Advances in Brief

Treatment of Human Malignant Meningiomas by G207, a Replication-competent Multimutated Herpes Simplex Virus 1

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Abstract

We have demonstrated that replication-competent attenuated mutants of herpes simplex virus type 1 (HSV-1) have therapeutic potential for malignant gliomas. Moreover, a recently described multiple mutant HSV (termed G207) has properties which may allow human clinical trials. G207 is able to replicate within and kill cells from three human malignant meningiomas in cell culture. In nude mice harboring s.c. human malignant meningioma (F5), G207 can inhibit growth in a dose-dependent fashion. In nude mice harboring intracranial subdural human malignant meningioma (F5), one injection of G207 caused significantly decreased tumor growth and one apparent cure with neither neurological dysfunction nor pathological changes in the surrounding brain. These results suggest that G207 should be considered for therapeutic trials in the treatment of malignant meningioma refractory to currently available therapies.

Introduction

Meningiomas are the most common non-neuroepithelial-derived brain tumors, constituting approximately 22% of all primary intracranial neoplasms (1). Although most benign convexity meningiomas are surgically curable, approximately 3–11% of meningiomas are rapidly growing aggressive or malignant tumors (2–5). Therapeutic methods for these meningiomas such as radiotherapy, chemotherapy, and/or hormonal chemotherapy are not very effective, and multiple recurrences requiring multiple surgeries are common (6–8). This led us to examine a novel therapeutic approach using genetically engineered viruses.

Previous studies demonstrated that attenuated, single-gene mutants of HSV-1 were effective in killing malignant glioma cells (9–14) and other nervous system tumors (15, 16). The herpes genes deleted or altered in these studies included: (a) thymidine kinase (UL23; Refs. 9, 12, 13, and 16); (b) ribonucleotide reductase (UL39; Refs. 11, 13, and 17); and (c) γ34.5 gene (RL1; Refs. 10 and 14). On the basis of our previous studies (9–11), we developed G207 (18), a multigene mutant of HSV-1 with deletions at both γ34.5 loci, which are required to replicate in the brain or cause encephalitis (19), and a lacZ gene insertion into the ICP6 gene which is required for efficient viral growth in nondividing cells but not in many dividing cells in tissue culture (20). These mutations make reversion to wild type highly unlikely and confer important advantages that are essential in considering clinical trials, including: (a) acyclovir/ganciclovir sensitivities; (b) temperature sensitivity; and (c) markedly attenuated neuropathogenicity. We demonstrated that G207 has the potential to treat human malignant glioma U87 cells both in vitro and in vivo (18). Malignant gliomas were initially chosen as a therapeutic target for viral therapy because of their lack of systemic metastasis and their lack of response to conventional therapies. Malignant meningiomas have these same properties as well as several others which may make them ideal for virus-based therapies: (a) location at the brain surface; (b) minimal invasion into brain; and (c) multiple intercellular junctions. This study expands the range of human nervous system tumors that might be treated with the second generation HSV-1 mutant G207 to include malignant meningiomas and demonstrates additional important features of this strategy, such as the relationship between effective dose and tumor size. The intracranial meningioma model described in this article provides a useful system for testing therapeutic approaches to malignant meningiomas.

Materials and Methods

Viruses and Cells. G207 was constructed as described (18) and contains deletions of both copies of the γ34.5 gene as well as a lacZ insertion into the ICP6 gene. Stocks of G207 were grown in African green monkey kidney (Vero) cell cultures, and virus titration was performed as described previously (9). One human malignant meningioma cell line (F5) was kindly provided by Anil G. Menon (University of Cincinnati, Cincinnati, OH; Ref. 21), and the other two human malignant meningioma cell lines (GPSM4, GPSM5) were kindly provided by Gabriel Pulido-Cejudo (Health Canada, Ottawa, Ontario, Canada). Original tumors of these cell lines were histologically diagnosed as malignant meningiomas. These cells were cultured in RPMI 1640 medium supplemented with 10% inactivated FCS and antibiotics. F5 cells were also maintained by monthly passaging in the s.c. space of nude mice. Histological diagnosis, immunohistochemical reactivity against S-100 and vimentin, and a karyotypic finding of a deletion of chromosome 22 of s.c. F5 tumors were the same as in the original tumor (5).

Cell Culture Cytotoxicity. Subconfluent monolayers of meningioma cells in 25-cm² tissue culture flasks were infected at a MOI of 0.1 pfu/cell while controls were mock infected with virus buffer (150 ml NaCl, 20 mM Tris, pH 7.5). Viable cell numbers were determined by trypan blue exclusion on days 1–6.

Animal Studies. Six-week-old female athymic BALB/c nude mice (nu/nu), purchased from the National Cancer Institute (Bethesda, MD), each weighing 12–20 g, were kept in groups of five or less, housed in sterile cages, and had free access to autoclaved food and water. All animal procedures were approved by the Georgetown University Animal Care and Use Committee. For surgical procedures, each mouse was anesthetized with a i.p. injection of a 0.25–0.30 ml solution consisting of 84% bacteriostatic saline, 10% sodium pentobarbital (1 mg/kg; Abbott Laboratories, Chicago, IL), and 6% ethyl alcohol.

s.c. Tumor Therapy and X-gal Staining. F5 tumors were removed aseptically from the flanks of host mice, minced into 1-mm pieces, and transplanted into additional mice for study. Mice harboring s.c. tumors (approximately 6 mm in diameter) were randomly divided into two groups (n = 8/group) and treated intracranially with either 5 ¥ 10⁷ pfu of G207 virus suspended in 50 µl virus buffer and DMEM (1:1) or with 50 µl mock-infected extract prepared from mock-infected cells using the same procedures as those used for virus inoculum. Mice harboring s.c. tumors (approximately 10 mm in diamet.
ter) were randomly divided into three groups (n = 8/group). Group 1 was treated intraneoplastically with 5 × 10⁶ pfu of G207 in 50 μl virus buffer and DMEM (1:1), group 2 was treated intraneoplastically with 5 × 10⁷ pfu of G207 in 50 μl virus buffer and DMEM (1:1), and group 3 was treated with mock-infected extract (50 μl) alone. The tumor diameter was determined by external caliper measurements. Tumor growth ratio was determined by (l × w × h)/(l₀ × w₀ × h₀) where l is length, w is width, and h is height.

Statistical differences in growth ratio were assessed by using an unpaired t test.

For pathological studies, one mouse in group 1, and three mice in groups 2 and 3 were sacrificed on day 24. Animals were perfused with 2% paraformaldehyde-5 mM [ethylenebis(oxyethylenenitriilo)tetraacetic acid]-2 mM magnesium chloride in 0.1 mM 1,4-piperazinediethanesulfonic acid buffer (pH 7.3). The s.c. tumors were removed and stained with X-gal as described previously (11). After being frozen on dry ice, tumors were sectioned on a freezing microtome. The sections (20 μm) were mounted onto gelatin-coated glass slides, stained with X-gal again overnight, and counterstained with Carmalum.

**Intracranial Tumor Therapy.** We established an intracranial tumor model to simulate treatment of meningioma patients. Tumors were generated by transplanting F5 tumor fragments 1 mm in diameter into the right frontal subdural space. Mice harboring tumors were randomly divided into two groups (n = 9/group). Fifteen days later, 5 × 10⁶ pfu of G207 in 5 μl virus buffer and DMEM (1:1) or mock extract (5 μl) were inoculated intraneoplastically, and animals were followed up until death. Because of limitations in virus titer and the larger volume that would be necessary and which could cause excessive leakage and damage to the brain, we could not test the 5 × 10⁷ pfu/ml dose in the subdural tumor model. For studies of tumor size, animals were sacrificed, perfused with 2% paraformaldehyde-5 mM [ethylenebis(oxyethylenenitriilo)tetraacetic acid]-2 mM magnesium chloride in 0.1 mM 1,4-piperazinediethanesulfonic acid buffer (pH 7.3), and brains were removed with skull bone. Brains were placed in 10% formalin and then decalcified for 6 h in decalci solution (8% hydrochloric acid, concentrated, 10% formic acid in distilled water). Microtome sections (5 μm) were mounted on glass slides and stained with hematoxylin and eosin.

**Results**

**In Vitro Cytopathic Efficacy.** To determine whether G207 could replicate in and destroy malignant meningioma cells, human malignant meningioma cell lines F5, GPSM4, and GPSM5 were infected with G207 at a MOI of 0.1. All three cell lines were efficiently destroyed by G207 within 6 days (Fig. 1a). The cytopathic effect appeared on day 1 postinfection, and >99% cytotoxicity was evident on day 6.

**In Vivo Treatment of s.c. Tumors.** The effect of G207 infection on s.c. tumor xenografts was examined. F5 tumor fragments were transplanted into BALB/c (nu/nu) mice. Growing tumors approxi mately 6 mm in diameter within 3 weeks, at which time each tumor was treated with one injection of G207 or mock extract. For 6-mm tumor fragments, there was limited tumor growth and no significant difference in tumor diameter between control and 5 × 10⁷ pfu of G207-treated tumors for the first 7 days after injection of G207. From day 10 onward the tumor size between the two groups diverged (Fig. 1b). When the experiment was terminated on day 28 because of the large tumor burden in the control animals, the mean tumor growth ratio was significantly different between G207-treated and control groups (P < 0.001; unpaired t test; Fig. 1b). Next we tested whether larger tumors could also be successfully treated. When tumors were allowed to grow to 10 mm in diameter before treatment, tumor growth was rapid from the time of treatment, and G207 was effective in a dose-dependent manner (Fig. 1c). On day 24 when the experiment was terminated due to tumor burden in the controls, the mean tumor growth ratio was significantly inhibited (P < 0.005 with 5 × 10⁷ pfu, P < 0.01 with 5 × 10⁶ pfu; unpaired t test) in G207-treated tumors when compared to control tumors treated with mock extract. There was also a significant difference between the 5 × 10⁷ pfu of G207-treated tumors and the 5 × 10⁶ pfu of G207-treated tumors on day 24 (P < 0.05; unpaired t test; Fig. 1c). Tumors larger than 10 mm could not be tested in this model system due to the large tumor burden that would be necessary in the control group.

To assess the spread of replication-competent virus in F5 tumors in vivo, animals in the 10-mm treatment group were sacrificed on day 24 after treatment. These tumors were fixed and stained with X-gal to examine the extent of β-galactosidase expression. Mock-infected tumors showed no β-galactosidase expression (data not shown). Tumors treated at a low dose (5 × 10⁶ pfu; Fig. 2, a and b) showed lacZ expression in the area adjacent to the needle tract. In contrast, tumors treated at doses one log higher (5 × 10⁷ pfu; Fig. 2, c and d) showed lacZ expression that was more diffuse with islands of lacZ-positive cells throughout the tumor and extending to the capsule. However, in both cases X-gal-positive cells were seen at a distance from infected foci, indicative of viral infection spreading a large distance from the site of injection. Within positive foci, necrotic areas were evident.

**In Vivo Treatment of Intracranial Tumors.** We next studied the effect of G207 infection on right frontal subdural F5 tumors that were treated with G207 (5 × 10⁶ pfu) or mock extract 15 days after tumor implantation. All animals treated with mock extract died by day 31. A statistically significant increase in survival was seen in G207-treated animals compared with controls (P < 0.01; Wilcoxon test and Cox-Mantel test; Fig. 3). Brains were removed from three animals at the time of death in the control group and from three survivors in the G207-treated group who were sacrificed at day 65. Sections stained

![Fig. 1. Effect of G207 on human malignant meningiomas in vitro and in vivo. a, cytopathic efficacy of G207 in vitro. Three human malignant meningioma cell cultures were infected with G207 at a MOI of 0.1. The data plotted are the mean of triplicate wells. b and c, s.c. tumor growth of F5 malignant meningioma cells in BALB/c (nu/nu) mice. Mice harboring s.c. F5 tumors (approximately 6 mm (b) and 10 mm (c) in diameter) were treated with G207 or mock-infected extract (n = 8/group). The mean tumor growth rate was significantly inhibited in G207-treated tumors compared to control tumors treated with mock extract. For rapidly growing 10-mm tumors, two different viral doses were tested (c).](https://cancerres.aacrjournals.org)
MULTIMUTATED HSV THERAPY FOR MENINGIOMAS

Fig. 2. Pathological examination of G207-treated s.c. malignant meningioma tumors after staining with X-gal. On day 24 postinfection, animals from 10-mm tumor fragment-implanted mice were sacrificed and tumors fixed. s.c. meningioma tumors were removed and stained with X-gal solution. Tumors treated at a low dose (5 × 10^6 pfu; a and b) showed lacZ expression in the area adjacent to the needle tract. In contrast, tumors treated at doses one log higher (5 × 10^7 pfu; c and d) showed lacZ expression that was more diffuse with islands of lacZ-positive cells throughout the tumor and extending to the capsule. Bars, 4.5 mm in a and c, 1.1 mm in b and d.

Fig. 3. Extended survival of mice with intracranial (subdural) malignant meningioma F5 tumors treated with G207. BALB/c (nu/nu) mice with subdural tumors (15 days postimplantation) received either 5 × 10^6 pfu G207 or mock extract (n = 9/group). G207-treated animals had a prolonged median survival and three were alive at day 65 (P < 0.01 with Wilcoxon or Cox-Mantel test).

Discussion

We previously demonstrated that HSV-thymidine kinase-deficient mutants, γ34.5-deleted mutants, and HSV-ribonucleotide reductase-deficient mutants have therapeutic potential for central nervous system tumors (9–11, 15, 16). However, each of these viruses has limitations that could preclude use in humans. Therefore, to provide adequate safeguards for possible clinical trials, we developed a multiple gene mutant of HSV-1, G207, with deletions at both γ34.5 loci and a lacZ gene insertion in the ICP6 gene (18). G207 was avirulent upon cultured primary rat neurons and astrocytes and also upon intracerebral inoculation of mice and HSV-
sensitive nonhuman primates (18). G207 inhibited s.c. glioblastoma U87 tumor growth in vivo and prolonged survival of mice with intracerebral tumors. G207 provides many advantages for clinical therapy including: (a) multiple large mutations make reversion to the wild type unlikely; (b) retained sensitivity to ganciclovir or acyclovir; (c) temperature sensitivity which would compromise viral replication in the presence of encephalitis and fever; and (d) the lacZ gene provides a sensitive means to track viral replication both in the tumor and outside the tumor.

We have now extended the use of G207 to the treatment of malignant meningiomas. In cell culture, spreading infection and monolayer destruction occurred in all three human malignant meningiomas tested at a MOI of 0.1. Intraneoplastic inoculation of G207 virus into s.c. xenografts of malignant meningioma F5 cells in nude mice resulted in a significant decrease of tumor growth rate in vivo. Using F5 cells, we were able to establish an intracranial subdural meningioma model which had histological characteristics similar to human meningiomas. Intraneoplastic inoculation of G207 virus into subdural xenografts of F5 in nude mice induced significantly prolonged survival, with three animals still alive on day 65 compared to 100% lethality in the control group at day 31. One G207-treated animal had no detectable tumor on histological examination. These results demonstrate that G207 has the potential to destroy malignant meningioma in situ. Moreover, our studies with two viral doses in larger (10 mm diameter) s.c. tumors suggest that for large, rapidly growing tumors, a larger dose of virus may be necessary. Since G207 is an attenuated replication-competent virus, its growth is slower than wild-type HSV. We therefore postulate that virus growth and tumor growth are in competition and that the virus dose and spacing of inoculations may need to be adjusted relative to the size of the tumor and its rate of growth. Additional studies in various tumor types of different sizes and growth rates are necessary.

Acknowledgments

We thank Dr. Anil G. Menon for providing F5 cells, Dr. Gabriel Pulido-Cejudo for providing GPSM4 and GPSM5 cells, and Dr. William D. Hunter, Dr. Toshihiro Mineta, Dr. Shin-Ichi Miyatake, Kent C. New, Anu Iyer, Dale Gibson, Dr. Joseph T. Newsome, and the Georgetown University Research Resource Facility staff for their technical assistance.

References


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