Molecular Pathways

Molecular Pathways: Multimodal Cancer-Killing Mechanisms Employed by Oncolytic Vesiculoviruses

Douglas J. Mahoney\textsuperscript{1,2,3,4} and David F. Stojdl\textsuperscript{5,6,7}

Abstract

Cancer is a heterogeneous disease that, for the most part, is not effectively managed with existing therapies. Oncolytic viruses are an attractive class of experimental cancer medicine because, unlike conventional chemotherapeutic and molecularly targeted drugs, they orchestrate tumor cell death in multiple ways simultaneously. In this review, we discuss the numerous cancer-killing "pathways" marshalled by oncolytic vesiculoviruses. From directly infecting and lysing malignant cells, to engaging the host’s innate and adaptive anticancer immune responses, to inducing vascular collapse within a tumor, oncolytic vesiculovirus therapy commandeers a coordinated, multipronged assault on cancer that is curative in numerous preclinical models. And as our appreciation of these mechanisms has progressed, so has our capacity to engineer improved outcomes. Notably, efforts to polarize the host’s immune system toward the tumor and away from the virus have been particularly effective in immunocompetent murine models, and hold tremendous therapeutic promise for human patients. With a first-in-man phase I trial recently initiated in the United States, the clinical significance of oncolytic vesiculovirus therapy, after nearly 15 years of development, may soon come into focus. Clin Cancer Res; 19(4); 758–63. ©2012 AACR.

Background

With few exceptions, cancer is a mosaic disease comprising a mixture of genetically and phenotypically distinct tumor cell populations (1). It is also a plastic disease, imparted by its genomic instability, that rapidly evolves beyond most therapeutic stressors (2). As a descendent of self, it is a disease that is difficult to distinguish from its host, despite its many abnormal behaviors. These three facts are at the heart of why, four decades after the initiation of our “war on cancer”, survival rates for most metastatic tumors remain largely unchanged.

So what is the path forward for cancer therapy? In our view, growing evidence points toward a class of experimental biologic medicines referred to as oncolytic virus therapy (3). Oncolytic virus therapy is attractive because it orchestrates the specific destruction of tumors in several distinct ways. From directly killing malignant cells, to breaking immunologic anergy towards the cancer, to blocking blood supply to tumors, oncolytic virus therapy commandeers a coordinated, multipronged assault on cancer that has the capacity to overwhelm the mosaic, plastic, and evasive nature of malignancy. Clinically, recent data from several oncolytic virus trials in humans have confirmed its preclinical promise. In one phase II study, for example, stand-alone herpes simplex virus (HSV)-based virotherapy led to a 16% complete response rate in patients with metastatic melanoma, efficacy far exceeding the existing standard of care (4). Critical phase III trials for several viruses are nearing completion, and regulatory approval in the United States for a first-in-class oncolytic virus medicine is expected in 2013.

For the past 15 years, we and others have been developing oncolytic agents based on viruses from the vesiculovirus genus of the rhabdovirus family such as vesicular stomatitis virus (VSV) and more recently Maraba virus (5–10). In this review, we discuss the numerous ways in which oncolytic vesiculoviruses (OV) marshal tumor destruction, and recent efforts to potentiate those mechanisms for improved therapeutic activity.

OVs selectively infect and kill malignant cells

The basis underlying OV therapy is their ability to selectively infect malignant cells. This therapeutic window exists because, in the process of mutating around host immune surveillance, cancer cells frequently delete components of their innate immune response (6). OVs are highly vulnerable to type I IFN (11); as such, tumors harboring type I IFN defects provide the only cellular reservoir for a productive infection (6). Once inside the cancer cell, OVs replicate quickly and to high titers by coopting the host’s biosynthetic machinery. Upon egress, OVs elicit cancer cell death, or oncolysis, via apoptosis, which is...

\textsuperscript{1}Alberta Children’s Hospital Research Institute; \textsuperscript{2}Southern Alberta Cancer Research Institute; \textsuperscript{3}Snyder Institute for Chronic Disease; \textsuperscript{4}Department of Microbiology, Immunology and Infectious Disease, Faculty of Medicine, University of Calgary, Calgary, Alberta, Canada; \textsuperscript{5}Children’s Hospital of Eastern Ontario Research Institute; \textsuperscript{6}Chile; \textsuperscript{7}Biochemistry, Microbiology, and Immunology, University of Ottawa, Ottawa, Ontario, Canada

Corresponding Author: David F. Stojdl, Children’s Hospital of Eastern Ontario Research Institute, 401 Smyth Road, Ottawa, Ontario K1H 8L1, Canada. Phone: 613-738-4179; Fax: 613-738-4833; E-mail: dave@arc.cheo.ca

doi: 10.1158/1078-0432.CCR-11-3149

©2012 American Association for Cancer Research.
triggered by both intracellular and extracellular pathways (12, 13).

Historically, the major barrier to VSV treatment efficacy was presumed to be its inability to infect every tumor cell in vivo. Numerous roadblocks were identified: rapid virus elimination in the vasculature, sequestration of virus in depot tissues such as the liver, inefficient virus spread through a tumor, and a strong innate and adaptive immune response towards the virus. As a result, great effort was put forth toward improving tumor delivery and spread, such as shielding the virus with cell-based carriers (14) and co-administering the virus with immunosuppressant drugs (15). Although these strategies often provided a survival benefit, they rarely improved durable cure rates in immunocompetent tumor models. Thus far, the obstacles impeding efficient VSV delivery to every tumor cell in vivo have proven insurmountable.

Fortunately, we have come to realize that OVs need not infect every tumor cell to induce a complete and durable response (16). In fact, in cases such as the CT26 carcinoma and B16ova melanoma models, VSV is curative despite limited productivity at the tumor site (16, 17). As it turns out, the bystander mechanisms evoked by oncolytic VSV may be the sine qua non of their therapeutic use (Fig. 1).

OVs break innate and adaptive immune tolerance toward tumors

A decade ago, we noted that immunocompetent tumor-bearing mice cured by VSV therapy rejected subsequent engraftment with those same tumor cells (6), and, more recently, we have seen the same with Maraba virus (Stojdl et al, unpublished observation). Other studies showed that the adoptive transfer of splenic cells derived from immunocompetent mice previously cured of tumors by VSV protects naïve animals challenged with those same tumor cells (18). These observations indicate that, at least in some immunocompetent tumor models, OVs elicit an adaptive immune response that provides memory for those cancer cells. Experiments showing T-cell infiltration into tumors and their activation towards model tumor antigens in response to VSV strengthened this assertion (10, 16). Importantly, acquired antitumor immunity induced by VSV therapy was shown to be integral to long-term efficacy. Those studies showed that VSV is more effective when treating tumors in mice with a complete immune system (17), and that depleting T cells blunts oncolytic activity (10, 19).

At the same time, complimentary work showed that innate antitumor immune responses are also evoked by oncolytic VSV treatment. Several reports identified natural killer (NK) cell, macrophage and neutrophil recruitment to VSV-infected tumors, and immune depletion experiments showed their involvement in VSV therapy (10, 16, 20–23). In the B16ova model, for example, it was shown that VSV treatment requires NK cells for tumor destruction (10, 22).

In this system, VSV elicits interleukin (IL)-28 secretion from a population of immune-sensing cells expressing the myeloid differentiation antigen Gr-1, a signal for tumors to increase their expression of NK cell receptors (22, 23), making them vulnerable to NK cell killing. In other experimental systems, VSV was shown to engage dendritic cells (DC) to upregulate IL-15, a potent cytokine for NK cell activation and an absolute requirement for treatment efficacy (24).

It has become clear, therefore, that in the context of treating cancer, OVs are strong immunontherapeutic adjuvants. Our running model for how this works is that VSV infects tumor cells, produces progeny, lyses the infected cells, and spreads to some extent through the tumor bed (Fig. 1). In the process, tumor-resident DCs are alerted to virus infection and relay that information to the host's innate immune effectors. Activated NK cells, macrophages, and neutrophils seek and destroy virus-infected tumor cells, and, while doing so, also kill noninfected cells. Concomitantly, activated DCs migrate into tumor-draining lymph nodes, and lymph node–resident DCs are alerted to tumor and viral antigens released during oncolysis, where they cross-present antigen to T cells and evoke an adaptive immune response. Those antigen-specific T cells then migrate back to the tumor to clear residual virus infection and eliminate the remaining tumor cells.

Admittedly, aspects of this model are debatable and almost certainly different from cancer to cancer. For instance, although several reports showed that oncolytic VSV potently activates DCs (22, 24–26), a recent study by Leveille and colleagues showed that, in contrast, VSV infects and kills DCs and thus impedes antitumor immunity (27). Moreover, while many studies have documented that VSV elicits antitumor immunity, Willmon and colleagues showed that VSV subverts host immunity by engaging a population of myeloid-derived suppressor cells (MDSC; ref. 28). While the reasons for these disparities are not entirely known, it may be due to different model systems used between studies. Other outstanding questions also remain. Within a given model, why do some tumor-bearing mice develop curative antitumor immunity following VSV therapy whereas others do not? Between model systems on the same mouse background, why is the immune response to VSV treatment so different? These and many other details are currently the subject of active investigation in several laboratories. But one thing is certain: VSVs have the capacity, at least in some animal models, to generate a complete tumor response that is durable and is dependent upon the host's immune system. Understanding how this happens will be the first step toward engineering better efficacy and predicting when the therapy is most likely to work.

OVs induce tumor-specific vascular shutdown

An additional bystander mechanism evoked by OVs is vascular collapse (20, 29). Breitbach and colleagues showed that vascular endothelial cells in the outer rim of some tumors are abnormally susceptible to VSV infection. They also showed that VSV therapy leads to blood...
clotting at the tumor’s outer edge, which is dependent upon neutrophil recruitment and severely limits perfusion to the tumor core. Widespread apoptosis and necrosis were observed throughout these tumors, despite VSV infection constrained to the periphery. Together, these data suggest a model in which VSV infects tumor vessels at the outer rim, which leads to the recruitment of neutrophils. Presumably, in the process of destroying virus-infected endothelial cells, these neutrophils cause clotting, which reduces blood flow to the tumor core and induces massive cancer cell death, contributing to tumor eradication (Fig. 1). Although the mechanism by which VSV infects tumor endothelial cells was not elucidated, recent data showing that VEGF sensitizes endothelial cells for reovirus infection may shed some light (30). Perhaps this VEGF sensitization effect extends more generally to other viruses including VSV. These details, as well as the scope of this phenomenon, as it pertains to other tumors, are needed to gain perspective on the importance of vascular shutdown to VSV-based therapy.

Clinical–Translational Advances

In September 2012, recombinant VSV-expressing human IFN-β was administered to a human patient with advanced hepatocellular carcinoma (HCC), the first participant in the inaugural clinical trial evaluating an oncolytic VSV (31). This landmark study will provide valuable safety data for VSV in humans, and, undoubtedly, be the first in a series of many to test iterations on the VSV platform. Our feeling is that the most successful agents will be those engineered to enhance the engagement of the patient’s immune system, a view that is consistent with recent preclinical work from several laboratories at the vanguard of oncolytic therapy (17, 32–35). Several strategies have been applied thus far, such as engineering immune modulating cytokines into virus backbones (32) and using OVs to enhance cell-based therapies (36, 37). However, in our opinion, the most promising strategies are those that engineer tumor antigens into OVs for use as an oncolytic vaccine (38).
Engineering oncolytic vaccines to prime or boost antitumor immune responses

In 2007, Diaz and colleagues reported that VSV engineered to express chicken ovalbumin (ova) could efficiently treat mice harboring transgenic B16 melanomas expressing ova (B16ova; ref. 10, 39). Mice inoculated with VSVova developed strong CD8 T-cell–directed anti-ova immunity and many of their tumors completely regressed. Although a tantalizing finding, the model system used was problematic in that ova is a foreign antigen expressed at very high levels. Taking a more biologically relevant approach, others have built VSVs that express viral genes from known carcinogens, for the treatment of virus-infected tumors. For example, intradermal vaccination with VSV-expressing cottontail rabbit papillomavirus (CRPV) proteins was shown to induce complete regression of CRPV-induced papillomas in rabbits (40–42), and similar observations were made in murine papilloma models (43). However, as most tumors lack strong viral antigens, this approach, while having the potential to treat a small subset of human cancers, does not have broad therapeutic use.

Clearly, the most clinically relevant strategy is to use viruses that express self-tumor antigens. For several reasons, however, this approach has proven more challenging, including the facts that (1) real self-tumor antigens are relatively weak as the host is tolerized toward them and contains very few T-cell precursors (39); (2) the selective pressure applied by immune therapies towards a single tumor antigen can lead to escape variants (44), and (3) OVs are extremely immunogenic themselves and may distract the immune system from the tumor (34, 45). To address aspects of these barriers, two innovative approaches have recently been developed. The first strategy uses replicating OVs to boost antitumor immunity primed by a nonreplicating adenovirus-based vaccine (34, 46). It is well known that adenoviruses are very capable vectors for generating a primary immune response towards a transgene (47). Bridle and colleagues therefore took a heterologous “prime boost” approach in a metastatic B16 melanoma model, where they first primed tumor-bearing animals intramuscularly with an adenovirus expressing the self-tumor antigen hDCT (Ad-hDCT) and then, days later, boosted those same mice with replicating VSV-hDCT administered intravenously (34, 46). Remarkably, while VSV-hDCT treatment alone elicited a strong T-cell response towards viral antigens, the prime boost regimen completely polarized the adaptive immune response towards the hDCT tumor antigen. And, importantly, a large percentage of mice were cured of tumors when treated with the prime-boost regimen in this very challenging tumor model.

The second strategy, developed by Kottke and colleagues, used replicating VSV to express a cDNA library of altered self antigens derived from normal tissue (33, 35). When injected intravenously into mice bearing tumors of the same histologic type, the VSV-cDNA library induced a tumor specific CD4 T helper-17 response that cured mice with established tumors. Importantly, those activated T cells could then be screened against the cDNA library to identify the dominant tumor antigens for those tumors. Using this strategy in their melanoma model, these authors identified 3 strong tumor antigens that, when coexpressed from VSV, evoked the same antitumor response as the library itself. Although awaiting validation, this approach holds tremendous promise for the development of personalized oncolytic vaccines for patients with cancer.

Conclusions

Cancer therapy is at a crossroads. While a single drug targeting one oncogenic pathway is almost universally ineffective, combination therapies are generally too toxic to maintain appropriate dosing. Is oncolytic virotherapy, platform medicines that are nontoxic and target cancer cells in multiple ways simultaneously, the answer? Time will tell, but the future looks bright. HSV-, vaccinia- and reovirus-based agents have all moved successfully through early to mid-stage clinical evaluation and are currently in registration stage trials. Many other platforms, including OVs, are also being tested in human patients. Each of these agents is inherently multimechanistic, and most can be readily engineered for additional cancer-killing properties. As we have come to understand them, in part, as immune therapy adjuvants, their potential to synergize with other emerging immunomodulating medicines such as anti-CTLA4, anti-PD1, or adoptive cell therapies is enormous. In theory, oncolytic immunotherapy is an ideal approach for treating heterogenous, plastic, and evasive cancers, in particular, as we move toward an era of personalized medicine.

But many questions and challenges remain. In this review, we have described the predominant mechanisms, to our knowledge, by which OVs evoke tumor destruction. But, for example, how is blocking blood supply to a tumor compatible with the recruitment of antitumor immune effector cells, and why doesn’t it generate highly invasive escape variants as does antiangiogenic VEGF therapy? And, given the duality of the host’s immune response to oncolytic therapy, as an impediment to viral productivity and yet a requirement for antitumor efficacy, how do we best influence this interaction for a therapeutic gain? Although tumor-selective infectivity and killing has been the foundation of oncolytic therapy for most of the past 2 decades, is it actually necessary? If so, how much, and can it sometimes be a distraction, in particular, when these viruses are engineered and used primarily as tumor vaccines as has been reported recently (16, 34, 46)? If we are to realize the enormous potential of oncolytic virus medicines, addressing these and other mechanistic questions must be a top priority.

Disclosure of Potential Conflicts of Interest

D.F. Stojdl has ownership interest (including patents) in and is a consultant for Jennerex Biotherapeutics Inc. No potential conflicts of interest were disclosed by D.J. Mahoney.

Authors’ Contributions

Conception and design: D.F. Stojdl
Writing, review, and/or revision of the manuscript: D.J. Mahoney, D.F. Stojdl
Gaddy DF, Lyles DS. Vesicular stomatitis viruses expressing wild-type
Gaddy DF, Lyles DS. Oncolytic vesicular stomatitis virus induces
282–91.
9. Gao Y, Whitaker-Dowling P, Griffin JA, Barnama MA, Bergman I. Recombinant vesicular stomatitis virus targeted to Her2/neu com-
19.
Whitaker-Dowling P, Griffin JA, Barnama MA, Bergman I. Recombinant vesicular stomatitis virus targeted to Her2/neu com-
bined with anti-CTLA4 antibody eliminates implanted mammary

Received September 27, 2012; revised November 19, 2012; accepted November 21, 2012; published OnlineFirst December 26, 2012.
Molecular Pathways: Multimodal Cancer-Killing Mechanisms Employed by Oncolytic Vesiculoviruses

Douglas J. Mahoney and David F. Stojdl


Access the most recent version of this article at:
doi:10.1158/1078-0432.CCR-11-3149

This article cites 45 articles, 12 of which you can access for free at:
http://clincancerres.aacrjournals.org/content/19/4/758.full.html#ref-list-1

This article has been cited by 1 HighWire-hosted articles. Access the articles at:
/content/19/4/758.full.html#related-urls

Sign up to receive free email-alerts related to this article or journal.

To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.