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Prevention of Fluorodeoxyuridine-Induced Cytotoxicity and DNA Damage in HT29 Colon Carcinoma Cells by Conditional Expression of Wild-Type p53 Phenotype

LESLIE A. PARSELS, RICHARD C. ZELLARS, TANIA L. LONEY, JOSHUA D. PARSELS, MICHAEL F. CLARKE, AUDREA K. MERCHANT, THEODORE S. LAWRENCE, and JONATHAN MAYBAUM

Departments of Pharmacology (L.A.P., T.L.L., J.D.P, A.K.M, J.M.), Radiation Oncology (R.C.Z., T.S.L.) and Internal Medicine (M.F.C.), University of Michigan Medical School, Ann Arbor, Michigan, 48109-0504.

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SUMMARY

We have examined the effects of conditionally expressing wild-type p53 activity in HT29 cells on DNA damage and cytotoxicity caused by exposure to fluorodeoxyuridine (FdUrd). Expression of wild-type p53 phenotype for 24 hr before FdUrd treatment provided HT29 cells with virtually complete protection from cytotoxicity caused by this drug. In addition, wild-type p53 expression also prevented FdUrd-induced DNA double-strand breaks and, unexpectedly, single-strand breaks in parental (mature) DNA. Temporary expression of wild-type p53 activity in the absence of drug treatment caused some loss of clonogenicity, although the magnitude of this cytotoxic effect was small compared with the level of cell kill obtained by treatment with cytotoxic drugs for similar periods of time, indicating that HT29 cells are not highly sensitive to induction of programmed cell death by wild-type p53. Because these observations conflict with previously suggested models for FdUrd-induced damage to parental DNA, we propose an alternative model to explain how incorporation of uracil into nascent DNA might result in single-strand breaks in the opposite (parental) strand and how these breaks might be converted to the double-strand breaks that produce cell death.

The fluoropyrimidines fluorouracil and FdUrd can cause multiple biochemical lesions in mammalian cells, including DNA synthesis inhibition, uracil and fluorouracil incorporation into DNA, and fluorouracil incorporation into RNA (1). In some cases, variation among cells in sensitivity to fluorouracil or FdUrd may arise from differences in the severity of these initial lesions. However, cells can also vary in their sensitivity to chemotherapeutic drugs because of differences in "distal events" in the cytotoxic process, i.e., how cells respond to the initial drug-induced lesions. Of particular importance has been the recognition that many (but not all) cells seem to be poised to initiate active cell death processes (i.e., PCD) upon exposure to various stresses, and that the ability of such stresses to trigger PCD in a given cell type may be a determinant of the sensitivity of that cell type to a wide range of cytotoxic treatments.

One factor that has been found to have great significance in determining the effectiveness of various stresses to induce PCD is p53 status. It has been shown that, in some cells, deletion of the wild-type p53 phenotype can suppress the PCD responses to a diverse panel of insults, and it has been proposed that the general pattern of chemoresistance of certain tumor types (such as colorectal carcinoma) may be related to widespread incidence of p53 mutations in these tumors (2). This proposal is consistent with our observations (3)[1] that in several colorectal tumor cell lines with mutant p53, treatment with a variety of agents that inhibit cell cycle progression failed to produce either apoptotic DNA ladders or loop-sized DNA fragments characteristic of PCD (4). Nevertheless, we also found that FdUrd, unlike most other drugs tested, induced nonrandom DNA double-strand breaks in these cell lines (despite their mutant p53 phenotype) and was significantly more cytotoxic than other treatments under conditions that caused similar degrees of growth inhibition (3). This result suggests that fluoropyrimidine cytotoxicity can occur, at least in part, through mechanisms that do not require wild-type p53 phenotype or a predisposition for PCD and might possibly explain the efficacy of fluoropyrimidines in tumor types in which p53 mutations are common. Further-

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[1] C. E. Canman and J. Maybaum, unpublished observations.

ABBREVIATIONS: FdUrd, fluorodeoxyuridine; PCD, programmed cell death; Neo, neomycin resistance marker; PBS, phosphate buffered saline; PFGE, pulsed field gel electrophoresis; ANOVA, analysis of variance; kb, kilobase pair(s).

more, recent studies have demonstrated a relationship between cytotoxicity and progression past a G1/S checkpoint during fluoropyrimidine treatment (5, 6). If it is true that progression into S-phase in the presence of single-strand breaks is a critical step in converting those single-strand breaks into lethal double strand lesions, then the absence of a wild-type p53 response (and the associated failure to halt progression into S-phase) may selectively predispose mutant p53 tumor cells to double-strand break formation, compared with host cells with wild-type p53. We have therefore designed experiments to test the hypothesis (that fluoropyrimidine treatment of human colorectal tumor cells that lack wild-type p53 phenotype leads to greater cytotoxicity than in comparable cells that possess intact wild-type p53 function) by comparing responses to FdUrd treatment in a parental cell line that has mutant p53 phenotype (HT29) to derivatives with a conditionally expressed wild-type p53 phenotype.

Materials and Methods

Cell culture and drug solutions. The derivation and initial characterization of HT29 clones expressing murine temperature-sensitive p53 (ts29-A and ts29-G) or Neo are described in detail elsewhere (7). Briefly, cells were maintained in RPMI 1640 medium supplemented with 10% calf serum, 2 nM L-glutamine, and 1 unit/ml of penicillin/μg/ml of streptomycin at 38° in a 5% carbon dioxide atmosphere. FdUrd (Sigma Chemical, St. Louis, MO) was dissolved in double-distilled water and stored at 4° for up to 2 months.

Flow cytometry. Cells were trypsinized, washed in PBS and fixed by dropwise addition of ice-cold 70% ethanol to a concentration of 10^6 cells/ml and stored at 4°. For flow analysis, 1 ml of cell sample was washed and resuspended in PBS containing 18 mg/ml propidium iodide and 40 mg/ml ribonuclease A. Samples were analyzed on an EPICS C flow cytometer (Coulter Electronics, Miami Lake, FL). Human leukocytes were used for the internal standard. Cell cycle phase distribution was estimated with CytoLogic software (Coulter), based on a multiple broadened rectangular S phase model.

Clonogenic assay. Cells were plated at a density of 1300 cells/cm^2 and allowed to recover for 2 days at 38°. Samples designated for expression of wild-type p53 phenotype were shifted to the permissive temperature (32°) 24 hr before and throughout drug treatment. Cells were treated with 100 nM FdUrd in medium supplemented with 10% dialyzed calf serum. For samples undergoing 48 hr drug treatment, medium was replenished after 24 hr. After drug treatment, cells were trypsinized, replated and grown at 38° for 12 days. Surviving colonies (>50 cells) were fixed with a 3:1 methanol/acetic acid solution and stained with trypan blue. Two sets of control samples were required for the clonogenic assay: a sample for the temperature shift from 38° to 32° and one for FdUrd treatment. Surviving fraction was calculated as the plating efficiency for drug-treated cells at either 38° or 32° normalized to the plating efficiency of non–drug-treated cells at the corresponding temperature. Similar controls were used for each of the following experiments.

PFGE. Cells were plated at 700 cells/cm^2 and treated as above, with the addition of a 24-hr labeling period with 0.1 μCi/ml [2-^{14}C]thymidine (0.15 mCi/ml; 56 mCi/mmol; Moravek Biochemicals, Brea, CA) and a 24-hr chase period before temperature shift and drug treatment. Cells were trypsinized, washed in PBS, and resuspended at 10^7 cells/ml in 0.7% low-melting-point agarose/PBS (Gibco/BRL, Grand Island, NY). Cell blocks were then incubated in 0.5 M EDTA, pH 8.0, 1.0% sarkosyl and 1 mg/ml proteinase K for 16–18 hr at 50° to digest proteins. Cell blocks containing 2–3 $\times$ 10^5 cells were loaded onto a 0.7% agarose gel and analyzed with PFGE using a CHEF DR-II apparatus (Bio-Rad, Hercules, CA). Samples were run in 14° recirculating 0.5 $\times$ Tris·borate/EDTA buffer (45 mM Tris·borate/1 mM EDTA, pH 8.0) at 1.9 V/cm with a reorientation angle of 120°. The switching interval was ramped linearly from 1200 to 2400 sec over 48 hr. Lanes were cut into 7-mm slices, melted in 0.1 N HCl, and analyzed by liquid scintillation counting. $F_{released}$ values represent the fraction of total radioactivity that migrated from the cell block into the gel. Control $F_{released}$ values ranged from 0.02 to 0.07 for all cell lines.

Alkaline elution. Neo and ts29-A cells were plated at 700 cells/cm^2, allowed to recover overnight, then labeled with 0.03 μCi/ml [2-^{14}C]thymidine for 36 hr at 38° and chased for 16 hr before temperature shift and drug treatment as described above. Alkaline elution was performed as described (8, 9). Briefly, cells were collected in ice-cold 15 mM EDTA/PBS and approximately 3 $\times$ 10^5 cells were loaded onto a Nucleopore polycarbonate filter (pore size 2 μM; Costar, Cambridge, MA), washed with ice cold PBS and lysed in a solution of 2% sodium dodecyl sulfate, 0.05 M glycine and 0.02 M Na_2-EDTA, pH 9.6. Cells were further digested with 0.5 mg/ml proteinase K in lysis solution for 30 min. DNA was eluted by perfusing the filter with tetrapropyl-ammonium hydroxide solution, pH 12.2 (Sigma), containing 0.02 M EDTA and 0.1% sodium dodecyl sulfate at a rate of 2 ml/hr for 10 hr. Eluted material was collected in fractions at 2-hr intervals. The radioactivity in each fraction, in subsequent wash fractions, and in the traces remaining on the filter was determined by liquid scintillation counting. Data are expressed as the percent of radioactivity in a given fraction relative to the total amount of radioactivity in the sample and are representative of at least three separate experiments.

Results

Alteration of cell cycle distribution in HT29 populations by temperature shift, p53 phenotype and FdUrd treatment. The cell cycle distribution of HT29 cells was indistinguishable from control transfected (Neo) cells or from cells transfected with the temperature-sensitive murine *p53* gene (ts29-A and ts29-G) when these lines were maintained at 38° (Fig. 1). No consistent changes in cell cycle distribution were observed when HT29 or Neo cells were shifted to 32° for 72 hr in the absence of drug treatment; however, incubation of ts29-A or ts29-G cells at 32° resulted in G1 and G2 arrest, and almost complete disappearance of S-phase cells, all of which are characteristic of the expression of wild-type p53 phenotype.

Exposure to 100 nM FdUrd at 38° for 48 hr resulted in accumulation of cells in the first half of S-phase in all four cell lines, which is consistent with our previous studies. Early S-phase arrest was also observed when HT29 or Neo cells were shifted to 32° for 24 hr before treatment and maintained at 32° during a 48-hr drug treatment. However, the extent of progression into S-phase was less than for the corresponding treatments at 38°. In contrast, when ts29-A or ts29-G cells were similarly shifted to 32° and exposed to FdUrd, no progression into S-phase was detected.

Inhibition of FdUrd-induced cytotoxicity and DNA damage by temperature shift. Before determining the effects of temperature shift on cytotoxicity caused by FdUrd treatment, we first evaluated the consequences of temperature shift in the absence of drug, using both acute and long term indicators of cytotoxicity. When grown continuously at 38°, the fraction of cells that detached spontaneously from exponentially growing monolayers during a 72-hr period ranged from 1.59% in HT29 cells to 4.69% in ts29-A cells (Table 1). When shifted to 32°, no change in morphology or detachment was detected for HT29 or Neo cell lines. Both ts29-A and ts29-G cells became rounded after being shifted to 32°, although after incubation at this temperature for 48 or

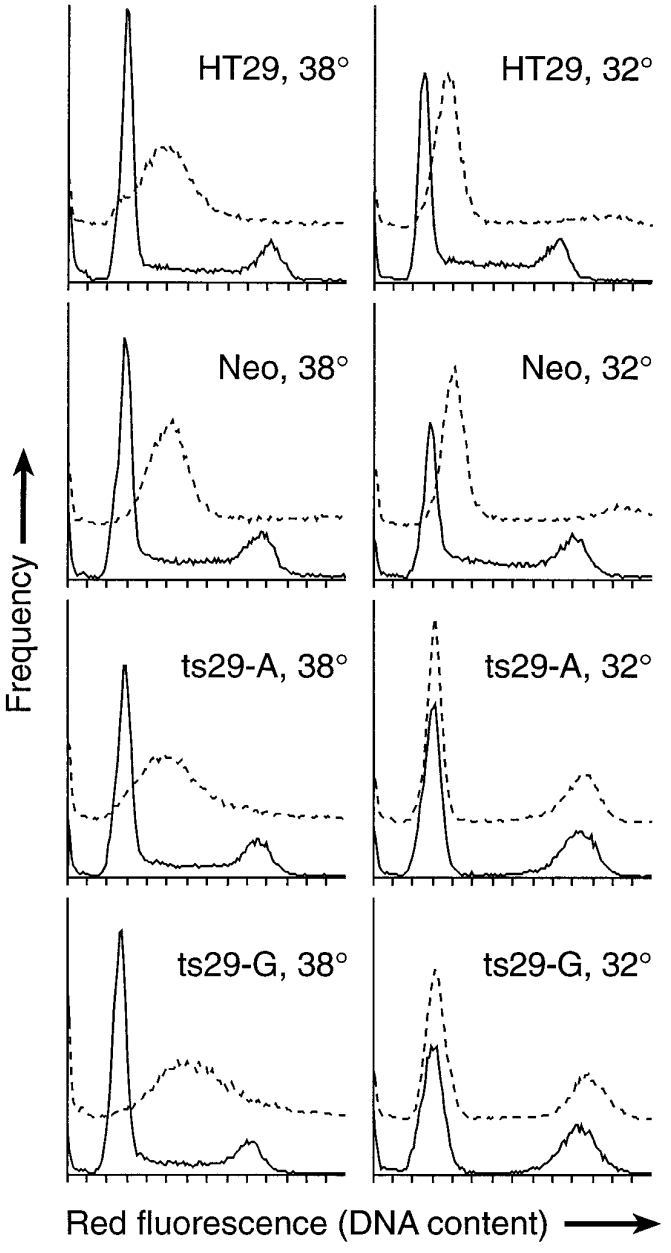

Fig. 1. Cell cycle analysis of control (——) and FdUrd (- - - - -) treated HT29, Neo, ts29-A and ts29-G cells. Samples maintained at 38° were treated with media alone or 100 nM FdUrd for 48 hr. Cellular DNA content was then measured with flow cytometry. Both control and FdUrd-treated 32° samples were shifted to the permissive temperature 72 hr before analysis and treated with either media alone or 100 nM FdUrd the last 48 hr of this 72-hr period. The flow diagrams shown are representative of two separate experiments.

72 hr, the fraction of detached cells did not exceed 6.52% (Table 1). Long term survival, as determined by a clonogenicity assay, was not compromised in HT29 or Neo cells by incubation at 32° for 72 hr, whereas in both the ts29-A and ts29-G lines, this treatment reduced plating efficiency to 54.2 ± 14.0% and 75.6 ± 12.7%, respectively, of the values seen at 38° (mean ± standard error of five experiments). We therefore conclude that, although expression of wild-type p53 phenotype in HT29 cells for 48–72 hr is toxic, the magnitude of this toxicity is much less than that obtained by exposure to FdUrd for similar time periods (see below).

TABLE 1

Detachment of HT29, Neo, ts29-A, and ts29-G cells maintained at 38° or 32° for 72 hr.

The percentage of detached cells in samples that grew at either 38° or 32° for 72 hr was determined by separately counting the number of cells in the sample media and the number of trypsinized cells that had been attached to the dish. Values shown are single determinations from two separate experiments.

Cell Line	Detached cells (38°)	Detached cells (32°)
	%	
HT29	2.31, 1.59	1.69, 0.78
Neo	2.92, 2.98	2.58, 1.43
ts29-A	3.47, 4.69	4.10, 6.52
ts29-G	2.13, 3.72	4.30, 3.90

Exposure of the four cell lines to 100 nM FdUrd at 38° (Fig. 2) resulted in reduction of clonogenicity similar to that seen previously in HT29 cells (3), about one-half logarithm unit for a 24-hr exposure and about two logarithm units for a 48 hr exposure. Although the surviving fraction of ts29-A cells was significantly lower than HT29 cells after 24 hr drug exposure at 38° ($p < 0.01$, one-way ANOVA), there was no significant difference between ts29-A and Neo or between ts29-G and either HT29 or Neo under these conditions. In addition, there was no significant difference among any of the four lines after a 48-hr drug treatment at 38°. In contrast to the results obtained at 38°, we found that there was significantly less cytotoxicity after a 48-hr treatment with FdUrd at 32° in both ts29-A ($p < 0.001$) and ts29-G cells ($p < 0.01$) than in HT29 or Neo cells (Fig. 2).

Analysis of DNA double-strand break formation using PFGE gave results consistent with the cytotoxicity data. FdUrd treatment for 24 hr did not cause significant DNA fragmentation under any of the conditions tested. In all four cell lines, 48-hr FdUrd treatment at 38° produced DNA fragments with a broad size distribution, ranging from about 50 kb to about 1–2 Megabase pairs (Fig. 3), as we reported earlier for parental HT29 cells (10). Upon inspection of the gel, fragmentation seemed to be reduced in all four cell lines treated at 32° compared with 38°. Although there was still an easily visible distribution of DNA fragments in HT29 and

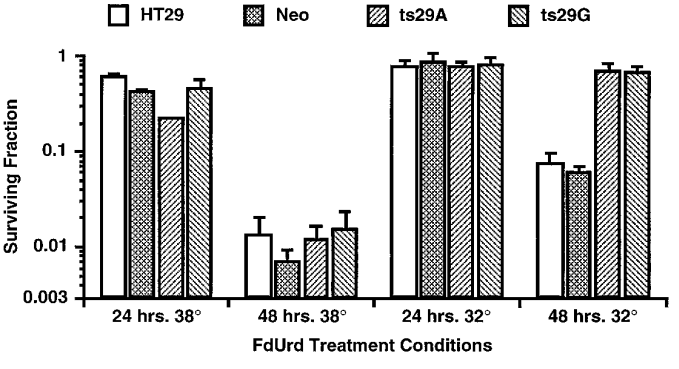

Fig. 2. FdUrd-induced loss of clonogenicity in HT29, Neo, ts29-A, and ts29-G cells. Samples were either maintained at 38° and treated with 100 nM FdUrd for 24 or 48 hr or shifted to the 32° permissive temperature 24 hr before and maintained at that temperature throughout drug treatment. Clonogenic survival was then measured as described in the text. There were no significant differences among samples after 24-hr treatment at 32° as determined by one-way ANOVA. However, after 48-hr drug treatment at 32°, both ts29-A and ts29-G clones were significantly less sensitive to FdUrd-induced loss of clonogenicity than either the HT29 or Neo cells ($p < 0.001$ and $p < 0.01$ for ts29-A and ts29-G cells, respectively). Data are expressed as the mean ± standard error of three or more experiments.

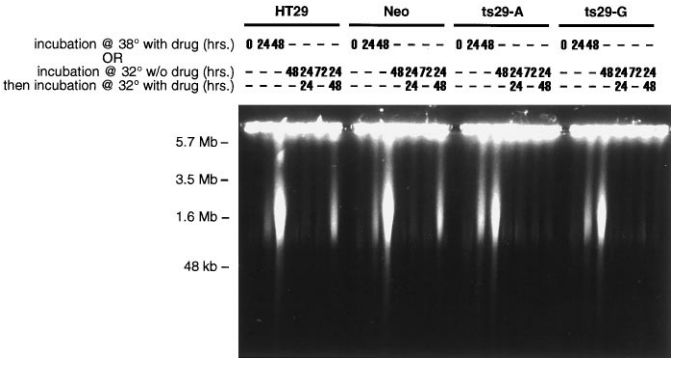

Fig. 3. FdUrd-induced DNA fragmentation in HT29, Neo, ts29-A, and ts29-G cells treated at either 38° or 32°. Cells were temperature-shifted and treated with FdUrd as indicated. The resultant DNA damage was then assessed by PFGE using a protocol that resolves a wide range of fragment sizes.

Neo cells treated for 48 hr at 32°, no fragments were seen in the ts29 clones treated under these conditions. These results were confirmed by measuring the fraction of DNA entering the gel in each sample, in replicate experiments (Fig. 4).

The ability of wild-type p53 to prevent FdUrd-induced double-strand breaks may result from either an inhibition of the accumulation of FdUrd-induced single-strand DNA breaks or an inhibition of the conversion of these single-strand breaks to double-strand breaks. To further characterize this protection from FdUrd-induced DNA damage, the effect of wild-type p53 expression on induction of single-strand breaks in parental DNA was assessed using alkaline elution analysis. Treating either Neo or ts29-A cells at 38° with 100 nM FdUrd for 24 hr gave rise to single-strand breaks in parental DNA at about the same level as that observed previously in normal HT29 cells (9). When Neo cells were preincubated and exposed to FdUrd at 32°, damage was still detected in parental DNA, although it was reduced from the amount seen at 38° (Fig. 5). In contrast, the elution curves for DNA from ts29-A cells similarly treated with FdUrd at 32° were coincident with (or even more slowly eluting than) the curves from cells without FdUrd treatment. Similar results were obtained with the ts29-G clone, which indicates that expression of wild-type p53 completely inhibited FdUrd-induced single-strand breaks in parental DNA.

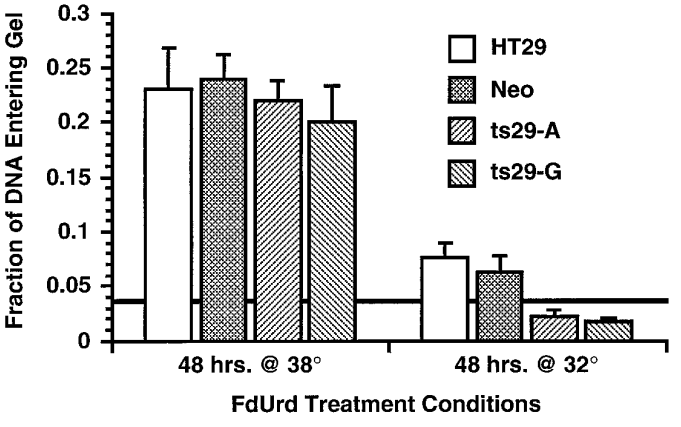

Fig. 4. Quantitation of FdUrd-induced DNA fragmentation in HT29, Neo, ts29-A, and ts29-G cells. Cells were temperature-shifted and treated with FdUrd as shown in Fig. 3. Data are expressed as the mean ± standard error (four or more experiments) of the fraction of DNA released into the gel ($F_{released}$). *Heavy line,* background level of fragmentation (0.039). Each cell line was significantly protected from FdUrd-induced DNA fragmentation when treated with drug for 48 hr at 32° instead of 38° ($p < 0.01$, one-way ANOVA). In addition, there was significantly less drug-induced DNA damage in ts29-G cells treated for 48 hr at 32° than in either HT29 or Neo cells treated at the same temperature ($p < 0.01$ and $p < 0.05$, respectively). When treated for 48 hr at 32°, ts29-A cells were also significantly less sensitive to FdUrd-induced double-strand breaks than HT29 cells ($p < 0.05$). Drug treatment for 24 hr did not cause a significant increase in fragmentation in any cell line at either temperature (not shown).

Discussion

Inhibition of FdUrd-Induced DNA Damage by wt p53

Mechanistic implications. Various lines of evidence demonstrate that cytotoxicity caused by FdUrd (and other agents that inhibit thymidylate synthase activity) is related to uracil misincorporation into DNA and to subsequent DNA damage (11–17), although the mechanism by which this damage occurs remains incompletely understood. It is generally agreed that incorporation of dUTP into nascent DNA during replication should give rise to breaks in the newly synthesized strand as a result of incomplete processing by uracil-DNA glycosylase and other members of the base excision repair system. However, it is unclear either how single-strand breaks arise in the *parental* strand or how double-strand breaks occur.

It has been proposed that parental strand breaks might be a result of interference with repair of spontaneous parental strand lesions because of the dUTP/deoxy-TTP imbalance caused by thymidylate synthase inhibition (14, 16). Under this scenario, double-strand breaks could be formed subsequently by attempted replication of unrepaired parental DNA. In this case, we would have predicted that inhibition of progression into S-phase by wild-type p53 should prevent double-strand break formation (by not allowing the cells to

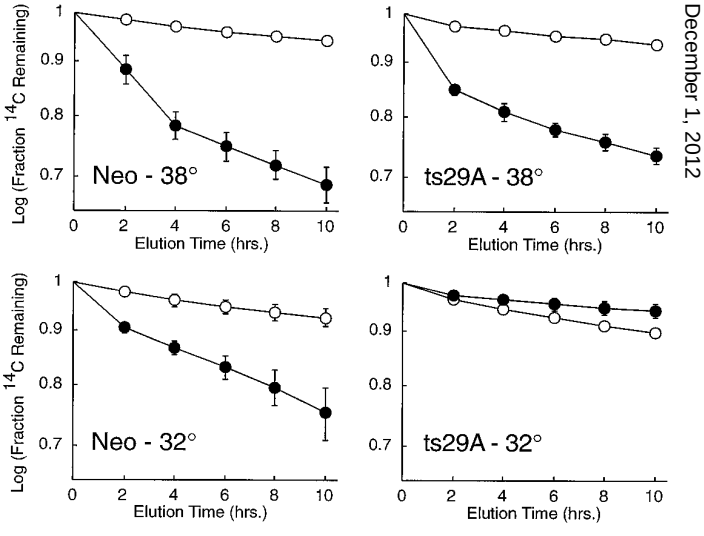

Fig. 5. Alkaline elution of DNA from control (○) and FdUrd-treated (●) Neo and ts29-A cells. For 38° samples, control Neo and ts29-A cells were treated with media alone (○) or 100 nM FdUrd (●) for 24 hr and the resulting parental DNA single-strand breaks measured with alkaline elution as described in the text. For 32° samples, Neo or ts29-A cells were shifted to the permissive temperature for 24 hr, then treated with 100 nM FdUrd for 24 hr at the same temperature. *Error bars,* range of duplicate samples from a single experiment. *Elution curves,* representative of at least three separate experiments.

attempt replication of a damaged template) but that single-strand breaks in parental DNA should be unaffected by wild-type p53 activity. Although we did find that wild-type p53 efficiently protected cells from double-strand break formation (Fig. 3 and 4), it also completely prevented single-strand breaks in parental DNA (Fig. 5). We interpret this result to contradict the model described above.

An alternative explanation for our observations may be suggested by the recent characterization of multiple mismatch repair activities in mammalian cells and by previous observations concerning the properties of uracil-containing DNA. Mammalian cells contain activities that nick DNA at sites of imperfect base pairing (18). Some of these activities recognize specific mismatches, whereas at least one activity (Topoisomerase I) nicks mismatched DNA nonspecifically (19). Although dUTP functions quite well in place of dTTP as a substrate for most DNA polymerases, the properties of DNA that contains uracil substituted for thymine are sufficiently different from normal DNA to significantly disrupt its biological function. For example, DNA homopolymers containing uracil substituted for thymine have considerably lower melting temperatures than normal homopolymers (20). In addition, the substitution of uracil for thymine can significantly disrupt sequence-specific DNA-protein binding interactions (21). We speculate that dA-dU base pairs that result from dUTP incorporation into nascent DNA might be recognized as abnormal and might therefore be subject to nicking by Topoisomerase I (or some other mismatch-sensitive enzyme) in either the A- or U-containing strand. In those cases where the A-containing (parental) strand is nicked first, subsequent excision of the opposing uracil by uracil-DNA-glycosylase followed by the action of apurine/apyramidinic endonuclease would result in double-strand break.

Although we have not yet evaluated this model experimentally, it makes some predictions that should be testable. First, inhibition of entry into S-phase by expression of an inhibitor of cyclin-dependent kinases (e.g., $p16^{INK}$ or $p21^{WAF1/CIP1}$) should have similar effects to those seen here with the expression of wild-type p53, i.e., protection of cells from FdUrd-induced parental strand breaks, double-strand breaks, and cytotoxicity. This prediction is consistent with a recent report in which conditional expression of $p16^{INK}$ was shown to protect cells from methotrexate cytotoxicity (although the effects of $p16^{INK}$ on DNA damage were not examined in that study) (22). The model also predicts that if cells having significantly reduced levels of uracil-DNA-glycosylase activity are challenged with FdUrd, there should be an increase in single-strand breaks in parental DNA (due to an increase in A-U base pairs), but a decrease in double-strand breaks (because of reduced uracil excision). We plan to test these predictions in the near future.

With regard to the relationship between cell cycle arrest, DNA damage and induction of p53 and p21, our results are complementary to a recent report that examined the responses of a wild-type p53 colon cancer cell line (HCT-8) after treatment with ZD-1694 (a folate-analog inhibitor of thymidylate synthase) (23). In contrast to our system, in which expression of wild-type p53 was induced before drug treatment, wild-type p53 levels in HCT-8 cells did not rise until after drug treatment, apparently as a consequence of drug-induced DNA damage. It was shown that under those conditions, despite the accumulation of significant levels of wild-type p53, cell cycle arrest was caused by the absence of thymidine nucleotides, rather than the expression of a p53 checkpoint function. Furthermore, similar patterns of cell cycle arrest and DNA damage were also noted in a mutant p53 line (SW480), indicating that by the time the cells had progressed far enough into S-phase for DNA damage to occur, they were past the point at which p53-induced arrest would be relevant. Taken together with the data presented here and with our previous report (6), a consistent picture emerges in which drug-induced cell cycle arrest and DNA damage occur at a point in early S-phase that is beyond the p53 checkpoint.

Therapeutic implications. We show here that expression of wild-type p53 in HT29 cells produces two opposing effects with regard to cytotoxicity. The first effect, which would be desirable in the context of a therapeutic strategy, is a modest loss of clonogenicity (about 50% after 72 hr at permissive temperature) in the absence of drug treatment. The second effect, which would be therapeutically undesirable, is the induction of resistance to FdUrd cytotoxicity (Fig. 2).

Because wild-type p53 is well known to induce PCD in many cell types, it is reasonable to consider that the first effect might reflect stimulation of a PCD response. The capacity for activation of a PCD response in unmodified (mutant p53) HT29 cells seems to be quite limited, however, based on previous studies in which they were challenged with various toxic treatments. For example, when HT29 cells were exposed to FdUrd, CB3717, or etoposide under conditions that reduce clonogenicity by 2 or more logarithm units, no oligonucleosomal ladders were observed (3, 24) and analysis of the high-molecular-weight fragments formed in FdUrd-treated HT29 cells revealed a pattern of fragmentation distinctly different from the 50–200 kb size distribution now recognized to be the precursors of oligonucleosomal ladders (3). In other cases, some features of PCD, such as oligonucleosomal ladders, have been observed in detached HT29 cells after treatment with teniposide (25), short chain fatty acids (26), and sulindac sulfide (27), although this fragmentation generally requires 48–72 hr to become evident and usually occurs in a small fraction of the total cell population. Therefore, although it is possible to produce a detectable PCD response in HT29 cells, the pathways responsible for this response are highly repressed in this line in its ordinary, mutant p53 state.

The basis for the relative resistance of HT29 cells to activation of PCD is not known. However, we show in a separate report (7) that these cells have easily detectable levels of the anti-PCD protein, Bcl-x_L, in their basal state, and that expression of wild-type p53 causes a significant up-regulation of Bcl-x_L protein level. It is possible that if Bcl-x_L activity were abolished or neutralized by expression of one of the pro-death members of the Bcl-2/Bcl-x family, then HT29 cells might exhibit a much stronger PCD response when treated with toxic agents or when expressing wild-type p53. However, without some intervention of this kind, it seems that the net cytotoxic effect of treatment of HT29 cells with FdUrd in the wild-type p53 state is considerably less than when they are treated with FdUrd in their base-line mutant p53 state and that, for this particular tumor cell type, a therapeutic strategy for expressing wild-type p53 in combination with fluoropyrimidines would be counterproductive.

As is always the case, these results must be viewed within

the context of constraints imposed by the experimental system used. Although the temperature-sensitive p53 mutant employed here allows us to use each clone as its own control, it does not allow us to exercise fine control over the magnitude of expression of the wild-type p53 phenotype. As a consequence, the levels of wild-type p53 activity present in ts29 cells at the permissive temperature are probably quite high compared with levels of endogenous wild-type p53 typically induced in response to DNA damage (although probably not in excess of acute levels of wild-type p53 that would result from treatment with an adenoviral expression vector for p53). Also, because phenotypic and genotypic heterogeneity is characteristic of tumor cells, it is impossible to know whether our observations in HT29 cells are idiosyncratic to that line or if they represent a common pattern of response to thymidylate synthase inhibition. Nevertheless, the findings presented here are consistent with a growing body of evidence indicating that wild-type p53 expression can antagonize fluoropyrimidine-induced DNA damage and cytotoxicity in tumor cells rather than potentiating these effects.

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Send reprint requests to: Dr. Jonathan Maybaum, Department of Pharmacology, 4701 Upjohn Center for Clinical Pharmacology, University of Michigan Medical School, Ann Arbor, MI 48109-0504. E-mail: maybaum@umich.edu