Advances in the mechanisms of action of cancer-targeting oncolytic viruses (Review)

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Abstract. Cancer virotherapy mediated by oncolytic viruses (OV), has emerged as a novel and effective strategy in cancer therapeutics. Preclinical models have demonstrated anticancer activity against numerous types of cancer. Currently, a number of recombinant viruses are in late phase clinical trials, many of which have demonstrated promising results regarding the safety and reliability of the treatments, particularly when combined with standard antineoplastic therapies. In addition to molecular-targeted therapeutics, genetic engineering of the viruses allows functional complementation to chemotherapy or radiotherapy agents. Co-administration of chemotherapy or radiotherapy is imperative for an effective treatment regime. Additionally, these approaches may be used in combination with current treatments to assist in cancer management. The near future may reveal whether this renewed interest in oncological virotherapy will result in meaningful therapeutic effects in patients. The aim of the present review was to highlight how the knowledge of oncolytic viral specificity and cytotoxicity has advanced in recent years, with a view to discuss OV in clinical application and the future directions of this field.

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1. Introduction

Oncolytic virotherapy is not a new concept; this area of research first arose as an observational science in the early 20th century, when it was occasionally reported that cancer regression occurred spontaneously in patients following certain viral infections (1). Numerous experiments were carried out following these reports; however, the topic of anticancer viral therapy languished, due to the deficiency of specificity and the harmful side effects, as well as the limited knowledge regarding the mechanisms of how cancer cells succumb to oncolytic viruses (OVs) (2). Since the 1990s, the genomes of wild-type viruses have been selectively engineered as a result of the development of molecular virology; this has reignited interest in the use of replicating viruses as cancer therapeutics. The manufactured viruses are capable of preferential replication and proliferation in cancerous cells, which may cause the cancerous cells to die at the end of replication cycles via lysis or the activation of an antitumor immune response, with minimal damage to normal cells (3). Several OVs have been tested in humans, and although the safety results were optimistic, their efficacy as single agents was limited (4). Administration of chemotherapy or radiotherapy in addition to the viruses is vital for creating an effective treatment. Over the past two decades, the study of oncolytic virotherapy has grown exponentially alongside the advancement of molecular biology, virology, immunology and, particularly, genetic engineering (5). In addition, combinations with other treatment options, particularly cancer gene therapy, has been demonstrated the potential to strengthen the antitumor efficacy through viral amplification of the therapeutic transgene inside tumor cells and tissues (6). Various cancer-targeted OVs carry a number of exogenous genes that have transitioned from preclinical studies into early phase clinical testing and more recently into randomized clinical trials (Table I) (1). These include vaccinia, adenovirus, herpes simplex virus, reovirus and Newcastle disease virus (7,8); additionally, novel OV species are being explored. The current status of clinical trials for such therapeutic OVs has recently been reviewed by Russell *et al* (9).

Compared with conventional treatments for cancer, including chemotherapy and radiotherapy, the engineered OV strain expressing transgenes exhibits a contrasting mechanism of action, specificity and cross-resistance, among other characteristics (Fig. 1) (10-12).

2. Specificity of OV in succumbing tumor cells

The progression of a tumor is generally considered a stochastic, dynamic process; genetic and epigenetic changes are also involved, including in the limitless proliferative potential, evasion of apoptosis, enhanced angiogenic capacity, tissue invasion and metastasis, and modification of the intracellular signaling pathway. These changes may make malignant cells susceptible to an infection, due to a number of the pathways subverted by the tumor also being necessary for efficient antiviral responses. Once a virus penetrates into a tumor cell, the tumor microenvironment provides abundant support for viral replication; therefore, these changes create the conditions required for oncolytic viral selective replication in cancer cells (11,12). The oncolytic viral targeting mechanism is divided into the following categories: Targeting transduction, targeting transcription, targeting translation and targeting pro-apoptosis mechanisms.

Targeting transduction. Tumor cells have specific and high surface expression of the oncolytic viral primary receptor, which is capable of binding to viral surface proteins via the receptor-ligand pathway, essential for transduction. The first step is absorption, followed by the viral surface adhesion protein combining with the target cell surface receptor, which initiates endocytic signaling pathways (13). A number of viruses have a natural tropism for tumor cells. This principle is illustrated by the susceptibility of malignant glioma cells to poliovirus, depending on the presence of CD155 on glial neoplasms (14,15). Alternatively, in order to infect the targeted tumor cells, the viral surface binding protein, which has the ability to serve as a receptor, must transform by introducing a single chain antibody or a polypeptide binding ligand: For example, the urokinase receptor (uPAR) is overexpressed in multiple malignancies and serves a role in tumor invasion and angiogenesis (16,17). Measles virus retargeted against human (MV-h-uPA) or mouse (MV-m-uPA) uPAR were constructed by superinducing the aminoterminal fragment of either human or mouse urokinase in the C-terminus of recombinant measles virus. *In vitro* experiments indicated that MV-h-uPA and MV-m-uPA were able to specifically infect cancer cells that overexpressed uPAR via the receptor-ligand pathway (18).

Targeting transcription. The approach to achieving tumor-selective viral replication has been to alter the function of the control of genetic transcription, which is essential in viral replication, to a tissue- or tumor-specific promoter. These promoters include human telomerase reverse transcription (hTERT) (19), hypoxia-inducible factor-1 (20), prostate-specific antigen (21) and α-fetoprotein (22), among others. hTERT has been identified as a major protein that functions to maintain telomere length in tumors; however, it demonstrates little or no expression in normal cells, allowing cancer cells to subvert

the Hayflick limit (23). An attenuated adenovirus 5 vector, OBP-301, was constructed, whereby the hTERT promoter element drives the expression of E1 genes: Due to only tumor cells with telomerase activity possessing the ability to activate this promoter, selective viral replication and oncolytic cell death was demonstrated (19).

In addition to this approach, a novel system, which may regulate oncolytic viral gene-targeted expression by exploiting microRNAs (miRNAs), has been developed. miRNAs are 20-22 nucleotides of small noncoding endogenously produced RNAs that can base pair to their target mRNAs. This enables them to guide post-transcriptional silencing of their target genes and determine the differential expression between cancerous and normal tissues (24). The negative regulation in gene expression makes it possible to utilize these miRNAs to inhibit viral replication in normal cells (25,26). Aberrant expression of miRNAs has previously been observed in numerous types of cancer (27). The downregulation of miRNA (miR)-199 occurs consistently in almost all hepatocellular carcinomas (HCC), when compared with a normal liver (28). Additionally, a conditionally replication-competent oncolytic adenovirus (OAd), ad-199T, was generated by introducing miR-199 target sites within the 3'untranslated region of the E1A gene, essential for viral replication, in order that miR-199 demonstrated the capacity to negatively regulate E1A gene expression. As a result, ad-199T replication was inhibited in normal miR-199-positive liver parenchyma, and unaffected in tumor cells with low expression of miR-199 (29).

Targeting translation. Type I interferons (IFNs), which are spontaneously produced in response to a viral infection, are an important cytokine (12). The mechanism of cancer evolution that causes the loss of antiviral responsiveness in the majority of cancer cell lines, particularly the activity of IFN-regulated signaling pathways, is not completely understood. Previous studies have revealed that tumor antiviral activity is incompatible with their own efficient cell growth, as IFN and IFN-responsive genes are known angiogenesis inhibitors (30), and are also recognized for their capacity to induce apoptosis (31). In addition, the deficiency of tumor antiviral activity renders it more susceptible to an infection compared with normal cells, which results in a survival advantage for viruses within tumor cells. The vesicular stomatitis virus (VSV) is sensitive to IFN and therefore has an advantage in cancer therapeutics, including using a defective IFN pathway, to preferentially infect and kill tumor cells (32). The targeting of cancer cells by the VSV can be improved by introducing mutations in the matrix (M) protein. The M protein of VSV is a major structural protein that functions in virus assembly, as well as in the suppression of host gene expression, causing inhibition of IFNs and other antiviral proteins. The ability of the M protein to subdue host gene expression is genetically distinct from its viral assembly function. Furthermore, various mutations render the M protein defective in its ability to suppress host gene expression, without subverting its ability to function in viral assembly (33). As a result, normal cells, due to the activated IFN pathway, inhibit the M protein mutant VSV replication; otherwise cancer cells with defects in the IFN pathway are killed by M protein mutant VSV selective replication (34).

Table I. A listing of clinical trials of oncolytic viruses provided by a previous review (1).

| Virus family	Genetic modifications	Target types of cancer	Clinic trial sponsor
Adenovirus			
Oncorine (H101)	Ad-E1b–	Liver, lung, head/neck and pancreas	Shanghai Sunway (approved in China)
CGTG-102	Ad-GMCSF+	Solid tumors	Oncos
DNX-2401	Ad-d24RGD	Brain	DNAtrix/Erasmus Medical Center
ICOVIR-5	Ad-DM-E2F-K-d24RGD	Melanoma	Institut Catala d'Oncologia
CG0070	Ad-GMCSF+	Bladder	Cold Genesys
Colo Ad1	Ad3::Ad11p hybrid	Metastatic solid tumors	PsiOxus
Herpesvirus			
T-VEC	HSV1-ICP34.5–/4–GMCSF+	Melanoma	Amgen/BioVex (completed Phase III)
Seprehvir	HSV1716-ICP34.5–	Lung, various solid tumors	Virttu Biologics and Children's Hospital
G207	HSV1-ICP34.5–/6–	Brain	MediGene
HF10	HSV-HF strain	Head/neck, skin, breast and melanoma	Takara Bio
Poxvirus			
Pexa-Vec/JX594	Vaccinia Wyeth TK–/GMCSF+	Liver, colorectal, head/ neck, others	Jennerex (multiple trials)
Paramyxovirus			
Measles virus	MV-NIS+, MV-CEA+	Ovarian, peritoneal, myeloma, others	Mayo Clinic/NCI (multiple trials)
Newcastle disease virus	Natural isolate (HUJ)	Glioblastoma multiforme, neuroblastoma, sarcomas	Hadassah Medical Organization
Reovirus			
Reolysin	Reovirus-serotype 3	Diverse types of cancer	Oncolytics Biotech Inc. (multiple trials)
Rhabdovirus			
Vesicular somatitis virus	VSV-IFNb+	Hepatocellular carcinoma	Mayo Clinic
Maraba virus	MAGE A3+/Matrix glycoprotein mutations	Lung, colon, melanoma	NCIC Clinical Trials Group
Picornavirus			
CAVATAK	Coxsackievirus-A21	Melanoma	Viralytics (multiple trials)
PVS-RIPO	Polio:Rhinovirus chimera	Glioblastoma	Duke University
Seneca Valley V.	Natural isolate (NTX-010)	Neuroendocrine tumors	Children's Oncology Group
Parvovirus			
H-1 PV	Natural isolate	Glioblastoma	Heidelberg University Hospital
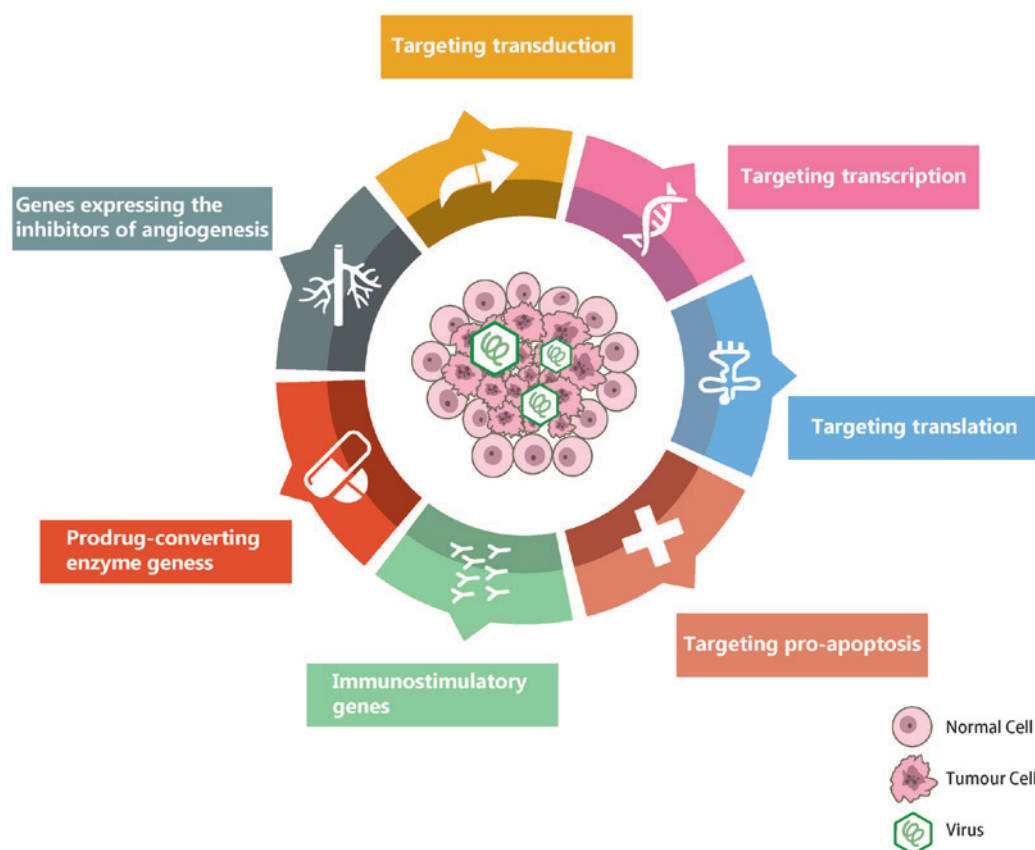
Figure 1. OV are designed to grow in the tumor niche. There are at least seven key action mechanisms that could be engineered or selected for OV specificity or cytotoxicity to tumor cells. OV, Oncolytic virus.

Targeting pro-apoptosis mechanisms. TP53 serves a key function in inducing apoptosis by prompting cell cycle arrest, due to activation in response to oncogene activation, DNA damage and other stress signals (35). *p53* is the most frequently mutated gene in cancer, being altered in >50% of all types of human malignancies (36). The mutated *p53* gene is an important hallmark of cancer (37). Viruses have evolved the ability to inhibit the apoptosis of host cells, in order to accomplish viral replication. The E1B55K adenovirus codes for the protein that inactivates the p53 protein of host cells, contributing to self-proliferation and virus replication (38). China approved the world's first OV therapy for cancer treatment (39). OV, also termed H101, has been approved for the treatment of numerous types of cancer clinically (40), H101 is a type of OAd with E1B-55KD and partial E3 deletion, and it cannot replicate in normal cells where p53 is active; therefore, H101 can selectively infect and kill tumor cells via the targeting of pro-apoptosis (41,42).

3. Lethal effect of OV on tumor cells

An important reason why OVs are currently being advanced as a promising antitumor modality is that they are able to transfer and amplify therapeutic genes, whilst simultaneously avoiding immunosurveillance by infected host cells (43). Virosomes are utilized as vectors for the delivery of toxic genes, including immunostimulatory genes [for instance, interleukin-12 (IL-12) (44)], anti-angiogenic genes (45), prodrug-converting enzyme genes and pro-apoptotic genes, to advance its potency, which can be used for oncotherapy (46).

Immunostimulatory genes. Delivery of immunostimulatory genes to cancer cells should boost immune responses against tumor antigens and the activity of cytotoxic effector cells, as a result of an abnormal increase of inflammatory infiltrates in the tumor milieu (47). Currently, a variety of immunostimulatory genes are used as part of recombinant oncolytic virusal vectors, which are utilized in viral gene therapy, including granulocyte-macrophage colony stimulating factor (GM-CSF) (48), IL-2, IL-12, IL-15 and IL-18 (49,50), IFN-α and IFN-β (51,52). Previously, Choi *et al* (49) generated IL-12- and IL-18-expressing (Ad-ΔE1Bmt7/IL-12/IL-18) OAd. IL-12 has been demonstrated to directly activate cytotoxic T cells and natural killer (NK) cells, which then produce high levels of IFN-γ, enhance their cytolytic activity and induce an antitumor effect (46); however, despite encouraging results in animal models, weaker antitumor effects of IL-12 were recorded in early clinical trials, and frequently accompanied by unacceptable levels of adverse events (53,54). This markedly dampened the hopes of the clinical application of this cytokine in patients with cancer. In order to minimize the adverse events of an IL-12-based therapy, a decrease in the systemic expression of IL-12 has been the selected approach. This approach involves patients who are injected intratumorally with Ad-RTS-hIL-12, an adenoviral vector engineered for the controlled expression of IL-12. Preliminary results were encouraging, with positive clinical efficacy observed in 5/7 patients treated with Ad-RTS-IL-12. These responses were associated with intratumoral IL-12 mRNA expression, reflected as a decrease in the size of the injected and distant lesions (55). IL-18 serves a

function in inducing the differentiation of antitumor effector T cells and the activation of NK cells, which exhibit potent cytotoxicity against tumor cells (56). In a B16-F10 murine melanoma model, IL-12, IL-18, IFN-γ and GM-CSF levels within the tumor tissues were significantly increased when treated with the intratumoral application of OAd co-expressing IL-12 and IL-18 compared with control virus-treated mice. Higher numbers of CD4^{+} T cells, CD8^{+} T cells and NK cells were detected via an immunohistochemical staining and the histological evaluation of tumor sections, which also exhibited large areas of necrosis compared with control virus-treated mice. These results further demonstrated the enhanced anti-tumor effects of OAd that express IL-12 and IL-18. The results were also validated in a murine colon adenocarcinoma model of MC38cea (48). IL-12- and IL-18-based immunotherapies were particularly efficacious in patients with cancer that contained inherited defects in IL-12 or IL-18 production, or with downregulated expression of IL-12 or IL-18. Compared with control virus-treated mice, the intratumoral administration of GM-CSF-expressing oncolytic MV markedly prolonged the median overall survival time (49).

Prodrug-converting enzyme genes. OVs were engineered to express prodrug-converting enzymes, which caused the conversion of the non-toxic prodrug into a toxic form, at the site of viral replication; therefore, causing selective tumor cell death. The prodrug-converting enzymes applied to the oncolytic virotherapy, including thymidine kinase (TK) (57), cytosine deaminase (CD) and purine-nucleoside phosphory-lase (PNP) (58-60). The mechanism of action involves the conversion of the prodrug ganciclovir (GCV), via TK, into ganciclovir triphosphate (GCV-TP), CD converts the prodrug 5-fluorocytosine (5-FC) into 5-fluorouracil (5-FU) and PNP converts the prodrug fludarabine phosphate into 2-fluoro-adenine. The transformed products are nucleoside analogs, which interfere with DNA replication in actively dividing tumor cells via the inhibition of DNA synthesis (61). A new recombinant herpes simplex virus type 1 thymidine kinase (HSV-1-TK), containing BoHV-4, has been constructed by Redaelli *et al* (62). The HSV-TK enzyme, which is an enzyme homologous to that of TK, has 1,000 times more affinity for the substrate GCV than the host cell TK (63). HSV-1-TK in combi-nation with GCV has been identified as a promising suicide gene system, whereby GCV is phosphorylated by HSV-1-TK to form GCV-TP. GCV-TP has the ability to inhibit DNA polymerases, resulting from competing with deoxyguanosine triphosphate to bind to DNA polymerases, causing DNA damage that leads to cell death (62,64). The active metabolite of TK, PNP and 5-FU can directly kill infected tumor cells. Furthermore, they promote a strong bystander effect on neigh-boring tumor cells that are not actively infected (59,65,66). In a previous study by Leveille *et al* (67), recombinant vesicular stomatitis virus (VSV-MD51) expressing the cytosine deaminase/uracil phosphoribosyl-transferase suicide gene with the 5-FC prodrug was combined for the treatment of cancer. It was observed that there was a synergistic effect on killing cancer cells with VSV-MD51 and 5-FU in several cancer cell lines. The high solubility of 5-FU is lethal to non-infected bystander tumor cells, which is an important advantage in using this combination.

Genes encoding the inhibitors of angiogenesis. In 1971, Folkman (68), to the best of our knowledge, first produced the hypothesis that tumor growth depends on angiogenesis, which also serves an essential role in tumor invasion and metastasis. Within four decades, anti-angiogenic therapy with broad targeting has rapidly evolved to become an integral component of current standard anticancer treatments (69,70). Endostatin and angiostatin are two endogenous and broad-spectrum angiogenesis inhibitors. These inhibitors only function when they are continuously transported to the tumor microenvi-ronment (71,72). Additionally, it is difficult to make the two endogenous inhibitors function in the traditional way, due to characteristics of short serum half-lives, low solubility and poor stability (73,74). Hutzen *et al* (73) developed recombinant MVs that express human and mouse variants of endostatin: Angiostatin (E:A) fusion proteins, known as MV-hE:A and MV-mE:A, respectively. The *in vitro* study revealed that there is active MV replication and concomitant continuous expression of target genes within the medulloblastoma cells; therefore, angiogenic factors were inhibited, leading to a significant decrease in endothelial cell growth, viability and migration (73).

4. New trends in OV based approaches

Gene-based virotherapy mediated by OV is currently the focus of numerous studies; however, little attention has been given to the dual gene virotherapy strategy, which could be utilized as a novel therapeutic approach for mediating triplex anticancer combination effects, particularly if the two suitable genes are well selected (75). A number of the previously published reports have stated that a single therapeutic modality, including those mediated by tumor necrosis factor-associated apoptosis-inducing ligand (TRAIL) or IL-12 alone, could not achieve sufficient antitumor responses; therefore, combina-tions of anticancer therapeutics, including those mediated by dual gene-based cancer therapy, hold great promise for killing cancer cells in the future (76-78). The anticancer therapeutic potential of OAd virotherapy strategy-mediated co-delivery of TRAIL and IL-12 genes has not been sufficiently investigated thus far. El-Shemi *et al* (79) suggested in their preclinical study that dual therapy with Ad-ΔB/TRAIL plus Ad-ΔB/IL-12 mark-edly suppressed human HCC via the promotion of antitumor apoptosis and immune activity, as well as by inhibiting tumor angiogenesis and neovascularization. Although further studies are warranted to evaluate this therapeutic combination, and also to explore the precise anti-tumor mechanisms, the OAd strategy-mediated co-delivery of TRAIL and IL-12 genes may be a potential therapeutic strategy for the treatment of human HCC, along with other types of cancer (79). Co-therapy with OAd-expressing TRAIL and another type of immunostimulant cytokine (IL-24) has been previously reported to be associated with potent activation in the caspase pathway, particularly of caspases-3 and -8, and apoptosis promotion in HCC (80). Comparatively, Han *et al* (81) demonstrated that treating patients with pancreatic cancer with gemcitabine and OAd armed with survivin shRNA and TRAIL greatly enhanced the cytotoxic death of pancreatic cancer cells (81). In addi-tion, OVs utilized to simultaneously express dual anticancer genes have attracted an increased interest, as it has provided a

multimodal method of killing cancer cells with an increased effectiveness, via a selective viral lytic effect on cancer cells and the additive or synergistic interaction between the two expressed anticancer genes (82,83).

5. Conclusions

The oncolytic virotherapy area of study for cancer therapeutics has experienced considerable progress in recent years. Preclinical models have demonstrated an anticancer activity against numerous types of cancer (84). Currently, several recombinant viruses, including adenovirus, herpes simplex virus, vaccinia virus, reovirus, Newcastle disease virus and Parvovirus are in late phase clinical trials. A number of the clinical trials have indicated promising results, including in the safety and reliability of the treatment, particularly when combined with standard antineoplastic therapies (85). There is hope that oncolytic virotherapy will be included in the armamentarium of anticancer agents, and that certain groups of patients will benefit from the treatment. Despite the renewed hope, the following key challenges remain: OV cannot be viewed as a stand-alone therapy for any type of cancer; the safety of systemic administration remains untested; and the optimal arming strategies that combine chemo-, radio- and immuno-therapies require investigation (2). With the rapid development of molecular biology and cell biology techniques, cancer is viewed as a systemic and heterogeneous disease; therefore, using OV as a single therapy would be difficult when attempting to achieve the desired therapeutic effect. Individual and comprehensive therapies have become new trends in cancer therapy. Conventional therapies, including radiotherapy and chemotherapy, have various disadvantages, such as limited efficacy low specificity, cross-resistance, severe adverse effects and an eventual inability to meet the requirements of the patient (2). Oncolytic virotherapy as a new modality of cancer therapy is promising, and may be used to complement conventional therapies; however, the efficacy of combination therapy regimens must be validated. In the foreseeable future, an ever-growing number of recombinant OV may be used in clinical trials and applied in clinical practice.

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