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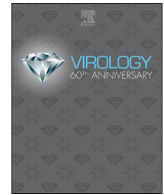

Chikungunya-vesicular stomatitis chimeric virus targets and eliminates brain tumors

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ABSTRACT

Vesicular stomatitis virus (VSV) shows potential for targeting and killing cancer cells, but can be dangerous in the brain due to its neurotropic glycoprotein. Here we test a chimeric virus in which the VSV glycoprotein is replaced with the Chikungunya polyprotein E3-E2-6K-E1 (VSVΔG-CHIKV). Control mice with brain tumors survived a mean of 40 days after tumor implant. VSVΔG-CHIKV selectively infected and eliminated the tumor, and extended survival substantially in all tumor-bearing mice to over 100 days. VSVΔG-CHIKV also targeted intracranial primary patient derived melanoma xenografts. Virus injected into one melanoma spread to other melanomas within the same brain with little detectable infection of normal cells. Intravenous VSVΔG-CHIKV infected tumor cells but not normal tissue. In immunocompetent mice, VSVΔG-CHIKV selectively infected mouse melanoma cells within the brain. These data suggest VSVΔG-CHIKV can target and destroy brain tumors in multiple animal models without the neurotropism associated with the wild type VSV glycoprotein.

1. Introduction

Vesicular stomatitis virus (VSV) is an enveloped, negative-sense, single-strand RNA virus in the *Rhabdoviridae* family. While rarely causing disease in humans, the virus can pose a potential threat to livestock including cattle, horses, and pigs (Lyles and Rupprecht, 2007). In recent years, recombinant altered versions of VSV have shown considerable potential as the molecular basis for live vaccines engineered to express antigenic proteins from other viruses (Kurup et al., 2015; Clarke et al., 2006; Geisbert et al., 2008, 2009). VSV has also shown promise as an oncolytic virus (Wongthida et al., 2011; Obuchi et al., 2003; Ozduman et al., 2008; van den Pol and Davis, 2013; Wollmann et al., 2005). However, a substantive limitation of VSV is that the VSV glycoprotein is highly neurotropic, and upon entering the brain, can lead to deleterious neurological consequences, including death (Huneycutt et al., 1993; Lundh et al., 1987, 1988; van den Pol et al., 2002).

Although substitution of glycoprotein genes from other viruses can reduce VSV neurotropism (Wollmann et al., 2015; van den Pol et al., 2017a), the attenuation of neurotropism is not necessarily a universal attribute of chimeric VSVs. Glycoproteins from some viruses that have been substituted for the VSV glycoprotein can be maladaptive and enhance neurotropism; for example the replication competent Nipah-VSV chimera is lethal in the brain (van den Pol et al., 2017a). Even for the potential treatment of non-brain cancers with oncolytic viruses, the importance of attenuating or eliminating the neurotropism of VSV is suggested by data showing that metastatic myeloma cancer cells can form a bridge from outside the brain across the meninges into the brain, potentially serving as a conduit through the blood brain barrier for a neurotropic virus to enter the brain (Yarde et al., 2013).

Chikungunya virus (CHIKV) is a positive-sense single-strand RNA virus of the alphavirus genus and Togavirus family. Prior to 2013 it was primarily found in Asia, Africa, and Europe; starting in 2013 the virus has been spread by mosquitoes through most of South America and parts of North America with primates as a potential reservoir (https://www.cdc.gov/chikungunya/geo/index.html; Vignuzzi and Higgs, 2017; Vu et al., 2017). There is currently no approved vaccine available; a number of different experimental vaccines are being tested (Chattopadhyay et al., 2013; Powers, 2018; Yang et al., 2017). CHIKV has generally been associated with fever and joint pain, but can also cause headache, muscle ache, and rash (Hua and Combe, 2017; Amdekar et al., 2017). The joint pain can persist for many months or longer. Chikungunya may bind to one of several surface proteins which have been suggested to include cholesterol transporters, prohibitin and others (Wichit et al., 2017; Wintachai et al., 2012) and appears to be internalized in clathrin coated pits (Bernard et al., 2010; Schwartz and

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Albert, 2010; Hoornweg et al., 2016). VSV has been proposed to utilize the LDL receptor as an entry port (Finkelshtein et al., 2013).

Here we test a CHIKV-VSV chimeric virus containing a portion of the CHIKV structural polyprotein that includes the E3-E2-6K-E1 glycoprotein sequence substituted for the VSV glycoprotein (Chattopadhyay et al., 2013). CHIKV E2 underlies receptor binding, and E1 is responsible for the low pH membrane fusion activity after endocytotic entry (Voss et al., 2010; Solignat et al., 2009). Together E2 and E1 constitute spike-like trimers on the virus surface. E3 is postulated to prevent premature virus fusion (Uchime et al., 2013), and 6K enhances virion release and titer (Taylor et al., 2016). VSV in which the normal glycoprotein gene G has been deleted and replaced by genes coding for the CHIKV envelope glycoprotein (VSVΔG-CHIKV) appears safe within the brain and, as tested in rodents, did not evoke neurological dysfunction or substantive negative consequences (van den Pol et al., 2017a).

Here we address the question of whether this VSVΔG-CHIKV chimera can selectively infect and cytolytically kill brain tumor cells without substantive damage to normal cells in the brain.

2. Results

2.1. Human cancer cells have high susceptibility to CHIKV

A CHIKV-VSV chimera VSVΔG-CHIKV was used in which the VSV glycoprotein was replaced with the glycoprotein sequence from CHIKV (Fig. 1A). To determine whether VSVΔG-CHIKV displayed a preferential infection of cancer cells, we compared a variety of different cell types including both cancer and non-cancer normal control cells. The cells used included human glioma U373, U118 and U87 and the mouse glioma CT-2A, along with normal human glia and normal mouse glia. Additional cancer types included the human melanoma cells YUMAC and 501mel, and the breast cancer cells MDA-MB-436, MDA-MB-231 and BT-549. Cells were inoculated using an MOI of 0.02 and VSVΔG-CHIKV infection was determined by immunostaining at 3 days post-infection (dpi). The percentage of infected human glioma cells (n = 6 samples/group) was substantially greater than that of normal human glia (U373 $p < 0.001$; U118 $p < 0.01$; U87 $p < 0.01$; ANOVA) (Fig. 1B,D). Additionally, the percentage of infected human melanoma and breast cancer cells was also significantly greater than control normal human cells (glia) (ANOVA) (Fig. 1C and D). Mouse glioma CT-2A cells also showed a greater percentage of infected cells than normal mouse glia, but displayed less infection than human gliomas (Fig. 1B,D).

To compare relative levels of infection and replication of VSVΔG-CHIKV in different cell types, we compared virus plaque size on glioma, melanoma, breast cancer and normal human brain cells at 3 days (Fig. 2A). All human glioma cell lines yielded large plaques (n = 20 plaques/group $p < 0.001$ vs normal human glia, ANOVA), whereas on normal human glia VSVΔG-CHIKV displayed significantly smaller plaques (Fig. 2B,D). Both mouse glioma (CT-2A) and normal mouse glia appeared less susceptible to VSVΔG-CHIKV. In comparisons of breast cancer cells, BT-549 displayed a significantly larger (n = 20 plaques; $p < 0.001$; ANOVA) plaque size than MDA-MB-231 or MDA-MB-436 cells. YUMAC and 501 human melanoma cells also yielded larger plaques than normal human cells (Fig. 2C,D). Infected cells ultimately showed a lethal response to virus infection as corroborated with ethidium homodimer labeling.

2.2. VSVΔG-CHIKV selectively infects a broad range of human glioma

In order to examine further the susceptibility of human glioma cells to VSVΔG-CHIKV infection, a panel of different glioma with different growth characteristics and mutational defects were infected with VSVΔG-CHIKV at a low MOI of 0.02. 24 h later, VSVΔG-CHIKV not only infected the inoculated cells (Fig. 3A, left) but additionally showed significant replication (Fig. 3A, right) after secondary inoculation (24 h) of fresh cultures with media collected from infected cultures, as suggested above in the plaque analysis. In contrast to the human glioma cells, normal human astrocytes showed attenuated infection and little evidence of virus replication. Similarly, normal mouse glia supported little infection or replication of VSVΔG-CHIKV whereas mouse CT-2A glioma cells displayed both infection and replication (Fig. 3B,D).

2.3. Comparison of recombinant VSV infection of glioblastoma

To determine the susceptibility of glioma cells to VSVΔG-CHIKV infection, two additional recombinant VSVs, one wild-type (VSVwt) and another chimeric VSV, VSV-LASV-GPC (VSVΔG-LASV) were compared with VSVΔG-CHIKV for their ability to infect and kill either mouse (CT-2A) or human (U118) derived glioma cells (Fig. 4A–D). Twenty-four hours after inoculation (MOI = 1) all three viruses showed good infection levels at 24 hpi (Fig. 4A,B) and evoked cell death at 48 hpi as determined with ethidium homodimer (Fig. 4C,D) in both mouse and human glioma cells. VSVwt showed greater infection and cell death than VSVΔG-CHIKV or VSVΔG-LASV. Non-infected control tumor cells showed no infection and little cell death.

To compare the relative propagation of these 3 viruses, viral plaque size was measured using monolayer cultures of human (U118, U87) and mouse (CT-2A) glioma (Fig. 4E, F). Forty-eight hours after infection both human glioma cell lines yielded robust large plaques; mouse CT-2A yielded smaller plaques for all 3 viruses. As expected the VSVwt plaques were larger than those generated by VSVΔG-CHIKV or VSV-LASV-GPC.

2.4. Type I interferon actions on VSVΔG-CHIKV infection of human glioma

Interferons (IFNs) are cytokines that play a critical role in the induction and maintenance of innate and adaptive immunity, and dysfunctional IFN signaling has been suggested as a key mechanism contributing to enhanced infection of cancer cells (Stojdl et al., 2000). To test whether type I interferon might play a role in the selectivity of VSVΔG-CHIKV infection of cancer cells, glioma cells and normal cells were cultured and pre-treated with 1 or 10 IU of a recombinant hybrid type I interferon (IFN-αA/D that activates both mouse and human type 1 IFN receptors) for 12 h prior to infection with VSVΔG-CHIKV at an MOI of 0.02 (Fig. 5). Twenty-four hours later, immunostaining was used to quantify the number of cells showing VSVΔG-CHIKV infection. IFN-α (1 IU, n = 6 samples/group) almost completely blocked VSVΔG-CHIKV infection of normal astrocytes compared to no IFN ($p < 0.01$ vs. control) (Fig. 5A,C). In contrast to IFN's block of infection in normal human astrocytes, viral infection of human glioma U118, U87, and U373 was attenuated but not completely blocked by IFN-α at 1 IU (U118, $p = 0.37$; U87, $p = 0.37$; U373, $p = 0.058$; vs no-IFN controls). As expected, a greater effect was observed at 10 IU in a dose-dependent manner ($p < 0.001$ vs control) (Fig. 5A,C). VSVΔG-CHIKV showed only modest inhibition by IFN-α in mouse CT-2A glioma (Fig. 5B,D).

2.5. VSVΔG-CHIKV targets glioma

To examine whether VSVΔG-CHIKV can act in vivo, the mouse brain was injected with glioblastoma rU118, and rU373 cells. Nine days after tumor injection into the striatum of SCID mice, VSVΔG-CHIKV (7×10^8 PFU) was injected intracranially in the area of the tumor. Mice were euthanized 4, 7, and 15 days later (Fig. 6A). Four days after VSVΔG-CHIKV administration (13 days after injection of cancer cells), the virus showed selective infection of all types of glioma including U118 (n = 3) (Fig. 6B–F) and U373 (n = 4) (not shown). At 7 and 15 dpi, a greater number of tumor cells were selectively infected (Fig. 6B–F). In contrast to the infection of glioma (Fig. 6B–F), little infection was detected in the normal host brain.

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