In Situ Prostate Cancer Gene Therapy Using a Novel Adenoviral Vector Regulated by the Caveolin-1 Promoter

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ABSTRACT

Caveolin-1, a structural component of caveolae, is overexpressed in metastatic and androgen-resistant prostate cancer and highly expressed in tumor-associated endothelial cells. The mouse cav-1 promoter was cloned and placed upstream of the HSV-tk gene in an adenoviral vector (Adcav-ltk) and compared with a cytomegalovirus (CMV) or Rous sarcoma virus (RSV) promoter-driven HSV-tk, AdCMVtk and AdRSVtk vectors, respectively. Mouse and human prostate cancer cells and mouse endothelial cells were infected with Adcav–ltk, AdCMVtk or control vectors without the HSV-tk gene (Adcav-1 and AdCMV) and subsequently treated with ganciclovir (GCV). GCV-mediated cytotoxicity induced by the Adcav-ltk vector was comparable to that for AdCMVtk in multiple mouse and human prostate cancer cell lines. To evaluate the activity of Adcav-ltk in vivo, orthotopic mouse prostate cancer tumors were generated with RM-9 cells and injected in situ with Adcav-ltk, AdCMVtk, AdRSVtk, or AdCMVBgal (control) and treated with GCV. All three HSV-tk transducing vectors produced statistically significant reductions in wet weight and increased apoptotic indices compared with the control vector. However, only Adcav-ltk produced significant necrosis, and only Adcav-ltk and AdRSVtk caused significant decreases in microvessel density. In conclusion, Adcav-ltk demonstrated efficacy in vitro and in vivo in preclinical models of prostate cancer. Our results suggest that the cav-1 promoter may have unique benefits in targeting gene therapy to prostate cancer and its associated vasculature.

INTRODUCTION

Prostate cancer is the most commonly occurring malignancy and the second leading cause of cancer deaths in men (1). At present, therapy with surgery or irradiation is aimed primarily at curing localized disease. However, despite improvements in early detection and aggressive treatment of localized disease, the mortality rate remains high (1). This is likely a result of the presence of micrometastatic disease in bone or lymph nodes at the time of treatment, which ultimately leads to further disease progression. Once prostate cancer becomes metastatic, there is no curative therapy, only palliation; therefore, novel strategies aimed at treating metastatic disease are probably necessary to have significant impact on the mortality rate of prostate cancer.

In situ suicide gene therapy using HSV-tk1 plus GCV is an experimental therapy for prostate cancer pioneered by our group in preclinical studies that have been extended into several ongoing clinical gene therapy trials. In our initial studies, we documented significant in vitro cytotoxicity in both mouse and human prostate cancer cells by adenoviral vector-mediated transduction of the HSV-tk gene under the control of a RSV promoter followed by treatment with GCV (2). We also used specific in vivo model systems to establish the safety (3) and efficacy of this therapeutic approach for localized disease (2, 4, 5).

One general limitation of in situ gene therapy is its incapacity to effectively transduce all of the cancerous cells within a solid organ. This is particularly important for prostate cancer because multiple tumor foci are commonly associated with this disease (6, 7). However, certain forms of gene therapy, such as with HSV-tk+GCV, have a “bystander effect” in which nontransduced cells in the adjacent area are also killed and thus the biological cytotoxic effect of gene therapy is enhanced. The mechanism of the bystander effect has been attributed to the diffusion of toxic GCV metabolites via GAP junctions to adjacent cells (8, 9), enhanced immune responses (9 –11), or direct cytotoxicity to endothelial cells, resulting in reduced tumor vasculature (12). Although bystander effects have been more extensively documented as localized activities, distant bystander effects that extend to experimentally induced metastatic disease have been reported (4, 9).

Interestingly, we documented HSV-tk+GCV-mediated antimetastatic effects under some conditions in an in vivo prostate cancer model (4). The results of many studies, including those from our laboratory, are consistent with an HSV-tk+GCV-

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1 The abbreviations used are: HSV, herpes simplex virus; tk, thymidine kinase; GCV, ganciclovir; RSV, Rous sarcoma virus; CMV, cytomegalovirus; cav-1, caveolin-1; MPR, mouse prostate reconstitution; FBS, fetal bovine serum; m.o.i., multiplicity of infection; pfu, plaque-forming unit(s); TUNEL, terminal deoxynucleotidyltransferase-mediated uridine 5'-triphosphate nick-end labeling; AI, apoptotic index.

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mediated antitumor immune response (10–13). More recently, we have developed more direct, cytokine-based immunostimulatory in situ gene therapy approaches to provide novel and effective treatments for localized and metastatic prostate cancer (14–16). In the first in situ gene therapy clinical trial for prostate cancer, we confirmed the relative safety of a single (17) or multiple and repeated (18) intraprostatic injections of an HSV-1tk gene therapy approach in prostate cancer patients failing radiotherapy. We hypothesized that the cav-1 promoter would provide a unique level of tissue selectivity and could enhance the efficacy of HSV-1tk+GCV gene therapy by maximizing local necrosis and enhancing the bystander effect. To test these hypotheses, we constructed a replication-defective adenoviral vector in which the HSV-1tk gene was transcriptionally controlled by the cav-1 promoter. We compared this promoter with either the RSV long terminal repeat promoter or the CMV promoter, both of which are widely used, constitutively active promoters. In this preclinical study, we document that the cav-1 promoter is generally as effective as the CMV promoter in vivo in prostate cancer and in endothelial cell lines that express variable amounts of cav-1. Using an in vivo orthotopic prostate cancer model, we also show that the cav-1 promoter may have unique advantages in maximizing the bystander activities of HSV-1tk+GCV gene therapy in vivo.

**MATERIALS AND METHODS**

**Cell Lines.** The mouse prostate cancer cell line RM-9 was derived from a primary prostate tumor induced in the Zipras/myc-9-infected MPR model system in C57BL/6 mice as described previously (32, 33). The cell lines 148-1 PA and 148-1 LMD were derived from a p53 nullizygous MPR from a primary tumor or a lung metastasis, respectively, as described previously (19). The 178-2 PA and 178-2 BMA cells were also derived from a p53 nullizygous MPR in an animal with a primary prostate tumor and a bone metastasis (34). All mouse prostate cell lines were grown in DMEM with 10% FBS, 10 mm HEPES, penicillin (100 IU/ml), and streptomycin (100 mg/ml); passaged by trypsinization with 0.025% trypsin; and maintained with routine changes of medium. The SV40-transformed mouse endothelial cell lines MS1 and SVEC4-10 were derived from mouse endothelial pancreatic islet cells and endothelial lymph nodes, respectively, and were purchased from American Type Tissue Culture (Rockville, MD). The MS1 cells were grown in DMEM with 4.5 g/L glucose, 4 mm L-glutamine, 1.5 g/L sodium bicarbonate, 1.0 mm sodium pyruvate, and 5% FBS. SVEC4-10 cells were grown in DMEM with 4.5 g/L glucose and 10% heat-inactivated FBS.

The human prostate cancer cell lines PC-3 and LNCaP, which were established from organ metastases, were obtained from American Tissue Type Culture Collection and were cultured as described previously (35). The human prostate cancer cell line ND-1 (36) was obtained from Dr. R. Dahiyet University of California, San Francisco, CA) and cultured in DMEM with 10% FBS. All chemicals for cell culture were obtained from Life Technologies, Inc. (Gaithersburg, MD).

**Western Blot Analysis.** Protein lysates were separated by 12% SDS-PAGE and transferred to nitrocellulose membranes. The membranes were blotted with rabbit polyclonal cav-1 antibody (Santa Cruz Biotechnology, Santa Cruz, CA) and mouse monoclonal β-actin antibody (Sigma, St. Louis, MO) for 12–18 h at 4°C with shaking. The membranes were washed and then reacted with rabbit antimouse sera (Dako Corp., Carpinteria, CA) followed by horseradish peroxidase-conjugated goat antirabbit sera (ICN/CAPPEL, Aurora, OH). Binding was detected by the super Signal enhanced chemiluminescence method (Pierce, Rockford, IL).

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Adenoviral Vectors. The HSV-tk gene was packaged in replication-defective recombinant adenoviral vectors under the transcriptional control of either the RSV long terminal repeat (AdRSVtk), the human CMV promoter (AdCMVtk), or 721 bp of the mouse cav-1 promoter (Ref. 27; Adcav-1tk), as depicted in Fig. 1. The AdRSVtk vector has been used previously in mouse models of prostate cancer (2, 4, 5, 16). Adenoviral vectors without the HSV-tk gene were constructed from either the CMV promoter (AdCMV) or the cav-1 promoter (Adcav-1) constructs and were used as controls. Another control vector, AdCMVβgal, with the bacterial β-galactosidase gene under the control of the CMV promoter was packaged in a replication-defective recombinant adenovirus as described previously (37). All recombinant adenoviruses were isolated from a single plaque, expanded in 293 cells, and purified by double cesium gradient ultracentrifugation (38). Virus titers were determined by plaque assay on 293 cells.

In Vitro Cytotoxicity. An in vitro cytotoxicity assay was performed essentially as described previously (2). Plates (24-well) were seeded with 2.5 × 10^5 cells/cm² in triplicate in regular medium with 10% FCS for 24 h. The cells were then infected at increasing m.o.i. (12.5, 25, 50, 100, and 200) with a viral vector (Adcav-1tk or AdCMVtk) or a control vector (Adcav-1 and AdCMV) in serum-free medium (regular medium containing 0.1% BSA). After 3 h, the serum-free medium was replaced with medium containing 10% FCS. Twenty-four h later the cells were treated with medium containing GCV, at a concentration of 10 μg/ml, or PBS. After further incubation for 72 h, the cells in each well were trypsinized, and surviving adherent cells were counted with a Coulter counter. In all cases, >90% of counted cells were viable as assessed by trypan blue exclusion. The percentage of survival was then calculated from the mean cell count divided by the mean cell count in cells infected with AdCMV at the same m.o.i. and treated with PBS. This controlled for any nonspecific adenoviral toxicity.

In Vivo Orthotopic Tumor Model. Following trypsinization, RM-9 cells were counted and resuspended in HBSS. C57BL/6 mice were anesthetized with sodium pentobarbital, shaved, and opened with a low transverse abdominal incision, exposing the dorsolateral prostate. Injection of 5000 cells in 10 μl directly into the right or left lobe of the dorsolateral prostate resulted in efficient and reproducible orthotopic tumor formation. Adenoviral vectors (5 × 10⁹ pfu of AdRSVtk, AdCMVtk, Adcav-1tk, or AdCMVβgal) were injected directly into established tumors on day 6 postinoculation. The mice were then randomized into treatment groups. Before viral injection, each tumor was measured with a vernier caliper, and volume was calculated. Beginning the following day, i.e. injections of GCV (10 mg/kg) were administered twice daily for 6 days. Animals were sacrificed on day 14 after tumor inoculation (day 8 post-virus injection). Primary tumors were excised, a wet weight was obtained, and a portion of the tumor was placed in formalin for standard histological analysis. The experiment was repeated twice independently.

Histological Analysis. Formalin-fixed, paraffin-embedded sections were stained with H&E for routine analysis. To determine apoptotic activities, sections of primary tumor tissue were prepared for staining for DNA fragmentation by the TUNEL technique (39). The extent of apoptosis was assessed quantitatively via computer-assisted image analysis of 15–20 microscopic fields at ×200 magnification. The data were recorded as apoptotic bodies per 1000 cancer cells (defined as AI). The extent of necrosis was also assessed quantitatively on H&E-stained slides via computer-assisted image analysis and recorded as percentage of necrosis. Microvessel density was determined by anti-factor VIII antibody staining and expressed as number of vessels per high-power field.

Statistical Analysis. An unpaired t test was used to compare tumor wet weight, apoptotic indices, percentage of necrosis, or microvessel density for each test vector with control vector. ANOVA using Fisher’s protected least significant difference was performed using Statview 4.02 (Abacus Concepts, Berkeley, CA).

RESULTS

In Vitro Cytotoxicity. To test the comparative cytotoxicity of Adcav-1tk, we used a panel of mouse and human prostate cancer cell lines and mouse endothelial cell lines that express variable levels of cav-1 (Fig. 2) in a series of in vitro assays (Figs. 3–5). Cells were infected with increasing concentrations of vector and grown with or without GCV for 3 days. Control vectors were identical vector promoter constructs lacking the HSV-tk gene, Adcav-1, or AdCMV. Uninfected cells were also tested with and without GCV treatment. In general, surviving cell numbers were not significantly altered by treatment with the control vectors, either with or without GCV, or by the treatment vectors, Adcav-1tk and AdCMVtk, without GCV. However, Adcav-1tk+GCV and AdCMVtk+GCV significantly reduced cell survival.

In the mouse prostate cancer cell line RM-9 (ras+myc

Fig. 1 Schematic representation of adenoviral constructs. All adenoviral vectors were generated in 293 cells as described previously (2).

Fig. 2 Western blot analysis of cav-1 expression in cell lines used in this study. Equivalent loading was confirmed by simultaneous analysis of β-actin levels.
transformed, wild-type p53), Adcav-1 tk+GCV treatment was less cytotoxic over the range of vector doses compared with AdCMV tk+GCV (Fig. 3A). At a m.o.i. of 100, the surviving cell fractions were 3% and 13% for AdCMV tk+GCV and Adcav-1 tk+GCV, respectively. In the mouse prostate cancer cell lines, 148-1 PA and 148-1 LMD (ras + myc transformed, nullizygous p53), the fractions of cells killed by either the Adcav-1 tk or AdCMV tk vectors when treated with GCV were comparable over the range of vector doses (Fig. 3, B and C). At a m.o.i. of 100, the surviving fractions for 148-1 PA cells were 31% (AdCMV tk) and 47% (Adcav-1 tk), whereas for 148-1 LMD cells, they were 46% (AdCMV tk) and 62% (Adcav-1 tk). Adcav-1 tk was also tested in two other ras + myc-transformed, p53 nullizygous mouse prostate cancer cell lines, 178-2 PA and 178-2 BMA, which express higher levels of cav-1 than the 148-1 cell lines (Fig. 2). Again, GCV-mediated cytotoxicity with Adcav-1 tk was comparable to that of AdCMV tk over the range of vector doses, and at a m.o.i. of 100, the surviving fractions were 30% (AdCMV tk) and 42% (Adcav-1 tk) for 178-2 PA and 31% (AdCMV tk) and 49% (Adcav-1 tk) for 178-2 BMA (Fig. 3, D and E).

Adcav-1 tk was then tested in human prostate cancer cell lines (Fig. 4). In the PC-3 cell line, which expresses relatively high levels of cav-1 (Fig. 2), GCV-mediated cytotoxicity with the Adcav-1 tk vector was higher than that with AdCMV tk at most m.o.i. levels (Fig. 4A). At a m.o.i. of 100, the fraction of surviving cells was 60% for Adcav-1 tk but 80% for AdCMV tk. At a m.o.i. of 200, the two vectors were comparable, with 62%
cell survival for Adcav-1 tk and 65% survival for AdCMV tk. In ND-1 cells, which also express relatively high levels of cav-1 (Fig. 2), both vectors effected comparable high levels of GCV-mediated cell killing, with 31% cell survival at a m.o.i. of 100 for both Adcav-1 tk and AdCMV tk (Fig. 4B). The LNCaP cell line expresses essentially nondetectable cav-1 at low passage numbers (see Fig. 2). The AdCMV tk vector induced more cytotoxicity with GCV than did the Adcav-1 tk vector with GCV at all but the lowest m.o.i. (12.5). At a m.o.i. of 100, the cell survival was 50% with AdCMV tk compared with 70% survival with the Adcav-1 tk vector (Fig. 4C).

We also tested Adcav-1 tk in mouse endothelial cell lines that express high levels of cav-1 (see Fig. 2), SVEC4-10 and MS1 (Fig. 5). GCV-mediated cytotoxicity with Adcav-1 tk and AdCMV tk was very effective at all m.o.i. in SVEC4-10 and had increasing effectiveness as the viral titer increased in MS1 cells. The fraction of cells surviving at a m.o.i. of 100 for SVEC4-10 was 2% for both AdCMV tk and Adcav-1 tk. The fractions surviving for MS1 at a m.o.i. of 100 were 20% (AdCMV tk) and 17% (Adcav-1 tk).

In Vivo Gene Therapy. Previous in vivo studies have determined that the optimal viral dose of HSV-tk transducing adenoviral vectors is \(5 \times 10^8\) pfu/tumor at multiple prostate cancer cell lines, including RM-9 (2, 4, 5, 16). In the RM-9 model system, 5000 cancer cells are injected orthotopically; 6 days later, when the tumor size is 20–30 mm\(^3\), the adenoviral vectors indicated were injected into the tumors. The next day the animals began receiving twice daily i.p. injections of GCV for 7 days. On day 14 after tumor initiation, the tumors were weighed (A) and tissue was collected for subsequent analysis of AI (B) and necrosis (C). Computer-assisted quantitative analysis of tissue sections was performed after immunohistochemical staining for factor VIII (D).

![Fig. 5 In vitro cytotoxicity in mouse prostate endothelial cell lines with increasing m.o.i. of each vector (symbols as in legend for Fig. 2). The cell lines are MS-1 (A) and SVEC4-10 (B). All results are expressed as the percentage of survival (% survival) relative to cell number for AdCMV-infected cells treated with PBS. Bars, SE.](image1)

![Fig. 6 In vivo gene therapy with RM-9 orthotopic model. Six days after tumor initiation with 5000 cells, when tumor volume was 20–30 mm\(^3\), the adenoviral vectors indicated were injected into the tumors. The next day the animals began receiving twice daily i.p. injections of GCV for 7 days. On day 14 after tumor initiation, the tumors were weighed (A) and tissue was collected for subsequent analysis of AI (B) and necrosis (C). Computer-assisted quantitative analysis of tissue sections was performed after immunohistochemical staining for factor VIII (D). **, \(P \leq 0.05\); ***, \(P \leq 0.01\); ***, \(P \leq 0.001\). Bars, SE.](image2)
To further evaluate the mechanism(s) of tumor suppression, the prostate tumor tissues were analyzed with factor VIII antibodies to microvessels (Fig. 6D). Statistically significant decreases in microvessel density were seen in tumors treated with Adcav-1tk ($P = 0.0031$, $t$ test) and AdRSVtk ($P = 0.0194$, $t$ test) compared with AdCMVβGal.

**DISCUSSION**

Screening of asymptomatic men for increased serum PSA has resulted in a dramatic increase in the diagnosis and treatment for prostate cancer over the last decade (1). Following a diagnosis of prostate cancer, the patient is faced with the treatment choice of radical prostatectomy or irradiation therapy. Although surgery or irradiation is potentially curative for localized disease, these treatments can have significant morbidity. Furthermore, in a significant minority of patients with prostate cancer detected by PSA screening and treated with curative intent, the disease may not have progressed to clinical significance in the absence of treatment. Given these limited treatment options, it would be highly advantageous to develop effective therapies for localized disease that would not put the patient at risk for serious morbidity. More importantly, many patients diagnosed with prostate cancer believed to be confined to the prostate on the basis of clinical staging modalities likely have micrometastatic disease at the time of diagnosis. In these cases, the available localized therapies have little chance to cure the patient of the disease. The serious deficiencies in staging and limitations in treatment of prostate cancer have led to the emergence of tens of thousands of men who had recurrence of disease after either radical prostatectomy or irradiation therapy. Many of these men have very limited disease that can be detected only by serum PSA over extended periods of time; whereas others rapidly develop extensive metastases. It is critical to develop novel therapies, including neoadjuvant/adjuvant approaches, that would benefit these men.

Recent and ongoing clinical trials have shown the safety and some degree of efficacy of the HSV-tk + GCV system in radiorecurrent prostate cancer (17, 18). This form of gene therapy relies heavily on the bystander effect (8–12). The underlying mechanisms for the HSV-tk + GCV bystander effect include drug diffusion, immune response, and effects on tumor vasculature (8–12). One approach to improving the HSV-tk + GCV bystander effect is to target gene expression to specific heterologous cells within the cancerous prostate to maximize specific bystander effects (13).

Cav-1 is up-regulated in androgen-resistant and metastatic prostate cancer. It is also highly expressed in tumor-associated endothelial cells. This was first discovered in a mouse model (21) and then confirmed in human specimens (21, 24, 25). The role of cav-1 in metastatic prostate cancer is under investigation at present in our laboratory and in part involves antiapoptotic activities (26–28). We cloned the mouse cav-1 promoter and hypothesized that using this promoter, we could direct the HSV-tk gene to be more selectively expressed in aggressive prostate cancer and its associated endothelial cells, thus maximizing both direct and indirect killing of potentially lethal malignant cells. We believed that the cav-1 promoter would be more effective than standard prostate epithelial cell-selective promoters such as the PSA promoter/enhancer (40, 41), the prostate-specific membrane antigen promoter (42), or the glandular kallikrein 2 promoter (43) in that the cav-1 promoter would be more active in prostate cancer cells with increased potential to generate metastatic, androgen-insensitive disease. In addition, we believed that cav-1 promoter-regulated viral vectors would have advantages with regard to a bystander effect when compared with standard constitutive CMV or RSV promoter-driven vectors via increased tumor necrosis secondary to tumor-associated endothelial cell cytotoxicity. Specifically, we were of the opinion that the cav-1 promoter would prove advantageous for the regulation of adenoviral vector delivered therapeutic genes such as HSV-1tk (2) or interleukin-12 (14) for *in situ* prostate cancer gene therapy.

We evaluated this approach in a series of *in vitro* cytotoxicity experiments in which Adcav-1tk was compared with AdCMVtk in a variety of cell lines that express varying levels of cav-1. In general, we found that the cav-1 promoter was comparable to the CMV promoter in effecting cell death in cell lines that express cav-1. In RM-9 mouse prostate cancer cells, which express intermediate levels of cav-1 protein, the AdCMVtk vector was generally more active with regard to cytotoxic activities compared with Adcav-1tk. However, other mouse prostate cancer cell lines [148-1 PA (low cav-1), 148-1 LMD (intermediate cav-1), 178-2 PA (high cav-1), and 178-2 BMA (high cav-1)] were sensitive to Adcav-1tk+GCV in a manner comparable to that of AdCMVtk. The mouse endothelial cell lines MS1 and SVEC4-10 express very high levels of cav-1 and were very efficiently killed by Adcav-1tk+GCV. The human prostate cancer cell line PC-3 expresses relatively high levels of cav-1 and showed more efficient cell killing with Adcav-1tk than with AdCMVtk at most m.o.i. levels. However, in this cell line the relative cytotoxicity of both vectors was low, possibly because of low transduction efficiency secondary to low levels of Coxackie adenoviral receptor (44). The ND-1 human prostate cancer cell line also expresses high levels of cav-1 and showed efficient cell killing comparable to that of AdCMVtk. In early-passage LNCaP cells, which do not express cav-1 (29), Adcav-1tk was not as effective as AdCMVtk. Overall the data were suggestive that endogenous expression of cav-1 was related to Adcav-1tk-mediated cytotoxicity. However, regulation of the endogenous cav-1 gene is likely complex, and additional studies will be required to establish a clear relationship between endogenous cav-1 expression and regulation of exogenous gene activities by the 721-bp mouse cav-1 promoter.

We also demonstrated the effectiveness of the Adcav-1tk vector in an *in vivo* orthotopic prostate cancer model. Here, the Adcav-1tk vector proved to be comparable to the AdRSVtk and AdCMVtk in decreasing tumor size and inducing apoptosis. It also caused significant decreases in microvessel density, as did AdRSVtk. Interestingly, Adcav-1tk was the only vector to induce a significant increase in necrosis compared with the control. These results support the concept of targeting tumor endothelial cells together with tumor cells *per se* for *in situ* gene therapy (13). Although HSV-1tk + GCV gene therapy can have a modest antimetastatic effect in orthotopic prostate cancer models (4, 16), we have not yet thoroughly evaluated the Adcav-1tk vector in this regard.

In summary, we have used mouse cav-1 promoter se-
quences to regulate expression of the HSV-tk gene in preclinical models of prostate cancer. Cav-1 expression has previously been shown to be up-regulated in metastatic, androgen-resistant prostate cancer (21, 26) and to be an independent prognostic marker for recurrence in men who have had a radical prostatectomy (24). In addition, cav-1 is commonly expressed in endothelial cells, including prostate cancer-associated endothelial cells (21). We hypothesized that this multipotential cell selectivity of cav-1 promoter sequences might have advantages for in situ adenoviral vector-mediated gene therapy applications. Our results support this hypothesis. In general, Adcav-1tk was comparable to AdCMVtk with regard to GCV-mediated cytotoxicity in vitro. Although our results suggested that Adcav-1tk-mediated activities were related to endogenous cav-1 expression, additional studies will be necessary to clearly determine this relationship. In vivo studies using RM-9 cells in an orthotopic tumor model indicated that Adcav-1tk was as effective as AdCMVtk in suppressing tumor growth and more effective than AdCMVtk in producing tumor necrosis. Overall, Adcav-1tk was shown to be an effective HSV-tk delivery vector for prostate cancer. These results support the concept of simultaneous promoter targeting of aggressive tumor cells and tumor-associated endothelia by in situ adenoviral vector-mediated gene therapy for prostate cancer. Further studies in this area will be focused on exploiting this concept in clinical trials for prostate cancer gene therapy.

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