted. Cotransfection experiments were performed in all cell lines with an albumin reporter plasmid and expression vectors for C/EBPβ and p53wt. Additionally, the hepatocellular carcinoma-derived p53 mutations p53–249M, p53–249S, and p53–220C, which are unable to transactivate p53-responsive genes (33), were included.

Cotransfection of wild type p53 represses C/EBP-dependent activation of the albumin promoter in a dose-dependent manner (Fig. 3, A and B). However, for p53 mutations, this effect was only evident in HepG2 and Hep3B cells, but not in the Huh7 cells, which implicates that the transcriptional repression through the intrinsic p53–220C mutation cannot be further enhanced by additional ectopic tumor-derived p53 mutations (Fig. 3A).

**The Transactivation Domain of p53 Is Essential to Repress C/EBPα- and β-dependent Target Gene Transcription**—Activation of target genes is an essential mechanism of wild type p53 to act as a tumor suppressor (43). Tumor-derived p53 mutations are mostly located in the DNA binding domain (3, 4), whereas other domains of the molecule are less frequently mutated. Therefore, in further experiments, we examined which p53 domains are involved in repression of C/EBP-de-
Repression of Albumin Transcription through p53

The transactivation of the albumin promoter by C/EBPβ was only repressed by p53wt, by the tumor-derived p53 mutants used in this analysis. The results showed that only p53 mutants with a wild type transactivation domain repress C/EBPβ-mediated transactivation of the albumin promoter.

To exclude a direct role of the p53 constructs on the expression of C/EBPβ and -β, Western blot analysis with nuclear extracts was performed (Fig. 4B). No change in nuclear expression of C/EBP proteins was evident when the different p53 mutants were cotransfected.

p53 Wild Type and Tumor-derived p53 Mutations Have No Impact on Sequence-specific DNA Binding of C/EBPβ or -β—We performed gel shift analysis, using the D-site of the albumin promoter, to determine whether sequence-specific DNA binding of C/EBP isoforms is altered by p53 expression and thus represses transcription of the albumin promoter.

Nuclear extracts derived from Hep3B cells cotransfected with C/EBPβ or -β and p53 mutants were incubated with a 32P-labeled oligonucleotide, representing the D-site of the albumin promoter, at 10% transfection efficacy (data not shown).

To further sensitize our analysis, recombinant C/EBPβ was incubated with nuclear extracts of 293T cells transfected with high amounts of p53 expression vectors. 293T cells were used, because of very high transfection efficacy (∼70% compared with 5–10% in Hep3B cells). Also, these studies clearly showed that p53 does not inhibit DNA binding of C/EBPβ (Fig. 5B), indicating that a different mechanism is relevant to explain repression of C/EBP-dependent gene transcription through p53.

Fig. 4. A, effect of p53 wild type and various p53 mutations on C/EBPα and -β activation of the albumin promoter. The CAT activity found when an expression vector for C/EBPα- and -β was transfected with albumin-CAT alone was set to 100%. The results shown represent the mean of three independent experiments. B, nuclear extracts were isolated from Hep3B cells cotransfected with equal amounts of p53- and C/EBP-expression vectors. 10 μg of nuclear protein were resolved on a denaturing 10% polyacrylamide gel and electroblotted onto Hybond N membrane. p53 and C/EBP proteins were detected with the primary antibodies p53 pAb240 (Dianova), C/EBPα 14/AA (Santa Cruz Biotechnology), and a polyclonal C/EBPβ antibody (30).

Fig. 5. p53 does not influence DNA binding of C/EBPα and C/EBPβ. C/EBPα- and C/EBPβ-specific complexes in the EMSA were identified as retarded bands, which supershifted in the presence of specific antibodies (black arrow). A, EMSA were performed with nuclear extracts (2 μg of protein) of Hep3B cells cotransfected with 3 μg of C/EBPα and 3 μg of p53 expression vectors. B, EMSA were performed using nuclear extracts (1 μg of protein) of 293T cells transfected with 6 μg of p53 expression vectors together with 1 ng of in vitro translated C/EBPβ.
Repression of C/EBPβ-dependent Gene Transcription through p53 Requires the Inhibitory Domain II, the Transactivation, and the Leucine Zipper Domain of C/EBPβ—We investigated whether specific C/EBPβ domains are involved in the repression mediated by p53. Besides the DNA binding, transactivation, and leucine zipper domain of C/EBPβ, two inhibitory regions were characterized (31). Therefore, four different constructs, in which all the functional domains (except the DNA binding) of C/EBPβ were replaced or deleted, were used. In the constructs C/EBPβD116–149 and D163–191, the inhibitory domain I and II were deleted, respectively. In the C/EBPβ/VP16 construct, the transactivation domain of C/EBPβ was replaced by the one of VP16 and in the C/EBPβ-GCN4 construct the leucine zipper was swapped by the one of GCN4. Each C/EBPβ mutant was cotransfected with the albumin reporter gene and the different p53 mutant constructs in Hep3B cells (Fig. 6).

In Fig. 6 (A and B), the results of the C/EBPβD116–149 and D163–191 are depicted. C/EBPβ D116–149 showed the same repression of the albumin promoter transactivation through p53 as C/EBPβ wild type (Fig. 6A). In contrast, differences were found with the C/EBPβ D163–191 construct where the inhibitory region is deleted which modulates DNA binding (31). Albumin promoter transactivation through C/EBPβ D163–191 is not repressed by p53 as 220C, 249M, and 249S; also, p53 wild type revealed a 2-fold reduced transcriptional repression compared with the experiments with C/EBPβ wild type (Fig. 6B).

These results showed that the inhibitory domain II, which modulates DNA binding, but not the inhibitory domain I, which modulates transactivation, is required for the repressive effect mediated by p53, although C/EBPβ binding to DNA is not altered by p53. However, the molecular function of the inhibitory domain II in p53-dependent transcriptional repression remains unknown.

In Fig. 6 (C and D), the mutants C/EBPβ/VP16 and C/EBPβ-GCN4 were studied. Both constructs showed results similar to those found with the C/EBPβ D163–191 deletion. Transactivation of the albumin promoter was not repressed through tumor-derived p53 mutations, only the p53-del 327 and p53wt decreased the transcriptional activity to 50% (Fig. 6, C and D). Therefore, our results indicate that wild type or mutant p53 repress C/EBP-dependent transactivation of the albumin promoter through the leucine zipper, the inhibitory domain II, and the transactivation domain of C/EBPβ.

DISCUSSION

After adenoviral infection of the liver, p53 expression and DNA binding increased, which was associated with a decrease in albumin gene transcription mediated through the albumin promoter. Interestingly, protein and mRNA levels of C/EBPα and -β remained constant during the time course of the virally induced stress response. Thus we hypothesized that p53 could be involved in the down-regulation of albumin promoter activity. Reporter gene analysis demonstrated that p53 could be directly involved in repressing C/EBP-dependent activation of albumin gene transcription. These results implicate that p53 mediates...
down-regulation of liver-specific gene transcription during stress response induced by nonimmunological mechanisms after viral infection of the liver. Thus, our study gives a molecular explanation for the phenomenon that liver-specific gene expression decreases during acute viral hepatitis although liver cell mass remained unchanged. Obviously, a different situation is evident during hepatocarcinogenesis. In the course of tumor development and tumor progression, tissue-specific gene transcription decreases. Therefore, it was also the aim of this study to investigate the role of p53 mutations on liver-specific gene transcription.

p53 is a strong transcriptional repressor. Complex formation of the general transcription factor TBP with wild type p53, but not with tumor-derived p53 mutations, results in repression of TATA box-mediated transcription (44, 45). Additionally, interaction of wild type p53 with a set of transcription factors has been shown, which may lead to TATA box-unrelated repression of gene transcription (46, 47). The property of mutants to alter gene transcription in a manner distinct from wild type p53, is thought to mediate the specific gain of function phenotype of some p53 mutations (5, 48–51). The impact of a particular p53 mutation on the transcriptional regulation may be cell type-dependent and as a consequence may determine, at least in part, the tumor-specific spectrum of p53 missense mutations.

The role of C/EBP proteins for cell proliferation has been studied before. Overexpression of either C/EBPα or β in liver cells blocks cell proliferation (52, 53). Additionally hepatocytes derived from C/EBPα−/− mice have a higher rate of DNA synthesis and immortalization (54). Therefore, C/EBP proteins, which contribute to cell differentiation, seem an attractive target of repression during neoplastic transformation. Li et al. (55) reported that transcriptional repression might contribute to neoplastic growth. More specifically it has been demonstrated that the repression of gene transcription is necessary for neoplastic transformation by c-Myc (56). Since c-Myc down-regulates genes by inhibiting the function of C/EBP transcription factors, a link between cell transformation and repression of C/EBP-mediated gene transcription is strongly suggested (57).

No uniform pattern has been reported for different p53 mutants in controlling C/EBP-dependent gene transcription in hepatoma cells. The p53 mutation Val-143 → Ala has been described to repress C/EBPβ-mediated transactivation, whereas Cys-135 → Val and Lys-132 → Phe enhance the transcriptional activity of C/EBPβ in hepatoma cells (26). In our study we show that the hepatocellular carcinoma-derived mutations p53-220C, -249M, and -249S retain a capacity to repress C/EBPα- and β-mediated transactivation of the human albumin promoter in hepatoma cells, which can be considered to be a factor that determines the selection during hepatocarcinogenesis. Besides the tumor-derived mutations, only p53 mutations (31). Therefore, further experiments seem promising to test our hypothesis that the same C/EBPβ domains required for hepatocyte differentiation are involved in the repression through p53 and this mechanism may thus contribute to hepatocarcinogenesis.

In summary, we show an increase of p53 expression after adenoviral infection, which is associated with a down-regulation of promoter-dependent transcription of the albumin gene. We demonstrate that different regions of C/EBP and p53 are involved in this regulation despite the lack of sequence-specific DNA binding of p53 to the albumin promoter. Transcriptional repression of C/EBP-mediated transactivation through p53 wild type might contribute to the down-regulation of liver-specific gene expression during liver failure. Since overexpression of p53 mutations is associated with dedifferentiation of human hepatocellular carcinomas, this mechanism may also contribute to the selection of p53 mutants during hepatocarcinogenesis.

Acknowledgments—We thank B. Vogelstein for providing the p53 expression vector pC53SN3. We thank A. Levine for providing the expression vector p53-22Q/22S. We thank P. Johnson for the generous gift of the expression plasmids pCRP2116–149, pCRP2163–191, pMexCRP2–1-47(VP16), and pMexCRP2-GCN4. We thank S. Deb for providing the plasmids p53del1–59 and p53del193–327. We thank M. Oren for providing the plasmids p53N315 and p53DASS. We thank D. Brenner for providing the adenoviral vector Ad5CMVlacZ.

REFERENCES

1. Selivanova, G., and Wiman, K. G. (1995) Adv. Cancer Res. 66, 143–180
2. Agarwal, M. L., Taylor, W. R., Chernov, M. V., Chernova, O. B., and Stark, G. R. (1998) J. Biol. Chem. 273, 1–4
3. Greenblatt, M. S., Bennett, W. P., Hollstein, M., and Harris, C. C. (1994) Cancer Res. 54, 4855–4878
4. Hussain, S. P., and Harris, C. C. (1998) Cancer Res. 58, 4023–4037
5. Dittmer, D., Pati, S., Zambetti, G., Chu, S., Teresky, A. K., Moore, M., Finlay, C., and Levine, A. J. (1993) Nat. Genet. 4, 42–46
6. Sun, Y., Nakamura, K., Wedel, E., and Colburn, N. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 2857–2861
7. Hsiao, M., Low, J., Dorn, E., Ku, D., Pattengale, P., Yaragin, J., and Haas, M. (1994) Am. J. Pathol. 145, 702–714
8. Pochel, F., Puisieux, A., Tahone, E., Michot, J. P., Froeschl, G., Morel, A. P., Frebourg, T., Fontanier, E., Oberhammer, P., and Ozturk, M. (1994) Canc. Res. 54, 2064–2068
9. Yin, L., Ghebrahun, N., Chakraborty, S., Sheehan, C. E., Ilic, Z., and Sell, S. (1998) Hepatology 27, 73–80
10. Blandino, G., Levine, A. J., and Oren, M. (1999) Oncogene 18, 477–485
11. Unsal, H., Yakicier, C., Marcais, C., Kew, M., Volkmann, M., Zentgraf, H., Isselbacher, K. J., and Ozturk, M. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 822–826
12. Kühnke, S., Trautwein, C., Schrem, H., Tillman, H., and Manns, M. P. (1999) J. Hepatol. 23, 412–419
13. Hsu, I. C., Mentel, I. R., Sun, T., Welsh, J. A., Wang, N. J., and Harris, C. C. (1991) Nature 350, 427–428
14. Aguilar, F., Hussain, S. P., and Cerutti, P. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 8586–8590
15. Ozturk, M. (1991) Lancet 338, 1356–1359
16. Ueda, H., Ullrich, S. J., Gangeri, J. D., Kappel, C. A., Ngo, L., Feistelson, M. A., and Jay, G. (1995) Nat. Genet. 9, 41–47
Repression of Albumin Transcription through p53

17. Oda, T., Tsuda, H., Scarpa, A., Sakamoto, M., and Hirohashi, S. (1992) Cancer Res. 52, 6358–6364
18. Tanaka, S., Tobi, Y., Adachi, E., Matsumata, T., Mori, R., and Sugimachi, K. (1995) Cancer Res. 55, 2884–2887
19. Teramoto, T., Satonaka, K., Kitazawa, S., Fujimori, T., Kozaburo, H., and Maeda, S. (1994) Cancer Res. 54, 231–235
20. Hayashi, H., Sugio, K., Matsumata, T., Adachi, E., Takenaka, K., and Sugimachi, K. (1995) Hepatology 22, 1702–1707
21. Wedel, A., and Ziegler-Heitbrock, H. W. (1995) Immunobiology 193, 171–185
22. Mischoulen, D., Rana, B., Bucher, N. L., and Farmer, S. R. (1992) Mol. Cell. Biol. 12, 2553–2560
23. Levrat, F., Vallet, V., Berbar, T., Miquerol, L., Kahn, A., and Antoine, B. (1993) Exp. Cell Res. 209, 307–316
24. Chen, P. L., Riley, D. J., Chen-Kiang, S., and Lee, W. H. (1996) J. Biol. Chem. 271, 307–316
25. Margulies, L., and Sehgal, P. B. (1993) J. Biol. Chem. 268, 15096–15100
26. Wang, L., Bayanade, R. J., Garcia, D., Patel, K., Pan, H., and Sehgal, P. B. (1995) J. Biol. Chem. 270, 23159–23165
27. Webster, N. J. G., Reznik, J. L., Reichart, D. B., Strauss, B., Haas, M., and Seely, B. L. (1996) Cancer Res. 56, 2781–2788
28. Pietrangelo, A., Panduro, A., Roy-Chowdhury, J., and Shafritz, D. A. (1992) J. Clin. Invest. 89, 1755–1760
29. Morris, G. F., Bischoff, J. R., and Mathews, M. B. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 895–899
30. Trautwein, C., Caelles, C., van der Geer, P., Hunter, T., Karin, M., and Chojkier, M. (1993) Nature 364, 544–547
31. Williams, C. W., Baer, M., Dillner, A. J., and Johnson, P. F. (1995) EMBO J. 14, 3170–3183
32. Trautwein, C., Rakemann, T., Pietrangelo, A., Plumpe, J., Montosi, G., and Manns, M. P. (1997) Hepatology 28, 867–873
33. Kubicks, S., Trautwein, C., Niehof, M., and Manns, M. P. (1997) Hepatology 28, 867–873
34. Subler, M. A., Martin, D. W., and Deb, S. (1992) Oncogene 9, 1351–1359
35. Shaulian, E., Haviv, I., Shaul, Y., and Oren, M. (1995) Oncogene 10, 671–680
36. Lin, J., Teresky, A. K., and Levine, A. J. (1995) Oncogene 10, 2387–2390
37. Bradham, C. A., Qian, T., Street, K., Trautwein, C., Brenner, D. A., and Lemasters, J. J. (1989) Mol. Cell. Biol. 18, 6353–6364
38. Graham, F. L., and van der Eb, A. J. (1973) Virology 54, 536–539
39. Lichtsteiner, S., Wurain, J., and Schihler, U. (1987) Cell 51, 963–973
40. Trautwein, C., van der Geer, P., Karin, M., Hunter, T., and Chojkier, M. (1994) J. Clin. Invest. 93, 2554–2561
41. Poli, V., Manzini, P. P., and Cortese, R. (1990) Cell 63, 643–653
42. Gorski, K., Carneiro, M., and Schiebler, U. (1986) Cell 47, 767–776
43. Pietenpol, J. A., Tokino, T., Thiagalingam, S., el-Deiry, W. S., Kinzler, K. W., and Vogelstein, B. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 1986–2002
44. MacK, D. H., Vartikar, J., Pipas, J. M., and Laimins, L. A. (1993) Nature 363, 281–283
45. Raginov, N., Krauskoft, A., Navot, N., Rotter, V., Oren, M., and Aloni, Y. (1993) Oncogene 8, 1383–1393
46. Itozova, V., Crepieux, P., Montpellier, C., Laudet, V., and Stehelin, D. (1996) Oncogene 13, 2331–2337
47. Dugimont, T., Montpellier, C., Adriaenssens, E., Lottin, S., Dumont, L., Itozova, V., Lagrou, C., Stehelin, D., Coll, J., and Curgy, J. J. (1998) Oncogene 16, 2395–2401
48. Chin, K. V., Ueda, K., Pastan, I., and Gottesman, M. M. (1992) Science 253, 459–462
49. Deb, S., Jackson, C. T., Subler, M. A., and Martin, D. W. (1992) J. Virol. 66, 6184–6170
50. Shiroyo, Y., Yamamoto, T., and Yamaguchi, Y. (1992) Proc. Natl. Acad. Sci. U. S. A. 89, 5206–5210
51. Ueba, T., Tosaka, T., Takahashi, J. A., Shibata, F., Florkiewicz, R. Z., Vogelstein, B., Oda, Y., Kikuchi, H., and Hatanaka, M. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 9009–9013
52. Buck, M., Turler, H., and Chojkier, M. (1994) EMBO J. 13, 851–860
53. Watkins, P. J., Condrey, J. P., Huber, B. E., Jakobs, S. J., and Adams, D. J. (1996) Cancer Res. 56, 1063–1067
54. Soriano, H. E., Kang, D. C., Finegold, M. J., Hicks, M. A., Wang, N. D., Harrison, W., and Darlington, G. J. (1998) Hepatology 27, 392–401
55. Li, J., Thurm, H., Chang, H. W., Iacovoni, J., and Vogt, P. K. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 10885–10888
56. Lee, L. A., Dolde, C., Barrett, J., Wu, C. S., and Dang, C. V. (1996) J. Clin. Invest. 97, 1687–1695
57. Mink, D., Mitchel, B., Weiskirchen, R., Bister, K., and Klempnauer, K. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 6635–6640
58. Liu, X., Miller, C. W., Koeffler, P. H., and Berk, A. J. (1993) Mol. Cell. Biol. 13, 3291–3300
59. Sang, B. C., Chen, J. Y., Minna, J., and Barbosa, M. S. (1994) Oncogene 9, 853–859