

Oncolytic virus delivery: from nano-pharmacodynamics to enhanced oncolytic effect

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Abstract: With the advancement of a growing number of oncolytic viruses (OVs) to clinical development, drug delivery is becoming an important barrier to overcome for optimal therapeutic benefits. Host immunity, tumor microenvironment and abnormal vascularity contribute to inefficient vector delivery. A number of novel approaches for enhanced OV delivery are under evaluation, including use of nanoparticles, immunomodulatory agents and complex viral-particle ligands along with manipulations of the tumor microenvironment. This field of OV delivery has quickly evolved to bioengineering of complex nanoparticles that could be deposited within the tumor using minimal invasive image-guided delivery. Some of the strategies include ultrasound (US)-mediated cavitation-enhanced extravasation, magnetic viral complexes delivery, image-guided infusions with focused US and targeting photodynamic virotherapy. In addition, strategies that modulate tumor microenvironment to decrease extracellular matrix deposition and increase viral propagation are being used to improve tumor penetration by OVs. Some involve modification of the viral genome to enhance their tumoral penetration potential. Here, we highlight the barriers to oncolytic viral delivery, and discuss the challenges to improving it and the perspectives of establishing new modes of active delivery to achieve enhanced oncolytic effects.

Keywords: oncolytic viruses, oncolytic virotherapy, drug delivery systems, tumor microenvironment

Introduction

Efficient delivery of oncolytic viruses (OVs) remains a major challenge in the field of oncology limiting their therapeutic effect. This may account for the disparity between in vitro and in vivo preclinical studies^{1,2} and the relatively modest antitumor effects observed thus far in clinical trials.³

Three major limitations need to be addressed to enhance delivery: first, virus bioavailability determined by the host vascular dynamics, perfusion parameters and innate immune responses;⁴⁻⁶ second, OV biodistribution and propagation,⁷⁻⁹ usually impaired by the intra-tumoral microenvironment heterogeneity; and third, the amplification of the virus bystander killing effect by cell-to-cell contact or by intrinsic vector enhancement.⁵ In this review, we discuss these three aspects and provide alternatives to improve them.

A number of prior studies have been done to optimize systemic delivery of OVs.¹⁰⁻¹² Although a systemic approach is still a major goal of therapy given its simplicity, it has been difficult, and clinical trials employing systemic delivery have had limited success thus far.^{3,4,13} Even locoregional approaches of administration – such as intraperitoneal

delivery for ovarian cancer,¹⁴ intrapleural delivery for mesotheliomas,¹⁵ intracavitary delivery for gliomas¹⁶ and intradermal delivery for melanomas¹⁷ – have yielded inconsistent results in both preclinical and clinical studies. Despite the difficulties for a viral vector to reach and eventually infect extravascular tumor cells, the main mode of administration has remained direct intravascular infusion through a major vessel supplying the tumor or by local intra-lesional injection in a solid tumor, with some studies employing both modes of administration.^{14,18} As a result, the intra-lesional approach has been preferred due to limited viral inactivation by the innate immune system, lower probability of systemic toxicity and optimal delivery of viral load in a single dose. Nonetheless, intra-tumoral delivery needs to be enhanced given the presence of a heterogeneous extracellular matrix (ECM) and the fact that transvascular perfusion may not be achieved to a therapeutic level. Inasmuch as tumor neovasculature is often abnormal, it severely impacts the intra-tumoral spread of OV and maintenance of viral propagation. Tumor angiogenesis results in heterogeneous pericyte coverage promoting transient perfusion, leading to a hypoxic and acidic microenvironment, with tendency to microvasculature coagulation. This leads to suboptimal infection, impacts treatment resistance and may result in tumor recurrence.^{19–21} In view of this, vector enhancement has been equally important to improve the potential of the virus platform.

The host complex and viral vector bioavailability

Strategies for improving vector delivery include shielding of the virus from host immune defenses, and the use of nanoparticles for active targeting and nanofilaments to improve vector propagation.

Shielding and surface modifications

Shielding is done by cell-based delivery approaches²² listed in Table 1, or through an interface with nanoparticle carriers.²³ The first relies on passive delivery, given that direction and flow of viral propagation is dependent solely on the cell-based properties towards the target organ or tissue. The latter relies on active delivery, where nanoparticle physical properties can be used to promote monitoring and targeting of a specific organ. In this case, shielding may be done by physical interface with biomaterials such as encapsulation and coating with polymers, or biodegradable nanoparticles, liposomes or copolymers.²⁴ It can also be achieved by chemical modification with biomaterials such as polyamidoamine, polyethylene glycol (PEG), poly-N-(2-hydroxypropyl) methacrylamide,

polysaccharides, bio-reducible polymers, arginine-grafted bio-reducible polymers, cationic polymers, poly-ethylenimine, poly-L-lysine and cationic lipids.^{24–26} Another way of shielding with biomaterials is through immobilization of the vector to a material's surface through a process termed reverse transfection, solid-phase delivery or substrate-mediated delivery. This may also provide controlled viral release rate, localizing the gene expression to the surroundings, diminishing systemic infectivity, maintaining an elevated local concentration and as such helping to overcome transport limitations. Other similar biomaterials include microporous scaffolds, hydrogels, silk-elastin-like polymers, recombinant polymers, alginate and poly (lactic-co-glycolic acid), chitosan, fibrin and collagen micelles.^{23,25} Both cell-based and biomaterial interface-based delivery approaches are summarized in Table 1.

Using physical properties of nanoparticles to enhance active delivery and vector propagation

Active delivery is desirable over passive delivery as transvascular extravasation leads to the same constraints for nanotherapeutic viral delivery that have been described for other nanotherapy approaches.^{19,51} In this sense, the modulation of the tumor microenvironment is fundamental to enhance tumor spread.

Specific biomaterials possess properties required to allow delivery of viral vectors through ultrasound (US) using microbubbles (MBs) and focused sonoporation.^{52–54} While using MBs, contrast is employed for US using inert gas to pre-produce MBs that are injected at the tumor followed by percutaneous US, with shocking waves propelling the MBs against the tumor matrix causing temporary cavitation which may considerably enhance OV delivery through increased extravasation.

Another strategy for improved real-time monitoring of delivery includes magnetic-viral complexes, detected by magnetic resonance^{55,56} which enables noninvasive therapy monitoring. From the active propagation perspective, nanofilaments⁵⁷ can be used to enhance viral propagation, in a way comparable to the spontaneously formed tunneling nanotubes in mesothelioma cells. The goal is to use ultrafine actin-based cytoplasmic extensions for increased bystander killing due to amplification of cell-to-cell contact.

Appropriate control of viral delivery can also be achieved by nanoparticles. Some techniques provide the particles with stimuli-responsive properties for enabling antitumoral effect. An example is photodynamic virotherapy, where the particle-viral ligand is armed with a genetically encoded photosensitizer, such as photofrin or talaporfin,⁵⁸ which

Table I Selected examples of shielding the OV from host barriers

Strategy	Approach	Viral platform	Tumor type	Outcome	References
Cell-based delivery	Mesenchymal stem cell (bone marrow derived)	MV	Liver cancer	Evasion of host immunity in setting of systemic delivery	27
	Mesenchymal stromal cell	Ad	Pancreatic tumor	Decreased expression of CD24 and Ki67 and enhanced activity of caspase-3	28
	Neural stem cell	Ad	GBM	Single administration of oncolytic virus-loaded NSCs allows for up to 31% coverage of intracranial tumors	29
	Activated T-cells	VSV	Ovarian cancer	Increased efficiency compared to nonactivated T-cells	30
	Immortalized cell line from solid tumor	VSV	Murine model metastatic tumors	Ease of manipulation and propagation in vitro, but has a tendency to arrest in the small capillary beds of the lungs and fail to recirculate in animal (mice) model	31
	HeLa (cervical carcinoma)				
	A549 (lung carcinoma)				
	MCF-7 (breast carcinoma)				
	CT26 (colorectal carcinoma)				
	SF268 (glioblastoma)				
	Dendritic cells	MV	Breast cancer	Prevention of pleural exudate in a xenograft model	32
	Sickle cell	Reovirus	Melanoma	Absorption and transfection despite presence of neutralizing antibodies	33
	Macrophages	Ad	Prostate cancer	Abolishment of tumor regrowth	34
	Myeloid-derived suppressor cells	VSV	Metastatic colon tumor	Robust immunosuppressive activity, preferential migration to tumor and decreased toxicity	35
Physical interface with biomaterials	Monocytes	Ad	Syrian hamster models of cancer	Antitumoral effect after multiple dosing	36
	Ghost erythrocytes	VSV-G	In vitro transfection	Improved transfection efficiency	37
	Encapsulation (within biomaterial) alginate	Ad	Model for shielding the adenoviruses	Enhanced transgene expression and reduced immune response	38
	Encapsulation (within biomaterial) PLGA	Ad	Model for shielding the adenoviruses	Enhanced transgene expression and reduced immune response	38
Chemical modification with biomaterials	Surface modification coating with biodegradable nanoparticles (PNLG)	Ad	Model for shielding the adenoviruses	Improved efficacy and safety	39
	PAMAM dendrimer-coated	Ad	EGFR+ cells	Increased transduction efficiency, especially in low-to-medium CAR-expressing cancer cell lines	40
	Cationic polymers* (form electrostatic interactions with anionic Ad, can also be classified as physical interface)	Ad	Model for shielding the adenoviruses	Permitted ligand attachment and manipulation of molecular weight	25
	PLL (cationic polymer*)	Ad	Model for shielding the adenoviruses	Caused Ad to bind and infect cells through a pathway other than classic CAR-mediated entry PEG-PLL-Ad had gene expression ~4× compared to naked Ad	41
	Cationic lipids*	Ad	Model for shielding the adenoviruses	Increased delivery ~80× compared to naked Ad	42
	Liposomes	Ad	Model for shielding the adenoviruses	Resulted in effective immune shielding	
	PEGylation (covalent chemical modification)	VSV	Model for shielding the adenoviruses	Increased circulation half-life	43
	Poly-HPMA	Ad	Model for shielding the adenoviruses	Protected from neutralization Increased half-life by diminishing hepatic transgene expression	44

(Continued)

Table 1 (Continued)

Strategy	Approach	Viral platform	Tumor type	Outcome	References
Substrate-mediated viral gene delivery	Polysaccharides	Ad	Model for shielding the adenoviruses	Unable to evade neutralizing antibodies	45
	Hydrogel	Ad	Model for shielding the adenoviruses	Minimized sequestration by the mononuclear phagocytic system	46
	Silk-elastin-like polymer	Ad	Model for shielding the adenoviruses	Increased viral gene expression but demonstrated some acute toxicity	47
	Chitosan	Ad	Model for shielding the adenoviruses	Infectivity was observed in cells that do not express CAR	48
	Biogels: fibrin and collagen micelle based	Ad	Model for shielding the adenoviruses	Sustained release of viral particles by fibrin	49
	Microporous scaffolds (could be considered as physical interface given that coaxial electrospinning is used to encapsulate vectors)	Ad	Model for shielding the adenoviruses	Reduced macrophage activation	50

Note: *Cationic polymers and cationic lipids may be classified as a way to establish physical instead of chemical interface because they are formed by electrostatic interactions with anionic adenoviruses rather than through chemical conjugation.

Abbreviations: OV, oncolytic virus; MV, measles virus; Ad, adenovirus; GBM, glioblastoma multiforme; NSCs, neural stem cells; VSV, vesicular stomatitis virus; VSV-G, vesicular stomatitis virus glycoprotein G; PLGA, poly(lactic-co-glycolic acid); PNLG, poly[2-(diethylamino)ethylamine-L-glutamate]; PAMAM, polyamidoamine; EGFR+, epidermal growth factor receptor positive; CAR, coxsackie adenovirus receptor; PLL, poly(L-lysine); PEG, polyethylene glycol; poly-HPMA, poly-N-(2-hydroxypropyl) methacrylamide.

provides light-induced antitumor effect when activated at a specific wavelength. Another approach is deployment of a synthesized pH-sensitive polymer with a bioreducible disulfide bond (methoxy-pegylated cystaminebisacrylamide) armed to release the viral particle load in an acidic environment.⁵⁹ Particle ligand approaches have also been used to target the tumor. An example of such an approach is the use of penetrating peptides or homing peptides⁶⁰ such as arginylglycylaspartic acid (Arg-Gly-Asp) which are frequently used as a targeting moiety in adenovirus/polymer complexes with high affinity for α_v integrins,⁶¹ E-selectins⁶² and vascular endothelial growth factor (VEGF) receptors.⁶³ Target ligands can also be growth factors or antibodies such as cetuximab, and even natural ligands such as folate and chitosan PEG-folic acid.⁶⁴ Active delivery approaches using virus-particle ligand complexes are summarized in Table 2. Some of those strategies may facilitate the induction of virus persistence by evasion of the DNA and RNA sensing systems and thereby help to improve oncolytic effect. On the top of that, further effects on viral replication and necessary viral load for each type of nanoparticle used and specific target organ still need to be evaluated in a case-by-case fashion.

Biomaterials shielding for improving monitoring and control of vector release

Biomaterial shielding has been important to diminish peripheral sequestration and to improve targeting by using

specific ligands, while ensuring that size and molecular shape remain fairly uniform. This allows to predict hemodynamic interactions at a certain body temperature and blood viscosity depending on the polymer type used such as molecule, protein⁷² or hydrogels. This strategy also allows for prediction of the potential rate of viral release at the tumor level.⁷³ In the long run, biomaterials may help track and predict viral delivery within reliable ranges. From this perspective, copolymerization can result in enhanced tumor penetration. Similarly, optimal water solubility has been achieved by introducing a pH-sensitive cleavable linker and target moiety to a multi-arm copolymer and ultimately complexing it with viral particles.^{74,75}

The tumor microenvironment: perfusion, permeability and retention

On one level, the more bioavailability a vector achieves inside the tumor, the more optimal the killing effect. On the other level, bioavailability can be increased by manipulation of host hemodynamics. Improving perfusion pressure may have an impact in a short window of time for locally administered compounds, as listed in Table 3. Animal studies have shown that promoting a hypertensive state through exercise also promotes extravasation for OV administration.⁷⁶ Mayo Clinic studies^{8,76} have proposed a mathematical model of radial expansion and conflation of intra-tumoral infectious centers.

Table 2 Selected examples of active delivery using complexes of virus–viral particle ligands

Strategy	Nanoparticle ligand	Virus	Results	References
Bioreducible disulfide bond	mPEG-PIP-CBA	Ad	Armed release of viral particle in hypoxic, acidic environment	59
Vascular zip code	Linear RGD (Arg-Gly-Asp)	Ad	Enhanced endocytic ability	65
	CD-PEG-cRGD	Ad	Downregulation of ICAM-1, VCAM-1, E-selectin, IL-6, IL-18, VEGF-A and Tie-2	66
	Cyclic CD-PEG-cRGD			
Natural ligand (folate)	PEG-folic acid	Ad	Enhanced cell entry through folate receptors	67
Targeting ligands (antibodies)	Trastuzumab (HER2/neu)	Ad	Retargeted viral receptor to breast cancer cells	68
	Ad-PEG-HER	Ad	Retargeted viral receptor to intraperitoneal ovarian cancer cells	69
	Cetuximab-pHPMA-PEG			
Targeting ligands (growth factors)	VEGF-pHPMA or bFGF-pHPMA	Ad	Retargeting evaded neutralizing antibodies	70
	Biotin-EGF	Ad	Enhanced tumor specificity and membrane permeability	71

Abbreviations: mPEG-PIP-CBA, methoxy-pegylated pH-sensitive polymer cystaminebisacrylamide; Ad, adenovirus; RGD, arginylglycylaspartic acid; CD-PEG-cRGD, cyclodextrin pegylated arginylglycylaspartic acid; ICAM-1, intercellular adhesion molecule 1; VCAM-1, vascular cell adhesion molecule 1; IL, interleukin; VEGF A, vascular endothelial growth factor A; PEG-folic acid, pegylated-folic acid; HER2/neu, human epidermal growth factor receptor 2/proto-oncogene neu; Ad-PEG-HER, pegylated adenovirus conjugated to herceptin; Cetuximab-pHPMA-PEG, cetuximab-pegylated conjugated with poly-N-(2-hydroxypropyl) methacrylamide; VEGF-pHPMA, vascular endothelial growth factor conjugated with poly-N-(2-hydroxypropyl)methacrylamide; bFGF-pHPMA, basic fibroblast growth factor conjugated with poly-N-(2-hydroxypropyl) methacrylamide; biotin-EGF, biotin conjugated with epidermal growth factor.

Table 3 Pharmacodynamic manipulation to enhance oncolytic virus bioavailability

Infusion type	Drugs	Effect	References
Local	Nitric oxide	Local improvement of vasodilation and perfusion for short period of time (normalization window) with impact in normal tissues	80
	Bradykinin		
	Nitroglycerin		
	Histamine		
	Local hyperthermia		
	Low-dose paclitaxel		
Systemic	Angiotensin receptor blockers	Decreased collagen deposition improving trans matrix propagation	83

This model also predicts the probability of tumor cell survival after the oncolytic phase and establishes perfusion pressure as a major determinant of intra-tumoral extravasation of OV. The major impact was seen upon density of viral infection within the tumor achieved by increase and decrease in the mean arterial pressure.^{8,76}

The focus on increasing permeability of tumor blood vessels has been critical since the enhanced perfusion and retention (EPR) effect was first described in 1986,⁷⁷ exploiting the leaky nature of tumor vasculature.^{9,78} Convection is compromised on account of the high intra-tumoral interstitial pressure, dense heterogeneous stroma, lack of fenestration in the tumor endothelium and heterogeneous basement membranes hindering lymphatic drainage and impeding viral extravasation, all diminishing the EPR effect.¹⁹ The extent to which the EPR effect observed in murine models may translate to the human disease setting remains unclear.²⁵

The tumor microenvironment as a barrier to OV delivery

Unlike normal vasculature, intra-tumoral vessels are immature, chaotic and mostly saccular, with a tortuosity that highly impacts effective blood perfusion.⁷⁹ Nonuniform endothelial structure that promotes leakage in nonspecific areas plus constant changes in the tumor environment due to tumor growth and treatment may partially explain why a primary tumor may respond to treatment whereas its metastases may be unresponsive. The leakier a tumor becomes, the higher the interstitial fluid pressure, as the endothelial cells may not maintain pressure gradients across the endothelial wall and the drainage by lymphatics is dysfunctional. Stasis increases local hypoxia triggering upregulation of HIF-1 alpha and VEGF pathway activity, leading to a vicious cycle.⁸⁰ Blocking VEGF has been shown to transiently “normalize” vascular structure and function,

perhaps explaining why anti-VEGF antibodies have shown clinical efficacy (increased overall survival) when combined with chemotherapy in patients with mesothelioma, colon, lung, ovarian or cervical cancers. It was hypothesized that anti-VEGF approaches improve tumor perfusion by normalizing functionality of the tumor vasculature. The use of anti-VEGF and VEGF receptor therapies could enhance viral delivery in selected patients as well, akin to how these therapies have been enhancing immunotherapy.⁸¹ Similarly, another approach to normalize nitric oxide gradients to recover vessel function has been described.⁸²

Another obstacle to viral penetration is the ECM, particularly in highly desmoplastic tumors, where nanoparticles injected directly inside the tumor are unable to move far from the injection site.^{84,85} One study co-injected local intratumoral bacterial collagenase with oncolytic herpes virus for melanoma treatment, and the distribution area of the OV was found to increase threefold.⁸⁶ Given that collagen is an integral structural component of the vascular wall, collagenase may never be used systemically. An alternate approach is to use an anti-fibrotic agent that can diminish collagen such as relaxin, which is known for reorganizing collagen during pregnancy, and has been shown to increase tumor penetration by OVs after 2 weeks.⁸⁷ Another option is to use the metalloproteinase-1 and -8, which have been shown to increase OV delivery, improving distribution and yielding improved efficacy.⁸⁸ A novel use for antihypertensive drugs from the angiotensin II receptor blocker class has been to modulate transforming growth factor beta activation and decrease collagen deposition.⁸⁹ This mode of action has been employed in the prevention of esophageal sclerosis due to eosinophilic esophagitis. Losartan was the first candidate drug to be evaluated for decreasing collagen deposition in the tumor microenvironment. Dramatic decreases in collagen deposits and increase in tumor penetration were observed after 2 weeks.⁸³ Another study in non-hypertensive patients with pancreatic

adenocarcinoma demonstrated that use of candesartan was associated with a 6-month longer survival compared with the control group.^{90,91} In patients with hepatocellular carcinoma, candesartan downregulated the expression of VEGF-A, via the angiotensin II type I receptor, suggesting that it might be useful to inhibit angiogenesis in liver cancer.⁹² Studies with prostaglandin-I2 analogs have also demonstrated their efficiency in promoting vascular blood flow enhancement and extravasation at the tumor microenvironment, but safety studies must be performed prior to evaluating their potential for drug development.⁹³

Intrinsic vector enhancement, selective replication and retargeting viral tropism

Therapeutic safety of vectors is proportional to their tumor target selectiveness. Selective targeting using tumor-specific promoters ensures that viral replication will be restricted to cancerous tissues while healthy tissues will remain unharmed. Examples of tumor-specific promoters are provided in Table 4. Another approach is to restrict tropism, and thereby enhance selectivity, by retargeting infection by a virus while ablating its ability to infect cells through its natural receptors.⁹⁴ Instead, viruses are hexon swapped⁹⁵ and pseudotyped with a more potent entry gene, or by fusing the entry gene to a single-chain antibody against upregulated tumor-specific receptors.⁹⁶⁻⁹⁸ To sum up, arming recombinant viruses with prodrug convertases, cytokines, and pH-releasing arms using a variety of envelopes, capsids and fibers may onset viral proteases only in a cancer-specific environment.⁹⁹ Arming the viruses with a prodrug convertase will enable them to transform a nontoxic substrate or metabolite into a lethal drug within the tumor environment. Using this approach, the inclusion of cytotoxic genes and suicide genes was instituted in a herpes simplex virus (HSV) encoding thymidine kinase

Table 4 Selected examples of tumor-specific promoters

Tumor target	Virus-encoded promoter	Viral platform	References
Bladder cancer	Uroplakin II	Ad	131
Brain tumors	Nestin	HSV-I	132
	Musashi-I	HSV-I	133
Breast cancer	Estrogen response element	Ad	134
Gastroenteropancreatic neuroendocrine tumors	Chromogranin-A	Ad	135
Glioma	Glial fibrillary acidic protein	Ad	136
Hepatocellular carcinoma	Alpha-fetoprotein	Ad, HSV-I	137
Melanoma	Tyrosinase	Ad	138
Mesothelioma	Mesothelin	Ad	139
Ovarian cancer and breast cancer	Mucin-I	Ad	140

Abbreviations: Ad, adenovirus; HSV-I, herpes simplex virus-I.

(TK) to sensitize dividing cells to ganciclovir.¹⁰⁰ Still another strategy involves introduction of single-stranded RNA tumor suppressor or lethal genes with enhanced cytopathic and apoptotic effect (ADP-overexpressed adenovirus).¹⁰¹ Likewise, induction of autophagy can be accomplished by telomere-specific replication.¹⁰² Moreover, approaches where the virus may encode sequences for relaxin,¹⁰³ decorin,¹⁰⁴ hyaluronidase,¹⁰⁵ heparanase¹⁰⁶ and elastase¹⁰⁷ from macrophages metalloelastase have tested the concept of tumor microenvironment targeting viruses with intrinsic capabilities.

Immunomodulation at the tumor microenvironment

Oncolysis in cancer cells may be limited by immune response. Although most tumor cells have defective immunomodulation and limited response to interferon (IFN) stimulation, normal cells can still sense the virus even if the progenies are replication deficient. Some viruses have developed mechanisms to evade or block the type-I IFN pathway at different levels.¹⁰⁸ The most potent OV's are often wild types. First generations of OV's have been attenuated in an effort to achieve therapeutic safety, which led to reduced oncolytic potency. Hence, next generations are being engineered by modifying molecular patterns in order to boost oncolytic effect without compromising safety. Therefore, particular attention has been given to viral evasion of the DNA or RNA cytoplasmic sensing mechanisms, antiviral IFN blockages and molecular or sub-particle interactions that may improve oncolytic efficacy.^{99,109,110} On one hand, the virus should escape recognition by the host. On the other hand, the virus genome can be made to enhance tumor cell killing by using stimulatory cytokines.¹¹¹ These include tumor necrosis factor-related apoptosis-inducing ligand,¹¹² cytosine deaminase (CD),¹¹³ and immune-stimulatory cytokines genes such as interleukin (IL)-2,¹¹¹ IL-12¹¹⁴ and IL-18.¹¹⁵ Some studies have employed a combination strategy with a TK-CD hybrid protein¹¹⁶ to enhance killing and cancer specificity.

At the transcriptional level, inhibition of angiogenesis by viral encoding genes has been achieved using short hairpin RNA-expressing oncolytic adenovirus-mediated inhibition of IL-8 resulting in antiangiogenesis and tumor growth inhibition.¹¹⁷ Similarly, a VEGF-specific short hairpin RNA-expressing adenovirus has been developed¹¹⁸ to block tumor growth and achieve potent inhibition of angiogenesis. These approaches exhibit ability of vector-

encoded RNA knockdown for OV delivery. In addition, a vaccinia virus armed with the soluble VEGF receptor 1 protein developed antiangiogenic effect in a renal cancer cell model.¹¹⁹

Micro-RNA (miRNA) is evolving as a regulator of vector tropism,^{120,121} which is in contrast to the initial descriptions of RNA viruses where vector tropism could not be controlled through transcriptional targeting. This was evident in the case of coxsackievirus type A21, which causes off-target severe, often fatal myositis.¹²² miRNA techniques can promote detargeting of OV's during systemic administration, reshaping tumor tropism. Several insertions of combined miRNA target sites can be adapted to a single vector to detarget pivotal organs at risk for off-target side effects.¹²³

Short-interfering RNA (si-RNA)¹²⁴ delivery systems have been designed to increase tumor specificity. Small double-stranded RNAs impact posttranscriptional gene silencing as they target mRNAs that are then taken up by the RNA-induced silencing complex.¹²⁵ They can be used to bind and guide cleavage of mRNA in a sequence homology-dependent manner.¹²⁶ These may limit side effects, and toxicity, and take oncolytic virotherapy to a new safety level.

At the translational level, OV replication can be targeted by internal regulation of viral protein translation. This control is made through the internal ribosome entry site (IRES) element and is becoming a powerful tool to co-express genes of interest from a single mRNA, as IRES appears to play the role of a translational enhancer and may soon expand perspectives for better vectors.¹²⁷ As such, multiple genes in viral payloads can be delivered using intrinsic genomic attributes, to impact viral delivery.

Local spread of the virus can be boosted by immunosuppressive drugs, such as cyclophosphamide.¹²⁸ Pulsed application of immunosuppressive drugs is preferred, as seen with the prodrug 5-fluorocytosine and measles vaccine virus.¹²⁹ Other immunomodulators such as cobra venom factor may also facilitate infection with HSV.¹³⁰

Future directions

There is a need in the field of OV delivery to explore natural tropism of therapeutically modified viral platforms, such as hepatitis viruses for hepatocellular cancer and encephalitic viruses for brain tumors. Thus far, natural tropism of viruses has not been vastly explored from an oncolytic viral perspective.

Delivery of multiple distinct therapeutic viral vectors at the same time using biomaterials to bypass the viral load sequestration and neutralization by the immune system has

not been attempted yet. This has the potential for simultaneous delivery of complementary viruses in an effort to achieve maximal synergy. Use of different viruses has been reported with better tumor penetration.¹⁴¹

Most current delivery approaches are passive in nature. Enhanced delivery could potentially be achieved using active delivery methods such as nanomachine-enabled propulsion of viral vectors. Simultaneously, the expectation is that active delivery could also contribute to the noninvasive monitoring process of the OV targeting and propagation.

Improved monitoring methods for real-time viral injections and tagging viral particles for better in vivo visualization is also a growing necessity to assess viral delivery, especially for evaluating propagation after the first viral replication. To date, the gold standard for monitoring is biopsy. Bioluminescence and fluorescence optical methods are being developed along with noninvasive monitoring that allows deep tissue imaging. Examples of agents used for monitoring are radiotracer-coupled surface transporters used as the sodium iodide symporter,¹⁴² human norepinephrine transporter meta-iodobenzylguanidine which can be imaged by positron emission tomography or single photon emission computed tomography¹⁴³ and human somatostatin receptor 2 radiolabeled with indium-111 along with vaccinia virus.¹⁴⁴

Conclusion

Delivery of OVs remains a major challenge in the field of oncology. Rapidly evolving, innovative bioengineering and molecular approaches, at the host, tumor and viral level, are currently being tested. Overcoming the barriers to OV delivery will be paramount to clinical translation. A multimodal strategy based on novel viral engineering, host defense manipulation and novel active delivery techniques is necessary for more successful cancer therapy.

Disclosure

The authors report no conflict of interest in this work.

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