In Vivo Molecular Chemotherapy and Noninvasive Imaging With an Infectivity-Enhanced Adenovirus


Background: Adenovirus-based gene therapy is a promising approach to treat advanced cancers that are resistant to other treatments. However, many primary cells lack the requisite cox sackie-adenovirus receptor (CAR), limiting the in vivo efficacy of gene therapy. Recently, a modified adenovirus that is not dependent on CAR expression for infectivity was developed. We used noninvasive imaging to investigate the in vivo antitumor efficacy of gene therapy using this adenovirus in an animal model of ovarian cancer. Methods: The adenoviral vectors RGDTKSSTR (CAR-independent) and AdTKSSTR (CAR-dependent) express herpes simplex virus thymidine kinase (TK) for molecular chemotherapy and the human somatostatin receptor subtype 2 (SSTR) for noninvasive nuclear imaging. Subcutaneous or peritoneal human xenograft ovarian cancers were established from highly aggressive SKOV3.ip1 cells in immune-deficient mice. Adenoviral constructs were infected intratumorally or intraperitoneally once a day for 3 days. Control mice received three injections, one per day, of Ad5Luc1, a CAR-dependent adenoviral vector that includes a luciferase marker gene. The somatostatin analogue 99mTc-P2045 was used for noninvasive in vivo imaging of RGDTKSSTR that was injected into subcutaneous tumors. For mice with peritoneal tumors, survival was compared among the different treatment groups using Kaplan–Meier analysis with the log-rank statistic. All statistical tests were two-sided. Results: Tumor-associated RGDTKSSTR could be detected 15 days after introduction of the vector. In the subcutaneous model, tumors injected with RGDTKSSTR were statistically significantly smaller than those injected with AdTKSSTR (P<.001). In the intraperitoneal model, mice treated with RGDTKSSTR lived longer (survival at day 45 = 63.6%; 95% confidence interval [CI] = 35.2% to 92.0%) than those treated with AdTKSSTR (survival at day 45 = 45%) or Ad5Luc1 (survival at day 45 = 18.1%; 95% CI = 0.0% to 41.0%). Discussion: RGDTKSSTR shows antitumor efficacy against ovarian cancer in vivo in animal models. The virus can be imaged noninvasively and may have the potential to be a useful agent for treating ovarian cancer. [J Natl Cancer Inst 2002;94:741–9]

Adenovirus-mediated gene therapy is a promising treatment option for advanced cancers refractory to other modalities. Although there are some preliminary results that suggest clinical efficacy (1–3), breakthroughs in cancer treatment comparable with those seen with treatment of genetic or cardiovascular diseases (4–6) are still pending. Of note, in a randomized trial for patients with glioma (2), an adenoviral-based herpes simplex thymidine kinase/ganciclovir (TK/GCV) strategy resulted in statistically significantly improved overall patient survival. An adenovirus-TK/GCV protocol has been evaluated in phase I trials as a treatment strategy for other cancers, including ovarian cancer, with good safety data (7,8). Although these studies were not planned to test for efficacy, evidence of gene transfer was marginal, suggesting the need to modify the approach.

Efficient gene transfer or tumor transduction continues to be the central limitation for achieving an antitumor effect with adenoviral vectors. This limitation occurs despite bystander effects, in which neighboring cancer cells are killed although not infected by the virus associated with adenovirus-TK/GCV vectors. Adenovirus serotype 5 is most commonly used for gene therapy applications, and it binds to the cox sackie-adenovirus receptor. Although cox sackie-adenovirus receptor expression is ubiquitous on most epithelial tissues (9), expression is frequently lost in various primary cancer cells (10–21). Loss of expression may be associated with tumor aggressiveness (22–24) or with high activity of the Harvey rat sarcoma viral oncogene homolog (RAS)/mitogen-activated protein kinase (MAPK) pathway (23). Moreover, it has been suggested that reintroduction of cox sackie-adenovirus receptor expression into malignant cells could...
have tumor-suppressing activity, perhaps related to a role for the receptor in cellular adhesion (22,24). Because there is increasing evidence that coxackie adenovirus receptor level is the main factor determining the infectivity of tissues with adenoviruses (14,16,20,21,25–29), the lack of expression on primary tumor tissue may have contributed to the unimpressive results achieved in many clinical trials.

For physicians, ovarian cancer continues to be a challenging disease to treat. Symptoms of early disease are often inconspicuous, and thus most patients present with advanced disease, for which 5-year survival rates are approximately 30%. Ovarian cancer spreads preferentially throughout the peritoneal cavity, which provides the rationale for local treatment. Local peritoneal adenoviral treatments have proven feasible and are well tolerated by patients in clinical trials (7,30–32). Unfortunately, ovarian cancer is similar to many other cancers in that coxackie-adenovirus receptor expression levels vary widely and are often low (10–13). Therefore, it has not been surprising that clinical trials with various adenoviral vectors have detected the presence of the vector in the peritoneal cavity, with limited evidence of transduction of tumor masses (7,30–32). A contributing factor to the low transduction efficiency could be that most cases of ovarian cancer are associated with the formation of malignant ascites, which often contains anti-adenovirus neutralizing antibodies (33–35).

One promising approach to improve transduction efficiency and overcome dependence on the coxackie-adenovirus receptor is genetic modification of the adenovirus fiber with an arginine–glycine–aspartic acid (RGD-4C) motif (10). Adenoviruses enter cells by first binding the coxackie-adenovirus receptor with the C-terminal portion of the fiber, called the knob. Binding is followed by the interaction of an RGD motif present at the base of the fiber (in the penton protein) with α,β class integrin receptors on the cell surface. The RGD-4C fiber knob modification allows the virus to use cellular integrins to bind and internalize, thus, circumventing the dependence on the coxackie-adenovirus receptor. α,β class integrins are regularly expressed and frequently overexpressed in ovarian cancers (11,36,37) and thus the RGD-4C modification results in a dramatic increase in transduction efficiency for ovarian cancer (10,12,35) and other tumor types (15,21,38,39). Importantly, the RGD-4C modification allows partial escape from pre-existing neutralizing antibodies (33,35). Although these modified adenoviral vectors have shown promise in vitro, no in vivo results have been available heretofore.

Central questions in the evaluation of the safety and efficacy of a gene therapy agent include the level, persistence, and location of transgene expression. For most cancers, the collection of repeated biopsy specimens is not a practical option. However, the use of noninvasive imaging, in which the treatment vector includes a second transgene encoding a receptor that can be detected with a radiolabeled ligand administered intravenously, permits consecutive assessments to be made. One such receptor, effective in noninvasive imaging, is the human somatostatin receptor subtype 2 (SSTR), the expression of which can be followed by the intravenous administration of radiolabeled somatostatin analogs (40,41). Previously, we reported the construction of RGDTKSSTR, an RGD-4C infectivity-enhanced adenoviral vector, containing two transgenes (TK and SSTR), each expressed from a cytomegalovirus (CMV) immediate early promoter (35). Furthermore, by comparison with an isogenic non-RGD-4C control virus (AdTKSSTR), we demonstrated the functionality of both transgene products and investigated in vitro transgene expression, imaging, and killing of ovarian cancer cell lines and of primary cancer cells (35).

In this article, we report our testing of the imaging and therapeutic effects of RGDTKSSTR in animal models of ovarian cancer that incorporate the potentially neutralizing effect of ascites. These preclinical findings set the stage for clinical testing of the vector.

**Materials and Methods**

**Cell Lines**

This study used the ovarian cancer cell lines Hey (from Timothy J. Eberlein, Harvard Medical School, Boston, MA) and SKOV3.ip1 (from Janet Price, The University of Texas M. D. Anderson Cancer Center, Houston). Human embryonic kidney 293 (293) cells were acquired from Microbix (Toronto, Canada). Hey cells were grown in RPMI-1640 (Mediatech, Herndon, VA), whereas SKOV3.ip1 and 293 cells were grown in Dulbecco’s Modified Eagle Medium/F12 (Mediatech). All culture media were supplemented with 2 nM α-glutamine, 100 IU/mL penicillin, 25 μg/mL streptomycin, and 10% fetal bovine serum (FBS; Hyclone Laboratories, Logan, UT). Cells were maintained in a humidified 37 °C atmosphere containing 5% CO2.

**Viruses**

Construction of RGDTKSSTR and AdTKSSTR has been described (35). A shuttle vector was constructed in which the SSTR and TK genes were placed between a CMV promoter and a simian virus 40 (SV40) polyadenylation signal. Each gene had separate control elements. The shuttle vector was cotransfected into Escherichia coli cells for homologous recombination with an E1-deleted rescue plasmid containing an RGD-4C modified (RGDTKSSTR) or unmodified (AdTKSSTR) fiber gene. The E1 deletion renders the adenoviruses’ replication incompetent. Then, recombinants were selected and propagated in 293 cells. Viral particles (VP) were purified on CsCl gradients by standard methods, and their concentration was determined by spectrophotometry at 260 nm using a conversion factor of 1.1 × 1012 VP/absorbance unit. Functional particles (plaque-forming unit [PFU]) were determined by a modification of the standard plaque assay, where 293 cells were infected overnight to ensure entry of all functional virions and to avoid confounding by receptor density. This protocol allowed us to use the PFU titer (functional virions) for dosing. Resulting titers for RGDTKSSTR were 1.1 × 1012 VP/mL and 2.6 × 1010 PFU/mL; for AdTKSSTR, the resulting titers were 1.0 × 1012 VP/mL and 5.3 × 1010 PFU/mL. AdsLuc1 is a replication-incompetent adenovirus that encodes the luciferase marker gene (42). AdsLucRGD is an RGD-4C fiber-modified isogenic version of AdsLuc1 (10). AdsLuc1 and AdsLucRGD viruses were propagated on 293 cells, purified by CsCl gradients, and titered as above.

**Radiolabeling and Imaging**

For imaging, 10⁷ Hey cells were injected into each flank of 7- to 8-week-old female CD1-NU athymic mice (Charles River Laboratories, Wilmington, MA). When the tumor surface area was approximately 10 mm² (11 days after tumor cell inoculation), the mice were randomly assigned to one of three groups (each containing three mice) and given two daily injections of
5 x 10^8 PFU of adenovirus (days 1 and 2). Each injection used three needle tracts introducing the virus into separate areas to maximize dissemination within the tumor. Groups 1 and 2 were injected with the RGDTKSSTR adenoviral vector, and group 3 was injected with the Ad5Luc1 control adenoviral vector. GCV (Roche Laboratories, Nutley, NJ) was diluted in OptiMEM (Media Preparation Shared Facility, University of Alabama at Birmingham), and mice in groups 1 and 3 were given daily intraperitoneal injections (50 mg/kg/day) on days 4–17.

P2045, a somatostatin analogue (1450 Da), was obtained from Diatide Research Laboratories (Londonderry, NH). Fifty micrograms of P2045, 0.1 mg of EDTA, 10 mg of glucoheptonate, and 100 μg of SnCl2 dihydrate were mixed with 1 mL of saline containing 1.9 GBq of 99mTcO4- (University of Alabama at Birmingham Central Pharmacy), and the mixture was boiled for 10 minutes. The radiolabeled P2045 peptide was analyzed by instant thin-layer chromatography analyses, in which the instant thin-layer chromatography silica gel strips (Gelman Sciences, Ann Arbor, MI) were spotted with the radiolabeled P2045 peptide at one end and eluted with saturated saline, methyl ethyl ketone, or a 50:50 mixture of methanol and ammonium acetate (1 M) as the mobile phase. The 99mTc-P2045 migrated only with the mixture of methanol and ammonium acetate. The radiochemical purity of the 99mTc-P2045 averaged 97 ± 3% for the imaging experiments, with a mean specific activity of 33 ± 2 MBq/μg. Chemicals were obtained from Fisher Scientific (Pittsburgh, PA) unless otherwise noted.

On days 4, 8, and 15 after adenovirus injection, each mouse was given an intravenous injection of 99mTc-P2045 (22.9 ± 2.5 MBq/mouse). After 5 hours, the mice were anesthetized with enfurane gas before the imaging. The mice were positioned with their dorsal surface adjacent to and beneath the pinhole collimator (model PHW; Ohio Nuclear, Solon, OH), which was attached to an Anger Sigma 410 Radiosotope Gamma Camera (Ohio Nuclear). Images (Fig. 1) were collected with the use of a Numa Station (Nuna, Amherst, NH). The planar static images were collected for 1–15 minutes per image. Images were processed with a modified version of NIH Image (NucMed_Image, Mark D. Wittry, St. Louis University, MO) using standard region of interest (ROI) analyses (40,41). The ROI included the entire animal and the tumor regions. Background regions for each image and imaging time were taken from an area that did not include the region occupied by each mouse. The program converts radioactivity to pixels, allowing detection of areas that contain the tracer. The mean activity in counts per pixel was recorded for all regions. The mean number of counts per pixel for the background region was subtracted from the mean number of counts per pixel in the body and tumor ROI. The data were then adjusted to normalize for the time elapsed after injection (and subsequent reduction in activity), and the percentage of doses in the tumor regions were calculated based on the initial dose. Stock solutions and syringses (before and after injection) were assayed using an Atomlab 100 dose calibrator (Biodex Medical Systems, Shirley, NY).

**Biodistribution Analysis**

For the biodistribution analysis, mice were killed after the third imaging session, the tumors and normal tissues were collected and weighed, and the amount of 99mTc-P2045 in each sample was measured in a Minaxi Auto-Gamma 5000 series gamma counter (Packard, Downers Grove, IL). Kidneys were not analyzed due to confounding by renal excretion of the tracer. The raw count rate data from the gamma counter were adjusted to account for the rate of decay from the time of injection. The precise injected dose was calculated on the basis of the difference of each injection syringe, measured before and after each injection. In this manner, the percentage of the dose injected per organ and the percentage of the dose injected per gram of tissue were calculated.

**Marker Gene Assays**

To determine the infectivity of Hey cells with Ad5Luc1 and Ad5LucRGD, 80,000 cells/well were plated in 24-well plates. After an overnight incubation, the cells were infected for 1.5 hours at a concentration of 0, 5, 20, and 200 PFU/cell. Cells were then washed with phosphate-buffered saline (PBS), and infection medium was replaced by complete growth medium. The luciferase...
assay was performed 24 hours later according to the protocol recommended by the manufacturer (Luciferase Assay System; Promega, Madison, WI). Results are displayed as the mean of five experiments, and error bars indicate 95% confidence interval (CI).

Murine Models for Treatment of Ovarian Cancer

For the subcutaneous model of ovarian cancer, flank tumors (one tumor per mouse) were established by subcutaneous injection of 10^7 SKOV3.ip1 cells into CD1-NU athymic mice. When tumors were considered large enough for reliable intratumoral injection of adenovirus (after approximately 7 days, when the tumors had an average diameter of 7 mm), 10^8 PFU of adenovirus was diluted with PBS in a volume of 10 µL and then mixed with 20 µL of pooled ascites. The ascites samples were obtained from patients receiving treatment for pathologically confirmed ovarian adenocarcinoma, according to an Institutional Review Board-approved protocol. On three consecutive days (from day 1), RGDTKSSTR (n = 8 mice), AdTKSSTR (n = 8 mice), Ad5Luc1 (n = 6 mice), or PBS (n = 6 mice) was injected intratumorally with three needle tracts per injection. A 14-day intraperitoneal GCV treatment (50 mg/kg/day) regimen was begun 48 hours after the last virus injection. The tumors were measured every three days with calipers, and the mean of the longest and shortest diameters was calculated and plotted as the average tumor size.

An orthotopic model of intraperitoneally spread ovarian cancer was created by injecting 10^7 SKOV3.ip1 cells into the peritoneal cavity of severe combined immunodeficient (SCID) mice (University of Alabama at Birmingham Center for AIDS Research SCID Mouse Core Facility). Two, 3, and 4 days later, 3 x 10^8 PFU of RGDTKSSTR (n = 11 mice), AdTKSSTR (n = 11), Ad5Luc1 (n = 11), or no virus (n = 8) was mixed with 40 µL of pooled ascites from ovarian cancer patient samples and diluted in OptiMEM (Mediatech) to a total volume of 1.5 mL/mouse. A 14-day intraperitoneal GCV treatment (50 mg/kg/day) regimen was begun 48 hours after the last virus injection. Mice were followed daily and killed when there was any evidence of pain or distress in accordance with Institutional Animal Care and Use Committee guidelines.

Statistical Analysis

To statistically compare groups in the ROI and biodistribution assays, preplanned two-way analysis of variance was performed by fitting the data into the general linear model with the Statistical Analysis System (SAS) version 8 (SAS Institute, Cary, NC). A log transformation was used to stabilize the variance when means were highly different and when the coefficient of variation was constant among the groups.

The analysis of the tumor size data was performed using a repeated measures growth model with PROC MIXED (SAS version 8.02), which treated the within-mouse effect of time as a continuous variable and the treatment group as a fixed effect. The observed curvature in the tumor growth data by time across the four treatment groups was included in the model as a quadratic term. The compound symmetry structure was used as the covariance matrix. The effects of treatment group, time, and the interaction of treatment group and time were evaluated by F tests. The differences in predicted treatment means at each time interval were compared with a t test using the Tukey–Kramer adjustment. Survival data were plotted on a Kaplan–Meier curve, and the LIFETEST procedure in SAS version 8.2 was used for log-rank calculations. For all analyses, a two-sided P value of <.05 was considered statistically significant.

RESULTS

Noninvasive Imaging of RGDTKSSTR In Vivo

Clinical ovarian cancers typically express low amounts of the coxsackie-adenovirus receptor. To develop a comparable animal model, we investigated the infectivity of Hey cells (Fig. 2, A). Infection of cells with 5, 20, and 100 PFU/cell of Ad5LucRGD resulted in 27-fold, 19-fold, and 41-fold higher luciferase expression levels, respectively, than did infection of cells with Ad5Luc1. This is in accord with previous findings, suggesting that the infectivity of cells expressing low levels of the coxsackie-adenovirus receptor can be improved with RGD-4C modification of the fiber (10, 12, 15, 21, 35, 38, 39).

An important aspect of the next generation of adenoviral vectors, such as RGDTKSSTR, is the possibility of noninvasive detection of gene expression. Imaging of subcutaneous Hey tumors injected with RGDTKSSTR was performed on days 4, 8, and 15 with a gamma camera (Fig. 1). Although treatment with GCV reduced binding of the tracer in tumors treated with RGDTKSSTR, somatostatin receptor expression could be detected at day 8 (Fig. 2, B) and, in some cases, up to day 15 (Fig. 1). In ROI analyses, tumors treated with RGDTKSSTR/GCV+ and RGDTKSSTR/GCV– bound more radiotracer than did those treated with the Ad5Luc1/GCV+ control (day 4: P<.001 and P<.001, respectively; day 8: P = .002 and P = .005, respectively). On day 15, tumors treated with RGDTKSSTR/GCV– bound more of the radiotracer than did the other groups (P = .027). Longer time points were not investigated because of tumor burden.

Biodistribution Analysis of 99mTc-P2045 in Tumor-Bearing Animals

Next, we determined the biodistribution of the radiotracer following intratumoral adenoviral treatment. The mice were killed after 15 days, the organs were collected, and the amount of radiotracer, normalized to the organ weight, was determined using a gamma counter (Fig. 2, C). The amount of 99mTc-P2045 bound to the tumor was not statistically significantly different among tumors treated with RGDTKSSTR/GCV+ or RGDTKSSTR/GCV– (P> .05). Both groups of RGDTKSSTR-treated tumors bound more of the radiotracer than did the control group (P = .008 and P = .023, respectively). Less than 1% of the total tracer dose bound to the tumor (Fig. 2, B). Therefore, most of the 99mTc-P2045 was excreted or detected in other organs (Fig. 2, C). Regardless of whether the mice were treated with GCV, the patterns of 99mTc-P2045 distribution were similar among the various groups. The highest concentration of 99mTc-P2045 was detected in the lungs, and the next highest, in the large intestine. The lowest concentration of 99mTc-P2045 was detected in the brain. Compared with mice treated with Ad5Luc1/GCV+, mice treated with RGDTKSSTR/GCV– had increased binding of 99mTc-P2045 to the liver, cecum, and spleen (P = .040, P = .030 and P = .012, respectively).
RGDTKSSTR or control vectors were mixed with pooled malignant ascites and injected intratumorally into established subcutaneous tumors consisting of the highly aggressive, treatment-resistant SKOV3.ip1 cells (Fig. 3). A transient antitumor effect was seen with RGDTKSSTR but not with AdTKSSTR. To compare differences among the groups, observations on measurements of tumor size were fit into a statistical model. A repeated measures model showed a statistically significant effect of time ($P < .001$), treatment group ($P < .001$), and an interaction of time and treatment group ($P < .001$) on tumor size. By day 29, the predicted mean size of the tumors injected with RGDTKSSTR was statistically significantly smaller than that of control tumors injected with PBS ($95\%$ CI for model-predicted difference in tumor size = $0.001590$ to $1.1918$ mm; $P = .049$), those injected with...
Ad5Luc1 (95% CI for model-predicted difference = 0.9947 to 2.1849 mm; \( P < .001 \)), and those injected with AdTKSSTR (95% CI for model-predicted difference = 0.1134 to 1.2295 mm; \( P = .003 \)).

**RGDTKSSTR in an Intrapertitoneal Model of Ovarian Cancer**

We also tested the efficacy of RGDTKSSTR in an orthotopic model of disseminated ovarian cancer, established by intraperitoneal inoculation of ovarian cancer cells. To simulate human intraperitoneal conditions and treatment protocols, the adenoviruses were mixed with pooled malignant ascites obtained from ovarian cancer patients and were injected into the peritoneal cavity (Fig. 4). Because it was not feasible to measure tumor burden in this model, we followed the overall survival of the mice. By comparison with the other groups, the overall survival of mice treated with RGDTKSSTR was statistically significantly improved (log-rank test \( P < .001 \)). By day 35, none of the 11 mice treated with RGDTKSSTR had died (survival = 100.0%; 95% CI = 100.0% to 100.0%), whereas two of the 11 mice treated with Ad5Luc1 (survival = 81.8%; 95% CI = 59.0% to 100.0%), five of the eight mice injected with medium without virus (survival = 37.5%; 95% CI = 4.0% to 71.0%), and 10 of the 11 mice treated with AdTKSSTR (survival = 9.0%; 95% CI = 0.0% to 26.1%) had died. By day 45, four of the 11 mice treated with RGDTKSSTR had died (survival = 63.6%; 95% CI = 35.2% to 92.0%), whereas nine of the 11 mice treated with Ad5Luc1 (survival = 18.1%; 95% CI = 0.0% to 41.0%), seven of the eight mice injected with medium without virus (survival = 12.5%; 95% CI = 0.0% to 35.4%), and all of the mice treated with AdTKSSTR (survival = 0.0%) had died. By day 55, all mice had died, with the exception of two mice (18%) treated with RGDTKSSTR. These two mice were still alive and healthy, without evidence of peritoneal cancer or ascites, at the end of the experiment (day 72).

**DISCUSSION**

Gene therapy is a promising treatment for malignant diseases, such as advanced ovarian cancers, that are refractory to more conventional modalities. Adenoviruses are emerging as the vector of choice because of their high capacity to transfer genes to cycling and dormant cells, their in vivo safety and stability, and the ease of high titer production (43,44). Adenoviral vectors have been used as gene delivery vehicles in five ovarian cancer trials (7,8,30–32). Although two of the trials used the Ad-TK approach (7,8) and both were phase I trials not specifically designed to test efficacy, none of the trials reported any evidence...
of clinical response. Furthermore, it is unclear whether cancer cells were transduced. The vector was detected in ascites (7,30,32). Only in one study were biopsy specimens collected; however, normal cells were not separated from cancer cells (31). Therefore, despite promising preclinical data in vitro and in animal models, there is no conclusive evidence that adenoviral-based clinical gene delivery strategies are efficacious for ovarian cancer. Because coxsackie adenovirus receptor levels are often low in primary ovarian and other tumor cells (10–21), improving the ability of the vectors to infect cancer cells may be required to achieve a clinical benefit.

The RGD-4C modification allows increased gene transfer into ovarian and other cancer cells in vitro (10,12,15,21,35,38,39). Our results are the first to show a therapeutic benefit in vivo. With the use of stringent subcutaneous (Fig. 3) and orthotopic (Fig. 4) models, we found the effect of RGDTKSSTR improved over that of controls, including the isogenic non-RGD-4C virus (AdTKSSTR), which had no effect in the orthotopic model. In the subcutaneous model, we also observed a small antitumor effect with AdTKSSTR, a virus that does not effectively transduce cells that express low levels of the coxsackie adenovirus receptor, such as SKOV3.ip1 cells (42). However, this virus could produce an antitumor effect by infecting and killing infiltrating nonmalignant stromal cells, perhaps helped by the high local concentrations of virus resulting from intratumoral injection. Although RGDTKSSTR was superior to controls in both animal models, it can be argued that the therapeutic effect was relatively modest. However, it should be noted that the orthotopic SKOV3.ip1 SCID mouse model is extremely resistant to treatment, and no cures have been previously reported (45,46). Thus, it is possible that modest therapeutic advances in this model could be amplified in immune-competent hosts with slower growing tumors, such as most human ovarian cancers.

For accurate analysis of the success of a novel approach, it is crucial to be able to determine which cells are infected. We used an adenoviral vector that contained the gene for the SSTR. This receptor can be used for noninvasive imaging of gene transfer in vivo with positron emission tomography (PET), single photon emission computerized tomography (SPECT), or gamma camera-based methods. $^{99m}$Tc-P2045 is closely related to the somatostatin analogs Neotec$^{TM}$ (Berlex, Richmond, CA) and Octreoscan$^{TM}$ (Mallinckrodt, Hazelwood, MO), which are approved by the U.S. Food and Drug Administration for use in patients. Although successful in vitro and in vivo imaging of adenovirus-based SSTR expression has been reported before in a variety of disease models (35,40,41), to our knowledge, this is the first time that an infectivity-enhanced virus has been imaged in vivo. Using SSTR for noninvasive imaging may improve the quality and quantity of correlative gene transfer data that can be obtained from clinical trials. In most trials, collection of multiple biopsy specimens is limited for practical reasons, which has led to an unsatisfying level of evidence on gene transfer into tumor tissue. Furthermore, there is a lack of longitudinal studies on the persistence and dynamics of gene expression. On the basis of our results with mice and the availability of clinical grade somatostatin analogs, RGDTKSSTR could allow noninvasive imaging in humans, thus improving the quality of correlative studies. Further improvements may involve using PET or SPECT, which have higher sensitivity than a gamma camera.

We were able to image SSTR expression conferred by RGDTKSSTR for 15 days in a murine model of ovarian cancer (Fig. 1). Hey cells resemble typical primary ovarian cancer cells in that they express a low level of the coxsackie adenovirus receptor, making them resistant to infection by unmodified adenoviruses but susceptible to infection by RGD-4C modified adenoviruses (Fig. 2). Conceivably, imaging could be compromised if the GCV treatment kills the cells expressing SSTR. In our experiments, detection of RGDTKSSTR in vivo was affected by, but not prevented by, GCV treatment, and SSTR expression could still be noninvasively detected at 8 days and at 15 days with a gamma counter (Fig. 2).

It is of interest that, by comparison with mice treated with Ad5Luc1, mice treated with RGDTKSSTR had increased binding of $^{99m}$Tc-P2045 to a variety of organs (Fig. 2, C). This could be the result of virus leaking from the tumor into the bloodstream and subsequently infecting other tissues. It is unclear what relevance this finding has for ovarian cancer patients. In completed trials, severe toxicity associated with adenoviral vectors has been rare (7,8,30–32) and there has been no clear evidence of clinically significant organ damage. Intraperitoneal administration of the adenovirus allows a degree of compartmentalization that may have helped reduce toxicity. Regardless, because RGDTKSSTR may have the potential to infect normal cells more effectively, toxicity should be monitored carefully in clinical trials.

All ovarian cancer adenoviral gene therapy trials published thus far have relied on intraperitoneal administration of the vector (7,8,30–32). Because ovarian cancer patients often present with malignant ascites (47) that contain anti-adenovirus antibodies (33–35), the ability of RGD-4C-modified adenoviruses to partially escape pre-existing neutralizing antibodies (33,35) may have contributed to the therapeutic efficacy seen in our study. Recently, advances in vector design have resulted in therapeutic benefits for patients with hemophilia, severe combined immune deficiency, and cardiovascular diseases (4–6). It remains to be seen whether improvements in adenoviral vector development, such as presented here, will help produce similar results in cancer gene therapy.

In conclusion, we used a novel infectivity-enhanced adenovirus with TK and SSTR transgenes for molecular chemotherapy and noninvasive imaging. With the use of a gamma camera, we could detect the virus for more than 2 weeks in a stringent ovarian cancer animal model. RGD-4C is well established as an infectivity enhancement motif for various types of human cancer in vitro, and we found that it conferred a therapeutic advantage in two animal models. This study is the first demonstration that the RGD modification can increase the in vivo effect of the TK/GCV approach. These results suggest that RGDTKSSTR may have the potential to be a useful agent for the treatment of ovarian cancer.

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NOTES

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