Retargeting of adenoviral vector using basic fibroblast growth factor ligand for malignant glioma gene therapy

WELJUN WANG, M.D., NIAN-LING ZHU, M.D., JASON CHUA, M.D., STEVE SWENSON, PH.D., FRITZ K. COSTA, M.S., STEPHANIE SCHMITTEINER, PH.D., BARBARA A. SOSNOWSKI, PH.D., TOSHIKI SHICHINOHE, M.D., PH.D., NORIYUKI KASAHARA, M.D., PH.D., AND THOMAS C. CHEN, M.D., PH.D.

Department of Pediatrics, University of Southern California School of Medicine; Departments of Neurosurgery and Pathology, Departments of Biochemistry and Molecular Biology, University of Southern California, Los Angeles, California; Institute for Clinical Chemistry and Laboratory Diagnostics, Medical School, Heinrich-Heine University, Düsseldorf, Germany; Selective Genetics, Inc., San Diego, California; School of Medicine, Hokkaido University, Sapporo, Japan; and Division of Digestive Diseases, Department of Medicine, University of California, Los Angeles, California

Object. Adenovirus vector (AdV)–mediated gene delivery has been recently demonstrated in clinical trials as a novel potential treatment for malignant gliomas. Combined coxsackievirus B and adenovirus receptor (CAR) has been shown to function as an attachment receptor for multiple adenovirus serotypes, whereas the vitronectin integrins (αvβ3 and αvβ5) are involved in AdV internalization. In resected glioma specimens, the authors demonstrated that malignant gliomas have varying levels of CAR, αvβ3, and αvβ5 expression.

Methods. A correlation between CAR expression and the transduction efficiency of AdV carrying the green fluorescent protein in various human glioblastoma multiforme (GBM) cell lines and GBM primary cell lines was observed. To increase transgene activity in vitro glioma cells with low or deficient levels of CAR, the authors used basic fibroblast growth factor (FGF2) as a targeting ligand to redirect adenoviral infection through its cognate receptor, FGF receptor 1 (FGFR1), which was expressed at high levels by all glioma cells. These findings were confirmed by in vivo study data demonstrating enhanced transduction efficiency of FGF2-retargeted AdV in CAR-negative intracranial gliomas compared with AdV alone, without evidence of increased angiogenesis.

Conclusions. Altogether, the results demonstrated that AdV-mediated gene transfer using the FGF2/FGFR system is effective in gliomas with low or deficient levels of CAR and suggested that FGF2-retargeting of AdV may be a promising approach in glioma gene therapy.

KEY WORDS • malignant glioma • gene transfer • adenoviral vector • basic fibroblast growth factor • mouse

Brain tumors are a major source of morbidity and death in both pediatric and adult populations. Gliomas account for more than half of these primary brain tumors. The most malignant type of glioma, the GBM, constitutes more than 50% of all gliomas. Although advances have been made in surgery, radiotherapy, and chemotherapy to combat malignant gliomas, the survival rate in patients with these lesions remains poor because tumor cell invasion and angiogenesis inevitably result in glioma recurrence; therefore, novel strategies at the molecular level have been developed to treat these malignancies more effectively. Gene therapy for malignant gliomas, despite a lack of efficacy in the Phase III trial using replication-incompetent retroviruses, remains a viable treatment option that may be improved as the biological features of the vectors become better understood. Recently, data from two trials have been published, demonstrating that AdV gene therapy may be a promising therapeutic intervention in patients with recurrent malignant gliomas. Immonen, et al., reported that the injection of a replication-incompetent AdV containing the herpes simplex thymidine kinase gene into the glioma resection bed, followed by the prodrug ganciclovir, increased the median survival time from 37.7 to 62.4 weeks in treated patients compared with patients receiving the best standard of care. Chiocca, et al., reported that a conditionally replicative oncolytic adenovirus, ONYX-015, may be safely injected into the peritumoral bed after glioma resection. In both cases, no significant immune reaction occurred against the AdV in the patients.

To improve the efficacy of AdV transduction, the biological characteristics of AdV binding and internalization in the glioma cell must be understood. The process of AdV entry into cells requires at least two different receptors, CAR and α(ν) integrin. The 46-kD transmembrane glycopro-
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The adenovirus (AdV) coat protein penton base contains the Arg-Gly-Asp (RGD) sequence recognized by αβ-heterodimeric cell surface proteins, the integrins.2,3,4 The vitronectin receptors, αvβ3 and αvβ5, have been shown to play a critical role in AdV internalization into host cells and are also believed to participate in tumor invasion, as they are found to be frequently overexpressed in glioma cells at the brain–tumor interface.2,3,5,6,7

Obstacles with respect to vector transduction efficiency due to the scarcity of CAR have been recently overcome by retargeting AdV to alternative cell surface receptors that are abundant on the target cells.9 The family of FGFs has been shown to be highly effective in retargeting AdV because of its cognate high affinity FGFRs.10 Importantly, it was demonstrated that AdV retargeted by FGF2 allows a higher level of transgene expression and in vivo transduction efficacy.11,12 Basic FGF can be either introduced onto viral capsids13 or chemically conjugated to a Fab fragment against the adenosovirus knob region.14

Based on the fact that both FGF2 and FGFR1 are also overexpressed in human gliomas,15,16 we hypothesized that improved targeting utilizing the FGF2/FGFR1 system would result in more uniform AdV transduction of malignant gliomas. In this study, we systematically examined gloma CAR and integrin expression both in vitro and in vivo and demonstrated that AdV-mediated gene delivery is often variable due to different CAR and integrin expression in various glioma cell lines and specimens. We present proof of the principle that delivery of a therapeutic gene using the FGF2/FGFR1 system may provide the selective transgene expression required for successful clinical application for glioma gene therapy.

Materials and Methods

Cells and Cell Culture

Primary human glioma cell lines (LA492 and LA567) were isolated from primary surgically removed glioma specimens. Established human GBM cell lines (A172, U87, U373, T98G, U138, and U118) were obtained from a commercial source (American Type Culture Collection, Rockville, MD). Stock cultures of these cells and primary glioma cells were maintained in Dulbecco modified Eagle medium supplemented with 10% fetal bovine serum, 1% penicillin/streptomycin, and 1% HEPES. All cells were kept in a humidified atmosphere containing 5% CO2 at 37 °C.

Adenoviral Vector and Ligand of Adenovirus to the FGF2-Fab’ Conjugate

The replication-incompetent adenoviral vector AdGFP, encoding the GFP gene under the control of the cytomegalovirus promotor/enhancer, was obtained from Selective Genetics, Inc. (San Diego, CA). The AdV was prepared as purified lots of known plaque forming units and particle numbers by using standard techniques. The FGF2-Fab’ conjugate was constructed by the chemical conjugation of FGF2 to a neutralizing antibody (ID6,14) to adenovirus 5 knob of the fiber protein, as described previously.9 Briefly, an FGF2-Fab’-AdGFP complex was formed by incubating AdV with the FGF2-Fab’ conjugate in binding buffer (20 mM HEPES, 100 mM NaCl [pH 7.6]) at room temperature for 30 minutes. Virus volume was determined based on the desired MOI, and FGF2-Fab’ conjugate volume based on the desired FGF2-Fab’ virus ratio.

Cell Transduction and Transgene Expression

Gliona cell lines (U87, U373, A172, T98G, U138, and U251) and primary glioma cell cultures (LA567 and LA492) were seeded into six-well plates at a density of 104 cells/well in complete medium and allowed to attach overnight at 37°C. The cells were transduced by unmodified or FGF2-retargeted AdGFP at different MOIs in Dulbecco modified Eagle medium containing 2% fetal bovine serum. Serial dilutions from 0.78 to 200 pfu/cell were performed to demonstrate that FGF2 retargeting can significantly improve the transduction efficiency of AdV in all cells tested. The MOI of 50 pfu/cell was found to be sufficient for all transductions. After a 6-hr incubation, the medium containing virus vectors was aspirated, cultures were rinsed twice with warmed PBS, and the complete medium was added. At 48 hours postinfection, transgene activity was determined by flow cytometry. Cells were harvested by brief trypsin/ethylene-diaminetetraacetic acid treatment, washed once, and resuspended in cold PBS prior to analysis with a FACSscan flow cytometer (Becton Dickinson, Heidelberg, Germany). Data are presented as the percentage of fluorescent-positive cells and the mean fluorescence intensity of positive cells.

Transduction of Gliomas With Undirected and FGF2-Retargeted AdGFP in Animal Models

Transgene efficiency of unmodified AdGFP and FGF2-retargeted AdGFP was tested and compared in intracranial U87 and U118 glioma models in nude mice. For treatment of experimental animals, a protocol approved by the Institutional Animal Care and Use Committee, University of Southern California, was used and the procedure was strictly followed. All animals were kept in a pathogen-free environment. Male athymic nude mice (nu/nu) 4 to 6 weeks of age were purchased from Harlan (Indianapolis, IN). The U87 and U118 glioma cells (3 × 105 and 5 × 105, respectively) were injected into the right frontal lobe in the mice. The cells were allowed to form a tumor mass for 1 week. At that time, PBS (10 μl), AdGFP (1.5 × 109 pfu/10 μl), or FGF2-retargeted AdGFP (1.5 × 108 pfu/10 μl) was stereotactically injected into the brain tumor using a 26-gauge Hamilton syringe. One week after infection, animals were killed and the brains were removed surgically. The brains were processed by fixing in phosphate-buffered 10% formalin, embedded in paraffin, and sectioned at 8 μm. Immunostaining was performed to detect the expression and distribution of GFP in normal and cancerous brains.

Immunostaining Procedures

The expression of the integrins αvβ3 and αvβ5, FGFRs (FGFR1 and FGFR2), factor VIII and CAR in primary human gliomas from surgical specimens, established human glioma cell lines, and primary glioma cultures was evaluated by immunohistochemical staining of the cells for these receptors. Cells from subconfluent monolayers were plated onto slides by cytocentrifugation. Samples were dried overnight and then fixed in acetone for 10 minutes. After being washed in PBS and quenched in 0.3% H2O2 for 5 minutes, the samples were blocked with 10% normal goat or calf serum (Vector Laboratories, Inc., Burlingame, CA) for 30 minutes. All primary antibodies to the receptors were diluted 1:200 in 2% normal goat or calf serum. Primary antibodies were as follows: 1) αvβ3, LM609 (Chemicon, Temecula, CA); 2) αvβ5, PIP6 (Chemicon); 3) FGFRs: 8E10 for FGFR1 and 11E11 for FGFR2 (Selective Genetics, San Diego, CA); 4) factor VIII (Zymed Laboratories, Inc., San Francisco, CA); and 5) CAR (kindly provided by J. M. Bergelson, University of Pennsylvania, Philadelphia, PA and now commercially available from Upstate Biotechnology, Lake Placid, NY). All primary antibodies were either incubated overnight at 4°C or for 1 hour at room temperature. After a PBS rinse, the samples were exposed to the secondary antibody, biotinylated anti–mouse or anti–rabbit immunoglobulin G (1:200, Vector Laboratories), for another hour. To visualize cells expressing any receptor, the samples were incubated in acetate buffer containing 2 mM 3-aminio-9-ethylcarbazole (Sigma Chemical Co., St. Louis, MO) and 3% H2O2, and then counterstained with Mayer hematoxylin.

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analysis of the slides was performed to determine the percentage of positive cells for immunostaining. Slides were classified by percentage of positive cells as follows: 0; +, less than 25%; +, 25 to 50%; ++, 51 to 75%; and ++++, 76 to 100%.

Quantification of GFP- and Factor VIII–Positive Cells on Stained Slides

Stained slides were subjected to “hot spot analysis” to examine areas of high vascular density. Hot spots were then selected in each sample slide with a magnification of 40 on an Olympus Vanox-S light microscope, while acquiring digital images with an Olympus C-5050 zoom digital camera (5-megapixel acquisitions; Olympus America, Melville, NY). Following selection and capture of the hot spot images, the areas showing positive staining were quantified in terms of the pixels within a given hot spot by using SimplePCI advanced imaging software (C-Imaging System, Cranberry Township, PA). To eliminate bias in the study, selection of the vascular hot spots and subsequent analysis of the slides were performed in a blinded fashion. The significance of the data was determined using descriptive statistics (means, standard deviations, 95% confidence intervals) and analysis of variance. 

Results

Expression of CAR, Integrins, and FGFR

Surface expression of CAR, integrins, and FGFR1 and -2 in human glioma tissue specimens, established glioma cell lines, and primary glioma cultured cells were determined by immunostaining. As shown in Table 1, glioma tissue specimens, most of which were pathologically diagnosed as GBM, express varying levels of CAR. No CAR was detected in three specimens (Cases 1–3), 0, whereas other specimens showed weak (Cases 4–6, +++) or strong (Case 7, ++++) staining for CAR, indicating low or high CAR expression levels in gliomas, respectively. The use of anti-αvβ3 antibody (P1F6) revealed that the αvβ3 integrin was present in all tissue specimens. In contrast, cell staining with anti-αvβ3 antibody (LM609) showed that all tissues except the specimen from Case 4 were positive for αvβ3. Specimens immunostained with antibodies to FGFR1 (8E10) and FGFR2 (11E11) indicated that all gliomas strongly expressed FGFR1, but were devoid of FGFR2 expression. Various established human glioma cell lines and primary glioma cultures were also tested for their expression of CAR, vitronectin receptors αvβ3 and αvβ5, and FGFRs, as shown in Table 2. The established glioma cell lines U118, U138, and U251 failed to bind to anti-CAR antibody, indicating that they lacked CAR. In contrast, CAR was detectable on other glioma cell lines and primary glioma cultured cells. Greater CAR expression was observed in A172, U373, and U87 compared with other CAR-positive cells. Furthermore, all glioma cells except the U118, U138, and U251 ones displayed αvβ3 on their cell surfaces; αvβ5 was present on all glioma cell lines except for U251 and A172. Similar to glioma tissue specimens, all glioma cell lines and primary glioma cultured cells were strongly positive for FGFR1 but lacked FGFR2 expression.

Transduction Efficiency of Unmodified and FGF2- Retargeted AdGFP in Glioma Cells

To determine the role of integrins, FGFRs, and CAR in adenoviral transduction in glioma cells, various glioma cells were infected with unmodified or FGF2-retargeted AdV carrying the gene for GFP (AdGFP or FGF2-AdGFP) at MOIs ranging from 0 to 200 pfu/cell. We also compared transduction efficiency of AdGFP in U87 cells correlates with greater MOIs (200 pfu/cell). The addition of FGF2-AdGFP to the cells did not significantly increase its transduction efficiency. In contrast, U118 cells were weakly transducible with unmodified AdGFP at MOIs between 0 and 200 pfu/cell. We also compared transduction efficiency of AdGFP and FGF2-AdGFP using the fluorescence microscope. Infection of U87 cells with either AdGFP or FGF2-AdGFP caused high expression of GFP (Fig. 1C and E). Only a few U118 cells showed transgenic activity when transduced with AdGFP alone (Fig. 1D). The addition of FGF2-AdGFP resulted in a marked increase in GFP expression in these cells, as shown by a high number of GFP-positive cells (Fig. 1F). The use of other glioma cell lines and primary glioma cultured cells demonstrated that greater than 55% of U373 and A172 cell populations expressed GFP when infected with unmodified AdGFP. In contrast, low transduction efficiency was achieved in other glioma cells given that only 8% or fewer cells expressed the GFP gene after infection with AdGFP. When considering expression levels of integrins and CAR in all glioma cells, we found a correlation between trans-
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Gene efficiency and CAR expression, demonstrating a role of CAR in AdV-mediated gene transfer. Adenoviral transduction in most CAR-deficient glioma cells was enhanced by more than 50% when AdGFP was retargeted by FGF2; whereas glioma cells (U87, U373, and A172) expressing high levels of CAR were as transducible with FGF2-AdGFP as with AdGFP (Fig. 2). Although all glioma cells strongly express FGFR1, FGF2 retargeting induced an increase in gene expression that was dependent on the cell line (~ 55% for U138 and U118 cells; > 75% for T98G, LA492, and LA567 cells). The U251 cells, which lack both vitronectin integrins (v3 and v5) and CAR, demonstrated little increase in transduction with FGF2-redirected AdV.

In Vivo Gene Delivery to Gliomas by Unmodified and FGF2-Retargeted Adenovirus

We next sought to determine whether increased transgene expression in gliomas could be achieved by FGF2-AdGFP in vivo. The U87 cells (strongly positive for CAR) compared with the U118 cells (CAR-negative) were injected intracranially into nude mice. One week after implantation, PBS, AdGFP, or FGF2-AdGFP was stereotactically administered to the tumors. One week post-delivery, the animals were killed and the brains removed to examine the expression and distribution of GFP by immunostaining. As shown in Fig. 3, specimens from PBS-treated U118 (Fig. 3A) and U87 (Fig. 3D) tumor-bearing animals were negative for GFP, indicating no transgene activity in these tumors. Delivery of the GFP gene to U118 tumor by stereotactic injection of AdGFP caused faint expression of GFP (Fig. 3B). Compared with AdGFP, the use of FGF2-AdGFP resulted in significantly enhanced transgene expression in U118 tumor in which GFP was widely dispersed inside the tumor (Fig. 3C). The U87 tumor was strongly positive for GFP when infected with FGF2-AdGFP. In contrast to the U118 tumor, AdGFP was as effective as FGF2-AdGFP in delivering the GFP gene to the U87 tumor (Fig. 3E and F). These findings are in agreement with those from our in vitro studies (Fig. 1). Importantly, positive GFP staining was seen only inside the tumors and not in the normal brain (Fig. 3B, C, E, and F). Moreover, factor VIII staining for endothelial cells demonstrated no increase in angiogenesis in tumors transduced with FGF2-retargeted AdV (Fig. 4A and B). Overall, these findings indicate that gene delivery to gliomas by unmodified and FGF2-retargeted AdV is feasible in vivo; enhancement of transduction efficiency by FGF2-retargeting compared with unmodified AdV depends on CAR and integrin expression in gliomas.

Discussion

Strategies for gene therapy using a nonviral delivery system are limited due to a lack of transduction specificity and efficacy. Recombinant AdV-mediated gene transduction is a powerful technology for gene therapy. Therefore, AdV delivery of therapeutic genes into target cells has been widely used to achieve high transgene expression levels in vitro and in vivo. The AdVs have been shown to infect both dividing and nondividing cells with relatively high transduction efficiency and to be potent vectors for gene therapy. Nevertheless, the application of AdVs in gene therapy...
may be limited by the following: 1) widespread cellular expression of receptors for adenovirus, leading to nonspecific transduction; 2) low receptor expression in target cells, resulting in resistance of these cells to adenoviral infection; 3) vector-related toxicity due to the high number of viral particles necessary to give adequate levels of gene transfer and to nonspecific tropism; and/or 4) antivector immune responses.\(^{12,37,42}\) In this study, we found that primary human gliomas from surgically removed human specimens express varying levels of CAR, including some malignant gliomas, which were absolutely CAR-negative. These findings are consistent with those of Fuxe, et al.,\(^{13}\) who found great variability in CAR expression, with the least expression in GBM. This finding of low CAR expression in GBM is especially unfortunate, as most of the patients undergoing adenoviral gene therapy will be those with recurrent GBM. Therefore, host cells can become less transducible with viruses because of a lack of CAR expression, limiting the applicability of AdV-based gene therapy for high-grade gliomas. This variability in CAR expression in human gliomas may explain the lack of a consistent response to successful AdV gene therapy in some patients, as previously reported.\(^{9,20}\) Recently, Immonen, et al.,\(^{20}\) demonstrated an approximately 10% transduction efficiency in patients with recurrent malignant gliomas injected with \(3 \times 10^{10}\) pfu AdV. Despite this relatively low transduction efficiency, survival time was significantly increased in these patients.

To increase transduction efficiency, several attempts have been made to alter normal adenoviral tropism to achieve cell-specific transduction.\(^{0,11,28,34,40,43}\) A retargeting strategy to ablate native tropism and redirect AdV infection via a receptor that is highly expressed on the target cell surface has been shown to enhance transgene expression significantly.\(^{17,30}\) Basic FGF is complexed with AdV via a neutralizing antianoviral antibody, and thus can serve as a targeting ligand. The FGF2 and its cognate receptor, FGFR1, have been shown to be overexpressed in gliomas and to be downregulated in normal neuronal and glial cells.\(^{36,38}\) Furthermore, Morrison, et al.,\(^{25}\) have demonstrated that FGFR1 is barely detectable in normal white matter, and its expression levels in human astrocytomas are in correlation with the degree of malignancy.\(^{32,38}\) In contrast, FGFR2 expression is abundant in normal white matter and in all low-grade astrocytomas but is absent in GBMs.\(^{36}\) Consistent with these findings, we found that all glioma cells used in our study exhibit high expression of FGFR1 and are devoid of FGFR2 expression. We had previously demonstrated that FGF2-retargeted AdV carrying \textit{herpes simplex thymidine kinase} significantly increased cytotoxicity in glioma cells on ganciclovir treatment compared with the nontargeted virus.\(^{44}\) Based on these findings, the use of FGF2 as a ligand for therapeutic gene delivery should be beneficial. Therefore, we used the FGF2/FGFR1 system to transduce glioma cells expressing varying levels of CAR with AdGFP and compared the transgene activity with FGF2-AdGFP vector. Glioma cells expressing high CAR levels were found to be as transducible with FGF2-AdGFP as with AdGFP alone. By contrast, glioma cells expressing intermediate levels of CAR and CAR-deficient cells infected with FGF2-AdGFP showed significantly enhanced transduction efficiency compared with those transduced with AdGFP alone. Nevertheless, CAR-negative and \(\alpha(v)\) integrin–negative U251 cells were not as transducible with FGF2-AdGFP as other CAR-deficient glioma cell lines, highlighting the importance of integrins in AdV internalization.

Although CAR expression varies considerably between glioma cells and all cells strongly express FGFR1, why does FGF2-retargeting not result in the uniform enhancement of gene transfer to all glioma cells? Our data suggest that the integrins, which are involved with AdV internalization, also play a key role in AdV gene transduction, independent of AdV binding to the glioma cell by CAR and/or

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**Fig. 2.** Bar graph depicting a comparison of transgene activity of unmodified and FGF2-redirected AdGFP in glioma cell lines and primary cultured glioma cells.
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Fig. 3. Transfer of the GFP gene by unmodified and FGF2-redirected AdGFP in a murine glioma tumor model. Photomicrographs showing GFP expression in U118 tumors injected with PBS (A), unmodified AdGFP (B), and FGF2-retargeted AdGFP (C). Bar graph (D) demonstrating a significant difference in GFP expression between U118 tumors transduced with AdGFP and those with FGF2-AdGFP (***p < 0.0001, Student t-test). Photomicrographs showing GFP expression in U87 xenografts transduced with PBS (E), unmodified AdGFP (F), and FGF2-retargeted AdGFP (G); no significant difference was observed on statistical analysis. Bar graph (H) demonstrating the quantification of GFP-positive cells in U87 tumors infected with AdGFP and FGF2-AdGFP. Black arrows indicate GFP-positive cells; blue arrow, the area of normal brain; and red arrow, the area of the U87 or U118 xenograft. Original magnification × 20.
FGFR1. Moreover, although all glioma cell lines express FGFR1, the functional affinity of FGFR1 to FGF2 in individual glioma cell lines is not clear. Doukas, et al.,\(^\text{11}\) demonstrated that FGF2 retargeting is dependent on high-affinity but not low-affinity FGFR receptors. Moreover, both free FGF2 and soluble FGFR1 antagonize FGF2-retargeted transduction. In control experiments, these reagents had no influence on nonretargeted viral transduction.

Data from our in vivo study using athymic nu/nu mice showed that intracranially implanted glioma is more transducible with FGF2-AdGFP than AdGFP alone when it lacks CAR expression. By contrast, in strong CAR-expressing glioma, there was no significant difference in transgene activity between unmodified and FGF2-redirected AdGFP. This finding is in good agreement with that from our in vitro study, suggesting that transduction efficiency of undirected AdV in gliomas is increased because of their high CAR expression, and enhanced transgene activity of FGF2-redirected AdGFP can be achieved in CAR-deficient gliomas. The degree of transfection efficiency was greater than 90% in all the glioma cells transduced (in vitro and in vivo). We did not see any GFP-positive staining outside xenografts of U87 and U118 tumors in the brain. We believe that this isolation of our transduction to the glioma cells may be attributed to two main factors. 1) Xenograft models involving human gliomas in nude mice are necessarily limited by the nature of the tumor growth in the host brain. The tumor tends to grow as a circumscribed ball within the host brain. Therefore, the possibility of FGF2-AdGFP extending into the tentacles of gliomas, which in turn might extend into the normal brain, would be limited in the animal model. 2) We are using a replication-incompetent adenovirus. The possibility of transduction outside the original injection site would be limited by poor adenovirus transduction given that it is replication incompetent. Future work in our laboratory will encompass a syngeneic brain tumor model together with the use of an oncolytic replication-competent adenovirus. Moreover, the possibility of the FGF2 ligand inducing an angiogenic response was not found, as gliomas transduced with FGF2-AdGFP did not demonstrate an increase in factor VIII staining. Additionally, vectors have been shown to display normal tissue toxicity when administered by unmodified adenovirus. A reduction in systemic and local toxicity could be achieved by redirecting AdV with FGF2, as decreased adenovirus titers would be needed to achieve satisfactory transduction. Because of the fear of long-term adenovirus immunogenicity, this reduction of adenovirus titers could be a tremendous advantage in the use of adenoviruses in the future.\(^\text{22}\) Recently, Chiocca, et al.,\(^\text{9}\) injected an E1B-attenuated adenovirus, ONYX-015, into the peritumoral region of recurrent malignant gliomas, and demonstrated that the injection can be well tolerated up to a dose of \(10^{10}\) pfu without evidence of toxicities attributable to ONYX-015.\(^\text{9}\) Furthermore, modification of the native adenoviral tropism, through the use of the neutralizing Fab fragment of an antiknob antibody conjugated to FGF2,
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may also reduce the immunogenicity of the native adenovirus; this possibility was not tested in our study.18,27

Conclusions

Data in the present study demonstrated that the use of FGF2 as a ligand to redirect AdV results in significant enhancement of transgene activity in both in vivo and in vitro gliomas with low or deficient levels of CAR. Given that FGF1 is usually upregulated in many tumors, including gliomas, the method for overcoming the problem related to varying CAR expression in gliomas, to increase transgene expression, to provide specificity, and to decrease systemic and local toxicity by redirecting AdV infection of target cells via this receptor, may offer significant therapeutic advantages in glioma gene therapy.

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References


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Address reprint requests to: Thomas C. Chen, M.D., Ph.D., Department of Neurosurgery and Pathology, University of Southern California, 1200 North State Street, #5046, Los Angeles, California 90033. email: TChen68670@aol.com.