Identification of a Functional Link for the p53 Tumor Suppressor Protein in Dexamethasone-induced Growth Suppression*  

Serine/threonine phosphatase 5 (PP5) can act as a suppressor of p53-dependent growth suppression and has been reported to associate with several proteins, including the glucocorticoid receptor/heat-shock protein-90 complex. Still, the physiological/pathological roles of PP5 are unclear. To characterize the relationship of PP5, glucocorticoid receptor activation and p53, here we describe the development of chimeric antisense oligonucleotides that potently inhibit human p53 expression. This allowed us to regulate the expression of either p53 (e.g. with ISIS 110332) or PP5 (e.g. with ISIS 15534) in genetically identical cells. Studies with ISIS 110332 revealed that the suppression of p53 expression is associated with a decrease in the basal expression of the cyclin-dependent kinase inhibitor protein, p21WAF1/Cip1, and a concomitant increase in the rate of cell proliferation. Suppression of p53 also blocks dexamethasone-induced p21WAF1/Cip1 expression and G1 growth arrest. Furthermore, treatment with ISIS 110332, but not the mismatched controls, ablates the suppression of growth produced by prior treatment with dexamethasone. Additional studies revealed that dexamethasem depedent p21WAF1/Cip1 expression occurs without an apparent change in p53 protein levels or the phosphorylation status of p53 at Ser-6, -37, or -392. However, dexamethasone treatment is associated with an increase in p53 phosphorylation at Ser-15. Suppression of p53 expression with ISIS 15534 also results in the hyperphosphorylation of p53 at Ser-15. Together, these findings indicate that the basal expression of p53 plays a functional role in a glucocorticoid receptor-mediated response regulating the expression of p21WAF1/Cip1 via a mechanism that is suppressed by PP5 and associated with the phosphorylation of p53 at Ser-15.

Serine/threonine phosphatase 5 (PP5) is an okadaic acid/calycin A-sensitive phosphatase that is expressed ubiquitously in mammals and has been reported to associate with the atrial natriuretic peptide receptor (1), the heat shock protein 90 (Hsp-90)1-glucocorticoid receptor (GR) heterocomplex (2, 3), the CDC16/CDC27 subunits of the anaphase-promoting complex (4), cryptochrome 2 (5), apoptosis signal-regulating kinase 6 (6), Hsp90-dependent heme-regulated eIF2a kinase (7), and the Gα12/Gα13 subunits of heterotrimeric G proteins (8). In estrogen-responsive breast carcinoma cells, the expression of PP5 is induced by treatment with estrogen, and the constitutive expression of PP5 converts MCF-7 cells into an estrogen-independent phenotype (9). Still, determining the physiological/pathological roles of PP5 has proven difficult for many reasons. First, in a crude cell homogenate PP5 exists predominately in an inactive state (for review see Ref. 10). Second, the physiological activators of PP5 are unknown. Third, when activated by the addition of polyunsaturated lipids or protease mediated cleavage of the N-terminal autoinhibitory domain (11, 12), the activity of PP5 cannot be distinguished from that of PP2A and PP1, which are both ubiquitously expressed at high levels in all eukaryotic cells. Finally, no selective small molecule inhibitors of PP5 have been reported, and genetic deletions studies in Drosophila suggest that the lack of PP5 expression results in an embryonic lethal phenotype (13). Therefore, to date there is no reliable method to measure changes in the enzymatic activity of PP5 in vivo or in crude cell homogenates.

Despite the difficulties associated with studying PP5, several studies suggest PP5 plays a functional role in both GR- and p53-dependent growth control. PP5 associates with the GR-Hsp-90 complex (2, 3), and mutational analysis has implicated the N-terminal tetratricopeptide repeat domains of PP5 in this interaction (3, 14). In GR-responsive A549 cells, treatment with ISIS 15534, a potent and specific suppresser of PP5 expression (9, 15, 16), induces the nuclear accumulation of GRs (17). ISIS 15534 treatment also markedly increases the association of GR with its cognate DNA binding sequence and induces GR-transcriptional activity in the absence of glucocorticoids (18). When combined, ISIS 15534 and dexamethasone, a potent GR agonist, have an additive effect, with dexamethasone-mediated induction of GR reporter plasmid activity elevated to a level that is 10 times greater than the maximal response obtainable in the presence of PP5 (18).

Treatment with ISIS 15534 also suppresses the growth of some, but not all, human tumor cells (9, 15, 18). In p53 wild-type lung carcinoma cells, treatment with ISIS 15534 produces G1 growth arrest, which occurs with a concomitant increase in the expression of the cyclin-dependent protein kinase inhibitors, p21WAF1/Cip1 (15, 18). In contrast, in p53-/- human fibroblast or p53-defective T24 bladder carcinoma cells, ISIS 15534 treatment does not suppress growth or induce the expression of p21WAF1/Cip1 (18). Studies with TR9–7 cells (p53-/-/cancer-activated cell sorting; PI, propidium iodide; E3, ubiquitin ligase; DOTMA/DOPE, N-[1-(2,3-dioleyloxy)propyl]-N,N,N-trimethylammonium chloride/dioleoylphosphatidylethanolamine.

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human fibroblasts that contain tetracycline-regulated transac-
tivator and operator plasmids to control the expression of wild-
type p53) revealed that ISIS 15534-mediated induction of p21WAPLC
requires a small amount of p53 protein, which becomes hyperphosphorylated but does not necessarily accumu-
late when PP5 expression is suppressed (15). In A549 cells both GR activation and p53 activation have been shown to induce the expression of p21WAPLC and induce G1 growth arrest. Therefore, the data with ISIS 15554 is consistent with PP5 acting as a suppressor of both a GR-dependent and a p53-dependent pathway leading to the induction of p21WAPLC expression. Alternatively, the data are also consistent with PP5 acting to suppress a single pathway in which p53 is a functional participant in GR-induced expression of p21WAPLC.

In the present study we further explored the relationship between PP5, GR, and p53 by developing chimeric antisense oligonucleotides that potently and specifically suppress the expression of p53. The data presented indicate that deksamethasone induces a response leading to the hyperphosphorylation of p53 at serine 15 and that Ser-15-phosphorylated p53 is a functional participant in a glucocorticoid-initiated response regulating the expression of p21WAPLC. The data are consistent with the basal expression of p53, in cells that have not encountered environmental stress, playing a functional role in glucocorticoid-mediated growth control via a pathway that was likely masked in previous studies by the ubiquitous expression of PP5.

EXPERIMENTAL PROCEDURES

Materials—Dexamethasone, all chemicals, and goat anti-mouse Ig were purchased from Sigma. Purified mouse anti-human p53 monoclonal antibody was purchased from BD Pharmingen. Antibodies recognizing specific sites of phosphorylation on p53 were purchased from Cell Signaling Technology (Beverly, MA). Phosphorothioate deoxyoligo-
nucleotides and 2′-O-(2-methoxy)ethylphosphorothioate oligonucleo-
tides were synthesized and purified as described previously (19, 20). The sequence of antisense oligonucleotides targeting p53 (ISIS 110332, ISIS 15554, and mismatched controls are as follows: ISIS110332, 5′-CCACGCCGACCGCAAGGT; ISIS 15554, 5′-GGGCGCATTGTCGTAGTTG; ISIS 116947, 5′-CACCGCAAGCGCCACCAAGGTA; ISIS122205, 5′-GCAACCACGCGACAAATGTA; ISIS 15521, 5′-GTGCCTA-
ATCGTTGCGAGTACG.

Cell Cultures and Transfection—Tissue culture medium, Lipofectin® and TRizol® were purchased from Invitrogen (Gaithersburg, MD). Hu-
man A549 lung carcinoma cells were obtained from the American Type Tissue Collection and cultured in DMEM supplemented with 10% fetal calf serum, streptomycin (0.1 μg/ml) and penicillin (100 units/ml). Cell cultures were passed when 90–95% confluent unless indicated otherwise. Antisense treatment was performed using a 1-ml solution of DMEM containing 15 μg/ml DOTMA/DOPE (Lipofectin®) and the indicated amount of oligonucleotides for 4 h at 37 °C, as described previously (15, 19, 20). Cell cultures were transfected with p53 mRNA and protein expression by Dr. P. Howe (Cleveland Clinic Foundation).

RESULTS

Antisense-mediated Inhibition of p53 mRNA and Protein Ex-
pression—To study the relationship of GR, p53, PP5, and p21WAPLC in human cells, we needed a system that would allow us to independently regulate the expression of either p53 or PP5 in cells that are p21WAPLC wild-type and sensitive to dexamethasone-induced growth suppression. Although dexamethasone has been reported to suppress growth in many types of cells, after testing numerous cell lines we failed to find a single p53-null cell line that was still sensitive to growth sup-
pression by dexamethasone. Indeed, a search of the published literature failed to reveal even a single report documenting dexamethasone-induced growth suppression in p53-defective or p53-null cells. Nonetheless, previous studies have shown that treatment with either dexamethasone (18) or ISIS 15534 can induce G1 growth arrest with a concomitant increase in the expression of p21WAPLC in A549 cells (15, 17). This suggests we could use A549 cells as a model if we could develop a method of suppressing p53 expression. Therefore, we developed potent

ferred to a Duralon-UV (Stratagene) membrane. Following UV cross-
linking and pre-hybridization, the membrane was hybridized overnight in the presence of 50% formamide at 42 °C with the indicated 32P-
labeled cDNA probe. cDNA probes were generated by random labeling with a DECAprime DNA labeling kit (Ambion) according to the protocol of the manufacturer. The membranes were subjected to two high stringency washes with 0.1 × SSC at 55 °C and two low stringency washes with 2 × SSC at room temperature (15). Hybridization was visualized by autoradiography. For quantification of hybridization signals, the radiograms were scanned and the scanned signals were integrated using Scion Image software, beta 3b release (Scion Corp.). The mem-
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and highly specific “chimeric” 2′-O-(2-methoxy)ethylphosphorothioate antisense oligonucleotides that suppressed the expression of human p53.

To identify a suppressor of p53 expression, oligonucleotides, 20 bases in length and predicted to hybridize to different regions of human p53 mRNA, were synthesized and tested for their ability to suppress the expression of human p53 employing Northern and Western blot analyses as described previously (15–19). The oligonucleotides tested were designed to target specific regions in the protein coding, the 5′-untranslated or the 3′-untranslated regions of human p53 mRNAs, and were chimeric 2′-O-(2-methoxy)ethylphosphorothioate oligonucleotides, containing 10 central phosphorothioate oligodeoxy residues (“oligodeoxy gap”) flanked by five 2′-O-(2-methoxy) residues on the 3′- and 5′-ends. These modifications have been shown previously to enhance the potency of antisense oligonucleotides targeting mRNAs encoding other proteins (12, 22, 23). Because phosphorothioate oligonucleotides commonly act through an RNase H-dependent mRNA cleavage mechanisms in cells (24), the ability of a particular oligonucleotide to inhibit the expression of p53 was originally identified based on their ability to inhibit p53 mRNA expression (15, 16, 20). The initial screen revealed that many oligonucleotides had little or no effect on the inhibition of p53 mRNA levels, whereas others had a moderate effect, and a few had pronounced effects. The activity of the active antisense oligonucleotides was verified with dose-response studies, utilizing Northern analysis to detect changes in p53 mRNA levels and Western analysis to detect changes in the level of p53 protein. Of the antisense oligonucleotides identified as having potent inhibitory activity against p53 in the initial screen, two were chosen for further characterization. Both of these antisense oligonucleotides produced a dose-dependent inhibition of p53 expression, and representative Northern and Western blots for ISIS 110332 are shown in Fig. 1.

To test the specificity of the response, several mismatched control oligonucleotides were also synthesized and tested. The mismatched control analogues contain the same base composition and same chemistry as the antisense oligonucleotides targeting p53. However, the sequences are scrambled and non-complementary to p53. None of the mismatched controls inhibited p53 protein expression. Time course analysis revealed that p53 protein levels could be effectively suppressed 4 h after the addition of antisense oligonucleotides (Fig. 1C). As observed previously with chimeric 2′-O-(2-methoxy)ethylphosphorothioate oligonucleotides that target other proteins (15–21), protein levels remained repressed for 3 days. The cationic lipids (DOTMA/DOPE; Lipofectin®) were used to facilitate the uptake of the oligonucleotides (25) and had no apparent effect on p53 mRNA or protein levels.

**Suppression of p53 Expression Is Associated with an Increase in the Rate of Cell Growth**—Having developed a method of specifically inhibiting the expression of human p53, we next tested the effect of a single treatment with ISIS 110332 at 300 nM on A549 cells. Similar to results obtained in studies employing p53-deficient human cells or cells derived from mice homozygously null for p53 (26, 27), A549 cell growth was not dependent on p53. In contrast, the suppression of p53 was associated with an increase (~26%) in the rate of cell proliferation (Table I).

**Dexamethasone Treatment Arrestrs A549 Cell Growth at the G0/G1-phase of the Cell Cycle**—The ability of glucocorticoids to suppress cell growth can be diminished or lost as cells are passed in culture. Therefore, to ensure our cell cultures were

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**Table I**

**Effects of ISIS 110332 on A549 cell growth**

| Time (mm control (ISIS 116947; 300 nM)) Anti-p53 (ISIS 110332; 300 nM) | Number of cells | Number of cells (% of controls) |
|---|---|---|
| 1 | 628,563 ± 33,391 | 601,783 ± 7,918 (96%) |
| 2 | 1,165,090 ± 13,217 | 1,463,940 ± 18,351 (126%)* |

* p ≤ 0.01.
responding as reported previously, we treated A549 cells with dexamethasone and measured both p21WAF1/Cip1 mRNA levels and proliferation rates. As reported previously, 50–100 nM dexamethasone was sufficient to suppress A549 cell growth (18). FACS analysis 24 h after treatment with a single dose of 100 nM dexamethasone revealed that the majority of the cells became arrested prior to the onset of DNA synthesis (Fig. 2), which is similar to the effect produced by treatment with ISIS 15534, which targets PP5 (15, 18). Northern analysis confirmed that dexamethasone treatment produced a dose-dependent increase in the expression of p21WAF1/Cip1 (Fig. 2, inset).

The Inhibition of p53 Expression Prevents Dexamethasone—induced p21WAF1/Cip1 Expression—Dexamethasone-induced p21WAF1/Cip1 expression was clearly evident after 24 h, and maximal induction occurred 48 h after continuous exposure to 100 nM dexamethasone (Figs. 2 and 3) (18). A single treatment with 300 nM ISIS 110332 inhibited dexamethasone-induced p21WAF1/Cip1 expression at all concentrations tested, even in cell cultures continuously exposed to 100 nM dexamethasone for 48 h (Fig. 3). The inhibition of p53 expression also suppressed the basal expression of p21WAF1/Cip1 (Fig. 3C).

In contrast, treatment with mismatched control antisense oligonucleotides did not suppress dexamethasone-induced p21WAF1/Cip1 expression or the basal expression of p21WAF1/Cip1. Transient transfection studies using a p21-luciferase reporter plasmid revealed that ISIS 110332 suppressed dexamethasone-induced p21-luciferase activity, suggesting dexamethasone-induced p21 expression is a transcriptionally regulated event (Fig. 3D). Similar results were observed with additional antisense oligonucleotides targeting different regions of p53 and their respective scrambled controls. Together, these studies suggest that the suppression of p53 expression by treatment with antisense oligonucleotides targeting p53 suppresses the basal expression of p21WAF1/Cip1 and prevents dexamethasone-induced p21WAF1/Cip1 expression.

Dexamethasone Induces the Phosphorylation of p53 at Ser-15—When A549 cells are cultured in media containing 32P, label and then treated with ISIS 15534 (to suppress the expression of PP5) and/or with dexamethasone (to activate GRs), immunoprecipitation studies revealed that the suppression of p53 expression is associated with the hyperphosphorylation of p53 (15). However, the site at which p53 becomes phosphorylated was not determined. Furthermore, because radiation-induced DNA damage can activate p53, it was also possible that the observed hyperphosphorylation of p53 resulted from the suppression of PP5 in combination with the activation of a stress response triggered by the 32P, used to label the cells. Therefore, in the present study we employed antibodies that recognized specific sites of p53 phosphorylation to assess changes in the phosphorylation status of p53. As seen in Fig. 4, treatment with 100 nM dexamethasone produced an increase in the phosphorylation of p53 at Ser-15 but had no apparent effect in the phosphorylation of p53 at Ser-15, with adriamycin (used as a positive control) also resulted in an increase in the phosphorylation at Ser-15, with adriamycin producing an marked increase in both phosphorylation and p53 protein levels. As reported previously, adriamycin-treatment also results in the hyperphosphorylation of p53 at several additional sites (data not shown).

The Suppression of p53 Expression Restores Growth to A549 Cells Arrested by Treatment with Dexamethasone—Treatment of A549 cells with dexamethasone produced a dose-dependent suppression of growth that became evident after 48–72 h (Fig. 4C). Because the suppression of p53 expression inhibits dexamethasone-induced p21WAF1/Cip1 expression, we tested the ability of ISIS 110332 to influence dexamethasone-induced growth suppression. To assess this, A549 cells in early log phase growth were treated with 100 nM dexamethasone. After 72 h, the growth-suppressed cell cultures were treated with 300 nM ISIS 110332 to inhibit the expression of p53 or with 300 nM ISIS 116947, a mismatch control for ISIS 110332. Cellular proliferation was then assessed by counting the number of cells in each dish over a period of 5 days. As seen in Fig. 4D, treatment with ISIS 110332, but not the mismatched control, ablates the suppression of growth produced by treatment with 100 nM dexamethasone. Together, these findings indicate that p53 can play a functional role in a GR-mediated response leading to the expression of p21WAF1/Cip1 and growth suppres-
The cells were treated with dexamethasone (100 nM), and 24 h later luciferase activity was measured. The data were normalized to protein and are expressed as the mean ± S.D. from three experiments conducted in triplicate.

**DISCUSSION**

Because the majority of tumors from late stage cancer patients contain mutations affecting the p53-protein, considerable effort has been devoted to determining how aberrations in the normal functions of p53 are associated with the pathology of human cancers. From these studies, it has become clear that p53 acts as a stress-induced transcription factor that plays a key role in activating and integrating a multitude of adaptive cellular responses to a wide range of environmental stresses (for review see Refs. 28–31). Nonetheless, the reason most cells continuously express low levels of p53 in the absence of stress is not clear.

Previous studies have shown that PP5 associates with the GR-Hsp-90 complex (2, 3) and that ISIS 15534-mediated suppression of PP5 enhances both dexamethasone-induced transcription and the expression of p21WAF1/CIP1 (18). In addition, ISIS 15534-induced p21WAF1/CIP1 expression does not require an increase in p53 protein levels, yet it is dependent on the presence of p53 (15, 16). The data presented here indicate that dexamethasone can induce the phosphorylation of p53 at Ser-15, as does ISIS 15534. When p53 expression is inhibited with ISIS 110332 in the absence of cellular stress, dexamethasone treatment no longer induces the expression of p21WAF1/CIP1. Furthermore, GR-induced growth suppression is ablated by the suppression of p53, and the suppression of basal p53 expression results in an increase in the rate of cell growth. Together, these observations suggest that basal p53 does more than provide a pool of readily available protein that allows for a rapid response to stress. Rather, p53 can act as an active participant in a GR-mediated signaling cascade that suppresses growth and is countered by the actions of PP5.

There is ample evidence that the induction of p21WAF1/CIP1 aids the onset of G1-growth arrest and that either p53 or GR activation can elicit a response leading to the induction of p21WAF1/CIP1 (26, 27, 32–35). Still, a functional role for p53 in the GR response leading to p21WAF1/CIP1 expression had not been established. In contrast, transient transfection studies conducted with rat hepatocytes indicate dexamethasone is capable of inducing transcriptional activity in p21WAF1/CIP1 reporter plasmids in constructs from which the core p53 response element had been deleted (33), possibly via an atypical GR porter plasmids (34). p53 has also been reported to physically interact with GRs, and dexamethasone-mediated transcription activity can be repressed by the co-transfection of p53 expression plasmids (36). Similarly, p53 and GR have been shown to form a complex that mutually inhibits the activity of both proteins, and it has been suggested that this p53-GR complex sequesters the active forms of both proteins in the cytoplasm (37, 38).

At first glance the above-mentioned studies contradict the data reported here. However, the previous studies addressed “cross-talk” between GR- and P53-induced responses under...
conditions that produced an increase in p53 protein levels (or an increase in p53 protein was used as an indicator of p53 activation). In contrast, the data presented here indicates that dexamethasone-induced p21WAF1/Cip1 expression is associated with p53 hyperphosphorylation at Ser-15 and occurs without an increase in p53 protein levels. Therefore, the requirement of Ser-15 phosphorylated p53 in a GR-induced pathway leading to growth suppression does not exclude another role for elevated p53 protein levels in the suppression of GR-induced gene transcription. Indeed, depending on the type and the severity of cellular stress, several studies indicate that p53 can elicit either a protective response (that is transient and suppresses growth) or a sustained response leading to irreversible growth arrest or apoptosis (28–31).

Determining how p53 is able to respond “appropriately” to a given stimuli and how p53 then drives a cellular response that is “tailored” to a specific insult is an area of great interest, and several studies indicate that phosphorylation is a key regulator of p53 (28, 39). For example, following extensive DNA damage, such as that produced by ionizing radiation, p53 promotes a death response by inducing apoptosis or irreversible growth arrest (for review see Refs. 28, 40, and 41). In such a response several protein kinases are activated and multiple sites on p53 become phosphorylated (42–44). Phosphorylation of Thr-18 and Ser-20 negatively regulates the interaction with Hdm2, a key regulator of p53 that binds its N-terminal domain and suppresses transcription. Hdm2 also acts as an E3 ubiquitin ligase, which catalyzes the ubiquitination of p53 and targets it for proteasome-mediated degradation. Therefore, the disruption of the interaction of Hdm2 with p53 by phosphorylation promotes both the accumulation of p53 and an increase in p53-dependent transcriptional activity.

The role of p53 phosphorylation in cells that have not encountered stress has been studied less extensively. Basal p53 expression can be detected in most, if not all cells, yet p53 knock-out mice are developmentally normal (45). The trans-expression of wild-type p53 in unstimulated p53−/− human fibroblasts is sufficient to induce both p21WAF1/Cip1 expression and growth arrest (26), suggesting that the transcriptional activity of p53 is not totally dependent on the activation of stress-induced kinases. Phosphorylation of Ser-15 has been shown to stimulate p53-dependent transcriptional activation, to increase the association of p53 with transcriptional co-activator proteins, and to aid the nuclear retention of p53 by blocking nuclear export (44, 47, 48). Consistent with the findings reported here, Bean and Stark (46) have shown that phosphorylation of p53 at Ser-15 is crucial to p53-mediated basal expression of p21 mRNA. Thus, although clearly additional studies will be needed to clarify the role of PP5 in GR- and p53-mediated processes, the data obtained to date are consistent with PP5 acting to suppress a GR-induced signaling cascade.

Fig. 4. Inhibition of p53 expression restores growth to dexamethasone-arrested cell cultures. A, dexamethasone treatment induces the hyperphosphorylation of p53 at Ser-15. A549 cells were treated with 100 nM dexamethasone or solvent (controls). 24–48 h later the relative phosphorylation of the indicated serine residues on p53 was assessed via Western analysis using phosphorylation-specific antibodies as described under “Experimental Procedures.” B, the suppression of PP5 expression enhances the phosphorylation of p53 at Ser-15. A549 cells were treated with 100 nM dexamethasone, ISIS 15534 (to suppress the expression of PP5), UV light, or 100 ng/ml adriamycin (as positive controls). 24 h under phosphorylation of the indicated serine residues on p53 was assessed via Western analysis using phospho-Ser-15-specific antibodies. The blots were also probed with an antibody that recognizes p53 to assess changes in the amount of total p53 protein. C, continuous exposure to dexamethasone suppresses A549 cell growth. A549 cells in log phase growth were treated with solvent (controls), 50 nM dexamethasone, or 100 nM dexamethasone ~16 h after the cells were plated (indicated by an arrow). Cell growth was then assessed daily for 5 days. The media in all cultures was changed after 72 h, at which time fresh dexamethasone was added to each dish at the concentrations indicated. D, the suppression of p53 expression restores A549 cell growth rates to control levels. A549 cells in log phase growth were treated with 100 nM dexamethasone as described above ~16 h after the cells were plated (indicated by a filled arrow). After 72 h, the cells were treated with 300 nM ISIS 110332 or a mismatched control (indicated by an open arrow) and placed in fresh media containing 100 nM dexamethasone. Growth rates were assessed at the time intervals indicated. To confirm that treatment with ISIS 110332 suppressed p53 expression, protein from identically treated dishes in each experiment were subjected to Western analysis. Insets show representative Western blots of samples from the 96- and 120-h time points. Each point represents the mean ± S.D. (n = 6).
that influences p53 phosphorylation at Ser-15 and the basal expression of p21WAF1/Cip1.

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