Targetting and Arming Oncolytic Viruses

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CHEMOTHERAPY AND RADIATION THERAPY HAVE BEEN BENEFICIAL CANCER TREATMENTS; HOWEVER, THEY LACK TISSUE SPECIFICITY AND INDUCES TOXIC EFFECTS ON HEALTHY CELLS. THE USE OF ARMED ONCOLYTIC VIRUSES PRESENTS AN ALTERNATIVE TO THE SIDE EFFECTS OF CURRENT TREATMENT AND IS AT THE FOREFRONT OF CURRENT CANCER THERAPIES.

Cancer is the abnormal and uncontrolled division of cells that have the ability to invade adjacent tissues and potentially metastasize to other regions in the body (Vogelstein & Kinzler, 2004). In 2008, approximately 166 400 Canadians were diagnosed with cancer, a number that will continue to increase along with our growing and aging population (Canadian Cancer Society/National Cancer Institute of Canada: Canadian Cancer Statistics 2008, Toronto, Canada, 2008). While chemotherapy and radiotherapy have been a beneficial treatment for many patients, they are relatively nonspecific to tumour tissue and present significant disadvantages. Rather than targeting cancerous cells, chemotherapy acts systemically, indiscriminately inducing toxic effects in healthy tissues (Neri & Schliemann, 2007). Traditional radiotherapy has caused extreme discomfort and produces undesirable side effects, among them infertility and fatigue (Harrison et al., 2000; Meirow & Nugent, 2001; Thachil et al., 2001). Consequently, there has been a push in the field to design treatments that attack defective pathways, while leaving normal tissue relatively unharmed, but are also applicable to a broad range of tumour types (Friedrich et al, 2004). Born amidst these research endeavors is the concept of viral oncolysis – using wild-type or recombinant viruses to selectively infect and kill cancer cells while leaving normal tissues viable (Goldman et al., 2008). This article will focuses on the current strategies used to isolate such viruses to tumour cells, ways through which to improve their destructive ability, as well as the state of their application in the clinic.

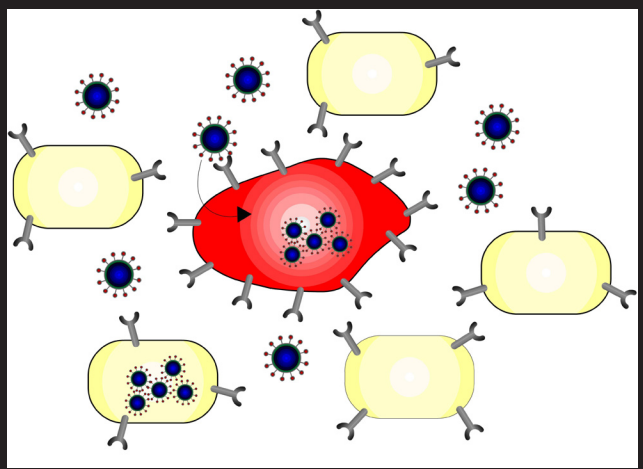


Figure 1
Receptor-based selective infection of cancer cells by an oncolytic virus. The virus interacts with a specific cell-surface receptor to mediate cell entry. This receptor is overexpressed on tumour cells (dark) relative to normal cells (light) which increases the probability of infecting malignant cells. Some normal cells may still be infected but to a much lesser extent.

Arguably, exquisite tumour targeting is the most valuable asset of oncolytic virotherapy. To create an effective recombinant oncolytic virus, the viral tropism must often be modified with the goal of increased viral affinity for replication in tumour cells. Three main strategies are most frequently used to target viruses to cancer cells: entry through receptors overexpressed on cancer cells, cancer-specific transcription and replication, and exploitation of cancer cell defects (Cattaneo et al., 2008).

The first mechanism by which viral tropism can be redefined requires adjusting the virus' selectivity for cell surface receptors (Figure 1). This can be achieved by genetically inactivating the residues that bind the virus' natural receptor and introducing alternate residues that enable the virus to bind receptors overexpressed on tumour cells (Vongpunsawad et al., 2004). As such, the probability of the virus attaching, infecting, and replicating within tumour cells is increased. A common approach to introduce this specificity is to use a single-chain fragment variable (scFv) antibody, composed of only the

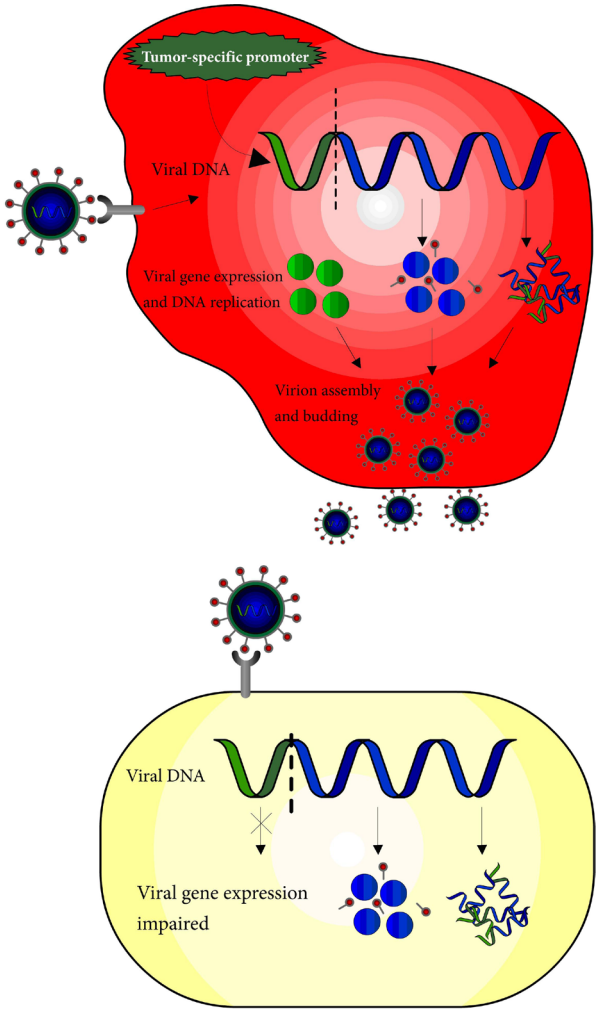
antigen-binding variable regions of the antibody, that is most easily applied to enveloped viruses such as herpes simplex virus (HSV) (Conner et al., 2008). Indeed, some normal cells also express these receptors and become infected to a much lesser extent.

Engineering the viral genome to selectively replicate in tumour cells is another effective way of altering viral tropism to achieve selective replication. Normally, after the attachment and injection of the viral genome, replication and propagation begin within the host cell (Kim et al., 2007). Primarily with DNA viruses, it is possible to manipulate the viral genome such that the transcription of essential viral gene products is controlled by a tumour-specific promoter (Figure 2). This strategy has been successfully employed in various models (Berk, 2005). This process results in a virus that is only capable of complete replication within tumour cells.

Lastly, the tumour-selective infection of oncolytic viruses can be mediated by the deficient antiviral responses of tumour cells (Figure 3). When normal cells are infected by an RNA virus, they immediately respond to invasion by secreting antiviral cytokines (Janeway et al., 2005). These signals recruit innate immune cells to combat the viral infection, while simultaneously protecting neighboring cells from further viral infection (Randall & Goodbourn, 2008). However, tumour cells display deficiencies in these antiviral responses, facilitating viral infection and lysis. For example, the rhabdovirus, vesicular stomatitis virus (VSV), with its high sensitivity to interferon (Lichty et al., 2004), has been demonstrated to selectively replicate in tumour cells which are often non-responsive to this cytokine (Grander & Einhorn, 1998).

In addition to targeting, strategies are often employed to amplify the cytolytic capabilities of oncolytic viruses to increase their efficacy (Cattaneo et al., 2008). Pro-apoptotic genes can be inserted to induce tumour cell death during the late stages of viral infection, as employed in an oncolytic adenovirus-expressing tumour-necrosis-factor-related apoptosis-inducing ligand (TRAIL) (Sova et al., 2004). However, this approach can limit viral spread and oncolysis, due to low virus production resulting from premature apoptosis (Cattaneo et al., 2008). Another strategy involves the expression of so-called prodrug convertases by the virus, which converts a harmless systemically delivered prodrug to a cytopathic compound. These genes have been shown to supplement tumour oncolysis in several viral systems, including an HSV-encoding thymidine kinase capable of activating the drug ganciclovir in infected tumour cells

Figure 2 Transcription-level selective replication. The oncolytic virus binds to both normal (light) and transformed (dark) cells and is taken up by endocytosis or membrane fusion. The genetic material of the virus, in this case double-stranded DNA, is released into the cell. Certain genes in the viral genome have been engineered to depend on a tumour-specific promoter for transcription. Cancer cells that express this promoter allow the synthesis of all viral proteins and facilitate viral replication. In normal cells, not all of the viral proteins can be transcribed, rendering the infection inconsequential.



(Boviatsis et al., 1994). In addition, oncolytic viruses can be made to express immune-stimulating molecules like GM-CSF (Lei et al., 2008; Malhotra et al., 2007) or tumour-associated antigens (TAA) (Diaz et al., 2007) to recruit antitumour effector cells from the host immune system (Prestwich et al., 2008).

With an abundance of candidate viruses and various mechanisms to retarget their tropism, oncolytic viruses present a promising therapeutic option for cancer treatment. The clinical application of oncolytic viruses is still in its infancy and lacks sufficient preclinical research. The first clinical trial took place only eleven years ago, and while a number of

subsequent trials have been performed, few have made it to the later stages (Parato et al., 2005). Concerns of toxicity have alleviated since the vast majority of dose escalation trials with oncolytic viruses have failed to reach the maximum tolerable dose (MTD) (Parato et al., 2005). There are currently a number of viruses being tested in the clinic on various tumour types. Recently, an oncolytic adenovirus (H101) has been approved for clinical use in combination with chemotherapy (Yu & Fang, 2007), (Liu et al., 2007), providing a promising outlook for this treatment method (Garber, 2006).

Over the next decade, it is likely that techniques for manipulating oncolytic viruses will continue to evolve, leading to successful clinical applications. It is quite possible that these viruses, which hold such great potential to yield high tumour-selectivity and limited therapeutic side effects, could ultimately fulfill the criteria of the ideal cancer treatment.

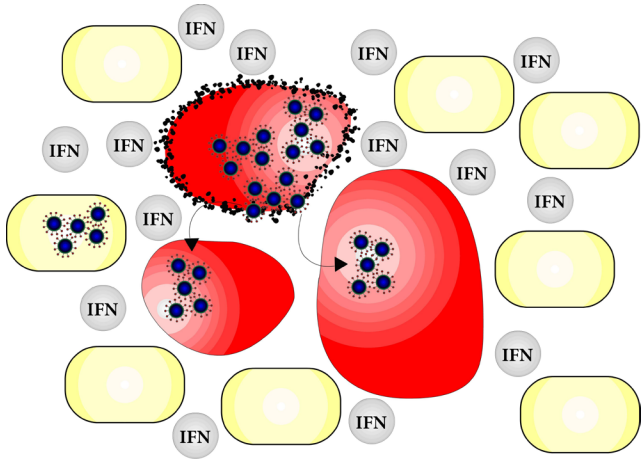


Figure 3 Selective infection based on deficiencies in antiviral pathways. Upon delivery, the oncolytic virus infects both normal (light) and cancer cells (dark). Shortly after infection, an antiviral response is initiated by the infected cell, which protects other normal cells in the vicinity from viral infection. Tumour cells, which often show impairments in their antiviral response, remain susceptible to viral infection and lysis. IFN = Interferon

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