A new transcriptionally driven oncovirus with Vstat120 expression has antiangiogenic and anti-tumorigenic effects

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Objectives

The purpose of this study was to: 1) create a novel oncolytic virus OV- 34.5ENVE transcriptionally driven to have increased virus replication in nestin-positive glioma cells also armed with anti-angiogenic Vstat120 gene to modulate tumor microenvironment, 2) examine anti-tumoral efficacy of 34.5ENVE in glioma tumor, 3) determine intracranial tumor perfusion by DCE-MRI technique.

Methods

Virus: To generate 34.5ENVE, the expression cassette encoding for Vstat120 gene under the control of viral IE4/5 promotor was generated using HSVQuik technology as previously described (Terada K et al, Gene Ther 2006). All viruses were propagated in Vero cells, and the titer (plaque forming units per ml, PFU/ml) used in this study was determined by plaque forming unit assay in Vero cells (Terada K et al, Gene Ther 2006) .

Animal model: Female athymic nu/nu (Charles River Laboratories, Frederick, MD) 6-8 week-old mice were used in all experiments. All animal experiments were performed in accordance with the Subcommittee on Research Animal Care of the Ohio State University guidelines.

Nude mice were implanted with $1.5x10^7$ U251T3 glioma cells into rear flank. When tumors reached an average size of 150-200 mm^3 (V=0.5*L*W*W) mice were administered with PBS, or the indicated virus by direct intratumoral injection ($5x10^5$ pfu) twice, two days apart.

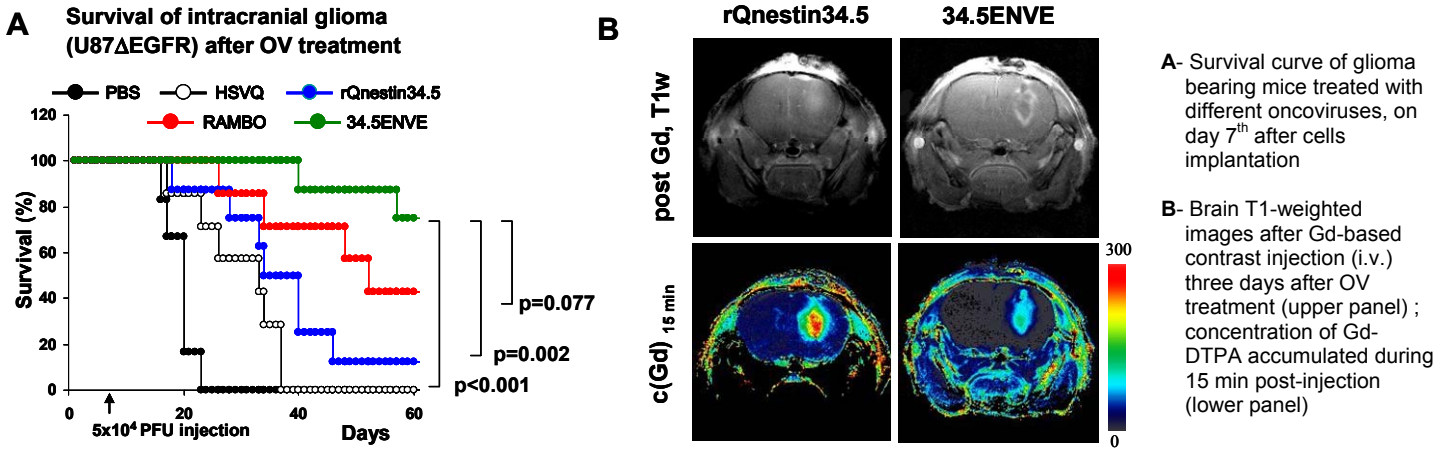
For intracranial tumor studies, anesthetized nude mice were fixed in a stereotactic apparatus, and a burr hole was drilled at 2 mm lateral to the bregma, to a depth of 3 mm. U87ΔEGFR cells ($1×10^5$ cells in 4 µl Hank's buffered salt solution) were implanted. Seven days later the mice were anesthetized again and stereotactically inoculated with $5×10^4$ pfu of PBS, rHSVQ, rQnestin34.5, RAMBO, and 34.5ENVE at the same location.

Perfusion study: Mice with intracranial tumors were treated with PBS or the indicated virus on day 10 after cancer cell implantation. Animals were imaged on day 9 (pre treatment), and on days 13, 16, 20 (3d, 6d, 10d post treatment) using dynamic contrast enhanced magnetic resonance imaging (DCE-MRI). The imaging was performed using a Bruker Biospin 94/30 magnet (Bruker Biospin, Karlsruhe Germany). Magnevist™ (Gd-DTPA, Bayer HealthCare) was used as a contrast agent. A 2.0 cm diameter receive-only mouse brain coil was placed over the head, and the mouse bed with surface coil was placed inside a 70 mm diameter linear volume coil. DCE data were collected using a FLASH sequence (TR/TE=135.8/2.4ms, flip angle=50°). Several baseline images were collected before the bolus of Gd-DTPA injection (0.5 mmol/kg) through the tail vein catheter and for 30 min post-injection. The acquisition parameters were as follows: FOV=20x20 mm^2, slice thickness=1 mm, matrix size=256x256, 1 average.
Gd-DTPA concentrations were calculated for all voxels within the region-of-interest ROI, manually outlined on the T2-weighted images, using the method described by Haacke EM et al (Magn Reson Med, 2007). A fixed value of T1(0) was chosen as 2029 based on T1 measurements made in normal mouse brain at 9.4T Kuo YT et al (J Magn Reson Imaging, 2005).

Results and Discussion

Treatment of tumor bearing mice (flank) with 34.5ENVE showed 3.5, 2.1 and 1.8 fold reduction in microvessel density compared to HSVQ, rQnestin34.5, and RAMBO treatment (respectively). Intratumoral injection with OVs of intracranial glioma (U87ΔEGFR) on day 7th, after cells implantation, significantly prolonged animal survival rate (see Figure A).

DCE MRI of mice with intracranial tumors revealed distinct spatial and temporal changes in vascular perfusion induced after treatment with 34.5ENVE compared to rQnestin34.5. Immediately after Gd injection the central tumor core of mice treated with 34.5ENVE showed absence of vascular perfusion/leakage. With a time contrast agent diffused from the rim surrounding the site of oncolysis and accumulated into the central 34.5ENVE treated core (Figure B). Thus it is possible that anti-angiogenic effects of Vstat120 combined with enhanced oncolysis resulted in large necrotic core in tumors treated with 34.5ENVE. In fact, large necrotic and avascular areas were observed in tumor sections treated with 34.5ENVE compared to rQnestin34.5. Collectively these results suggest that increased antitumor and anti-angiogenic effects of 34.5ENVE in the tumor core resulted in initial occlusion of the vasculature and subsequent necrosis. However, over a period of time the contrast agent leakage in to the tissue surrounding the site of oncolysis diffused into the necrotic/avascular core where it accumulated due to absence of capillaries what reduced re-absorption.



A- Survival curve of glioma bearing mice treated with different oncoviruses, on day 7th after cells implantation

B- Brain T1-weighted images after Gd-based contrast injection (i.v.) three days after OV treatment (upper panel) ; concentration of Gd-DTPA accumulated during 15 min post-injection (lower panel)

Conclusions

This study reports the construction and anti-tumor efficacy of a novel transcriptionally driven OV armed with Vstat120 expression. 34.5ENVE has shown unsurpassed anti-tumor efficacy and encourages its development as a potent therapeutic agent for patients.