Inhibition of glioma using a novel non-neurotoxic vesicular stomatitis virus

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OBJECTIVE The aim of this study was to demonstrate the *in vivo* safety and antitumor effect of a novel recombinant vesicular stomatitis virus (VSV): G protein less (GLESS)–fusion-associated small transmembrane (FAST)–VSV.

METHODS Viral infection efficiency and cell proliferation were detected using an inverted fluorescence microscope and alamarBlue assay, respectively. To evaluate the safety of the virus, different doses of GLESS-FAST-VSV and a positive control virus (VSV-ΔM51) were injected into normal F344 rats and C57BL/6 mice, and each animal's weight, survival time, and pathological changes were examined on the following day. To evaluate the efficacy of the virus, RG2 and GL261 cells were used to construct rat and mouse glioma models, respectively, via a stereotactic method. After multiple intratumoral injections of the virus, tumor growth (size) and the survival time of the animals were observed.

RESULTS *In vitro* experiments showed that GLESS-FAST-VSV could infect and kill brain tumor cells and had less toxic effects on normal cells. After direct injection of GLESS-FAST-VSV into the animal brains, all animals tolerated the virus well, and no animal death, encephalitis, or ventriculitis was observed. In contrast, all animals that received brain injections of VSV-ΔM51 in the brain died. Moreover, multiple injections of GLESS-FAST-VSV in brain tumors significantly prolonged the survival of normal-immunity animals harboring brain tumors.

CONCLUSIONS GLESS-FAST-VSV exhibited little neurotoxicity and could be injected directly into the tumor to effectively inhibit tumor growth and prolong the survival of normal-immunity animals, laying a theoretical foundation for the early application of such viruses in clinical trials.

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KEYWORDS glioma; G protein less (GLESS)–fusion-associated small transmembrane (FAST)–vesicular stomatitis virus (VSV); neurotoxicity

GLIOMAS, the most common malignant tumor in the central nervous system with invasive properties, are one of the biggest challenges in neurosurgical treatment. Gliomas account for about 60% of primary intracranial tumors, and nearly 600,000 people worldwide die from glioma each year.¹ Although surgery, radiotherapy, and chemotherapy temporarily relieve symptoms in most patients, the majority of patients usually experience a relapse almost 1–2 years later.² The main reason is that glioma cells invasively grow in normal brain tissue. Surgery can only remove tumor masses and temporarily relieve symptoms but cannot remove infiltrating tumor cells in the

brain. Therefore, the tumor cannot be completely cured. In such cases, the exploration and evaluation of new therapeutic strategies that can effectively kill invasive brain tumor cells have become some of the most important tasks in the field of glioma therapy. Recently, in-depth studies of various oncolytic viruses (OVs) have provided new hints on and methods for the treatment of glioma.^{3–5} OV is a kind of tumor-killing virus with a self-replication ability, meaning that it can rely on itself to specifically replicate in tumor cells to kill and directly lyse tumor cells and that the new virus subsequently released from cells then further infects the surrounding infiltrating tumor cells without impacting

ABBREVIATIONS DV = dead virus; GLESS-FAST-VSV = G protein less–fusion-associated small transmembrane–VSV; MOI = multiplicity of infection; OV = oncolytic virus; PBS = phosphate-buffered saline; RFP = red fluorescent protein; VSV = vesicular stomatitis virus.

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normal cells.^{6–10} In addition, the OV can further achieve the desired antitumor effects by generating an antitumor immune response or enhancing the effects of radiation or chemotherapy.^{11–14}

Vesicular stomatitis virus (VSV), a prototype virus with enveloped negative-strand RNA, is one of the interesting OVs found in recent years. Many studies have confirmed that the virus selectively and efficiently replicates in tumor cells, and the virus exhibits strong antitumor effects in a variety of preclinical trials.^{7–10,15} The systemic administration of VSV can also specifically target multiple tumors in the brain, invasive tumors, and systemic metastatic tumors,^{9,16–19} making VSV one of the most promising OVs for the treatment of various types of tumors. So far, there have been many in vitro and preclinical animal experiments using VSV to treat glioma, and the therapeutic effect is encouraging.^{9,19–22} However, all of these studies have used systemic medication, including an intravenous or intraperitoneal injection of the virus. There appear to be no reports on the direct injection of such viruses into brain tumors and no clinical studies of such viruses entering brain tumors. The absence of such studies can be attributed to the potential safety risks involved, especially the neurotoxicity when the wild-type or recombinant attenuated VSV is used in a brain tumor in a clinical trial. The fact that the VSV cannot be injected directly into brain tumors greatly limits its widespread application.^{21,22}

In this study, we aimed to validate the in vitro and in vivo safety of a newly constructed VSV (G protein less [GLESS]–fusion-associated small transmembrane [FAST]–VSV) that can be directly injected into brain tumors and its therapeutic effect on brain tumors.

Methods

This study was approved by the Ethics Committee of Qilu Hospital of Shandong University. All animal experiments were performed according to the principles of the National Institutes of Health (NIH) *Guide for the Care and Use of Laboratory Animals* (NIH Publication No. 80-23, revised 1978).

OVs and Glioma Cell Lines

VSV^{AM51} is a genetically recombinant attenuated virus (methionine deleted in the M-protein region [M51R])^{23,24} with the insertion of a green fluorescent protein gene (*GFP*).^{9,25} GLESS-FAST-VSV is also a genetically recombinant virus, with the deletion of the G protein and the fusion of the p14 FAST membrane fusion protein for enhanced viral transmission and the new envelope protein JSRVenv for reduced viral neurotoxicity. To better understand the efficiency of virus-infected cells, a red fluorescent protein gene (*RFP*) was also inserted. All of these viruses were originally constructed and kindly supplied by Dr. John Bell's laboratory (Ottawa Regional Cancer Centre Research Laboratories, Ottawa, Ontario, Canada), and subsequent culture and propagation of these viruses and titer determination (using BHK cells) were performed by Dr. Xueqing Lun in Drs. Peter Forsyth's and Zhigang Wang's laboratories.

All glioma cells (RG2, GL261, U87, SF767) and nor-

mal fibroblasts (HS68) were derived from ATCC and cultured in DMEM supplied with 10% fetal bovine serum. They were then incubated in a 37°C incubator with 5% CO₂ and subjected to subcultivation at ratios of 1:4 to 1:8.

In Vitro Experiments

Cell Proliferation Assay

A total of 5×10^3 cells were plated in a 96-well plate and then infected with different multiplicities of infection (MOIs) of GLESS-FAST-VSV for 1 hour in a 37°C incubator with 5% CO₂. Then the virus-containing medium was removed, and fresh glioma cell culture medium was added and cultured for another 48 hours. Subsequently, alamarBlue (10 µl/well) was added to measure the survival (proliferation) of the cells by enzyme-linked immunosorbent assay (570–600 nm). Ultraviolet-inactivated virus (dead virus [DV]) and normal fibroblasts (HS68) were used as negative controls. The cell survival rate (%) = (experimental group value/control group value) × 100%.

Viral Infection and Cytopathology

A total of 2×10^4 glioma cells were seeded in 24-well plates, and gliomas and normal fibroblasts were infected with GLESS-FAST-VSV (MOI = 0.1) on the next day. The viral infection efficiency and cell pathology were observed 48 hours later under an inverted fluorescence microscope (Axiovert 200M, Zeiss; AxioCam MRc camera, Carl Zeiss).

In Vivo Study

Experimental Animals

Immunologically normal female rats (F344) and mice (C57BL/6) were provided by the experimental animal center of the University of Calgary and the School of Medicine of Shandong University, respectively, and were fed a normal diet and maintained in a special pathogen-free animal feeding room.

Virus Safety Assessment

The intracranial injections of phosphate-buffered saline (PBS), different doses of GLESS-FAST-VSV (10^6 pfu/rat, 10^7 pfu/rat, 10^4 pfu/mouse, 10^6 pfu/mouse), or VSV^{AM51} (10^5 pfu/rat, 10^4 pfu/mouse) were administered using a stereotactic method. Briefly, each animal was anesthetized, and an incision was made in the middle of the head. Then the periosteum was separated, followed by drilling (0.5 mm behind the coronal suture, 2.0–2.5 mm along the right side of the sagittal suture). Finally, PBS or virus was slowly injected into the striatum through a microinjector (depth: 2.5–3 mm for mice, 3.5–4.0 mm for rats). All the animals were weighed daily. A portion of the animals (3 animals/group) was sacrificed at a defined time point for pathological examination. The survival of the animals was recorded.

Evaluation of Oncolytic GLESS-FAST-VSV

RG2 glioma cells were inoculated intracranially using the stereotactic method (10^4 cells/rat) to establish the intracranial glioma model. Briefly, the rat was anesthetized, a midline incision was made in the head, the periosteum

was peeled, and drilling was performed (0.5 mm behind the coronal suture, 2.0–2.5 mm on the right side of the sagittal suture). Next, 2 μ l of RG2 cells (10^4 cells) was slowly injected into the rat striatum through a microinjector (depth: 3.5–4.0 mm). Three days after tumor implantation, the animals were randomly divided into a control group and treatment group (10 rats/group). Then, the same method was used for the intratumoral injection of DV or GLESS-FAST-VSV (10^6 pfu/rat or 10^7 pfu/rat). The injection was performed every 3 days for a total of 6 injections. Four animals per group were sacrificed and their brain tissue was removed for histopathological examination to determine tumor size. The remaining 6 animals in each group were observed for survival until the animals were sacrificed with abnormal symptoms and signs, and the survival times were recorded.

The same survival study was performed in C57BL/6 mice with the intracranial injection of GL261 glioma cells. The cells were implanted using a stereotactic apparatus (Kopf Instruments). A 0.5-mm hole was made 2 mm right of the sagittal suture and 2.5–3 mm posterior of the coronal suture through a scalp incision. The stereotactic injection was administered using a 10- μ l syringe (Hamilton Co.) with a 25-gauge needle and mounted on a Kopf stereotactic apparatus. The needle was inserted into the frontal lobe to a depth of 3 mm. The needle was then withdrawn slowly, and the incision was closed using a wound Autoclip kit. Three days after the GL261 cells were implanted into the brain, animals were randomly divided into a control group and treatment group (6 rats/group). In the same manner as the intracranial injection of GL261 glioma cells, DV or GLESS-FAST-VSV (10^6 pfu) was injected intratumorally every 5 days for a total of 3 injections. The survival of the animals in the different treatment groups was then observed, and the time of death was recorded.

Immunohistochemical Analysis

Frozen sections of brain tumor were made according to the usual steps. Frozen brain tumor sections were fixed with 4% polyformaldehyde for 20 minutes, washed 3 times with PBS, and blocked with PBS containing 0.1% Triton X-100 and 10% normal goat serum for 1 hour. Then the polyclonal rabbit anti-VSV antibody (1:2500, provided by Dr. John Bell's laboratory) was added and incubated overnight at 4°C. After washing 3 times with PBS, the tumor tissue sections were incubated with biotin-labeled anti-rabbit secondary antibody (1:300, Cedarlane) for 30 minutes. After the sections were developed through the avidin-biotin-peroxidase complex (ABC) treatment, diaminobenzidine (DAB) was used for color developing. The results were observed under the microscope after coverslip treatment.

Statistical Analysis

The statistical analysis software (SAS Institute Inc.) and GraphPad Prism 5 (GraphPad Software Inc.) were used for statistical data analysis. Data are representative of three independent experiments, and error bars represent standard errors. The Student t-test was used for com-

parison between two groups. One-way analysis of variance was used for comparison between multiple groups. The survival data were analyzed using the Kaplan-Meier method. Significant differences were defined as $p < 0.05$.

Results

Inhibition of Glioma Cell Growth by GLESS-FAST-VSV In Vitro

First, we evaluated the infection efficiency of GLESS-FAST-VSV in different glioma cells (U87, SF767, RG2, and GL261) and normal fibroblasts (HS68) 48 hours after infection. The data showed that GLESS-FAST-VSV efficiently infected U87, SF767, and RG2 cells, as indicated by RFP expression. However, the GLESS-FAST-VSV infection was not efficient in GL261 and HS68 cells (Fig. 1A). In addition, we also analyzed the cell viability of the different cells infected with GLESS-FAST-VSV. The data revealed that GLESS-FAST-VSV significantly inhibited the growth of glioma cells, including U87, SF767, and RG2, with the majority of cells dead. The cell death in the GL261-infected group was significantly increased with an increase of the MOI (Fig. 1B). However, cell death was observed in only a small amount of cells in the HS68-infected group.

Absence of Neurotoxicity of GLESS-FAST-VSV In Vivo

Next, we evaluated the safety of GLESS-FAST-VSV in vivo. First, we compared the neurotoxicity of GLESS-FAST-VSV versus that of VSV Δ M51. After injection, significant weight loss was observed in normal F344 rats and C57BL/6 mice whose brains were injected with VSV Δ M51, followed by posterior limb paralysis or death (Fig. 2A and C). In contrast, none of the rats or mice injected with the same dose or even higher doses of GLESS-FAST-VSV displayed significant weight loss (Fig. 2A and C), physical paralysis, or death (Fig. 2B and D). Histopathological examination revealed only limited inflammatory reactions 1 week postinjection in the high-dose GLESS-FAST-VSV group (10^6 pfu), without ventricular inflammation or signs of total brain infection such as meningitis and with a small amount of virus staining (Fig. 2E). On the contrary, VSV Δ M51 infection led to total brain inflammation, signs of brain tissue necrosis, and bleeding, with extensive viral staining (Fig. 2E).

Inhibition of Glioma Growth and Prolongation of Survival Time by GLESS-FAST-VSV

To further determine the efficacy of GLESS-FAST-VSV in vivo, we established the RG2 intracranial tumor model in normal-immunity rats and then intratumorally injected the GLESS-FAST-VSV. The data demonstrated significant inhibition of tumor growth and tumor volume in the treatment group (Fig. 3A). Meanwhile, we found that direct injection of GLESS-FAST-VSV significantly prolonged the survival time of tumor-bearing rats, where the median survival time of the treatment group (28 days) was significantly longer than that in the control group (19.5 days; Fig. 3B).

The same experiment was repeated using the GL261 intracranial tumor model in normal-immunity mice. We

FIG. 1. Effects of GLESS-FAST-VSV on glioma cell lines in vitro. **A:** All glioma cell lines were infected with GLESS-FAST-VSV (MOI = 1) for 48 hours (h), and the expression of RFP was then examined. **B:** Effects of GLESS-FAST-VSV (MOIs = 0, 0.01, 0.1, 1, and 10) on cell viability at 48 hours after infection were examined via an alamarBlue assay. U87 and HS68 are positive and negative controls, respectively. All data were normalized to the negative control group. * $p < 0.05$; ** $p < 0.01$.

also found that the GLESS-FAST-VSV treatment significantly extended the survival time of tumor-bearing mice, where the median survival time of mice in the treatment group and that in the control group were 29.5 and 22 days, respectively (Fig. 3C).

Discussion

In this study, we confirmed that the newly constructed OV, GLESS-FAST-VSV, was not neurotoxic in normal-

immunity rats and mice and had a strong therapeutic effect in the normal-immunity rat and mouse glioma models. The following experiments were performed. First, we used GLESS-FAST-VSV to infect different types of glioma cell lines and normal fibroblasts in vitro and found that GLESS-FAST-VSV could infect and kill brain tumor cells with a less toxic effect on normal cells. Next, we injected different doses of GLESS-FAST-VSV and a positive control virus (VSV^{ΔM51}) into normal F344 rats and C57BL/6 mice for further evaluation of the safety of the virus. Each

FIG. 2. Safety/toxicity evaluation of GLESS-FAST-VSV in vivo. **A:** Representative body weights of the rats infected with different doses of GLESS-FAST-VSV and VSV Δ M51. **B:** Survival time of non-tumor-bearing normal rats (F344) injected with GLESS-FAST-VSV and VSV Δ M51. **C:** Representative body weights of the mice infected with different doses of GLESS-FAST-VSV and VSV Δ M51. **D:** Survival time of non-tumor-bearing normal mice (C57BL/6) injected with GLESS-FAST-VSV and VSV Δ M51. **E:** Representative images of brain tissue in rats inoculated with GLESS-FAST-VSV and VSV Δ M51, on the 7th day after intracranial injection (left and middle columns: H&E; right column: immunohistochemistry for viral antigen). Arrows indicate inflammation.

animal's weight, survival time, and pathological changes were examined on the following day. The results showed that all animals tolerated the GLESS-FAST-VSV injections well, and no animal deaths, encephalitis, or ven-

triculitis was observed. In contrast, all the animals whose brains were injected with VSV Δ M51 died. Finally, RG2 and GL261 cells were used to construct rat and mouse glioma models, respectively, using a stereotactic method, fol-

FIG. 3. Intratumoral administration of GLESS-FAST-VSV inhibited tumor growth and prolonged survival in vivo. **A:** Representative histological images (left) of brain tumor in rats treated with DV or GLESS-FAST-VSV (10^7 pfu/rat/time, every 3 days, 6 injections total) and quantification (right) of tumor size in DV- and GLESS-FAST-VSV-treated rats. $**p < 0.01$. **B:** Analysis of the survival time of RG2-bearing rats after treatment with DV or GLESS-FAST-VSV (10^7 pfu/rat/time, every 3 days, 6 injections total). Data showed that the VSV-treated rats survived longer than the DV-treated control rats ($p = 0.0007$, log-rank test). **C:** Kaplan-Meier survival curves for C57BL/6 mice harboring GL261 tumor treated with DV or GLESS-FAST-VSV (10^6 pfu/mice/time, every 5 days, 3 injections total; $p = 0.008$, log-rank test). Arrows indicate the time points of viral inoculation.

lowed by multiple injections of the viruses into brain tumors. The results demonstrated reduced tumor sizes and prolonged survival times of animals in the GLESS-FAST-VSV-injected group. Collectively, our results confirmed that GLESS-FAST-VSV could specifically target the tumor, effectively inhibit tumor growth, and then prolong the survival of normal-immunity rats and mice in the absence of neurotoxicity. These findings would provide a strikingly improved OV platform for the treatment of brain tumor with the injection of non-neurotoxic VSV, uncovering a potential vehicle for future gene therapy for brain cancer, as GLESS-FAST-VSV specifically and efficiently infects brain tumor cells and does not induce neurotoxic damage to the normal brain tissue.

As mentioned above, to remove infiltrating brain tumor cells, various types of OVs have been evaluated in diverse glioma models. At present, only preclinical studies on glioma have included nearly 10 OVs. The results of these studies have shown that most OVs are safe and have strong tumor-killing ability. Some viruses have even cured tumor-bearing immunodeficient animals.^{5–7,15–18} Among them, Newcastle disease virus (PV701, OV001),⁵ adenovirus (ONYX-015),⁶ reovirus-T3D,⁷ and herpes simplex virus (HSV-1, HSV G207)²⁵ have been used in the clinical phase I-II trials for the treatment of glioma patients, and the preliminary results have confirmed that these viruses

are safe for use in patients with brain tumors. Therefore, the OV may become another important method for the treatment of glioma, in addition to surgery, radiotherapy, and chemotherapy, or it may become a beneficial auxiliary treatment measure. VSV is widely recognized as an OV with a strong, selective tumor-killing ability, and it is one of the OVs with great potential for the treatment of various types of tumors. However, until now, there were no reports on the direct injection of VSV into brain tumors and no relevant clinical studies because of the safety risks, especially its neurotoxicity. Previous studies have shown that VSV cannot be injected directly into brain tumors.^{9,25} Even if a very low dose of attenuated VSV (VSV Δ M51) was injected into the brain, the animal would quickly die.^{9,26} Another study reported that some mice and primates developed hind limb paralysis or other neurological symptoms with the intravenous administration of high doses of VSV.²⁷ Thus, the toxicities of VSV greatly limit its widespread use, particularly its clinical application for brain tumors. Brain tumors are confined to the brain and rarely spread outside the brain. At the same time, the central nervous system is an immune preferential area. Therefore, brain tumors may be a good indication for local OV treatment, especially direct intratumoral injection of OV after surgery. Therefore, it is very important to further improve the safety of VSV directly applied in the brain. Through an in-depth

understanding of the structure of VSV, the viral matrix protein (M) and cystic membrane protein (G) were found to play an important role in VSV-induced cell damage and neurotoxicity. Our collaborators from Dr. John Bell's laboratory successfully constructed a novel stomatitis virus, GLESS-FAST-VSV, through the pseudo-transformation of VSV. In the present study, we initially demonstrated that this virus not only significantly reduced the neurotoxicity of wild viruses but also retained the powerful oncolytic effect. Thus, the virus can be injected directly into the brain tumor, and it can extend the survival time of the animals with brain tumors. Therefore, GLESS-FAST-VSV would become the first choice for direct intratumoral injection of VSV for brain tumor treatment. So far, there have been no reports on the treatment of glioma by direct intracerebral injection of VSV.

Currently, most preclinical in vivo models evaluating OV treatment of brain glioma use immunodeficient mice carrying human glioma cell lines.^{8,9,19,21,22} However, not only does this animal model lack a normal immune system, but the gliomas in most of these models are dissimilar to human glioma given the loss of many valuable genes or molecular markers during long-term cultivation and passage. Thus, the use of these animal models of glioma means that many drugs and OVs may be very effective in in vivo studies but ineffective in clinical studies. Therefore, it is particularly important to study the therapeutic effects of OV and to explore new drugs using the appropriate animal models of glioma, such as using normal-immunity animals carrying gliomas. The animals' normal innate immune system or acquired immunity may regulate the therapeutic effect of OVs.²⁷ In this study, we evaluated the safety and efficacy of GLESS-FAST-VSV using rat and mouse glioma models with normal immune systems. The results are relatively reliable, laying a certain experimental foundation for the future application of this type of VSV in clinical trials. Moreover, recent studies have found that stem cells cultured from fresh brain tumor specimens have molecular markers very close to those of the patients' brain tumor cells (more than 80%), and glioma stem cells are likely to be the origin of various brain tumor cells and also the main reason for glioma recurrence and resistance to various treatments (radiotherapy and chemotherapy).^{28–31} Therefore, the glioma model based on brain tumor stem cells may become the main focus of and may be extremely useful in the preclinical studies on OVs.³²

Conclusions

In summary, our results confirmed that GLESS-FAST-VSV treatment can specifically target tumors, effectively inhibit brain tumor growth, and then prolong the survival of normal-immunity animals without neurotoxicity. These findings provide new ideas and methods for the treatment of brain tumors. They also lay a theoretical foundation for the future application of such VSVs in clinical trials.

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References

1. Penas-Prado M, Armstrong TS, Gilbert MR. Glioblastoma. *Handb Clin Neurol*. 2012;105:485–506.
2. Kesari S. Understanding glioblastoma tumor biology: the potential to improve current diagnosis and treatments. *Semin Oncol*. 2011;38(suppl 4):S2–S10.
3. Kirn D, Martuza RL, Zwiebel J. Replication-selective virotherapy for cancer: biological principles, risk management and future directions. *Nat Med*. 2001;7(7):781–787.
4. Liu TC, Galanis E, Kirn D. Clinical trial results with oncolytic virotherapy: a century of promise, a decade of progress. *Nat Clin Pract Oncol*. 2007;4(2):101–117.
5. Freeman AI, Zakay-Rones Z, Gomori JM, et al. Phase I/II trial of intravenous NDV-HUJ oncolytic virus in recurrent glioblastoma multiforme. *Mol Ther*. 2006;13(1):221–228.
6. Chiocca EA, Abbed KM, Tatter S, et al. A phase I open-label, dose-escalation, multi-institutional trial of injection with an E1B-attenuated adenovirus, ONYX-015, into the peritumoral region of recurrent malignant gliomas, in the adjuvant setting. *Mol Ther*. 2004;10(5):958–966.
7. Forsyth P, Roldán G, George D, et al. A phase I trial of intratumoral administration of reovirus in patients with histologically confirmed recurrent malignant gliomas. *Mol Ther*. 2008;16(3):627–632.
8. Lun X, Yang W, Alain T, et al. Myxoma virus is a novel oncolytic virus with significant antitumor activity against experimental human gliomas. *Cancer Res*. 2005;65(21):9982–9990.
9. Lun X, Senger DL, Alain T, et al. Effects of intravenously administered recombinant vesicular stomatitis virus (VSV(ΔM51)) on multifocal and invasive gliomas. *J Natl Cancer Inst*. 2006;98(21):1546–1557.
10. Lun X, Chan J, Zhou H, et al. Efficacy and safety/toxicity study of recombinant vaccinia virus JX-594 in two immunocompetent animal models of glioma. *Mol Ther*. 2010;18(11):1927–1936.
11. Peters C, Rabkin SD. Designing Herpes viruses as oncolytics. *Mol Ther Oncolytics*. 2015;2:150.
12. Kaufman HL, Kohlhapp FJ, Zloza A. Oncolytic viruses: a new class of immunotherapy drugs. *Nat Rev Drug Discov*. 2015;14(9):642–662.
13. Zamarin D, Palese P. Oncolytic Newcastle disease virus for cancer therapy: old challenges and new directions. *Future Microbiol*. 2012;7(3):347–367.
14. Chan WM, Rahman MM, McFadden G. Oncolytic myxoma virus: the path to clinic. *Vaccine*. 2013;31(39):4252–4258.
15. Lun XQ, Jang JH, Tang N, et al. Efficacy of systemically administered oncolytic vaccinia virotherapy for malignant gliomas is enhanced by combination therapy with rapamycin or cyclophosphamide. *Clin Cancer Res*. 2009;15(8):2777–2788.
16. Yang WQ, Lun X, Palmer CA, et al. Efficacy and safety evaluation of human reovirus type 3 in immunocompetent animals: racine and nonhuman primates. *Clin Cancer Res*. 2004;10(24):8561–8576.
17. Lun X, Alain T, Zemp FJ, et al. Myxoma virus virotherapy for glioma in immunocompetent animal models: optimizing administration routes and synergy with rapamycin. *Cancer Res*. 2010;70(2):598–608.
18. Myers R, Harvey M, Kaufmann TJ, et al. Toxicology study of repeat intracerebral administration of a measles virus derivative producing carcinoembryonic antigen in rhesus macaques in support of a phase I/II clinical trial for patients with recurrent gliomas. *Hum Gene Ther*. 2008;19(7):690–698.
19. Ozduman K, Wollmann G, Piepmeyer JM, van den Pol AN. Systemic vesicular stomatitis virus selectively destroys multifocal glioma and metastatic carcinoma in brain. *J Neurosci*. 2008;28(8):1882–1893.
20. Alain T, Lun X, Martineau Y, et al. Vesicular stomatitis virus oncolysis is potentiated by impairing mTORC1-dependent

- type I IFN production. *Proc Natl Acad Sci U S A*. 2010; 107(4):1576–1581.
21. Wollmann G, Rogulin V, Simon I, et al. Some attenuated variants of vesicular stomatitis virus show enhanced oncolytic activity against human glioblastoma cells relative to normal brain cells. *J Virol*. 2010;84(3):1563–1573.
22. Wollmann G, Tattersall P, van den Pol AN. Targeting human glioblastoma cells: comparison of nine viruses with oncolytic potential. *J Virol*. 2005;79(10):6005–6022.
23. Varela-Guruceaga M, Tejada-Solís S, García-Moure M, et al. Oncolytic viruses as therapeutic tools for pediatric brain tumors. *Cancers (Basel)*. 2018;10(7):E226.
24. Felt SA, Grdzlishvili VZ. Recent advances in vesicular stomatitis virus-based oncolytic virotherapy: a 5-year update. *J Gen Virol*. 2017;98(12):2895–2911.
25. Aghi MK, Chiocca EA. Phase I trial of oncolytic herpes virus G207 shows safety of multiple injections and documents viral replication. *Mol Ther*. 2009;17(1):8–9.
26. Stojdl DF, Lichty BD, Tenover BR, et al. VSV strains with defects in their ability to shutdown innate immunity are potent systemic anti-cancer agents. *Cancer Cell*. 2003;4(4): 263–275.
27. Bi Z, Barna M, Komatsu T, Reiss CS. Vesicular stomatitis virus infection of the central nervous system activates both innate and acquired immunity. *J Virol*. 1995;69(10):6466–6472.
28. Singh SK, Clarke ID, Terasaki M, et al. Identification of a cancer stem cell in human brain tumors. *Cancer Res*. 2003; 63(18):5821–5828.
29. Kelly JJ, Stechishin O, Chojnacki A, et al. Proliferation of human glioblastoma stem cells occurs independently of exogenous mitogens. *Stem Cells*. 2009;27(8):1722–1733.
30. Bao S, Wu Q, McLendon RE, et al. Glioma stem cells promote radioresistance by preferential activation of the DNA damage response. *Nature*. 2006;444(7120):756–760.
31. Liu G, Yuan X, Zeng Z, et al. Analysis of gene expression and chemoresistance of CD133+ cancer stem cells in glioblastoma. *Mol Cancer*. 2006;5:67.
32. Wakimoto H, Kesari S, Farrell CJ, et al. Human glioblastoma-derived cancer stem cells: establishment of invasive glioma models and treatment with oncolytic herpes simplex virus vectors. *Cancer Res*. 2009;69(8):3472–3481.

Disclosures

The authors report no conflict of interest concerning the materials or methods used in this study or the findings specified in this paper.

Author Contributions

Conception and design: Wang, Jiang, Lun. Acquisition of data: Jiang, Huang, Guo, Yin, Forsyth. Analysis and interpretation of data: Jiang, Huang, He, Forsyth. Drafting the article: Jiang. Critically revising the article: Wang, Forsyth, Lun. Reviewed submitted version of manuscript: Wang, Jiang, Lun. Approved the final version of the manuscript on behalf of all authors: Wang. Statistical analysis: He. Administrative/technical/material support: Wang. Study supervision: Wang, Lun. Conducted experiments: Huang, He.

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