Suppression of Gastric Cancer Cell Growth by Targeting the β-catenin/T-cell Factor Pathway

Hadas Dvory-Sobol, PhD1,2
Eyal Sagiv, BSc1,2
Eliezer Liberman, MD1,2
Diana Kazanov, MSc1
Nadir Arber, MD, MSc, MHA1,2

1 Integrated Cancer Prevention Center, Tel Aviv Sourasky Medical Center, Tel-Aviv, Israel.
2 Sackler School of Medicine, Tel-Aviv University, Tel-Aviv, Israel.

BACKGROUND. Functional activation of β-catenin/T-cell factor (Tcf) signaling plays an important role in the early events of carcinogenesis. Recently, it was demonstrated that adenomatous polyposis coli or β-catenin genes are mutated frequently in gastric cancer cells. The objective of the current study was to use a gene-targeting approach to kill human gastric cancer cells selectively with activated β-catenin/Tcf signaling.

METHODS. A recombinant adenovirus that carries a lethal gene (p53 up-regulated modulator of apoptosis [PUMA]) under the control of a β-catenin/Tcf-responsive promoter (AdTOP-PUMA) was used selectively to target gastric cancer cells (AGS) that possess an active β-catenin/Tcf pathway. The combined effect of AdTOP-PUMA and several chemotherapeutic agents (5-flourouracil, doxorubicin, paclitaxel) also was evaluated. Cell viability was measured by methylene blue assay, protein expression was measured by Western blot analysis, and cell cycle and apoptosis were evaluated by fluorescent-activated cell sorter analysis.

RESULTS. The TOP-PUMA adenovirus inhibited AGS cell growth in a dose- and time-dependent fashion. Growth inhibition was associated with the up-regulation of PUMA expression and the induction of apoptosis. Chemotherapy synergistically enhanced the killing effect of AdTOP-PUMA.


KEYWORDS: T-cell factor, β-catenin, gastric cancer, adenovirus, gene therapy.

Gastric cancer is among the most common and deadly cancers worldwide, accounting for >870,000 new diagnoses and >650,000 deaths annually.1 Mortality from gastric cancer is second only to lung cancer. Despite advances in conventional cancer treatment strategies, the prognosis for patients with gastric cancer is poor, with reported overall 5-year survival rates that rarely exceed 20%. Thus, gastric cancer is a disease that requires the development of new therapeutic modalities.

β-Catenin is a multifunctional protein that exerts 2 important functions in epithelial cells. These diverse functions are realized through interaction with various binding partners. In nonstimulated cells, β-catenin is associated largely with E-cadherin and connects it, through α-catenin, to the actin cytoskeleton.2 It is well known that tyrosine phosphorylation of β-catenin regulates adherence junction complex formation and cell-cell adhesion.3,4 When it is not bound to E-cadherin, β-catenin enters the nucleus, acting as a transcriptional coactivator, through association with the T-cell factor...
(Tcf)/lymphoid-enhancer factor (Lef) transcription factor. Large numbers of genes that are relevant for tumor formation and progression have been identified as activated transcriptionally by the β-catenin/Tcf complex. Some of those genes are implicated in growth control and cell cycling (c-Myc, c-Jun, fra-1, cyclin D1, and gastrin), some are relevant for cell survival (Id2, MDR1), and some are implicated in tumor invasion and metastasis (matrilysin, vascular endothelial growth factor).5–11

In normal cells, most β-catenin protein is present at the cell-cell junctions, and very little is present in the cytoplasm or the nucleus because of its rapid degradation by the proteasome. In the cytoplasm, in the absence of the secreted factor, Wnt, β-catenin is rapidly phosphorylated and degraded by the Axin-adenomatous polyposis coli (APC)-glycogen synthase kinase (GSK) 3β complex. This phosphorylated β-catenin is recognized by β-TrCP, which is an F-box component of the E3 ubiquitin ligase complex that promotes ubiquitination of β-catenin and increases its susceptibility to degradation by ubiquitin-proteasome system.12 Disheveled (Dsh) activation by Wnt blocks the ability of GSK3β to phosphorylate β-catenin. This stabilization of β-catenin is detected in different types of cancer.13 Cytosolic β-catenin protein also can be stabilized by mutational inactivation of the APC gene, usually leading to a truncated protein product, or by β-catenin mutations at regulatory amino-terminal serine residues. When the failure of this degradation in cells occurs, β-catenin proteins are accumulated in cytoplasm, and some of them translocate into the nucleus, leading to activation of the Tcf/Lef transcription factor. Activated β-catenin/Tcf signaling by accumulation of β-catenin in the nucleus has been implicated in human carcinogenesis, including melanoma, hepatoma, and gastric carcinomas.13–15 These data strongly suggest that the dysregulation of β-catenin/Tcf signaling may be involved in the development of a broad range of human malignancies. Some studies on the effect of β-catenin/Tcf signaling in gastric cancer recently were performed. Nakatsuru et al.16 reported APC mutations in 12 of 46 gastric cancers. Clements et al.17 reported that β-catenin nuclear localization occurred in approximately 33% of gastric tumors and that β-catenin mutations occur in both diffuse- and intestinal-type gastric cancers. p53 Up-regulated modulator of apoptosis (PUMA) is a potent mediator of the p53 apoptotic response.18,19 It belongs to the group of BH3-only proteins, which have been shown to function by dimerization with other BH3 domain-containing proteins, including Bcl-2 and Bcl-XL, that results in cytochrome C release from the mitochondria and induction of apoptosis by the activation of caspasas 3 and 9.20

Although a growing number of cancer patients have benefited from modern therapeutic methods, there remains a great need to increase the efficacy and reduce the toxicity of cancer therapy. Human clinical trials have suggested that gene therapy is expected to join surgical, radiologic, and chemotherapeutic strategies in future methods.21–24 To make the treatment effective and safe, the transgene should be expressed only in tumor cells and not in normal gastric epithelial cells. In the current study, we demonstrated that induction of the PUMA gene under the control of promoter containing wild-type Tcf/Lef-binding sites resulted in preferential killing of gastric cancer cells with hyperactive β-catenin/Tcf activity. Combining this strategy with standard chemotherapy resulted in a synergistic growth inhibition of gastric cancer cells.

MATERIALS AND METHODS

Cell Culture

Human gastric cancer cells lines (AGS and N87), human colorectal cancer (CRC) cell lines (HCT116 and LS174T), human pancreatic cell lines (Colo357 and Panc-1), and embryonic kidney cell lines (293) were obtained from the American Type Culture Collection (Manassas, VA). The cells were grown in Dulbecco modified Eagle medium (Sigma, Rehovot, Israel) that contained from 5% to 10% fetal bovine serum (FBS) (Biological Industries, Beit Haemek, Israel), 1% penicillin, and 1% streptomycin at 37°C in an atmosphere of 95% oxygen and 5% CO2 (complete medium). KATO III human gastric cancer cells were grown in RPMI medium (Sigma) that contained 15% FBS, 1% penicillin, and 1% streptomycin.

Construction of Plasmids and Adenoviral Vectors

To construct the Tcf-responsive promoter (TOP) and the corresponding control plasmid that contains mutant Tcf-binding oligomers (FOP) (TOP/FOP)-cFos-Luciferase (Luc) plasmids, an Xbal fragment that contained the TOP-cFos (wild-type sequence of Tcf/Lef-binding site) and the FOP-cFos (corresponding control plasmid that contains mutant Tcf-binding oligomers) from TOPFLASH or FOPFLASH plasmids (generous gifts from Hans Clevers, Utrecht University, Utrecht, the Netherlands) was cloned into the NheI site upstream to the Luc gene in the pGL3-basic plasmid (Promega, Rehovot, Israel) (see the TOP and FOP sequences, Fig. 1A). The AdEasy system25 was used to generate the AdTOP-PUMA and AdFOP-PUMA (AdTOP/FOP-PUMA) adenoviruses.
The TOP and FOP sequences were obtained from the TOP-cFos-Luc and FOP-cFos-Luc plasmids and were cloned into the shuttle vector, pAd-Track. The pAdTrack also contains a green fluorescent protein (GFP) gene under the control of the cytomegalovirus (CMV) promoter. The blunted HA-PUMA fragment (containing the human PUMA combinational DNA [cDNA] fused to a double hemagglutinin [HA]-epitope tag) from pCEP4-PUMA (a generous gift from Bert Vogelstein, The Johns Hopkins Oncology Center, Baltimore, MD) was cloned downstream to the TOP/FOP elements in the pAd-Track vectors. The resultant plasmids were designated pAdTrack-TOP/FOP-PUMA. These shuttle vectors were linearized with PmeI and cotransformed with E1-deleted adenoviral backbone AdEasy-1 into the competent bacterial strain BJ5183, which enables efficient recombination. Panels of adenoviruses Ad-TOP-PUMA and Ad-FOP-PUMA recombinants were generated.

**Adenovirus Production and Titering**

To produce viruses, 4 μg of PacI-linearized adenoviral DNA was transfected into 50% to 70% confluent 293 cells in 10-cm dishes using LipofectAMINE and Plus Reagents (Invitrogen Life Technologies, Carlsbad, CA). Between 5 and 7 days post-transfection, colonies that expressed GFP were observed under a fluorescent microscope, the cells were harvested and lysed in phosphate-buffered solution (PBS) by 4 cycles of freeze/thaw/vortex. The supernatant was collected, and 50% of it was used to reinfect 50% to 70% confluent 293 cells. Viruses were collected 2 to 3 days postinfection when a cytopathic effect became evident. Further amplification and concentration of virus stocks were achieved through several rounds of infections. To titer the viruses, 50% to 70% confluent 293 cells in 96-well dishes were infected with serial dilutions of the virus stocks. GFP-positive colonies were counted 5 days postinfections.
The control Ad-CMV-GFP adenovirus containing the GFP gene under the control of a full-length CMV promoter (Fig. 1B) was a kind gift of Hila Giladi (Hadassah School of Medicine, Jerusalem, Israel), and it was amplified in 293 cells. Human cDNA of caspase-8, Bak, and Bax were a kind gift from Atan Gross (Weizmann Institute of Science, Rehovot, Israel). The expression construct of PUMA was generous gifts from Bert Vogelstein (The Johns Hopkins Oncology Center, Baltimore, MD). PKGIb, which encodes a mutant PKG sequence with an N-terminal truncation,26 was a gift from I. Bernard Weinstein (Columbia University, New York, NY). This deletion renders PKG independent of cGMP, and it is constitutively active.

Luc Assay
Transfections were performed using LipofectAMINE and Plus Reagents (Invitrogen) according to the manufacturer’s instructions. In total, 5 \times 10^5 cells were seeded in 6-well plates. The next day, 50% confluent dishes were cotransfected with 1 \mu g of vectors plus 0.1 \mu g of pRL-TK (Promega). Luc assay was performed 24 hours post-transfection. Briefly, cells were washed once with PBS and lysed in 400 \mu L of lysis buffer for 15 minutes at room temperature. The lysates were centrifuged at 14,000 revolutions per minute for 5 minutes, and 20 \mu L of each lysate were used to measure Luc reporter gene expression. Luc activity was normalized to Renilla Luc activity from a parallel cotransfection of pRL-TK (Dual Luc system; Promega). All experiments were performed in triplicate at least 3 times and yielded similar results.

Cell Viability Assay and Chemicals
Between 2 \times 10^4 and 5 \times 10^4 cells in 100 \mu L of complete media were plated in 96-well dishes. The next day, 6 wells were infected with each adenovirus at a different multiplicity of infection (MOI) (from 0.1 MOI to 50 MOI). Cell viability was assessed by methylene blue staining after 48 hours. The cells were washed once with PBS and fixed in 150 \mu L of 0.1 M HCl to dilute the cell-bound dye. Absorbance was measured at 590 nm. Cell viability was expressed as the percentage absorbance relative to mock-infected cells. The average of at least 2 independent experiments with 6 replicates was recorded.

Chemicals
Paclitaxel, doxorubicin, and 5-florouracil (5-FU) were obtained from Sigma. Cells were infected with AdTOP-PUMA, AdFOP-PUMA, or Ad-CMV-GFP (5 MOI). After 5 hours, they were treated with 0.05 \mu M paclitaxel, 1 \mu M doxorubicin, or 0.05 \mu M 5-FU, and the cells were cultured for 48 hours.

Western Blot Analysis
Infected cells were harvested and protein concentrations determined using the Bio-Rad protein assay kit (Bio-Rad, Hercules, CA). An equal amount of protein from each lysate was analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and the proteins were transferred to Hybond-C extra nitrocellulose membranes (Amersham Life Science). Membranes were blocked with buffer containing 5% low-fat milk and 0.05% Tween-20 in PBS for 1 hour, incubated with primary antibodies for 1 hour with peroxidase-conjugated secondary antibodies, and developed with a Supersignal West Pico chemiluminescent substrate (Pierce, Rockford, IL). Antibodies against HA and actin were purchased from Santa Cruz Biotechnology (Santa Cruz, CA).

Apoptosis Analysis
Flow cytometry
Cells were plated at 5 \times 10^6/10 cm per dish 24 hours before infection and were infected with recombinant adenoviral vectors at 5 and 10 MOI. Twenty-four hours later, both adherent and floating cells were harvested, washed with PBS, fixed in 80% ethanol for 1 hour, and stained with propidium iodide for analysis of DNA content. The number of subdiploid cells, representing apoptotic cells, was quantified by a fluorescent-activated cell sorter (FACScan) using CellQuest software (Becton-Dickinson Immunocytometry Systems, San Jose, CA). Necrotic cells were excluded by staining with Trypan blue. The average of at least 3 independent experiments with 2 replicates was recorded.

Single-stranded DNA
For the single-stranded DNA (ssDNA) assay, 10^4 cells were seeded in 96-well microplates and, after 24 hours, infected with recombinant adenoviral vectors at 5 MOI. The next day, the ssDNA apoptosis enzyme-linked immunosorbent assay kit was used (Chemicon International Inc., Temecula, CA). Based on the selective, formamide-induced denaturation of DNA, this method identifies apoptotic cells27 by staining ssDNA using a mixture of anti-ssDNA monoclonal antibody and peroxidase-conjugated antimouse immunoglobulin M. The average of at least 2 independent experiments with 2 replicates was recorded.
Statistical Analysis

Statistical analysis was performed using InStat software (version 3.01; GraphPad Software Inc., San Diego, CA). In the tissue-culture experiments, comparisons between 2 samples were performed using the Student t test, and comparisons between >2 samples were performed using a 1-way ordinary parametric analysis of variance followed by a Tukey-Kramer multiple-comparison test. For all statistical tests, preliminary evaluation of the homoscedacity and normality of the compared samples was performed using Bartlett and Kolmogorov-Smirnov tests, respectively.

RESULTS

Up-Regulation of the β-Catenin/Tcf Signaling Pathway in Gastric Cancer

β-Catenin/Tcf-dependent activity in different cell lines is shown in Figure 1C. β-Catenin/Tcf-dependent activity was determined in human CRC cell lines (LS174T and HCT116), in a gastric cancer cell line (AGS), and in pancreatic cancer cell lines (Colo357 and Panc-1). LS174T cells harbored mutant APC protein, HCT116, and AGS, have mutant β-catenin protein, and Colo357 and Panc-1 cells have intact Wnt signaling.28–32

PUMA Induces Cell Death

To identify the most potent proapoptotic gene, we tested several full-length cDNAs, including Bak, caspase-8, PUMA, PKGβ, Bax, and Bid. The proapoptotic effect of the different genes was evaluated in 293 cells, because the transfection efficacy in transformed CRC and gastric cell lines is very low (≈7%). All the genes mentioned above were expressed under a strong human CMV promoter to allow the most efficient expression. Forty-eight hours after transfection, the number of subdiploid DNA-containing cells, representing apoptotic cells, was quantified by FACSscan (Table 1). PUMA induced the highest apoptotic activity.

AdTOP-PUMA Selectively Induces Cell Death in Gastric Cancer Cells

Next, we employed an adenoviral vector selected for gene delivery with PUMA placed downstream to the cFos minimal promoter. The promoter contained either the wild-type (AdTOP-PUMA) or the mutant (AdFOP-PUMA) Tcf/Lef binding sites. The Ad-CMV-GFP vector was used as a control for viral toxicity. The ability of AdTOP-PUMA and AdFOP-PUMA adenoviral vectors to kill AGS cells was evaluated by cell-viability assays 48 hours after infection with adenoviruses at varying doses (Figs. 1D,E, 2). AGS cells that displayed elevated β-catenin transactivation (Fig. 1C) were killed efficiently by infection with AdTOP-PUMA in a dose-dependent manner. The number of viable cells after infection with AdTOP-PUMA was proportional to the methylene blue color intensity, as shown for AGS cells infected with adenoviral constructs (Fig. 1D,E). N87 cells, which were derived from liver metastasis that originated from gastric cancer, and KATO III cells, which are gastric cancer cells that do not harbor an active β-catenin pathway and do not display the protein in the nucleus,32 also were exposed to the 3 viral constructs. Their growth was not inhibited by any of the viral constructs (Fig. 1D). The ability of AdTOP-PUMA (but not AdFOP-PUMA) adenoviral vectors to kill cells selectively with active Wnt signaling was confirmed in CRC and hepatic cancer cells.33

Activated β-Catenin/Tcf Signaling Specifically Induces Apoptosis by Up-Regulating PUMA Expression

To further confirm that AdTOP-PUMA induced apoptosis, the cells were stained with propidium iodide and were analyzed for the fraction of hypodiploid cells by flow cytometry. Apoptosis could be detected in AGS cells as early as 24 hours postinfection. The AGS cell line showed only a low background level of cell death after treatment with AdFOP-PUMA or Ad-CMV-GFP (Fig. 3A). The effect of the AdTOP-PUMA was dose-dependent, as shown in Figure 3, 24 hours and 48 hours postinfection (Fig. 3A,B, respectively). KATO III cells, which do not harbor an active β-catenin pathway, did not show any changes in their cell cycle distribution 48 hours after infection (Fig. 3C).

Apoptosis induced by AdTOP-PUMA also was detected in AGS cells 48 hours after infection by the ssDNA assay (Fig. 4A). The number of apoptotic cells was proportional to the color intensity of cell, as

TABLE 1

<table>
<thead>
<tr>
<th>Proapoptotic gene</th>
<th>% of Apoptotic cells ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bak</td>
<td>5.95 ± 1.8</td>
</tr>
<tr>
<td>Casp-8</td>
<td>17.0 ± 1.9</td>
</tr>
<tr>
<td>Bid</td>
<td>10.2 ± 1.9</td>
</tr>
<tr>
<td>PUMA</td>
<td>23.5 ± 3.6</td>
</tr>
<tr>
<td>PKGβ</td>
<td>18.0 ± 0.7</td>
</tr>
<tr>
<td>Bax</td>
<td>22.3 ± 4.5</td>
</tr>
<tr>
<td>Control</td>
<td>4.6 ± 1.0</td>
</tr>
</tbody>
</table>

SD indicates standard deviation; PUMA, p53 up-regulated modulator of apoptosis.

* Two hundred ninety-three cells were transfected transiently with human combinational DNA of caspase-8, Bid, Bak, Bax, PUMA, PKGβ and pCDNA. Forty-eight hours after transfection, the percent of apoptotic (sub-G1) cells was determined by fluorescent-activated cell sorter analysis.

1 Significantly different from control (P < .05).

2 Significantly different from control (P < .01).
FIGURE 2. Cell killing by the AdTOP-PUMA adenovirus. AGS human gastric cancer cells 48 hours after infection with either AdFOP-PUMA or AdTOP-PUMA. AdTOP-PUMA and AdFOP-PUMA also contain a GFP gene under the control of a CMV promoter. GFP expression was visualized by fluorescence microscopy. Cells were treated with phosphate-buffered saline (control).

FIGURE 3. AdTOP-PUMA suppresses the survival of gastric cancer cells. AGS cells were infected with AdTOP-PUMA, AdFOP-PUMA, or Ad-CMV-GFP adenovirus constructs at 5 MOI and 10 MOI. A percent apoptotic (sub-G1) cell was determined by fluorescent-activated cell sorter analysis 24 hours (A) and 48 hours (B) after treatment. Subdiploid phase significantly different from the control groups (no treatment, AdFOP-PUMA, and Ad-CMV-GFP) (single asterisk, \( P < .05; \) double asterisks, \( P < .01; \) triple asterisks, \( P < .001 \)). (C) KATO III gastric cancer cells were infected with the same viral constructs under the same terms described above, and the cell cycle distribution was assessed after 48 hours. In these cells, no significant interference in the normal cell cycle was caused by any of the viruses.
shown for AGS cells infected with adenoviral constructs (Fig. 4B).

To demonstrate that the observed cell death was caused by the controlled expression of PUMA, we analyzed PUMA expression by Western blot. High levels of PUMA expression were detected in AdTOP-PUMA infected cells, but not in AdFOP-PUMA or Ad-CMV-GFP infected cells (Fig. 4C). No increase in PUMA levels was seen in N87 gastric cancer cells with any of the viral constructs (Fig. 4C), which confirms that the AdTOP-PUMA is a β-catenin/Tcf inducible expression vector. Taken together, these data suggest that AdTOP-PUMA is capable of inducing selective death in gastric cancer cells that have elevated β-catenin/Tcf transcriptional activity.

**AdTOP-PUMA and Chemotherapy Synergistically Induced Cell Death**

We investigated whether the combination of AdTOP-PUMA adenovirus and chemotherapeutic agents induced apoptosis more efficiently in AGS cells than each treatment alone. AGS cells were infected with either AdTOP-PUMA, AdFOP-PUMA, or Ad-CMV-GFP adenoviruses (at 5 MOI) and were cultured for 48 hours in the presence or absence of paclitaxel (0.05 μM), doxorubicin (1 μM), or 5-FU (0.05 μM) (Fig. 5). The AdTOP-PUMA adenovirus dramatically enhanced the killing effects by 3 chemotherapeutic agents in AGS cells. The efficacy of the chemotherapeutic agents was not augmented by exposure of the different cells to either Ad-CMV-GFP or AdFOP-PUMA adenovirus constructs (Fig. 5).

**DISCUSSION**

The current study demonstrates that targeting β-catenin/Tcf responsive transcription can kill gastric cancer cell lines selectively and efficiently. Cell death was achieved by inducing the expression of the
potent proapoptotic gene PUMA. This gene-therapy approach, combined with standard chemotherapy, may pave the way for a novel therapy modality for patients with this devastating cancer.

One of the major hurdles in gene therapy is difficulty in delivering the appropriate gene to the target cells. Adenoviruses may be the right solution. Adenoviruses are easy to produce: They have a very high, effective nuclear-entry mechanism with a very low pathogenicity in humans.34 The virus does not integrate into the host cell genome; thus, it has the capability to incorporate relatively larger segments of DNA into the adenovirus vector.

Similar strategies in the setting of colon cancer recently were reported.35,36 In the work by Chen and McCormick,35 an adenoviral vector AdWt-Fd, which contained the thymidine kinase (TK) promoter carrying the proapoptotic gene Fadd, selectively killed CRC cells in vitro. Kwong et al.36 used an in-vitro/in-vivo animal model similar to the model used in the current study. Those authors demonstrated selective killing of DLD-1 CRC cells in an ex-vivo animal model by using the adenoviral vector AdTOP-CMV-TK, which contains a herpes simplex virus TK gene under the control of a β-catenin/Tcf-responsive promoter that is linked to a minimal CMV promoter. Lipinski et al.37 optimized the activity and specificity profile of a synthetic β-catenin-dependent promoter by varying its basal promoter, the number of Tcf binding sites, and the distance between them and the basal promoter. The optimal promoter showed virtually undetectable expression in cells with normal β-catenin regulation but displayed high levels in cells that expressed deregulated β-catenin. Malerba et al.38 inserted Tcf binding sites into the viral P4 promoter and showed that reduction of the number of Tcf sites from 4 to 2 leads to an increase in the efficiency of replication and toxicity of the virus in Co115 colon cancer cells.

In the current study, we took this strategy further in several aspects: Several genes that have known associations with the induction of apoptosis (Bax, Bak, Bid, caspase 8) were evaluated for their apoptotic effect. Among them, PUMA was identified as the most effective. Only 2 more recent reports have demonstrated the therapeutic efficacy of PUMA in the setting of malignant glioma39 and esophageal carcinoma cells.40 Adenovirus-mediated p53 gene transfer has been studied extensively to evaluate its clinical application in gene therapy for various cancers. However, some cancer cells are resistant to p53 treatment.41,42 PUMA, which is a critical mediator of p53-dependent apoptosis after DNA damage,18,19 may serve as a good alternative to p53-based gene therapy, because it may kill cancer cells directly.

In the current study, we demonstrated that the combination of a gene therapy approach with chemotherapeutic agents that have distinct mechanisms of action may be an effective strategy for achieving better antitumor response. We demonstrated that the combination of AdTOP-PUMA adenovirus and paclitaxel, doxorubicin, or 5-FU dramatically enhanced the cell-killing effects. This combination strategy may be very useful in the treatment of chemotherapy-resistant cells and may minimize the toxicity of this regimen. In a clinical setting, combination therapy using adenoviruses and chemotherapeutic agents is most suitable in the setting of metastatic disease. However, it may be used as adjunct therapy in patients with stage II and III disease with the objec-
tive of preventing residual tumor growth by destroying microscopic cancer foci.

Recently, we showed a proof of concept in a similar model. Using a Ras-responsive element, selective killing of ras transformed (but not normal) enterocytes was achieved in an in vitro model of CRC.23

In conclusion, the usefulness of a recombinant adenovirus that expresses the lethal gene PUMA and selectively kills gastric cancer cells was demonstrated in the current study. This strategy is not restricted to a cell or organ but can be used in practically all transformed cells with an active β-catenin/Tcf pathway.44,45

REFERENCES


