Modulation of Interleukin-6-induced Plasma Protein Secretion in Hepatoma Cells by p53 Species*

Ling Wang‡, Ravi J. Rayanade‡, Dorys Garcia†, Kirit Patel‡, Heng Pan†, and Pravin B. Sehgal‡

From the Departments of Cell Biology & Anatomy and of Medicine, New York Medical College, Valhalla, New York 10595

The ability of p53 species (wild-type and mutant) to modulate the “differentiated” response of human hepatoma cell lines Hep3B and HepG2 to interleukin-6 (IL-6) was investigated. Transient transfection experiments were carried out in Hep3B and HepG2 cell cultures in which IL-6 was used to activate a β-fibrinogen (βFib) enhancer/reporter construct containing two copies of the 36-base pair IL-6-response element (IL-6RE) (βFibCAT). Cotransfection with constitutive expression vectors for wild-type (wt) human or murine p53 inhibited the activation of the βFibCAT reporter by IL-6 in both Hep3B and HepG2 cells. Several mutant p53 species either did not inhibit the activation of βFibCAT or up-regulated the response. Hepatoma cell lines expressing the Val-135 temperature-sensitive mutant of murine p53 (wt-like at 32.5 °C and mutant-like at 37 °C) were derived from Hep3B cells and tested for the temperature-sensitive phenotype of their ability to synthesize and secrete fibrinogen and α1-antichymotrypsin in response to IL-6. In an experimental protocol in which the parental Hep3B cells did not show a significant difference in plasma protein secretion at the two temperatures, hepatoma line 3 (p53Val-135Δ) had a greater response to IL-6 at 37 °C than parental Hep3B cells, while line 3 cells had a reduced response to IL-6 at 32.5 °C. Similarly, hepatoma lines 1 and 2 (both p53Val-135Δ) had reduced IL-6 responsiveness at 32.5 °C, whereas line 22 (transfected with pSVneo alone) and the parental Hep3B cells did not. These data indicate that mutations in p53 contained in tumor cells can modulate the “differeniated” response of these cells to cytokines.

Mutations in the transcription factor p53 are among the commonest alterations observed in human cancer (1). These tumor cell-derived mutations in p53 can reflect both an abrogation of the function(s) of wild-type (wt) p53 as well as “gain in-function” mutations (2–7). Mutational “hot-spots” have been demarcated within p53 derived from particular cancers, e.g. hepatocellular carcinoma (1, 3). The biological functions of p53 and their alterations by mutations have been largely discussed in the context of the regulation of cell proliferation, apoptosis, and of repair of DNA damage (1, 3, 5). We have investigated the ability of p53 species to modulate the “differeniated” function of tumor cells treated with cytokines in a context distinct from that of the regulation of cell proliferation or cell death. Can mutations in p53 in hepatoma cells confer altered responsiveness of plasma protein gene expression to cytokines such as interleukin-6 (IL-6)? The question posed takes on particularly broad significance as cytokines enter the mainstream of cancer therapy. To what extent do alterations in p53 present in tumor cells alter cytokine-responsive gene expression in these cells?

Wild-type p53 can enhance transcription from promoters that contain DNA-binding sites for p53, whereas wt p53 can repress many promoters that do not contain p53-DNA-binding sites (1–5). Transforming mutations in p53 alter the ability of this molecule to modulate transcription. Mutant p53 species lack the ability to enhance or repress transcription of test enhancer/reporter constructs. Additionally some tumor-derived mutants (e.g. the murine p53 mutants Val-135 and Phe-132) can exhibit a gain in function that is manifest as the ability to enhance transcription from reporter constructs that are otherwise repressed by wt p53 (6, also see Ref. 7 for a review of gain in function mutations in p53). Transcriptional modulation by p53, particularly repression, is thought to occur due to its interactions with cellular proteins such the TATA-binding protein (8) or CAAT-binding factor (9) (the “squelching” model). A variety of other cellular and viral proteins interact with p53 and these, in turn, affect the stability or the transcriptional activity of p53 (10–18). There is little information about functional or structural interactions of p53 species with cell-surface receptors for cytokines or with molecules implicated in the signal transduction pathways triggered by cytokines or with transcription factors implicated in cytokine-mediated responses.

The negative regulation of some but not other cellular promoters by wt p53 and the lack of repression by transforming mutants was first pointed out in 1991 (4, 30). In transient transfection experiments, we showed that reporter plasmids containing regulatory elements from the IL-6, c-fos, β-actin, or from a major histocompatibility complex I promoter were strongly repressed by wt p53 but less so by the transforming mutants tested (4). Numerous investigators have confirmed and extended the ability of wt p53 to repress various cellular promoters in transient transfection experiments; these include the c-fos, β-actin, heat shock protein-70, c-jun, c-myc, p53 itself, the retinoblastoma gene promoter, proliferating cell nuclear antigen, and the multi-drug resistant gene promoters (8, 9, 30, 37–40). Additionally a variety of viral promoters derived from
the Rous sarcoma virus long terminal repeat (RSV-LTR), herpes simplex virus (thymidine kinase gene), simian virus 40 (early promoter), and human T-cell leukemia virus (p53-responsive element) have also been reported to be repressed by wt but not mutant p53 species (41–43).

In an earlier study, we observed the up-regulation of the IL-6 promoter by the murine p53 mutants Val-135 and Phe-132 (2). An intact CAAT enhancer binding protein (C/EBP)-binding site (alias nuclear factor-1L6 [NF-1L6] or C/EBPβ site) in the IL-6 promoter or in the herpesvirus thymidine kinase promoter was a requirement for up-regulation by these p53 mutants suggesting that the transcription factor C/EBPβ might be a target for p53 modulation (2). In functional experiments, wt p53 blocked transcriptional activation of the IL-6 promoter by C/EBPβ. In contrast, the p53 mutant species Val-135 and Phe-132 enhanced C/EBPβ-mediated gene activation. Stimulated by prior investigations implicating the C/EBP family transcription factors in the cascade of transcription factors activated by cytokines (e.g. by IL-1 or by the IL-6-type cytokines) in differentiated cells (e.g. the hepatocyte) (44–47), we posed a more general question: can p53 species modulate the response of cells to cytokines in a context distinct from the regulation of cell proliferation? Could p53 species modulate IL-6-induced activation of plasma protein gene expression in an hepatoma cell? Could mutations in p53 alter the response of a hepatoma cell to cytokines such as IL-6 that could contribute to an altered ability of these cells to synthesize and secrete acute-phase plasma proteins?

We have addressed these questions in two different ways. First, in a series of transient transfection experiments in Hep3B and HepG2 hepatoma cells we investigated the effect of p53 species on the function of the 36-bp IL-6-response element (IL-6RE) derived from the rat β fibronogen (βFib) promoter. Second, we derived Hep3B hepatoma lines stably transfected with a constitutive expression vector for a temperature-sensitive mutant of p53 (Val-135; wt-like at 32.5 °C and mutant-like at 37 °C), and investigated the ability of IL-6 to enhance the synthesis and secretion of Fib and of α1-antichymotrypsin (ACT) at the two temperatures in these p53Val-135 hepatoma cell lines.

MATERIALS AND METHODS
Cell Culture and Transient DNA Transfection Assays—Human hepatoma cell lines Hep3B and HepG2 were obtained from the American Type Culture Collection, Rockville, MD. Hep3B cells do not have an intact endogenous p53 gene, while HepG2 cells have low level expression of wt endogenous p53 gene (22, 48, 49). Both Hep3B and HepG2 cell lines were cultured in T-75, T-175, or 100-mm plasticware (Falcon, Franklin Lakes, NJ) in Dulbecco’s modified Eagle’s medium (MEM) supplemented with non-essential amino acids, 2 mM sodium pyruvate, and 10% (v/v) heat-inactivated fetal bovine serum (FBS) (Life Technologies, Inc.), 2 mM sodium pyruvate, and 10% (v/v) heat-inactivated fetal bovine serum (FBS) (Life Technologies, Inc.). Procedures for DNA transfections into cells growing in 100-mm plastic dishes using the calcium-phosphate method were essentially as described (4, 50). Each 100-mm culture was transfected with 25 µg of total plasmid DNA consisting of 10 µg of reporter plasmid (p55CAT or p50), together with, as appropriate, 5 µg of a p53 expression vector, 5 µg of a C/EBP expression vector, and, in each case 5 µg of pRSVgal as a marker for the overall effectiveness of transfection and to control for the effects of p53 on basal transcription factors, with the total amount of DNA made up to 25 µg using the control expression vector pCMV. In all experiments, transfected cultures were kept in serum-containing medium (10% (v/v) FBS) for 24 h and shifted to medium containing IL-6 (Escherichia coli-derived human IL-6, Sandz, East Hanover, N.J.) as appropriate. Cells were harvested for preparation of extracts approximately 24 h later. All cell extracts were first assayed for protein content using a microassay (Bio-Rad) and for β-galactosidase (gigalt) activity as described previously (4, 50). Two sets of chlamydomenial acetyltransferase (CAT) assays were performed for each experiment: in one set, the total protein in extracts was used as the basis for normalization between groups, in the other, β-galactosidase activity was used as the basis for normalization between extracts (2, 6); no significant difference was observed using the two procedures. Following thin layer chromatography and autoradiography, the intensity of spots corresponding to acetylated and non-acetylated (14C) chloramphenicol was monitored by densitometry. All transient transfection assays were repeated at least three times.

DNA Plasmids—The two enhancer-CAT reporter plasmids p55CAT and p50 (Fig. 1) were obtained from Drs. Heinz Baumann and Moshe Oren, respectively. p55CAT contains two copies of the 36-bp IL-6- and C/EBP-responsive element (IL-6RE) located between −168 and −134 in the rat β fibronogen promoter (44–46). The plasmid p50 contains two copies of the 50-bp p53-binding element located between −3182 and −3133 from the muscle-specific creatine kinase promoter (51). Both plasmids contain the adenosine virus type 2 major late promoter (and TATA-box) linked to CAT. For transient experiments in hepatoma cells, a series of constitutive expression plasmids for human wt p53 (p53-SCX3), human p53 mutant Val-143 to Ala (p53-SCX3), murine wt p53 (pCMVNc9), the murine mutants Glu-168 to Gly/Met-234 to Ile (pCMVc5), Ala-135 to Val (p53Val135), and Cys-132 to Phe (p53Phe-132) were used. The human p53 expression vectors were obtained from Dr. Bert Vogelstein (52), while the murine p53 expression vectors were obtained from Dr. Moshe Oren (21, 28, 30). In previous experiments we have confirmed by [³H]methionine labeling and immunoprecipitation that transient transfection of these plasmids leads to the synthesis and accumulation of the appropriate p53 species in the transfected cells (see Ref. 4 for examples). As a control the plasmid pCMV derived from the p53-SCX3 by removal of the p53 cDNA insert was used. Constitutive expression vectors using the Moloney sarcoma virus LTR for rat C/EBP α, β and γ were obtained from Dr. Steven L. McKnight (53).

Derivation of Stably Transfected Hep3B Cell Lines—Stably transfected Hep3B cell cultures in T-75 flasks were cotransfected with the constitutive expression vector for murine p53Val-135 together with the plasmid pSVneo using the calcium-phosphate procedure as indicated above. Three days after transfection, G418 (Sigma) was added to the culture medium at a concentration of 400 µg/ml, and the cultures maintained in this medium for 3 weeks. Clones of cells derived from these transfections that survived the G418 selection were subcultured either as single clones or as pools of two to four clones. Sixteen hepatoma cell lines were derived from transfection with p53Val-135 together with pSVneo, and seven lines were derived from transfection with pSVneo alone. After expansion and subculture, the cell lines derived were assayed for presence of p53 by immunofluorescence, and three of the p53Val-135-containing hepatoma cell lines (lines 1–3) as well as one p5SVneo control cell line (line 22) were used in further experiments.

Immunofluorescence Analyses for p53—Hepatoma cell lines were cultured in 8-well Chamber Slide (Nunc Inc, Naperville, IL) for 1 day so as to form confluent cultures. The cultures were maintained in this medium for 3 weeks. Thereafter, the cultures were washed with phosphate-buffered saline, fixed with chilled acetone for 2 min, and air-dried. p53 antigen was detected using the mouse-monoclonal antibody, PA2028 (pan-reactive to human and murine wt and mutant p53 species) (Santa Cruz Biotechnology, Inc., Santa Cruz, CA) as described by the manufacturer. The second Ab was fluorescein-labeled rabbit anti-mouse IgG. The cell lines used were examined both before commencement of the series of experiments depicted in Figs. 6–8 as well as near the conclusion of the experiments (see below).

IL-6-inducible Synthesis and Secretion of ACT and Fib by Hepatoma Cell Lines—The procedure using hepatoma cell cultures in 24-well plates has been described earlier (54, 55). Briefly, cells were plated at 2.5 × 10⁵ cells/well in 1 ml of culture medium (in Dulbecco’s MEM supplemented with non-essential amino acids, 2 mM sodium pyruvate, and 10% FBS) for 24 h at 37 °C. After that, the cultures were washed with phosphate-buffered saline and replenished with serum-free and methionine-free Dulbecco’s MEM (0.5 ml/well) containing [³H]methionine (0.1–0.2 mCi/ml) (ICN Radiochemicals, Irvine, CA) for 4 h, and then ACT added at either 0.1 or 10 ng/ml for 24 h. Thereafter, the cultures were washed with methionine-free Dulbecco’s MEM (0.5 ml/well) containing [³H]methionine (0.1–0.2 mCi/ml) (ICN Radiochemicals, Irvine, CA) for 24 h. The secretion of methionine-labeled plasma proteins into the culture medium was monitored by sequential immunoprecipitation using rabbit antibody to human α1-antichymotrypsin and to human fibrinogen (both from Calbiochem-Novabiochem Corp., La Jolla, CA), followed by SDS-polyacrylamide gel electrophoresis and autoradiography. The synthesis and secretion of labeled ACT and of the major β-fibrogenin were quantified from these autoradiograms by densitometry.

Statistical Analyses—Statistical evaluations were carried out using as appropriate the Student’s t test, matched analysis of variance for
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RESULTS

The β-fibrinogen and the α1-antichymotrypsin genes are so-called Type II acute-phase plasma protein genes in that their expression at maximal levels in hepatocytes requires only IL-6 (reviewed in Refs. 6, 46). More generally, the group of IL-6-type cytokines that include IL-6, leukemia inhibitory factor, oncostatin M, interleukin-11, ciliary neurotrophic factor, and cardiotrophin-1 can stimulate hepatic fibrinogen gene expression provided that the hepatoma cell lines tested harbor the appropriate α-chain or cytokine-binding component of the cell-surface receptor complexes (reviewed in Refs. 6, 46, 56, 57).

In each instance the signal-transducing chain is the gp130 β-chain of the receptor (reviewed in Refs. 57, 58). IL-6 enhances fibrinogen gene expression in both Hep3B and HepG2 cells. The 36-bp IL-6RE in the rat β-fibrinogen gene consists of the consensus CTTGGA sequence present in all Type II plasma protein genes that constitute binding sites for various STAT-family transcription factors (59–61). The βFib IL-6RE is also functionally responsive to C/EBP family transcription factors (44–46). Thus the reporter construct pβFibCAT (Fig. 1) allows two questions to be answered: (i) the ability of p53 species to modulate a response driven by IL-6, and (ii) the ability of p53 species to modulate a response driven by C/EBPα, β, or δ per se following cotransfection of appropriate constitutive expression vectors.

Modulation by p53 Species of the Response of βFibCAT to IL-6—IL-6 activated the pβFibCAT reporter when the latter was transfected into Hep3B (Fig. 2A) or HepG2 cells (Fig. 2B). In each cell type the cotransfection of expression vectors for murine or human wt p53 inhibited this IL-6-driven activation of the βFibCAT reporter (Fig. 2). Mutant p53 species did not inhibit IL-6-driven activation of the βFib enhancer, although, in some experiments, the mutants p53-CX3 and p53-C5 appeared to enhance βFib CAT expression (Fig. 2). As a contrast control we used the plasmid p50-2 that contains the Ad MLP (and the Ad TATA-box) (Fig. 1). Fig. 3 shows that wt p53 species enhanced expression from p50-2 in Hep3B cells mutant p53 species did not have this property. Similar results were obtained in HepG2 cells (data not shown). A comparison of Figs. 2 and 3 readily shows the dramatic difference in the effect of wt p53 on the βFibCAT and p50-2 reporters. The data show that p53 species can functionally modulate IL-6-driven activation of an acute-phase plasma protein enhancer in hepatoma cells.

Modulation by p53 Species of the Response of βFibCAT to C/EBP Transcription Factors—Fig. 4 is a compilation of transient transfection experiments carried out in HepG2 cells in which the reporter βFibCAT was driven by cotransfection with constitutive expression vectors for C/EBPα, β, or δ. Human and murine wt p53 species inhibited the activation of βFibCAT by all three C/EBP species. In contrast mutant p53 species either did not inhibit or enhanced expression of βFibCAT (Fig. 4A, Val-135 and C/EBPδ combination and Fig. 4C, Phe-132 and C/EBPδ combination). Similar results were obtained in Hep3B cells (data not shown). The enhancement of C/EBP-driven transcription of pβFibCAT is reminiscent of the ability of p53Val-135 and Phe-132 to markedly enhance C/EBPδ-driven activation of the IL-6 promoter and of the herpesvirus thymidine kinase promoter provided that the C/EBP-binding site was intact (6).

In order to confirm that the inhibitory effect of wt p53 on activation of the βFibCAT enhancer by the C/EBP transcription factors was specific, the control reporter p50-2 was used in HepG2 cells. The reporter p50-2 was activated to a small extent by cotransfection with C/EBPα, β, or δ expression vectors in HepG2 cells. However, the combination of wt p53 species (SN3 or Nc9) with C/EBP isoforms enhanced this expres-
The binding of recombinant baculovirus expression vector-derived murine wt p53 to the IL-6RE in FibCAT was tested in electrophoretic mobility shift assays. The data obtained indicate that when compared to authentic p53-binding DNA elements, such as p50–2, the binding of p53 to the βFib IL-6RE oligonucleotide in gel-shift assays was, at best, relatively weak (data not shown).

Fig. 4. Modulation by p53 species of C/EBP-induced activation of βFibCAT in hepatoma cells. HepG2 cultures in 100-mm dishes were cotransfected with βFibCAT (10 μg), expression vectors for C/EBPα, β, or δ (5 μg) together with various p53 expression vectors (5 μg) in duplicate. Cells were harvested 24 h after the beginning of transfection and the level of CAT expression monitored essentially as described in legend to Fig. 2. Inset in panel C shows an autoradiogram of a CAT assay from one experiment. p53 constructs: SN3, human wt; CX3, human mutant; Nc9, murine wt; c5, Val135, and Phe132, murine mutants.

Fig. 5. p53 immunostaining in hepatoma cell lines. Parental Hep3B cells (panel A), three p53Val-135-expressing hepatoma lines (lines 1–3, respectively, panels B–D), and a hepatoma line transfected with pSVneo alone (line 22, panel D) were tested for p53 by immunostaining using a panreactive anti-p53 monoclonal antibody (Pab 240) (reactive with wt and mutant human and murine p53 species). The length marker equals 20 μm.

Derivation of p53Val-135-expressing Hep3B Cell Lines—In order to assess the biological importance of the ability of p53 species to modulate the response of hepatoma cells to IL-6, we derived cell lines from the parental p53-free Hep3B cells that had been cotransfected with a constitutive expression vector for a ts mutant of murine p53. Several Hep3B cell lines that expressed p53Val-135 constitutively were derived (Fig. 5, lines 1–3). As a control we derived Hep3B lines containing only pSVneo (Fig. 5, line 22).

Temperature-sensitive Phenotype of Plasma Protein Secretion in Response to IL-6 of p53Val-135 heateroma Cells But Not of the Parental or of p53Val-135 Cells—The Val-135 mutant of murine p53 is known to be ts in its phenotype (6, 7, 28, 30). It is wt-like at 32.5 °C and transforming mutant-like at 37 °C. We have previously confirmed that p53Val-135 as studied in this laboratory is ts in its phenotype with respect to transcriptional regulation of various promoters (6). What is the ts phenotype of the response to IL-6 by p53Val-135 hepatoma cell lines? In these experiments the objective was to investigate the ts phenotype of IL-6-induced synthesis and secretion of the Type II acute-phase plasma proteins fibrinogen and ACT in cultures of p53Val-135+ hepatoma cells.

In order to address this question, it was first necessary to devise an experimental protocol for a ts shift experiment that would lead to a minimal, if any, difference in the response of the parental Hep3B cells to IL-6. Briefly, Hep3B cells first plated at 37°C at a density of 2.5 × 10^5 cells/well in 24-well plates for 24 h, washed with phosphate-buffered saline at 37°C, incubated at 32.5°C for 4 h with medium containing insulin and dexamethasone that was at 37°C when it was added to the cultures (thus ensuring a slow transition of the cells from 37 to 32.5°C), then adding IL-6 to that medium for 18–20 h, followed by subsequent labeling with [35S]methionine also at 37°C, incubated at 32.5°C for 4 h with medium containing insulin and dexamethasone that was at 37°C when it was added to the cultures (thus ensuring a slow transition of the cells from 37 to 32.5°C), then adding IL-6 to that medium for 18–20 h, followed by subsequent labeling with [35S]methionine also at 32.5°C, revealed little difference in the response of parental Hep3B cells to IL-6 at 32.5°C compared to cells kept at 37°C throughout (Figs. 6 and 7). Under these experimental conditions, it was possible to investigate the ts phenotype of the p53Val-135+ hepatoma cell line 3. Figs. 6 and 7 represent a summary of data from a series of experiments in which the ts phenotype of the p53Val-135+ hepatoma cell line 3. Figs. 6 and 7 represent a summary of data from a series of experiments in which the ts phenotype of the p53Val-135+ hepatoma cell line 3. Figs. 6 and 7 represent a summary of data from a series of experiments in which the ts phenotype of the p53Val-135+ hepatoma cell line 3. Figs. 6 and 7 represent a summary of data from a series of experiments in which the ts phenotype of the p53Val-135+ hepatoma cell line 3. Figs. 6 and 7 represent a summary of data from a series of experiments in which the ts phenotype of the p53Val-135+ hepatoma cell line 3. Figs. 6 and 7 represent a summary of data from a series of experiments in which the ts phenotype of the p53Val-135+ hepatoma cell line 3. Figs. 6 and 7 represent a summary of data from a series of experiments in which the ts phenotype of the p53Val-135+ hepatoma cell line 3. Figs. 6 and 7 represent a summary of data from a series of experiments in which the ts phenotype of the p53Val-135+ hepatoma cell line 3. Figs. 6 and 7 represent a summary of data from a series of experiments in which the ts phenotype of the p53Val-135+ hepatoma cell line 3. Figs. 6 and 7 represent a summary of data from a series of experiments in which the ts phenotype of the p53Val-135+ hepatoma cell line 3. Figs. 6 and 7 represent a summary of data from a series of experiments in which the ts phenotype of the p53Val-135+ hepatoma cell line 3. Figs. 6 and 7 represent a summary of data from a series of experiments in which the ts phenotype of the p53Val-135+ hepatoma cell line 3. Figs. 6 and 7 represent a summary of data from a series of experiments in which the ts phenotype of the p53Val-135+ hepatoma cell line 3. Figs. 6 and 7 represent a summary of data from a series of experiments in which the ts phenotype of the p53Val-135+ hepatoma cell line 3. Figs. 6 and 7 represent a summary of data from a series of experiments in which the ts phenotype of the p53Val-135+ hepatoma cell line 3.
Modulation of IL-6 Response by p53

The hypothesis that p53 species may have a role in modulating the response of differentiated cells to cytokines (2) was tested in a context devoid of considerations of the regulation of cell proliferation. The effect of p53 species on the rapid activation by IL-6 of the enhancer derived from the acute-phase plasma protein gene β-fibrinogen was investigated in hepatoma cell lines Hep3B and HepG2 in transient transfection assays. Wild-type p53 inhibited the activation of the IL-6-responsive enhancer, whereas several tumor cell-derived mutants of p53 had lost this inhibitory property. Since the 36-bp IL-6RE derived from βFib is also activated by C/EBP transcription factors, this experimental system was used to investigate the functional interaction between p53 species and C/EBP transcription factors. Again, wt p53 species inhibited the activation of the βFib IL-6RE by C/EBPα, β, or δ, whereas several tumor cell-derived mutants had lost this inhibitory property. Some mutants, e.g. p53Val-135 and p53Phe-132, appeared to enhance C/EBP species-driven activation of the βFib IL-6RE.

The biological consequences of modulation by p53 of the acute-phase plasma protein response of hepatoma cells were examined by deriving Hep3B cell lines constitutively expressing p53Val-135. This mutant of p53 is previously known to have a ts phenotype (28). Derivation of p53Val-135− hepatoma lines allowed us to examine the influence of p53 on synthesis of acute-phase proteins.

**DISCUSSION**

The biological consequences of modulation by p53 of the acute-phase plasma protein response of hepatoma cells were examined by deriving Hep3B cell lines constitutively expressing p53Val-135. This mutant of p53 is previously known to have a ts phenotype (28). Derivation of p53Val-135− hepatoma lines allowed us to examine the influence of p53 on synthesis of acute-phase proteins.
and secretion of plasma proteins by hepatoma cells as a function of temperature. At 37°C, hepatoma line 3 had an increased response to IL-6 than did the parental Hep3B cells, whereas at 32.5°C, line 3 had a reduced response than did the parental cells. Two additional p53Val-135 hepatoma lines (lines 1 and 2), but not the p53Val-135–line 22 displayed a similar ts phenotype with respect to their response to IL-6. The data show that certain tumor-derived mutations in p53 can alter the ability of hepatoma cells to respond to IL-6. From a broader perspective, the data suggest that mutations in p53 can modulate gene expression elicited in tumor cells in response to cytokines.

In previous experiments we had observed that the p53 mutants Val-135 and Phe-132 enhanced C/EBP-β-driven activation of the IL-6 promoter and of the herpesvirus thymidine kinase promoter (2). Mutagenesis studies showed that an intact C/EBP-binding site in the two target promoters was required for this enhancing effect of Val-135 and Phe-132. In the present study, Fig. 4 shows that Val-135 and Phe-132 also enhanced C/EBP-driven pβFbCAT expression in a C/EBP-species and cell-type-specific manner. While we have observed that recombinant adenovirus-activated p53 can coprecipitate recombinant C/EBP-α, β, or ς species in protein interaction studies in vitro, it is not yet clear that this interaction represents a regulatory event that occurs in intact cells.

That p53 species may stably expressing lines can modulate the response of hepatoma cells to IL-6 such as depicted in Figs. 7 and 8 leads to a broad range of questions with respect to the interactions of p53 species with molecules and transcription factors involved in the signal transduction pathways triggered by cytokines. That plasma protein secretion in response to IL-6 can be modulated by the presence of a mutant p53 species in a hepatoma cell is clear from our data. The p53Val-135–expressing hepatoma cell lines developed by us provide a unique substrate for further studies of the interplay between mutations in p53 and the biological response of tumor cells to cytokines.

This molecular mechanisms by which p53 modulates the response to IL-6 in the p53Val-135–expressing hepatoma lines may well include interactions between p53 and target molecules at the level of the cytokine receptor, at the level of the signal transduction cascade, and/or at the level of the transcription factors activated by IL-6. A systematic exploration of each of these possibilities now becomes feasible because of the availability of the p53Val-135–expressing Hep3B-derived lines and their ts phenotype with respect to the response to IL-6. With cancer patients being increasingly administered cytokines, these tumor cells need to be understood.

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