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Strategies and Innovations in Oncolytic Virus Delivery

Mingyue Zhou

Faculty of Health Science, The National University of Malaysia, Kuala Lumpur, Malaysia
zhou.mingyue316@gmail.com

Abstract. Oncolytic viruses (OVs) are a promising therapy in cancer treatment, thanks to their ability to selectively replicate in cancerous cells, induce apoptosis and trigger immune responses. Despite the therapeutic potential of OVs, the clinical application of oncolytic virotherapy (OVT) is very limited, primarily because of the challenges in achieving safe and efficient drug delivery. Conventional intratumoral (IT) injection can establish high local viral concentrations but is restricted to accessible tumors and suffers from inadequate IT distribution. Meanwhile, systemic delivery enables reach of metastatic and deep-seated malignancies but is hindered by immune clearance, nonspecific organ sequestration, and inefficient tumor targeting. This review paper summarizes the current OV delivery strategies, which are the clinical IT injection and preclinical systemic injection, and evaluates the advantages and limitations of these approaches. In addition, novel delivery strategies, such as platelet-membrane coatings and micro-/nanorobots, are discussed. Collectively, the continued advancement of OV delivery techniques is essential for promoting therapeutic efficacy and expanding clinical applicability of OVT.

Keywords: oncolytic virus, virotherapy, drug delivery

1. Introduction

With the emergence of various novel bioengineering methods, the twenty-first century has witnessed the rapid evolution of cancer therapies, one of which is OVs. OVs are natural or bioengineered viruses, which preferentially replicate in cancer cells, and simultaneously spare healthy cells [1]. As a promising therapy for cancer, OVT has dual benefits - its selective replication in cancer cells is able to lead to direct oncolysis, while its ability to induce immunogenic cell death (ICD), a particular form of apoptosis, can amplify its therapeutic performance [1]. In ICD, the apoptosis leads to the release of damage-associated molecular patterns, specifically calreticulin, ATP, and HMGB1, which can be recognized by the antigen-presenting cells in the tumor microenvironment (TME) and subsequently trigger the immune response [2]. Moreover, OVT can be applied in parallel to other routine cancer treatments such as radiotherapy and chemotherapy. Combined with other advanced cancer therapies, chimeric antigen receptor T cells (CAR-T cells) and immune checkpoint inhibitor (ICI) therapy, for instance, OVT may generate enhanced therapeutic effects [3].

Despite these advantages, obstacles in OV application cannot be simply neglected. Since the first OVT, Oncorine (H101) for nasopharyngeal carcinoma, was approved in China in 2005, only a few others have gained regulatory approvals in different countries and areas. Additionally, the delivery of

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these drugs is mainly restricted to IT injection. Even though direct IT administration can yield a higher therapeutic index, it also sets many limitations to its practices clinically [4]. Firstly, IT injection remains uncommon and lacks standardized procedures. Secondly, the feasibility of IT injection primarily depends on the location of the tumors. For example, IT injection is not feasible for liquid tumors or advanced metastatic tumors. Thirdly, the unique physical characteristics of solid tumors, such as dense tumor extracellular matrix (ECM) and elevated tumor interstitial fluid pressure (TIFP), set hurdles that limit the OV penetration [5, 6]. Systemic delivery strategies are needed aside from IT injection for optimal therapeutic effects across diverse cancer types. Intravenous (IV) injection appears an attractive delivery strategy due to its ease of administration, simple dosing, and natural systemic distribution [7]. Nonetheless, IV of naked OVs is also not practical due to rapid clearance by the immune system, development of neutralizing antibodies, poor tumor accumulation, and possibly systemic toxicity [7]. Therefore, safer and more efficient delivery strategies for both IT and systemic administration of OVs are still a landscape waiting to be expanded. Currently, nanoparticles (NPs), cell-based carriers, and engineering of viral capsids are some delivery strategies studied and examined to overcome the hurdles in the delivery of OVs [7].

This review paper summarizes the advantages and disadvantages of clinical IT injection, obstacles in the development of systemic delivery methods, several promising preclinical delivery strategies and advanced techniques that might facilitate better OV delivery in the future.

2. Intratumoral injection

The most used and oldest way of OV administration is through IT injection [4]. The technique is associated with direct delivery of viral particles to the tumor tissue and eliminates multiple physiological obstacles in systemic delivery, such as hepatic clearance, complement activation, and the existence of neutralizing antibodies [4]. The rationale for choosing IT administration is straightforward. By ensuring the virus immediate access to the target lesions, viral replication, oncolysis, and subsequent antitumor immune response can be optimized and off-target effects can be reduced [8]. This technique has found wide application in preclinical research and clinical practice, particularly in clinical trials in which convenient solid tumors are studied [4]. In particular, the only FDA-approved OV, Talimogene laherparepvec (T-VEC) for advanced melanoma treatment, is primarily administered via IT injection [4].

The major benefit of IT delivery is that it can offer high local viral concentrations. Direct IT injection enables a sufficient amount of viral load to arrive at the tumor site, thereby facilitating effective replication in the malignant cells. IT delivery also bypasses numerous barriers to directly penetrate the TME, including hepatic clearance and complement activation, and neutralizing antibodies. Therefore, systemic toxicity is diminished, and a more localized therapeutic effect can be achieved. Furthermore, this method provides clinicians with a high level of control, as using ultrasound, fluoroscopy, CT scanning, and others, one will be able to precisely target the dose and site of injections [4].

Despite such benefits, IT injection has a number of limitations that prohibit it to a wider clinical use. Only superficial tumors or lesions that can be accessed using image guidance can be treated with IT injection. Tumors that are deep seated or in areas that are anatomically sensitive are again difficult or unsafe to inject. Therefore, IT delivery is not a sufficient option to address patients with metastatic diseases, as the uninfected lesions might not be exposed to the virus sufficiently. Another significant difficulty is the constrained IT spread of OVs mainly due to the solid tumor physical barriers. A tumor develops dense ECM as cancer cells and associated fibroblasts remodel it by depositing excess, highly cross-linked collagen and other proteins, creating a stiff, fibrous scaffold

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that promotes tumor growth, invasion, and metastasis by acting as a physical barrier [5]. This barrier also distorts cell signaling, and establishes hypoxic and nutrient-poor microenvironment, both of which shield tumors from immune attack and drugs [5]. In the meantime, the disorganized and leaky blood vessels in the tumor drain slower than leakage, along with poor lymphatic drainage, and the rigid ECM that prevents fluid movement, creating elevated TIFP that traps fluid in TME and affects drug delivery [6]. The dense ECM structures, increased TIFP and the highly dissimilar architecture are obstacles to uniform distribution of viral particles [8]. As such, specific tumor regions might not be infected, thus resulting in suboptimal therapeutic outcomes.

Immune-mediated clearance is also a limitation to viral persistence after IT administration. Innate immune cells infiltrate the injection site rapidly and may destroy viral particles before extensive replication occurs [2]. This problem can be partially reduced by repeated injections. Nevertheless, they can also cause local inflammation and discomfort to patients. Technical factors such as needle selection, errors in needle placement, variation in injection depth, and potential needle-track leakage may further contribute to inconsistent therapeutic responses [3].

To enhance the effectiveness of IT delivery, a few strategies have been explored. Including preclinical testing of viruses engineered to express ECM-degrading enzymes such as hyaluronidase, and device-assisted approaches like electroporation or convection-enhanced delivery to enhance viral distribution [9]. The use of multi-site or repeated injections is common in clinic to enhance tumor coverage. Potentials have also been shown through combination therapies, especially IT OVs administered with ICIs, CAR-T cells or radiotherapy to boost both local and systemic antitumor immunity [10].

In brief, IT injection will continue to be a cornerstone in OVT with its safety, practicability, and the capacity to generate impactful local responses. Nevertheless, it has a restricted applicability to accessible tumors and poor viral distribution, which underlines the significance of alternative strategies. Such restrictions have prompted an increasing focus on systemic delivery approaches which are designed to treat disseminated disease more effectively.

3. Systemic delivery

Systemic administration represents a promising delivery route for OVT, particularly for treating metastatic or deep-seated tumors that are inaccessible to direct IT injection. In contrast to IT delivery, which restricts viral spread to the injected lesions, IV administration distributes the virus throughout the circulation, thereby enabling the targeting of both primary and metastatic tumors while bypassing the dense tumor ECM [7]. Moreover, IV administration procedures do not need the high level of injection technique from the healthcare professionals as compared to IT injection [7]. This broad therapeutic reach makes systemic delivery a critical component of the development of clinically applicable OV modalities. However, systemic administration exposes OVs to numerous immunological and physiological hurdles that significantly restrict their ability to reach and replicate within tumor tissues, namely the innate and adaptive immune responses, the sequestration of OVs by non-target tissues and the physical barriers of tumors.

A major barrier to successful systemic delivery is the high rate at which viral particles are eliminated by the innate immune mechanisms. After IV injection, the viruses are immediately exposed to complement proteins, natural antibodies and serum opsonin. All these components can neutralize or lyse OVs before they reach the actual tumor lesions [2]. Moreover, reticuloendothelial system (RES) phagocytic cells, especially Kupffer cells in liver and macrophages in the spleen and the lungs, are very competent in trapping viral particles released to circulation. This innate immune

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surveillance significantly reduces the viral load delivered to TME, thereby reducing treatment effectiveness.

Besides the innate immunity, adaptive immune responses also pose major problems on systemic delivery of OV. There are numerous clinical and preclinical OVs that are linked to large proportions of pre-existing immunity in humans, for example, adenovirus, measles virus, herpes simplex virus, and reovirus [2]. Neutralizing antibodies can rapidly bind viral capsids after IV injection, and therefore prevent successful infection of tumor cells. Even in cases where pre-existing immunity is low, the first systemic dose of an OV typically induces a strong humoral response that renders subsequent doses markedly less effective [2]. This effect complicates rationale behind repeated doses and makes it necessary to establish methods to protect the viral particles against humoral immunity, especially the effects of antibody-mediated neutralization [11].

Other than immune barriers, physiological obstacles also limit the physiological ability of systemically administered OVs to travel and proliferate within tumors. Most of the circulating virions are sequestered by non-target tissues, reducing the fraction that can reach the tumor site while causing severe toxicity and side effects. Tumor vasculature also poses barriers to effective systemic delivery. Although tumors often exhibit abnormal, leaky vessels, extravasation of viral particles remains inefficient due to elevated TIFP and a dense ECM [8]. Therefore, poor penetration of a virus into the tumor core may also diminish the therapeutic effect, even in case a virus manages to exit the circulation.

In an effort to address such challenges, different systemic delivery strategies have been developed. One of the key categories involves the use of NPs to protect viral particles against immune detection and increases the time of circulation. Depending on natural extracellular vesicles, biomimetic nanovesicles or inorganic polymers, NP coatings can protect OVs effectively and reduce recognition by complementation proteins and neutralizing antibodies [2, 7]. Hybrid nanovesicles, such as magnetic nanovesicles, have also been investigated to increase the targeting accuracy [12]. The strategies not only have the effect of shielding the virus but can also enhance tumor tropism by exploiting the increased permeability and retention (EPR) effect to enhance the accumulation of the tumor. However, EPR effect model is restricted in a certain way. The model has been only established in mouse models whose tumors have a leaky and immature vasculature when compared to human beings, and therefore makes the preclinical promise unreliable [13].

Cell carrier systems constitute another widely studied strategy for systemic delivery. Some of cancer cell lines, immune cells, neural stem cells, and mesenchymal stem cells (MSCs) can serve as cargos to package the viral particles and deliver them to tumor locations whilst avoiding clearance by the immune system [14]. MSCs are especially attractive carriers due to their natural ability to migrate toward hypoxic or inflamed regions, which are typical characteristics of solid tumors [14]. Besides, autologous T cells, monocytes, and macrophages have been developed to deliver OVs to tumor by utilizing their intrinsic migration mechanisms [14]. These cell-based strategies not only prevent viral particles from neutralization, but also facilitate the process of viral entry through cell fusion mechanisms once the carriers infiltrate the TME. Despite these benefits, inadequate tumor targeting, inefficiency of immune evasion by the virus, low release of the virus by cells, potential side effects of the carrier, and variable infection in the carrier cells remain some of the limiting factors to the use of cell carrier systems.

Genetic and capsid engineering approaches have also been employed to enhance systemic delivery. Modifications to viral surface proteins may lead to lower recognition by neutralizing antibodies, redirect viral tropism to tumor-specific receptors or enhance attachment of viral particles to coating materials. As an example, adenoviruses modified with the RGD can bind to integrins that

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are expressed in abundance by tumor vasculature and enhance tumor localization [15]. Additionally, genetically engineered albumin is able to interact with the surface of the adenovirus, enabling this plentiful protein within the plasma to act as a protein carrier and protect against immune recognition [16].

Combination therapies may also be used to facilitate systemic OV delivery. I Immune-modulating agents, including ICIs and transient immunosuppressants like cyclophosphamide, have been shown to boost viral persistence and reduce antibody-mediated clearance. Radiotherapy or vascular-modifying agents can also enhance extravasation as they elevate tumor vascular permeability which helps in viral infiltration [10].

Clinical studies of systemic OV administration have demonstrated that viruses can reach metastatic lesions and induce antitumor responses, although efficacy remains limited primarily by rapid immune clearance [3]. Reovirus, vaccinia virus and adenovirus preclinical trials demonstrate acceptable safety profiles, but the therapeutic effect stress the need for better delivery modalities. Thus, while systemic administration is a non-invasive approach to treat metastatic disease, its efficiency depends entirely on the design of improved engineering strategies.

In conclusion, OVT can be more therapeutically extended by systemic delivery. In spite of the biological and immunological obstacles, new delivery approaches including NPs, cell carriers, and engineered viral capsids, offer promise of optimal systemic delivery. Systemic delivery innovation in the future is significant to realize the full potential of OVs in advanced and disseminated malignancy treatment.

4. Novel delivery techniques

Recent advances in bioengineering and micro-robotics have promoted the development of novel delivery strategies of OVs, which aim at overcoming the major limitations of conventional IT and systemic administration. Platelet membrane-based coatings and micro-/nanorobotic delivery systems have emerged as two of the promising approaches, standing out with their unique features, which are increased immune evasion, active targeting, and controlled delivery of viral loads.

Platelet membrane-coated delivery systems constitute a biomimetic strategy that makes use of the intrinsic biological functions of platelets to enhance OV delivery. Many physiological features of platelets can benefit OV delivery. Firstly, platelets have prolonged circulation time and intrinsic immune evasion capability. By coating OVs or OV-loaded NPs with platelet membranes, viral particles can be protected from complement activation and neutralizing antibodies [17]. At the same time, there is less uptake of OVs by the RES. Secondly, platelets are characterized by an inherent tendency to accumulate during the location of the vascular injury and inflammation. This natural tumor-homing property increases tumor targeting while reducing systemic toxicity. Thirdly, platelet membranes contain surface adhesion molecules hence the interaction with tumor cells and activated endothelium. Recent preclinical studies have proven that platelet membrane-coated platforms can significantly enhance the tumor accumulation and therapeutic efficacy compared to viral formulations that are not platelet coated [17]. Even though OV delivery by platelet membranes is largely pre-clinical, its good immune-shielding property and biocompatibility make it worthy of application.

Micro- and nanorobots are a new frontier to active guided drug delivery. They can be programmed to navigate complex biological conditions with external stimuli, such as magnetic, acoustic, and chemical gradient and facilitate accurate spatial delivery of therapeutic agents [18]. Recent research investigated the application of magnetically driven microrobots and biohybrid nanorobots to ship viral vectors or virus-containing cargos to tumor tissues to overcome such

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obstacles as poor extravasation and poor tissue penetration. In contrast to passive carriers, micro-/nanorobots have the ability to actively enter dense ECM and access hypoxic or poorly vascularized tumor regions. Janus cell robots represent a novel class of biohybrid nanobots that combine living cells with asymmetric surface functionalization to enable externally guided transport of therapeutic cargo [18]. When applied in OV delivery, tumor-tropic cells (e.g., MSCs or cancer cells) are typically partially coated with magnetic NPs, thereby creating an asymmetric ("Janus") structure. Janus cell robots are capable of performing spatial movement as compared to traditional cell carriers due to its asymmetry. This design allows enable the cells to maintain their biological properties such as tumor homing, but at the same time the cells have the capacity to be directed by external magnetic fields. Janus cell robots loaded with oncolytic adenoviruses have been demonstrated to display preclinical capabilities of being steered magnetically to tumor sites [18]. Active delivery boosts spatial control and efficiency that is difficult to achieve with traditional cell carriers. Although it is at a relatively early stage in its experimental development, this technique solves significant problems in the traditional cell carriers, and is an important advance towards the possibility of actively and selectively directed OVT.

In summary, platelet membrane-based coatings and micro-/nanorobotic systems illustrate the shift from passive to actively guided and biomimetic OV delivery strategies. A proof-of-concept study of Janus platelet cell robots, the combination of platelet coating and Janus cell robots, has further demonstrated the promising potential of these novel techniques in optimizing OV delivery [19]. Despite that there are still technical and regulatory obstacles, these advanced methods show promise to overcome the persistent hurdles of immune clearance, inadequate targeting, and ineffective tumor penetration in OVT. Moreover, these innovative methods show that the challenges of OV delivery are transforming from addressing drug delivery barriers to fixing bioengineering flaws.

5. Conclusion

The therapeutic outcomes of OVT are significantly tied to delivery methods. Despite having intrinsic tumor selectivity and ability to induce antitumor immune responses, the clinical efficacy of OVs is strongly influenced by the efficiency, specificity and biological compatibility of their delivery to tumor tissues.

IT injection is still the foundational delivery strategy for OVT. Its advantages are the ability to establish high local viral concentrations while minimizing systemic toxicity. However, its dependence on tumor accessibility, restricted IT spread, and limited reach to metastatic disease restrict its broader clinical application.

Systemic delivery provides a valuable non-invasive alternative to IT injection. IV injection makes targeting disseminated and deep-seated malignancies possible. Nevertheless, there are significant biological barriers to this approach, such as immune-mediated clearance, sequestration of off-target organs, and ineffective tumor extravasation. Despite extensive efforts, including NPs, cell carriers, and capsid engineering, current systemic delivery strategies remain limited due to safety issues, poor targeting precision, and inconsistent therapeutic efficacy. Such failures highlight an enduring gap between preclinical promise and clinical translation in OV delivery. Immune recognition, clearance, and lack of adequate tumor targeting are barriers which may be overcome through engineering approaches but there are inherent biological constraints, which include host immunity and complex TME which cannot be entirely avoided by novel techniques.

Current development of bioengineering and micro-robotics indicate that new delivery systems have the potential to influence the future of OV delivery. Platelet membrane coating takes advantage of natural immune-evasion mechanisms of platelets to prolong circulation time and enhance the

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tumor accumulation. In the meantime, active delivery platforms such as micro-/nanorobots combine external guidance and controlled transport mechanisms to allow more accurate navigation through complex biological environments. It can be predicted that the future of OV delivery would probably imply mixed, active, and personalized methods that combine multiple delivery modalities and adapt to patient-specific tumor characteristics.

Although advanced OV delivery technologies largely remain experimental or clinical and confront major regulatory and manufacturing challenges, their potential to overcome long-standing barriers is evident. OVT does not involve intracellular delivery as compared to other therapies, such as gene therapy and RNA therapy. OVs are capable of self-replication, and interact with host immunity and the TME dynamically. Although these unique biological features distinguish the OVs from conventional therapy, they aggravate the challenges of delivering the agent, which is excessive immune system clearance, biodistribution regulation, and safety issues. These experimental strategies will require interdisciplinary research to be performed in the future incorporating virology, material science, and robotics to transform them into a viable clinical therapy. In due course, the delivery strategies of OVs will be optimized, contributing significantly to the full therapeutic potential in cancer treatment.

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