In Vivo Selective Gene Expression and Therapy Mediated by Adenoviral Vectors for Human Carcinoembryonic Antigen-producing Gastric Carcinoma

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ABSTRACT
Previously, we reported that adenoviral vectors carrying the carcinoembryonic antigen (CEA) promoter sequences to direct the Escherichia coli β-galactosidase gene (AdCEA-lacZ) or cytosine deaminase (CD) gene (AdCEA-CD) confer selective gene expression on a CEA-positive gastric cancer cell line (MKN45) in vivo. Here, adenoavrus-mediated tumor-specific gene therapy for CEA-positive gastric carcinoma in vivo was investigated. Using an animal model with i.p. disseminated MKN45 tumors, adenovirus-mediated tumor-specific transgene expression and therapeutic efficacy were analyzed. After an i.p. injection of AdCEA-lacZ, β-galactosidase activity was confined to tumor xenografts. Moreover, CD mRNA was expressed exclusively in MKN45 tumor xenografts after infection with AdCEA-CD, despite the fact that an adenovirus-mediated transfer of CD DNA was detected in all tissues tested. In contrast, CD mRNA was not detected not only in tumor xenografts but also in other organs of mice infected with AdCA-CD, in which CD gene expression is governed by an ubiquitous promoter. Suppression of tumor growth and prolongation of survival were noted in tumor-bearing mice treated with AdCEA-CD and 5-fluorocytosine (5FC). These results reveal that the CEA promoter restricts CD gene expression to CEA-positive tumor cells in the adenoviral context in vivo, along with the beneficial therapeutic effects of 5FC treatment, suggesting the potential of the AdCEA-CD/5FC system may provide a novel approach to treatment of i.p. disseminated gastric cancer.

INTRODUCTION
Gastric carcinoma, the most frequently occurring cancer in Japan, accounts for 20–30% of all incident cancers. Surgical treatment of gastric cancer without distant metastasis leads to a relatively good prognosis. However, even aggressive treatment through surgery or chemotherapy has not been able to sufficiently improve the prognosis of gastric cancer with distant metastasis, such as peritoneal dissemination and liver metastasis (1). Peritoneal metastasis is the most frequent type of recurrence in patients, even in those patients who show no evidence of residual tumor after surgery, and is a serious obstacle in the curative treatment of gastric cancer (2); therefore, the prevention and treatment of peritoneal dissemination are vital therapeutic targets for gastric cancer. A promising genetic approach to the treatment of cancer is the delivery of a "suicide" gene, such as the Escherichia coli CD3

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3 The abbreviations used are: CD, cytostine deaminase; 5FC, 5-fluorocytosine; 5FU, 5-fluorouracil; CEA, carcinoembryonic antigen; Ad, adenovirus; FBS, fetal bovine serum; pfu, plaque-forming unit(s); X-gal, 5-bromo-4-chloro-3-indolyl-b-D-galactopyranoside; RT-PCR, reverse transcriptase-PCR; ALT, alanine transaminase; AST, aspartate transaminase; HSV-1, herpes simplex virus thymidine kinase; GCV, ganciclovir.

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Inability to specifically deliver sufficient levels of a therapeutic agent to all tumors, while sparing their normal, nontransformed counterparts (3). Localized generation of 5FU was achieved by surgical implantation of encapsulated CD into s.c. tumors growing in rats in the presence of 5FC (4). Through the approach, considerable antitumor activity without observable toxic effect was evident. With the advancement of recombinant DNA techniques, a tumor-specific gene can be expressed by the use of promoter elements of genes usually transcribed by tumors, such as tumor markers; i.e., CEA, which is often expressed at high levels in gastrointestinal malignancies, including colon, pancreatic, and gastric tumors (5). Although CEA is also expressed in some normal tissues of epithelial origin, we think the difference in the amount of CEA production between malignant and normal tissue may make CEA's application in cancer gene therapy possible. By coupling the CEA promoter with a CD gene, CD gene expression can be restricted in cancer cells that produce high levels of CEA. Consequently, in the presence of 5FC, a high local concentration of 5FU can be produced in the CEA-positive tumor to kill cancer cells, while sparing the surrounding normal tissues (6).

To fully understand the clinical potentials of gene therapy, it is necessary to produce efficient strategies for delivering therapeutic genes to somatic cells in vivo (7). Ads have numerous attractive features for this development. Adenoviral particles are relatively stable and amenable to high purification and concentration, the serotypes most widely used in vector design (Ad2 and Ad5) are associated with only benign pathologies in humans, and, most importantly, they are attractive vehicles for gastrointestinal studies because of their known tropism for this organ (8). For all these reasons, Ad vectors hold great promise for the development of in vivo gene therapy against gastrointestinal cancer. Our previous study rendered the specificity and selectivity of CD gene transduction, directed by CEA promoter, into CEA-positive MKN45 gastric cancer cells by an adenoviral vector, producing transduced MKN45 cells that were susceptible to 5FC. Furthermore, together with the bystander effect, a significant growth inhibition of both CD-transduced and nontransduced MKN45 cells was observed in vitro (9). Here, we investigated the efficacy of Ad-mediated tumor-specific gene therapy using the AdCEA-CD/5FC system for the treatment of established i.p. MKN45 gastric carcinoma in an immunodeficient animal. Our results show that adenoviral vectors can successfully transduce gastric cancer in vivo and that the AdCEA-CD/5FC system is effective in reducing tumor burden and prolonging survival without substantial host toxicity. These results have direct implication for the application of the AdCEA-CD/5FC in the treatment of i.p. carcinomatosis derived from a CEA-positive human gastric carcinoma.

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MATERIALS AND METHODS

Cell Culture and Animal Model. MKN45, a cell line derived from a human gastric carcinoma with a high level of CEA production, was obtained from Japanese Cancer Research Resources (Tokyo, Japan). The cells were maintained in RPMI 1640 supplemented with 10% FBS (Cansera International Inc., Rexdale, Ontario, Canada), 2 mM L-glutamine, 50 units/ml penicillin, and 50 µg/ml streptomycin. For the development of an animal model that closely mimics human advanced gastric carcinoma, male, 8-week-old athymic BALB/cAiccl nu/nu mice (CLEA, Tokyo, Japan) received an injection of MKN45 i.p. (1 × 10⁶ cells/mouse). Procedures in animal experiments were performed with approved protocols and in accordance with recommendations for the proper care and use of laboratory animals.

Recombinant Adenoviral Vectors. Recombinant replication-defective Ad vectors were prepared, purified, and titrated by a standard protocol as described previously (10, 11). Four vectors were used: AdCEA-lacZ, an adenoviral vector with an E. coli lacZ gene driven by a human CEA promoter; AdCEA-CD, an adenoviral vector with an E. coli CD gene driven by a human CEA promoter; AdCA-lacZ, an adenoviral vector with an E. coli lacZ gene driven by a CAG promoter (composed of a cytomegalovirus immediate early enhancer and a modified chicken β-actin promoter; Ref. 12); and AdCA-CD, an adenoviral vector with an E. coli CD gene driven by a CAG promoter (9). Recombinant Ad vectors were stored in a DMEM containing 10% FBS in −80°C. All viral stocks were evaluated for the presence of replication-competent Ad by PCR as described previously (13). None of the stocks of Ad vectors used in our studies contained detectable replication-competent viruses.

Ad-mediated lacZ Expression in Vivo. To evaluate the ability and selectivity of Ad-mediated gene transduction in disseminated gastric carcinoma in vivo, 1 × 10⁶ pfu of adenoviral vector (AdCEA-lacZ, AdCA-lacZ, or AdCEA-CD) were injected into the peritoneal cavities of mice bearing i.p. MKN45 tumor xenografts 10 days after tumor implantation. Animals were sacrificed 48 h after viral vector injection. The peritoneal cavities were washed with PBS and fixed by immersion in cold PBS containing 2% formaldehyde and 0.2% glutaraldehyde for 3 h. To detect the expression of β-galactosidase, the peritoneal cavities were stained with X-gal for 4 h. After staining and photography, tumors were postfixed in the same fixative, embedded in paraffin, cut into 5-µm sections, and counterstained with eosin.

Ad-mediated CD Gene Expression in Vivo. To analyze the target organs following the i.p. infusion of the recombinant Ad, genomic DNA from various tissues of mice receiving AdCEA-CD or AdCA-CD was extracted (Qiagen, Hilden, Germany) and examined by PCR for CD gene distribution. The PCR primers were 5'-GAATAACGCGTACAcCAGCA and 5'-TAACGTACCAGAACCTGACG, resulting in an amplified CD DNA fragment of 1166 bp. The distribution of CD mRNA in the organs of mice receiving AdCEA-CD or AdCA-CD by RT-PCR was then examined. Total RNA was isolated from mouse tissues using the Isogen kit (Wako, Ltd., Osaka, Japan) and treated for 15 min at 37°C with 25 units of DNase I (Pharmacia, San Diego, CA). RNA was then extracted with phenol/chloroform and precipitated with ethanol in the presence of 1 µg of glycogen (Boehringer Mannheim, Indianapolis, IN). Purified total RNA (3 µg) was used for cDNA synthesis by using murine leukemia virus reverse transcriptase (Perkin-Elmer, Foster City, CA) with oligo(dT)₁₂₋₁₈ as the primer. Twenty µl of reverse transcriptase products from each sample were amplified for 4 cycles with AmpliTaq Gold (Perkin-Elmer) in PCR with an annealing temperature of 50°C. Sixteen µl of each PCR product were analyzed by 1% agarose gel electrophoresis and visualized by ethidium bromide staining.

Ad-mediated Gene Therapy for Animals with Disseminated Gastric Carcinoma. To investigate the efficacy of Ad-mediated tumor-specific gene therapy on established tumors in vivo, an animal model with i.p. disseminated MKN45 tumors that closely resemble human advanced gastric carcinoma was used. The mice receiving an i.p. injection of MKN45 cells (1 × 10⁶ cells per mouse) were randomly divided into the following six groups according to treatment schedules: (a) AdCEA-CD followed by SFC (500 mg/kg) treatment (n = 5); (b) AdCA-CD followed by SFC treatment (n = 5); (c) AdCEA-CD followed by PBS administration (n = 5); (d) AdCA-CD injection followed by PBS administration (n = 5); (e) AdCA-lacZ followed by SFC treatment (n = 5); and (f) “vehicle” (DMEM with 10% FBS) followed by PBS administration (n = 5). The Ad vectors (1 × 10⁶ pfu) or vehicles were injected i.p. into the mice 10 days after tumor implantation. From the following day, SFC or PBS was administered i.p. once daily for the next 14 days. Animals were sacrificed at the end of 4 weeks after tumor inoculation, following which the i.p. tumor mass of each animal was collected and weighed. Blood samples of mice from each group were also collected for the purpose of measuring serum CEA levels.

To further evaluate whether the therapeutic effects of reduction in tumor burden were also reflected on prolongation of animal’s survival, an additional set of animals inoculated and treated with the same protocols described above (n = 8 in each group) was followed for the purpose of survival time evaluation. Whenever possible, each animal was necropsied at time of death and evaluated for the cause of death.

Evaluation of Adenoviral Vectors and SFC Treatment. For evaluating the adverse effects of Ad-mediated suicide gene therapy, serum biochemical parameters, including bilirubin, ALT, AST, blood urea nitrogen, and creatinine, of mice from each treatment group described above were measured at the end of 4 weeks after tumor inoculation. In addition, major organs of mice from each group were removed for gross and histological analysis. One set of sections was stained with H&E and examined by a pathologist who was blinded to the identities of the slides.

Dose Responses of Adenoviral Vectors versus Treatment Efficacy and Toxicity. To further elucidate the treatment efficacy and toxicity mediated by adenoviral vectors, dose response effects of both AdCA-CD and AdCEA-CD on tumor suppression and toxicity of therapy were evaluated. To accomplish this, the AdCA-CD or AdCEA-CD vector (10⁵, 10⁶, or 10⁷ pfu), with either SFC or PBS, was delivered i.p. into MKN45 tumor-bearing animals (n = 5 in each group) using the same protocol mentioned above. Control animals received no vector. If possible, animals were followed until day 28 after tumor inoculation. Tumor weights, serum biochemical parameters, and histology of major organs were evaluated as described above.

Data Presentation and Statistical Analysis. Results are expressed as the mean ± SD. Statistical analysis was performed by ANOVA, Fisher’s test, and Kaplan-Meier method using Statview software (Abacus Concepts, Inc.), and P < 0.05 was considered to be statistically significant.

RESULTS

Selective Expression of β-galactosidase by AdCEA-lacZ in Vivo. In vivo Ad-mediated expression of the lacZ gene was evaluated in animals with i.p. MKN45 tumors after an i.p. administration of AdCEA-lacZ, AdCA-lacZ, or AdCEA-CD. Successful transfer and expression of the lacZ gene was demonstrated by the presence of lacZ product, β-galactosidase, which was detectable by X-gal staining. Selective expression of β-galactosidase (blue staining) was confined to the tumor (not found in the surrounding normal tissue) of the mouse injected with AdCEA-lacZ (Fig. 1A). In contrast, blue staining was detectable not only in the tumor masses but also in the adenoviral vector-injection site and intestine of the mouse receiving AdCA-lacZ (Fig. 1B). The mouse with AdCEA-CD injection served as a negative control, showing no detectable background after X-gal staining (Fig. 1C). Tumors were then embedded in paraffin, cut into 5-µm sections, and counterstained with eosin. Expression of β-galactosidase was evident not only superficially but also within tumor masses of mice infected with AdCEA-lacZ (data not shown).

Selectively CEA Promoter-directed CD Gene Expression by Adenoviral Vectors in Vivo. To test the ability of the CEA promoter to direct transgene expression selectively in vivo, we evaluated CD gene distribution and expression from the tissues of mice infected with AdCA-CD or AdCEA-CD. AdCA-CD or AdCA-CD (1 × 10⁶ pfu) was injected into the peritoneal cavity 10 days after MKN45 tumor inoculation. Three days later, total DNA and RNA of various organs were extracted and subjected to PCR to detect CD gene existence and mRNA expression. As shown in Fig. 2A, the CD gene was detected in all organs of animals infected with either AdCA-CD or AdCA-CD. However, by using RT-PCR, CD mRNA was expressed exclusively in CEA-producing tumor xenografts of mice infected with AdCA-CD (Fig. 2B, left). By contrast, CD mRNA was expressed in...
a broad range of tissues of mice infected with AdCA-CD, in which CD gene expression was directed by the constitutive CAG promoter (Fig. 2B, right). Samples with intact total RNA were confirmed by electrophoresis on a formaldehyde gel to show sharp bands of 28S and 18S rRNA before being subjected to RT-PCR (data not shown).

**Therapeutic Effect of CD-expressing Adenoviral Vectors and 5FC in Vivo.** To evaluate the therapeutic effects of an in vivo gene transduction with Adcea-CD and 5FC treatment on i.p. metastases of CEA-positive gastric carcinoma, an animal model of metastatic gastric carcinoma was used, as described in “Materials and Methods.” Within 1 week after implantation of the tumor cells, disseminated tumor foci were visible in the peritoneal cavity. Tumor-bearing mice were randomly grouped for treatment with AdCEA-CD/5FC, AdCA-CD/5FC, AdCEA-CD/PBS, AdCA-CD/PBS, AdCEA-lacZ/5FC, or vehicle/PBS. Ad vectors and vehicles were injected i.p. 10 days after tumor inoculation. 5FC or PBS administration was initiated on the following day for a 2-week period. Four weeks after tumor inoculation, i.p. tumor masses and blood samples were collected from each animal. As indicated in Fig. 3A (left), tumor weights in animals treated with AdCEA-CD/PBS or AdCEA-lacZ/5FC were similar to those of untreated animals (vehicle/PBS), whereas tumor weights of those treated with AdCEA-CD/5FC or AdCA-CD/5FC were significantly reduced as compared to those in the untreated group (P < 0.0001). In addition, there was a small but statistically significant difference (P = 0.0464 and 0.0087, respectively) in mean tumor weight between the AdCA-CD/PBS control group (0.37 ± 0.08 g) and the AdCEA-lacZ/5FC group (0.50 ± 0.13 g) or vehicle/PBS controls (0.55 ± 0.13 g), suggesting that the CD gene product itself may impart toxicity to tumor. Serum CEA levels were also measured for addressing the treatment effects on tumor-bearing mice, indicating that the decrease of serum CEA level correlates well with the reduction of tumor burden (Fig. 3A, right).

The beneficial effects of AdCEA-CD plus 5FC on tumor burden were also reflected in the overall survival. Animals treated with AdCEA-CD/5FC had survival prolongation (101 ± 15 days, P < 0.0001) as compared to those treated with vehicle/PBS (47 ± 6 days), AdCEA-lacZ/5FC (48 ± 6 days), or AdCEA-CD/PBS (44 ± 6 days; Fig. 3B). In addition, animals treated with AdCA-CD, combined with either 5FC or PBS, showed average survival prolongations of 88 ± 43 days and 70 ± 20 days, respectively. However, some of the treated animals died as early as 17–30 days after tumor inoculation, which is earlier than the average survival time of untreated animals, suggesting that CD gene transduction by the ubiquitously strong CAG promoter may induce toxicity not only to tumor xenografts but also to the treated host itself. The remaining animals died directly of tumors suggesting that CD gene transduction by the ubiquitously strong CAG promoter may induce toxicity not only to tumor xenografts but also to the treated host itself. The remaining animals died directly of tumors or metastases, which were confirmed by necropsies.

**Treatment-related Toxicity.** To address the possibility of adverse effects in animals receiving adenoviral vectors and/or 5FC, organ toxicity was analyzed through evaluation of gross inspection, H&E-stained histological sections, and serum biochemical examinations of each treated animal. Necropsy of animals showed no gross abnormal-
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Dose-dependent Effects of Adenoviral Vectors on Treatment Efficacy and Toxicity. There were no deaths observed in any treatment groups except that with AdCA-CD (10^10 pfu)/5FC, of which all animals died by the 10th day after viral inoculation. Necropsy showed no overt tumor burden (0.05 ± 0.02 g) in the animals treated with AdCA-CD (10^10 pfu)/5FC. However, evaluation of liver-related parameters in serum samples and histological liver tissue revealed a significant increase in ALT and AST levels, as well as severe hepatic necrosis. There was a dose-dependent effect on both treatment efficacy, as represented in tumor burden reduction, and hepatic toxicity in the animals by AdCA-CD or AdCEA-CD plus 5FC (Table 2). Both effects are more significant in animals with AdCA-CD treatment than those with AdCEA-CD when same amount of vector was applied.

DISCUSSION

For gene therapy to be a useful clinical modality for gastric carcinoma, it is important to target tumor cells, while sparing other normal cells. Here, we used an animal model bearing a MKN45 tumor to imitate human advanced metastatic gastric carcinoma. The present study revealed β-galactosidase expression in CEA-positive MKN45 xenografts after an i.p. injection of AdCEA-lacZ, indicating that the CEA promoter, in the context of a recombinant Ad vector, can confer selective expression of a transduced gene in human CEA-positive gastric carcinoma in vivo. Consistent with the results reported previously (14, 15), the expression of β-galactosidase was also found within the tumor masses, which is speculated to be caused by the penetration of Ad vectors through immature tumor capillaries. In addition, transduction of CD gene, under the control of CEA promoter by an adenoviral vector, was found to bestow the disseminated CEA-positive human gastric carcinoma that is susceptible to the prodrug 5FC in vivo. Significant suppression of tumor growth and prolonga-

Fig. 3. Effect of CD/5FC therapy on the growth of established i.p. gastric carcinoma and survival in nude mice. Animals with established i.p. MKN45 tumor were treated with AdCA-CD, AdCEA-CD, AdCA-lacZ, or vehicle i.p. 10 days after initial tumor inoculation. From the following day, animals were administered with either 5FC (500 mg/kg) or PBS for 14 days. A, 4 weeks after initial tumor inoculation, animals were necropsied, and all i.p. tumors were weighed. Blood samples were also collected to determine serum CEA levels. Columns, mean tumor weights and CEA levels; bars, SD. All groups were compared using ANOVA analysis and subjected to Fisher's test. B, survival data (Kaplan-Meier plots) for i.p. MKN45 tumor-harboring mice treated with vehicle followed by PBS (n = 8), AdCEA-lacZ followed by 5FC (n = 8), AdCEA-CD followed by PBS (n = 8) or 5FC (n = 8), and AdCA-CD followed by PBS (n = 8) or 5FC (n = 8). Animals were inoculated and treated with the same protocols described above. Data points, survival.

Fig. 4. Histological findings of livers from animals treated with AdCA-CD/5FC or AdCEA-CD/5FC. A, representative photomicrograph of liver from mice with AdCA-CD plus 5-FC treatment demonstrates zonal necrosis around periportal area, in which reduction of eosinophilic granules of hepatocyte is evident. B, photomicrograph of a liver section from animals treated with AdCEA-CD/5FC showed normal histological features, which are the same as those seen in untreated animals. Magnification, ×25.
different from clinical manifestations. Chen et al. (16) reported that gene therapy (16–20), most are in vitro studies or utilization of MKN45 tumors following i.p. treatment of AdCEA-CD and 5FC. Observation; that is, even in the absence of 5FC, about 20% of gastric tumors had prolonged survival or had suffered from adverse effects. However, their report did not unveil the details of whether the treated animals had prolonged survival or had suffered from adverse effects. The elucidation of these questions is important because safety is a major issue for the clinical application of gene therapy.

The finding that treatment with only AdCA-CD has a mild effect on tumor growth inhibition is consistent with our previous in vitro observation; that is, even in the absence of 5FC, about 20% of gastric cancer cells (MKN45, MKN28, and MKN1) died of CA-CD infection at a multiplicity of infection of 50 (9). However, the finding of expression of CD mRNA in tissues other than tumor xenografts by AdCA-CD raises important safety concerns. Although the treatment of AdCA-CD, either in the presence or absence of 5FC, affected tumor growth inhibition and prolongation of average survival, some treated animals suffered a toxic death during or soon after treatment. In addition, when animals received high dose of AdCA-CD (1010 pfu) and 5FC, none of them could survive more than 10 days after viral inoculation. Autopsy revealed severe histological abnormalities in the liver, whereas other organs were not affected, suggesting that toxicity to the liver was the cause of death. Hepatic injury is most likely to be caused by a CD product, the expression of which was directed by a CAG promoter in an Ad vector and/or 5FC itself around the hepatic vessels, through which this adenoviral vector and/or 5FC are induced by converted 5FU and/or transduced CD itself around the tumor mass is not achievable with currently available vectors, including the adenoviral vector. Therefore, the bystander killing effect, the killing of nontransduced tumor cells in the presence of a minority of cells expressing the suicide gene, is thought to play an important role in achieving successful gene therapy. The bystander killing effect has been demonstrated in such suicide gene strategies as the HSV-tk/GCV and CD/5FC systems. Recently, Trinh et al. (21), Hoganson et al. (22), and Rogers et al. (23) reported that CD/5FC regimen has a better bystander effect as well as a greater therapeutic margin than the HSV-tk/GCV or doxycytidine kinase/1-β-D-2-arabinofuranosylcytosine combination. In the HSV-tk/GCV system, the bystander effect appears to rely on the presence of gap junctions of cells or components of the immune system. However, the CD/5FC bystander effect, which is mediated by rapid diffusion of 5FU through cell membranes (24), is especially useful for tumor-specific promoter application, which is usually weaker than the constitutive promoter, to direct suicide gene transduction.

Although the data presented here appear promising, a number of problems still exist. First, the reduction in tumor burden by AdCEA-CD/5FC, although statistically significant, was not as great as that induced by AdCA-CD/5FC. In fact, our preliminary results using the β-galactosidase assay showed that the CEA promoter is about 200 times less active than the CAG promoter in MKN45 cells (data not shown), which may account for the difference of reduction in tumor burden between AdCEA-CD/5FC and AdCA-CD/5FC. Second, nude mice are not natural hosts of Ad and are bred in a pathogen-free environment, which may reduce the opportunity of contamination by wild-type Ads. Further investigations of potential complementation of recombinant replication-deficient Ad vectors are necessary before clinical application. Third, because the CEA promoter constructed in our Ad vectors are derived from human genomic DNA, the activation of this element may not occur in normal nude mice tissues. Thus, not surprisingly, there was no detectable CD mRNA expression in tissues other than tumor xenografts in animals treated with AdCEA-CD/5FC. However, the potential of 5FC-induced toxicity, mediated by CD gene product, to normal human tissues should not be ignored in the administration of AdCEA-CD/5FC to human beings because a few human normal tissues also produce a small amount of CEA protein, such as antral mucosa.

This study has demonstrated the therapeutic efficacy and selectivity of AdCEA-CD/5FC for the treatment of established i.p. CEA-producing gastric carcinoma in an animal model. Because exclusive targeting of viral vectors to the tumor is presently impossible, the use of tumor-specific promoters to drive expression of therapeutic genes is an essential alternative. It has also been reported that the utilization of CEA promoter in the context of adenoviral vectors directs specific targeting of CD gene expression to CEA-positive MKN45 tumor and

### Table 1 Serum biochemical data of animals with various treatment protocols

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<tr>
<th>Treatment groups</th>
<th>ALT*</th>
<th>AST*</th>
<th>Blood urea nitrogen*</th>
<th>Creatinine*</th>
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<tr>
<td>AdCEA-CD/5FC</td>
<td>46 ± 20</td>
<td>110 ± 16</td>
<td>18 ± 3</td>
<td>0.4 ± 0.2</td>
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<td>AdCA-CD/5FC</td>
<td>122 ± 22</td>
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<td>54 ± 24</td>
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<td>0.4 ± 0.2</td>
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<td>AdCA-CD/PBS</td>
<td>128 ± 88</td>
<td>221 ± 116</td>
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<td>AdCA-CD/5FC</td>
<td>39 ± 6</td>
<td>147 ± 19</td>
<td>19 ± 7</td>
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<td>Vehicle/PBS</td>
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<td>47 ± 15</td>
<td>110 ± 16</td>
<td>18 ± 3</td>
<td>0.4 ± 0.2</td>
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</table>

* Data expressed as the mean ± SD of five animals.

### Table 2 Dose responses of adenoviral vectors on treatment efficacy and toxicity

<table>
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<th>Treatment groups</th>
<th>Tumor reduction rate (%)</th>
<th>Increasing of ALT levels</th>
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<td>AdCA-CD (1010 pfu)/5FC</td>
<td>88</td>
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<td>1.3</td>
<td>±</td>
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<td>±</td>
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<td>±</td>
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<td>12</td>
<td>1.1</td>
<td>±</td>
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</table>

* Data were calculated as: [1 – (average tumor weight of treated animals/average tumor weight of untreated control animals)] × 100.

* Data were calculated as: average serum ALT value of treated animals/average serum ALT value of untreated control animals.

* Data were calculated as: average serum ALT value of treated animals/average serum ALT value of untreated control animals.
enhances the treatment to toxicity ratio in vivo. These data, together with beneficial therapeutic effects on tumor-bearing animals, support the potential clinical use of the i.p. AdCEA-CD/5FC system in the treatment of CEA-producing gastric cancer with peritoneal metastasis.

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