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EFFICACY OF ADENOVIRAL P53 DELIVERY WITH SCH58500 IN THE INTRACRANIAL 9L AND RG2 *MODELS*

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1. ABSTRACT

Malignant gliomas remain incurable entities that provide fertile ground for experimental therapy. The observation that impaired p53 expression is present in a proportion of these tumors suggests that reconstitution of this ability may impart some degree of tumor control. In this investigation, the 9L and RG2 intracranial rodent tumor models are utilized to assess SCH58500, an adenoviral p53 delivery system. The RG2 tumors demonstrate a greater propensity for transfection with this vector *in vitro* than the 9L tumors. *In vivo*, little tumor transfection beyond the immediate area of the needle tract used for direct SCH58500 injection was observed in either tumor type. Intracarotid injection resulted in no tumor transfection. Even at high concentrations of SCH58500 or control virus, injections resulted in no apparent toxicity in terms of weight gain, eating habits or activity in normal animals. The intratumoral administration of SCH58500 enhances the survival of animals with established 9L tumors. Both SCH58500 and its control viral construct not containing the p53 gene enhance survival in animals with RG2 tumors. None of the injected viral constructs caused an alteration in the markers used to detect the character of the white cell infiltrate in either of these tumors. Utilization of SCH58500 provides measurable efficacy in these preclinical brain tumor models without significant toxicity.

2. INTRODUCTION

Malignant gliomas account for 50% of newly diagnosed brain tumors each year. In their most aggressive form median survival with treatment is less than twelve months. (1) Current treatment modalities include surgical resection, radiation therapy, and a variety of chemotherapeutic regimes. The use of chemotherapy has yielded some enhancement of survival but is constrained by the potential systemic and neurologic toxicity presented by each agent. (2,3) In addition, the recurrence of glial tumors also coincides with the generation of clonal populations, which are more resistant to treatment, particularly treatments with the same general mechanism of tumor control as those used previously in the same individual. (4) As a result, new therapies are currently being investigated in order to provide alternate approaches to treating this difficult disease.

A greater understanding of the regulation of the cell cycle in tumor cells has yielded a variety of new targets

for cancer therapy. The best known of these is the p53 gene and protein that functions in growth arrest and apoptosis in response to cellular stress DNA damage, growth factor withdrawal and hypoxia. (5-11) Mutations of the tumor suppressor gene p53 inactivating the normal function of the expressed protein have been implicated in the malignant progression of approximately one third of primary brain tumors. (12) Additionally, inhibitors of p53 function, such as the mdm2 gene can be over expressed with the same functional result. (13) The reintroduction of functional p53 protein has been shown to suppress tumor growth (14, 15) stimulate apoptosis (16-19) and inhibit telomerase activity (20) both *in vitro* (21, 22) and in animal models (6, 21) in which p53 is absent or mutated. (23) Tumors are known to have heterogeneity, between tumors and within the same tumor, in p53 expression (24) and less effect on intracranial tumor proliferation seems to be present when the tumor cells already has wild type p53 present. (6) In patients, the challenge lies in determining a safe and efficient means of p53 delivery to the tumor site. (25) Currently a variety of delivery vehicles including adenoviral vectors are being assessed to determine maximum transfection efficiency with limited toxicity. Selectivity in transfection is also being explored utilizing the ONYX-015 virus, which selectively replicates in cells that do not produce the p53 protein normally, though its true ability to do so remains a subject of some controversy. (26, 27, 28) Additionally, infection of p53 negative tumor cells with adeno-associated virus has been reported to result in transient G2 arrest followed by cell death. (29) Finally, a retroviral construct utilizing the herpes simplex virus thymidine kinase gene that is self-deleting, but only when the p53 gene is present and expressing normally, has been developed that will leave p53 deficient cells sensitive to ganciclovir. (30)

Even if an adenoviral vector provides efficient delivery *in vitro* (21), the efficiency of adenoviral mediated gene transfer to intracranial glial tumors is limited by several factors. (6, 31, 32) First and foremost, the method of viral delivery must be optimized in order to ensure that the tumor is the primary site of infection, and that the infecting agent is delivered at high enough dose to ensure gene transfer to a significant number to tumors cells. Additionally, optimization of viral doses is necessary to limit toxicity to surrounding normal brain. SCH58500 is a recombinant adenovirus modified to carry and deliver the wild-type p53 gene. (15) We have investigated two different delivery methods of this virus in intracranial tumor model in order to assess it's toxicity, efficiency of gene transfer, and resulting inhibition of tumor growth.

3. MATERIALS AND METHODS

3.1. Cell Lines

Rat 9L Glioma cells were cultured in 4.5 g/mL glucose DMEM supplemented with 10% Fetal Bovine Serum, penicillin (100 U/ml), and streptomycin (100mg/ml). Rat RG2 Glioma cells were grown in DMEM/F12 50:50 media supplemented with 10% FBS and penicillin (100 U/ml), and streptomycin (100mg/ml). When used for implantation, cells were grown to 80% confluence in a 75 cm^2 flask. Following trypsinzation, cell suspensions were centrifuged at 1500 rpm for 5 minutes at room temperature and the resulting cell pellets were resuspended in phenol-red free culture media without FBS or additives to a final concentration of 10,000 cells/? l. One ml of the resulting cell suspension was then placed on ice prior to implant.

3.2. Tumor Implantation

Male Fischer 344 rats weighing 125-175 grams were obtained from Charles River Laboratories and housed in accordance with Emory's Institutional Animal Care and Use Committee regulations utilizing an approved protocol. Animals were anesthetized using a mixture of 0.1 cc ketamine (500 mg/ml) and 0.025 cc xylazine (20 mg/ml) per 100 grams. Animals were then placed in a standard stereotactic frame and a sagittal midline incision was made starting 5mm anterior of the bregma and ending at the occiput. The scalp was then bluntly elevated in the subperiosteal plane and a small burr hole was made 3mm to the right and 1mm anterior to the bregma. Five ? l of the cell suspension was injected over a period of approximately three minutes at a depth of 4mm. The needle was then withdrawn slowly, and the burr hole closed with sterile bone wax. The incision was closed with running 6-0 absorbable suture, and the animal allowed to recover in isolation.

3.3. Viral Vectors

Adenoviral constructs containing p53 tumor suppressor protein (SCH58500), green fluorescent protein (Ad-GFP), or β-Galactosidase (Ad-βG) were supplied by Schering Plough Pharmaceuticals (Kenilworth, NJ). The adenoviral vectors were derived from human adenovirus type 5 and constructed so that the E1 region, necessary for viral replication, was replaced either with a gene expression cassette containing the human cytomegalovirus immediate early gene promoter directing expression of the wild-type p53 cDNA, green fluorescent protein, or β-galactosidase. (15) Virus was supplied at a concentration of 3.2×10^{11} - 6.0×10^{11} viral particles /ml in sterile PBS and was stored at -80^{0}C prior to use.

3.4. Determination of *In Vitro* Transfection Efficiency

Both 9L and RG2 were exposed to the Ad-betaG or Ad-GFP suspended in DMEM to serve as reflection of optimum infection efficiency. Cells were grown to 80% confluence and subjected to a range of concentrations of virus each virus. Ad-betaG transfection was determined with X-Gal staining. (30) Ad-GFP transfection was visualized with fluorescence microscopy. In each case, efficiency was determined by cell counting in ten representative microscopic fields within the culture flask and averaging them.

3.5. Intracranial Vector Delivery

Ten days post tumor implant animals were anesthetized as previously described and again placed in a stereotactic frame. The incision was re-opened, and 5 microliters of Ad-betaG or SCH58500 at a range of concentrations (1×10^{8} – 1×10^{11} viral particles/ml of

sterile saline) was injected at the same site at which the tumor injection had occurred and with the same methods as for tumor implantation. A sham injection of 5 microliters of sterile saline was given to the same site to parallel to serve as a control for this injection. The incision was closed as previously described, and the animal allowed to recover.

3.6. Intracarotid Vector Delivery

Animals were anesthetized as described and placed supine on a surgery board for rodents. The neck was shaved, prepared in an aseptic fashion, draped, and a 3 cm incision was made from jaw line to sternum. The skin was retracted using sutures. Following the natural plane dividing the parapharyngeal muscles and the right sternomastoid muscle, the right common carotid artery was exposed just proximal to its bifurcation. The right external carotid artery was ligated using 7-0 resorbable suture, the pterygopalatine artery occluded with coagulation and virus was injected into the right internal carotid using a 25 microliters Hamilton syringe equipped with a 33 gauge needle. Varying concentrations of Ad-GFP ranging from 1 x 10^6 to 1 x 10^{11} viral particles/ml in a volume of 20-50 microliters were used in the infusion. A total of 10 animals were used for this purpose. The needle was withdrawn, and hemostasis was obtained through the application of direct pressure and occasional use of microfibrillar collagen. The wound was closed with running 6-0 resorbable suture, and the animal allowed to recover in isolation.

3.7. Determination of *In Vivo* Transfection Efficiency

Stereotactic intratumoral injections of Ad-GFP and SCH58500 were carried out after 9L/RG2 (n=20 for each tumor type) tumors had been established for fourteen/ten days, respectively, in male Fisher 344 rats. Doses of the control vector were delivered in 5 microliters of sterile phosphate buffered saline over a range of 2 x 10^4 viral particles (4.0 x 10^9 viral particles/ml) to 3 x 10^6 viral particles (6.0x 10^{11} viral particles/ml). The same doses and volumes of the SCH58500 were delivered. Animals were sacrificed at intervals of 6, 24, 48, 72 and 96 hours later. Two animals were sacrificed for each tumor type at each time interval. Histologic analysis of the needle tracks was carried out by staining for each respective marker with to assess for transfection and eosin to identify background tissue architecture. Serial 6 microM frozen sections were made using a cryotome and β-gal expression was determined through an established protocol for fixed frozen tissue. (33, 34) Expression of GFP was again determined through fluorescence microscopy, and p53 expression detected by immunohistochemistry. The p53 primary antibody was obtained from Neomarkers (Fremont, CA) and all other chemicals were obtained from Sigma (St. Louis, MO).

3.8. Assessment of Viral Toxicity

Male Fischer 344 rats were injected intracranially (n=3) with 5 microliters a suspension of 1 x 10^{11} viral particles/ml of Ad-betaG or SCH58500 as described above. Similarly, normal male Fisher 344 rats underwent intracarotid injection (n=3) with 50 microliters of a suspension of 1 x 10^{11} viral particles/ml of Ad-betaG or SCH58500. They were subsequently weighed daily, and a record was kept of their activity level, appetite, and weight. At the end of two months, animals were sacrificed by CO_2 asphyxiation, and brains were removed. 6 microM sections were made, and sequential representative samples from the injection site stained with hematoxylin and eosin for anatomic analysis or Luxol fast blue to assess for demyelination.

3.9. Survival Analysis

In order to assess the therapeutic efficacy of adenoviral p53 delivery, 18 rats were implanted with the 9L glial tumor as described. Ten days following the implant, animals were injected intratumorally with 5 ? l of 1 x 10^{11} viral particle/ml suspension (the highest concentration used for the toxicity studies) of either Ad-GFP (n=6), SCH58500 (n=6), or saline control (n=6). Using identical methods, Ad-GFP, SCH58500, and saline were injected ten days following implantation of RG2 tumor cells.

Following tumor implant and subsequent adenoviral administration, animals were observed and weighed daily to determine toxicity. Established endpoints were set to coincide with a loss of 20% of total body weight, an inability to reach food/water for one day, or frequent debilitating seizures. Sacrifice was carried out via CO_2 asphyxiation.

3.10. Identification of Infiltrating Lymphocytes.

Using standard Immunohistochemistry techniques described by DAKO, identification of white cells labeled with CD4, CD8, CD25, and CD28 infiltrating the 9L and RG2 tumors was determined on 6 microM fresh frozen sections. Rat spleen and human tonsil were used as control tissue. CD4 and CD25 antibodies were obtained from Pharmacia (Piscataway, NJ) all other antibodies and reagents were obtained from DAKO (Carpinteria, CA)

4. RESULTS

4.1. *In Vitro* Transfection Efficiency

Rat 9L and RG2 cells were exposed to culture media with Ad-betaG and Ad-GFP at 1 x 10^9, 1 x 10^{10}, and 1 x 10^{11} viral particles/ml over a period of 96 hours in order to determine optimum conditions for transfection. The beta-Gal expression in the RG2 cells by percentage was 91.2 $\pm$ 4.34% (mean $\pm$ SD) at 72 hours and 89.2 $\pm$ 3.91% at 96 hours. In contrast, 9L cells appeared to transfect at a lower efficiency with values of 44.7 $\pm$ 6.5% at 72 hours and 51.4 $\pm$ 6.38% at 96 hours. (Figure 1) GFP expression in both cell lines peaked at 72 hours, with expression levels just below 80% for the RG2 cells, and at 50% for 9L. Based on this information the Ad-betaG and Ad-GFP were accepted as essentially equivalent controls in terms of *in vitro* transfection efficiency in this experiment.

4.2. *In Vivo* Transfection Efficiency: Intratumoral Injection

By inspection of needle tracks, the most intense X-GAL staining occurred in the 48 and 72 hour specimens regardless of dosage. Increasing amounts of virus in the

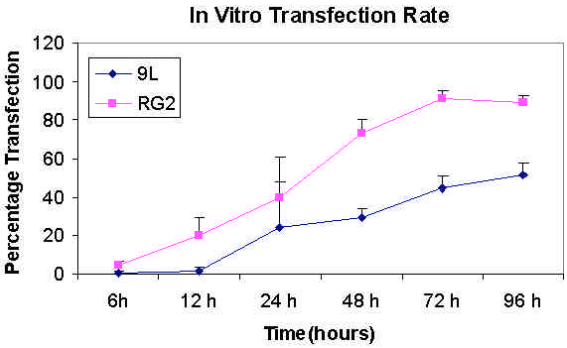

Figure 1. This graph demonstrates that the rate of transfection over 96 hours is greater in the RG2 cell line than the 9L cell line. Significant variability (standard deviation represented in error bars) in the rate of transfection occurs at 24 hours. This variability decreases thereafter with the means beginning to plateau at about 72 hours.

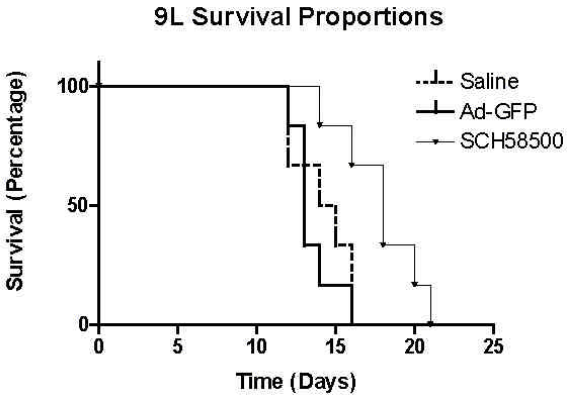

Figure 2. Survival curves of rats with 9L tumors treated by intratumoral injection with saline, Ad-GFP or SCH58500 10 days after tumor establishment.

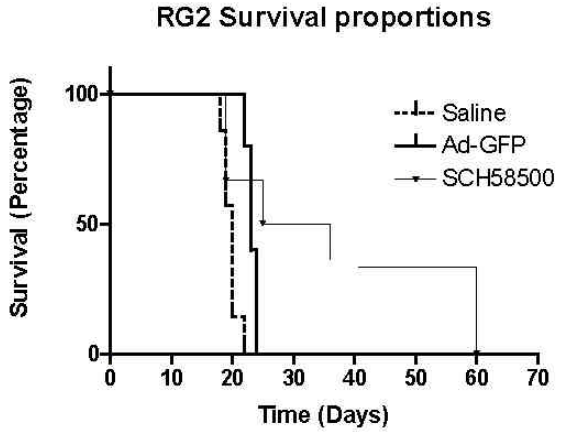

Figure 3. Survival curves of rats with RG2 tumors treated by intratumoral injection with saline, Ad-GFP or SCH58500 10 days after tumor establishment.

injectate was not associated with any greater degree of penetration of tissue. The greatest distance from the needle track that transfection could be detected was approximately two 40X fields, or 1.2 mm. By inspection an estimate of transfection efficiency could be obtained in any specific high-powered field in terms of percentage of positively staining cells. However, it was totally dependent on distance from the needle track. Because there was so little apparent functional tumor penetration by virus, no further pursuit of the transfection efficiency in this model was carried out. Along the needle track at the junction of the tumor and the brain rare neurons were seen with degenerated and fragmented nuclei consistent with apoptotic death. By simple observation this was similar in control untreated animals sacrificed for weight loss or inability to eat and this group of animals being assessed for transfection efficiency.

4.3. *In Vivo* Transfection Efficiency: Intracarotid Injection

Seven to ten days post 9L/RG2 tumor implant, animals were injected with 20-50 microliters of Ad-GFP in sterile saline at a maximum concentration of 6 x 1011 viral particles/ml into the right internal carotid artery as previously described. Brains were harvested at 12, 24, 48, and 72 hours post injection and 6 microM frozen sections were made of the tumor and surrounding brain. Fluorescence microscopy revealed no expression of green fluorescent protein in any of the brain sections. Additional sections were made of liver, kidney, and bladder. A similar microscopic examination again yielded no evidence of transfection or gene transfer.

4.4. Assessment of Viral Toxicity

Neither intracranial (5 microliters of a suspension of 1 x 1011 viral particles/ml) or intracarotid (50 microliters of a suspension of 1 x 1011 viral particles/ml) injection of Ad-betaG or SCH58500 resulted in significant alteration in observed activity level, appetite, or weight in normal animals. Histologic analysis of the needle tracks revealed no unusual evidence of hemorrhage, vacuolization, vascularization, demyelination or white cell infiltrate.

4.5. Treatment of Brain Tumors: Intratumoral Injections

The Ad-GFP treated set of animals implanted with 9L tumors had a mean survival of 13.5 $\pm$ 1.4 (standard deviation) days and showed no significant enhancement of mean survival over the saline control value of 14.2 $\pm$ 0.8 days. In contrast, rats receiving SCH58500 by intratumoral injection demonstrated an increase in mean survival to 17.8 $\pm$ 2.6 days. This is significantly longer than the Ad-GFP control (p=.0051). The survival curves of these three groups are shown in Figure 2.

A more pronounced effect was seen when the same experiment was conducted using the RG2 tumor model. Injections of Ad-GFP showed a mean survival of 23.2 $\pm$ 1.0 days that was better than the 19.3 $\pm$ 0.8 days for the saline control (p=0.01). The mean survival of the animals treated with SCH58500 was 36.5 $\pm$ 19.2 days. Two out of the animals receiving the same amount survived for 60 days. These animals were sacrificed at that time, and upon removal and examination of their brains it was noted that a small, apparently viable tumor mass was still present. Overall, the animals with RG2 treated with SCH585800 demonstrated enhanced survival over Ad-GFP group (p=0.045). The survival curves of these three groups are shown in Figure 3.

4.6. Treatment of Brain Tumors: Intracarotid Injections

Regardless of tumor type or dose level, no significant enhancement of survival was noted following intra-carotid delivery of virus. Fluorescence microscopy and immunohistochemical analysis of brain and brain tumor 1, 2, 3 and 4 days following injection yielded no evidence of gene transfection of either GFP or p53, respectively.

4.7. Phenotypic Alterations of Lymphocytic Infiltration

Regardless of the viral agent delivered, CD4 populations, while abundant in normal spleen, were virtually absent in both tumor types as well as surrounding normal brain. In contrast, CD8 populations were high in both treated and untreated brain tumors. However, there was no difference between tumor types. CD25 and CD28 labeled white cell populations were also absent in treated and untreated 9L and RG2 tumors.

5. DISCUSSION

Recent evidence has pointed to adenoviral mediated gene transfer as a promising treatment modality for brain tumors. It has been shown that a replication deficient adenovirus-mediated gene delivery can be accomplished efficiently to cells of the brain and brain tumors. (25, 31. 35-38) Unlike retroviral vectors, it is not necessary for the gene of interest to be inserted into the host genome prior to expression. Adenoviral DNA persists in a functional state outside the host genome, thus limiting the possibility of an induced insertional mutation. (39) As the virus can infect cells in G_0, the status of a number of cells in brain tumors, this method of therapy has a functional advantage over those dependent on tumor cell division. Non-replicating adenovirus vectors are also superior to retroviruses in that their capacity for gene inserts (>7.5 kb) and ease of production, thus permitting a broad range of potential therapeutic agents to be introduced. (6)

In our experiments, the p53 tumor suppressor gene was evaluated as a therapeutic agent in response to it's reported frequency of p53 mutations in glial tumors, as well as evidence suggesting that regaining p53 function can control tumor progression. (12, 21) Additionally, SCH58500 has been shown to be effective in controlling glioblastoma growth *in vitro* and in a nude mouse flank tumor model using the G122 cell line derived from a human glioblastoma. (40) In the current report, using two similarly implanted rat brain tumor models, intratumoral delivery of an adenoviral vector containing the p53 gene yielded a statistically significant, though modest, enhancement of survival. This confirms observations by other investigators using adenoviral p53 delivery in the 9L tumor model. (31) In the RG2 model, there was a small increase in survival using the control virus alone. This may be related to virus-induced tumor cell lysis. (41) Others have voiced concern over extent of functional transfection by a p53 containing adenovirus that can realistically be obtained. (25) It has been estimated that the rate of tumor cell transfection may be 40% or less. (31) This is certainly supported in our both of our tumor types where transfection only occurred in the immediate area of the needle injection track itself. The exact mechanism for the prolonged survivals in this pair of tumor models remains speculative. Transfection does occur over short distances and SCH58500 injection was carried out in a small tumor only 10 days after establishment. Thus even partial transfection may result in temporary slowing of a portion of the tumor growth due to either temporary cell cycle arrest or cell death. This would in turn result in temporary prolongation of survival as unaffected and viable tumor would proceed with proliferation

Contrary to others observations, no spread of the tranfection into the surrounding brain appeared to occur. (42) This lack of efficiency may be due to the need for the spread of the viral infection to occur by progressive movement via relatively large distances through contiguous components of solid tumor tissue and brain parenchyma. Areas of viability are known to be heterogeneous in malignant tumors, ranging from vigorous proliferation to necrosis. Thus, all cells within the lesion may not be able to serve as effective sites for adenovirus proliferation due their active involvement in the mitotic process or, on the other hand, due to limitations in nutrients and oxygen. This is a problem not faced in other settings, such as intraperitoneal delivery of adenoviral vectors for pancreatic cancer where only short distances must be traveled by the adenoviral vector within tumor nodules themselves. (18) It must be kept in mind that prior practical experience with gene therapy in the form of retroviral delivery of the thymidine kinase gene also met with significantly small amounts of apparent activity. Polymerase chain reaction analysis of samples from the human tumors injected with the producer cells demonstrated less than one percent of cells had thymidine kinase expression. No specific distances from the location of injection were calculated, however, the tumors were small in general and this finding demonstrates the limitations in transfection efficiency with that small virus system suggesting that our findings of limited transfection with a larger virus could have been foretold. (43) Of course the reason for the lack of detection of transfection beyond a short distance from the needle track may be related to technical issues. Very small amount of fluorescence or immunopositivity may be present more than 1.2 mm distant from the needle track that was simply not seen. Alternative methods of detection such as *in situ* hybridization, viral coat immunohistochemistry or PCR analysis may be more sensitive and in fact show a greater spread of viral transfection. Though interesting, this would not necessarily provide an exact explanation of the reason for the prolonged survival in these animal models.

Though not curative, by itself, this modality has been shown to be potentially useful, and possibly synergistic, in conjunction other forms of therapy such as chemotherapy and/or radiotherapy. (39, 44, 45) The toxicities these combined forms of treatment could also be synergistic and will need to be subjected to careful scrutiny in application to humans.

In this report, we saw no value in intracarotid injections. It remains to be seen whether or not other alternative delivery methods to direct inoculation can yield efficacy with this particular vector. Other investigators have demonstrated that when chemical agents such as RMP-7 and mannitol are used to further compromise the blood-tumor and blood-brain barrier in tumor-bearing rats, adenoviral infection can be mediated through carotid delivery. (46, 47) This may prove to be of value in the use of SCH58500 also.

Replication deficient adenoviral vector injection into normal brains of experimental animals has been shown to induce a local inflammatory response. (48-51) Thus there is concern that efficacy, if observed, may be due to a more general inflammatory reaction to the presence of the virus than to the therapeutic effect of p53 reintroduction. (15) Examination of the brains of the experimental animals showed a fairly typical population of lymphocytes around the injection sites with no notable difference between control and treated rats. This event did not seem to be occurring in either of these models. The RG2 tumor system has been reported as less "immunogenic" than the 9L tumors. (52) Therefore both tumor types were used in this study to see if this reported difference would result in a difference in the tumor response to the adenoviral injection. No significant difference in the phenotype of the lymphocytic response between saline, or viral injections was seen between the two tumors types even though the injection of the SCH58500 resulted in extended mean survival. Thus the reported difference in elicitation of an immune response between the two tumors, though maybe important in some contexts, had no impact on lymphocytic or therapeutic response in this study. It has been suggested that the tumor control that occurs with this therapy is primarily due to apoptotic death and not necrosis, and therefore the usual inflammatory response seen due to tumor death is in not elicited. (40) The presence of lymphocytes limited to expression of CD8 is consistent with a prior report in human malignant gliomas. (53) Additionally, CD8 cells have previously been observed to be the predominant lymphocyte type in RG2 tumors. (54) The failure of CD4, CD25 and CD28 expressing cells to infiltrate into the tumors may be partly responsible for continued tumor growth despite the clear presence of CD8 expressing cells. The reason for this failure remains to be defined completely.

The use of the vector as constructed appears to induce no overt untoward effects in this model system. No toxicity over a period of 60 days was seen in normal animals treated with intracerebral or intracarotid inoculations of the virus as evidenced by continued growth, maintenance of normal activity and unremarkable histologic exam of brain, and systemic organs.

This work confirms previous studies indicating the efficacy of wild-type p53 delivery by an adenoviral vector inhibits tumor growth. (25, 31) The results from the models as used here do not address concerns raised by *in vitro* work that p53 transfection may be associated with neuronal apoptosis as no significant amount of this was seen. (55) However, no exhaustive search for such activity was carried out and therefore this may have been occurring in other regions of the brain. This lack of observed local toxicity may be because the amount of viral delivery does not truly reach the intensity utilized *in vitro* and also because the primary target of the injection was within an established tumor, and not in the cerebral parenchyma itself. In the clinical study of adenoviral p53 delivery for recurrent malignant gliomas as reported by Lang et al, significant limitation of the extent of p53 transfection was noted and little overall efficacy was observed in the initial dose finding studies. (56) By itself, adenoviral delivery of p53 may not have a large effect. Consideration of it's combined utilization with radiation therapy, chemotherapy, or with other tumor suppressor genes, such as $p33^{ING1}$, may be useful in augmenting its efficacy. (19, 57-59) Delivery of adenoviral constructs that selectively replicate in p53 deficient cells may also provide a benefit if enough can be delivered to proper locations. (60) Additionally, our findings indicate a minimal amount of local immune cell response to high levels of adenoviral administration, suggesting that toxicity on this basis is not a serious concern as it is used in this model system. This work does not present reasons to become discouraged with viral delivery of potentially therapeutic agents, as other viruses and molecules have been show to have preclinical efficacy in brain tumors and are certainly worthy of pursuit. (61)

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