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p53 Antisense Oligonucleotide Inhibits Growth of Human Colon Tumor and Normal Cell Lines

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We examined the relationship between the expression of mutant p53 proteins and tumor cell growth using a p53 antisense oligonucleotide (5′-CCCTGCTCCCCCCTGGCTCC-3′). The oligonucleotide inhibited the growth of three human colon tumor cell lines (DLD-1, SW620 and WiDr), which produce only mutant p53 proteins with different mutation sites. Treatment of DLD-1 cells with the p53 antisense oligonucleotide caused a decrease in the level of p53 mutant protein. Synthesis of DNA in DLD-1 and SW620 cells was inhibited more potently than that of RNA or protein after antisense treatment. Furthermore, these cells were accumulated in the S phase when DNA synthesis was inhibited. Meanwhile, the antisense oligonucleotide also inhibited the growth of three human normal cell lines (WI-38, TIG-1 and Intestine 407). While treatment of WI-38 and TIG-1 cells with the antisense oligonucleotide inhibited synthesis of DNA more potently than that of RNA or protein, these normal cells were accumulated in the G0/G1 phase. These results suggest that p53 proteins, either with or without mutation, play a pivotal role in the growth of tumor and normal cells, but that mutant and wild-type p53 proteins may function differently in cell growth.

Key words: p53 Antisense — Oligonucleotide — Cell cycle — Cell growth

The *p53* gene has been suggested to be a tumor suppressor gene. Genetic abnormalities of the gene are observed in a wide variety of human tumor cells, at least in those derived from colon, lung, breast, brain and liver tissues. Missense mutations and loss of the short arm of chromosome 17 are common genetic alterations in the *p53* gene of human tumors,[1,2] e.g., colon carcinoma.

A number of studies have indicated that overexpression of wild-type p53 protein arrests cell-cycle progression at the G1 phase. p53 protein may also enhance the repair of damaged DNA since DNA-damaging agents cause overexpression of wild-type p53 protein.[3,4] It also inhibits transformation induced by *myc* and *ras*, and by this mechanism inhibits the growth of human tumor cells in culture. As it activates or represses gene transcription, wild-type p53 protein may regulate cell growth at the level of gene transcription. It was indicated that p53 protein may regulate the expression of *p21/waf 1/cip 1*, a universal inhibitor of cyclin-dependent kinases.[5–7] Wild-type p53 protein also binds to some viral and cellular proteins, including SV40 large T antigen, adenovirus 5 E1B and E6 of human papillomavirus, *mdm*-2 protein, TATA-binding protein and replication protein A. Since these proteins are closely related to gene transcription and DNA replication, wild-type p53 protein may also induce cell-cycle arrest via protein-protein interaction.

Mutant p53 proteins appear to lose some of the functions of wild-type p53 protein,[2,8–10] including its short half-life and the DNA-binding activity, and to act in a dominant negative fashion. They tend to inactivate wild-type p53 protein by forming oligomeric protein complexes with it.[10,11] However, as most human tumor cells express only mutant p53 proteins,[1,2] mutant p53 proteins may confer growth advantages on tumor cells without the dominant negative effect. This possibility, the so-called gain-of-function mutation of the p53 gene,[12] is supported by a number of studies: mutant p53 increases tumorigenicity,[12–14] saturation density in culture[15] and plating efficiency in agar in p53-negative cells,[12] and reduces serum requirements for growth of oncogene-transformed cells.[16]

It is known that four different p53 antisense oligonucleotides inhibit the growth of primary acute myeloblastic leukemia cells *in vitro*.[17,18] One type has been administered systemically to patients with acute myeloblastic leukemia in a phase I clinical study.[18] It is unclear, however, whether the target of the oligonucleotides is wild-type or mutant p53. They can hybridize to sequences where no mutations have thus far been found, and the target was primary acute myeloblastic leukemia cells, whose p53 status has not been determined.[18] In the present study, we used a p53 antisense oligonucleotide identical to one used in a phase I clinical study, to investigate the relationship between the amount of p53 protein and the growth of tumor cells. Interestingly, p53 antisense oligonucleotide inhibited the growth of human tumor and normal cells, suggesting that p53 protein, either with or without mutation, may stimulate cell growth. Further, wild-type and mutant p53 proteins may function in different phases of the cell cycle.

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MATERIALS AND METHODS

Materials Lipofectin was purchased from Gibco BRL (Gaithersburg, MD). [*Methyl*-^{3}H]thymidine, [5-^{3}H]-uridine and L-[4, 5-^{3}H(N)]leucine were purchased from NEN Research Laboratories (Boston, MA). Mouse anti-p53 monoclonal antibody (PAb1801) was purchased from Oncogene Science (Uniondale, NY). Horseradish peroxidase-conjugated sheep anti-mouse immunoglobulin was purchased from Amersham (Buckinghamshire, England). Chemiluminescence reagents for the Western blot, Renaissance, were purchased from DuPont (Ontario, Canada). CycleTEST for the analysis of nuclear DNA was purchased from Becton Dickinson (Erembodegem, Belgium).

Cell lines Human colon tumor DLD-1, SW620 and WiDr cells and human ileum Intestine 407 cells were maintained in RPMI 1640 (Nissui, Tokyo) containing 10% fetal bovine serum (FBS, Hyclone, Rogan, UT), 0.3 mg/ml L-glutamine and 0.1 mg/ml kanamycin. Human diploid fibroblast WI-38 cells were maintained in minimum essential medium alpha modification (α-MEM, Gibco) containing 15% FBS, 1% nonessential amino acids, and L-glutamine and kanamycin as above. Human diploid fibroblast TIG-1 cells were cultured in Eagle's MEM (Nissui) containing 10% FBS and 0.06 mg/ml kanamycin. All cells were grown in an atmosphere of 5% CO_2 at 37°C.

Oligonucleotides Phosphorothioate antisense (5′-CCC-TGCTCCCCCCTGGCTCC-3′) and sense (5′-GGAG-CCAGGGGGGAGCAGGG-3′) oligonucleotides (20-mer) were synthesized by using an automated DNA synthesizer (349 DNA/RNA synthesizer, Applied Biosystems, Foster City, CA). The oligonucleotides were purified twice by reversed-phase high-performance liquid chromatography on a Capcell Pak C18 column before and after detritylation. This antisense oligonucleotide is complementary to position 1071–1090 of exon 10 of the *p53* gene. This complementary sequence is included in the carboxy-terminal region, which is capable of forming oligomers,[19] of p53 protein. The antisense sequence is the same as that of OL(1)p53 administered systemically to patients with acute myeloblastic leukemia.[18]

Treatment with oligonucleotides Cells were washed once with serum-free RPMI 1640 medium and treated with oligonucleotides for 6 h in freshly prepared serum-free medium containing Lipofectin (10 μg/ml). After treatment, the medium was replaced with growth medium. For the simultaneous addition of sense and antisense oligonucleotides, equal concentrations of the oligonucleotides were combined and pre-incubated for 10 min at room temperature prior to the treatment.

Determination of cell growth To measure the growth rate of cells treated with antisense oligonucleotide, DLD-1 cells (5×10^3/well) and other cells (2×10^4/well) were plated in 24-well plates on day 0. After 1 day of incubation, the cells were treated for 6 h with oligonucleotides as described above. The number of cells was then counted using a Coulter multisizer II (Coulter Electronics, Bedfordshire, England) on days 1, 2, 4 and 6. All experiments were performed in triplicate. No cells of any type had reached confluence on day 6.

Western blot analysis DLD-1 cells were plated at 8×10^4 cells in 60-mm dishes on day 0. The next day (day 1), the cells were treated with oligonucleotides at 0.5 μM for 6 h. On day 2, they were washed once with ice-cold phosphate-buffered saline (PBS) and lysed directly in 2% sodium dodecylsulfate (SDS). The concentration of total cellular protein was measured by Bio-Rad protein assay (Richmond, CA). These samples were mixed with 2× loading buffer and boiled. Next, 20 μg each of the protein preparation was separated by electrophoresis on a 10% to 20% gradient SDS-polyacrylamide gel and transferred to nitrocellulose membranes. The filter was incubated at room temperature sequentially with an PAb1801 and a horseradish peroxidase-conjugated anti-mouse immunoglobulin, and washed with PBS containing 0.05% Tween 20. Horseradish peroxidase activity was then detected with chemiluminescence reagents. All experiments were performed in duplicate.

Incorporation of thymidine, uridine and leucine DLD-1 and other cells were plated at 5×10^3 and 2×10^4 cells/well, respectively, in 24-well plates on day 0 and treated with oligonucleotides as described above. The cells were treated with [^{3}H]thymidine (185 kBq/ml), [^{3}H]uridine (46 kBq/ml) and [^{3}H]leucine (66 kBq/ml) for 2 h on the indicated day. On day 1, the radioactive tracers were added to the cells 2 h after oligonucleotide treatment. The cells were washed with ice-cold PBS and trypsinized, and radioactivity incorporated into the cells was measured using a liquid scintillation counter (Liquid Scintillation System LSC-700; Aloka Co., Ltd., Tokyo). All experiments were performed in triplicate.

Analysis of cell cycle Cells were treated with oligonucleotides as described above. On day 2, the samples were prepared for the analysis of nuclear DNA content using CycleTEST (Becton Dickinson, Erembodegem, Belgium). Flow cytometric analysis was carried out on a FACScan flow cytometer (Becton Dickinson, Mountain View, CA). The data obtained were analyzed for the cell-cycle distribution using the SFIT mathematical algorithm of the FACScan/CellFIT software.

RESULTS

To clarify the relationship between mutant p53 protein and tumor cell growth, we first examined the effect of the p53 antisense oligonucleotide on cell growth of human

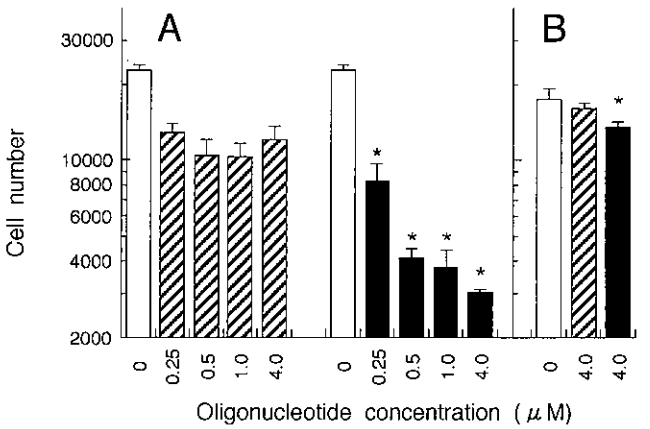

Fig. 1. Effect of p53 antisense oligonucleotide on growth of human colon tumor DLD-1 cells. DLD-1 cells were treated with either antisense or sense oligonucleotide at various concentrations in the presence (A) or absence (B) of Lipofectin (10 μg/ml). The number of cells was counted on day 4. All experiments were performed in triplicate. Each bar in the figure represents the mean±SD (n=3). Symbols: □, no oligonucleotide; ▨, sense oligonucleotide; ■, antisense oligonucleotide. The significance of differences between sense and antisense-treated groups at the same concentration was examined by use of Student's t test. * $P<0.05$.

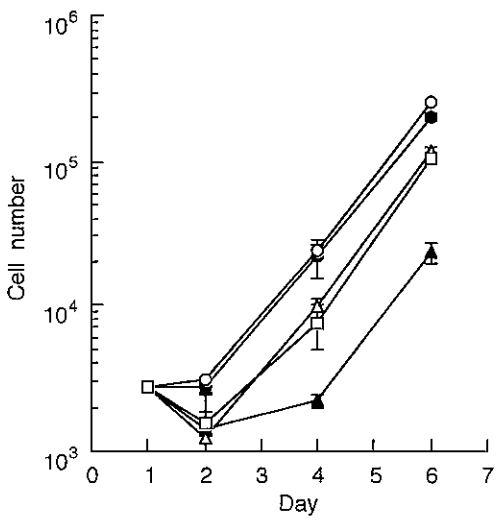

Fig. 2. Duration of p53 antisense oligonucleotide-induced growth inhibition of DLD-1 cells. DLD-1 cells were treated with oligonucleotides (0.5 μM) for 6 h. In one group, simultaneous addition of sense and antisense oligonucleotides was done after mixing 0.5 μM of each oligonucleotide; total oligonucleotide concentration in this group was therefore 1.0 μM. The number of cells was counted on days 1, 2, 4 and 6. All experiments were performed in triplicate. Bars: SD (n=3); ○, control; ●, Lipofectin alone; △, sense oligonucleotide with Lipofectin; ▲, antisense oligonucleotide with Lipofectin; □, both sense and antisense oligonucleotides with Lipofectin.

colon tumor DLD-1 cells, which produce mutant (S241F) but not wild-type p53 protein.[20] The cells were treated for 6 h with the antisense or sense oligonucleotide in the presence of Lipofectin and the cell number was counted 3 days later (Fig. 1 A). While sense oligonucleotide (0.25–4.0 μM) induced a 30% decrease in cell growth, the antisense oligonucleotide further inhibited cell growth in a dose-dependent manner (Fig. 1A). The inhibitory activities of the antisense and sense oligonucleotides disappeared in the absence of Lipofectin, and Lipofectin itself had no effect on cell growth (Figs. 1B and 2).

We next measured the growth rate of DLD-1 cells treated with 0.5 μM antisense or sense oligonucleotide for 5 days (Fig. 2). Although the antisense oligonucleotide inhibited growth more strongly than the sense oligonucleotide and Lipofectin alone on day 4, no difference in growth rates was observed after day 4, suggesting the inactivation of the oligonucleotides in the cells. We further examined whether hybridization ability of the antisense oligonucleotide was required for its growth-inhibitory effect. Simultaneous addition of an equivalent amount of the sense oligonucleotide completely abolished the growth-inhibitory effect of the antisense oligonucleotide; the inhibitory activity was comparable to that seen with the sense oligonucleotide, not with the Lipofectin control (Fig. 2). It thus seems likely that sense oligonucleotide-induced growth inhibition may be due to nonspecific cytotoxicity of phosphorothioates, as previously suggested.[21] These results suggest that the p53 antisense oligonucleotide selectively inhibits the growth of DLD-1 cells.

To confirm that the p53 antisense oligonucleotide delays growth of DLD-1 cells by inhibiting production of p53 protein, p53 protein in DLD-1 cells treated with the antisense oligonucleotide for 6 h was detected by means of Western blot analysis. As shown in Fig. 3, treatment with the antisense oligonucleotide (lanes 5 and 6), but not with Lipofectin (lanes 1 and 2) or with the sense oligonucleotide (lanes 3 and 4), reduced the expression of p53 protein in the cells. This reduced p53 production disappeared when an equal amount of the sense oligonucleotide was added simultaneously (lanes 7 and 8). The growth inhibition induced by the antisense oligonucleotide seen in Figs. 1 and 2 is thus associated with reduced production of p53 protein.

We next examined the effect of the antisense oligonucleotide on the growth of other human colon tumor (Fig. 4A) and normal (Fig. 4B) cells. SW620 and WiDr cells produce only mutant p53 protein with mutation sites (R273H and P309S in SW620 cells and R273H in WiDr

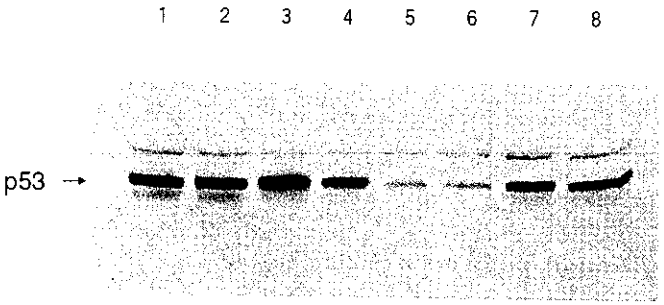

Fig. 3. Effect of p53 antisense oligonucleotide on expression of mutant p53 protein in DLD-1 cells. Total cellular protein of DLD-1 cells was prepared 20 h after oligonucleotide treatment: 20 μg was separated by electrophoresis on a gradient sodium dodecylsulfate-polyacrylamide gel (10% to 20%) and transferred to a nitrocellulose membrane. Expression of p53 protein was detected by use of a mouse anti-p53 monoclonal antibody (PAb1801). All experiments were done in duplicate. Lanes 1 and 2, Lipofectin alone; lanes 3 and 4, sense oligonucleotide (0.5 μM) with Lipofectin; lanes 5 and 6, antisense oligonucleotide (0.5 μM) with Lipofectin; lanes 7 and 8, combined sense and antisense oligonucleotides at 0.5 μM each with Lipofectin.

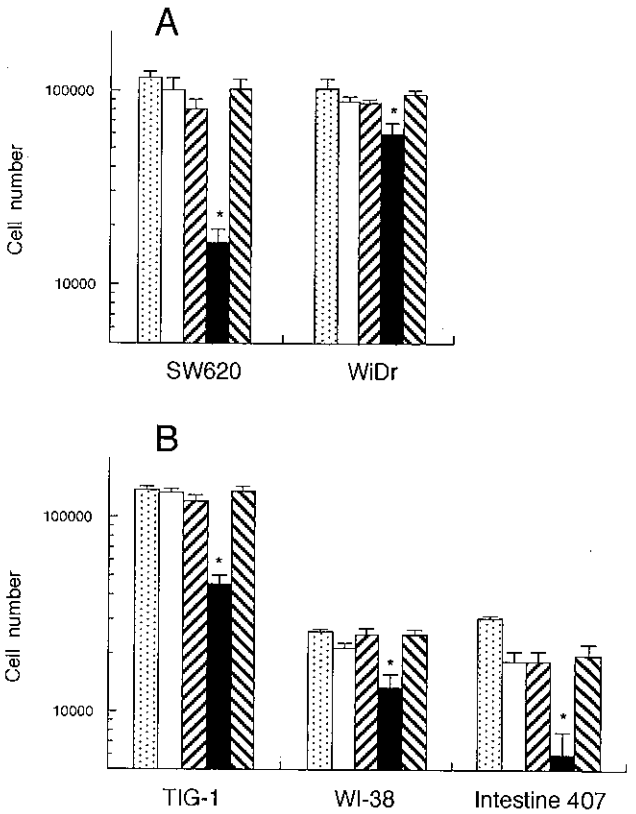

Fig. 4. Effect of p53 antisense oligonucleotides on growth of human colon tumor and normal cell lines. Human colon tumor (A) and normal (B) cell lines were used. All cells were treated for 6 h in the presence of Lipofectin with oligonucleotides at 0.5 μM. Addition of both sense and antisense oligonucleotides was done after mixing them at the same concentration. Cell numbers were counted 3 days after treatment. All experiments were performed in triplicate. Each bar in the figure represents the mean±SD (n=3). Symbols: ▨, control; □, Lipofectin alone; ▨, sense oligonucleotide; ■, antisense oligonucleotide; ▧, both oligonucleotides. The significance of differences of the antisense- and both-treated groups from the sense-treated group was examined by use of Student's t test. $* P<0.05$.

cells) different from that of DLD-1 cells.[20, 22] It is known that WI-38 cells produce only wild-type p53 protein.[3] It is not known whether p53 proteins of TIG-1 and Intestine 407 cells have any mutations. Cells were treated with each oligonucleotide for 6 h at the concentration of 0.5 μM and the cell number was counted 3 days later. The growth of these cells was significantly inhibited by treatment with the antisense oligonucleotide, and this inhibition was abolished by simultaneous treatment with sense and antisense oligonucleotides. Treatment with Lipofectin alone partially inhibited growth of Intestine 407, but not other cell lines. These results imply that both mutant and wild-type p53 proteins are essential for the growth of cells producing p53 protein.

To understand better the mechanism of the growth inhibition of the antisense oligonucleotide, we next examined its effect on DNA, RNA and protein syntheses by measuring the amount of radio-labeled precursors incorporated into DLD-1 cells on day 2 (Fig. 5 A, B and C). Treatment with antisense oligonucleotide for 6 h remarkably inhibited DNA synthesis and slightly reduced protein synthesis, but had less effect on RNA synthesis. Inhibition of the cell growth induced by the sense oligonucleotide (Figs. 1 and 2) may thus be due to the mechanism other than the inhibition of DNA, RNA or protein synthesis. While DNA synthesis was inhibited for 3 days by treatment with antisense oligonucleotide, the inhibitory potency was decreased as the cells were treated longer (Fig. 5 D). Further, the inhibition of DNA synthesis was abolished by simultaneous addition of an equal amount of the sense oligonucleotide. These results suggest that the growth inhibitory effect of the p53 antisense oligonucleotide is associated with reduced DNA synthesis.

Treatment with p53 antisense oligonucleotide reduced DNA and RNA syntheses in other human tumor and normal cell lines (Table I). DNA synthesis in those cells was inhibited more strongly than RNA and protein syntheses. These inhibitory activities were abolished by simultaneous addition of the sense oligonucleotide; the results were similar to those obtained with DLD-1 cells.

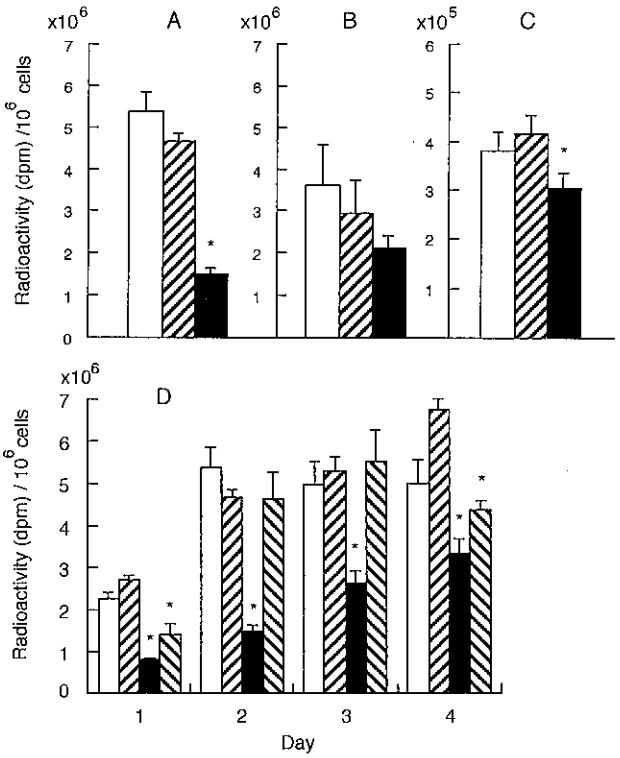

Fig. 5. Effect of p53 antisense oligonucleotide on DNA, RNA and protein synthesis in DLD-1 cells. Incorporations of [^{3}H]thymidine (A), [^{3}H]uridine (B) and [^{3}H]leucine (C) into DLD-1 cells were measured 19 h after treatment with oligonucleotides (0.5 μM). In panel D, incorporation of [^{3}H]-thymidine into DLD-1 cells was measured daily until day 4. All experiments were performed in triplicate. Each bar in the figure represents the mean±SD (n=3). Symbols: □, Lipofectin alone; ▨, sense oligonucleotide; ■, antisense oligonucleotide; ▧, both oligonucleotides. The significance of differences of the antisense- and both-treated groups from the sense-treated group in each column was examined by use of Student's t test. * $P<0.05$.

We finally analyzed by using flow cytometry whether the antisense oligonucleotide could arrest the cell cycle of these cells in selected phases (Table II). As compared to Lipofectin alone, treatments of DLD-1 and SW620 tumor cells with the antisense oligonucleotide induced a slight increase in the cell ratio in S phase and a decrease in other phases. Treatment with the sense oligonucleotide or with both oligonucleotides did not alter the distribution pattern of the cell cycle. In contrast, treatment of WI-38 and TIG-1 normal cells with the antisense oligonucleotide increased the cell ratio in G0/G1 phase and decreased that in S phase, as compared to Lipofectin alone. Treatment with the sense oligonucleotide or with both oligonucleotides slightly changed the cell-cycle distribution, causing an increase in the population in the

Table I. Effects of p53 Antisense Oligonucleotide on DNA, RNA and Protein Syntheses

Cell line	Precursor	Incorporation rate (% of control)[a]		
		Sense	Antisense	Both
SW620	Thymidine	89	50[b]	81
	Uridine	90	70	94
	Leucine	98	114	104
WI-38	Thymidine	119	40[b]	112
	Uridine	120	67[b]	109
	Leucine	121	80[b]	110
TIG-1	Thymidine	86	44[b]	78
	Uridine	95	66	90
	Leucine	105	109	89

All cells were treated for 6 h with oligonucleotides (0.5 μM) in the presence of Lipofectin. Addition of both sense and antisense oligonucleotides was done after mixing them at the indicated concentration. Radioactivities incorporated into these cells 18 h (TIG-1) and 20 h (SW620 and WI-38) after oligonucleotide treatment were measured in triplicate.
a) Percent incorporation compared to cells treated with Lipofectin alone.
b) The difference from the value obtained in the sense-treated group is statistically significant (Student's t test, $P<0.05$).

Table II. Cell-cycle Progression Following Treatment with p53 Antisense Oligonucleotide

Cell line	Treatment	Percent of total		
		G0/G1	S	G2/M
DLD-1	Lipofectin	15.2	61.5	23.3
	+Sense	16.1	62.1	21.9
	+Antisense	13.2	70.5	16.4
	+Both	16.7	57.5	25.9
SW620	Lipofectin alone	26.0	62.3	8.0
	+Sense	26.4	61.5	12.2
	+Antisense	25.1	68.0	7.0
	+Both	29.3	60.2	10.5
WI-38	Lipofectin alone	26.6	60.6	12.9
	+Sense	32.8	55.4	11.9
	+Antisense	56.6	32.8	10.7
	+Both	34.5	53.2	12.4
TIG-1	Lipofectin alone	37.1	49.5	13.5
	+Sense	27.8	51.2	21.0
	+Antisense	55.0	37.7	7.4
	+Both	27.2	50.0	22.9

All cells were treated for 6 h with oligonucleotides (0.5 μM) in the presence of Lipofectin. Addition of both sense and antisense oligonucleotides was done after mixing them at the indicated concentration. Samples were prepared 18 h (WI-38), 20 h (DLD-1) and 21 h (SW620 and TIG-1) after oligonucleotide treatment.

G0/G1 phase in WI-38 cells and a decrease in that phase in TIG-1 cells. These data indicate that the p53 antisense oligonucleotide induces growth delay of human tumor and normal cells predominantly in the S and G0/G1 phases, respectively.

DISCUSSION

p53 Antisense oligonucleotide inhibited the growth of human tumor and normal cells expressing mutant and/or normal p53 proteins more potently than the complementary sense oligonucleotide. Using fluorescence-labeled antisense oligonucleotide, we confirmed that Lipofectin greatly enhanced the uptake of the antisense oligonucleotide and that simultaneous treatment with the sense oligonucleotide did not affect incorporation of the antisense oligonucleotide into the cells (data not shown). In DLD-1 cells, growth inhibition induced by p53 antisense oligonucleotide was associated with reduced expression of p53 protein, inhibition of DNA synthesis and alteration of the cell-cycle distribution. These growth-inhibitory effects of the antisense oligonucleotide were completely abolished by simultaneous treatment with the sense oligonucleotide. Our antisense oligonucleotide is identical to that reported by Bayever *et al.*[18] to have no influence on the growth of HL-60 cells, which lack p53 expression. We also examined the effect of the antisense oligonucleotide on the growth of Saos-2 cells, which lack p53 expression (data not shown). Although this cell line was more sensitive to cytotoxicity induced by the sense oligonucleotide than other cell lines, growth inhibitions induced by the antisense and the sense oligonucleotides were comparable. These results suggest that the antisense effects occur inside the cells, presumably via hybridization to the target molecule (p53 mRNA and/or *p53* gene) in a sequence-specific fashion.

Hara *et al.* reported that treatment with p53 antisense oligonucleotide in the absence of Lipofectin had no effect on the growth of TIG-1 cells, although it reduced the expression of p53 protein.[23] Further, treatment with Rb antisense oligonucleotide induced an increase in proliferation lifespan. However, our p53 antisense oligonucleotide inhibited the growth of TIG-1 cells (Fig. 4). These inconsistencies may be due to different potency in p53 reduction between the two experiments. In addition, the sequence, size and treatment method of the oligonucleotides are not identical.

Our findings indicate that both mutant and wild-type p53 proteins are related to cell growth. These results are supported by previous findings by others: microinjection of anti-p53 antibody into murine and human cells producing either wild-type or mutant p53 proteins inhibited DNA synthesis,[24–26] and expression of p53 antisense RNA reduced DNA synthesis in murine Meth A fibrosarcoma cells, which produce mutant p53 proteins.[27] Moreover, it is known that microinjection of anti-p53 antibody into resting WI-38 cells inhibits serum-induced DNA synthesis.[26] We also showed that WI-38 cells were arrested in the G0/G1 phase after treatment with p53 antisense oligonucleotide (Table II). Taken together, our findings provide evidence that human mutant p53 proteins are required for the growth of human tumor cells.

The requirement of p53 protein for cell growth, however, is not universal. For example, p53 knock-out mice grow normally[28] and p53-deficient cells such as Saos-2 cells are able to proliferate. Therefore, p53 protein may not always be essential for cell growth, though there is no evidence suggesting that the function of p53 protein in cell growth can be duplicated by other proteins. Such functional replacement may not be easy in somatic cells, in view of the low incidence of p53-deficient cells.

We next examined the possible mechanism of antisense oligonucleotide-induced growth inhibition. In both tumor and normal cell lines, the antisense oligonucleotide inhibited synthesis of DNA, rather than that of RNA or protein. These results indicate that both mutant and wild-type p53 proteins may play a pivotal role in DNA replication. Further examination indicated that p53 antisense oligonucleotide induces mild cell-cycle arrest in the S phase in DLD-1 and SW620 cells (Table II). Decreased DNA synthesis observed in these cells may be responsible for this S phase arrest. In contrast, normal WI-38 and TIG-1 cells were accumulated in the G0/G1 phase (Table II). These data suggest that mutant and wild-type p53 proteins may act at different phases of the cell cycle. However, it seems possible that this difference in the cell cycle population between normal and tumor cell lines is due to delayed recovery from the antisense effect in normal cells. To clarify this point, the effect of antisense oligonucleotide on specific phases of the cell cycle should be examined.

Since DLD-1 and SW620 cells were arrested in the S phase after treatment with p53 antisense oligonucleotide, it seems likely that mutant p53 protein is associated with the progression of DNA synthesis. It has also been suggested, although wild-type p53 protein was used in most studies,[29–31] that p53 protein has a domain in the molecule which stimulates DNA replication.[31] Mutation has never been found in this domain in human or murine p53 proteins. The p53 protein also binds to replication protein A, a part of the DNA replication machinery, and stimulates DNA replication of bovine papilloma virus *in vitro*. Further investigation is necessary to clarify whether mutant p53 protein functions to stimulate cellular DNA replication.

It has been shown that overexpression of wild-type p53 protein induces G1 phase arrest[15, 20, 32–34] and that this effect may be mediated by a p53-induced inhibitor of

cyclin-dependent kinases, such as p21/WAF1/CIP1.[5-7] However, we have shown in the present study that reduced production of p53 protein in WI-38 and TIG-1 cells causes similar cell-cycle arrest in the G0/G1 phase. Wild-type p53 protein may thus regulate transition of the cell cycle from G1 to S phase.

With respect to p53 function, an interesting model has been proposed, based on analyses of p53 conformation and oligomerization changes, to the effect that wild-type p53 protein has a dual role as both a stimulator and inhibitor in the regulation of cell growth.[35-38] Thus, mutant p53 proteins may lose only the growth inhibition function. In addition, wild-type and mutant p53 proteins may also have different mechanisms of growth stimulation, as the cell cycles of human tumor and normal cells were differently arrested.

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