Syncytia Induction Enhances the Oncolytic Potential of Vesicular Stomatitis Virus in Virotherapy for Cancer

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ABSTRACT

Vesicular stomatitis virus (VSV) selectively replicates in tumor but not in normal cells and is being developed as an oncolytic agent for cancer therapy. Here we report the construction of a recombinant VSV capable of inducing syncytia formation between tumor cells through membrane fusion at neutral pH, which led to enhanced oncolytic properties against multifocal hepatocellular carcinoma (HCC) in the livers of immunocompetent rats. Recombinant VSV vectors were constructed by insertion into their genome a transcription unit expressing a control or fusion protein derived from Newcastle disease virus. In vitro characterization of the recombinant fusogenic VSV vector on human and rat HCC cells showed extensive syncytia formation and significantly enhanced cytoxic effects. In vivo, administration of fusogenic VSV into the hepatic artery of Buffalo rats bearing syngeneic multifocal HCC lesions in their livers resulted in syncytia formation exclusively within the tumors, and there was no collateral damage to the neighboring hepatic parenchyma. The fusogenic VSV also conferred a significant survival advantage over a nonfusogenic control virus in the treated animals (P = 0.0078, log-rank test). The results suggest that fusogenic VSV can be developed into an effective and safe therapeutic agent for cancer treatment in patients, including those with multifocal HCC in the liver.

INTRODUCTION

Oncolytic viruses provide an attractive new tool for cancer treatment because of their ability to replicate selectively within the tumor and kill neighboring cancer cells upon tumor lysis and secondary infection (1). In addition to viruses that are molecularly engineered to replicate preferentially in tumor cells, a number of RNA viruses, including reovirus, Newcastle disease virus (NDV), measles virus, mumps virus, and vesicular stomatitis virus (VSV), are currently being developed as a novel class of oncolytic agents because of their inherent preference for replication in tumor cells (2–5). VSV is a negative-strand RNA virus of the Rabdoviridae family with inherent specificity for replication in tumor cells because of their attenuated antiviral responses mediated by type 1 IFNs (5). The natural host for VSV infection is cattle, horses, and swine. Infections in humans are asymptomatic in most cases or result in a mild febrile illness (6). VSV is not endemic to the North American population, implying that there will not be preexisting neutralizing antibodies and/or memory cellular immune responses in most patients to interfere with its clinical application (7). Recently, we have demonstrated the oncolytic potential of VSV against human and rat hepatocellular carcinoma (HCC) in vitro and in vivo (8). Even when applied to cultured human and rat HCC cells at a multiplicity of infection (MOI) as low as 10−4, all HCC cells were eradicated within 48 h. In contrast, normal primary human and rat hepatocytes were refractory to VSV infection, even at a MOI of 1. Intratumoral injection of the vector in immunocompetent rats bearing large solitary HCC tumors in their livers also caused massive tumor destruction that led to significant prolongation of survival (8). We have further shown that hepatic arterial administration of VSV resulted in efficient tumor-selective viral transduction and oncolysis of multifocal HCC in the rat liver without causing damage to the normal hepatic parenchyma (9). Our results, although encouraging, also demonstrated that viral replication and spread in the solid tumors are limited, and the treated animals eventually succumbed to relapse.

We envisioned that intratumoral virus spread could be improved by syncytia-inducing VSV, which can lead to substantially improved treatment outcome. VSV normally propagates from cell to cell by virions that bud out from infected cells and become internalized by endosomes in neighboring cells, where the VSV glycoprotein (VSV/G) protein, upon acidification of the endosome, mediates fusion between the membrane of the virus and the membrane of the endosome. As a result, viral nucleocapsids are released into the cytoplasm, where they start viral replication and transcription (7). Other viruses have evolved a different spreading mechanism that involves fusion of the infected cell with the adjacent cells, resulting in the formation of multinucleated giant cells or syncytia (10). Plasmid-mediated expression of viral fusogenic membrane glycoproteins efficiently killed tumor cells through induction of fusion to form large multinucleated syncytia (11). In vitro, this “bystander effect” was reported to be at least 10-fold greater than that observed with suicide gene/prodrug systems such as herpes simplex virus thymidine kinase + ganciclovir or cytosine deaminase + 5-fluorocytosine (11). The antitumor efficacy of an oncolytic herpes simplex virus was also significantly enhanced in vivo by inclusion in its genome the COOH-terminal truncated form of the Gibbon ape leukemia virus envelope glycoprotein, which is a fusogenic membrane glycoprotein (12, 13). We hypothesized that expression by VSV of a fusogenic membrane glycoprotein able to induce membrane fusion at neutral pH would result in a syncytia-forming VSV with significantly increased oncolytic activity in vivo.

Membrane fusion mediated by most paramyxoviruses, such as NDV, requires two virion-associated glycoproteins, the attachment or hemagglutinin-neuraminidase protein and the fusion protein [F (10)]. Interestingly, Sergel et al. (14) reported that a single amino acid substitution (L289A) in the heptad repeat motif (HR3) of the NDV fusion protein (NDV/F) allows this protein to induce syncytia alone, whereas the wild-type (wt) NDV/F protein requires coexpression with the hemagglutinin-neuraminidase protein. In this article, we report the generation of a novel fusogenic VSV vector, which expresses mutant (L289A) F protein from NDV, and demonstrate its superior oncolytic activity against multifocal HCC in an orthotopic and immunocompetent rat model.

MATERIALS AND METHODS

Cell Lines. Hep3B, HepG2, McA-RH7777, and BHK-21 cell lines were obtained from American Type Culture Collection (Manassas, VA). The human HCC Hep3B and HepG2 cells were maintained in MEM-Eagle (Mediatech, Herndon, VA) and supplemented with 10% fetal bovine serum (Sigma-Aldrich, St. Louis, MO). The rat HCC McA-RH7777 and BHK-21 cells were maintained in 10% fetal bovine serum-DMEM (Mediatech). All media used in this study contained 100 units/ml penicillin-streptomycin (Mediatech).

Plasmid Construction. To create recombinant VSV vector expressing wt-NDV/F protein, the sequence encoding NDV/F (1.6 kb) was PCR amplified from pNDV/B1/F3 containing the F protein of NDV strain Hitchner B1 with a modified
protease cleavage site (112R-R-Q-R-R/F117; modified amino acids are underlined) recognized by ubiquitous proteases (15, 16). PCR amplification was performed using the forward 5'-GGCGCGGCTGAGTGTTGCTCCAGCTTCTAC-3' and reverse primers 5'-AAGCTGCAAGTCTACATTTGGTTAGTGGCCT-3' with unique restriction sites (shown in bold) XhoI and NheI, respectively. The PCR product was digested with XhoI and NheI and cloned into the full-length pVSV-XN2 plasmid (a gift from Dr. J. Rose, Yale University, New Haven, CT). The resulting plasmid was designated pVSV-NDF/V(FV). To generate recombinant VSV vector expressing mutant (L289A) F protein, the pVSV-NDF/V(FV) plasmid was modified by PCR site-directed mutagenesis. To this end, the sequence encoding the NDV/F protein was assembled from two overlapping PCR fragments. The first PCR fragment was generated by using the forward 5'-GCTACATCGTACGACCTTGCTGC-3' and reverse primers 5'-AGCTGCAAGTCTACATTTGGTTAGTGGCCT-3'. The second overlapping PCR fragment was joined in a third PCR, and the resulting fragment, which contained the mutant (L289A) F protein sequence, was cloned into pVSV-XN2 to generate pVSV-NDF/V(L289A). Sequencing of the plasmids was conducted in the DNA Sequencing Core Facility at Mount Sinai School of Medicine.

Generation of Recombinant Viruses. For recovery experiments of recombinant VSV, the established method of reverse genetics was used (17, 18). Control wtVSV not expressing a transgene was generated using the pVSV-XN2 plasmid encoding the full-length VSV cDNA. Briefly, BHK-21 cells infected previously with vaccinia virus expressing T7 RNA polymerase (vTF-7.3) were transfected with 10 µg of full-length VSV cDNA in addition to plasmids encoding VSV N, P, and L proteins by using LipofectAMINE 2000 reagent (Invitrogen, Carlsbad, CA). Two days after transfection, supernatants were filtered through a 0.22 µm filter to remove the majority of vaccinia virus and transferred onto fresh BHK-21 cells. Vaccinia virus was eliminated by plaque purification, and the titers of recombinant VSV stocks were determined by plaque assays on BHK-21 cells.

Indirect Immunofluorescence Assay. BHK-21 cells were seeded onto plastic coverslips (Nalge Nunc, Naperville, IL) at 10^5 cells/6.4-cm² plate. The following day, cells were infected with wtVSV or recombinant VSV expressing NDV fusion protein (rVSV-NDF/V) vectors at a MOI of 10. Cells were incubated at 37°C for 14 h, washed with PBS, and fixed with 3% paraformaldehyde for 20 min at room temperature, and washed twice with PBS-glycine (10 mM glycine and 5 mg/ml BSA) and PBS-glycine-BSA. As a control, expression of VSV/G protein was detected in both wtVSV- and rVSV-NDF/V-infected cells by using a monoclonal antibody specific for VSV/G protein (Alpha Diagnostic). Immunohistochemistry sections were counterstained with hematoxylin.

For comparison of individual time points, unpaired t test was applied to determine statistical difference. Survival curves of animals were plotted according to the Kaplan-Meier method, and statistical significance in different treatment groups was compared using the log-rank test. Results and graphs were obtained using the GraphPad Prism 3.0 program (GraphPad Software, San Diego, CA).

RESULTS

Construction and Functional Characterization of Recombinant VSV Vectors Expressing NDV/F Protein. The cDNAs corresponding to the wt or mutant (L289A) F protein from NDV were cloned into the full-length genome of VSV as a new transcription unit after the G glycoprotein gene (Fig. 1A). We chose this cloning strategy for two reasons: first, the expression level of the NDV/F protein would be similar to levels achieved for endogenous VSVG protein; and second, relatively low levels of expression were expected to be sufficient to induce cell fusion with neighboring cell membranes. Reverse genetics was used to generate rVSV-NDF/V(L289A) and rVSV-NDF/V(FV) as described previously (17, 18). The latter virus would not be able to form syncytia and is therefore an excellent control virus. Expression of the NDV/F protein was monitored in infected BHK-21 cells by indirect immunofluorescence assay, using monoclonal antibodies specific for NDV/F protein (Fig. 1B). As a control, expression of VSV/G protein was detected in both wtVSV- and rVSV-NDF/V-infected cells by using a monoclonal antibody specific for VSV/G protein. In contrast, NDV/F protein expression was clearly localized to the cell surface and cytoplasm in BHK-21 cells infected with rVSV-NDF/V, but not in those infected with wtVSV.

One concern about recombinant VSV expressing a fusogenic membrane glycoprotein was that this could be detrimental to viral morphogenesis and infectivity. To compare the replication kinetics of rVSV-NDF/V(L289A), rVSV-NDF/V(FV), and wtVSV in detail, a multicycle growth curve was performed by infecting BHK-21 cells at a MOI of 0.01 (Fig. 2A). Both rVSV-NDF/V viruses grew very similarly and reached approximately the same titer at the same time points, indicating that there were no differences in viral replication and spread. The replication kinetics of wtVSV appeared to be slightly faster, but all three viruses reached a similar titer at 24 h after infection. We also performed a one-step growth curve by infecting BHK-21 cells at a MOI of 10 to analyze a single cell cycle of viral replication (Fig. 2B). All three viruses showed approximately the same viral titer at all four time points. Therefore, incorporation of F protein into the genome of VSV did not appear to have any major impact on viral life cycle and viral yield.

Phenotypic Characterization of rVSV-NDF/V Vectors in Rat and Human HCC Cells in Vitro. To investigate the ability of wtVSV, rVSV-NDF/V(L289A), and control rVSV-NDF/V(FV) to form syncytia
in HCC cells in vitro, we infected two human HCC cell lines (Hep3B and HepG2) and the rat HCC cell line (McA-RH7777) with the viruses at a MOI of 10 (Fig. 3). At 14 h after infection, large multinucleated syncytia were observed for all three tumor cells infected by rVSV-NDV/F(L289A). In contrast, the same tumor cells infected with wtVSV or control rVSV-NDV/F(wt) lacked this phenotype.

**Fusogenic VSV Displays Enhanced Oncolytic Properties of Rat and Human HCC Cells in Vitro.** We next examined whether syncytia formation, in the context of fusogenic VSV infection, would result in enhanced tumor cell killing. To this end, we infected the three human and rat HCC cell lines with wtVSV, fusogenic rVSV-NDV/F(L289A), or control rVSV-NDV/F(wt) vector at a low MOI of 0.01. The cytotoxic effects on the cells were quantified by a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay and expressed as a fraction of mock-infected cells at each time point. The two human HCC cell lines, Hep3B and HepG2 (Fig. 4, A and B), were killed by the fusogenic VSV-NDV/F(L289A) much more efficiently than by the control nonfusogenic rVSV-NDV/F(wt) vector or wtVSV. In fact, fusogenic VSV killed most cells within 36 h, at which time point 50–75% of the control vector-infected cells were still viable. On the other hand, rat McA-RH7777 cells tend to shed from the growth surface before achieving confluence, and the degree of viral killing through syncytia induction, which is known to be cell density dependent (11), was not as extensive as that seen in the human HCC cell lines, and statistical significance was achieved only 48 h postinfection (Fig. 4C).

**Fusogenic VSV-Induced Syncytia Formation in Multifocal HCC Tumors in Rats without Liver Damage.** To determine whether syncytia formation contributes to the oncolytic process of fusogenic VSV-mediated antitumor activity in vivo, rats bearing multifocal HCC tumors in their livers were infused with a previously determined maximum tolerable dose of 1.3 × 10^7 plaque-forming units of rVSV-NDV/F(L289A) or control rVSV-NDV/F(wt) vector via the hepatic artery. The animals were sacrificed 1 day later, and tumor-containing liver sections were examined by H&E staining. Syncytia, which are represented by giant cells with multinucleated nuclei, were frequently and exclusively observed in HCC tumors infused with rVSV-NDV/F(L289A) but not with the control virus (Fig. 5A, top panel). Immunohistochemical staining of consecutive sections demonstrated abundant expression of VSV/G pro-
tein that localized at the plasma membrane of individual tumor cells but surrounded the multinucleated structure in animals infused with the control and fusogenic virus, respectively (Fig. 5A, bottom panel).

To demonstrate the tumor specificity of rVSV-NDV/F(L289A) vector-induced syncytia formation and the absence of liver damage, histopathological sections showing the border region between tumor and liver tissues (Fig. 5B) and neighboring hepatic parenchyma (Fig. 5C) were examined. The liver histology was completely normal, and, in particular, no evidence of inflammatory cell infiltrate or syncytia formation of hepatocytes was found.

**Prolonged Survival of Multifocal HCC-Bearing Rats Treated with Fusogenic versus Control VSV.** To assess the potential of fusogenic VSV as an improved oncolytic agent, rats bearing more than 10 visible HCC tumors in their livers with sizes ranging from 1 to 10 mm in diameter were randomly assigned to a single infusion with 1.3×10⁷ plaque-forming units of either rVSV-NDV/F(L289A) (n = 11), rVSV-NDV/F(wt) (n = 9), or PBS (n = 9) via the hepatic artery, and survival was followed (Fig. 6). Buffer-treated rats started to die of tumor progression in 5 more days, and all of them expired at 18 days (median survival, 10 days). In contrast, rats treated with control rVSV-NDV/F(wt) survived for up to 24 days (median survival, 18 days), which was a significant improvement over the PBS group (P = 0.0016). In the fusogenic rVSV-NDV/F(L289A) treatment group, animals survived for up to 31 days, and median survival was further extended to 23 days (P < 0.0001 versus PBS). In pairwise comparison with the nonfusogenic control virus rVSV-NDV/F(wt), survival of rVSV-NDV/F(L289A)-treated animals was further improved (P = 0.0078).

**DISCUSSION**

In our previous study (9), we have shown that VSV vector administered via the hepatic artery in tumor-bearing rats resulted in efficient viral transduction of multifocal lesions in their livers, tumor-selective viral replication, and extensive oncolysis. However, intratumoral virus spread and treatment efficacy appeared to be limited, implying that improvements in the potency of the oncolytic virus will be beneficial. In this study, we have investigated a potential strategy for enhancement of the inherent oncolytic activity of VSV based on the generation of syncytia formation. To this end, we have constructed a novel VSV vector expressing the mutant (L289A) F protein from NDV and demonstrated its ability to induce strong syncytia formation in human and rat HCC cells in vitro and in vivo. A major concern regarding this approach was that the replication efficiency of this virus might be compromised due to deficient viral morphogenesis or other factors. For example, expression of the F protein of measles virus by VSV decreased viral replication, and this F gene was rapidly eliminated by deletion mutations from the recombinant viruses (19). Although syncytia formation is not a natural part of the VSV infection cycle, we demonstrated here that incorporation of mutant (L289A) NDV/F protein into the VSV genome does not appear to have any major

![Fig. 3](image-url) Phenotypic characterization of recombinant vesicular stomatitis virus (VSV) vectors in hepatocellular carcinoma (HCC) tumor cells in vitro. Human HCC cell lines (Hep3B and HepG2) and the rat HCC cell line (McA-RH7777) were infected with either rVSV-NDV/F(L289A), rVSV-NDV/F(wt), or control wtVSV at a multiplicity of infection of 10. Photomicrographs of H&E-stained cells were taken at 14 h after viral infection (original magnification, ×20).

![Fig. 4](image-url) Comparison of the cytotoxicity of recombinant vesicular stomatitis virus (VSV) vectors in hepatocellular carcinoma tumor cells in vitro. Hep3B (A), HepG2 (B), or McA-RH7777 (C) tumor cells were infected with either rVSV-NDV/F(L289A), rVSV-NDV/F(wt), or control wtVSV at a multiplicity of infection of 0.01. At the time points indicated, cell viability was measured using a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay. The A₅₇₀ values of mock-infected cells were set as 100% at each time point. Data are expressed as means ± SE of triplicate experiments [*P < 0.05; **P < 0.01; ***P < 0.001; wtVSV versus rVSV-NDV/F(L289A) or rVSV-NDV/F(wt) versus rVSV-NDV/F(L289A), respectively].

[Image URLs]

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**Fig. 4.** Comparison of the cytotoxicity of recombinant vesicular stomatitis virus (VSV) vectors in hepatocellular carcinoma tumor cells *in vitro*. Hep3B (A), HepG2 (B), or McA-RH7777 (C) tumor cells were infected with either rVSV-NDV/F(L289A), rVSV-NDV/F(wt), or control wtVSV at a multiplicity of infection of 0.01. At the time points indicated, cell viability was measured using a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay. The A₅₇₀ values of mock-infected cells were set as 100% at each time point. Data are expressed as means ± SE of triplicate experiments [*P < 0.05; **P < 0.01; ***P < 0.001; wtVSV versus rVSV-NDV/F(L289A) or rVSV-NDV/F(wt) versus rVSV-NDV/F(L289A), respectively].
impact on the viral life cycle and yield of VSV. In addition, there was no loss of viral titer over multiple passages in vitro (data not shown), indicating that it is a stable vector.

In the present study, we provide conclusive evidence that fusogenic VSV displays increased oncolytic activity over its nonfusogenic counterpart, which translated into highly significant prolongation of animal survival ($P = 0.0078$, log-rank test). This enhanced oncolytic activity of fusogenic VSV was achieved after a single administration of the virus, and the tumor burden at the time of treatment (15 days after tumor cell implantation) was quite extensive, resembling the actual situation in HCC patients with end-stage multifocal disease. In addition, the orthotopic locale of our tumor model more realistically recapitulates the liver environment and results in a locally aggressive, spontaneously metastatic tumor. On the basis of these considerations, the observed prolongation of median survival from 18 to 23 days posttreatment conferred by fusogenic VSV over its nonfusogenic counterpart is indeed impressive.

This benefit may be attributable to various factors. Our in vitro analysis has shown that fusogenic VSV kills tumor cells much more efficiently than wtVSV or nonfusogenic control viruses. The process of syncytial formation is known to involve a significant bystander cell killing effect through recruitment of adjacent untransduced cells into the evolving syncytium (11). In addition, fusogenic VSV kills tumor cells through both replication with subsequent cell lysis and direct cell membrane fusion, and these two cytolytic mechanisms may have produced a synergistic effect that has facilitated the spread of fusogenic VSV in the solid tumor tissue in vivo. Limited dispersion of viral particles and antiviral immunity are known contributing factors to the relative inefficiency of viral spread of current nonfusogenic oncolytic viruses within a solid tumor mass in vivo (20, 21). It is possible that by inducing cell-cell fusion, the virus may have propagated more efficiently in vivo because several steps in the life cycle of VSV, including viral budding and virus endocytosis, are bypassed. Fusogenic VSV may also have avoided humoral immune mechanisms of virus neutralization because syncytia-forming viruses can spread from cell to cell within the solid tumor mass that is refractory to the presence of neutralizing antibodies (10).

Fig. 5. Virus-induced syncytial formation in tumor tissues in vivo and absence of liver damage. A, H&E staining (top panels) and immunohistochemistry (IHC; bottom panels) against VSV/G protein of representative tumor sections obtained 1 day after infusion with either rVSV-NDV/F(L289A) or control virus rVSV-NDV/F(wt) via the hepatic artery (original magnification, ×40). H&E staining of representative sections showing (B) the border region between tumor and liver tissues and (C) neighboring hepatic parenchyma obtained from VSV NDV/F(L289A)-infused animals (original magnification, ×20).

Fig. 6. Prolonged survival of tumor-bearing rats treated with fusogenic vesicular stomatitis virus (VSV) vector. Kaplan-Meier survival curves of rats with multifocal hepatocellular carcinoma after hepatic arterial infusion with $1.3 \times 10^7$ plaque-forming units of either fusogenic rVSV-NDV/F(L289A) ($n = 11$), nonfusogenic control rVSV-NDV/F(wt) ($n = 9$), or PBS ($n = 9$) via the hepatic artery on day 0. The difference in survival advantage for the rVSV-NDV/F(L289A) vector-treated animals was statistically significant compared with PBS ($P < 0.0001$) or the nonfusogenic control virus ($P = 0.0078$). The results were combined from two consecutive sets of animals with stratification.
We and others have shown previously that wtVSV primarily induces tumor cell apoptosis in vivo (22, 23), which is mediated by the VSV matrix protein and by another viral component that has yet to be identified (24). Cell death by apoptosis is a highly ordered process, which escapes detection by the immune system and is actually immunosuppressive (25). In contrast, cancer cell death through synctium formation mediated by fusogenic VSV is a cytopathic effect that can lead to tumor necrosis in vivo, which, in contrast to apoptotic cell death, may result in highly efficient immune activation (26). Fusing tumor cells release large numbers of tumor-derived, exosome-like vesicles, which may be associated with an effective release of tumor antigens, their efficient transfer into immature dendritic cells, and subsequent cross-presentation to T cells to generate systemic antitumor immune responses (27, 28). Therefore, fusogenic VSV-mediated oncolysis may combine very effective local effects with the potential to be a highly immunogenic method of cytoxic gene therapy. In the present study, we have focused on the use of fusogenic VSV as an effective and safe oncolytic agent, which will permit more detailed mechanistic studies in our immunocompetent animal tumor model in the future.

Although we detected large necrotic regions within multiple tumors, which led to significant prolongation of animal survival, there were no long-term survivors. Repeated intrahepatic artery infusions would likely result in further improved survival over a single injection. Although VSV administration through the hepatic artery is technically not feasible in the rat, percutaneous catheterization of the hepatic artery for local-regional therapy of liver tumors is commonly used in the clinic, thereby potentially allowing readministration of the oncolytic virus before onset of humoral immune response. Moreover, the ability to express foreign sequence of at least 4.0 kb makes it possible to insert more than one foreign gene into the VSV genome (29). In this regard, recombinant VSV vectors encoding for the interleukin-4 cytokine or the herpes simplex virus thymidine kinase suicide gene were generated and reported to exhibit considerably more oncolytic activity than a control virus expressing green fluorescent protein (30). Recently published data (31, 32) presented the generation of IFN-inducing VSV variants or recombinant VSV vectors expressing IFN-β with greatly improved therapeutic indices over wtVSV after systemic delivery. These and other transgenes can be integrated into fusogenic VSV vectors to further increase their oncolytic potential.

Uncontrolled induction of synctium formation by fusogenic viruses raises potential safety concerns (12, 13). In the case of VSV, expression of viral and any accompanying foreign genes depends directly on viral RNA replication (33). Therefore, it was anticipated that fusogenic VSV would be compromised in its ability to induce fusion in normal cells because expression of an F protein is unlikely to result in a better ability of VSV to escape the innate IFN response. Indeed, hepatic artery infusion of fusogenic VSV in rats bearing multifocal HCC lesions in their livers resulted in extensive synctium formation within tumors but caused no damage to surrounding hepatocytes. Thus, the fusogenic VSV vector can be developed into effective and safe therapeutic agents for HCC and other types of cancer in the future.

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