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p53 regulates the minimal promoter of the human topoisomerase IIα gene

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ABSTRACT

DNA topoisomerase IIα is an essential enzyme for chromosome segregation during mitosis. Consistent with a cell division-specific role, the expression of the topoisomerase IIα gene is strongly influenced by the proliferation status of cells. The p53 protein is one of the most important regulators of cell cycle progression in mammals, with an apparent dual role in the induction of cell cycle arrest following cytotoxic insults and in the regulation of the apoptotic cell death pathway. We have analysed whether p53 plays a role in regulating expression of the human topoisomerase IIα gene. We show that wild-type, but not mutant, p53 is able to decrease substantially the activity of the full length topoisomerase IIα gene promoter. Using a series of constructs comprising various deleted or mutated versions of the promoter lacking critical *cis*-acting elements, we show that this p53-specific regulation of the topoisomerase IIα promoter is independent of all characterised transcription factor binding sites and is directed at the minimal gene promoter. We conclude that expression of wild-type p53 induces downregulation of the human topoisomerase IIα promoter by acting on the basal transcription machinery. These findings implicate topoisomerase II as one of the downstream targets for p53-dependent regulation of cell cycle progression in human cells.

INTRODUCTION

Topoisomerase II is an essential nuclear enzyme that catalyses changes in the topological state of DNA (for reviews see 1–4). Genetic studies in yeast have indicated that topoisomerase II plays a critical role during mitosis, being required for the disentanglement of newly-replicated sister chromatids (5–8). As a consequence of this role, chromosome segregation during cell division is prevented in the absence of active topoisomerase II, leading to chromosome breakage during an abortive anaphase (for a review see 9). Other roles for topoisomerase II include relief of torsional stress during DNA replication and transcription, and the suppression of hyperrecombination within repetitive DNA sequences (2,10–12). In humans, topoisomerase II is also the primary cellular target for many of the most effective antineoplastic drugs, including etoposide, doxorubicin, mitoxantrone and epirubicin (for reviews see 13–15). The relationship between the cytotoxicity of these drugs and the intracellular level of the target protein (topoisomerase II) is unusual, in that high levels of topoisomerase II confer relative drug sensitivity, because the enzyme participates in the formation of the cytotoxic DNA lesions.

There are two closely related isoforms of topoisomerase II in mammalian cells that are designated topoisomerase IIα (170 kDa form) and topoisomerase IIβ (180 kDa form) (16–20). The respective functions of the two isoforms remain to be identified, although the α isoform is a well-established marker of proliferation both in cultured cell lines and in tissues *in vivo*, while the β isoform appears to be expressed in both proliferating and quiescent cells (21–24).

The factors that regulate topoisomerase II gene expression have not been defined in detail. The level of expression of topoisomerase IIα mRNA is low in quiescent cells, but accumulates to a much higher level as cells traverse the cell division cycle with peak levels in late S phase (21,25). Levels of topoisomerase IIα protein are very low in G_1 phase cells, reflecting the degradation of this protein that occurs during late M phase. Previous studies using constructs of the human topoisomerase IIα gene promoter fused to a chloramphenicol acetyl transferase (CAT) reporter gene have localised the elements required for maximal expression of the gene to the 350 bp region immediately upstream of the major transcription start site (26). A similar core region required for high level expression of the hamster topoisomerase IIα promoter has been identified (27). However, it is known that a minimal region of ~100 bp still retains substantial promoter activity (26) suggesting that many of the *trans*-acting factors required for basal expression of human topoisomerase IIα mRNA interact with a short region of the promoter immediately 5′ to the CAP site.

The p53 protein is a sequence-specific DNA binding protein capable of activating transcription from a set of genes that contain

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a consensus p53 binding element in either their promoter region or elsewhere in the gene (28–30). For example, p53 positively regulates expression of the cyclin-dependent kinase inhibitor p21 (also known as CIP1/WAF1) (31) as well as the growth arrest and DNA damage-inducible gene, GADD45 (32,33). In this latter case, the p53 binding element is located within one of the introns of the gene, not directly in the promoter region (32). Conversely, wild-type p53 protein has been shown to repress the activity of certain cellular and viral promoters that do not contain p53 binding sites (34–36). In this paper, we have investigated whether the p53 protein is able to regulate expression of the topoisomerase IIα gene. We show that wild-type p53, but not mutant p53, represses transcription from the topoisomerase IIα promoter by targeting the minimal sequences required for promoter activity.

MATERIALS AND METHODS

Cells

The human ovarian cancer cell line SKOV3, which does not express p53 mRNA or protein (37), was grown in RPMI-1640 medium supplemented with 10% foetal calf serum (FCS). The SK23a and SKN cell clones (38) were both derived from SKOV3 cells. To generate the SK23a derivative, SKOV3 cells were co-transfected with a plasmid encoding murine temperature-sensitive mutant p53 (39) and the pSV2neo vector containing the neomycin selectable marker genes. The SKN cell line was derived by transfection of SKOV3 cells with the neomycin expression vector alone, and served as a negative control. The human osteosarcoma cell line Saos-2, which expresses no p53 (40) and no functional pRb protein (41) as well as a derivative (designated Saos-2 ts p53) that stably expresses a temperature-sensitive human p53 protein due to a valine to alanine substitution at amino acid 138, were maintained in RPMI-1640 supplemented with 10% FCS. Where required for transfection using calcium phosphate (see below), cells were grown in Dulbecco's modified Eagles's Medium (DMEM) for 24 h prior to addition of the precipitate. All cells were grown in a humidified atmosphere in the presence of 5% CO_2, and were regularly screened for the presence of mycoplasma.

General plasmid vectors

The construct encoding the wild-type human p53, designated pLSVhp53c62 (42) utilises the SV40 early promoter in the vector pLSV. The construct for expression of mutant murine p53 (pLTRp53cGVal 135; kindly supplied by Dr M. Oren) has been described in detail elsewhere (39), and encodes a temperature-sensitive p53 that adopts a wild-type conformation at 32°C, but a mutant conformation at 37°C due to a mutation at position 135 (valine to alanine). The construct (designated pCMVtsp53Val 138) for expression of a human p53 that adopts a mutant conformation at 37°C and a wild-type conformation at 32°C, was kindly provided by Dr J. Jenkins and is essentially identical to that described previously (43). pnlsLacZ is an expression plasmid encoding the bacterial β-galactosidase gene under control of SV40 promoter. Plasmids encoding the *Escherichia coli* CAT gene under the control of different fragments of the human topoisomerase IIα gene promoter, have been reported previously (26). Plasmids carrying the mouse ferritin (FGH) and the human phosphoglycerate kinase 1 (PGKGH) gene promoters linked to the human growth hormone (HGH) coding region (44,45) were kindly provided by Dr J. Firth (Oxford, UK). The pKV461/CD2 (kindly provided by Dr C. J. Norbury) contains a truncated rat CD2 cDNA and was generated by excising the CD2 cDNA from pERCD2-2 (46) and cloning it into the *Bgl*II site of pKV461 (kindly supplied by Dr M. Sowden; 47).

Constructs encoding human growth hormone (HGH) from truncated or mutated versions of the human topoisomerase IIα gene promoter

The plasmid used for all of the HGH constructs was PGEM7Zf+ (PROMEGA), containing the 1.8 kb HGH cDNA as a reporter gene. This plasmid, designated pSVGH, was a generous gift from Dr J. Firth, Oxford, UK. The SV40 promoter in pSVGH was replaced by the topoisomerase IIα promoter to generate pHGH, as described previously (48). Deletion constructs containing various truncated forms of the topoisomerase IIα promoter were generated by PCR from the full length 2.5 kb fragment of the promoter (26). PCR primers incorporated restriction sites to enable directional cloning via the *Xba*I and *Hin*dIII, or the *Bam*HI sites in pHGH. Sequences for the 5′ primers (with the 5′ limit indicated on the left) used in generating the promoter truncations were as follows. Numbering begins at +1, the transcription start site.

5′-GATCTCTAGAGCCACCGCACACAGCCTACTT-3′
5′-GATCTCTAGATTTGAAGCCTCTCTAGTCC-3′
5′-GATCTCTAGAAGCCGTTCATAGGTGGATAT-3′
5′-GATCTCTAGACTTCTGGACGGAGACGGTGA-3′
5′-GATCTCTAGAGCTTCGGGCGGGCT-3′

In each case, the 3′ primer, which ran up to the translation start site, was as below:

ATG 5′-GGATCAAGCTTATGGTGACGGTCGTGAAGG-3′

After directional cloning of the PCR products into pHGH, the orientation and sequence of all promoter fragments was confirmed.

Transfection of SKOV3 cells

Exponentially growing cells were transfected with 1–15 μg of the p53 expression vectors and 10 μg topoisomerase IIα promoter/reporter gene constructs using the $CaPO_4$ co-precipitation procedure of Graham and van der Eb (49). Forty-eight hours after transfection, cells were harvested and lysed by three successive cycles of freezing and thawing. Where indicated, an expression plasmid encoding the bacterial β-galactosidase gene was included in the transfection, both to monitor transfection efficiency and to standardize the amount of extract to be used in subsequent CAT assays. β-galactosidase assays were performed as described by Herbomel *et al*. (50). An equivalent concentration of DNA was used in all transfections by adjusting the level of control vector DNA.

Transfection of Saos-2 cells

Exponentially growing Saos-2 or Saos-2 ts p53 cells were transfected by the $CaPO_4$ co-precipitation method of Graham and van der Eb (49). Precipitates (1.5 ml) which contained 30 μg of the appropriate pGEM plasmid carrying the topoisomerase IIα promoter linked to the HGH reporter gene, and, where required, 15 μg pKV461/CD2 (51) or 15 μg of a control plasmid, were applied to subconfluent cells for 16 h. The cells were then washed in phosphate buffered saline, trypsinized, and seeded at a ratio of

1:2 in RPMI-1640 medium. Cultures were then maintained either at 32 or 37°C for up to 120 h.

CAT assays

CAT activity was measured by the conversion of ^{14}C-labelled chloramphenicol to its acetylated forms using standard techniques. Briefly, cells were harvested and lysed by three cycles of freezing and thawing. Aliquots of extract were incubated for 2 h at 37°C with 4 mM acetyl co-enzyme A and [^{14}C]deoxychloramphenicol. The acetylated reaction products were separated from the substrate by thin layer chromatography. The percent conversion was determined by excising radioactive spots from the thin layer chromatography plates and measuring the level of radioactivity in each sample in a scintillation counter.

HGH assays

Promoter activity was determined by measuring the level of the HGH gene product in the media of transfected cells in culture, as described previously (48). All assays were kindly performed by Gillian Campling at Littlemore Hospital, Oxford, UK.

Site-directed mutagenesis

This was performed using the Muta-gene *in vitro* mutagenesis system (BioRad), which utilizes oligonucleotide primers containing the appropriate point mutations, and a single-stranded template into which uracil residues have been incorporated to permit selection against the template strand of DNA. This protocol was based on the method of Kunkel *et al.* (52). The pHGH derivative containing the –617 promoter fragment was used to generate single-stranded DNA templates. Primers used for mutating the first inverted CCAAT box (ICB1) and the first GC box consensus (GC1) elements (Fig. 4a), were as follows:
Mutant GC1: 5′-GGTCTGCTTCGTGCGTGCTAAAGG-3′
Mutant ICB1: 5′-AGTCAGGGATTCCCTGGTCTGCTT-3′

DNA sequencing

Nucleotide sequencing was performed on double-stranded plasmid templates using the dideoxy chain termination method and Sequenase enzyme, as recommended by the suppliers (US Biochemical Corp.).

Flow cytometry

The detection of the CD2 cell surface antigen was achieved using a FITC-conjugated anti-rat CD2 monoclonal antibody (OX-34; SeroTec), as described by O'Connell *et al.* (51).

RESULTS

p53 negatively regulates the topoisomerase IIα gene promoter

In order to determine whether p53 has a role in regulating expression of the topoisomerase IIα gene, we analysed the ability of wild-type p53, expressed from pLSVhp53c62, to modulate expression of a CAT reporter gene that was linked to the full-length, 2.5 kb, topoisomerase IIα gene promoter (26). Following transient transfection of SKOV3 cells with different concentrations of the human wild-type p53 expression construct, together with a constant amount of the full length topoisomerase IIα promoter/CAT plasmid, a concentration-dependent repression of promoter activity was observed. A reduction in CAT activity >90% was found in the presence of 15 μg of the p53 expression construct (Fig. 1). Co-transfection of the pLSV vector alone did not influence CAT expression from the topoisomerase IIα promoter (data not shown).

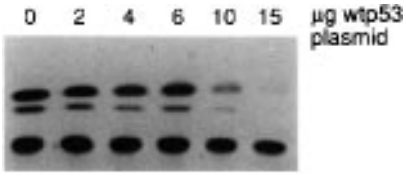

Figure 1. Concentration-dependent inhibition of human topoisomerase IIα promoter activity by wild-type p53. Different concentrations of the wild-type p53 expression plasmid were co-transfected with 10 μg of topoisomerase IIα-CAT construct in SKOV-3 cells. After 48 h, cells were lysed and a CAT assay was performed on the extract. The percent conversion of chloramphenicol to its acetylated forms for the samples containing 0, 2, 4, 6, 10 and 15 μg of p53 plasmid was 100, 85, 61, 58, 20 and 4%, respectively.

Downregulation of topoisomerase IIα promoter activity is specific for wild-type p53

In the same SKOV3 cell line, we also tested whether the observed repression of topoisomerase IIα promoter activity showed any specificity for wild-type p53. In co-transfection experiments using expression constructs containing either wild-type p53 (human and murine) or mutated versions of human and murine p53 containing amino acid substitutions in the DNA binding domain of the protein, significant repression of CAT activity was observed only in cells transfected with the constructs encoding wild-type p53 (data not shown). This suggested that the repression of topoisomerase IIα promoter activity was specific for wild-type p53. However, a number of alternative explanations for these data were possible. For example, p53 might be directly influencing transfection efficiency, or the expression of the β-galactosidase reporter gene used to control for differences in transfection efficiency between experiments.

To circumvent these potential problems, we took advantage of an expression construct, designated pLTRp53cGVal 135, encoding a murine p53 protein that assumes a wild-type conformation at 32°C, but a mutant conformation at 37°C (39). The construct containing the CAT reporter gene under the control of the 2.5 kb fragment of the topoisomerase IIα promoter was transfected into a clone of SKOV3 cells (designated SK23a), into which the construct encoding the temperature-sensitive p53 had been stably integrated. Following transfection, the cells were cultured at either 32 or 37°C, and the level of CAT expression was quantified. A clone of SKOV3 cells stably transfected with the neomycin-containing vector alone (SKN) was studied in parallel as a control. Figure 2 shows that the temperature shift had little or no effect on expression of CAT in the control SKN cells. In contrast, while CAT activity was readily detectable in extracts of SK23a cells grown at 37°C, a dramatic reduction in CAT activity was seen in the cells maintained at 32°C. Because of the potential regulation of β-galactosidase gene expression from the SV40 promoter in pnlsLacZ by p53, we excluded this reporter as a means of quantifying transfection efficiencies. Instead, in these

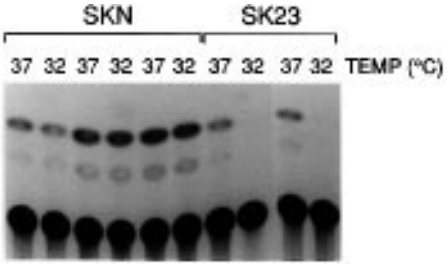

Figure 2. Topoisomerase IIα promoter activity in cells expressing no p53 (SKN), mutant p53 (SK23 at 37°C) or wild-type p53 (SK23 at 32°C). In each case, the transfected cells were divided between two dishes, one of which was grown at 32°C and the other at 37°C. After 48 h, cells were lysed and a CAT assay was performed on the extract. CAT activity catalyzed by three independent extracts from SKN cells and two independent extracts from SK23 cells are shown.

experiments, a single population of cells was divided equally and incubated at the different temperatures only after transfection, and therefore the effects of wild-type p53 on promoter activity could not be explained by differences in the ability of DNA constructs to transfect the host cell line. We conclude that p53 downregulates expression from the topoisomerase IIα gene promoter and that this effect is specific for p53 in its wild-type conformation.

p53 acts on the minimal topoisomerase IIα gene promoter

The results of the above experiments raised a number of questions: does p53 act through interaction with a specific binding sequence in the topoisomerase IIα gene promoter, or could the observed effects of p53 be mediated via perturbations of cell growth, or cell cycle progression? To address these issues, and to confirm the results presented above, we utilised a $p53^{null}$ human cell line, Saos-2, into which a CMV-based construct expressing a temperature-sensitive human p53 (Val138-Ala) had been stably integrated (designated Saos-2 ts p53). As before, this p53 protein adopts a wild-type conformation at 32°C and a mutant conformation at 37°C. Immunohistochemical staining confirmed that the p53 protein was expressed in these cells at both temperatures, but that it localised predominantly to the nucleus in cells incubated at 32°C (data not shown). Moreover, the wild-type p53 was shown to be functional since expression of the p53-regulated p21 protein (WAF1/CIP1) was seen in cells grown at 32°C but not at 37°C (data not shown). For the analyses of Saos-2 ts p53 cells, we used a series of topoisomerase IIα promoter constructs generated for analysis of the growth-state regulation of the promoter (48). Instead of the CAT gene, these constructs contain human growth hormone (HGH) as a reporter gene cloned downstream of various different fragments of the human topoisomerase IIα promoter. Figure 3A shows a diagrammatic representation of the different promoter constructs employed. Following transient transfection, the level of expression of HGH from constructs containing 617, 210, 144 and 101 bp of the promoter was in each case substantially lower in Saos-2 ts p53 cells cultured at 32°C, than in those cells cultured at 37°C (Fig. 3B). To exclude effects of incubation temperature on cell cycle transit times, we measured cell numbers at the end of each experiment. HGH expression as a function of cell number still showed a substantially reduced level at 32°C as compared with 37°C (Fig. 3C). Indeed, temperature shift did not dramatically

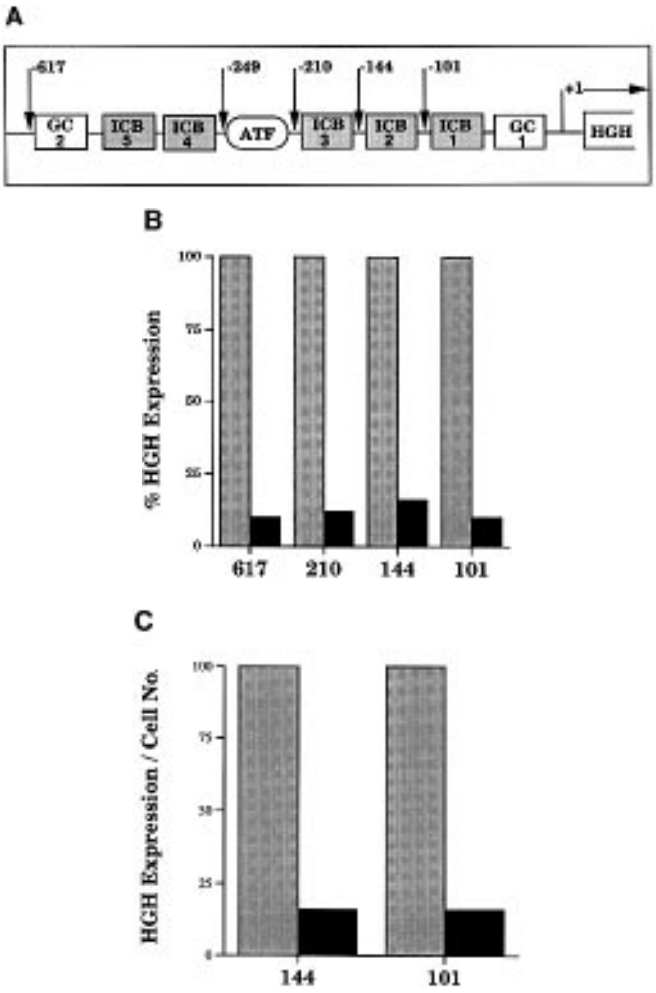

Figure 3. (A) Diagrammatic representation of the promoter constructs employed. The 5′ limit of each promoter fragment is indicated on the left. The position of the GC boxes (GC), inverted CCAAT boxes (ICB), ATF site (ATF) and growth hormone reporter gene (HGH) are indicated. The major transcription start site is marked with an arrow. **(B)** Percentage of HGH expression 120 h after transfection of Saos-2 ts p53 cells with various different lengths of the topoisomorase IIα promoter (indicated on the x-axis). In each case, the transfected cells were divided between two dishes, one of which was grown at 32°C and the other at 37°C. The shaded bars represent the expression when the cells were cultured at 37°C (p53 mutant) and the black bars represent cells at 32°C (p53 wt). In each case, the level of growth hormone at 37°C has arbitrarily been given a value of 100%. **(C)** Percentage of HGH expression as a function of cell number 120 h after transfection of Saos-2 ts p53 cells with 144 and 101 bp topoisomerase IIα promoter constructs. The shaded bars represent those cells cultured at 37°C (p53 mutant) and the black bars represent cells grown at 32°C (p53 wt).

affect cell proliferation over the time-course of these experiments, since cell numbers generally differed by no more than 50% in the transfected cell populations incubated at 32 and 37°C. Moreover, the downregulation of topoisomerase IIα promoter activity seen at 32°C was not simply a function of the temperature shift, since we demonstrated that the 101 bp promoter fragment was expressed at similar levels in Saos-2 parental cells (not transfected with p53) at 37 and 32°C (data not shown). These data confirm those generated using CAT reporter constructs in the SKOV3 cell line, and indicate that the activity of the previously described minimal promoter of the topoisomerase IIα gene

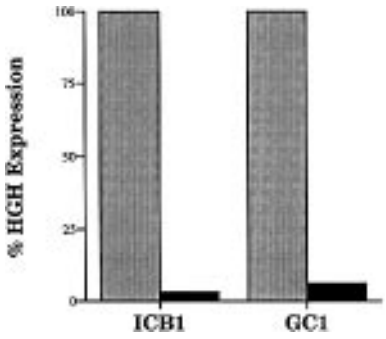

Figure 4. Percentage of HGH 120 h following transfection of Saos-2 cells with mutated forms of the topoisomerase IIα promoter (ICB1 and GC1) alone (shaded bars), or following co-transfection with the wild-type p53-expressing construct pLSVhp53c62 (black bars). The HGH expression level in the absence of p53 was arbitrarily given a value of 100%.

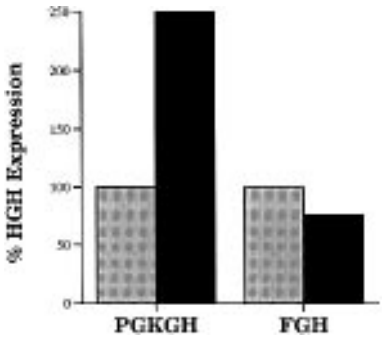

Figure 5. Percentage of HGH 120 h after transfection of Saos-2 ts p53 cells with the control promoters PGKGH and FGH. The shaded bars represent expression of HGH when the cells were cultured at 37°C (mutant p53) and the black bars cells cultured at 32°C (wt p53). The expression level of HGH at 37°C in each case was arbitrarily given a value of 100%.

(~100 bp of sequence upstream of the CAP site) is negatively regulated by wild-type p53.

The results presented thus far demonstrate that many of the *cis*-acting elements previously identified within the topoisomerase IIα promoter, which lie in the region between –101 and –617 (Fig. 3A), can be eliminated as candidates for the target of p53 action on promoter activity. However, the 100 bp minimal promoter does contain two well-defined consensus transcription factor binding elements, a GC box (a potential Sp1 binding site) at position –44, and an inverted CCAAT box (ICB1) at position –64 (Fig. 3A). To study whether these elements were implicated in the regulation of promoter activity by p53, we mutated the potential GC box and ICB1 elements in the 617 bp promoter/growth hormone fusion construct and co-transfected Saos-2 cells with these mutated constructs and the wild-type p53 expressing construct, pLSVhp53c62. The ICB1 element was mutated from CCAAT to GGAAT, which has been shown previously to lead to loss of binding to the transcription factor NF-Y (48). The GC1 element was converted from GGGCGGG to GTGCGTG, which has no significant similarity to the consensus binding motif for the Sp1 transcription factor. Figure 4 shows that mutation of these elements did not abrogate the downregulation of promoter activity seen in Saos-2 cells when wild-type p53 was expressed.

p53 does not appear to act via perturbing the cell cycle

We next addressed the possibility that the wild-type p53 protein was influencing the cell cycle distribution of the transfected cells and that this was indirectly affecting the activity of the topoisomerase IIα promoter. To study this, Saos-2 ts p53 cells were co-transfected with the pKV461/CD2 expression vector encoding the CD2 cell surface protein (to act as an antigenic tag for transfected cells) and the 101 bp topoisomerase IIα promoter fragment linked to HGH. The control promoters, FGH and PGKGH, were also co-transfected with the pKV461/CD2 vector into Saos-2 ts p53 cells. Following transfection, the cells were cultured at either 32 or 37°C. The cell cycle distribution of the cells cultured at 32°C (when p53 would be wild-type) and at 37°C (when p53 would be mutant) was found to be similar (data not shown). Thus, cell cycle perturbations are unlikely to account for the dramatic downregulation of promoter activity by p53 wild-type.

p53 acts specifically on the topoisomerase IIα gene promoter

In order to eliminate the possibility that wild-type p53 was having a general negative influence over gene transcription in the transfected cells, we analyzed the effect of p53 on expression of the HGH reporter gene from two control promoters. Figure 5 shows that expression of HGH from the phosphoglycerate kinase and ferritin gene promoters in Saos-2 ts p53 cells was not substantially downregulated by temperature shift from 37 to 32°C, indicating that the activity of these promoters was not negatively regulated by co-expression of wild-type p53 protein. Indeed, the activity of the phosphoglycerate kinase gene promoter was somewhat higher in cells grown at 32 than at 37°C.

DISCUSSION

We have shown that wild-type p53 is a negative regulator of the activity of the human topoisomerase IIα promoter and that this effect is mediated through the minimal sequences required for topoisomerase IIα promoter activity. This regulation is apparently independent of a perturbation in the cell cycle distribution of the transfected cells, and has been demonstrated in different cell lines using different constructs encoding either human or murine p53. Moreover, we have shown that p53 has some apparent specificity for the topoisomerase IIα promoter, in that the activity of two control gene promoters was not downregulated by expression of wild-type p53.

The p53 protein is a key regulator of gene expression in mammalian cells. In its wild-type conformation, p53 activates the transcription of those genes that contain a consensus p53 binding element in their promoter or other regulatory sequences (28–30). Conversely, wild-type p53 is able to repress the transcription of certain genes that lack a consensus binding element in their regulatory sequences (34–36). Many studies have focused on the ability of p53 to stimulate or repress the activity of genes important either for the control of cell proliferation, or for the response of cells to DNA damaging agents. For example, p53 has been reported to activate the transcription of both the $p21^{CIP1/WAF1}$ gene, which encodes an inhibitor of cyclin-dependent kinases (53–55), and the GADD45 gene, which encodes a 18 kDa protein whose expression is induced by DNA damaging agents, and which

may play a role in regulating cell cycle progression and/or DNA repair through its interactions with the proliferating cell nuclear antigen (56,57). Similarly, wild-type p53 has been shown to activate the transcription of the *BAX* gene, which heterodimerises with Bcl-2, and consequently antagonises the anti-apoptotic role of Bcl-2 (58). In contrast, wild-type p53 represses the activity of the interleukin 6 gene promoter, apparently in combination with the retinoblastoma susceptibility gene product (35).

p53 interacts with DNA in a sequence-specific fashion binding to DNA containing two contiguous monomers of the sequence 5′-PuPuPuC (T/A) (T/A) GPyPyPy. These two elements are generally separated by between 0 and 13 bp of non-conserved sequence (59). The topoisomerase IIα promoter does not contain a precise match for this consensus p53 binding element. However, a similar sequence (AAGCTTTCCG-7 bp-AAACAAGTGA) is present between –269 and –295 bp of the topoisomerase IIα promoter, but this motif contains two changes from the consensus in each unit of the p53 binding sequence (indicated above by underscoring). Consistent with this motif lacking any functional significance, we have been unable to detect any binding of wild-type p53 protein to oligonucleotide containing this sequence using gel retardation assays (unpublished observations). Moreover, expression of the HGH reporter gene from a topoisomerase IIα promoter construct lacking this element has been shown still to be negatively regulated by wild-type p53.

Because of the cell cycle regulatory role of p53, we addressed whether the topoisomerase IIα promoter could be responding to cell cycle perturbation induced by p53, and not to a direct effect of p53 on the transcription machinery. No evidence was obtained for a substantial accumulation of cells in any particular cell cycle phase, in those cells in which wild-type p53 was expressed, at least over the time course of these experiments. Clearly, the other genetic changes that accompany the acquisition of a transformed state, such as alteration in the retinoblastoma susceptibility gene (which is not expressed in Saos-2 cells) or cyclin-dependent kinase inhibitors, could be influencing the efficiency with which wild-type p53 can mediate cell cycle arrest in the cell lines chosen for study here. Yamato *et al.* (43) showed that expression of wild-type p53 caused some accumulation of Saos-2 cells in the G_1 and G_2 phases of the cell cycle 20 h after initiating protein expression. In our study, cell cycle distribution was analysed at both 96 and 120 h after temperature shift and in neither case did we observe an obvious cell cycle perturbation, suggesting that any arrest that might have occurred was transient in nature. However, it is extremely unlikely that cell cycle perturbations induced by expression of p53 are responsible for altering the activity of the topoisomerase IIα promoter, since we have shown that promoter activity varies <2-fold during cell cycle traverse in human cells (unpublished observations). Indeed, recent data indicate that the variation in topoisomerase IIα mRNA expression that occurs during the cell cycle traverse is almost exclusively due to changes in transcript stability, not promoter activity (25). We have shown elsewhere that the topoisomerase IIα promoter responds to growth arrest signals (48). However, the regulatory elements in the promoter that respond to changes in growth state are distinct from those responding to p53, and are located upstream of the minimal topoisomerase IIα gene promoter defined in this work. In particular, the 101 bp minimal promoter, which we have shown to be regulated by p53, lacks normal negative regulation brought about by inhibition of proliferation (48). Topoisomerase II protein is a key target for many clinically used anticancer drugs, with the cellular level of topoisomerase II being an important determinant of the response of tumour cells to these agents. As a consequence, the identification of factors that control topoisomerase IIα gene expression may be important in determining the clinical efficacy of several classes of antineoplastic agents. We would suggest that the differential sensitivity of tumour cells to killing by topoisomerase II-targeting drugs may be dependent upon the p53 status of individual tumour cells. Since p53 is frequently mutated in human cancers, it is possible that a dysregulation of topoisomerase IIα gene expression will be evident in those tumour cells that lack expression of wild-type p53. If stable overexpression of topoisomerase IIα were to occur under these circumstances, it might be expected that this would lead to an increased susceptibility of these cells to killing by topoisomerase II-targeting drugs. This may be one explanation for the differential sensitivity of tumour and normal tissues to topoisomerase II-targeting drugs. Further analysis of this phenomenon is now warranted.

At least one action of p53 as a negative regulator of gene transcription appears to be directed towards components of the basal transcription machinery. An important feature of this effect may be the formation of complexes between p53 and TATA box-binding protein (TBP)-associated factors, possibly TBP itself. Moreover, Liu and Berk (60) have shown recently that p53 may act through direct or indirect interactions with both TFIIB and TFIID, which act as basal transcription factor complexes. Our data are consistent with the hypothesis that wild-type p53 acts via negatively regulating the basal transcription machinery required to effect expression of the topoisomerase IIα gene. In other cases, such as in the regulation of SV40 and hsp70 gene transcription, p53 appears to disrupt the ability of a protein complex to bind to DNA that includes either the transcription factor Sp1 (GC box binding factor) (61), or CBF (CCAAT box binding factor) (62). Although consensus Sp1 and CCAAT boxes lie close to the CAP site in the topoisomerase IIα gene promoter, mutation of these sites did not prevent p53 from negatively-regulating promoter activity. This suggests that Sp1 and CBF are unlikely to be important targets for p53 action in regulating the expression of the topoisomerase IIα gene.

In summary, we have shown that wild-type p53 can specifically down-regulate the activity of the human topoisomerase IIα gene promoter. The challenge is now to delineate the precise downstream consequences of this regulation and to ascertain whether topoisomerase IIα gene expression is an important target for other regulators of cell cycle progression in human cells.

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