Varicella Zoster Virus Infection of Malignant Glioma Cell Cultures: A New Candidate for Oncolytic Virotherapy?

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Abstract. *Background: Glioblastoma multiforme is a highly aggressive tumor with a median survival of 14 months despite all standard therapies. Focusing on alternative treatment strategies, we evaluated the oncolytic potential of varicella zoster virus (VZV) in malignant glioma cell cultures. Materials and Methods: Replication of wildtype and mutant VZV was comparatively analyzed in glioma cell lines (U87, U251 and U373) and in primary malignant glioma cells (n=10) in vitro by infectious foci assay, immunofluorescence microscopy and western blot analysis. Additionally, the tumor-targeting potential of VZV-infected human mesenchymal stem cells was evaluated. Results: VZV replicated efficiently in all the glioma cells studied here followed by rapid oncolysis in vitro. The attenuated vaccine VZV mutant rOKA/ORF63rev[T171] exhibited most efficient replication. Human mesenchymal stem cells were found suitable for targeting VZV to sites of tumor growth. Conclusion: VZV exhibits an intrinsic oncolytic potential in malignant glioma cell cultures and might be a novel candidate for virotherapy in glioblastoma multiforme.*

Glioblastoma multiforme (GBM) of World Health Organization (WHO) grade IV is the most common and most malignant primary brain tumor in adults (1). The high proliferation rate and invasive growth pattern of the tumor, as well as its broad resistance to conventional therapies, critically limit clinical prognosis and necessitate exploration of alternative treatment strategies.

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Oncolytic virotherapy has been explored as a potential anticancer modality for many tumor types. Efficacy in malignant glioma, however, frequently suffers from i) poor penetration of the viral particles across the blood-brain barrier, ii) ineffective transduction of sufficient numbers of malignant glioma cells, iii) poor oncolytic potential, iv) limited tumor cell selectivity of stable gene delivery, as well as v) uncontrolled host immune reaction and considerable side-effects of viral infections (2-13). Some viruses, however, have properties that might help to overcome some of these common difficulties.

Varicella zoster virus (VZV) is an ubiquitous human alpha-herpesvirus that causes varicella (chicken-pox) and herpes zoster upon reactivation from latency in sensory ganglia. A highly cell-associated propagation which may facilitate efficient viral spread within cell-rich tissues and may allow for targeted delivery by cellular carriers, as well as a lytic capacity in several non glial cell types make this neurotropic virus an interesting candidate for use in oncolytic virotherapy of malignant glioma (14-16). Moreover, recombinant mutants may be developed in order to enhance safety and efficacy, if needed.

The purpose of our study was to identify oncolytic properties of wild-type VZV and three recently engineered VZV mutants in human malignant glioma cell cultures and to evaluate human mesenchymal stem cells (hMSCs) as potential carriers for targeted VZV delivery *in vitro* (17, 18).

Materials and Methods

Cell culture. Human melanoma (MeWo), fibroblast (MRC5) and glioma (U87, U251 and U373) cell lines were obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA); hMSCs were obtained from Lonza (PT2501, Basel, Switzerland). Primary cell cultures were generated from surgical specimens of untreated malignant glioma (5 GBM WHO grade IV and 5 anaplastic astrocytomas WHO grade III) according to standard protocols (19). All donors gave their written informed consent. Cell

cultures were maintained in standard culture medium (SCM) consisting of Dulbecco's modified Eagle's minimal essential medium (DMEM) supplemented with 10% heat-inactivated fetal calf serum (FCS), 1% penicillin/streptomycin, 1% glutamine, 0.2% amphotericin B, 0.1% gentamicin, 1% $NaHCO_3$ and 1% non essential amino acids. Cultures were cultivated in a humidified atmosphere at 37˚C, with 5% CO_2.

Viruses. Wild-type and mutant VZV were obtained from Dr. Ann Arvin (Stanford University, CA, USA). The VZV mutant rOKA/ORF63rev[T171] is based on a vaccine OKA (vOKA) backbone and contains a point mutation (T171A) within the transcriptional regulatory protein IE63 (20). The VZV mutant rOKA/47ΔC is based on a vOKA backbone and contains a C-terminal truncated version of the viral serine/threonine kinase ORF47 (21). Finally, the VZV mutant pOKA66S is based on a parental OKA (pOKA) backbone and contains a deletion of the viral serine/threonine kinase ORF66 (22). All viruses were propagated in MRC5. Viral titers within infected MRC5 cells, indicated as plaque forming units (pfu), were determined by single dilution assays on MeWo cells.

Viral infection of malignant glioma cell cultures and spheroids. Viral replication and phenotypic effects of VZV infections were analyzed in glioma cell lines and in primary human malignant glioma cell cultures and compared to non-glial cell lines MeWO and MRC5 as well as in hMSCs. Cell cultures were seeded on slides (Becton Dickinson, Bedford, MA, USA) for two days in SCM and subsequently infected with 120 pfu of VZV. After three days, the infected cells were processed for immunofluorescence microscopy.

Comparative analysis of viral replication kinetics was performed in U251 and two primary malignant glioma cell cultures (TB402 and TB426). Cells were seeded in 6-well plates and infected with 250 pfu of wild-type or mutant VZV. Viral replication was assayed several days post infection (*p.i.*) by serial dilution plaque assays on MeWo cells, as described recently (20).

Sensitivity towards virostatic treatment was assessed in semi-confluent malignant glioma cells cultured in 10 μg/ml acyclovir containing SCM. Replication foci of VZV were investigated at day 6 *p.i.* by crystal violet staining.

The spheroids used in our experiments were prepared by liquid overlay cultures as described previously (23). Briefly, 96-well plates were coated with 60 μl of 1% Agar noble diluted in PBS. Four thousand cells were dissociated in SCM and seeded into each well. After three days, spheroids were selected and infected with 240 pfu of wild-type or mutant VZV. Infected spheroids were selected after four and 14 days, respectively, and embedded in Cryo Tissue Tek medium for subsequent cryosectioning at –20˚C (15μm slices; Reichert Jung 1800; Leica, Vienna, Austria).

Immunofluorescence microscopy. Slides with cells or spheroid sections prepared for immunostaining were fixed in ice-cold 4% paraformaldehyde for 10 min, washed several times in phosphate-buffered saline (PBS), blocked at room temperature for one hour in PBS containing 30% goat serum and 0,3% Triton X-100 and incubated overnight with polyclonal antiserum (human anti-VZV; 1:1000; a generous gift from Dr. Ann Arvin, Stanford University, CA, USA). Incubated probes were washed 3 times in PBS and a secondary antibody including mouse anti human (Alexa 488, 1:500; invitrogen, Eugene, Oregon, USA) in a solution of PBS, 3% goat serum and Triton X-100 was applied for one hour. Probes were washed and stained with 4',6-diamidino-2-phenylindole (Dapi) using standard protocols. Slides were mounted with Vectashield (Vector Laboratories, Burlingame, CA, USA). Immunofluorescence microscopy was performed with an Olympus microscope.

Western blot. Lysates of wild-type (pOKA) and mutant (rOKA/ORF63rev(T171), rOKA47ΔC or pOKA66S) VZV-infected U251 cells were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis in 10% gels. Viral protein loads were adjusted to ORF4 expression (20). Analysis included the expression profiles of the major transactivator IE62, the transcriptional regulatory protein IE63, the two serine/threonine kinases ORF47 and ORF66, as well as the membrane-bound surface glycoprotein ORF68. The respective polyclonal antibodies were kindly provided by Dr. Ann Arvin, Stanford University, CA, USA.

Confrontational assay. Glioblastoma and MRC5 spheroids were created as described above. Spheroids of 1,1'-dioctadecyl-3,3,3'3'-tetramethylindocarbocyanine perchlorate (DiI)-labeled hMSCs were generated with 3000 cells and infected with 120 pfu of wild-type VZV. After three days, the infected hMSC spheroids were confronted with the GBM or MRC5 spheroids on a Agar noble-covered 96-well plate. After seven days, spheroid aggregates were selected, embedded and prepared for cryosectioning.

Results

Viral replication foci reveal phenotypic differences in various malignant glioma and non-glial cell cultures. Replication of wild-type and mutant VZV was observed in monolayer cultures of both established glioma cell lines (U87, U251 and U373) and various primary malignant glioma cells of WHO grade III (TB477, TB414, TB372, TB275, and TB022) and WHO grade IV (TB428, TB426, TB403, TB402, and TB389), as well as in three-dimensional malignant glioma models. Expression of surface glycoprotein ORF68 (aka glycoprotein E), indicating permissive viral replication, was observed as early as two days *p.i.* (Figure 1). Notably, foci of viral replication exhibited phenotypic differences in monolayer cell cultures: Whereas MeWo and MRC5 typically presented syncytia and plaque formation (Figure 1A), the viral replication foci within the glioma cell lines U87, U251, and U373 rather presented a cauliflower-like phenotype, with rounded cell conglomerates and only a marginal tendency towards cell-to-cell fusion (Figure 1B). In contrast, all assayed primary human malignant glioma cell cultures exhibited a spider web-like reorganization. Here, the integrity of the cell monolayer was strongly impaired and infected cells were contracted (Figure 1C). This latter phenotype was also observed within VZV-infected hMSCs (data not shown). Of note, similar phenotypic effects were observed for cells infected with mutant VZV (data not shown). Phenotypic alterations were commonly followed by effective cytolysis within 6-14 days *p.i.*. As exemplified in Figure 1D (here TB426), efficient replication of VZV in

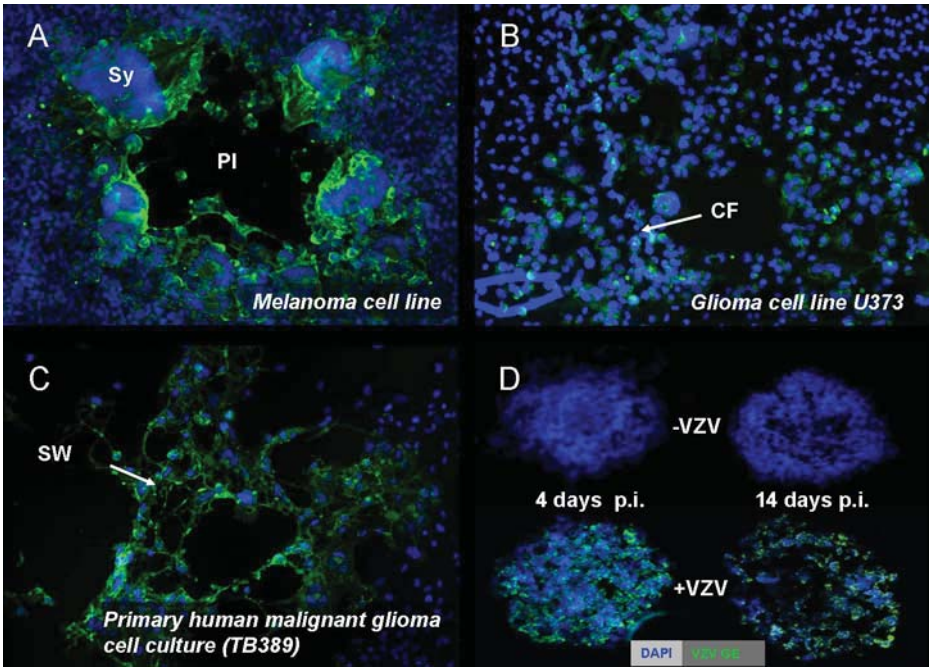

Figure 1. *Phenotypes of varicella zoster virus (VZV) replication foci in different human cell lines. Cell cultures were infected with VZV. Viral protein expression was determined two days post inoculation (p.i.) in monolayer cultures and four days, as well as 14 days, p.i. in spheroid models by immunocytochemistry (Alexa 488). Viral replication caused different phenotypic alterations including typical syncytia (Sy) and plaque formation (Pl) in MeWo (A), a cauliflower-like phenotype in U373 (B) and a spiderweb-like formation in primary human malignant glioma cells (C). In the three-dimensional model, strong viral protein expression was observed throughout the entire spheroid volume (+VZV) followed by disintegration of the spheroids as compared to non-infected tumor controls (-VZV). Nuclei were counterstained with DAPI (blue) (original magnification: ×100).*

three-dimensional primary WHO grade IV spheroids was observed within four days *p.i.*. Immunocytochemistry against VZV proteins confirmed viral propagation throughout the spheroids. Disintegration of spheroids was observed within 14 days *p.i.*, whereas uninfected glioblastoma spheroids remained aggregated.

Wild-type and mutant VZV reveal different replication kinetics in malignant glioma cell cultures. Replication kinetics of wild-type (pOKA) and mutant (rOKA/ORF63rev(T171), rOKA47ΔC and pOKA66S) VZV infections were comparatively assessed in the established glioma cell line U251 (Figure 2A) and the two primary GBM cell cultures TB402 (Figure 2B) and TB426 (Figure 2C), respectively. Replication rates of wild-type and mutant VZV were comparable in all cell lines. Replication kinetics of mutant pOKA66S was similar to that of wild-type VZV (bold line). In comparison, the mutant rOKA47ΔC consistently exhibited a qualitatively lower rate, and the mutant rOKA/ORF63rev[T171] increased growth kinetics by half a log (Figure 2). Notably, maximum replication of wild-type and mutant VZV was observed four days *p.i.*, followed by a constant decline due to effective lysis of entire cell cultures thereafter.

Replication of wild-type and mutant varicella zoster virus is accompanied by stable expression of viral transcription factors, kinases and cell surface proteins. Replication of wild-type and mutant (rOKA/ORF63rev[T171], rOKA47ΔC, and pOKA66S) VZV in the glioma cell line U251 was comparatively evaluated by western blot analysis of viral indicator proteins. Viral loading was adjusted based on the detection of equal amounts of the immediate early protein ORF4 (Figure 3). Four days *p.i.* stable expression of early, intermediate and late viral proteins indicated permissive replication of wild-type and mutant VZV strains. However, some differences in protein expression profiles were noted: In both mutants VZV pOKA66S and rOKA/ORF63rev[T171], protein levels of the serine/threonine kinase ORF47 tended to be higher as compared to wild-type VZV (Figure 3), respectively. The serine/threonine kinase ORF66 was absent from mutant VZV pOKA66S, due to the stop mutation within this ORF (Figure 3). In comparison, VZV mutant rOKA47ΔC (Figure 3) exhibited a slightly lower expression of the immediate early major transactivator ORF62 (Figure 3) and late surface glycoprotein ORF68 as compared to wild-type VZV (Figure 3). Integrity of this VZV mutant was verified by expression of the truncated ORF47 kinase (Figure 3, asterisk).

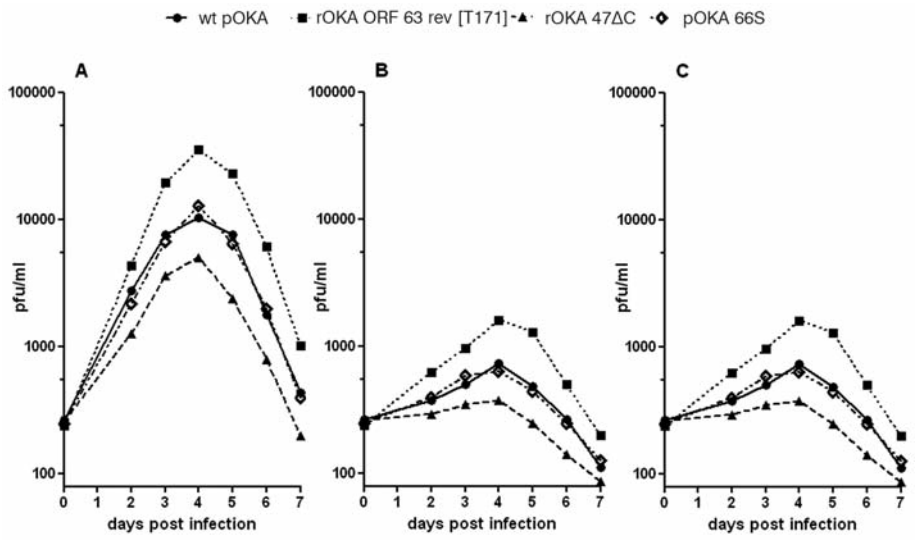

Figure 2. *Replication of wild-type and mutant varicella zoster virus in glioma cells. Replication kinetics of wild-type and mutant (rOKA/ORF63rev[T171], rOKA47ΔC and pOKA66S) VZV in the glioma cell line U251 and in primary malignant glioma TB402 and TB426 cell cultures were analyzed by serial dilution plaque assay on MeWo cells. Each time point represents the mean of results for at least three wells.*

Virostatic treatment inhibits viral replication in vitro. In order to test the effects of virostatics on viral replication, cultures of the glioma cell line U373 (Figure 4, first row), of non-glial fibroblasts MRC5 (second row), of primary human malignant glioma cells (TB428 is exemplarily shown in the third row), and hMSC culture (fourth row) were infected with VZV and were kept in culture with or without addition of acyclovir (Figure 4). All cells were cultivated in 24-well plates and inoculated with 120 pfu of wild-type VZV at day zero (Figure 4, first column). Typical wild-type VZV replication foci and early tumor cell lysis were observed around six days after inoculation in the absence of acyclovir (Figure 4, second column). No replication focus was observed when cells were cultivated in media supplemented with 10 μg/ml acyclovir (third column) or in the absence of wild-type VZV (fourth column), confirming that viral replication was effectively inhibited by acyclovir treatment in all cell cultures investigated.

hMSCs serve as cellular vehicles for tumor targeted delivery of VZV in vitro. In order to evaluate the tumor-targeting potential of hMSCs as a putative carrier for cell-bound VZV to the tumor site, an *in vitro* spheroid confrontation assay was designed (Figure 5). For this purpose, DiI-labeled and wild-type VZV-infected hMSC spheroids were confronted with native glioblastoma spheroids (TB426 is exemplarily shown in Figure 5). After seven days, infected hMSCs were found along the surface of the glioblastoma spheroids and even opposite to the site of primary confrontation indicating a migratory capacity of VZV-infected hMSCs (Figure 5A, B). Moreover, individual DiI-negative glioblastoma cells became positive for

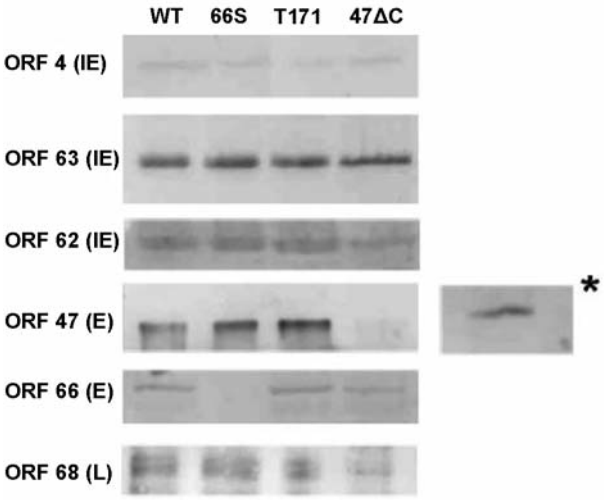

Figure 3. *Qualitative analysis of viral protein expression patterns in malignant glioma cells. Expression analysis of viral transcriptional activators ORF62/63, the kinases ORF47/66 and ORF68 (aka glycoprotein E) after malignant glioma cells (U251) were infected with either wild-type (wt) or mutant (rOKA/ORF63 rev[T171], rOKA47ΔC or pOKA66S) VZV. Expression of late surface protein ORF68 was increased in cultures infected with ORF63 rev[T171] as compared to wt VZV and pOKA66S-infected cultures, whereas rOKA47ΔC infected cultures exhibited a reduced expression pattern.*

VZV protein expression, indicating permissive viral replication within infected glioma spheroid cells. In contrast, wild-type VZV-carrying hMSCs did not show active migration towards control MRC5 spheroids and viral transmission was limited to the primary site of confronation (data not shown).

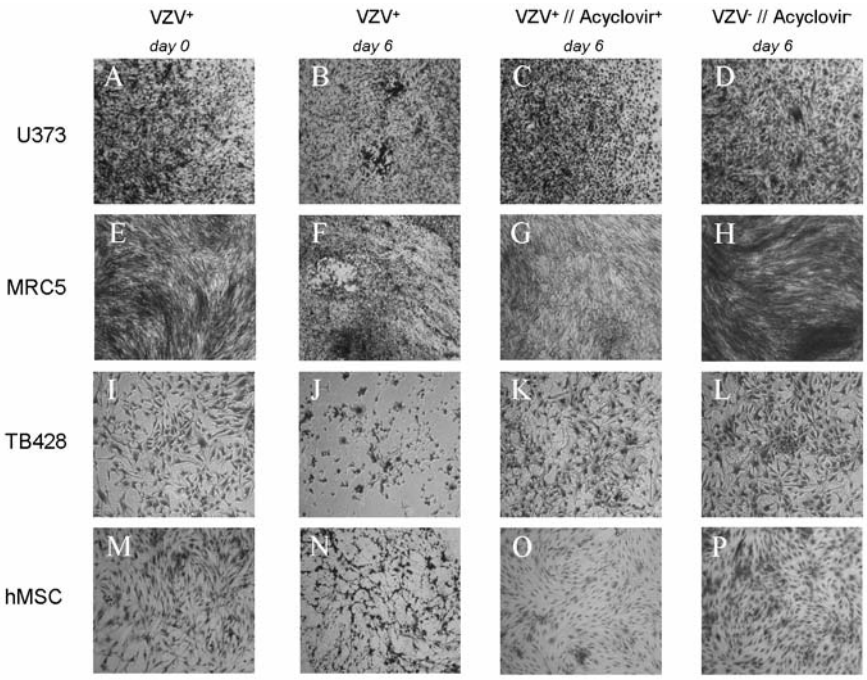

Figure 4. *Inhibition of wild-type varicella zoster virus (VZV)-replication with acyclovir treatment. Each cell population (U373, MRC5, TB428 and hMSC) was cultured for six days in standard culture medium (SCM) either infected with 120 plaque forming units (pfu) of wild-type VZV (first and second row) or exposed to both wild-type VZV and 10 μg/ml acyclovir (third row). In comparison to mock controls (fourth row), VZV infections were accompanied by cytomorphological changes, including plaque formation, cell aggregation and lytic foci, in all cell populations analyzed, which were efficiently inhibited by acyclovir treatment (original magnification: ×40).*

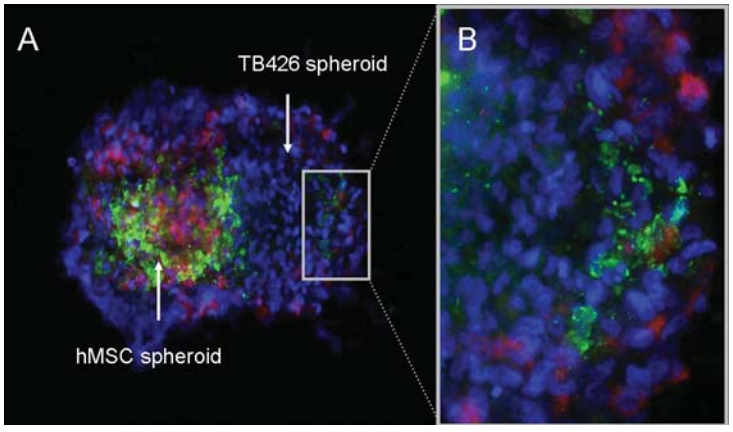

Figure 5. *Infected human mesenchymal stem cells (hMSCs) are able to deliver wild-type varicella zoster virus (VZV) to malignant glioma spheroids. VZV infected (120 pfu) and DiI-labeled hMSC spheroids were confronted with glioblastoma spheroids (TB426 shown) as well as MRC5 fibroblast spheroids for seven days. A: The DiI-labeled hMSCs exhibit strong viral protein expression (Alexa 488). B: Human MSCs selectively migrated along the entire surface of the tumor spheroid and DiI-unlabeled tumor cells became positive for VZV protein expression (Alexa 488). Nuclei were counterstained with DAPI (blue) (original magnification: A 100; B 400).*

Discussion

To our knowledge, VZV has never been used for oncolytic virotherapy of brain tumors. This study introduces VZV replication in established glioblastoma cell lines and primary malignant glioma cultures. The reason for phenotypic differences between various cell cultures remains unclear but might be at least partially caused by heterogeneous *in vitro* growth properties (16, 24).

In oncolytic virotherapy, the velocity of viral replication critically determines the therapeutic efficacy: viral spread and subsequent cell lysis should be more rapid than tumor

growth. Our growth experiments showed effective viral replication and subsequent malignant glioma cell lysis in monolayer cultures and spheroids within about one week *p.i.* Viral protein expression was present even at the core of three-dimensional spheroids (Figure 4D), indicating that viral spread, consisting of replication and penetration was sufficiently rapid to infect dense aggregates of tumor cells followed by their efficient disintegration *in vitro*.

Of note, viral replication was efficiently inhibited by virostatic treatment, which might be of importance in order to prevent undesired dissemination of VZV infection *in situ*. Efficient acyclovir treatment, however, might be hampered in heavily immune compromised patients (as described for stem cell transplant recipients but rarely in a patient with malignant glioma) making alternative treatment, *e.g.* with foscarnet necessary (25, 26).

A comparative analysis of replication of wild-type VZV and mutant VZV indicated differences in glioma cell tropism. The first mutant, rOKA/ORF63rev[T171], has a point mutation within the regulatory protein ORF63 which is necessary for optimal expression of early and late gene products and therefore causes a dramatic reduction of virulence and *e.g.* an attenuated replication phenotype within human skin tissue *in vivo* (20). The second mutant rOKA47ΔC is characterized by a C-terminal deletion of the viral protein kinase ORF47, which also results in a markedly attenuated virulence as ORF47 is required for viremia and cutaneous replication (21, 27). The third assayed parental virus mutant, pOKA66S, has a stop mutation within the gene encoding for the viral protein kinase ORF66 and has been characterized by attenuation within human T-cells *in vivo* (22). Interestingly, in our experiments, rOKA/ORF63rev[T171] revealed an increased replication of half a log in comparison to wild-type VZV (Figure 2). This effect was validated by western blot analysis which indicated the up-regulation of ORF47 and strong ORF68 expression, both being important for viral replication. Notably, the same mutant exhibits reduced replication potential and plaque formation in MeWo cells and a dramatic reduction of virulence in differentiated human skin cells (21). The reasons for these differences in growth rate and protein expression in glioma cell lines remain unclear, but suggest intrinsic glioma cell tropism, and might favor this mutant for further investigation *in vivo*.

Since viral propagation of VZV is strictly cell associated (and have not been described for the infectious form) (28), cellular carriers that bear an intrinsic tumor cell tropism might be exploited for targeted delivery and enable broad infection of cell-rich tumors while reducing the risk of uncontrolled dissemination within normal brain parenchyma. Moreover, the virus might be shielded against the host immune system. Interestingly, hMSCs exhibit an intrinsic glioma cell tropism and tend to migrate even towards invading glioma cells (17, 18). This characteristic has already been exploited for oncolytic adenoviruses (29). In monolayer cultures, hMSCs facilitated efficient replication of VZV followed by early cytolysis. Here, the potential application of hMSC as local carriers for VZV delivery was evaluated in the three-dimensional spheroid confrontation model. VZV-infected, DiI-labeled hMSCs were stable enough to transmit their viral load to the neighboring cells. Moreover, hMSC maintained their intrinsic tumor cell tropism and migrated along the surface of the three-dimensional tumor cell spheroids, an effect that was not observed in MRC5 fibroblast spheroids (data not shown). Hereby, viral replication was found from the rim to the core before disintegration of the spheroids occurred. Even though these *in vitro* results are auspicious, it remains unclear whether VZV-infected hMSCs survive long enough to deliver the viral package and transfect enough viruses to tumor cells for therapeutic effects *in vivo*.

The efficacy and safety of viral replication and VZV-induced tumor cell lysis *in vivo* has not been addressed so far. However, the preference of VZV for human hosts critically interferes with animal models in order to asses important aspects such as the influence of the host immune response and associated side-effects (28). In turn, viral replication, lytic capacity and potential neurotoxicity will have to be analyzed *in vivo* before this human pathogen can be further evaluated for oncolytic purposes in patients with brain tumors. As wild-type VZV can cause meningitis and encephalitis, especially in immune-suppressed patients, future experiments should rather concentrate on less virulent strains, such as the vaccine VZV mutant rOKA/ORF63rev[T171] that combines an increased oncolytic efficacy in malignant glioma cell cultures with a dramatic reduction of virulence and most probably fewer side-effects in humans. Similar aspects were described for the genetically modified herpes simplex virus G207, which also efficiently replicates within glioma cells but without evidence of viral encephalitis in humans (30, 31) Therefore, even though our preliminary results indicate favorable oncolytic effects of VZV in malignant glioma, further experiments are needed to confirm VZV as a novel candidate for oncolytic virotherapy.

Disclosure

There is nothing to be disclosed.

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References

1 Louis DN, Ohgaki H, Wiestler OD, Cavenee WK, Burger PC, Jouvet A, Scheithauer BW and Kleihues P: The 2007 WHO classification of tumours of the central nervous system. Acta Neuropathol *114*: 97-109, 2007.

2 Chen B, Timiryasova TM, Andres ML, Kajioka EH, Dutta-Roy R, Gridley DS and Fodor I: Evaluation of combined vaccinia virus-mediated antitumor gene therapy with p53, IL-2, and IL-12 in a glioma model. Cancer Gene Ther *7*: 1437-1447, 2000.

3 Chiocca EA, Abbed KM, Tatter S, Louis DN, Hochberg FH, Barker F, Kracher J, Grossman SA, Fisher JD, Carson K, Rosenblum M, Mikkelsen T, Olson J, Markert J, Rosenfeld S, Nabors LB, Brem S, Phuphanich S, Freeman S, Kaplan R and Zwiebel J: 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 *10*: 958-966, 2004.

4 Dobrikova EY, Broadt T, Poiley-Nelson J, Yang X, Soman G, Giardina S, Harris R and Gromeier M: Recombinant oncolytic poliovirus eliminates glioma *in vivo* without genetic adaptation to a pathogenic phenotype. Mol Ther *16*: 1865-1872, 2008.

5 Forsyth P, Roldan G, George D, Wallace C, Palmer CA, Morris D, Cairncross G, Matthews MV, Markert J, Gillespie Y, Coffey M, Thompson B and Hamilton M: A phase I trial of intratumoral administration of reovirus in patients with histologically confirmed recurrent malignant gliomas. Mol Ther *16*: 627-632, 2008.

6 Freeman AI, Zakay-Rones Z, Gomori JM, Linetsky E, Rasooly L, Greenbaum E, Rozenman-Yair S, Panet A, Libson E, Irving CS, Galun E and Siegal T: Phase I/II trial of intravenous NDV-HUJ oncolytic virus in recurrent glioblastoma multiforme. Mol Ther *13*: 221-228, 2006.

7 Hellums EK, Markert JM, Parker JN, He B, Perbal B, Roizman B, Whitley RJ, Langford CP, Bharara S and Gillespie GY: Increased efficacy of an interleukin-12-secreting herpes simplex virus in a syngeneic intracranial murine glioma model. Neuro Oncol *7*: 213-224, 2005.

8 Lun X, Senger DL, Alain T, Oprea A, Parato K, Stojdl D, Lichty B, Power A, Johnston RN, Hamilton M, Parney I, Bell JC and Forsyth PA: Effects of intravenously administered recombinant vesicular stomatitis virus (VSV(deltaM51)) on multifocal and invasive gliomas. J Natl Cancer Inst *98*: 1546-1557, 2006.

9 Ochiai H, Campbell SA, Archer GE, Chewning TA, Dragunsky E, Ivanov A, Gromeier M and Sampson JH: Targeted therapy for glioblastoma multiforme neoplastic meningitis with intrathecal delivery of an oncolytic recombinant poliovirus. Clin Cancer Res *12*: 1349-1354, 2006.

10 Ozduman K, Wollmann G, Piepmeier JM and van den Pol AN: Systemic vesicular stomatitis virus selectively destroys multifocal glioma and metastatic carcinoma in brain. J Neurosci *28*: 1882-1893, 2008.

11 Parker JN, Gillespie GY, Love CE, Randall S, Whitley RJ and Markert JM: Engineered herpes simplex virus expressing IL-12 in the treatment of experimental murine brain tumors. Proc Natl Acad Sci USA *97*: 2208-2213, 2000.

12 Tyminski E, Leroy S, Terada K, Finkelstein DM, Hyatt JL, Danks MK, Potter PM, Saeki Y and Chiocca EA: Brain tumor oncolysis with replication-conditional herpes simplex virus type 1 expressing the prodrug-activating genes, CYP2B1 and secreted human intestinal carboxylesterase, in combination with cyclophosphamide and irinotecan. Cancer Res *65*: 6850-6857, 2005.

13 Wohlfahrt ME, Beard BC, Lieber A and Kiem HP: A capsid-modified, conditionally replicating oncolytic adenovirus vector expressing TRAIL Leads to enhanced cancer cell killing in human glioblastoma models. Cancer Res *67*: 8783-8790, 2007.

14 Hayward AR, Herberger M and Lazslo M: Cellular interactions in the lysis of varicella-zoster virus infected human fibroblasts. Clin Exp Immunol *63*: 141-146, 1986.

15 Li Q, Ali MA and Cohen JI: Insulin degrading enzyme is a cellular receptor mediating varicella-zoster virus infection and cell-to-cell spread. Cell *127*: 305-316, 2006.

16 Santos RA, Hatfield CC, Cole NL, Padilla JA, Moffat JF, Arvin AM, Ruyechan WT, Hay J and Grose C: Varicella-zoster virus gE escape mutant VZV-MSP exhibits an accelerated cell-to-cell spread phenotype in both infected cell cultures and SCID-hu mice. Virology *275*: 306-317, 2000.

17 Birnbaum T, Roider J, Schankin CJ, Padovan CS, Schichor C, Goldbrunner R and Straube A: Malignant gliomas actively recruit bone marrow stromal cells by secreting angiogenic cytokines. J Neurooncol *83*: 241-247, 2007.

18 Schichor C, Birnbaum T, Etminan N, Schnell O, Grau S, Miebach S, Aboody K, Padovan C, Straube A, Tonn JC and Goldbrunner R: Vascular endothelial growth factor A contributes to glioma-induced migration of human marrow stromal cells (hMSC). Exp Neurol *199*: 301-310, 2006.

19 Miebach S, Grau S, Hummel V, Rieckmann P, Tonn JC and Goldbrunner RH: Isolation and culture of microvascular endothelial cells from gliomas of different WHO grades. J Neurooncol *76*: 39-48, 2006.

20 Baiker A, Bagowski C, Ito H, Sommer M, Zerboni L, Fabel K, Hay J, Ruyechan W and Arvin AM: The immediate-early 63 protein of Varicella-Zoster virus: analysis of functional domains required for replication *in vitro* and for T-cell and skin tropism in the SCIDhu model *in vivo*. J Virol *78*: 1181-1194, 2004.

21 Besser J, Sommer MH, Zerboni L, Bagowski CP, Ito H, Moffat J, Ku CC and Arvin AM: Differentiation of varicella-zoster virus ORF47 protein kinase and IE62 protein binding domains and their contributions to replication in human skin xenografts in the SCID-hu mouse. J Virol *77*: 5964-5974, 2003.

22 Schaap A, Fortin JF, Sommer M, Zerboni L, Stamatis S, Ku CC, Nolan GP and Arvin AM: T-cell tropism and the role of ORF66 protein in pathogenesis of varicella-zoster virus infection. J Virol *79*: 12921-12933, 2005

23 Haji-Karim M and Carlsson J: Proliferation and viability in cellular spheroids of human origin. Cancer Res *38*: 1457-1464, 1978.

24 Harson R and Grose C: Egress of varicella-zoster virus from the melanoma cell: a tropism for the melanocyte. J Virol *69*: 4994-5010, 1995.

25 Brink AA, van GM, Wolffs PF, Bruggeman CA and van Loo IH: Compartmentalization of acyclovir-resistant varicella zoster virus: implications for sampling in molecular diagnostics. Clin Infect Dis *52*: 982-987, 2011.

26 Snoeck R, Andrei G and De CE: Current pharmacological approaches to the therapy of varicella zoster virus infections: a guide to treatment. Drugs *57*: 187-206, 1999.

27 Besser J, Ikoma M, Fabel K, Sommer MH, Zerboni L, Grose C and Arvin AM: Differential requirement for cell fusion and virion formation in the pathogenesis of varicella-zoster virus infection in skin and T cells. J Virol *78*: 13293-13305, 2004.

28 Gershon AA, Chen J and Gershon MD: A Model of Lytic, Latent, and Reactivating Varicella-Zoster Virus Infections in Isolated Enteric Neurons. JID *2008*: 197, 2008.

29 Sonabend AM, Ulasov IV, Tyler MA, Rivera AA, Mathis JM and Lesniak MS: Mesenchymal stem cells effectively deliver an oncolytic adenovirus to intracranial glioma. Stem Cells *26*: 831-841, 2008.

30 Martuza RL, Malick A, Markert JM, Ruffner KL and Coen DM: Experimental therapy of human glioma by means of a genetically engineered virus mutant. Science *252*: 854-856, 1991.

31 Mineta T, Rabkin SD, Yazaki T, Hunter WD and Martuza RL: Attenuated multi-mutated herpes simplex virus-1 for the treatment of malignant gliomas. Nat Med *1*: 938-943, 1995.

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