Oncolytic Therapy: Curing Cancer With Viruses

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When we think of viruses, they are often stigmatized as something that is harmful to our bodies. However, the genetic amenability of viruses allows researchers to easily manipulate them to specifically target cancer cells. This new approach to cancer treatment is referred to as oncolytic virus therapy. In China, a new oncolytic strain of adenovirus has recently been approved for treating cancer, but the technology is lagging in North America. This article discusses some of the barriers and novel findings in the exciting field of oncolytic virus therapy.

Due to emerging cancers that are resistant to conventional chemotherapy, the field of oncolytic therapy is touted as the future of cancer treatment. Oncolytic therapy involves the use of a virus to specifically replicate and destroy tumour cells found throughout the body. Within the past decade, researchers have experimented with the poliovirus, influenza virus, and measles virus as potential candidates for oncolytic virus therapy (Russell, 2002). This may seem counter-intuitive since these viruses are generally viewed as something that is harmful. Several decades ago, Peyton Rous was awarded the Nobel Prize for discovering that viruses are the causative agents of certain tumours. Many virologists today, however, have reversed this conventional view by trying to eliminate tumours with viruses.

China's Food and Drug Administration (FDA) recently approved the first oncolytic virus called H101, created by Shanghai Sunway Biotech, for oncolytic treatment of head and neck cancers (Garber, 2006). Although a wide range of these recombinant viruses exist in the United States, oncolytic therapy is still considered an experimental procedure because of tight safety regulations imposed by the American FDA. This article discusses the current barriers that oncolytic therapy faces, followed by a review of new directions that this field is taking, with particular focus on the use of the vesicular stomatitis virus.

Barriers to Overcome

Although several viruses have been shown to possess oncolytic abilities, there are still many unanswered questions and problems that need to be addressed. Getting viruses to the site of the tumour has been problematic since most experiments require injecting high viral titers directly into the tumour site. The efficacy of eliminating metastasized cancer by using oncolytic therapy may be very low since all cancerous cells must be removed to prevent relapses. Systemic delivery of oncolytic viruses via intravenous injection is difficult because of viral tropism and activation of the immune system upon viremia. Getting the virus into specific tumour areas is the challenge that lies ahead. The fear of oncolytic viruses evolving into pathogens is another concern that is deeply rooted in the belief that viruses are harmful (Bell, Lichty, & Stojdl, 2003). The human body can place selective pressures on these viruses, but clinical trials for oncolytic viral therapy are designed to ensure that patients do not inadvertently infect other individuals. By preventing transmission or "serial passage" of oncolytic viruses, the probability of either mutating or recombining, and eventually reverting into its original pathogenic form, is reduced. Occasionally, administration of an oncolytic virus might cause an adverse response. Although this often is not lethal, one method of administration that has been used in some experiments is to gradually increase viral dosage over the course of several injections (Bell et al., 2003). Even though adverse symptoms may appear after the first inoculation, the host will eventually become desensitized and the virus can continue its anti-tumour activity unimpeded by the immune system.

Further complicating matters are the ethical and economic factors that need to be considered with regards to oncolytic therapy. As the United States biotechnology companies continue to be impeded by the FDA, they may relocate their research branches to China. This raises some ethical concerns regarding practices in recruiting test subjects. However, it is economically feasible to relocate since the bulk of their market resides in China, where oncolytic therapies seem to earn government approval faster. The economic perspective is an important consideration since it has

become extremely costly for biotechnology companies to research, produce, test and market these products. To date, only a handful of oncolytic viruses have made it into the advanced stages of FDA testing (e.g. human test trials), only to be withdrawn or rejected (Garber, 2006).

The Difference Between Using DNA and RNA Viruses

When compared to the adenovirus, a double-stranded DNA virus, RNA viruses are better candidates for oncolytic therapy. This is because they do not rely on the host nucleus and polymerases for transcription and translation of the viral genome. The requirement for viral transcripts to be exported out of the nucleus also complicates and diminishes the expediency of the DNA virus life cycle. However, the lifecycle of an RNA virus is less restrictive in terms of the host cell environment that is required for its replication. RNA viruses either carry their own polymerases or polymerase transcripts are immediately transcribed upon entry into the host cell. Furthermore, RNA viruses generally replicate faster than DNA viruses. This is a desirable characteristic since the goal of oncolytic therapy is to rapidly eliminate tumour cells before these cells can spread or become resistant (Russell, 2002). One setback is that RNA virus genomes tend to be more prone to errors during replication, possibly leading to an evolved pathogenic form of the oncolytic virus. Viral evolution is a problem that is shared by both DNA and RNA viruses even though it might be more rapid with RNA viruses.

Selectively Targeting Tumour Cells

There are currently three approaches used to ensure that viruses are able to specifically target tumour cells: (1) complementing a viral mutation with a tumour mutation, (2) fusing the viral genome to a tumour-driven promoter so that it can only be expressed in tumours, and (3) allowing the virus to express recombinant receptors that show high affinity to cell surface markers found on tumour cells (O'Shea, 2005).

The first approach can be further divided into two ways that allow mutant viruses to exploit tumour cell-specific defects. One defect includes an inability to respond to interferon (IFN), a crucial antiviral compound that activates a whole subset of genes responsible for "arming" cells during infection. It is believed that tumour cells possess a defective IFN response because IFN normally diminishes uncontrolled growth by shifting cellular resources away from the cell and towards antiviral response. Nonetheless, this defect results in reduced cellular fitness as the cell becomes more vulnerable to viral infections.

Another tumour-specific defect that can be exploited is Ras hyperactivation, resulting in uncontrolled cell proliferation (tumours). The Ras pathway is closely related to the protein kinase R (PKR) mechanism, which usually suppresses cellular translation upon detecting foreign RNA. In cells with Ras-induced cell proliferation, PKR must either be mutated or inhibited so that cellular translation can proceed in tumours (Norman, Farassati, & Lee, 2000). In normal cells, viral infection results in long, double-stranded RNA that activate PKR and arrest cellular translation in the

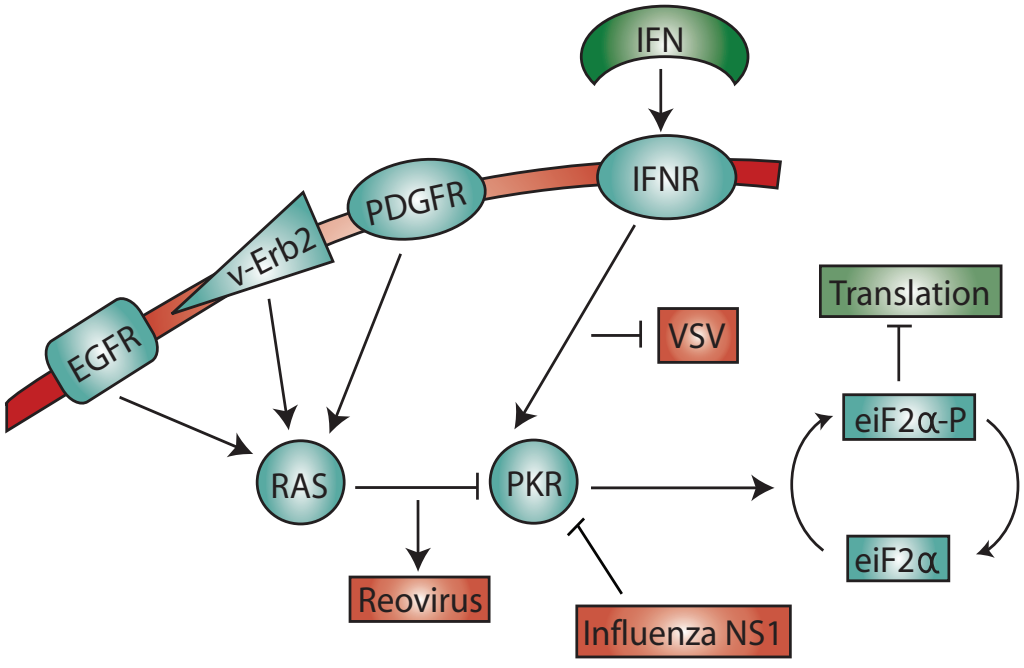

Figure 1 This diagram shows how the Ras, PKR, and IFN pathways converge in a normal (non-cancerous) cell to shut down cellular translation in response to an infection. The influenza virus typically encodes an NS1 protein that can inhibit the PKR-antiviral response to ensure that translation continues (Chiocca, 2002).

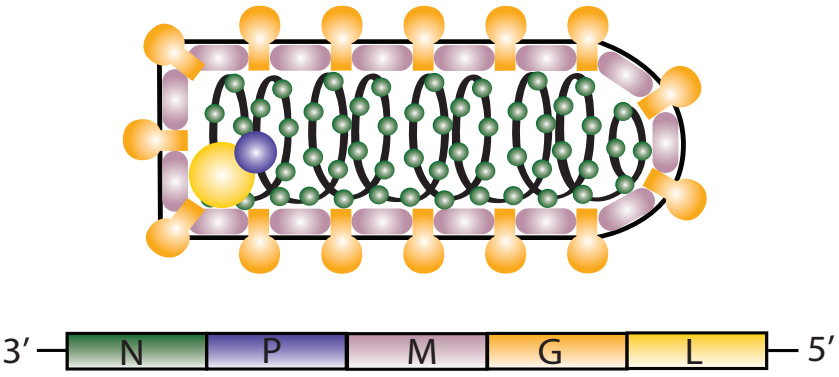

Figure 2 The vesicular stomatitis virus is an enveloped negative-strand RNA virus. Its genome is segmented into five genes that encode the following proteins: large polymerase protein (L), glycoprotein (G), matrix protein (M), phosphoprotein (P), and nucleoprotein (N). The coiled structure found within the phospholipids bilayer represents the ribonucleoprotein containing RNA and proteins to ensure the viral genome is protected (Lichty et al., 2004).

host cytoplasm, thus preventing the virus from replicating and spreading. Viruses such as influenza encode a protein that can counteract this PKR-antiviral response. However, if this viral protein is deleted, the virus will only be able to persist in cells with a defective PKR response and this is how tumour cells can be selectively targeted.

Vesicular Stomatitis Virus

A group of Canadian researchers decided to approach the problems facing oncolytic viruses from another perspective. Rather than taking a human viral pathogen and subjecting it to specific mutations, this group decided to start with a non-pathogenic virus called the vesicular stomatitis virus (VSV). This virus is a primary candidate for oncolytic therapy because it typically does not infect humans, meaning most individuals will not be seropositive for VSV (Lichty et al., 2004). Without pre-existing neutralizing antibodies, this virus is able to reach target sites (e.g. tumours) more effectively. VSV is a negative-strand RNA virus that is spread by an arthropod vector and causes ulcerations in cattle, swine, and horses. It is part of the rhabdoviridae family, which also includes the rabies virus (Figure 2). Individuals that are frequently in contact with livestock may become infected, but the symptoms are not lethal and are similar to those of a mild flu (Lichty et al., 2004).

The broad tropism of VSV seen within humans is another characteristic that contributes to the viability of using this as an oncolytic virus (Lichty et al., 2004). The ability to infect a variety of tissues is an important property if VSV is to be used as an oncolytic treatment for metastatic cancers. One other notable characteristic of VSV is its ability to encode for a matrix (M) protein. Once translated, the M protein is capable of localizing to the nuclear pores of the host cell to block transcripts for immune system factors (e.g. cytokines, interferons) from accessing the cytoplasm. This not only prevents an antiviral response from mounting, but also induces cellular apoptosis resulting in VSV pathogenesis. The M-protein has also been studied for its ability to contribute towards viral budding and virion assembly (Jayakar, Murti, & Whitt, 2000).

Here at McMaster University, Dr. Brian Lichty has continued the quest of optimizing the use of VSV for oncolytic therapy. An earlier paper by Lichty and his colleagues studied the effect of single amino acid substitutions in the M protein of VSV (referred to as AV1 and AV2). Through microarray data, the authors discovered that VSV strains deficient in M protein function allowed carcinoma cells to upregulate genes involved with antiviral response (interferon stimulated response elements or ISREs). The normal cellular response that prevents viral infection is divided into three distinct transcriptional waves. Wild-type VSV with functional M protein blocks transcripts at the second wave. The authors were able to quantify IFN sensitivity of various cancer cell lines by IFN pre-treatment, followed by infection with wild-type VSV. Since cancer cells did not respond properly to IFN, these cells were vulnerable to infection as measured by the multiplicity of infection. *In-vivo* testing with mice infected by the AV1/AV2 strains showed significant reduction in tumour size while side effects were minimal. Mice were more tolerant to AV1/AV2; the lethal dose increased more than 10 000-times compared to wild-type VSV and multiple doses of AV1/AV2 improved the outcome. Another notable finding was that systemic administration of the virus, rather than direct injection into the site, was equally effective at reducing the size of xenographically-implanted cancers (Stojdl et al., 2003). This highlights the possible systemic use of VSV and capitalizes on the virus's broad tropism. How does all this work prove that VSV can selectively target carcinoma cells? The logic is as follows (Figure 3):

1. Mutant VSV strain (AV1/AV2) can infect any tumour cell type.
2. The infected cell releases IFN since the virus cannot produce the counter-acting M protein.
3. IFN reaches surrounding tissue and cells that can respond to IFN and will mount an antiviral response to prevent viral entry. This ensures that normal cells are protected.

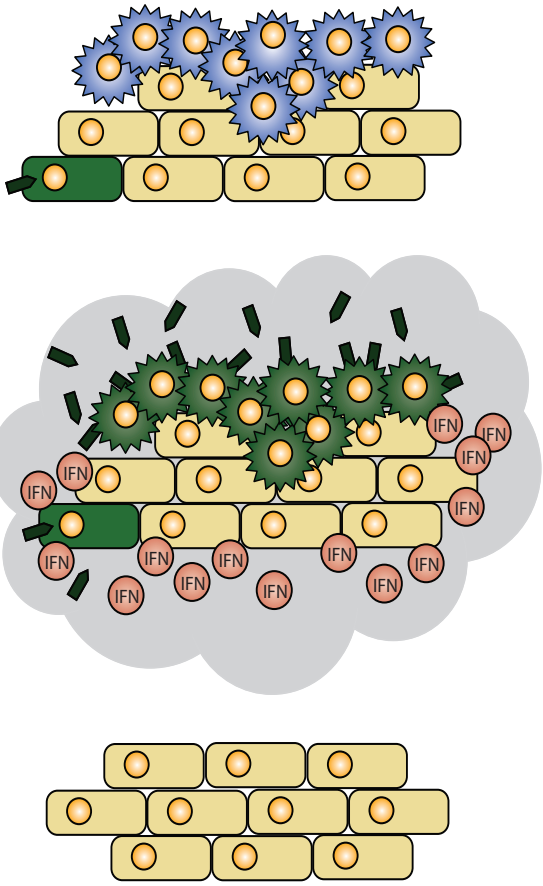

Figure 3 Diagram of VSV infection of tumour cells. Cells that are infected by VSV are depicted in the darkest shade. (A) VSV infects a cell and causes the release of interferon and other cytokines. (B) Normal cells (non-spiked) are protected by interferon but cancer cells (spiked) are susceptible to infection. (C) Malignant cells are lytically removed, leaving behind the normal cells (Lichty et al., 2004).

4. Tumour cells are not sensitive to IFN, therefore no antiviral response is produced and these cells remain vulnerable to infection by VSV.

Although our understanding of VSV is derived from current studies on oncolytic viruses in murine models, preliminary results show that it is a promising and novel approach to overcoming the barriers surrounding oncolytic virus therapy.

Conclusion

The challenge in creating the ideal oncolytic virus lies in finding the proper balance between two competing requirements. On one hand, there is the need to mutate the subset of viral genes that is normally responsible for pathogenesis. Conversely, there is the need for the virus to counteract immune responses in order to persist in the human body long enough to reach the target site and kill tumour cells. The idea of taking a virus and using it to treat cancer still seems abnormal due to the stigma that is associated with viruses. Public concern over the safety of this technology offers one explanation as to why the American FDA has emphasized such unyielding guidelines. This is the first of many roadblocks to come. Whether or not this method of treating cancer will continue to capture the interests of researchers depends on public response and the willingness for continual investigation. As health expenditure continues to increase, Meropol and Schulman (2007) raise an interesting point: how much is too much? Cancer has recently surpassed heart disease as the number one cause of death for Americans under the age of 85 and cancer expenditure totaled $74 billion in 2005 without any signs of slowing down. A dire need exists for a new approach to treating cancer. Oncolytic virus therapy is a promising start for a technology that can potentially save millions of lives and reduce the increasing cost of healthcare. M

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