

Clinical challenges and strategies on delivery of Oncolytic Virus

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Abstract. Oncolytic Virus therapy, including HSV-1 and adenovirus, is a new type of tumour immunotherapy that uses specific viruses to directly kill tumour cells or to induce the body's intrinsic and acquired immunity to generate anti-tumour immune responses. In addition, Oncolytic Virus is capable of recruiting immune cells to improve the tumour microenvironment, so the development of The clinical significance of oncolytic virus therapy lies in its potential to enhance the efficacy of other immunotherapeutic approaches. However, the delivery of Oncolytic Virus in current clinical trials has huge defects, which greatly limits the benefits of Oncolytic Virus and even produces certain side effects. Therefore, this review discusses the definition and mechanism of Oncolytic Virus and focuses on the defects in Oncolytic Virus delivery, such as systemic delivery and intratumoural delivery. Further, this review also summarizes some improvement strategies against the challenges of intratumoural heterogeneity and vascular complexity.

Keywords: Oncolytic Virus; Systematic delivery; Viral vector.

1. Introduction

Cancer has always been one of the most deadly and difficult to treat diseases in the world. For this reason, scientists have researched a variety of treatment modalities for tumours. In the past decades, chemotherapy and radiotherapy have been the mainstream ways to treat tumours, but since these two treatments are not specific, they tend to kill other normal and healthy cells indiscriminately, producing great irreversible side effects and even the opposite treatment effect. Over the last several years, notable advancements have been made in the fields of molecular biology and cancer immunology, resulting in the development of a therapeutic strategy known as cancer immunotherapy. The therapeutic modality under consideration entails the modification of the body's immune system with the purpose of recognising and combating cancer cells. Notable examples of cancer immunotherapy, including immune checkpoint blockers, adoptive cell therapy, and Oncolytic Virus therapy. The ability of immunotherapy to specifically attack tumour cells makes the treatment process more effective and safer than chemotherapy and radiation. The ability of immunotherapy to specifically attack tumour cells makes the treatment process more effective and safer than chemotherapy and radiation. Among them, Oncolytic Virus therapy has aroused great interest among researchers for its unique treatment modality - using viruses to attack tumour cells - and remarkable therapeutic effects. In fact, Oncolytic Virus is not only effective in directly killing tumour cells, but also in recruiting immune cells to create a favorable tumour microenvironment for other immunotherapies. However, one significant challenge encountered in the field of Oncolytic Virus therapy pertains to the existing deficient delivery method, which imposes constraints on the therapeutic effectiveness of Oncolytic Virus. This review aims to analyze the deficiencies observed in the delivery of Oncolytic Virus and propose potential options for enhancement.

2. Definition of Oncolytic Virus

Oncolytic virus is a new type of cancer immunotherapy drug. It can take advantage of the activation of oncogenes and suppression of oncogenes in cancer cells, preferentially replicate and spread in cancer cells without infecting normal cells (**Figure 1**): When normal cells are infected with an Oncolytic Virus, the viral PAMPs stimulate the cellular TLRs, which in turn initiate signalling

pathways to trigger the synthesis of type I IFNs via the NF- κ B. RIG-1 recognises the virus's dsRNA upon entrance into the cell, and this, together with signals from TLR activation, stimulates the creation of type I IFNs. Through the JAK-STAT signalling pathway, type I IFNs cause an increase in the expression of cell-cycle regulators, which in turn triggers the activation of type I IFN transcriptional networks. This activation subsequently facilitates the death of infected cells, thereby restricting viral replication and impeding the intracellular propagation of the virus. In contrast, activation of oncogenes and inhibition of oncogenes in cancer cells downregulate RIG-1, limiting virus detection by cancer cells. Furthermore, in order to prevent apoptosis, cancer cells down-regulate essential elements of the type I IFN signalling pathway, which creates an environment that is conducive to viral replication in cancer cells [1]. Therefore, a large number of different types of DNA and RNA viruses have been selected for preclinical studies as candidates for oncolytic viruses. Actually, there is no standard method for Oncolytic Virus selection in research. Because some viruses exhibit preferential replication in tumours cells while others demonstrate improved replication in tumour cells after genetic modification. Currently, the most common viruses studied are Herpesvirus, Adenovirus, Vaccinia Virus, and many more.

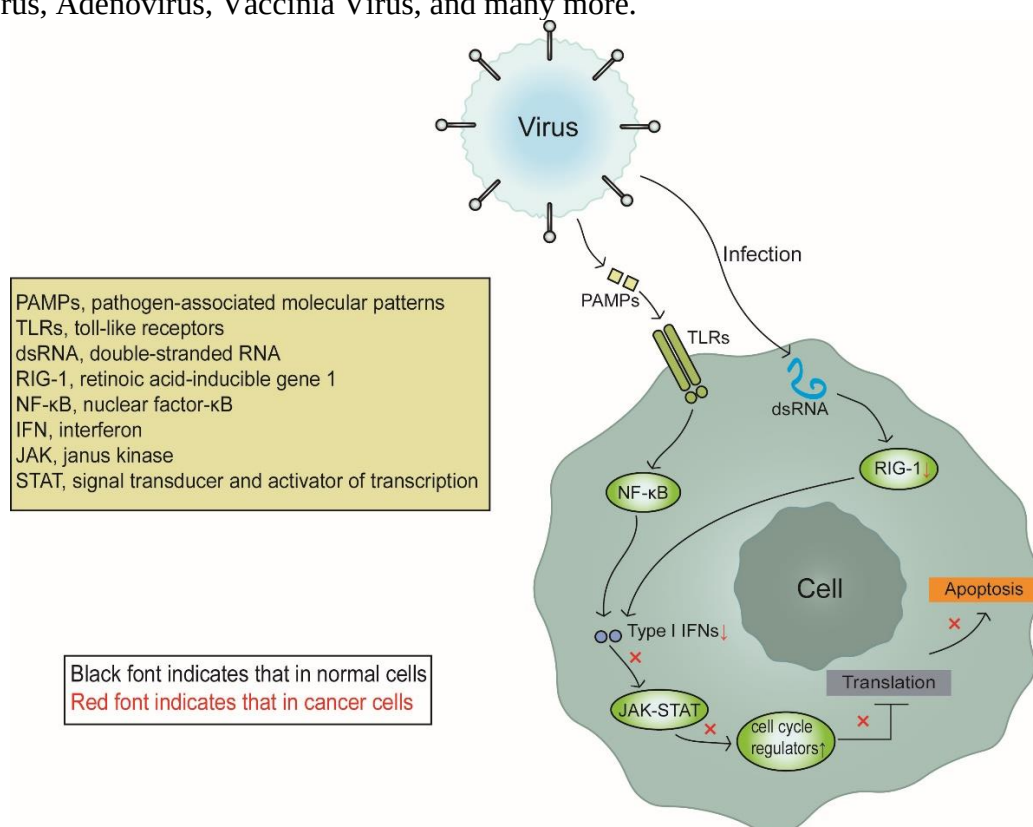


Fig. 1 Mechanism of Oncolytic Virus's preferential infection

3. Cilinical Application

3.1. G47 Δ

The strain of HSV-1, referred to as G47 Δ , has been subject to genetic alterations that have led to the elimination of the ICP34.5 genes and ICP47 genes. This genetic alteration aims to enhance the virus's ability to replicate and disseminate, while also augmenting its anti-tumour properties. The therapeutic efficacy of G47 Δ is significant in the treatment of glioblastomas characterized by a poor prognosis. Data from sixteen clinical trials examining the effectiveness of various chemotherapeutic drugs in the treatment of glioblastoma were analysed for this research. According to the data, the median OS of the study patients was 5.0 months and the PFS was 1.8 months. However, 84.2% of patients who got G47 Δ survived after a year. Furthermore, the median OS and median PFS for such

individuals were 20.2 and 4.7 months, respectively [2]. According to this research, patients' prognoses are significantly improved by G47 Δ intervention.

3.2. T-VEC

T-VEC is a novel therapeutic agent derived from the HSV-1. It exerts its therapeutic effects by deleting two specific genes, ICP34.5 and ICP47, which are associated with the pathogenicity of neuroviruses. Additionally, T-VEC incorporates GM-CSF gene, which serves to enhance the local production of GM-CSF. Consequently, this makes it easier for antigen-presenting cells to be drawn into the cancer microenvironment. T-VEC is mostly used to treat primary cutaneous B-cell lymphoma and melanoma. GM-CSF is frequently employed as a therapeutic intervention for the management of melanoma. Nevertheless, when considering the management of stage IIIB-IVM1a melanoma, it was observed that individuals who received T-VEC exhibited a median OS of 19.5-29.6 months, surpassing the median OS of 16.0-23.7 months observed in patients treated with GM-CSF [3]. This finding provides evidence that T-VEC has the potential to extend the overall survival duration for patients.

3.3. H101

H101, an adenovirus with deleted E1B and E3 genes. This virus can replicate and spread by targeting tumour cells with dysfunctional RB/P53 pathways. It was among the earliest medications available for the treatment of cancerous tumours. Presently, H101 is predominantly employed in clinical settings in conjunction with chemotherapy for the purpose of managing individuals afflicted with HNSCC and NPC. Its main purpose is to make cancer cells more vulnerable to the effects of chemotherapy. When compared to the use of chemotherapeutic drugs alone, the H101-Chemotherapy combination treatment significantly boosted the response rate in a clinical study including patients with advanced HNSCC. Out of the 123 patients who were eligible for participation in the clinical trial, the overall response rate in the group receiving cisplatin plus 5-fluorouracil (PF) plus H101 was found to be 78.8% (41 out of 52 patients), which exceeded the overall response rate observed in the group receiving PF alone, which was 39.6% (21 out of 53 patients) [4].

4. Clinical challenges on Oncolytic Virus delivery

Depending on the nature of virus and tumour, there are several methods to administer oncolytic virus; nevertheless, the effectiveness of oncolytic virus cancer immunotherapy depends on choosing the right route. The research did discover, however, that various therapeutic techniques of oncolytic virus delivery faced various difficulties.

4.1. Systematic delivery

Systemic delivery involves the intravenous delivery of Oncolytic Virus into the organism. The treatment option provides the potential to address primary tumours as well as metastatic deposits that are difficult to detect. This offers a promising therapeutic outlook for patients with advanced metastatic tumours and untreatable diseases that are constrained by the blood-brain barrier. Furthermore, the systemic delivery of this treatment has demonstrated notable effectiveness in inhibiting tumour growth and ensuring patient safety, as evidenced by a phase I clinical trial [5]. The vasculature of the human body is intricate and interconnected, which leads to a delayed transit of Oncolytic Virus towards the tumour. Additionally, the entry of Oncolytic Virus into tumour cells is hindered by vascular abnormalities within the tumour caused by tumour formation. These anomalies include the appearance of fenestrations of about 50 to 80 nanometers [6]. Furthermore, the fast elimination of Oncolytic Virus from the bloodstream occurs as a result of the abundant presence of antibodies, complement, and antiviral cytokines in the blood, as well as non-specific absorption by many organs including the lungs, liver, spleen, and tissue-resident macrophages [7]. The Oncolytic Virus exhibits suboptimal enrichment within the tumour location over time.

4.2. Intratumoural delivery

Intratumoural injection is currently the most common clinical delivery method, which can precisely control the enrichment site of Oncolytic Virus and rapidly produce anti-tumour response. Therefore, a variety of viruses, such as HSV-1 and T-VEC, have been treated with intratumoural injection for diseases such as melanoma. However, intratumoural tissues are complex and have a certain internal pressure, resulting in difficulty in injecting Oncolytic Virus into the tumour, and the procedure of single intratumoural delivery is complicated and costly, which is not suitable for long-term repeated delivery. Furthermore, the delivery of intratumour injections may prove to be unattainable in certain exceptional scenarios, such as instances when cancerous lesions are located within deep organs or when malignant tumors exhibit extensive diffusion. The reasons for this are that it is difficult for the needle to reach the deeper organs and there is a high risk of harming the surrounding normal tissues when injecting; intratumoural delivery is localized and it can only clear a very small percentage of diffuse cancer cells. In addition, Oncolytic viruses are impeded during intratumoural drug delivery. Tumours have more extracellular matrix compared to normal cells, which results in tougher tumour tissue and increased interstitial tumour pressure, preventing effective spread and penetration of oncolytic viruses within the tumour [8]; A significant proportion of solid tumor cells are linked together through cellular adhesion sites, specifically tight junctions. These tight junctions effectively seal the gaps between adjacent cells, creating a barrier that limits the movement of medications with molecular weights above 400 Da. [8]. Thus, intratumoural injections of oncolytic viruses, especially adenoviruses, are likely to fail to affect the majority of tumour cells and have very limited spread.

4.3. Other delivery

In addition to the aforementioned two prevalent forms of oncolytic virus delivery, researchers have explored other delivery strategies tailored to specific tumour types and viral agents. Clinical trials have provided evidence that pancreatic cancer can be treated through the intraperitoneal injection of low-dose CF33 virus. Additionally, advanced cancer can be addressed by employing the subcutaneous injection of ONCOS-102 virus. Furthermore, oncogenic meningitis in patients with glioblastoma multiforme can be treated by administering PVS-RIPO virus therapy through intrathecal means [9]. **Table 1** summarizes the current clinical applications and advantages and disadvantages of these delivery modalities.

Table 1. Other routes of delivery

	Intraperitoneal delivery	Subcutaneous delivery	Intrathecal delivery
Clinical applications	Angiosarcoma Gastrointestinal stromal tumour Epithelioid sarcoma Leiomyosarcoma Liposarcoma Kaposi's sarcoma [9]	Melanoma; Soft tissuesarcoma [9]	Glioma Ependymoma Primitive neuroectodermal tumour Central nervous system lymphoma [9]
Advantages	Faster absorption than subcutaneous delivery Relatively easy operation with few specialized skills	Relatively easy operation	Ideal delivery for central nervous system tumours
Disadvantages	Slower absorption than intravenous delivery	Only for small animals where veins are difficult to find	Only for central nervous system tumours

5. Strategies

Given the different deficiencies in different modes of Oncolytic Virus delivery, researchers have developed, or are developing, improved delivery modes that address the different deficiencies.

5.1. Facilitating adenovirus translocation within tumours after intratumoural injection

Clinical findings suggest that intratumoural injections of adenovirus have minimal effect on tumours and that the spread of Oncolytic Virus is very limited. Therefore, researchers have proposed some strategies to ameliorate this phenomenon. Tumour cells have greater interstitial fluid pressure compared to normal cells, which interferes with efficient adenovirus propagation within the tumour. Given this characteristic of tumours, researchers have incorporated extracellular enzymes which can cause the degradation of matrix, such as hyaluronidase, desmoteplase, relaxin, and matrix metalloproteinases, among others, into adenoviral expression cassettes to reduce extracellular matrix and interstitial fluid pressure in tumours, creating more space for adenovirus propagation [8]. In a set of experiments with mouse tumour models, mice were injected with AdwtRGD adenovirus and AdwtRGD adenovirus with the addition of PH20 which is a hyaluronan hydrolase (AdwtRGD-PH20), respectively. Twenty-seven days after delivery, it was found that AdwtRGD-PH20-treated tumours showed 10%-70% regression, while no regression was observed in AdwtRGD-treated tumours [10]. At the same time, the researchers found that intercellular connections, especially the transient opening of tight junction structures, enhance adenovirus penetration in tumours. Moreover, the interaction between PtDd and desmoglein-2 formed during infection could promote the opening of tight junctions. Therefore, researchers have developed a novel recombinant protein named Junction Opener-1 (JO-1), including structural domains derived from PtDd. The combination of JO-1 and adenovirus enhanced the intratumoural dissemination of the virus. The study revealed that the injection of Ad3 mutant adenovirus into the tumours of mice with tumours, either individually or in conjunction with JO, resulted in a much greater tumour-delaying impact compared to the delivery of Ad3 mutant alone [8]. Furthermore, the researchers accelerated the dispersion of the viral particles within the tumour and mitigated the infection of healthy cells in the vicinity of the tumour by promoting the production of fusion proteins in the adenovirus. At now, scholars have opted to utilise the fusion protein of the measles virus, specifically the C-terminal shortened variant of the Gibson ape leukemia virus envelope glycoprotein, together with several Fusion-Associated Small Transmembrane proteins, as the target proteins for induced expression in adenovirus. Research findings have indicated that the utilisation of adenoviral vectors that produce hemagglutinin (H)/fusion proteins (F) in conjunction with adenoviral vectors that express various cytokines can effectively augment the immune response directed against targeted tumour cells. The observed combinations yielded a substantial decrease in tumour size, ranging from 87% to 98%. In comparison, the single use case resulted in reductions of 51% and 10% to 47%, respectively [8].

5.2. Utilising Carriers to Deliver Oncolytic Virus

Direct intravenous injection of Oncolytic Virus can easily lead to rapid removal of the virus in the blood vessels. Therefore, scientists have invented a number of viral vectors to help transport Oncolytic Virus in the bloodstream. These viral vectors can be natural cells, bioengineered cells or abiotic vectors. In terms of natural cells, researchers have primarily used mesenchymal stem cells, monocytes/macrophages and natural killer cells as Oncolytic Virus vectors. These vector cells attach Oncolytic Virus to cell membranes, which not only enhances the tumour tropism of Oncolytic Virus, but also protects Oncolytic Virus from neutralization by antibodies during blood transport. Among them, T-lymphocytes, although less frequently used as carriers of Oncolytic Virus, cytotoxic T-cells can synergize with Oncolytic Virus to achieve anti-tumour effects. Compared to natural cell carriers, bioengineered cells not only have similar functions but also extracellular vesicle mimetics (EVM) are more readily available. In addition, nanocarriers constructed using bioengineered donor cells can also protect oncolytic viruses from antibody neutralization, and adenoviruses encapsulated in

nanoparticles can also promote anti-tumour effects in adenoviral HepG2 and Huh7 cells (hepatocellular carcinoma cell lines) [7]. The abiotic carriers can be categorised into two distinct groups, namely polymers and biomineralization. Polymers, such as PEG, possess the ability to bind to the surface of adenoviruses through electrostatic interactions. This association can enhance the selectivity of Oncolytic Virus and hinder its deactivation. Biomineralization, exemplified by PLC-ADs, refers to the process of incorporating calcium phosphate co-precipitated with ZD55-IL-24 into lipidized and PEGylated structures. This technique effectively extends the duration of circulation of Oncolytic Virus inside the bloodstream, while also exhibiting an anti-neutralizing impact and demonstrating non-cytotoxic properties [7]. The study revealed that the utilisation of cellular vectors for delivering Oncolytic Virus not only does not compromise the virus's infection efficiency, but significantly enhances its infectivity. The Ad5-GFP adenovirus, which was encapsulated with EVM and infected with VSV-G protein, did not exhibit a reduced infection rate in spite of the existence of anti-Ad5 antibody in the performed trials. It was discovered that the encapsulated adenovirus had an infection effectiveness of 80.30–87.04%. On the other hand, unencapsulated Ad5-GFP's infection efficiency dropped dramatically to 3.56–7.66%. Furthermore, when the virus was exposed to tumour cell lines expressing low levels of adenoviral receptors, the infection efficiency of the encapsulated adenovirus was considerably higher compared to that of naked Ad5-GFP. The increase in infection efficiency ranged from a minimum of 2.83 ± 0.04 -fold to a maximum of 14.99 ± 1.09 -fold [11].

5.3. Loading Oncolytic Virus onto CAR-T cells

It was demonstrated that CD8⁺ T cells can not only serve as cellular carriers for Oncolytic Virus, but CAR-T cells can also piggyback on Oncolytic Virus for co-delivery and optimize the generation of DS CAR-T cells. Oncolytic Virus piggybacked with CAR-T cells showed higher titers at the tumour compared to intravenous injection alone, and the recovery of CAR-T cells from lymph nodes was increased after preloading with oncolytic viruses in vitro. In addition, DS CAR-T cells equipped with Oncolytic Virus have a more potent function compared to single CAR-T therapies because Oncolytic The virus helps CAR-T cells produce an abundance of cytokines, such IFN γ , which enhances the anti-cancer activities of CAR-T cells. Experimental evidence of improved viral delivery to lymph nodes and tumours was demonstrated by higher VSV viral titers detected in vivo two days after delivery of CAR-T extracellularly loaded viruses and in subcutaneous tumours compared to intravenous injection alone[12].

6. Summary

Clinically, there is a wide variety of Oncolytic Viruses under research experimentation, which offers the possibility of specific treatment for different kinds of tumours. However, this diversity also poses significant challenges for the delivery of different species of Oncolytic Virus due to the complexity of the human vasculature and the heterogeneity of tumours. This paper summarizes the shortcomings of the different delivery modalities currently available in the clinic, and although the data are not exhaustive, it provides a snapshot of the types of Oncolytic Viruses used in the clinic and the associated state of research on delivery modalities. At the same time, the paper summarizes some targeted strategies for improvement. Overall, however, the current research on Oncolytic Viruses and their delivery modalities is far from adequate. More studies of molecules within Oncolytic Virus and related signaling pathways are needed to better determine the causes of activation and inactivation of Oncolytic Virus during delivery, and more standardized definitions of viral distributions and appropriate biomarkers are needed for clinical studies to provide information about antiviral and antitumour immune responses during delivery. In addition, since Oncolytic Virus is self-toxic, there is a greater need for scientists to rigorously establish appropriate clinical trial design and pharmacological testing and to strictly regulate the production pathways of Oncolytic Virus in order to avoid irreversible toxicity to patients. It is also important to carefully select Oncolytic Virus candidates for treatment based on the patient's medical history and autoimmunity.

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