Antisense Bcl-xl Down-Regulation Switches the Response to Topoisomerase I Inhibition from Senescence to Apoptosis in Colorectal Cancer Cells, Enhancing Global Cytotoxicity

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ABSTRACT

Purpose: To identify determinants of the effect of antisense-mediated Bcl-xl down-regulation (Bcl-xl knockdown) on the response of colorectal cancer cells to SN38, the active metabolite of irinotecan, a topoisomerase I inhibitor licensed for colorectal cancer chemotherapy.

Experimental Design: Using wild-type HCT116, p53 null, Bax null, or p21/WAF1 null isogenic derivatives, we measured expression of regulators of cellular response, and associated growth arrest or apoptosis, after SN38 treatment, with or without antisense-mediated Bcl-xl knockdown.

Results: A modified phosphorothioate antisense oligonucleotide (ISIS15999) reduced Bcl-xl protein expression by ~90%. SN38 induced p53, Bax, Bcl-xl, and p53-dependent p21/WAF1 protein accumulation. The Bax:Bcl-xl ratio changed little. In wild-type HCT116, but not in Bax null cells, Bcl-xl knockdown induced a shift in response from drug-induced senescence to apoptosis, and enhanced the global cytotoxicity of SN38. In p53 null or p21/WAF1 null cells marked apoptosis occurred after SN38 alone, and was additionally enhanced by Bcl-xl knockdown in p21/WAF1 null cells but not in p53 null cells.

Conclusions: Drug-induced senescence is associated with late relapse after therapy in transgenic models of cancer in vivo. We have shown that abolition of p21/WAF1-mediated drug-induced senescence or antisense-mediated Bcl-xl knockdown can both, independently, enhance the apoptotic response of colorectal cancer cells to SN38 in vitro. The growth arrest suppresses a p53-independent apoptotic pathway, whereas Bcl-xl induction suppresses a p53 and Bax-dependent apoptotic pathway. The combination of irinotecan and Bcl-xl antisense merits testing in models of colorectal cancer in vivo.

INTRODUCTION

It has been conclusively shown recently that downstream determinants of apoptosis (specifically Bcl-2 in a murine transgenic lymphoma model) can profoundly influence the response of malignant cells to chemotherapy in vivo, and that prolonged drug-induced growth arrest can suppress apoptosis, leaving a residual pool of viable malignant cells from which late relapsing clones may ultimately emerge with lethal results (1). Other solid tumors display similar properties in vitro. For example, colorectal cancer cell lines undergo a prolonged senescent-like growth arrest when treated with topoisomerase I inhibitors in vitro (2). A therapy which, when combined with a topoisomerase I inhibitor, could switch the chemotherapeutic response from growth arrest to apoptosis, might improve the outcome of colorectal cancer treatment. In this paper we demonstrate that antisense down-regulation of Bcl-xl (Bcl-xl knockdown) can enhance the apoptotic response in vitro to the topoisomerase I inhibitor SN38 in colorectal cancer cells otherwise destined to undergo drug-induced senescence. We identify p53 and Bax as important genetic determinants of this effect of Bcl-xl knockdown. Both p53 and Bax are frequent targets of genetic disruption in particular subtypes of human colorectal cancer (3, 4) so that elucidation of their role in chemotherapeutic response will help to guide more effective therapy.

Topo I inhibitors predominantly produce S phase-dependent DNA damage by stabilizing the cleavable complex of topo I with DNA and, thus, facilitate DNA double-strand breakage by the collision of a precessing replication fork with the cleavable complex. SN38 is the active metabolite of irinotecan, which is licensed for the treatment of patients with colorectal cancer (5).

Bcl-2 family members are of importance in colonic development and pathophysiology (6). Human colonic adenomas overexpress Bcl-2 relative to surrounding normal mucosa (7, 8). Associated with the transition to invasive malignancy, Bcl-2 expression tends to fall, and elevated Bcl-xL expression appears (9–11). Clinical studies have associated this transition with worsening prognosis in colorectal cancer (12). In some tumors the switch to Bcl-xL expression may relate to deletion of the Bcl-2 locus, by virtue of its close linkage to both the DCC and...
in a variety of other tumor types. Determinants of this effect were not identified. Bcl-xl or Bcl-2, shown recently to enhance 5FU-induced apoptosis (34). Genetic might be expected to enhance the apoptotic response to DNA damage in models of colorectal cancer (2, 16). On the other hand, in the presence of wild-type p53 and intact checkpoints, cell death can be mediated via the mitochondrial apoptotic pathway (15, 20). Indeed, colorectal cancer cells rendered null for p53 by targeted gene knockout are resistant to 5FU-induced apoptosis (14). Similarly, Bax null cells are highly resistant to apoptosis induced by nonsteroidal anti-inflammatory drugs, and are partly resistant to 5FU-driven apoptosis (21). Additional proapoptotic proteins regulated by p53 include Noxa, Bak, and PUMA, as well as Bax (20, 22, 23).

Additional proapoptotic proteins regulated by p53 include Noxa, Bak, and PUMA, as well as Bax (20, 22, 23). Each of these, like Bax, localizes to mitochondria, and can heterodimerize with Bcl-xl through a BH3-containing domain (24–28). The relative ratio of pro- to antiapoptotic Bcl-2 family members is believed to determine the threshold for induction of mitochondrial-dependent apoptosis (27, 29, 30). Thus, overexpression of exogenous Bcl-xl suppresses mitochondrial-mediated apoptosis and enhances cancer cell survival in several cancer models (31–33). Therefore, down-regulation of Bcl-xl might be expected to enhance the apoptotic response to DNA damage in wild-type p53 containing cancer cells. Indeed, antisense targeting of Bcl-xl in colorectal cancer cells has been shown recently to enhance 5FU-induced apoptosis (34). Genetic determinants of this effect were not identified. Bcl-xl or Bcl-2 antisense oligonucleotides enhance the effect of chemotherapy in a variety of other tumor types in vitro and in vivo (35–38).

Bcl-2 antisense has entered clinical trials in melanoma and lymphoma (39, 40).

Thus, Bcl-xl is highly expressed in colorectal cancer, correlates with invasiveness, regulates apoptotic responses to chemotherapy in several cancer models, and may provide a therapeutic target. We used a chemically modified Bcl-xl antisense oligonucleotide to down-regulate Bcl-xl protein expression (37). Using an isogenic set of cell lines derived from the HCT116 colorectal cancer cell line, we identified genetic determinants of response to SN38 alone or in combination with Bcl-xl knockdown. The parental HCT116 line carries wild-type p53, p21/WAF1, and Bax, and is mismatch repair-deficient (by virtue of silencing of the hMLH1 gene). The derivative lines we used were generated by selective knockout of p53, p21/WAF1, or Bax, respectively (data not shown). Cell lines were maintained in RPMI 1640 with 10% FCS and 1% penicillin/streptomycin (all from Life Technologies, Inc.) in a 37°C, 5% CO₂, fully humidified incubator and passed once or twice weekly.

Oligonucleotides. Oligonucleotides with 2′O-methoxyethyl modification of the ribose ring in flanking nucleotides and a fully phosphorothioate backbone were provided by ISIS Pharmaceuticals. The antisense agent ISIS 15999 (TCCCGGTTCGCTCTGAGACAT) is complementary to the first 20 Bcl-xl coding nucleotides. An 8-bp mismatched oligonucleotide ISIS 16971 (TCACATGCGCGTTACCGGT) provided a missense control, of which the single agent cytotoxic effects were similar to those of the antisense agent over a wide dose range (data not shown). Oligonucleotides were transfected using lipofectin (Life Technologies, Inc.), and antisense-specific Bcl-xl knockdown sought for a variety of lipofectin:oligonucleotide ratios. For experiments combining oligonucleotide and SN38 treatment, the transfection protocol was optimized to minimize nonspecific cytotoxic oligonucleotide effects. Thus OPTIMEM I was preincubated for 45 min at room temperature with lipofectin, in the ratio of 3 μl/ml lipofectin per 400 nM final oligonucleotide concentration. Oligonucleotides were added and the mixture incubated for an additional 15 min (room temperature) before transfections were performed in triplicate on six-well trays containing 0.75–1.25 × 10⁶ cells/well, with washing with 1 ml PBS at pH 7.5, adding 1 ml of transfection mix and incubating for 6 h. It proved essential to closely control the number of cells present on the day of treatment, to obtain quantitatively reproducible results.

Cytotoxic Agents. Stock solutions of SN38 (gift of Aventis) were prepared in DMSO at 100 μg/ml and stored frozen. Treatment solutions were made by serial dilution in growth medium such that the final concentration of DMSO was always <0.02%, inactive in the assays described. Drug-containing medium was added to washed cells and incubated without replacement for a maximum of 72 h or until cells were harvested.

Growth Assays by Coulter Counting. Adherent cells were washed in PBS, harvested in trypsin/EDTA (Life Technologies, Inc.), and counted in a Z2 Coulter counter.

Immunoblots. For actin, p53, p21/WAF1, Bax, and Bcl-xl, immunoblot pellets of adherent cells were washed with cold PBS and stored at −20°C, resuspended in 100 μl lysis buffer [50 mM HEPES (pH 7.4), 1% Triton X-100, 0.5% sodium deoxycholate, 150 mM sodium chloride, 5 mM EDTA with protease inhibitors pepstatin A 2 μg/ml, aprotinin 10 μg/ml, leupeptin 10 μg/ml, and phenylmethylsulfonyl fluoride 100 μg/ml] per million cells and mixed (25 min at 4°C). Lysate supernatants were subjected to a Bradford assay for protein concentration. Twenty μl aliquots of total protein were denatured in loading buffer (95°C, 5 min), electrophoresed on a 10% SDS-PAGE gel, and transferred to a polyvinylidene fluoride Immobilon-P transfer membrane (Millipore). Protein loadings were checked by Ponceau staining. Membranes were blocked in

MATERIALS AND METHODS

Cell Lines and Tissue Culture. The parental HCT116-wt colorectal cancer cell line was obtained from the European Collection of Cell Cultures. The p53 null, p21/WAF1 null, or Bax null derivatives of HCT116 were a generous gift from Professor Bert Vogelstein (Johns Hopkins Oncology Center, Baltimore, MD). Immunoblots confirmed the expected lack of expression of p53, p21/WAF1, or Bax, respectively (data not shown). Cell lines were maintained in RPMI 1640 with 10% FCS and 1% penicillin/streptomycin (all from Life Technologies, Inc.) in a 37°C, 5% CO₂, fully humidified incubator and passed once or twice weekly.
Bcl-xl Antisense Enhances SN38 Cytotoxicity

5% fat-free milk powder (Marvel; Premier Brands) in TBS (pH 7.5) with 0.1% TBS-T for 1 h at room temperature, incubated with primary antibody in 5% Marvel in TBS-T (1 h at room temperature for actin, p53, Bcl-xl, and Bax, or overnight at 4°C for p21/WAF1), washed in TBS-T, and incubated with secondary antibody conjugated to horseradish peroxidase (1 h at room temperature). After additional washing in TBS-T and TBS, blots were visualized by chemiluminescence (Santa Cruz Biotechnology) and photographed. Actin immunoblots confirmed near equal protein loading per lane. Expression for each protein was quantified by measuring the appropriate IOD using Labworks software (Ultra-Violet Products). The IOD for each band was corrected for variations in protein loading (which were small) using the corresponding IOD for the actin band. For each independent experiment and for each protein band of interest, the corrected IOD derived for that band was expressed as a proportion of the total IOD obtained for that band summed for all of the samples. The mean and SEs for the proportion of protein expression in drug-treated compared with untreated cells were, thus, derived from at least three independent experiments, each in duplicate. These results were normalized to give a mean value of 1 for each protein in untreated cells.

PARP immunoblots were used to detect caspase-mediated PARP cleavage during apoptosis. Floating and adherent cells were pooled and counted, washed in PBS, then ultrasonicated on ice in a volume of reducing loading buffer proportional to the cell count [62.5 mM Tris (pH 6.8), 6 mM urea, 10% glycerol, 2% SDS, 0.003% bromphenol blue, and 5% 2-mercaptoethanol] and electrophoresed on a 7.5% gel. Ponceau staining and actin immunoblotting confirmed near equal protein loadings. PARP immunoblotting followed the protocol above (substituting PBS for TBS at each step) using the antibodies outlined below. The IOD of uncleaved and cleaved PARP bands was measured using Labworks software (Ultra-Violet Products) and extent of cleavage expressed as a ratio of cleaved to total PARP. The mean and SEs for this ratio were derived from at least three independent experiments for each treatment condition.

Antibodies used were: ascites antiactin IgM CP01 (at 1:120,000 dilution), anti-p53 IgG OP43 (1:200), and anti-p21/WAF1 IgG OP64 (1:100; all mouse monoclonals from Oncogene Research Products); anti-Bcl-xl IgG sc-634 (1:200) and anti-Bax IgG sc-493 (1:500; both rabbit polyclonals from Santa Cruz Biotechnology); and mouse monoclonal anti-PARP C2-10 (1:10,000; R&D Systems). Secondary antibodies were goat antiantibody conjugated to horseradish peroxidase (1 h at room temperature for actin, p53, Bcl-xl, and Bax, or overnight at 4°C for p21/WAF1), washed in TBS-T, and incubated for 30 min at 37°C. Fluorescence was measured on a Becton Dickinson FACSCaliber flow cytometer within 1 h. The collected events were gated on the forward and side scatter plots to exclude cellular debris. Three discrete cell populations identified using standard cutoffs in each experiment represented viable (unstained), early apoptotic (Annexin-V but no PI staining), and late apoptotic/necrotic cells (Annexin-V and PI staining). Thus, the mean and SEs for the proportion of early apoptotic cells were derived from at least three independent experiments, each in duplicate.

**Definition of Supra-Additive Enhancement of Response in Growth Inhibition Assays.** Single agent dose response curves were measured in at least three independent experiments, each in duplicate for antisense oligonucleotide (6-h exposure to 0, 400, 800, 1600, or 4800 nM) or for SN38 (0, 15, 30, or 60 nM for 72 h), with counts of adherent cell numbers at 72 h. The dose response curves were concave upwards. Therefore, in the nomenclature of Steel and Peckham, the mode I expected combination effect defines the appropriate limit of the envelope of additivity at every level of effect and is calculated by multiplication of the corresponding single agent effects (42).

Combination experiments were performed at fixed antisense exposure (0 or 400 nM for 6 h) followed by SN38 (0, 15, or 30 nM for 72 h) with counts at 72 h. Therefore, for each experiment the mode I expected combination effects could be calculated from the single agent effects and compared with the measured combination effects. The mean measured or expected combination effects, with SEs, were thus derived from four independent experiments, each in triplicate. The measured combination effect was classified as supra-additive if greater than the expected combination effect by a statistically significant margin. Controls lipofectin/SN38 or nonsense/SN38 combination experiments were included to exclude supra-additive interactions among these agents.

**Statistical Analysis.** The significance of differences between experimental conditions was determined using the two-tailed Student’s t test.

**RESULTS**

**SN38 Induced Effects in HCT116-derived Cell Lines.** A brief dose-dependent pulse of apoptosis occurred among HCT116-wt cells on day 2 after SN38 treatment, as demon-
SN38-induced apoptosis was Bax dependent but p53 and p21/WAF1 independent. Cells were treated with SN38 as indicated. Floating and adherent cells were harvested at indicated times and pooled. A, early apoptotic changes as determined by Annexin V apoptosis assay on day 2. B, representative histograms demonstrating a population with sub-G1 DNA content as indicated by flow cytometry on day 2. Each graph records on the Y axis (linear scale) the number of fluorescent events with the given fluorescence intensity (X axis, linear scale, arbitrary units). C, onset of PARP cleavage: cells were counted and ultrasonicated on ice in a volume of reducing loading buffer proportional to the count. Lysates were immunoblotted for actin or PARP. Blots were visualized using chemiluminescence and photographed. Results for actin and Ponceau staining confirmed equal protein loadings. The graph displays mean for three independent experiments for which the ratio of cleaved to total PARP integrated absorbance was quantified using Labworks Software. Representative photographs are shown; bars, ±SE.
treatment to 85% ± 9% (mean ± SE; n = 3; not significant) of untreated control levels. Bax expression was unaffected (Fig. 4). Antisense mediated Bcl-xl knockdown was equally effective in p53 null HCT116 (data not shown).

Table 1  Mean (SE) for ratio of cleaved/total PARP

Mean and SE with associated P from an unpaired Student’s t test, for comparison of the degree of PARP cleavage (on the indicated day) after treatment A (OPTIMEM I followed by 30 nm SN38) versus treatment B (400 nm antisense followed by 30 nm SN38). Between three and eight independent experiments were performed (as indicated) for each cell line.

<table>
<thead>
<tr>
<th>Cell line</th>
<th>A (Optimem + 30 nm SN38)</th>
<th>B (Antisense + 30 nm SN38)</th>
<th>t test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wild-type (n = 3)</td>
<td>0.11 (0.04)</td>
<td>0.41 (0.03)</td>
<td>0.004</td>
</tr>
<tr>
<td>P53 null (n = 3)</td>
<td>0.22 (0.04)</td>
<td>0.25 (0.04)</td>
<td>0.49</td>
</tr>
<tr>
<td>P21 null (n = 8)</td>
<td>0.08 (0.02)</td>
<td>0.48 (0.11)</td>
<td>0.002</td>
</tr>
<tr>
<td>Bax null (n = 3)</td>
<td>0.002 (0.001)</td>
<td>0.03 (0.008)</td>
<td>0.06</td>
</tr>
<tr>
<td>Day 3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wild-type (n = 5)</td>
<td>0.05 (0.01)</td>
<td>0.23 (0.04)</td>
<td>0.007</td>
</tr>
<tr>
<td>P53 null (n = 5)</td>
<td>0.20 (0.05)</td>
<td>0.28 (0.03)</td>
<td>0.17</td>
</tr>
<tr>
<td>P21 null (n = 4)</td>
<td>0.28 (0.15)</td>
<td>0.74 (0.10)</td>
<td>0.002</td>
</tr>
<tr>
<td>Bax null</td>
<td>Not done</td>
<td>Not done</td>
<td></td>
</tr>
</tbody>
</table>

Effect of Prior Bcl-xl Knockdown on SN38-induced Apoptosis and on Global SN38 Cytotoxicity. In HCT116-wt cells antisense Bcl-xl knockdown but not missense treatment enhanced the extent of SN38-induced apoptosis, as demon-
Induction of p53, and p53 dependence of Bax and Bcl-xl induction after SN38 treatment. Lysates from HCT116-wt or p53 null cells, treated with 0, 15, or 30 nM SN38, and harvested at indicated times, were immunoblotted for actin, p53, p21/WAF1, Bcl-xl, or Bax. Blots were visualized using chemiluminescence and photographed. Results for actin and Ponceau staining confirmed equal protein loadings. The graph shows mean for three or four independent experiments for which the IODs of the relevant bands were measured using Labworks Software, corrected for the corresponding actin IOD, and normalized to the untreated state; bars, ±SE.

**DISCUSSION**

Using HCT116-wt cells or p53 null, p21/WAF1 null, or Bax null HCT116 derivatives (21, 41), we have studied genetic determinants (p53, p21/WAF1, or Bax) of the effect of SN38, and of Bcl-xl knockdown, on an otherwise isogenic background, and, therefore, in a well-controlled fashion. We chose to use moderate (but clinically relevant) concentrations of SN38 (44, 45) to avoid a pitfall of in vitro work, namely the generation of dramatic effects by the use of drug concentrations so high as to be irrelevant to clinical practice (2). Nevertheless, we have been able to draw statistically sound conclusions by careful quantitation of our data. For precise quantitation of the extent of apoptosis we used the PARP cleavage assay (46) in preference to measurement of the sub-G1 fraction or to the Annexin V assay. The sub-G1 assay does not readily detect cells dying from the G2 phase of the cell cycle. The Annexin V assay detects early apoptotic changes specifically but cannot discriminate late apoptotic from necrotic cells. Nevertheless, both of these apoptosis assays provided useful confirmation of our more detailed PARP cleavage results. Indeed, the PARP cleavage assay has been shown to correlate well with other measures of apoptosis in vitro and in vivo (46).

In these and in all of the other assays, the lack of effect of missense controls argues that the observed antisense-specific effects are because of Bcl-xl down-regulation and are not because of nonspecific effects of oligonucleotides on SN38 sensitivity. However, our experiments were optimized to reduce such nonspecific effects. In addition, nonspecific effects have been described for the combination of irinotecan with oligonucleotides in vivo (47). In these circumstances our use of p53 null or Bax null isogenic cell lines provide important additional controls. Because Bax is a major mediator of p53-dependent apoptosis, and a proapoptotic heterodimeric partner for Bcl-xl, we reasoned (and ultimately observed) that the specific effect of Bcl-xl knockdown on SN38-induced apoptosis should be attenuated in p53 or Bax null cells, whereas nonspecific effects should be p53 or Bax independent (because they are not known or expected to be mediated solely through p53 or Bax-dependent pathways).

SN38 induced the accumulation of p53 and of the cell cycle inhibitor p21/WAF1 in a p53-dependent manner. Accumulation of the proapoptotic protein Bax was also induced to a greater degree in HCT116-wt than in p53 null HCT116. Bax expression is induced under the transcriptional control of p53 (48), and Bax can induce mitochondrial-mediated apoptosis (21, 49, 50). Despite Bax induction in our system, the dominant cellular response to SN38 in HCT116-wt was a prolonged growth arrest (at least 10 days), and drug-induced senescence developed.
The maintenance of viability during the growth arrest after SN38 treatment was dependent on wild-type p53 and on p21/WAF1, such that apoptosis supervened as the major cellular response in p53 or p21/WAF1 null HCT116 after day 2, despite the initial onset of a G2 growth arrest in these cells. Thus, maintenance of a stable p53- and p21/WAF1-dependent growth arrest contributes to the protection of HCT116-wt cells from SN38-induced cell death, as has been observed with chemotherapeutics of various cytotoxic mechanism in similar model systems (2, 14, 16–19, 41, 43, 51–53). Clearly, in p53 null HCT116 cells, the apoptosis observed from day 2 onwards must occur via a p53-independent apoptotic pathway.

In HCT116-wt cells Bcl-xl was induced in parallel with Bax after SN38 treatment, such that the Bax:Bcl-xl ratio changed little. The accumulation of both Bax and Bcl-xl after exogenous p53 expression has been reported in colorectal cancer cells (54). In our study, prior Bcl-xl knockdown markedly enhanced the apoptotic response of HCT116-wt cells to SN38, switching the ultimate cellular fate from drug-induced senescence to apoptosis in a significant proportion of cells, as demonstrated by Annexin V assays, reflecting an early and reversible step in the apoptotic pathway (55), and by PARP cleavage assays, reflecting caspase activation in late apoptosis (46). Even after SN38-induced senescence was well established in HCT116-wt cells, 7 days after the initial chemotherapeutic insult, Bcl-xl antisense treatment could still switch arrested cells to apoptosis. Thus, Bcl-xl expression suppresses apoptosis during the initial establishment of senescence and during its maintenance after topoisomerase I inhibition by SN38 in HCT116-wt cells.

In contrast, SN38-induced apoptosis after Bcl-xl knockdown was markedly attenuated in Bax null cells. In p53 null cells the marked SN38-induced apoptosis occurring from day 2 onwards was not enhanced significantly by Bcl-xl knockdown, consistent with activation of a mitochondrial independent, and, hence, Bcl-xl independent, apoptotic pathway in p53 null HCT116 (56). On the other hand, in p21/WAF1 null cells, SN38-induced apoptosis was markedly enhanced by Bcl-xl...
switch the chemotherapeutic response from apoptotic cell death
SN38. Independently and additively, enhance the apoptotic response to
growth arrest or antisense-mediated Bcl-xl knockdown can both,
the way. Thus, abolition of the p21/WAF1-mediated sustained
induction suppresses a p53- and Bax-dependent apoptotic path-
cells from a p53-independent apoptotic pathway, whereas Bcl-xl
dependent SN38-induced senescent-like growth arrest protects
cells. In HCT116-wt cells a sustained p53- and p21/WAF1-
determinants of response to SN38 in HCT116 colorectal cancer
in assays of total surviving adherent cell numbers.

In summary, our data allow construction of a model for the
determinants of response to SN38 in HCT116 colorectal cancer
cells. In HCT116-wt cells a sustained p53- and p21/WAF1-
dependent SN38-induced senescent-like growth arrest protects
cells from a p53-independent apoptotic pathway, whereas Bcl-xl
induction suppresses a p53- and Bax-dependent apoptotic path-
way. Thus, abolition of the p21/WAF1-mediated sustained
growth arrest or antisense-mediated Bcl-xl knockdown can both,
independently and additively, enhance the apoptotic response to
SN38.

The capacity of antiapoptotic Bcl-2 family members to
switch the chemotherapeutic response from apoptotic cell death
to drug-induced senescence has been demonstrated recently in vivo,
after cyclophosphamide treatment of Bcl-2 overexpressing lymphoma in a transgenic murine model, and drug-induced
senescence was associated with late relapse after therapy (1).
Our results demonstrate that this chemotherapy resistance mech-
anism is not restricted to lymphoma or to cyclophosphamide
treatment only, but is a more general phenomenon. In addition,
we have identified a potential therapeutic approach to circum-
venting such resistance. Disruption of Bcl-xl function, if
achieved in tumors in vivo, using antisense technology or using
small molecule inhibitors currently under development (57, 58),
might provide a means of enhancing the chemotherapeutic ef-
effect, especially in those cancer patients whose tumors retain
wild-type p53 and Bax function. Therefore, the combination of
irinotecan and Bcl-xL antisense merits testing in models of
colorectal cancer in vivo.

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Fig. 6 Antisense Bcl-xl knockdown resulted in supra-additive
enhancement of SN38 cytotoxicity. HCT116-wt cells were treated in
six-well trays with OPTIMEM 1 alone (con), lipofectin in OPTIMEM 1
(lipo), or lipofectin with missense or antisense oligonucleotides (400
nm) in OPTIMEM 1, followed by 0, 15, or 30 nM SN38. After 72 h
adherent cells were harvested and counted. The surviving proportion of
cells for each treatment, with each dose of SN38, was expressed relative
to the surviving number of cells treated with OPTIMEM 1 and the
responding dose of SN38. The graph displays mean for three inde-
pendent experiments each in triplicate; bars, ±SE.

Fig. 7 Bcl-xl antisense knockdown enhanced apoptosis in senescent
HCT116-wt cells 7 days after SN38 treatment. HCT116-wt cells were
treated in duplicate with 15 nM SN38 in six-well trays. After 72 h wells
were replenished with fresh drug-free growth medium. Four days later
cells were treated for 24 h with OPTIMEM 1 alone (C) or OPTIMEM
1 with lipofectin and missense (M), or antisense (A) oligonucleotides
(800 nM), followed by return to fresh drug-free growth medium. After an
additional 72 h, floating and adherent cells were harvested, counted,
and ultrasonicated on ice in a volume of reducing loading buffer propor-
tional to the cell count. Lysates were immunoblotted for actin or PARP
using specific antibodies. Blots were visualized using chemilumines-
cence and photographed. Results for actin as well as Ponceau staining
confirmed equal protein loadings (data not shown). A representative gel
is shown. The graph displays mean for three independent experiments
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