Vector Targeting Makes 5-Fluorouracil Chemotherapy Less Toxic and More Effective in Animal Models of Epithelial Neoplasms

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ABSTRACT

**Purpose:** 5-Fluorouracil (5-FU) has been combined in the past with other drugs for the combination chemotherapy for cancers of the breast, ovary, and colon. These drug regimens were limited by the fact that 5-FU fails to kill nondividing cancer cells at the doses that are safe to deliver. The goal of the present study is to test the feasibility of replacing 5-FU in established 5-FU combination chemotherapy with the Ad-LpCDIRESE1A/5-fluorocytosine (5-FC) system for the purpose of reducing toxicity and increasing efficacy.

**Experimental Design:** We have replaced 5-FU in the weekly combination of CPT-11, folic acid (FA) and 5-FU chemotherapy by 5-FC and an adenoviral vector that carries the L-plastin (Lp) tumor-specific promoter-driven transcription unit encoding the cytosine deaminase gene linked to the E1A gene by an internal ribosomal entry site element. This combination is called “genetic combination therapy.” The goal of using the vector was to decrease the toxicity to normal tissue and to increase the efficacy of therapy in the cancer cells by increasing the concentration of 5-FU sufficiently high that even nondividing cancer cells would be killed by 5-FU through its incorporation into mRNA and consequent inhibition of synthesis of functional proteins. We compared the in vivo efficacy of the genetic combination therapy with the conventional combination chemotherapy in a mouse colon cancer model.

**Results:** Both replication-competent and -noncompetent adenoviral vectors carrying an L-plastin–driven cytosine deaminase transcription unit when combined with 5-FC, CPT-11, and FA showed increased in vitro therapeutic activity that was significantly higher than that of the conventional chemotherapy combination. Tumor-bearing mice treated with the genetic combination therapy showed a statistically significant advantage in terms of increased response rate, response duration, survival, and reduced toxicity when compared with tumor-bearing mice treated with the conventional combination chemotherapy.

**Conclusions:** Replacement of 5-FU in 5-FU–based combination chemotherapy with the Ad-LpCDIRESE1A vector and 5-FU reduces toxicity and increases efficacy. This is a concept that could be potentially applied widely for many forms of cancer treatment.

INTRODUCTION

5-Fluorouracil (5-FU) is a component of many chemotherapy regimens that have been used in the past for advanced carcinomas of the breast, ovary, and colon as well as the programs used for the adjuvant therapy for carcinomas of the colon, ovary, and breast (1). One of the limiting factors of a regimen like the CPT-11, 5-FU, and FA is its gastrointestinal toxicity. When 5-FU is given at the maximal doses that are safe to administer systemically, it is usually considered to be toxic only for dividing cells through incorporation of the 5-FU into DNA and binding of 5-FU to thymidylate synthase (1, 2).

The failure of existing 5-FU–based chemotherapy in many advanced cancer patients may be attributable in part to the fact that <10% of neoplastic epithelial cells are proliferating at any given time and therefore most of the cancer cells escape control by 5-FU. If it were possible to safely increase the levels of intravenously administered 5-FU to those at which RNA is sufficiently substituted with 5-FU to suppress protein synthesis (1), then one could kill nondividing cancer cells as well as dividing cancer cells. Unfortunately, the dose increments of systemically administered 5-FU that would be required to prevent protein synthesis and thereby kill nondividing cancer cells would generate unacceptable levels of toxicity to the normal cells of the bone marrow and gastrointestinal tract.

We have therefore proposed to use the L-plastin tumor-specific transcriptional promoter to regulate the expression of the transcription units of an adenoviral vector that is selectively cytolytic to tumor cells, on the basis of the levels of the cytosine deaminase (CD) protein that it produces in vector-infected tumor cells. The L-plastin promoter (3, 4) has been shown to drive the expression of genes in tumor cells but not in normal cells (5–7). The Escherichia coli or yeast cytosine deaminase (CD) gene (8, 9) catalyzes the conversion of the relatively...
harmless drug, 5-fluorocytosine (5-FC), into the cytotoxic agent, 5-FU. The levels of 5-FU, which are generated by the CD/5-FC system (2) within tumor cells (>300 μmol/L), are much higher than those possible when 5-FU is systemically administered (5 μmol/L).

Adenoviral vectors carrying the CD gene driven by the L-plastin promoter have been shown in our laboratory to sensitize breast, ovarian, and colon cancer cells to the effects of 5-FU (5, 6). We have also placed the gene for E1A, which is necessary for viral replication, downstream of the L-plastin promoter to create a vector that is selectively cytolytic to cancer cells (10). A vector that contains both the CD and E1A genes under control of the L-plastin promoter (AdLpCDIRESE1A) can be used to kill cancer cells through two mechanisms: vector replication within the tumor cells and sensitization of the cancer cell infected by this vector to the effects of 5-FU. The concentration of 5-FU generated in 5-FC-exposed cells infected with the AdLpCDIRESE1A vector is 50 times those possible to safely generate (5 μmol/L) when the 5-FU is administered intravenously and sufficiently high (300 μmol/L) to kill even nondividing cancer cells. This double gene vector has already been shown in our laboratory to have a tumor selective cytotoxic effect that is greater than vectors carrying either the CD or the E1A genes alone (11).

In this report, we have compared the combination of the AdLpCDIRESE1A vector added to CPT-11, 5-FC, and FA chemotherapy (genetic combination therapy) with the conventional combination of CPT-11, 5-FU, and FA. The results of these experiments that are summarized in this report show that "genetic combination therapy" is less toxic and much more effective in suppressing the growth of cancer and extending the survival of mice than is the conventional combination chemotherapy.

MATERIALS AND METHODS

Cells and Reagents. The human cancer cell lines of colon (HTB-38), breast (MCF-7) and prostate (Lncap) were purchased from the American Type Culture Collection (Manassas, VA), and the human epithelial ovarian cancer cell line (Ovcar-5) was obtained from Dr. Thomas C. Hamilton (Fox Chase Cancer Center, Philadelphia, PA). All drugs and chemicals were purchased from Sigma (St. Louis, MO), 2'-Deoxyuridine 5'-monophosphate and diammonium salt, [5-3H] (15.0 Ci/mmol) were purchased from Moravek (Brea, CA); IgG-FITC was purchased from eBioScience (San Diego, CA). α,βs and α,β1 monoclonal antibodies were purchased from Covance (Richard, CA). Mouse anti-αcox sackie-adenoviral receptor (anti-CAR) antibody was kindly provided by Dr. Robert W. Finberg (University of Massachusetts Medical School). Wild type Adenovirus type 5 (Ad5WT) was purchased from the American Type Culture Collection. The construction of the following vectors, AdLpE1A, AdCMVE1A, AdLpCD, AdLpCDIRESE1A, and AdCMVCDIRESE1A, has been described in previous publications from our laboratory (5–7, 11) and others (12).

Carboxylesterase and Thymidylate Synthase Activities of Tumor Cells. Carboxylesterase (CE) activity of all cell lines was assessed by measuring the hydrolytic conversion of paranitrophenolic acid to paranitrophenol that is catalyzed by CE (13). Thymidylate synthase activity of tumor cells was measured according a tritium-release assay as described previously (14). The incubations were done in quadruplicate.

Immunofluorescent Analysis. Using an antismouse IgG-FITC antibody, we measured the level of expression of CAR, α,βs, and α,β1 integrin receptors on tumor cells by flow cytometry as described in previous publications (6, 7, 11). To show the in vivo tumor specificity of Lp-driven vector, normal hepatic tissue and subcutaneous tumor nodules caused by inoculating HTB-38 cells were infected with AdLpCDIRESE1A or AdCMVCDIRESE1A vectors by percutaneous injection. We then studied the expression of E1A protein by immunofluorescent staining (Vector M.O.M. Immunodetection kit, Vector Laboratories, Burlingame, CA) using adenovirus type 5E1A antibody from NeoMarkers (Fremont, CA).

In vitro Studies of Vector-Infected Cell Lines. The tumor cells were seeded at a density of 100,000 cells/well in 6-well plates. The attached cells were infected with the several vectors. The first is the AdLpE1A conditionally replication-competent vector with the tumor-specific L-plastin promoter regulating the viral E1A transcription unit (7). The second is the AdLpCDIRESE1A conditionally replication-competent vector carrying a bicistronic transcription unit composed of the cytosine deaminase chemotherapy sensitization gene linked by an internal ribosomal entry site (IRES) to the viral E1A replication gene under the control of the tumor-specific L-plastin promoter (11). The third is the AdCMVE1A replication-competent vector carrying the viral E1A replication gene governed by the cytomegalovirus (CMV) tumor nonspecific transcriptional promoter (7). The fourth is the AdCMVCDIRESE1A replication-competent vector carrying the CDIRESE1A bicistronic transcription unit regulated by the CMV promoter (11). The fifth is the wild-type replication-competent adenovirus (AdWT).

After 2 days of incubation of the infected cells, the supernatant medium of the wells was discarded, and SDS sample lysis buffer (including DTT) was added to the cells. The cells were processed as outlined in a previous publication (11). The steady-state levels of 5-FU generated in the medium from AdLpCD-infected tumor cells were measured.

Effect of Vectors on the Drug Concentration Needed for Half-Maximal Growth Inhibition (IC50) of 5-Fluourouracil, CPT-11, and SN-38. We plated 1–5 × 104 cells/well in 96-well plates in 100 μL of culture medium. After an overnight incubation, fresh medium supplemented with various amounts of the test drug were added: for 5-FU, from 0.05 to 410 μmol/L of 14 different concentrations; for CPT-11, from 5 nmol/L to 1.3 mmol/L of 10 different concentrations; and for SN-38, from 10 pmol/L to 10 μmol/L of 7 different concentrations. After a 72-hour incubation period with drug, the medium was exchanged for fresh medium without drug. The next day, 10 μL of MTT reagent as provided in the commercial kit (American Type Culture Collection) were added to each well containing cells. After an incubation period of 4 hours, which was continued until a purple precipitate was visible at 37°C, the plates were then incubated further overnight at room temperature. The results were expressed as the average percentage (in quadruplicate) of the population of cancer cells present before treatment that were left surviving at any time point.
Animal Model 1. HTB-38 colon cancer cells (3 × 10^6) were injected subcutaneously into female nude/nude mice (4–6 weeks of age). We injected 1 × 10^8 plaque-forming units of the AdLpCDIRESE1A vector or the AdLpCD vector intratumorally into subcutaneous nodules (50 mm^3) of the HTB-38 colon cancer cells that developed from the subcutaneous injection of tumor cells in female nu/nu mice (Fig. 1). The vector or PBS control injections into the tumor nodules were repeated on days 1, 8, 15, and 22. All of the drugs were given to test mice at doses that were equivalent to human doses: 500 mg/kg/day 5-FU intraperitoneally for 10 days; 150 mg/kg 5-FU intravenously on days 1, 8, 15, and 22; 6 mg/kg/day FA intravenously daily starting on the day of intratumoral vector injection for the vector groups and on days 1, 8, 15, and 22 for the CPT-11 + 5-FU + FA group; and 40 mg/kg CPT-11 intravenously on days 1, 8, 15, and 22 (15). A total of two complete cycles of therapy (each 6 weeks long) were given to all animals. Please see Table 1 in which the treatment groups are summarized. Tumor volumes were measured every 2 days (6, 7).

Data are represented as the mean change in tumor size relative to the tumor size at the beginning of treatment of each animal. Animals were evaluated for toxicities of the treatment regimens every day. The following parameters for toxicity were measured daily: activity, skin color, hunching, and fur status, and the cage bedding was inspected daily for residues of diarrhea. The weight of mice was measured three times a week. A separate set of experiments was carried out in which the vector was injected twice a week (instead of the once a week described above), and the tumor dose injected was 2 × 10^5 cells instead of 3 × 10^6 cells.

Animal Model 2. To test the efficacy of the vector in a way that was not limited by diffusion and spread of the vector from the intratumoral injection sites of the vector particles to each of the tumor cells in the tumor nodule, we first incubated the HTB-38 cells in vitro with vector at a multiplicity of infection 30 (100% of the HTB-38 cells were shown previously to be infected at a multiplicity of infection 30) with either the AdLpCDIRESE1A vector or AdWT for 60 minutes. After subcutaneous injection of the vector-infected tumor cells (5 × 10^5 cells/mouse), we administered the drugs at the same doses used in animal experiment 1 to the assigned groups (Table 1). Two cycles of therapy were given in all groups except group 1. In group 1, intraperitoneal 5-FU injections were given only in the 1st and 2nd week because no tumor appeared. Please see Table 1 in which the treatment groups are summarized.

Statistical Analysis. IC_{50} values were calculated according to the median effect principle. The differences among the results of the various groups were compared by the Student’s t
test. One-way ANOVA (with Least Significant Difference post hoc comparisons) and Mann-Whitney tests were used for the comparison of tumor volumes. Tumor growth rates were evaluated by regression analysis. Survival analyses were done according to the Kaplan-Meier method, and the log-rank test was used for survival comparisons.

RESULTS

In vitro Studies in Cell Lines

To evaluate the reasons for differences in response to the CPT-11, 5-FU, and FA chemotherapy and the genetic combination therapy (the AdLpCDIRESE1A vector, CPT-11, 5-FC, and FA) in the cell lines and animal models, we studied the CE and thymidylate synthase activity of the human tumor cell lines. The CE activity of Ln-CaP (human prostate cancer), MCF-7 (human breast cancer), and Ovcar-5 (human ovarian cancer) cells were similar. However, the HTB-38 human colon carcinoma cells had six times more CE activity than the other cell lines, which is consistent with the finding that this cell line converts more CPT-11 into SN-38 (Table 2). The thymidylate synthase activity (14), which was expressed as the amount of 3H2O (fmol) formed in 1 minute/mg of protein, was lowest in the HTB-38 colon cancer cell line and highest in the Ovcar-5 ovarian cancer cell line (Table 2).

To characterize the cell lines with respect to differences which might alter the infectibility of the target cell lines, we studied the expression of α5β1 and α6β1 integrin receptors on tumor cells (16). The percentage of α5β1, α6β1 integrin receptor, as well as the CAR-positive cells was measured by flow cytometry. The percentage of cells positive for CAR and α5β1 integrin receptors varied among the tumor cell lines. More than half of the tumor cell lines have significant percentages of CAR, α5β1, and α6β1 integrin receptor-positive cells (Table 3). According to these results, Ln-CaP seems to be the most sensitive cell line in terms of taking up the adenoviral vectors. We have previously shown that the E1A gene expression of the human tumor cell lines infected by Lp-driven vectors carried the E1A transcription unit (7). The results suggest that the HTB-38 cell line should be infectable by the adenoviral vectors as well.

Specificity of Expression of the L-plastin Promoter–Driven Vector Transcription Units

We then used Western blot analysis (Fig. 2) to study the E1A expression after exposure of cell lines to various vectors and 2 days of incubation of all vector-infected tumor cell lines. The results of these experiments showed bands specific for E1A polypeptides (35–46 kDa). No protein bands are visible from the control cells that were not exposed to E1A-containing vectors.

To test whether the L-plastin–driven bicistronic CDIRESE1A transcription unit was expressed in a tumor-specific manner in the AdLpCDIRESE1A vector-infected cells (5–7), we injected percutaneously either the AdLpCDIRESE1A or the AdCMVCDIRESE1A vectors into normal liver (Fig. 3A and D) or subcutaneous tumor nodules (Fig. 3A and B) and stained histologic sections of the injected tissue for E1A expression (green color). The tumor nodules were positive for E1A whether injected by the AdLpCDIRESE1A or AdCMVCDIRESE1A vectors (Fig. 3A and B, respectively) whereas the normal liver tissue was positive for E1A after injection with the AdLpCDIRESE1A vector (Fig. 3C) but negative for E1A after injection with the AdLpCDIRESE1A vector (Fig. 3C). These results show that the expression of the transgenes in the AdLpCDIRESE1A vector-infected cells is tumor specific.

Table 3  The percentage of tumor cells positive for the CAR, α5β1, and α6β1 receptors as measured by fluorescence-activated cell sorter analysis

<table>
<thead>
<tr>
<th>Cell lines</th>
<th>CAR (%)</th>
<th>α5β1 (%)</th>
<th>α6β1 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ln-CaP</td>
<td>82.0 ± 13.8</td>
<td>64.1 ± 0.8</td>
<td>39.3 ± 1.6</td>
</tr>
<tr>
<td>MCF-7</td>
<td>37.5 ± 7.5</td>
<td>94.7 ± 5.8</td>
<td>19.1 ± 0.8</td>
</tr>
<tr>
<td>Ovcar-5</td>
<td>63.6 ± 19.6</td>
<td>88.2 ± 4.6</td>
<td>46.8 ± 1.4</td>
</tr>
<tr>
<td>HTB-38</td>
<td>77.9 ± 17.6</td>
<td>65.6 ± 5.8</td>
<td>29.5 ± 2.2</td>
</tr>
</tbody>
</table>

* fmol/minute/mg protein.
† mU/mg protein.
These levels are similar to those reported previously to result in the inhibition of protein synthesis (1) and thereby to the death of nondividing cancer cells.

We then tested whether the IC_{SS0} of 5-FU, CPT-11, and SN-38 was decreased by exposure of the test cells to the AdLpCDIRESE1A vector. When the AdLpCD vector plus 5-FC and FA were added to the test cells at a multiplicity of infection of 10, the IC_{SS0} value of CPT-11 decreased 65 to 2,200 times (Table 4). When the AdLpCDIRESE1A replication-competent vector plus 5-FC and FA was added to the cells, the decrease in

Table 4 The IC_{SS0} values of CPT-11 and SN-38 in tumor cells after exposure to the AdLpCD or AdLpCDIRESE1A vectors at a multiplicity of infection of 10.

<table>
<thead>
<tr>
<th>Tumor cells</th>
<th>CPT-11 IC_{SS0} (µmol/L)</th>
<th>SN-38 IC_{SS0} (µmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AdLpCD</td>
<td>AdLpCD-IRESE1A</td>
</tr>
<tr>
<td>Ln-CaP</td>
<td>3.5 ± 1.4 × 10^{-3}</td>
<td>2.5 ± 0.9 × 10^{-4}</td>
</tr>
<tr>
<td>MCF-7</td>
<td>6.2 ± 2.3 × 10^{-3}</td>
<td>1.4 ± 1.5 × 10^{-3}</td>
</tr>
<tr>
<td>Ovcar-5</td>
<td>2.8 ± 1.1 × 10^{-3}</td>
<td>8.2 ± 4.4 × 10^{-3}</td>
</tr>
<tr>
<td>HTB-38</td>
<td>8.8 ± 1.7 × 10^{-3}</td>
<td>4.3 ± 2.3 × 10^{-3}</td>
</tr>
</tbody>
</table>

These data show that the normal cells can be infected by the vector and can express E1A peptides in the presence of the CMV promoter.

Fig. 3 Tumor-specific expression of the E1A gene following injection with vectors carrying the CMV or L-plastin promoters. In this study, green color indicates expression of E1A peptides. A, in the tumor nodule injected with the AdLpCDIRESE1A vector, the green fluorescence is detectable showing that the E1A polypeptides (which are stained green) are produced by the L-plastin–driven E1A gene and are present with the nuclei of the tumor cells that are stained red. B, the tumor nodule that was injected with the AdCMVCDIRESE1A vector also showed expression of the E1A polypeptides. C, in the normal liver injected with the AdLpCDIRESE1A vector, there is no green fluorescence of E1A polypeptides. D, the liver was injected with the AdCMVCDIRESE1A vector. The nuclei of the liver cells are stained red whereas the E1A polypeptides are stained green in the cells injected with the vector carrying the tumor nonselective CMV promoter. These data show that the normal cells can be infected by the vector and can express E1A peptides in the presence of the CMV promoter.
with CPT-11, the decrease of the IC50 value of CPT-11 was 5 to 20 times the decrease seen with the AdLpCD replication-deficient vector. The decrease in the CPT-11 IC50 with both AdLpCD and AdLpCDIRESE1A plus 5-FU and FA was significantly higher when compared with 5-FU and FA (P < 0.05; data not shown). In contrast, when the AdLpCDIRESE1A vector plus FA without 5-FC was combined with CPT-11, the decrease of the IC50 value of CPT-11 was between 7- and 200-fold (data not shown). These studies show that the in vitro activity of CPT-11 is potentiating by the addition of the AdLpCDIRESE1A vector. Moreover, this vector sensitization does not depend on the replication competency of the vector but on the presence of the CD protein and 5-FC.

Replacement of 5-FU in the Conventional CPT-11, 5-FU, and FA Combination Chemotherapy by Intratumoral Injection of the AdLpCDIRESE1A Vector and In vivo 5-FC (Genetic Combination Therapy), Animal Model 1

5-FC–based chemotherapy is the choice for the treatment of colorectal cancer in humans. Therefore, we used the HTB-38 cells for the in vivo studies of the genetic combination therapy. Test mice were given the treatments outlined in Table 1.

Response Studies. The growth of the HTB-38 colon cancer cell line in nude mice was suppressed more by intratumoral injection of the AdLpCDIRESE1A vector given in concert with intraperitoneal 5-FC, intravenous FA, and intravenous CPT-11 chemotherapy (group 1) than it was by conventional CPT-11, 5-FU, and FA combination chemotherapy (group 6) as shown in Fig. 4. The duration of tumor response among the animals treated with the genetic combination therapy (AdLpCDIRESE1A/5-FC/FA/CPT-11, group 1) was statistically significantly longer than the duration of the response among animals treated with regimens not containing the AdLpCDIRESE1A/5-FC combination (P < 0.001).

Survival Studies. The mice treated with AdLpCDIRESE1A/5-FC/FA/CPT-11 (the genetic combination therapy or group 1) lived much longer than did the mice treated with conventional combination chemotherapy (group 6) or the other control groups (P < 0.01, Fig. 5). We then tested for the effect of increasing the frequency of the AdLpCDIRESE1A vector injections from once a week to twice a week. A survival advantage was seen in this latter model, but this advantage was lost by 12 weeks (data not shown). This suggested that additional cycles of therapy might be one way to increase the success of the outcome.

Toxicity Studies. As shown in Table 5, the mice given the conventional CPT-11, 5-FU, and FA combination chemotherapy had statistically significantly more diarrhea than that observed in mice treated with the genetic combination therapy, which involves the combination of AdLpCDIRESE1A, 5-FC, FA, and CPT-11 (32.5 versus 2.5%, respectively, P = 0.001).

Response of the HTB-38 Colon Cancer Cells to In vitro Infection with the AdLpCDIRESE1A Vector Infection and In vivo CPT-11, 5-FC, and FA Chemotherapy, Animal Model 2

To test if we could improve the outcome of the genetic combination therapy, we infected the HTB-38 cells with AdLpCDIRESE1A vector in vitro under conditions that would result in infection of 100% of the HTB-38 cancer cells before the injection of the tumor cells into the subcutaneous space of the test animals. After subcutaneous injection of HTB-38 tumor cells that had been infected in vitro with either the AdLpCDIRESE1A vector, the AdLpCD vector, or the AdWT virus, we treated the mice with the programs outlined in Table 1.

Response Studies. None of the mice treated with the genetic combination therapy (group 1) exhibited regrowth of the tumor cells at the injected sites during the 5 months of follow-up whereas the other treatment groups showed regrowth of tumor after chemotherapy (Fig. 6). In each of the groups in which the colon cancer cell line HTB-38 was infected in vitro with a replication-competent vector (groups 1 and 4) and in which in vivo 5-FC (in the case of the AdLpCDIRESE1A) or 5-FU (in the case of AdWT) was given, there was a statistically significant reduction in the tumor growth rates (P < 0.05, Fig. 6).

Survival Studies. There was a survival advantage of the genetic combination therapy (group 1) as compared with the use of in vivo administration of the conventional CPT-11, 5-FU, and FA combination chemotherapy (group 5) as shown in Fig. 7. When 5-FC was deleted from the genetic combination therapy,
the survival advantage of the genetic combination therapy over the conventional CPT-11, 5-FU, and FA combination chemotherapy was lost (e.g., group 3 in Fig. 7). These results indicated that the outcome of therapy depended on the conversion of 5-FC to 5-FU within the tumor cells.

**DISCUSSION**

We have tested whether it is possible to increase the efficacy and decrease the toxicity of 5-FU–based combination chemotherapy for advanced cancer by using a vector to target 5-FU therapy to tumor cells and spare the normal cells of the body. The combination of the AdLpCDIRESE1A conditionally replication-competent adenoviral vector with CPT-11, 5-FC, and FA chemotherapy, which is called the genetic combination therapy, is statistically significantly superior to the conventional CPT-11, 5-FU, and FA combination chemotherapy with respect to tumor response and survival. These effects may be attributable to the high levels of 5-FU generated by the AdLpCDIRESE1A vector/5-FU treatment within tumor cells that are not possible to safely achieve by systemic

![Fig. 5](image)

Survival of mice after treatment in animal model 1. The effect of the intratumoral injection of the AdLpCDIRESE1A vector along with 10 days of intraperitoneal 5-FC and one day of intravenous FA and CPT-11 (genetic combination therapy, group 1) on prolongation of survival of mice carrying subcutaneous nodules of HTB-38 cells is greater than that of the conventional CPT-11, 5-FU, and FA combination chemotherapy, group 6 ($P < 0.01$).

![Fig. 6](image)

Tumor response of colon cancer in mice after treatment in animal model 2. The effect of in vitro infection of HTB-38 cells with the AdLpCDIRESE1A vector along with 10 days of intraperitoneal 5-FC and 1 day of intravenous FA and CPT-11 (genetic combination therapy, group 1) on tumor growth is greater than that of the conventional CPT-11, 5-FU, and FA combination chemotherapy, group 5 ($P < 0.05$).
administration of 5-FU chemotherapy. In addition, we show that the toxicity of the genetic combination therapy is statistically significantly less than that seen with the conventional CPT-11, 5-FU, and FA combination chemotherapy.

Mice that were given the genetic combination therapy had a tumor volume that was almost one-fourth that seen in the mice treated with the conventional CPT-11, 5-FU, and FA combination chemotherapy \((P < 0.001)\) at the end of the first month. The mice given the genetic combination therapy also had statistically significantly prolonged survival compared with the mice given the conventional combination chemotherapy \((P < 0.001)\). The addition of FA to the AdLpCDIRESE1A vector and 5-FC (genetic combination therapy without CPT-11) also enhanced tumor control and survival more than the conventional CPT-11, 5-FU, and FA combination chemotherapy.

Animal model 2 was designed to test the efficacy of the vector under conditions that permitted infection of 100% of the tumor cells. This was accomplished by infecting the tumor cells with either \(CD\)-carrying vectors or wild-type adenovirus before subcutaneous inoculation of the tumor cells into the test mice. All of the mice given the genetic combination therapy were free of tumor nodules, whereas six of seven mice given AdLpCDIRESE1A + CPT-11 (intravenously) + FA (intravenously), which is the genetic combination therapy without 5-FC, developed tumor nodules.

One of the major limiting factors of the conventional combination chemotherapy is its gastrointestinal toxicity. Importantly, there was a statistically significantly decreased incidence of diarrhea in the animals treated with the genetic combination therapy as compared with conventional CPT-11, 5-FU, and FA combination chemotherapy. We generated this reduction in toxicity and increase in efficacy of the regimen by using conditionally replication-competent adenoviral vectors that are tumor specific in the delivery of 5-FU to cancer cells, thus sparing the normal tissues of the body from the toxicity of the regimen.

The results of these studies suggest that the combination of AdLpCDIRESE1A and 5-FC system with CPT-11 and FA is more effective and less toxic than the traditional combination of CPT-11, 5-FU, and FA. Because of the limited number of tumor cells infected when this vector is injected intratumorally, the goal of clinical translation of the genetic combination therapy will be feasible when the vector has been engineered so that it only infects tumor cells and tumor vascular endothelial cells. In that case, the vector will be suitable for administration in the bloodstream, and under these conditions, it is possible that a far greater number of tumor cells can be accessed by the AdLpCDIRESE1A vector. To accomplish this, our laboratory has created adenoviral vectors carrying the CDIRESE1A transcription unit that can be targeted to the tumor cells and their vasculature, providing that the nonselective uptake of the vector by the reticuloendothelial cells has been blocked. The feasibility of taking these vectors into the clinic for use in systemic administration for tumor vascular targeting therapy is currently being studied in our laboratory.

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REFERENCES

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