Chemotherapeutic agents targeting thymidylate synthase (TS) are effective against human tumors. Efficacy is limited by drug resistance, often mediated by TS overexpression. Treatment of HeLa cells in vitro with an antisense oligodeoxynucleotide (ODN 83) targeting human TS mRNA reduces TS mRNA and protein levels, inhibits cell proliferation, and sensitizes cells to TS-targeting drugs (Ferguson et al., 1999). The present study investigates the mechanism by which ODN 83 inhibits cell proliferation and examines its antitumor efficacy in vivo. ODN 83 treatment did not induce apoptosis in HeLa cells in vitro but caused accumulation of cells at G2/M. In contrast, TS-targeting chemotherapeutics arrest at G1 or S. Antisense down-regulation reduced TS mRNA levels in human colon cancer (HT29) cells by 40% in vitro, resulted in G2/M arrest, and reduced proliferation without enhanced cell death. Growth of HT29 tumors in immunocompromised mice was significantly inhibited when antisense ODN 83 treatment began promptly after tumor implantation and was accompanied by a 40% reduction in TS protein levels. Growth of tumors allowed to reach 400 mm3 prior to ODN administration was unaffected by antisense ODN 83. Radiolabeled ODNs were localized to the tumor periphery but evenly distributed in normal tissue. Thus, down-regulation of TS mRNA and protein by antisense ODN treatment exerts a novel G2/M cell cycle block without increasing cell death and inhibits HT29 tumor cell growth in vivo. Antisense ODN 83 may be an effective therapy for colon carcinoma, alone or in combination with TS-targeting cytotoxic drugs.
vitro with an antisense ODN targeting TS decreases TS mRNA and protein levels, inhibits cell proliferation, and sensitizes HeLa cells to 5-FU, 5-fluorodeoxyuridine (5-FDUR), and raltitrexed (Ferguson et al., 1999). Antisense ODNs against TS might be expected to block DNA synthesis and arrest cells in G1, or early S phase, similar to the action of 5-FU (Inaba and Mitsuhashi, 1994) and raltitrexed (Matsumi et al., 1996; Yin et al., 1999). However, TS protein binds to a variety of mRNA molecules, including those encoding p53 (Chu et al., 1996; Ju et al., 1999), c-myc (Chu et al., 1995), and TS itself (Chu et al., 1991, 1994). This has raised the possibility that post-transcriptional regulation of mRNA metabolism by TS protein might control not only TS protein production but also that of cell cycle regulatory proteins. Treatment of cells with TS antisense ODNs to decrease both TS mRNA and protein levels may lead to cell cycle perturbations that are not predicted from experiments using protein-targeting drugs that act after mRNA translation.

To examine the cytotoxic and cytostatic effects of targeting TS with antisense ODNs, we have measured apoptosis in HeLa cells and analyzed the cell cycle distribution of HeLa and HT29 carcinoma cells treated in vitro with TS antisense ODN 83. In addition, to test the hypothesis that antisense down-regulation of TS would be an effective antitumor strategy in vivo, we have assessed the antitumor activity of TS antisense ODN 83 on the growth of HT29 tumor explants in immunocompromised mice. We report that the TS antisense ODN 83 has novel and potentially therapeutically exploitable effects on human tumor cell cycle and viability in vitro and in vivo.

Materials and Methods

Cell Culture. HeLa and HT29 cells (from the American Type Culture Collection, Manassas, VA) were grown in Dulbecco’s modified Eagle’s medium and RPMI1640 (respectively) containing 10% fetal bovine serum at 37°C in a humidified atmosphere of 5% CO2. All tissue culture reagents were from Invitrogen Canada (Burlington, ON, Canada).

Oligodeoxynucleotides and Transfection. The TS antisense ODN 83 (5'-GGCGAGTTGGCAACATCTTATAA-3') is complementary to the sequence 136 to 155 base pairs downstream of the translation stop site in the 3' untranslated region of human TS mRNA. There are no other known mRNAs (including mouse TS) with sequences complementary to ODN 83. The control scrambled ODN 32 (5'-ATGCAGGCACGCTTTCTAA-3') has the same base composition in random order. For in vivo studies, ODNs with phosphorothioate linkages between the six nucleotides at both the 5'- and 3'-ends were purchased from BioCorp Inc. (Montreal, QC, Canada). For in vitro studies, fully phosphorothioated ODNs with 2'-methoxy-ethoxy modification on the six nucleotides at both the 5'- and 3'-ends were generously provided by Dr. N. Dean (ISIS Pharmaceuticals, Carlsbad, CA).

HeLa and HT29 cells were transfected with ODNs using LipofectAMINE (Invitrogen Canada), as described (Ferguson et al., 1999), or with LipofectAMINE 2000 (Invitrogen Canada) as described below. For HT29 cells, preliminary experiments indicated that LipofectAMINE 2000 was the superior lipid formulation and was effective at 1 to 5 μg/ml, although nonspecific toxicity was apparent at the higher doses. Concentrations of ODN 83 from 50 to 200 nM were found to be effective at inhibiting HT29 cell proliferation, with improved activity at the higher concentrations. For proliferation assays, HT29 cells were plated at 2 × 10^5 cells per 25-cm^2 flask in 2 ml of medium. On the following day, a 6× transfection mix was prepared containing 600 nM ODN and 6 μg/ml LipofectAMINE 2000 in serum-free medium. After incubation for 15 min at room temperature, 5 volumes of medium with 10% serum were added, and the medium on the cells was replaced with the 1× transfection mix. For flow cytometry samples and RNA preparation, cells were plated at 1 to 2 × 10^6 cells per 75-cm^2 flask in 5 ml of medium. The next day, 1 ml of a DNA/lipid mixture in serum-free medium was added directly to each 75-cm^2 flask to yield final concentrations of 200 nM ODN and 2 μg/ml lipid.

Animal Studies. Female nude mice (N:NIH-bg-nu-xid), purchased from Charles River Laboratories (St. Constant, QC, Canada), were housed and cared for according to standards of the Canadian Council for Animal Care and were used under a protocol approved by the University of Western Ontario Council on Animal Care. TS-inhibitory cytotoxic drugs are a mainstay in the treatment of colon cancer (Papamichael, 1999), and preliminary studies indicated that human HT29 colon carcinoma cells grow well in these immunocompromised mice (E. Behrend, unpublished observations). To assess the effect of ODN on tumor growth, 4- to 6-week-old mice were injected subcutaneously in the right flank with 5 × 10^6 HT29 cells on day 0. Every 2nd day beginning on day 1, mice were injected intraperitoneally with ODNs dissolved in 150 mM NaCl. Tumors were measured in two perpendicular dimensions with a caliper every 4 days, and tumor volume was calculated using the formula volume = length × width^2 × π/6. To measure their effect on larger, established tumors, ODNs were administered to mice when the tumors achieved a size of 400 mm^3.

Oligodeoxynucleotide Labeling and Distribution Analysis. ODNs (2 μg) were end-labeled to a specific activity of 2 × 10^6 cpm/μg with [γ-32P]adenosine triphosphate (specific activity, 7000 Ci/mmol, ICN Pharmaceuticals, Inc., Costa Mesa, CA) using 20 units of T4 polynucleotide kinase (New England Biolabs, Beverly, MA) for 10 min at 37°C. Unincorporated radionucleotide was removed using a Sephadex G50 nickel column (Amersham Pharmaccia Biotech AB, Uppsala, Sweden). Labeled ODN (200 ng) was mixed with 0.5 μg of unlabeled ODN for each injection. Mice that had been treated with ODN every 2nd day beginning the day after tumor cell implantation, and who were bearing HT29 tumors approximately 800 mm^3 in volume, were injected intraperitoneally two times 4 days apart with the labeled ODN and then sacrificed 2 days later. Various tissues were fixed in neutral-buffered formalin and embedded in paraffin, and 5-μm sections were stained with hematoxylin and eosin. PhosphorImage screens were exposed to tissue sections for 3 days and radioactive decay images captured on a PhosphorImager (Molecular Dynamics, Sunnyvale, CA).

Thymidylate Synthase Quantitation. A [6-H]-5-FdUMP binding assay (Spears and Gustavsson, 1988) was used to quantitate TS in tumor samples, as described (Ferguson et al., 1999). Tumors were obtained from mice treated with ODN every second day for 28 days, beginning on day 1 after tumor cell implantation. Cell lysates from frozen tumors were prepared by homogenization in 100 mM potassium phosphate, pH 7.4, followed by one cycle of freezing, thawing, and sonication. Supernatants were obtained following centrifugation at 6500g for 30 min at 4°C. Protein concentrations were determined using a protein assay kit (Bio-Rad, Hercules, CA). Aliquots containing 100 μg of total protein were incubated with 75 μM methylene tetrahydrofolate, 100 mM 2-mercaptoethanol, and 15 nM [6-H]-FdUMP (specific activity, 18.6 Ci/mmol, Moravek Biochemicals, Brea, CA), in 50 mM potassium phosphate (pH 7.4) for 30 min at 37°C, and the reaction was stopped with 1 ml of albumin-coated, acidified charcoal for 10 min at room temperature. The slurry was centrifuged two times at 5000g for 30 min at 22°C to remove particulate matter, and 400-μl aliquots of the cleared supernatant were assayed by scintillation counting. [6-H]-5-FdUMP bound to TS, and therefore unavailable for precipitation with charcoal, was quantitated.

Apoptosis Assay. HeLa cells were treated with 4 μg/ml LipofectAMINE and 50 nM ODN for 6 h, trypsinized, and plated onto coverslips in 12-well plates at 1 × 10^4 cells/well in culture medium.
Because ODN 83 effectively suppresses, within 24 h, the normal increase in HeLa cell number resulting from proliferation (Ferguson et al., 1999), an earlier time-point was chosen to assess apoptosis. After 15 h, cells were air dried and fixed in 4% paraformaldehyde for 30 min, washed with phosphate-buffered saline, permeabilized with 0.1% Triton X-100 in 0.1% sodium citrate for 2 min at 4°C, and washed with phosphate-buffered saline. Apoptotic cells were detected using the In Situ Cell Death detection kit (Roche Diagnostics, Laval, QC, Canada) and Fast Red (Sigma, St. Louis, MO) as the chromogenic substrate. More than 500 cells were scored as either apoptotic (stained red) or nonapoptotic for each condition.

**Flow Cytometry.** Cells were collected at 24, 36, 48, and 72 h after ODN treatment, washed with phosphate-buffered saline, fixed in 75% ethanol for 15 min at room temperature, and washed again. Cells treated with 1 μM raltitrexed or 0.1 mM 5-FU for 2 to 4 h, then washed and cultured for 2 days in drug-free medium, were similarly collected. The cells were stained with propidium iodide [0.02 mg/ml in phosphate-buffered saline with 0.1% (v/v) Triton X-100 and 0.2 mg/ml deoxyribonuclease-free ribonuclease A] and analyzed on a Beckman Coulter XL-MCL flow cytometer. At least 10,000 single cells were analyzed for each condition. Analysis regions were set manually to determine the proportion of cells in G0/G1, S, and G2/M cell cycle phases. Alternatively, MultiCycle (version 3.0) software (Phoenix Flow Systems, San Diego, CA) was used to analyze the cell cycle distribution.

**Quantitation of TS mRNA by RT-PCR.** Total RNA was isolated from HT29 cells treated with ODNs using TRizol (Invitrogen Canada), and 2 μg of RNA were reverse transcribed using Moloney murine leukemia virus reverse transcriptase (Invitrogen Canada). Two percent of the cDNA produced was used as template for PCR with the primers GAP-for (5′-TATTGGGCGCCTGGTCACCA-3′) and GAP-rev (5′-CCACCTTCTTGTGATGTCATCA-3′) for GAPDH, or TS-for (5′-TTTTGGAGGAGTTGCTGTGG-3′) and TS-rev (5′-CCACCTTCTTGTGATGTCATCA-3′) for TS. PCR cycling parameters were 3 min at 94°C, followed by 23 cycles of 30 s at 94°C, 30 s at 58°C, 45 s at 72°C, and a 7-min 72°C extension. Products were resolved on 1.5% agarose gels and stained with ethidium bromide. Quantitation of images captured using the ImageMASTER VDS gel documentation system (Amersham Pharmacia Biotech) was done with ImageQuant version 5.1 (Molecular Dynamics).

**Statistical Analysis.** Statistical significance within experiments was determined using Student’s t test (p<0.05). All experiments were repeated at least twice.

**Results.**

**Apoptosis of HeLa Cells is Unaffected by Oligodeoxynucleotide Treatment.** In order to examine more closely the mechanism by which treatment with ODN 83 inhibits cell proliferation, the terminal deoxynucleotidyl transferase dUTP nick-end-labeling assay was used to determine whether apoptosis was induced in HeLa cells. Cisplatin treatment was included as a positive control and induced apoptosis in 85% of exposed cells after a 15 h treatment (Table 1). The low levels of apoptosis in HeLa cells were unchanged at 15 h after treatment with ODN 83, suggesting that the antiproliferative effects of the antisense ODN 83 might be due to a cytostatic, rather than an apoptotic, mechanism. Apoptosis levels were also not increased at 24 or 39 h after ODN 83 treatment (data not shown).

**TS Antisense Oligodeoxynucleotide Induces a G2/M Cell Cycle Block in HeLa Cells.** Flow cytometric analysis was used to examine the cell cycle distribution of HeLa cells treated for various times with ODNs. There were substantial increases in the fraction of cells in G2/M at 24, 36, and 48 h after treatment with the TS antisense ODN 83, compared with the control scrambled ODN 32 (25–46 versus 18–22%, respectively) (Fig. 1). Increases in the fraction of cells in S phase at 24 and 36 h (19 and 12% in ODN 32-treated cells compared with cells treated with the control scrambled ODN 32) were essentially reversed by 48 h. Accumulation of cells in G2/M was seen as early as 24 h, whereas the profile at 72 h closely resembled that of control cells. In contrast, treatment of HeLa cells with raltitrexed (1 μM for 2 h, followed by culture in drug-free medium for 48 h) resulted in cell cycle arrest exclusively in early S phase: 76% of raltitrexed-treated cells versus 22% of control cells were in S phase, whereas 23% of raltitrexed-treated cells were in G0/G1, versus 61% of control cells.

**Effects of TS Antisense ODN 83 on HT29 Cells In Vitro.** To determine whether the observed G2/M arrest was p53-dependent, the effects of ODN 83 on a p53 mutant cell line, the HT29 human colon carcinoma, were examined. RT-PCR analysis showed that treatment of HT29 cells with the TS antisense ODN 83 reduced the levels of cytoplasmic TS mRNA but not GAPDH mRNA (Fig. 2A). Quantitation of the TS PCR product normalized to the GAPDH product from two independent RT-PCR experiments revealed 37% (±1.53) and 43% (±4.78) reductions in TS mRNA at 24 and 48 h, respectively, in cells treated with the TS antisense ODN 83 compared with cells treated with the control scrambled ODN 32. Flow cytometric analysis showed that HT29 cells, similar to HeLa cells, were blocked in G2/M at 48 h after treatment with TS antisense ODN 83 (Fig. 2C). Quantitation of these data indicated that 43.0% of the ODN 83-treated cells were in the G2/M phase, compared with 62.3, 25.2, and 12.5%, respectively, for ODN 32-treated cells. Again similar to HeLa cells, raltitrexed and 5-FU both induced G2/M phase arrest in HT29 cells (Fig. 2, D and E).

**Effect of TS Antisense ODN 83 on HT29 Tumor Growth In Vivo.** Growth of human HT29 colon carcinoma cells in immunocompromised mice was examined to determine the antitumor efficacy of TS antisense as a single agent therapy. Compared with mice treated with the control scram-
bled ODN 32, treatment with the TS antisense ODN 83 significantly inhibited tumor growth over the course of 4 weeks (Fig. 3). Growth of tumors in mice treated with the control scrambled ODN 32 was not significantly different from those injected with saline (p = 0.217 to p = 0.982 for differences in tumor volume on days 7–27, Student’s t test, n = 5). Tumor growth delay was caused by the antisense ODN 83 at 7.5 or 11.25 mg/kg of body weight when the ODN was administered every 2 days commencing the day after tumor implantation. However, there was no effect at either concentration of ODN against tumors that had been allowed to grow untreated to a size of 400 mm³ prior to commencing treatment (Fig. 4). The ODNs were well tolerated, without significant delays in normal weight gain (p = 0.37 to p = 0.76 for differences in mean body weight of mice treated for 28 days with saline, antisense ODN 83, or control ODN 32, Student’s t test, n = 10).

Distribution of Oligodeoxynucleotides in Vivo. We hypothesized that ODN delivery or penetration into larger tumors might be a limiting factor in this therapy. To examine this question, end-labeled ODN was used as a tracer for injection into mice bearing HT29 tumors. The radiolabel was evenly distributed in normal spleen, kidney, and liver, but it was relatively concentrated around the periphery of the tumor (Fig. 5A). There was a higher degree of cellularity around the periphery of the tumors (Fig. 5C) compared with the interiors (Fig. 5D), which were necrotic, as evidenced by cell shrinkage, increased extracellular space, and decreased hematoxylin-staining nuclei. The morphology of tumors from mice treated with the control and the antisense ODNs was similar. There was no significant difference in the mitotic index between tumors in the two groups of animals.

Decreased TS Levels in HT29 Tumors in Nude Mice Treated with Antisense Oligodeoxynucleotide. To test whether in vivo administration of TS antisense ODN 83 down-regulates TS expression in HT29 tumors, a [6-³H]5-FdUMP binding assay was used to measure TS levels. Tumors were dissected from mice that had been treated with ODN every 2nd day for 28 days (the experiment shown in Fig. 3B) and the levels of TS were measured as described under Materials and Methods. Systemic administration of TS antisense ODN 83 caused a 43% decrease in TS protein levels within the tumors, compared with tumors from mice treated with the control scrambled ODN 32 (Table 2).

Discussion

We report that use of an antisense strategy to deplete human tumor cells of TS mRNA, rather than inhibiting the activity of existing TS enzyme with cytotoxic drugs, induced a distinctive response in cultured tumor cell lines. Antisense ODN treatment blocked cells at G₂/M without appreciable arrest at G₁/S and suppressed proliferation in the absence of a measurable increase in apoptosis. The reduced fraction of cells in G₂/M at an early time following antisense ODN treatment (24 h) and the progressive increase in the fraction in G₂/M up to 48 h indicate that blockage at G₁/S followed by synchronous progression through the cell cycle cannot be invoked as an explanation. Between 48 and 72 h after antisense ODN 83 treatment, the proportion of cells in each cell...
cycle phase returned to control levels. We previously reported that the proliferation rate of antisense ODN 83-treated cells returned to normal at that time (Ferguson et al., 1999). Thus, the antisense ODN-mediated decrease in TS mRNA and protein corresponds directly with G2/M arrest and reduced proliferation.

Several molecules are known to play critical roles in mediating arrest at the G2/M boundary, particularly p53 (Agarwal et al., 1995; Stewart et al., 1995) and its downstream effector p21waf1 (El Deiry et al., 1993). The two human cell lines studied here, HeLa (cervical carcinoma) and HT29 (colon carcinoma), express widely disparate p53 levels. p53 is exceptionally low in HeLa cells, possibly related to unusual instability of the protein due to papillomavirus E6 protein expression (Hamada et al., 1996). Like many other human tumor cell lines, HT29 cells express high levels of mutant p53 (Rodrigues et al., 1990). The observation of a G2/M block induced by antisense ODN treatment in cells with both high and low p53 expression suggests a p53-independent mechanism, reminiscent of the lack of dependence on p53 of thymineless death induced by direct inhibitors of TS protein (Munoz-Pinedo et al., 2001). The accumulation of cells in G2/M, as opposed to G1/S arrest induced by raltitrexed and 5-FU (Fig. 2) (Inaba and Mitsuhashi, 1994; Matsui et al., 1996), suggests that TS antisense treatment has consequences other than simply limiting the supply of thymidylate for DNA synthesis. Direct inhibitors of TS reduce enzyme activity by inactivating pre-existing TS protein, without directly influencing TS mRNA levels, and often result in increased TS mRNA translation. Antisense targeting, on the other hand, decreases both protein and mRNA levels. The association between decreased TS mRNA and protein levels and G2/M cell cycle arrest observed in response to antisense targeting suggests that TS mRNA, TS protein, or both, mediate functions additional to catalysis of thymidylate production.

The complexity of control of TS expression provides multiple points where antisense treatment could interfere with additional functions to influence cell cycle and apoptotic pathways in novel ways. For example, TS mRNA levels and enzyme activity increase 10- to 20-fold as cells progress through cell cycle (Navalgund et al., 1980), while TS gene transcription rate is up-regulated only two to four times, suggesting that multiple post-transcriptional mechanisms play a major role in TS regulation (Johnson, 1994). TS protein has been reported to interact with its own and other mRNAs (p53 and c-myc), inhibiting mRNA translation and leading to decreased specific protein levels (Van Triest et al., 2000; Schmitz et al., 2001). In addition, antisense nucleic acids targeted to the TS mRNA translation start site stimulate TS gene transcription (DeMoor et al., 1998), possibly by interfering with associations between that region of TS mRNA and cellular components that function to suppress TS
peared to be poorly vascularized. This might result in poor...ultimately be of greatest value in adjuvant therapy of resid...
gest that treatment with TS antisense ODN in vivo may...
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cycle arrest at G2/M following TS antisense ODN treatment.

to dissect the molecular signaling pathways that lead to cell...

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In contrast to apoptosis induced by direct inhibitors of TS...
Considering that imbalances in dTTP/dUTP levels and DNA
damage caused by inhibitors of TS enzyme activity can ...
result in induction of downstream events leading to apop...
this indicates that antisense targeting has novel conse...
due simply achieve the same results as TS protein targeting by a different route. The potential of treat...
with antisense reagents to induce G2/M arrest and inhibit cell proliferation without enhancing cell death, and the mechanism by which this occurs, is a previously undescribed but important area of future investigation.

In the in vivo experiments described here, mice bearing H...
HT29 tumors were treated every 2nd day with ODN, resulti...
depending difference in response to TS antisense treat...
the greatest proportion of cells whose appearance was consis...
the presence of high antisense ODN levels suggests that the antisense reagent has diminished capacity to inhibit cell proliferation in larger tumors, or that decreased TS levels might have less influence on cell proliferation in macroscopic versus microscopic tumors. TS levels in tumor extracts were reduced by more than 40% by antisense TS treatment, and the possible presence of TS derived from mouse tissue or HT29 cells that failed to internalize ODN suggests that the observed reduction in TS protein underestimates the true effectiveness of antisense treatment. Although detailed examination of ODN distribution and effectiveness at the cellular level is required to resolve this issue, these data indicate a potential tumor size-dependent difference in response to TS antisense treatment.

The present results show clearly that systemic treatment with TS antisense ODN 83 as a single agent significantly delays HT29 tumor growth in nude mice (Fig. 3), similar to the effect of raltitrexed alone (data not shown). We demonstrated previously that HeLa cells treated in vitro with TS antisense ODN 83 are sensitized to cytotoxicity of 5-FUdR or raltitrexed (Ferguson et al., 1999), and further studies are under way to test the hypothesis that sensitization occurs in vivo in microscopic and/or macroscopic tumors. Such a combination treatment may be necessary to overcome drug resistance mediated by TS upregulation in tumors in response to anti-TS chemotherapy (Gorlick and Bertino, 1999). We have observed in vitro that antisense ODN 83 effectively sensitized drug-selected, highly resistant HeLa cells to 5-FUdR cytotoxicity (P. J. Ferguson, in preparation), supporting this hypothesis. Antisense down-regulation of several specific mRNA targets sensitizes cells to chemotherapeutic drugs in vitro. For example, antisense to protein kinase Co enhances sensitivity to mitomycin C, vincristine, and 5-FU (Chakrabarty and Huang, 1996), and antisense to c-myb sensitizes cells to cisplatin (Del Bufalo et al., 1996). The use of antisense ODNs to down-regulate Bel-2 (Miyake et al., 2000) or tubulin (Kavalaris et al., 1999; Kyu-Ho Han et al., 2000) enhances paclitaxel sensitivity in drug-resistant tumor cells. This rational approach to combat tumor cell chemotherapy resistance, also demonstrated for TS in vitro (Ferguson et al., 1999; P. J. Ferguson, in preparation), is an important ongoing area of investigation.

In summary, an antisense ODN targeting TS was shown to...
be an effective single-agent antitumor therapy in vivo. Dis...
ODNs into solid tumors may be a limiting factor in efficacy against larger tumors. Further in vivo studies to examine the potency of combination therapies using this antisense ODN in combination with raltitrexed and 5-FU are in progress. In HeLa and HT29 cells in vitro, down-regulation of TS mRNA and protein by treatment with TS antisense ODN 83 resulted in inhibition of proliferation, in the absence of increased cell death, via an immediate and sustained G2/M cell cycle block. This was in contrast to the effect of TS-targeting chemotherapeutic drugs, which block cells in G1/S and lead to increased apoptosis. Further studies are required to determine the molecular signaling pathways that mediate
the G2/M arrest and to ascertain how this phenomenon might be further exploited in novel antitumor therapies.

Acknowledgments

We thank Dr. Nicholas Dean (ISIS Pharmaceuticals) for supplying ODN, Mike Keeney and Dr. Ian Chin-Yee for flow cytometry analysis, and Dr. Subrata Chakrabarti for analysis of tumor sections. We also thank Charlene Stirling and Olga Collins for excellent technical assistance.

References


Fig. 5. Distribution of radiolabeled ODNs in HT29 tumors and normal mouse tissue. Mice bearing HT29 tumors that had been treated as described for Fig. 3B with control scrambled ODN 32 (left panels) or TS antisense ODN 83 (right panels) were injected with a trace amount of end-labeled ODN. A, radioactive decay images from sections of mouse spleen (S), kidney (K), liver (L), and HT29 tumors (T). B, hematoxylin and eosin staining of adjacent sections. Photomicrographs of the periphery (C) and the interior (D) of the tumors show cellular morphology. Scale bars, 5 mm (A and B) and 40 μm (C and D).

TABLE 2

<table>
<thead>
<tr>
<th>Treatment</th>
<th>TS Levels</th>
<th>% Reduction</th>
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<tbody>
<tr>
<td>ODN 32</td>
<td>73.8 ± 8.6</td>
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<tr>
<td>ODN 83</td>
<td>42.2 ± 13.9*</td>
<td>42.8</td>
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* Significant difference from ODN 32-treated mice (p = 0.003, Student’s t test).

TS Antisense ODN Blocks at G2/M and Inhibits Tumor Growth 483

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