Molecular Pathways: multimodal cancer-killing mechanisms employed by oncolytic vesiculoviruses

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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 tumour 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 anti-cancer immune responses, to inducing vascular collapse within a tumour, oncolytic vesiculovirus therapy commandeers a coordinated, multi-pronged assault on cancer that is curative in numerous preclinical models. And as our appreciation of these mechanisms has progressed, so to has our capacity to engineer improved outcomes. Notably, efforts to polarize the host’s immune system toward the tumour and away from the virus have been particular 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.
Background

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

So what is the path forward for cancer therapy? In our view, growing evidence points towards a class of experimental biological medicines referred to as oncolytic virus (OV) therapy (3). OV therapy is attractive because it orchestrates the specific destruction of tumours in several distinct ways. From directly killing malignant cells, to breaking immunologic anergy towards the cancer, to blocking blood supply to tumours, OV therapy commandeers a coordinated, multi-pronged assault on cancer that has the capacity to overwhelm the mosaic, plastic and evasive nature of malignancy. Clinically, recent data from several OV trials in humans has confirmed its preclinical promise. In one phase II study, for example, stand-alone HSV-based virotherapy led to a 16% complete response rate in patients with metastatic melanoma, efficacy far exceeding 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 OV medicine is expected in 2013.

For the past 15 years, we, and others, have been developing oncolytic agents based off vesiculovirus platforms (5-10). In this review, we discuss the numerous ways
in which oncolytic vesiculoviruses marshall tumour destruction, and recent efforts to potentiate those mechanisms for improved therapeutic activity.

**Oncolytic vesiculoviruses selectively infect and kill malignant cells**

The basis underlying oncolytic vesiculovirus 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). Vesiculoviruses are highly vulnerable to type I IFN (11); as such, tumours harbouring type I IFN defects provide the only cellular reservoir for a productive infection (6). Once inside the cancer cell, vesiculoviruses replicate quickly and to high titres by co-opting the host’s biosynthetic machinery. Upon egress, VSV elicits cancer cell death, or oncolysis, via apoptosis, which is triggered by both intracellular and extracellular pathways (12,13).

Historically, the major barrier to vesiculovirus treatment efficacy was presumed to be its inability to infect every tumour 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 tumour; and a strong innate and adaptive immune response towards the virus. As a result, great effort was put forth towards improving tumour delivery and spread, such as shielding the virus with cell-based carriers (14) and co-administering the virus with immunosuppressant drugs (15). Yet although these strategies often provided a survival benefit, they rarely improved durable cure rates in immunocompetent tumour models. Thus far, the obstacles impeding efficient vesiculovirus delivery to every tumour cell *in vivo* have proven insurmountable.
Fortunately, we have come to realize that oncolytic vesiculoviruses need not infect every tumour 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 in spite of limited productivity at the tumour (16,17). As it turns out, the bystander mechanisms evoked by vesiculoviruses may be the *sine qua non* of their therapeutic utility (Figure 1).

**Oncolytic vesiculoviruses break innate and adaptive immune tolerance towards tumours**

A decade ago we noted that immunocompetent tumour-bearing mice cured by VSV therapy rejected subsequent engraftment with those same tumour cells (6), and more recently we’ve seen the same with Maraba virus (unpublished observation). Other studies showed that the adoptive transfer of splenic cells derived from immunocompetent mice previously cured of tumours by VSV protects naive animals challenged with those same tumour cells (18). These observations indicate that, at least in some immunocompetent tumour models, vesiculoviruses elicit an adaptive immune response that provides memory for those cancer cells. Experiments demonstrating T-cell infiltration into tumours and their activation towards model tumour antigens in response to VSV strengthened this assertion (10,16). Importantly, acquired anti-tumour immunity induced by VSV therapy was shown to be integral to long-term efficacy. Those studies showed that VSV is more effective when treating tumours in mice with a complete immune system (17), and that depleting T-cells blunts oncolytic activity (10,19).

At the same time, complimentary work demonstrated that innate anti-tumour
immune responses are also evoked by oncolytic VSV treatment. Several reports identified natural killer cell, macrophage and neutrophil recruitment to VSV-infected tumours, and immune depletion experiments demonstrated 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 tumour destruction (10,22). In this system, VSV elicits IL-28 secretion from a population of immune sensing cells expressing the myeloid differentiation antigen Gr-1, a signal for tumours 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 (DCs) to up-regulate 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, vesiculoviruses are strong immunotherapeutic adjuvants. Our running model for how this works is that VSV infects tumour cells, produces progeny, lysed the infected cells and spreads to some extent through the tumour bed (Figure 1). In the process, tumour-resident DCs are alerted to virus infection and relay that information to the host’s innate immune effectors. Activated natural killer cells, macrophages and neutrophils seek and destroy virus-infected tumour cells, and while doing so also kill non-infected cells. Concomitantly, activated DCs migrate into tumour-draining lymph nodes (TDLN), and lymph node resident DCs are alerted to tumour 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 tumour to clear residual virus infection and eliminate the remaining tumour 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 (2011) demonstrated that, in contrast, VSV infects and kills DCs and thus impedes anti-tumour immunity (27). Moreover, while many studies have documented that VSV elicits antitumour immunity, Willmon et al. (2011) showed that VSV subverts host immunity by engaging a population of myeloid-derived suppressor cells (MDSC) (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 tumour bearing mice develop curative anti-tumour immunity following vesiculovirus therapy while others do not? Between model systems on the same mouse background, why is the immune response to vesiculovirus treatment so different? These and many other details are currently the subject of active investigation in several laboratories. But one thing is certain: vesiculoviruses have the capacity, at least in some animal models, to generate a complete tumour response that is durable and is dependent upon the host’s immune system. Understanding how this happens will be the first step towards engineering better efficacy and predicting when the therapy is most likely to work.

Oncolytic vesiculoviruses induce tumour-specific vascular shutdown

An additional bystander mechanism evoked by vesiculoviruses is vascular collapse (20,29). Breitbach and colleagues (2007) demonstrated that vascular endothelial cells in the outer rim of some tumours are abnormally susceptible to VSV infection. They also showed that VSV therapy leads to blood clotting at the tumour’s outer edge, which
is dependant upon neutrophil recruitment and severely limits perfusion to the tumour core. Wide-spread apoptosis and necrosis were observed throughout these tumours, in spite of VSV infection constrained to the periphery. Together, these data suggest a model in which VSV infects tumour 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 tumour core and induces massive cancer cell death, contributing to tumour eradication (Figure 1). Although the mechanism by which VSV infects tumour 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 the this phenomenon as it pertains to other tumours, are needed to gain perspective on the importance of vascular shutdown to vesiculovirus-based therapy.

Clinical-Translational Advances

In September 2012, recombinant VSV expressing human IFNβ was administered to a human patient with advanced hepatocellular carcinoma, the first participant in the inaugural clinical trial evaluating an oncolytic vesiculovirus (Stephen Russell, personal communication). 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 vesiculovirus 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 pre-clinical work from several laboratories at the vanguard of
oncolytic therapy (17,31-34). Several strategies have been applied thus far, such as engineering immune modulating cytokines into virus backbones (31) and using vesiculoviruses to enhance cell-based therapies (35,36). However, in our opinion the most promising strategies are those that engineer tumour antigens into vesiculoviruses for use as an oncolytic vaccine (37).

**Engineering oncolytic vaccines to prime or boost anti-tumour immune responses**

In 2007, Diaz and colleagues reported that VSV engineered to express chicken ovalbumin (ova) could efficiently treat mice harbouring transgenic B16 melanomas expressing ova (B16ova) (10,38). Mice inoculated with VSVova developed strong CD8 T cell directed anti-ova immunity and many of their tumours 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 vesiculoviruses that express viral genes from known carcinogens, for the treatment of virus-infected tumours. For example, intradermal vaccination with VSV expressing cottontail rabbit papillomavirus (CRPV) proteins was shown to induce complete regression of CRPV-induced papilloma’s in rabbits (39-41), and similar observations were made in murine papilloma models (42). However, as most tumours lack strong viral antigens, this approach, while having the potential to treat a small subset of human cancers, does not have broad therapeutic utility.

Clearly, the most clinically-relevant strategy is to use viruses that express self-tumour antigens. For several reasons, however, this approach has proven more challenging, including the facts that (1) real self-tumour antigens are relatively weak as
the host is tolerized towards them and contains very few T-cell precursors (38); (2) the selective pressure applied by immune therapies towards a single tumour antigen can lead to escape variants (43), and (3) vesiculoviruses are extremely immunogenic themselves, and may distract the immune system from the tumor (33,44). To address aspects of these barriers, two innovative approaches have recently been developed. The first strategy uses replicating vesiculoviruses to boost antitumour immunity primed by a non-replicating adenovirus-based vaccine (33,45). It is well known that adenoviruses are very capable vectors for generating a primary immune response towards a transgene (46). Bridle and colleagues (2010) therefore took a heterologous “prime-boost” approach in a metastatic B16 melanoma model, where they first primed tumour-bearing animals intramuscularly with an adenovirus expressing the self-tumour antigen hDCT (Ad-hDCT) and then, days later, boosted those same mice with replicating VSV-hDCT administered intravenously (33,45). 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 tumours when treated with the prime-boost regimen in this very challenging tumour model.

The second strategy, developed by Kottke and colleagues (2011), used replicating VSV to express a cDNA library of altered self antigens derived from normal tissue (32,34). When injected IV into mice bearing tumours of the same histological type, the VSV-cDNA library induced a tumor specific CD4 T\textsubscript{H}17 response that cured mice with established tumours. Importantly, those activated T-cells could then be
screened against the cDNA library to identify the dominant tumour antigens for those tumours. Employing this strategy in their melanoma model, these authors identified three strong tumour antigens that, when co-expressed from VSV, evoked the same antitumour response as the library itself. Although awaiting validation, this approach holds tremendous promise for the development of personalized oncolytic vaccines for cancer patients.

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 non-toxic 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 vesiculoviruses, are also being tested in human patients. Each of these agents is inherently multi-mechanistic, and most can be readily engineered for additional cancer-killing properties. And as we have come to understand them, in part, as immune therapy adjuvants, their potential to synergize with other emerging immune modulating 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 towards 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 vesiculoviruses evoke tumour destruction. But, for example, how is blocking blood supply to a tumour compatible with the recruitment of anti-tumour immune effector cells, and why doesn’t it generate highly invasive escape variants as does anti-angiogenic 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 anti-tumour efficacy -- how do we best influence this interaction for a therapeutic gain? Although tumour selective infectivity and killing has been the foundation of oncolytic therapy for most of the past two 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 tumour vaccines as has been reported recently (16,33,45)? If we are to realize the enormous potential of OV medicines, addressing these and other mechanistic questions must be a top priority.
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Figure Legends

Figure 1. Oncolytic vesiculoviruses target tumour destruction in multiple ways.
Schematic diagram depicting the known mechanisms of tumour destruction marshalled by oncolytic vesiculoviruses. See text for in-depth description.
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Figure 1:

1. **Virus-induced recruitment**
2. **Direct virus-mediated lysis**
3. **“Vascular shutdown”**
4. **NK-cell attack**
5. **T-cell attack**
6. **Cross-presentation**
7. **Virus-induced recruitment**

- **Vasculature**
- **Stroma**
- **Tumor**
- **Virus**
- **T cell**
- **DC**
- **Neutrophil**
- **NK cell**
- **Treg**
- **MDSC**
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