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Negative growth regulation in a glioblastoma tumor cell line that conditionally expresses human wild-type p53

(cell cycle/cell proliferation)

W. EDWARD MERCER*†, MICHELLE T. SHIELDS*, MAMTA AMIN*, GORDON J. SAUVE*, ETTORE APPELLA‡, JOSEPH W. ROMANO‡, AND STEPHEN J. ULLRICH‡

*Department of Pathology and Fels Research Institute, Temple University School of Medicine, Philadelphia, PA 19140; and ‡Laboratory of Cell Biology, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892

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ABSTRACT **To investigate the effect that human wild-type p53 (wt-p53) expression has on cell proliferation we constructed a recombinant plasmid, pM47, in which wt-p53 cDNA is under transcriptional control of the hormone-inducible mouse mammary tumor virus promoter linked to the dominant biochemical selection marker gene *Eco gpt*. The pM47 plasmid was introduced into T98G cells derived from a human glioblastoma multiforme tumor, and a stable clonal cell line, GM47.23, was derived that conditionally expressed wt-p53 following exposure to dexamethasone. We show that induction of wt-p53 expression in exponentially growing cells inhibits cell cycle progression and that the inhibitory effect is reversible upon removal of the inducer or infection with simian virus 40. Moreover, when growth-arrested cells are stimulated to proliferate, induction of wt-p53 expression inhibits G_0/G_1 progression into S phase and the cells accumulate with a DNA content equivalent to cells arrested in the G_0/G_1 phase of the cell cycle. Taken together, these studies suggest that wt-p53 may play a negative role in growth regulation.**

The gene encoding the cellular nuclear phosphoprotein p53 has been implicated in normal cell proliferation and neoplastic transformation (reviewed in ref. 1). Overexpression of mutant p53 protein can contribute to a more transformed phenotype in a number of different cell systems (2–4), and mutant murine p53 (but not wild type) can cooperate with an activated *ras* oncogene in transforming primary baby rat kidney cells (2, 3, 5). Recently, a high incidence of lung, bone, and lymphoid tumors has been reported in transgenic mice overexpressing mutant alleles of murine p53 (6).

Although there are many examples of p53 overexpression in cells transformed by a variety of agents (reviewed in refs. 1 and 7), there are other cases in which p53 expression is altogether absent. The most striking example is inactivation of the p53 gene through proviral integration in Friend virus-induced murine leukemia (8, 9). Rearrangements and deletions of the p53 gene also occur in the human leukemia cell line HL-60 (10) and in the osteogenic sarcoma cell line SAOS-2 (11). In these human tumor cell lines no p53 gene products (mRNA or protein) are detectable. Recently, alterations of the p53 gene have been identified in human colon (12) and lung (45) carcinomas. These findings have led to the suggestion that loss of normal p53 function may be conducive to tumorigenicity implying that normal p53 may function as a tumor suppressor or antioncogene (reviewed in ref. 13).

Given the implication of human p53 involvement in an ever increasing variety of malignancies, we focused our attention on studies dealing with the biology of human wild-type p53 (wt-p53). Evidence is presented in this communication that induction of human wt-p53 expression in a cell line derived from a human glioblastoma tumor (14) has a negative effect on cell proliferation.

MATERIALS AND METHODS

Plasmids. The vector plasmid pMSG was obtained from Pharmacia. It contains a 1450-base-pair (bp) long terminal repeat sequence encoding the hormone-inducible mouse mammary tumor virus (MMTV) promoter and polylinker together with the linked dominant biochemical selection marker gene *Eco gpt* (xanthine-guanine phosphoribosyltransferase) driven by the simian virus 40 (SV40) promoter (15). The *Xba* I insert of human wt-p53 was obtained from php53c-1 kindly provided by D. Givol (The Weizmann Institute of Science, Rehovot, Israel). pM47 was derived by inserting the *Xba* I p53 cDNA fragment into the *Nhe* I restriction site of the pMSG polylinker. The orientation of the p53 insert relative to the MMTV promoter was ascertained by restriction mapping. The nucleotide sequence of the *Xba* I p53 fragment used in this study is identical to the human p53 sequence described by Harlow *et al.* (16) except that amino acid codon 273 is arginine rather than histidine. The plasmid encoding human histone H3 has been previously described (17).

Cell Culture and DNA Transfections. T98G cells were cultured in Earle's minimal essential medium containing 10% fetal calf serum (GIBCO) at 37°C as described (18). The transfection procedure used in these experiments was the calcium phosphate suspension technique described by Shen *et al.* (19) except that the posttransfection polyethylene glycol shock was not employed. Briefly, cells were transfected in suspension with plasmid DNA, incubated for 48 hr in growth medium, and then shifted to gpt^+ selection medium (15) containing 15 μg of hypoxanthine per ml, 10 μg of thymidine per ml, 250 μg of xanthine per ml, 25 μg of mycophenolic acid per ml, and 2 μg of aminopterin per ml. Two weeks after shifting to selection medium individual gpt^+ colonies were isolated.

RNA Isolation and Northern Blot Analysis. Total RNA was extracted from cells by the method described in ref. 20. RNA (10 μg per lane) was denatured with 6.3% formaldehyde/50% formamide and then size fractionated on a 1.2% agarose gel containing 6.6% formaldehyde. Blotting of RNA to nitrocellulose was done as described (21). Radiolabeled probes were prepared by oligolabeling to high specific activity (22). Prehybridization, hybridization, and posthybridization washes were essentially as described (23).

Metabolic Labeling and Immunoprecipitation. Cell cultures were labeled in 5 ml of methionine-free medium containing 0.375 nCi of [^{35}S]methionine (DuPont/NEN, 1100 μCi/

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Abbreviations: SV40, simian virus 40; Dex, dexamethasone; MMTV, mouse mammary tumor virus.

†To whom reprint requests should be addressed.

mmol; 1 Ci = 37 GBq) and 2.5% dialyzed fetal calf serum for 4 hr. Cells were washed three times in cold phosphate-buffered saline and lysates were prepared as described (24). Prior to immunoprecipitation, lysates were precleared twice for 45 min with protein A-Sepharose (Pharmacia) and an irrelevant monoclonal antibody, Ma22. Lysates, containing equivalent amounts of incorporated radioactivity, were subject to immunoprecipitation by incubating for 30 min with either anti-human p53 monoclonal antibody, 1801 (Ab-2; Oncogene Sciences, Manhasset, NY), anti-p53 monoclonal antibody 122 (25), or control monoclonal antibody, Ma22. Immune complexes were collected by incubation with protein A-Sepharose for 30 min, washed as described (46), analyzed on 10% SDS/PAGE, and then subjected to fluorography.

Southern Blot Analysis. High molecular weight DNA was isolated by standard procedures, digested to completion with the restriction enzyme *Hin*dIII, and size fractionated a 0.8% agarose gel. Transfer of DNA to nitrocellulose filters was as described (23).

Cell Cycle Studies. Cells were arrested in G_0/G_1 phase of the cell cycle by allowing them to grow to confluency followed by incubation for 3 days in medium containing 0.5% fetal calf serum. The cells were stimulated to reenter the cell cycle by trypsinizing and replating at a 1:4 split ratio in medium containing 10% fetal calf serum with and without the addition of dexamethasone (Dex). Dex was obtained from Sigma, diluted in absolute ethanol, and stored at 4°C as a ×1000 stock solution. Dex was diluted in Hanks' balanced salt solution just prior to each experiment and added to cells at a final concentration of 1 μM. DNA synthesis was analyzed by standard autoradiography after labeling with 0.5 μCi of [^{3}H]thymidine (6.7 μCi/mmol) per ml obtained from New England Nuclear. Computer-operated microfluorimetry was used to determine the relative amount of DNA in individual cells stained with acridine orange (Polysciences) as described (26). Green fluorescence intensity was determined using a barrier filter in the range of 540–560 nm.

SV40 Infection. SV40 stocks were grown and titered as described (27). Cells were infected with purified virus at a multiplicity of infection of 250, and the percentage of infected cells was determined by indirect immunofluorescence staining using monoclonal antibodies specific for SV40 large tumor antigen (SV40 TAG) as described (27). In the experiments described in this communication the percentage of infected cells (TAG$^+$) ranged from 60% to 70%.

RESULTS

Isolation of Glioblastoma Cells That Express Wild-Type p53. The human cell line T98G was originally derived from a glioblastoma multiforme tumor (14). This cell line exhibits a unique combination of normal and transformed aspects of growth control. T98G cells are like normal cells in that they become arrested in G_0/G_1 phase under stationary phase conditions, yet they also exhibit the transformed phenotype of anchorage-independent growth and immortality (ref. 14; our present results). T98G cells were transfected with pM47 plasmid DNA and gpt$^+$ colonies were selected as described in *Materials and Methods*. Clonal cell lines were isolated and Southern blot analysis was performed on high molecular weight DNA of parental and individual gpt$^+$ colonies following digestion with the restriction enzyme *Hin*dIII.

The results of this analysis are shown in Fig. 1. The human p53 gene is identified on Southern blots by two fragments of ≈2.5 and ≈6.7 kb after *Hin*dIII digestion (29). *Hin*dIII digestion of the pM47 plasmid produces a 4.7-kb fragment containing the MMTV promoter, p53 cDNA insert, and the SV40 early promoter. Digestion of high molecular weight DNA from T98G cells produces two fragments of the endogenous gene. The four gpt$^+$ cell lines derived from pM47 transfection also contain these two fragments; however, three of the four cell lines also contain an additional fragment of ≈4.7 kb. The other cell line contains an additional fragment slightly smaller than 4.7 kb, suggesting that some deletion of the pM47 transcription unit (*Hin*dIII fragment) has occurred. The relative intensity of the hybridization signal of the band at 4.7 kb in GM47.11, GM47.18, and GM47.23 compared to the endogenous p53 gene suggests that these clonal cell lines contain one or two copies of the pM47 plasmid.

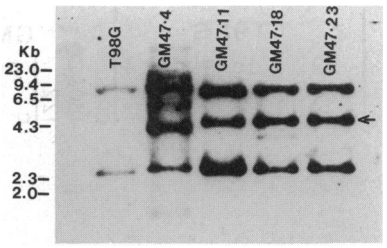

FIG. 1. Southern blot analysis of genomic DNA from GPT$^+$ human glioblastoma cell lines. High molecular weight genomic DNA was isolated from GPT$^+$ clones of human T98G cells transfected with pM47 plasmid DNA. The genomic DNA was digested with the restriction enzyme *Hin*dIII and a Southern blot containing 15 μg of DNA per lane (except for parental T98G, which contains 5 μg) was prepared. The blot was probed with the *Xba* I fragment of p53H. The arrow indicates the position of the *Hin*dIII fragment of pM47. Sizes are indicated in kilobases (kb).

The expression of p53 mRNA in parental T98G and GM47.23 cells in the presence or absence of the hormone Dex was examined by Northern blot analysis. The results are shown in Fig. 2. In normal human cells such as peripheral blood T lymphocytes the size of p53 mRNA is ≈2.8 kb (30). Dex treatment of GM47.23 cells induced the expression of a second p53 2.0-kb RNA band below the endogenous p53 mRNA of 2.8 kb; however, this lower p53 mRNA band is not observed in parental T98G cells after Dex treatment.

Expression of p53 proteins in GM47.23 under uninduced (without Dex) and induced (with Dex) conditions and in parental T98G cells was examined by immunoprecipitation of lysates from cells metabolically labeled with [^{35}S]methionine. Two different p53 specific monoclonal antibodies were employed: PAb 122 (25), which recognizes antigenic determinants localized to the C terminus (31), and PAb 1801, which recognizes antigenic determinants localized to the N terminus (32). For these experiments parental T98G and GM47.23 cells were first growth arrested and then subsequently stimulated to proliferate as described in *Materials and Methods*. The results are shown in Fig. 3. Both monoclonal antibodies precipitate p53 proteins of 53 kDa from quiescent and stimulated cells with and without Dex treatment. When GM47.23 cells were induced to express wt-p53, there was a 7-fold increase in the amount of p53 protein (major inducible p53 recognized by PAb 1801 relative to uninduced cells). More-

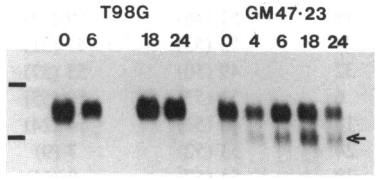

FIG. 2. Northern blot analysis of p53 mRNA expression. Total RNA was isolated from parental T98G and GM47.23 cells at the times indicated (in hours after treatment with 1 μM Dex), and a Northern blot was prepared using 10 μg of RNA per lane. The blot was probed using the *Xba* I fragment of p53H. Zero time is untreated. The arrow indicates the 2.0-kb transcript of human p53 in GM47.23 cells.

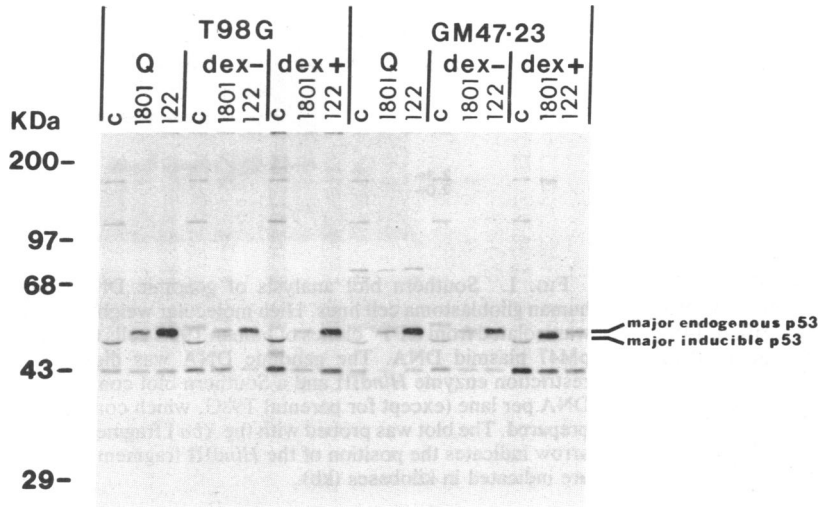

FIG. 3. Immunoprecipitation of p53 proteins. Cells were growth arrested in G_0/G_1 phase (Q) and subsequently stimulated to reenter the cell cycle with (dex+) and without (dex−) addition of Dex. At 18 hr after stimulation the cells were metabolically labeled with [^{35}S]methionine for a 4-hr period. Lysates were prepared and immunoprecipitated with p53 monoclonal antibodies PAb 1801 and PAb 122 or nonspecific control antibody. The immunoprecipitates in each lane represent equivalent amounts of trichloroacetic acid-precipitable radioactivity.

over, the major inducible p53 protein has a faster mobility (52 kDa) than the major endogenous p53 protein present in uninduced GM47.23 cells or parental T98G cells. Interestingly, the increase for the p53 1801 epitope protein was not found for the p53 122 epitope protein. Apparently the wt-p53 is poorly recognized by the PAb 122 monoclonal antibody, probably due to posttranslational changes. In fact, when wt-p53 was synthesized it reduced the amount of the 122 epitope protein that could be precipitated by ≈70%.

Effect of wt-p53 Induction on the Cell Cycle of Exponentially Growing Cells. The effect that induction of wt-p53 expression has on the cell cycle of exponentially growing GM47.23 cells was examined. Parental T98G cells and a gpt$^+$ clonal cell line derived from transfection with the vector pMSG (GMSG.26) were included in this study as a control for nonspecific effects of the hormone. Exponentially growing cells were treated with Dex, and at different times after addition of the hormone the cells were labeled for 1 hr with [^{3}H]thymidine and then fixed and processed for standard autoradiography. By this procedure, the percentage of S-phase cells was determined at different times after induction of wt-p53 expression. The results are shown in Table 1. Dex treatment of control T98G and GMSG.26 cells has no significant effect on the percentage of S-phase cells in exponentially growing cultures. On the contrary, induction of wt-p53 expression in GM47.23 cells results in a marked inhibition in the percentage of S-phase cells beginning at 18 hr after addition of the hormone.

Table 1. Cell cycle effects of wt-p53 in human glioblastoma cells

Cell line	Time, hr	% S-phase cells		% inhibition
		Without Dex	With Dex	
T98G	6	49 (45)	52 (49)	None
	18	47 (43)	50 (45)	None
	24	56 (52)	49 (46)	6
	32	52 (63)	42 (58)	8
GMSG-26	6	48 (50)	54 (52)	None
	18	51 (46)	59 (53)	None
	24	53 (53)	47 (51)	0.3
	32	49 (50)	53 (57)	None
GM47-23	6	51 (53)	58 (55)	None
	18	46 (51)	14 (24)	53
	24	53 (52)	7 (9)	83
	32	54 (57)	8 (11)	81

Cells were labeled for 1 hr with 10 μCi of [^{3}H]thymidine per ml at the times indicated. The cells were fixed and processed for autoradiography and the number of labeled cells was determined by light microscopic counting (500 cells per time point). The numbers represent two separate experiments. The numbers in parentheses were used to calculate % inhibition.

Histone RNA expression is tightly coupled with S phase and DNA replication (17). The expression of histone H3 RNA after induction of wt-p53 expression was examined by Northern blot analysis. In this experiment the blot shown in Fig. 2 was rehybridized with a probe for histone H3. The results are shown in Fig. 4. The steady-state levels of histone H3 RNA are significantly decreased in GM47.23 18 hr after induction of wt-p53 expression. By 24 hr, histone H3 RNA is barely detectable. In contrast, no reduction in H3 levels occurs in control T98G cells at 18 or 24 hr after Dex treatment. This rules out a nonspecific effect of the hormone on histone expression.

Effect of wt-p53 Expression on G_0/G_1 Progression into S Phase. The effect that wt-p53 expression has on the progression of cells from a resting to growing transition was also examined. For these studies, parental T98G cells and GM47.23 cells were arrested as described in *Materials and Methods*. [^{3}H]Thymidine was added at the time of replating and the percentage of cells able to enter S phase during a 24-hr period of culture was determined. The results of these experiments are shown in Table 2. The percentage of GM47.23 cells able to enter S phase following induction of wt-p53 expression is inhibited by 86% relative to untreated control cells. In contrast, Dex treatment of parental T98G cells does not significantly inhibit entry into S phase under these experimental conditions.

The nuclear DNA content of individual cells in each population (resting or stimulated by replating with or without Dex) was determined by acridine orange microfluorimetry as described (26). The results are presented in Fig. 5. Thirty-two hours after replating without Dex the mean nuclear DNA content per cell in GM47.23 cells (Fig. 5*b*) and control T98G cells (Fig. 5*e*) increases ≈2-fold relative to resting cells (Fig. 5 *a* and *d*). The DNA content of control T98G cells replated in the presence of Dex (Fig. 5*f*) also increases by nearly the same magnitude. On the contrary, in GM47.23 cells under the same conditions (Fig. 5*c*) the mean nuclear DNA content is nearly equivalent to that of resting cells (Fig. 5*a*).

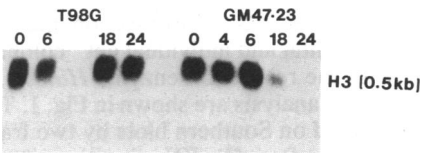

FIG. 4. Northern blot analysis of histone H3 mRNA expression. The blot shown in Fig. 2 was reprobed using the *Eco*RI fragment of pFo422 carrying a human histone H3 gene (17). Times are indicated in hours after Dex treatment.

Table 2. Effect of wt-p53 induction on G_0/G_1-phase progression into S phase

Experimental condition	% [^{3}H]thymidine-labeled cells	
	T98G	GM47.23
Confluent, 0.5% FCS	5 ± 1.7	8 ± 2.1
Replated, 10.0% FCS		
Control	84 ± 7.1	83 ± 9.5
Dex	80 ± 5.9	11 ± 1.7

Cells were treated as described in the text and labeled continuously with 0.5 μCi of [^{3}H]thymidine per ml for 24 hr. The cells were then processed for standard autoradiography and the percentage of labeled cells was determined by light microscopic counting (500 cells per experimental condition). The percentage of labeled cells is the mean of three separate experiments ± 1 SD. FCS, fetal calf serum.

Specificity of Growth Inhibition by wt-p53. Baserga (33) has pointed out that identification of growth stimulatory factors is easier than identifying negative regulatory or inhibitory factors. With the latter, one always has to consider nonspecific toxic effects. Reasonable criteria to rule out nonspecific toxic effects are that the observed inhibition should not be immediately toxic and that it should be reversible. In order to address the issue of specificity of growth arrest by wt-p53, the following experiments were conducted. GM47.23 cells were growth arrested as described above by Dex induction of wt-p53 expression for 24 hr. After induction, the hormone was removed and the percentage of cycling cells in the population was determined by continuous [^{3}H]thymidine labeling and autoradiography for a 24-hr period (roughly equivalent to one cell cycle) on consecutive days after removal of the hormone. The results of these experiments are shown in Table 3. During the 24-hr labeling period following removal of the hormone, the number of cycling cells (i.e., cells capable of entering S phase) increases 5-fold relative to those maintained in Dex. Further, the percentage of labeled cells is nearly equivalent to that of untreated exponentially growing control cells. This result indicates that the growth inhibitory effect of wt-p53 expression is reversible.

The biological relevance of the growth inhibition observed in GM47.23 cells induced to express wt-p53 protein was also addressed. It is well established in the literature that SV40 infection of quiescent cells induces cellular DNA replication (see, for example, ref. 33). Furthermore, it is known that SV40 large tumor antigen (SV40 TAG) can form a specific complex with wt-p53 (34), and it has been suggested that complex formation with SV40 TAG may neutralize the negative effects of p53 (for discussion, see ref. 35). Therefore, GM47.23 cells whose growth was inhibited by induction of wt-p53 expression were infected with purified SV40 and the percentage of S-phase cells was determined at different times after infection. The results of these experiments are also shown in Table 3. Twenty-four hours after infection of growth-inhibited GM47.23 cells there is a 4.7-fold increase in the percentage of labeled cells relative to uninfected control cells (with Dex). This result indicates that SV40 can overcome the inhibition of entry into S phase by wt-p53 expression.

Table 3. Specificity of growth inhibition

Time, hr	% [^{3}H]thymidine-labeled cells			
	UNT	With Dex	Without Dex	With Dex/SV40
0–24	56 ± 5.4	10 ± 1.6	58 ± 5.6	56 ± 5.3
48–72	63 ± 4.5	17 ± 4.3	68 ± 5.2	60 ± 7.0

Cells were labeled continuously for 24 hr with 0.5 μCi of [^{3}H]thymidine per ml at the times indicated. The percentage of labeled cells was determined as described in the legend to Table 2. Experimental conditions were as follows. UNT, untreated exponentially growing. With Dex, cells induced 24 hr with Dex and subsequently labeled with Dex present. Without Dex, cells induced 24 hr with Dex and subsequently labeled without Dex. With Dex/SV40, cells induced 24 hr with Dex, infected with SV40 with DEX present, and subsequently labeled. The percentage of labeled cells is the mean of three separate experiments ± 1 SD.

DISCUSSION

In this study we examined the biology of human wild-type p53 expression in a cell line (T98G) derived from a human glioblastoma multiforme tumor (14) as it relates to the regulation of cell proliferation. A plasmid, pM47, that encodes wt-p53 cDNA under transcriptional control of the hormone-inducible MMTV promoter was transfected into T98G cells, and individual clonal cell lines were established by selection for the linked biochemical selection marker gene *Eco gpt* (15). One clonal cell line designated GM47.23 that conditionally expressed wt-p53 following exposure to the hormone Dex was studied in detail. wt-p53 protein is synthesized in these cells and at 18–22 hr after mRNA induction, the levels of the major inducible p53 protein recognized by monoclonal antibody PAb 1801 is at least 7-fold higher than that of uninduced cells (Fig. 3). Expression of wt-p53 in this cell line resulted in inhibition of cell cycle progression and growth arrest.

The inducible wt-p53 protein expressed in GM47.23 cells appears to be a lower molecular weight (faster migrating) form than the major endogenous p53 protein in parental T98G or uninduced GM47.23 cells. This has been observed in five different independent experiments using different preparations of antibodies. Differences in the electrophoretic mobilities between the two forms of p53 protein could be due to posttranslational modifications. Both forms, the inducible wt-p53 and the endogenous p53 in GM47.23 cells, are phosphorylated (unpublished results). It is tempting to suggest

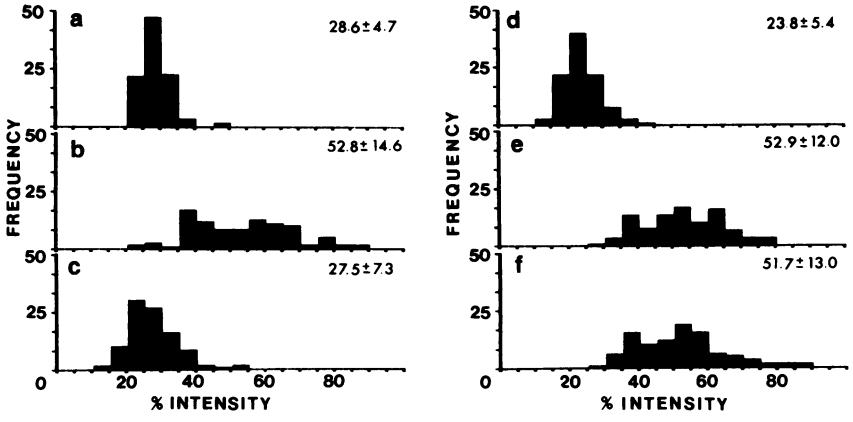

FIG. 5. DNA content histograms of GM47.23 and parental T98G cells. The DNA content of individual cells was determined after acridine-orange staining by computer-operated microfluorimetry (26). The abscissa gives the intensity of green fluorescence (540–560 mn), which is an accurate measure of DNA content per individual cell. The ordinate gives the frequency (no. of cells). (*a* and *d*) GM47.23 and T98G cells growth arrested in G_0/G_1-phase. (*b* and *e*) GM47.23 and T98G cells 32 hr after stimulation without Dex. (*c* and *f*) GM47.23 and T98G cells 32 hr after stimulation with 1 μM Dex. The numbers in the right-hand corner of each panel are the mean ± 1 SD.

that the faster migrating inducible form of p53 may be phosphorylated differently from the endogenous form of p53. Differences in the levels of phosphorylation for the retinoblastoma (Rb) protein that result in alterations in electrophoretic mobilities of the protein have been reported (36). Moreover, it has been proposed that the underphosphorylated form of the Rb protein may be the biologically active form in growth suppression (36, 37). Experiments are necessary to examine this intriguing possibility for wt-p53 protein in this model.

Induction of wt-p53 expression in GM47.23 cells has a very striking inhibitory effect on cell proliferation. We argue that the inhibitory effect observed is specific based on the following lines of evidence. (*i*) Dex induction of wt-p53 expression in exponentially growing cells inhibits cell cycle progression; however, the inhibitory effect is reversible upon removal of the inducer or infection with SV40. (*ii*) When growth-arrested cells are stimulated to proliferation, Dex induction of wt-p53 expression inhibits G_0/G_1 progression into S phase, and the cells accumulated with a nuclear DNA content equivalent to cells arrested in G_0/G_1 phase of the cell cycle. (*iii*) Finally, long-term expression of wt-p53 (6 days) results in a 5- to 7-fold reduction in the final saturation density of the culture without any apparent signs of cytotoxicity (data not shown). Taken together, these studies strongly suggest that the inhibitory effect of wt-p53 expression is specific (i.e., not simply due to overexpression of a toxic protein) and that it is a biologically relevant model.

A number of studies support the idea that the normal function of p53 protein is coupled with entry of cells into the cell cycle. For example, in serum-stimulated BALB/c 3T3 mouse cells, p53 mRNA and protein reach maximum levels of expression in middle to late G_1 phase during the G_0 to S-phase transition (38). Human p53 mRNA and protein are expressed in mitogen-stimulated peripheral blood T lymphocytes (24, 30, 39) and in serum-stimulated WI-38 human diploid fibroblasts (30) with similar kinetics. Further, functional studies imply that p53 protein might be important for cell cycle regulation by demonstrating that deregulating p53 expression by anti-p53 antibody microinjection can inhibit serum-stimulated entry into S phase (28, 40), and down-regulating its expression by antisense RNA transcripts can lead to growth arrest (41). The results presented in this communication are seemingly in conflict with these earlier studies. One possible explanation consistent with these apparently conflicting results is that p53 may behave as a positive and a negative regulator of cell growth in different phases of the cell cycle by association with different proteins and/or by posttranslational modifications. Perhaps appropriate regulation of cell proliferation is dependent upon a balance of the forms of p53 present, and, in the system studied here, the overexpressed wt-p53 protein may not be properly regulated. Further, mutant mouse p53 (4, 5, 42) behaves as an oncogene, whereas wild-type mouse p53 has recently been found to inhibit the oncogenic transformation by mutant p53 (43, 44). These observations are consistent with the inherent ability of wt-p53 to suppress cell growth, as shown in this report. Although p53 expression in normal cells is growth-regulated (24, 30, 38, 39), the results presented here show that overexpression of wt-p53 protein can have a negative effect on cell growth. Further experiments will be needed to elucidate the exact mechanism whereby p53 exerts these effects on the cell cycle.

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