Chondroitinase ABC I–Mediated Enhancement of Oncolytic Virus Spread and Antitumor Efficacy

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Abstract

Purpose: The inhibitory role of secreted chondroitin sulfate proteoglycans on oncolytic viral (OV) therapy was examined. Chondroitinase ABC (Chase-ABC) is a bacterial enzyme that can remove chondroitin sulfate glycosaminoglycans from proteoglycans without any deleterious effects in vivo. We examined the effect of Chase-ABC on OV spread and efficacy.

Experimental Design: Three-dimensional glioma spheroids placed on cultured brain slices were utilized to evaluate OV spread. Replication-conditional OV-expressing Chase-ABC (OV-Chase) was engineered using HSQuik technology and tested for spread and efficacy in glioma spheroids. Subcutaneous and intracranial glioma xenografts were utilized to compare antitumor efficacy of OV-Chase, rHsVQ (control), and PBS. Titration of viral particles was performed from OV-treated subcutaneous tumors. Glioma invasion was assessed in collagen-embedded glioma spheroids in vitro and in intracranial tumors. All statistical tests were two sided.

Results: Treatment with Chase-ABC in cultured glioma cells significantly enhanced OV spread in glioma spheroids grown on brain slices (P < 0.0001). Inoculation of subcutaneous glioma xenografts with Chase-expressing OV significantly increased viral titer (≥10 times, P = 0.0008), inhibited tumor growth, and significantly increased overall animal survival (P < 0.006) compared with treatment with parental rHsVQ virus. Single OV-Chase administration in intracranial xenografts also resulted in longer median survival of animals than rHsVQ treatment (32 vs. 21 days, P < 0.018). Glioma cell migration and invasion were not increased by OV-Chase treatment.

Conclusions: We conclude that degradation of glioma extracellular matrix with OV-expressing bacterial Chase-ABC enhanced OV spread and antitumor efficacy. Clin Cancer Res; 17(6); 1362–72. ©2010 AACR.
Materials and Methods

Cells, viruses, and antibodies

U1343, U87, U87ΔEGFR, LN229, Gli36ΔEGFR-H2B-RFP, X12 human glioma cells, and Vero cells were maintained as described (20–22). Mouse monoclonal anti-chondroitin-4-sulfate antibody (clone BE-123; MP Biomedicals Inc.) was used to probe for Chase functionality. Tumor-bearing sections were labeled with Wisteria floribunda lectin (WFL; Vector Labs Inc.), antivimentin (clone SP20; Lab Vision). OVs rhSVQ, rhSVQ-Luc, and rQNestin34.5 have been previously described (23, 24). Genomic DNA from Proteus vulgaris (ATCC number 9920D) was used as a template to clone Chase-ABC I cDNA as described (25). The PCR product was subcloned into pSecTag/FRT/V5 His-Topo vector (Invitrogen) and used to generate OV-Chase as previously described (24). Cytotoxicity of viruses was assessed by a standard crystal violet assay (26).

Viral spread/replication assays

Spread of viral particles was visualized by fluorescence microscopy of OV-encoded green fluorescent protein (GFP) in infected cells, using a Zeiss LSM 510 Meta confocal microscope and quantified using the software ImageJ (NIH). For replication assays, infected cells and supernatant or tissues were collected and viral stocks were titrated by a standard plaque assay (21).

Invasion assays

To perform collagen invasion assays, glioma cell spheroids were treated with OV-Chase, rhSVQ, or vehicle and embedded in collagen (22). Migration of cells away from the core aggregate was monitored using a Nikon Eclipse TE2000-U fluorescent microscope.

Animal studies

All experiments with animals were performed according to the guidelines of the Subcommittee on Animal Research and Care of The Ohio State University. Female athymic mice (nu/nu) were used for all xenografts studies. For intracranial xenografts, tumor cells were implanted stereotactically at a position 2 mm lateral to bregma at a depth of 3 mm. Ten days after tumor cell implantation, animals were stereotactically inoculated with PBS or virus. Mice bearing subcutaneous tumors received intratumoral injections of HBSS or the indicated OV. Tumor volumes were measured regularly as described (26). Animals were observed daily and were euthanized at the indicated time points or when they showed signs of morbidity (intracranial) or when tumor volumes exceeded 1,600 mm³ (subcutaneous).

Statistical analysis

Two-tailed Student’s t test were used to assess effects of Chase-ABC on viral infection/replication, cytotoxicity, and titration from subcutaneous tumors (Origin 7 statistical software; OriginLab Corporation). Two-way ANOVAs for repeated measures were used to evaluate viral spread on glioma spheroids. Kaplan–Meier curves were compared using the log-rank test, generalized Wilcoxon and Tarone–Ware tests (SPSS statistical software, version 17.0; SPSS, Inc.). The t test from fitting linear mixed model was used to test slope difference in the tumor volume

Translational Significance

Despite the current standard of care, malignant astrocytomas remain a devastating disease with a median survival of less than 15 months. Thus, there is an urgent need to improve current therapeutic modalities. Oncolytic viruses (OV) represent a promising biological therapy currently being evaluated in patients for safety and efficacy. Here we show that tumoral chondroitin sulfate proteoglycans (CSPG) present a formidable barrier for the spread of OV through the tumor. On the basis of these results, we have created a novel OV armed with bacterial chondroitinase ABC (Chase-ABC). Chase-ABC–mediated removal of chondroitin sulfate glycosaminoglycan sugar chains from glioma CSPG enhances virus spread without increasing glioma invasion. This is the first report investigating the effects of Chase-ABC on tumor biology. These preclinical results will facilitate future clinical testing of this OV.

collagenase-mediated ECM disruption can cause hemorrhagic necrosis of brain (15), and hyaluronidase elicits astrocytic reactivity, which can promote optic glioma growth (16).

Secreted and membrane-bound chondroitin sulfate proteoglycans (CSPG) linked to extracellular hyaluronan form a major component of the ECM in the brain (17). In central nervous system (CNS) tumors, expression of several CSPGs such as versican, brevican, phosphacan, and NG2 is increased and associated with increased tumor growth, angiogenesis, and invasion (18). Apart from molecular signaling, the sugar side chains of chondroitin sulfate glycosaminoglycans (CS-GAG) on CSPGs are responsible for biophysical properties that limit interstitial diffusion. Chondroitinase ABC (Chase-ABC) is a bacterial enzyme that can cleave and remove the CS-GAG from CSPG, leaving the core protein intact (19). While Chase-ABC has been studied for its effect on neuronal regeneration after injury, its impact on tumor ECM has not been previously examined. Here, we hypothesized that Chase-ABC–mediated digestion of glioma CS-GAGs would open glioma ECM, enhancing OV dissemination and efficacy without detrimental effects to surrounding brain. Treatment of glioma spheroids grown in organotypic cultures with purified Chase-ABC enhanced spread of OV into the sphere. To investigate the inhibitory role of tumor CSPGs on OV therapy, we have created a novel armed Chase-ABC I–expressing herpes simplex-1 OV and tested its spread and antitumor effects.

This is the first study investigating the use of bacterial chondroitinase to enhance anticancer therapy.
growth over the time. A value of $P < 0.05$ was considered to be statistically significant for all tests.

Results

CSPGs detected in glioma cell lines and tumor samples interfere with OV spread in glioma spheroids

The presence of CSPG in glioma cell lines and tumor tissue was confirmed by Western blotting and immunofluorescent staining of tumor tissue (Supplementary Fig. 1). To test whether purified Chase-ABC could affect the ability of oncolytic HSV-1 to infect and replicate in glioma cells, we first analyzed the ability of OVs: rQNestin34.5 (multiplicity of infection (MOI) = 1; Fig. 1A) and rHsvQ-Luc (MOI = 0.5; Fig. 2B) to infect glioma cells treated with purified Chase-ABC (black bars) or vehicle (white bars). OV infection was evaluated either by comparing the number of GFP-positive cells/view field 12 hours postinfection (Fig. 1A) or by measuring the amount of virus-encoded luciferase per milligrams of protein in cell lysates harvested 6 hours after infection in the wells treated or untreated cells (Fig. 1B). The results indicated that treatment of cells with Chase-ABC did not affect ability of virus to infect glioma cells grown in monolayer.

Next we tested whether Chase-ABC treatment of OV could affect the ability of OVs to infect and replicate in glioma cells. The indicated glioma cells were infected with buffer (white bars) or Chase-ABC–treated (black bars) OV, and the amount of infectious viral particles obtained was quantified by a standard titration assay (Fig. 1C). The results indicated that Chase-ABC treatment of rHsvQ did not affect its ability to infect and/or replicate in glioma cells in vitro. Collectively, these results indicated that Chase-ABC treatment of glioma or OV did not affect viral infection or replication in vitro.
To evaluate the effect of Chase-ABC treatment of glioma spheroids on OV spread in 3-dimensional matrix, glioma spheroids were cultured on organotypic brain slices. This reproduced most of the ECM organization of the tumor. U87ΔEGFR spheroids treated with purified Chase-ABC or vehicle were infected with rQNestin34.5 (n = 6/group), and OV spread was visualized by fluorescence microscopy of OV-encoded GFP. Visually, all Chase-treated spheroids showed more efficient spread of the OV throughout the spheroid than in control-treated spheroids (Fig. 1D). Quantification revealed a statistically significant increase in OV spread after Chase-ABC treatment of glioma spheroids (P < 0.0001; Fig. 1E).

Creation of OV-Chase–encoding functional recombinant Chase-ABC

On the basis of the observed lack of detrimental effects of purified Chase-ABC treatment on OV infection of monolayer cells, combined with the enhanced spread of OV observed in treated glioma spheroids, we created OV-Chase, an oncolytic HSV-1–expressing bacterial Chase-ABC driven by the HSV-1 IE4/5 promoter within the backbone of the double attenuated rHsvQ (24). Figure 2A shows the genetic maps of wild-type HSV-1, indicating the location of both the copies of γ34.5 and the ICP6 locus. Middle, map of control rHsvQ with deletion of both copies of γ34.5 and in-frame gene-disrupting insertion of GFP within the ICP6 gene. Bottom, map of OV-Chase showing the insertion of Chase-ABC cDNA under the viral IE4/5 promoter within the ICP 6 locus. B, glioma spheroids derived from U87ΔEGFR were treated with purified Chase-ABC (Chase) or infected with GFP-encoding OV-Chase or rHsvQ. Spheroids were fixed 72 hours postinfection and processed for immunohistochemistry with antibody against CS-stubs. Results show the appearance of red immunoreactive CS-stubs (arrows) in spheroids treated with purified Chase or OV-Chase, indicating that the virus produced a functional enzyme (scale bar, 5 μm).

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To evaluate whether expression of Chase-ABC affected the ability of the OV to infect, replicate, and lyse glioma cells in vitro, we compared the cytotoxicity of OV-Chase and rHsvQ in 3 different glioma cell lines grown as monolayers. To discriminate whether the observed properties were a true reflection of OV-Chase or occurred because of a random, nonspecific mutation that may have occurred during virus isolation, we compared 2 different isolates of OV-Chase. Glioma cells were inoculated with rHsvQ or 2 different isolates of OV-Chase at the indicated MOI. Five days postinfection, cell viability was assessed by a standard crystal violet assay (Fig. 3). No significant difference was observed between rHsvQ and either of the two independent viral isolates of OV-Chase (P values >0.14 for all rHsvQ-treated cells vs. OV-Chase1- or OV-Chase2–treated cells).

We next compared the spread of rHsvQ and OV-Chase in U87ΔEGFR glioma cells grown as 3-dimensional spheroids on organotypic brain slices. Briefly, glioma spheroids were infected with 10⁴ pfu (plaque forming units) of rHsvQ or OV-Chase (n = 6/group) and infected cells were visualized by appearance of OV-encoded GFP over time. Quantitative analysis of GFP fluorescence from spheres 24 and 60 hours postinfection (Fig. 4A) showed that rHsvQ infection did not spread within the spheroids. In contrast, OV-Chase spread to the core of the tumor spheroids in all cases, resulting in a wide distribution of GFP fluorescence. These results were statistically significant at all time points.
Figure 3. Chase expression from OV-Chase does not interfere with viral cytotoxicity. Glioma cells (U343, LN229, and U87ΔEGFR) were inoculated with rHsvQ or 2 different isolates of OV-Chase (OV-Chase 1 and OV-Chase 2) at MOI = 0, 0.05, 0.1, 0.5, or 1. Two independent isolates of OV-Chase were purified and used in this assay to test whether changes in virus infection or replication were specific to the inserted Chase ABC expression cassette. Cell viability was assessed during 5 days postinfection, using a standard crystal violet assay. Columns (% of surviving cells at day 5), mean values for each treatment (±95% CIs; n = 4/treatment for each cell line). Results indicate that in vitro cytotoxicity of both the independent isolates of OV-Chase is comparable with control rHsvQ.
Taken together, our results indicated that OV-Chase infected and replicated in glioma cells as efficiently as the control rHsvQ virus and exhibited a highly improved spread in 3-dimensional glioma cultures.

**OV-Chase has improved antitumor efficacy in vivo compared with control OV**

To test the antitumor efficacy of OV-Chase, we assayed its effect on subcutaneous tumors formed by injection of Gli36ΔEGFR-H2B-RFP glioma cells in athymic mice. Animals were treated with PBS or 10⁶ pfu of rHsvQ or OV-Chase when tumors reached a volume of 80 to 150 mm³. The mice were closely monitored for tumor growth and sacrificed when tumors reached a volume of 1,600 mm³ (endpoint condition). Survival analysis (Kaplan–Meier curves, Fig. 5A) showed a significant increase in survival of animals treated with OV-Chase compared with rHsvQ (median survival = 28 and 16 days, respectively;
Figure 5. OV-Chase has improved efficacy against glioma in vivo. A and B, mice injected with Gli36ΔEGFR-H2B-RFP cells subcutaneously were left untreated until the tumors reached an average volume of 80 to 150 mm³. Animals were then injected with PBS (triangles), 10⁶ pfu rHsvQ (squares), or 10⁶ pfu OV-Chase (diamonds) 6 times (day 0, 2, 4, 6, 8, and 10, arrows). Tumors were monitored daily and animals were sacrificed when the tumors reached a volume of 1,600 to 2,000 mm³. Results show the Kaplan–Meier survival curves (A) and tumor growth (B) (95% CI). Animals treated with OV-Chase survived significantly longer in average than those treated with vehicle or the control OV (P < 0.0001). Tumor volume values were log-transformed to make the trend over time linear. Slope difference between OV-Chase and PBS was 0.119 (P = 0.0001), whereas slope difference between OV-Chase and rHsvQ was 0.094 (P = 0.0008). Trends were not different between PBS and rHsvQ (P = 0.162). C and D, mice injected with U87ΔEGFR cells subcutaneously were left untreated until the tumors reached an average volume of 80 to 150 mm³ and then treated with PBS (triangles), rHsvQ (squares), or OV-Chase (diamonds) as above. Animals were monitored and tumors were harvested using the same criteria as before for Gli36ΔEGFR tumors. Results show (C) Kaplan–Meier survival curves and (D) tumor growth (95% CI). Animals treated with OV-Chase survived again significantly longer in average than those treated with vehicle or the control OV (P < 0.006 by log-rank test). OV-Chase-treated tumors had statistically significant lower slope values than both rHsvQ (P = 0.0008) and PBS-treated tumors (P = 0.0006). The slope difference between rHsvQ and PBS-treated tumors was not significant (P = 0.297). E, U87ΔEGFR cells were injected intracranially in mice and animals treated with a single dose of PBS, rHsvQ, or OV-Chase 10 days after tumor implantation. Kaplan–Meier survival curves indicate improved antitumor efficacy of OV-Chase over the control treatments (P < 0.05).
with OV-Chase lived more than 80 days, at which point they were sacrificed and no evidence of intracranial tumor was found by histologic examination. Collectively, these results indicated that OV-Chase treatment had a significant antitumor effect in vivo compared with control rHsvQ.

OV-Chase has enhanced spread in vivo compared with control OV

We compared the spread and replication of OV-Chase with control rHsvQ in tumors. Mice with subcutaneous tumors (Gli36AEGFR-H2B-RFP cells) that reached a volume of 200 to 270 mm³ were treated with 10⁷ pfu of OV-Chase or rHsvQ on day 0 and day 2 as described in the Materials and Methods section. Five days later, the animals were sacrificed and the amount of infectious virus particles in each tumor was evaluated. Results (Supplementary Fig. S2A) showed a statistically significant increase in the titer of infectious particles from OV-Chase–treated tumors compared with rHsvQ-treated tumors (P = 0.0008). Representative microphotographs of 50-μm-thick sections from these tumors (Supplementary Fig. S2B) depict the wider distribution of viral-encoded GFP in OV-Chase–treated tumors than in rHsvQ-treated tumors. In a parallel experiment, we tested the spread of OV-Chase in intracranial (U87AEGFR) tumors in mice. HSV-1 staining of tumor-bearing brain sections from mice 3 days after treatment with 1.5 × 10⁵ pfu of rHsvQ or OV-Chase was performed to visualize viral spread in intracranial tumors. Supplementary Figure 2C shows representative images of tumor sections from rHsvQ (top panel)- or OV-Chase (bottom panel)-treated tumors. Marked increase in the spread of OV-Chase was observed in all the sections compared with rHsvQ-treated tumors (Supplementary Fig. S2C).

Chase-ABC and OV-Chase do not increase glioma cell dispersal

CSPGs form large macromolecular complexes that collectively inhibit cell and axon motility in normal brain. In contrast, CSPGs are more soluble in gliomas and have been clearly shown to increase glioma cell dispersion (17). Because degradation of chondroitin sulfate (CS) chains by Chase could solubilize CSPGs from the tumor ECM and facilitate cell dispersion, we investigated whether treatment with OV-Chase would affect the invasiveness of glioma cells into their surrounding matrix in vivo and in vitro. First, we evaluated the effect of OV-Chase infection on invasion of glioma cells grown in a 3-dimensional matrix. Spheroids of LN229, U87AEGFR, and X12 glioma cells (n = 4/group) were treated with PBS, OV-Chase, or rHsvQ, implanted in collagen and imaged at different time points (Fig. 6A, and Supplementary Fig. S3). Glioma cells from PBS-treated spheroids derived from all 3 cell lines dissociated from the spheroid and migrated into the collagen matrix. However, this migration was largely reduced in both rHsvQ- and OV-Chase–treated spheroids compared with PBS-treated spheres. In general, OV-Chase–treated spheroids showed a trend toward reduced migration compared with rHsvQ and this may be a reflection of better

P < 0.0001). Two of the 8 animals treated with OV-Chase were long-term survivors (>80 days) and found to be tumor free upon sacrifice and histologic evaluation. The longer survival times correlated with smaller flank tumor burden, with PBS-treated tumors increasing more than 14.7-fold in volume by 12 days posttreatment versus 9.3-fold for rHsvQ-treated tumors and 2.7-fold for OV-Chase–treated tumors (Fig. 5B).

In a separate experiment, mice were inoculated with the highly proliferative U87AEGFR glioma cells and treated similarly with PBS (n = 6) or rHsvQ or OV-Chase. Comparison of slopes revealed that OV-Chase has statistically significant lower slope values than both rHsvQ and PBS (slope difference = -0.1976, P = 0.0008). The median survival of OV-Chase–treated mice was significantly more than that of mice treated with rHsvQ (median survival = 16 days for OV-Chase–treated tumors and 10 days for rHsvQ-treated tumors, P < 0.006).

To confirm that OV-Chase had efficient antitumoral activity in an orthotopic xenograft model, we tested the virus against intracranial gliomas derived from U87AEGFR cells. Tumor-bearing animals were treated with PBS or 3 × 10⁵ pfu of rHsvQ or OV-Chase 10 days after tumor cell implantation. Survival analysis (Fig. 5E) again showed a significant improvement in survival of animals treated with OV-Chase (median survival = 32 days vs. 21 days for rHsvQ-treated animals, P < 0.05). Two of the 8 mice treated

Figure 6. OV-Chase infection reduces glioma cell dispersion in vitro and does not affect tumor spread in vivo. A, spheroids of LN229 glioma cells were treated with PBS, rHsvQ, or OV-Chase and embedded in collagen as indicated in the Materials and Methods section. The images show representative bright-field (BF) and fluorescent (FL) images revealing total spheroid dispersal (bright-field) and total viral spread in the spheroid (scale bar, 500 μm). B, representative images of mouse brain sections bearing U87AEGFR glioma treated with PBS, rHsvQ, or OV-Chase. The sections were stained with anti-human vimentin to highlight human glioma cells in mouse brain. Infiltrating human glioma cells are visualized as brown staining cells. There was no difference in invasion of glioma cells after treatment with PBS, rHsvQ, or OV (scale bar, 100 μm).
tumor cell killing by OV-Chase. Importantly, the results indicated that OV-Chase did not increase glioma cell invasion in vitro. To test whether in vivo treatment of glioma with OV-Chase affected glioma invasion in vivo, we treated mice bearing U87AGFEG (day 7) or Gli36AGFEG-H2B-RFP (day 10) with PBS or 3 × 10⁷ pfu of HSVQ or OV-Chase (Fig. 6B and Supplementary Fig. S3C, respectively). Mice were sacrificed when they showed signs of tumor burden, and their brains were sectioned and stained for human vimentin. There was no obvious sign of increased glioma invasion in mice treated with OV-Chase compared with PBS- or HSVQ-treated mice.

Discussion

Malignant gliomas are resistant to traditional therapeutic approaches and, despite significant advances in research, their prognosis remains poor with a median survival of less than 15 months. Conditionally replicating OVs with the ability to replicate and destroy tumor cells are a promising therapeutic approach for this disease (3). Enzymatic degradation of some tumor ECM constituents has been shown to enhance viral spread and efficacy in vivo, suggesting that tumor ECM is one of the major limiting factors in viral spread and antitumor efficacy (4). However, concerns about damaging normal brain tissue have limited the usage of matrix-modulating enzymes in intracranial models (8, 10, 12). Here we show for the first time the ability of bacterial Chase-ABC–mediated digestion of glioma ECM to enhance OV spread and antitumor efficacy in vitro and in vivo. The composition and organization of the ECM directly affect tissue structural integrity, cell–cell communication, availability of trophic factors, and diffusion of macromolecules in the interstitial space. CSPGs are major structural components of the neural ECM, produced mostly by astrocytes, and are known to accumulate in aged brain and neural tissues after acute or chronic injury (27). These molecules are well-known inhibitors of cellular motility and axonal extension in the normal CNS (28) and can also regulate the bioavailability of growth factors and the biophysical properties of the extracellular space that facilitates particle diffusion (29). Removal of these CS-GAGs by Chase-ABC has been shown to result in improved regrowth of acutely injured axons in vivo and has been associated with some functional recovery with no evidence of toxicity (30–32). However, the impact of modifying tumoral ECM with Chase-ABC on tumor biology or therapeutic efficacy has not been previously studied.

Malignant gliomas exhibit a dense ECM that is rich in the components that are found in neural ECM, including HA and CSPGs (7). Several CSPGs such as versican, brevican, phosphacan, and NG2 are highly upregulated in gliomas compared with normal adult neural tissue and are associated with increased tumor growth, angiogenesis, and invasion (18). Thus, while CSPGs in normal brain are inhibitory toward axonal regeneration, they have been shown to promote glioma cell invasion and angiogenesis (33, 34).

In addition to their novel functions in glioma, accumulation of CSPGs in the glioma matrix leads to increased tortuosity of the extracellular space and increased interstitial pressure in the tumor. These changes cause resistance to diffusion and limit the spread of large-sized therapeutic agents (35). For these reasons, ECM macromolecules have been repeatedly identified as potential therapeutic targets for adjuvant therapy (36–38). Targeting of CSPGs, using blocking antibodies against versican (36) and RNA interference against phosphacan (39), has shown that reduction or inhibition of these molecules may impair tumor proliferation and dispersion. In addition, degradation of CSPGs with MMP-1 and MMP-8 has been shown to increase hydraulic conductivity and particle diffusion in solid tumors (40). Together, these results suggest that reduction of CSPG levels in gliomas by enzymatic manipulation could inhibit their protumoral effects and at the same time improve diffusion of therapeutic agents within the tumor.

The bacterial enzyme Chase-ABC I (chondroitin lyase), derived from Proteus vulgaris, has been shown to selectively depolymerize CS-GAG chains present on CSPGs into soluble disaccharides. Digestion of these glycosaminoglycans in normal brain reduces the level of glycanated CSPGs (41), overcomes many inhibitory effects of CSPGs, and enables part in the ability of neuronal processes to extend old site of injury to form new synaptic connections (19). This enzyme has been widely used to promote regeneration of injured axonal tracts in rodent models and has been shown to provide a long-lasting “loosening” effect on the ECM scaffold, promoting synaptic plasticity without noticeable deleterious effects (42). On the basis of extensive evidence from preclinical models, highly purified, protease-free preparations of recombinant Chase-ABC I have recently been used in phase 1 and 2 trials to treat patients with herniated lumbar disks in Japan (43). Collectively, these studies highlight the safety of Chase-ABC I in preclinical models and human trials.

In the present work, we hypothesized that Chase-ABC I–mediated digestion of CSPGs could enhance viral dissemination in gliomas without the deleterious effects associated with protease treatment of neural tissue. To test our hypothesis, we first studied the effect of Chase-ABC treatment on viral infection/replication and spread on cultured glioma cells and glioma spheroids. Our results showed that enzymatic digestion of CS-GAGs by Chase-ABC did not affect the ability of OVs to infect/replicate and lyse glioma cells and significantly enhanced OV diffusion through matrix-containing glioma spheroids. This result is particularly relevant because other studies have suggested that CSPGs can modulate virus–cell interactions and affect viral infection and gene transfer efficiency (44–47). Our observation that CS removal did not reflect in changes in viral efficiency in glioma cells suggests that the predominant effect of the enzyme was an improvement in viral spread through the tumor matrix.
On the basis of our initial results, we generated an armed OV-expressing recombinant Chase-ABC under the control of an immediate-early viral promoter. This strategy allowed us to directly remove CS from the surrounding matrix of infected cells independently of coinjection with purified enzyme. Expression of recombinant Chase-ABC by this approach did not interfere with viral oncolysis and enhanced the spread of OV-Chase in glioma spheroids. More important, the enhanced spread of OV-Chase observed in organotypic cultures translated into increased viral efficacy against subcutaneous and intracranial glioma xenografts compared with a control OV.

Because CS degradation in the brain may remove major components which inhibit cellular movement in the adult neural tissue, we further investigated whether Chase-ABC treatment could affect glioma cell motility and invasion. Cell migration assays performed in modified Boyden chambers (Transwell assay) revealed that treatment of CSPGs with Chase-OV did not increase glioma cell motility (not shown). This was an interesting result and suggested that exogenous CSPGs do not impair glioma cell migration compared with migration of neural growth cones (17). Therefore, removal of CSPGs should likely not affect tumor cell dispersal. Our results suggest that treatment of glioma spheroids with OV-Chase did not increase glioma cell dispersion through a 3-dimensional collagen matrix. Further tests in vivo indicated that direct treatment of intracranial xenografts with OV-Chase did not increase cell dispersion and tumor borders were comparable between tumors treated with OV-Chase and a control OV.

In summary, our results show for the first time that degradation of glioma CS-GAGs by OV-expressed recombinant Chase-ABC is sufficient to increase viral spread through the tumor ECM and underscore the potential of utilizing Chase-ABC–armed viruses for the enhancement of tumor oncolysis.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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References

8. Ganesh S, Gonzalez Edick M, Idamakanti N, Abramova M, Vanroey M, Robinson M, et al. Relaxin-expressing, fiber chimeric oncolytic adenovirus independently of coinjection with purified enzyme. Expression of recombinant Chase-ABC by this approach did not interfere with viral oncolysis and enhanced the spread of OV-Chase in glioma spheroids. More important, the enhanced spread of OV-Chase observed in organotypic cultures translated into increased viral efficacy against subcutaneous and intracranial glioma xenografts compared with a control OV.

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Kaur B, Brat DJ, Devi NS, Van Meir EG. Vasculostatin, a proteolytic fragment of brain angiogenesis inhibitor 1, is an antiangiogenic and antitumorigenic factor. Oncogene 2005;24:3832–42.


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