Future Application of Oncolytic Viruses for Cancer Treatment

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Abstract

Cancer treatments have developed over the years. A particular improvement is the utilization of oncolytic viruses to treat cancers. Oncolytic viruses are one of the immunotherapeutic tools that potentially could provide good results and benefits to the patients. Oncolytic viruses could mediate antitumor effects. Indeed, the connection between viral infections and cancer treatment have been reported historically. It is known that oncolytic viruses prefer to infect cancer cells rather than normal cells, resulting in the presentation of tumor-associated antigens to the immune system, boosting immunological activity in the tumor microenvironment, as well as assisting in the expression of inflammatory and immunomodulatory cytokines. Oncolytic viruses are a novel regimen in the cancer therapy, in which knowledge and technology of utilizing oncolytic viruses to treat cancer are still evolving. Importantly, clinical trials demonstrated that the viruses were well tolerated by cancer patients. Considering its potency and prospect, oncolytic viral treatments could be a useful additional tool for cancer therapy.

Introduction

Cancer is one of the leading causes of mortality worldwide with nearly 10 million deaths in 2020, in which the most common death-causing cancers in 2020 were lung, colon and rectum, liver, stomach and breast cancers.¹,² Multiple treatments have been developed in treating cancer, however its prevalence, morbidity and mortality are still high. The conventional treatments, including surgery, chemotherapy, hormonal therapy and radiotherapy, mostly provide a limited durable effect in patients with advanced cancer. The exception presumably applies for hematological and testicular cancer, in which they can be cured with the current therapies if they are detected at the early stage.³,⁴ Therefore, the cancer treatments are continuously advanced to create a better, more effective regimen in treating cancers. Oncolytic virus is one, arguably, of such innovations. Surprisingly, the concept of oncolytic viruses is not exactly novel in the medical field. There have been numerous case reports, suggesting that there is a connection between infections by microbes and the spontaneous regression of tumor.⁵

The first evidence might be the writing in the Ebers Papyrus around 1550 BC, stating that the Egyptian physicians used poultice followed by incision to induce infection in order to treat tumor.⁶ Another evidence is from the year 1320, when Peregrine Laziozi had suffered from cancer in his tibia, which then needed to be amputated. The cancer had grown through his skin, causing an infection in the area. Something intriguing occurred after the infection, nevertheless, in which the tumor started to disappear and no recurrence observed afterward. The phenomenon was known as ‘St. Peregrine tumor’.⁷ In the 17th and 18th centuries, a
procedure of creating open surgical wounds to allow infections occurred were considered to be useful. Reports had also shown that several leukemia patients became disease-free after viral infections. A female patient with acute leukemia in 1904 and a female patient with cervical cancer in 1912 reported a reduction of tumor proliferation and demonstrated tumor necrosis after viral infection. However, using viruses as a cancer treatment was unheeded. In addition, the very strict regulation in testing and implementing a new treatment’s method have impeded the clinical adoption of this concept. Indeed, it took three decades for this concept to re-emerge with a novel name as ‘oncolytic viruses’.

Oncoytic viruses are viruses that able to infect and lyse tumor cells, naturally or artificially. The aim of the artificial modification is to increase efficacy and safety of using oncoytic viruses. Oncolytic viruses have been suggested to be a novel cancer therapy’s advancement, as they provided a durable and effective responses in the clinical trials. Oncolytic viruses have also shown to be able to stimulate the immune system against tumor cells, which eventually modulate the development of antitumor response. It is postulated that the immune stimulation occurs due to several mechanisms that happen in the tumor microenvironment, which will be subsequently discussed. There have been numerous clinical trials involving oncoytic viruses with different modifications and in combination with other antitumor therapies thus far. The usage of oncoytic viruses is an attractive concept, hence it could explain why there have been more than 100 clinical trials using those viruses. Most of the reported trials were in phase I and II, while some were already in phase III. Taken together, this would be an exciting period to witness whether those findings would support the clinical implementation of using oncoytic viruses to treat cancers.

Oncoytic viruses and cancers

Certain DNA viruses that might have the potential to be oncoytic are adenovirus (family: Adenoviridae), vaccinia virus (family: Poxviridae), herpesvirus (family: Herpesviridae) and parvovirus H1 (family: Parvoviridae). Adenovirus, vaccinia virus and herpesvirus are double-stranded DNA virus, while parvovirus H1 is single-stranded DNA virus. The replication’s site for adenovirus, herpesvirus and parvovirus H1 are in the nucleus and cytoplasm, while vaccinia virus only replicates in the cytoplasm. Unsurprisingly, adenovirus, herpesvirus and parvovirus H1 have the nuclear integration ability, while vaccinia virus does not have it. The cell receptor for adenovirus is coxsackie-adenovirus receptor (CAR); the ones for herpesvirus are herpesvirus entry mediator (HVEM), nectin 1, and nectin 2; while the cell receptor for parvovirus H1 is sialic acid residues. Adenovirus, vaccinia virus and herpesvirus do not show immunogenicity upon re-exposure and penetration across the blood-brain barrier, while parvovirus H1 exhibits the immunogenicity. Table 1 describes properties of the mentioned DNA viruses.

Table 1. Properties of the mentioned DNA viruses (Kaufman et al., 2015).

<table>
<thead>
<tr>
<th>Properties</th>
<th>Adenovirus</th>
<th>Vaccinia virus</th>
<th>Herpesvirus</th>
<th>Parvovirus H1</th>
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<tbody>
<tr>
<td>Baltimore classification</td>
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<td>Group I: dsDNA</td>
<td>Group I: dsDNA</td>
<td>Group I: ssDNA</td>
</tr>
<tr>
<td>Replication site</td>
<td>Nucleus and cytoplasm</td>
<td>Cytoplasm</td>
<td>Nucleus and cytoplasm</td>
<td>Nucleus and cytoplasm</td>
</tr>
<tr>
<td>Cell receptor</td>
<td>CAR</td>
<td>Unknown</td>
<td>HVEM, Nectin 1, Nectin 2</td>
<td>Sialic acid residues</td>
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<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Immunogenicity</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Blood-brain barrier penetration</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>
Several RNA viruses that could be used as oncolytic virus are reovirus (family: Reoviridae), coxsackievirus (family: Picornaviridae), Seneca Valley virus (family: Picornaviridae), poliovirus (family: Picornaviridae), measles virus (family: Paramyxoviridae), Newcastle disease virus (family: Paramyxoviridae) and vesicular stomatitis virus (family: Rhabdoviridae). Reovirus is double-stranded RNA virus. Coxsackievirus, Seneca Valley virus and poliovirus are positive-sense, single-stranded RNA virus. Measles virus, Newcastle disease virus and vesicular stomatitis virus are negative-sense, single-stranded RNA virus. The replication site for those RNA viruses are in the cytoplasm, hence they do not possess the nuclear integration ability. The cell receptors for coxsackievirus are CAR, intercellular adhesion molecule 1 (ICAM-1) and decay accelerating factor (DAF); the one for poliovirus is CD155; the cell receptors for measles virus are signaling lymphocytic activation molecule (SLAM) and CD46; while the one for vesicular stomatitis virus is low-density lipoprotein receptor (LDLR). Reovirus, coxsackievirus, measles virus, Newcastle disease virus and vesicular stomatitis virus do not show immunogenicity upon re-exposure. While Seneca Valley virus exhibits the immunogenicity upon re-exposure, poliovirus might show the immunogenicity. Reovirus, Seneca Valley virus, poliovirus and Newcastle disease could penetrate the blood-brain barrier, while coxsackievirus, measles virus and vesicular stomatitis virus cannot penetrate it. Table 2 describes properties of the mentioned RNA viruses.

<table>
<thead>
<tr>
<th>Properties</th>
<th>Reovirus</th>
<th>Coxsackievirus</th>
<th>Seneca Valley virus</th>
<th>Poliovirus</th>
<th>Measles virus</th>
<th>Newcastle disease virus</th>
<th>Vesicular stomatitis virus</th>
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</thead>
<tbody>
<tr>
<td>Replication site</td>
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<td>Cytoplasm</td>
<td>Cytoplasm</td>
<td>Cytoplasm</td>
<td>Cytoplasm</td>
<td>Cytoplasm</td>
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<tr>
<td>Cell receptor</td>
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<td>CAR/ICAM-1/DAF</td>
<td>Unknown</td>
<td>CD155</td>
<td>SLAM and CD46</td>
<td>Unknown</td>
<td>LDLR</td>
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<tr>
<td>Nuclear integration</td>
<td>-</td>
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Oncolytic viruses indeed could infect neoplastic cells. A neoplasm comprises cells with an abnormal growth’s regulation system, results in cellular abnormalities. Neoplastic cells could expand disproportionately and proliferate in an abnormal way, causing problems to their surroundings. Neoplastic cells could also migrate from their original position via circulatory or lymphatic systems, inducing secondary cancers or metastasis. This characteristic is the hallmark of malignant neoplasms or cancers, in contrast to benign neoplasms that remain to its original location and do not metastases. The metastatic property indeed causes cancers to induce significant morbidity and mortality. In addition, these cancer cells could secrete toxic factors as well, causing systemic illness.
The development of cancer cells is based on the clonality of tumor, i.e., the development from a single cell to proliferate abnormally. For a cell to become cancerous, it must develop a series of alterations. This multistep process involves gene mutations (the driver mutations) to activate oncogenes and to select cells that have the properties as a neoplastic cell. The first step is the tumor initiation, in which an alteration in a single cell causing an abnormal proliferation. The second step is the tumor progression, in which additional mutations lead to more cancerous cells. The third step is the clonal selection, in which several mutated cells having selective advantages would become the dominant cancer cells. In addition, cancer cells do not exhibit density-dependent inhibition and contact inhibition, hence they are able to proliferate continuously, eventually migrating over the underneath cells and forming multilayered patterns of cells. Cancer cells display an autocrine growth stimulation, leading to continuous autostimulation of cell division without depending on growth factors produced by other cells. Cancer cells could also secrete growth factors promoting new blood vessels' formation (i.e., angiogenesis) to supply nutrients and support the metastasis. Cancer cells have a longer life span as well, compared with normal cells, due to the resistance to apoptosis.

There are several major groups of cancer, including carcinomas, sarcomas, leukemias and lymphomas. Carcinomas are malignant neoplasm of the epithelial tissues, comprising approximately 90% of human cancers. Sarcomas are malignant neoplasm of the connective tissues (muscle, bone, cartilage and fibrous tissue) in humans. Leukemias and lymphomas are cancers of white blood cells and cancers of the gland or nodes of the lymphatic system, respectively, comprising approximately 8% of human cancers.

**Mechanism of action of oncolytic viruses in treating cancer cells**

Oncolytic viruses could be administered to the patient via an injection directly to the tumor (intratumoral), subcutaneous, intraperitoneal, intravenous or intratechal (an injection into the spinal canal). After the administration, the viruses would infect cancer cells by targeting the cell receptors to enter the cells. Within the cancer cells, oncolytic viruses started to create their particles using the host’s cell machinery. As major characteristics of cancer cells include immune evasion and abnormal apoptotic regulation, the oncolytic viruses could exploit these properties to obtain an abundant time to complete their life cycle. Furthermore, the innate signaling pathway, including retinoic acid-inducible gene 1 (RIG-1), interferon regulatory factor 7 (IRF-7), interferon regulatory factor 3 (IRF-3) and Janus kinase-signal transducer and activator of transcription (JAK-STAT), of the cells is downregulated, thus minimizing the detection of viral particles by the host’s innate immunity (e.g., Toll-like receptors and RIG-1) as well as suppressing the cellular antiviral pathway’s mechanism. As a result, the production of type-1 interferon (IFN), inflammatory cytokines and protein kinase R (PKR) are downregulated. Of note, functions of type-1 IFN are to promote immune response, to reduce cellular proliferation, and to activate the pro-apoptotic protein p53. In addition, functions of PKR are to inhibit protein translation and to prevent viral particles’ production, which will eventually stop the viral spreading.

The viral replication within the cancer cells would eventually induce cell lysis and cell death, such as apoptosis, pyroptosis and necrosis. The viral infection induces dysfunction of cellular organelles and incites the oxidative stress. The oxidative stress is caused by the production of reactive nitrogen species and by the endoplasmic reticulum stress due to an elevated levels of intracellular calcium. Furthermore, the cell lysis would release new viral progeny to
infect other tumor cells and induce the antitumor immunity systematically by releasing several proteins, such as tumor-associated antigens. The released tumor-associated antigens could activate the adaptive immune response, which results in tumor regression, including cancer cells at distant sites (i.e., metastatic cancer).

Furthermore, pathogen-associated molecular patterns (PAMP), danger-associated molecular patterns (DAMP) and cytokines are released after cell death, promoting the maturation of antigen-presenting cells, such as dendritic cells. The activated dendritic cells would process tumor-associated antigens and present them to activate CD4+ and CD8+ T cells.3,13,20 The activated CD4+ and CD8+ T cells would subsequently recognize and destroy the corresponding neoplastic cells. CD4+ T cells would also stimulate B cells to mature into plasma cells to release specific antibodies. Those specific antibodies would facilitate the antibody-dependent cellular cytotoxicity (ADCC) on tumor cells by natural killer (NK) cells as well as the phagocytosis by M1 macrophages. B cells could also be activated by the interaction between B-cell receptors with the oncolytic viruses. In addition, DAMP could also activate NK cells to kill neoplastic cells that downregulated their major histocompatibility complex (MHC) class I expression. CD8+ T cells will target tumor cells that express MHC class I on the cell surface. After the interaction between T-cell receptor and peptide-MHC class I, CD8+ T cells would be activated and release cytotoxic molecules (such as Granzyme B and Perforin) and IFN-gamma. These concerted actions increase the immunological activity within tumor microenvironment.3,13 The summary of antitumor immunity’s induction by oncolytic viruses could be seen in Figure 1.

![Figure 1. Antitumor immunity of oncolytic Viruses (Hemminki et al., 2020).](image)

The tumor microenvironment of advanced cancers naturally inhibits the antitumor immune response. This activity could be enhanced, nonetheless, following lysis of cancer cells (oncolysis) by oncolytic virus. A. viral progeny, pathogen-associated molecular patterns (PAMPs), danger-associated molecular patterns (DAMPs) and cytokines are released after the oncolysis, which activating dendritic cells (DCs). B. Mature DCs activate CD4+ and CD8+ T cells. C. B-cells activation, through support of CD4+ T cells, would allow plasma cells (not shown) to secrete high-affinity, specific antibodies. D. CD8+ T cells and natural killer (NK) cells would subsequently target and destroy the tumor cells.

Limitation and advancement of using oncolytic viruses to treat cancers

Limitations of using oncolytic viruses for cancer treatment are the safety, efficacy and cancer cell’s susceptibility to cell death (apoptosis, pyroptosis and necrosis). In terms of safety, wild-type oncolytic virus might able to infect healthy cells as well. In terms of efficacy, the viral ability to infect and methods of administration are the challenges. In terms of susceptibility to cell death, the candidate oncolytic virus must be evaluated whether it is effective in inducing lysis of cancer cells (i.e., oncolysis). Therefore, advancement must be conducted on the oncolytic viruses to tackle those limitations.
Many oncolytic viruses have a natural tropism for cancer cell’s surface proteins. For example, while herpesvirus recognizes cancer receptor HVEM and selected nectins, coxsackievirus recognizes ICAM-1 and DAF, as well as poliovirus recognizes CD155 for cell entry. But oncolytic viruses could be engineered to target specific cell receptors, hence increasing their specificity. As an example, the modified adenovirus Ad5/3-Δ24 would bind to integrins that are highly expressed on the surface of ovarian cancer cells. Oncolytic virus could also be engineered to enhance tumor tropism for cancers that have a low receptor’s expression. For example, the adenovirus DNX-2401 showed a durable response in 20% of glioma patients due to the increase in tumor tropism.

Another purpose of the modification is to exploit the cancer property and its molecular mechanisms (such as immune evasion and apoptotic resistance mechanism), to reduce the pathogenicity, to increase the antitumor immunity, to enhance the lytic activity and to reduce the antiviral immune responses. Normal infected cells would activate PKR, which inhibits protein translation, eventually preventing the production of viral particles. In contrast, cancer cells have an abnormal PKR activation. A modified herpesvirus with gene deletion encoding ICP34.5 and US11 preferably would lyse tumor cells than normal cells. The gene deletion results in the viral inability to inhibit the PKR activation, thus it can only replicate well within cancer cells. Next, inserting promoters that are preferentially more active in cancer cells could help oncolytic viruses to exploit the inner mechanism of cancer cells. For example, a modified adenovirus with E1A gene promoter for PSA would facilitate a selective targeting to prostate cancer cells, as normal cells do not express E1A.

Viral genome modification by gene deletion or transgene expression could enhance the antitumor immunity within the tumor microenvironment. The deletion of ICP47 gene in herpesvirus permits transporter associated with antigen processing (TAP) complex to function, thus the infected cells could present antigen to CD8+ T cells. Transgene expression of granulocyte-macrophage colony-stimulating factor (GM-CSF) within genomes of herpesvirus, adenovirus and vaccinia virus could promote the maturation and accumulation of dendritic cells, hence improving the presentation of tumor-associated antigen and the stimulation of T-cell responses. Transgene expression could also enhance the lytic activity through an inclusion of ‘suicide genes’, which expressed by tumor-enriched/tissue-specific promoters. For example, transgene expression of cytosine deaminase (CD) and adenovirus death protein (ADP) would increase the lytic efficiency, in which the CD could convert 5-fluorocytosine into 5-fluorouracil, while the ADP, the nuclear membrane glycoprotein, is used for the efficient cell lysis and the release of viral particles.

While the immune activation would mainly eliminate cancer cells, it could also generate the antiviral immunity to eliminate the oncolytic virus. Prevention of the viral neutralization could increase the administrative efficiency. One strategy is to use alternative viral serotypes to limit viral neutralization. Another strategy is to perform viral coat PEGylation and polymer coating to suppress viral neutralization. Using cells as a carrier, e.g., mesenchymal stem cells, to protect oncolytic viruses had been tested as well. These strategies could circumvent the issue of administration’s efficiency. An intratumoral administration would be more efficient as it is directly administered into the cancer mass, hence minimizing the probability of viral neutralization. However, this method could not be used for inaccessible or multifocal cancers, e.g., pancreatic or brain tumors. In these cases, the systemic administration would be required, as the systemic administration would distribute viruses to the primary and
metastasized cancers. The efficiency could be unsatisfactory, however, as the viruses could be rapidly neutralized before reaching the cancer mass.\textsuperscript{42}

Another advancement is to combine oncolytic viruses with other modes of cancer treatment, such as chemotherapy, radiotherapy, adoptive cell therapy or immune checkpoint inhibitors. The most common combination to date is with immune checkpoint inhibitors. Briefly, immune checkpoint is the negative regulation of the immune response.\textsuperscript{43} Immune checkpoint inhibitors would attenuate the negative regulation, thus activating the immune response. The current popular targets for immune checkpoint inhibition are CTLA4 and PD-1/PDL1.\textsuperscript{13}

Clinical trials of oncolytic viruses for treating cancers

Cook & Chauhan (2020) reported that 86 trials on oncolytic viruses were found in the PubMed clinical trial database.\textsuperscript{12} There were 60 trials in phase I, 5 trials in phase I/II, 19 trials in phase II, as well as 2 trials in phase III. They observed the utilization of different types of oncolytic viruses with various modification and of various types of cancer cells as targets. Different outcomes on patient responses were reported from those trials as well. In general, no severe toxicity was observed during the clinical trials and some trials even demonstrated moderate to high responses for oncolytic viruses, as indicated by tumor necrosis.

Chaurasiya et al. (2021) summarized several trials utilizing different types of viruses.\textsuperscript{44} On each viral category, the authors described the transgene expression, combination with other cancer treatments (conventional and immunotherapy), types of cancers, the clinical trial's phases and their status (recruiting, ongoing, or completed). In general, the treatments were well tolerated at the maximum permitted doses with mild adverse events, such as flu-like syndromes and local reactions (e.g., pain, rash and peripheral edema).

Interestingly, there are several oncolytic viral treatments that have been approved to be used for certain cancer patients. For example, Rigvir\textsuperscript{®}, an oncolytic picornavirus, was approved in 2004 to be used in Latvia for melanoma.\textsuperscript{46} Adenovirus H101 (Oncorine\textsuperscript{®}) has been used in China since 2005 for solid tumors in head and neck, such as nasopharyngeal carcinoma.\textsuperscript{29,46} Herpesvirus, Talimogene laherparepvec or T-vec (Imlygic\textsuperscript{®}), has been approved by FDA and EMA in 2015 for melanoma patients.\textsuperscript{3,47}

Conclusion

Oncolytic viruses have been known for centuries but been only developed in the recent years as one of cancer treatments. The oncolytic viral treatment shows a promising outcome for cancer patients. The oncolytic viral treatment could also be used in a combination with other cancer treatments in order to boost the treatment efficiency. In recent years, advancements and clinical trials using oncolytic viruses for treating various cancers have flourished. The results are expected to support the concept of using oncolytic virus to treat certain cancers.

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