Viral delivery for gene therapy against cell movement in cancer

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Abstract

Viral delivery for cancer gene therapy is a promising approach, where traditional radiotherapy or chemotherapy to limit proliferation and movement of cancer cells has met resistance. Based on the new understanding of the biology of the viral vectors, therapeutic viral vectors for cancer gene therapy have been improved for greater safety and efficacy as well as transitioned from being non-replicating to replication-competent. Traditional oncolytic vectors have focused on eliminating tumor growth, while novel vectors simultaneously target epithelial-to-mesenchymal transition (EMT) in cancer cells, which could further prevent and reverse the aggressive tumor progression. In this review, we highlight the illustrative examples of cancer gene therapy in clinical trials as well as preclinical data and include proposals on methods to further enhance the safety and efficacy of oncolytic viral vectors in cancer gene therapy.

1. Introduction

Cancer metastasis is the leading cause of death in most cancer patients due to tumor burden and organ dysfunction. During cancer metastasis, tumor cells gain the ability to migrate throughout the body, seed and proliferate in distant organs to establish secondary tumors within normal tissues. The process of cancer metastasis is dependent on the motility of tumor cell, where mutations accumulated in tumors eventually endow tumors with migrating capacity. Metastatic tumors can be lethal, and are difficult to be treated clinically. Gene therapy is a technique for correcting defective genes responsible for disease development and is an attractive approach as cancer therapeutics.

In cancer gene therapy, therapeutic agents including functional normal tumor suppressor genes, inflammatory immune cytokine genes, and microRNAs are delivered to the tumor cell using a carrier. The delivery systems generally can be divided into two categories: viral vector and non-viral vector. Compared to non-viral vector, viral vector is more efficient in gene delivery to target cells [1]. The viral vectors expressing the carried gene in host cells can be replication-competent or non-replicating. Due to their safety compared to replicating vectors, non-replicating viral vectors have been widely investigated in vaccine development as well as in gene replacement therapy in last twenty years. The viruses commonly modified to be used as replication-defective therapeutic vectors are adenovirus (Ad), adeno-associated virus, lentivirus and herpesvirus. Factors for consideration for the choice...
of a particular vector include the packing capacity of transgenes, host range, tropism of vectors, inflammatory potential of the viral vectors, and serotypes [2,3].

In recent years, therapeutic viral vectors for cancer gene therapy have transitioned from being non-replicating to being replication-competent, or oncolytic. The selective replication of the viruses in cancer cells amplifies the initial viral seeding dose, leading to killing of cancer cells by virus-mediated cytolysis. The replicating viruses spread through the tumor mass to infect other cancer cells, resulting in self-perpetuating cycles of infection, replication, and oncolysis. The major limitation of replication-deficient viral vectors for cancer gene therapy is that they are unable to infect the majority of the cancer cells in a solid tumor mass, so the metastasis and growth of the cancer cells could not be maximally restricted and destroyed. The replicating vectors have the potential to overcome such limitation. Replication-competent oncolytic viral vectors have been widely tested in preclinical studies and translated into human clinical trials as monotherapy or in combination therapy for cancer patients. Traditional viral oncolytic therapeutics have focused on reducing tumor growth, which can antagonize the eventual tumor metastasis as cancer metastasis is dependent on continuous growth conditioning/signaling, such as the EGFR pathway [4,5]. Many cellular gene targets are important in epithelial-to-mesenchymal transition (EMT) in cancer cells, which help cancer cells metastasize and attain the “stem cell” phenotype. They can also be directly targeted to inhibit cancer cells’ invasiveness, “stemness” and resistance to death promoting agents [6,7].

In this review, we highlight some of the illustrative examples in clinical trials as well as preclinical data on oncolytic viral vectors and include proposals on methods to further enhance the safety and efficacy of replicating viral vectors in cancer gene therapy against cell movement and tumor progression.

2. Adenovirus

Adenovirus, first isolated in 1953 from adenoid tissue, is a non-enveloped, double stranded DNA virus. Ad has a number of attractive characteristics as cancer gene therapy vector because of its broad tropism and high transduction efficiency. 51 human serotypes of Ad have been isolated and identified, and the most commonly used vectors are based on human serotype 5 (AdHu5) [8]. To date, the replication-deficient AdHu5 vectors expressing p53, named Gendicine, has been approved in China for treating head and neck cancer [2].

As a replicating virus, Ad has been developed as a selective oncolytic viral vector through the dysregulation of p53 pathway and Rb pathway in the tumor cell. Ad E1A gene blocks the normal cellular tumor suppressor Rb gene function and E1B gene antagonizes the p53 to support viral replication in an infected cell [9]. Vectors deficient in E1A or E1B are developed to not replicate in normal cells but can replicate in cancer cells which often have impaired p53 or Rb pathways. Replicating Ad vectors deficient in E1A gene with compromised Rb antagonizing function can also mediate oncolysis in both p53 mutated or non-mutated tumors [10].

Other approaches to target replication-competent adenovirus to tumors include the use of cancer specific promoters such as survivin promoter or human telomerase (hTERT) promoter in the vector [11,12], as shown in Fig. 1. Human telomerase is activated in ~90% cancers, but is not expressed in somatic tissues [13,14]. Replication-competent Ad under the hTERT promoter control outperformed the non-replicating Ad vector expressing herpes virus thymidine kinase (hsvTK) with prodrg of gancicovir treatment in a fibrosarcoma model [15]. In addition, the co-injection into tumors with tumor-selective replicating Ad and replication-incompetent Ad vector carrying hsvTK led to replication of both vectors and synergistic efficacy [15]. Coupling oncolytic Ad with cell suicide genes has shown better efficacy compared to oncolytic virus alone or single suicide gene therapy vector [15,16].

Fig. 1. Construction of viral vectors to inhibit tumor metastasis and growth. Suitable transgenes and promoters are two major components of therapeutic viral vectors.

Ad vectors deficient in E1B (Onyx-015) have been shown in phase I and phase II clinical trials to replicate preferentially in tumor sites in oral carcinoma and liver cancers [17,18]. In another phase II trial, intra-arterial infusion of oncolytic Ad Onyx-015 in combination with chemotherapy drug 5-FU also demonstrated regression of metastatic gastrointestinal carcinoma as well as enhanced anti-tumor immune responses induced by the virus [19]. A genetically modified Ad expressing normal human gene p53 named H101 by Shanghai Sunway Biotech is the first oncolytic vector to be approved by a regulatory agency. It gained approval as a clinical treatment for head and neck cancer in China in 2005. With the treatment of H101 plus chemotherapy, the short-term responses are nearly doubled when compared to chemotherapy alone, while the overall survival rates are not known yet [2].

Although Ad shows high transduction efficiency in many types of cells, the most commonly used Ad, AdHu5 infect cells through cокsackie adenovirus receptor (CAR). Many cancers such as ovarian cancer, pancreatic cancer, and gastrointestinal cancer do not express CAR, which reduces the efficacy of Ad based vectors in cancer gene therapy. However, some serotypes of Ad are not dependent on CAR to target the cell, such as AdHu35 and chimpanzee Ad C1, which use CD46 for initial binding [20]. CAR independent Ad can be generated as a replicating viral vector in cancer gene therapy with efficient target transduction and efficient replication, as shown in Fig. 2. Chimpanzee Ad has a comparable excellent immunogenicity to AdHu5 virus, and also has many serotypes that could be used for heterologous re-administration to bypass pre-existing neutralizing antibodies in vivo [21,22]. Our lab has

Fig. 2. Approaches for improving therapeutic viral vectors. New viral vectors for the treatment of metastasis and growth of cancers can be improved by combination therapy with other anti-tumor agents, novel anti-cancer targets and more regulations, and enhanced tropism for tumors.
developed Ad vectors including rare human serotype such as AdHu26 and Ads from chimpanzee [23,24]. The virus yields of our Ad vectors normally can be greater than $2 \times 10^{11}$ virus particles from cultures of commonly available HEK 293 cells (40 T175 flasks), which carry adenoviral gene E1 supporting viral replication. These viruses can be produced to high purity with a viral particle–infectious unit ratio below 200 [24]. Compared to other viral vectors such as adeno-associated virus vectors, the expression of therapeutic transgene is transient with adenoviral vectors due to vigorous immune response against Ad vector-transduced cells [3]. Thus, as a cancer gene therapy agent, Ad vectors are more suitable as replication-competent oncolytic vectors or alternatively as vaccines to stimulate immune responses to tumors, which have widely been tested by other groups and ours.

3. Herpes simplex virus

Herpes simplex virus (HSV) belongs to herpesviridae family. It’s a double-stranded, linear DNA virus. HSV can infect and replicate in a wide scope of epithelial cells and neurons, including cancer cells, via binding to host cellular receptors HVEM, nectin 1 or heparin sulfate moieties [25].

Attenuated herpes virus strains have been adapted as oncolytic viral vectors against tumors while reducing cytopathogenicity in normal cells. Some virulence genes that were mutated in oncolytic HSV vectors include ICP4 and γ34.5. HSV immediate early gene ICP4 is required for lytic gene expression and for viral reactivation from latency, and US3 blocks apoptosis of the infected cell to support viral replication with similar functions as the cellular enzyme PKR [26–30]. γ34.5 is a major determinant of viral replication in CNS and neuro-pathogenicity of HSV, and it interacts with cellular phosphatase PTPα to dephosphorylate eIF-2α to support protein synthesis during infection [31,32]. UL39 encodes the large subunit of ribonucleotide reductase, and mutant virus lacking UL39 can only replicate productively in dividing cells [33–36]. Intratumoral injections of HSV vectors G207 lacking γ34.5 and UL39 functions have been shown to kill tumors and spare normal cells in models of human glioblastoma, gallbladder carcinoma, and breast cancer metastasis [37–39]. Variant of the vector with deletion of γ34.5 and LAT, a gene which promote cell survival and viral reactivation during HSV latency, also reduces glioma tumor progression while not lysing normal cells [40].

Promoter targeting of oncolytic HSV has similarly been shown to provide more selective viral replications in tumors than in normal cells. Mucin gene (MUC) transcripts are overexpressed in a variety of carcinomas such as pancreas, ovarian, and breast cancers [41–43]. Mutant HSV vector expressing γ34.5 under the control of MUC1 promoter (DF3 γ34.5) showed restricted replication in human liver tumors, as well as less cytotoxicity in MUC1-negative cells compared to attenuated virus deleted of γ34.5 or UL23 [32]. In MUC-negative cells, mutant DF3 γ34.5 virus did not exceed the toxicity of HSV viruses deficient in both UL39 and γ34.5, but in MUC-1 overexpressing tumor cells resulted in greater oncolysis compared to the double mutant HSV vectors. These results demonstrate cancer-specific promoter-restricted oncolytic HSV vector can be safer than attenuated HSV as well as increasing cytopathogenicity on cancer cells expressing the promoter [32]. In addition, combination of oncolytic HSV vectors with cytokine gene therapy (second-generation HSV vectors) can further enhance the therapeutic efficacy. In murine model of lung squamous cell carcinoma and metastatic colorectal cancer, treatment with oncolytic HSV carrying IL-12 is shown to be most effective in primary tumor and metastases regression as well as to be able to elicit production of IFN-γ or infiltration of CD8+ T cells in tumors compared to vectors carrying GM-CSF or no cytokine [44–46]. Oncolytic HSV vectors expressing IL-12 (NV-1042) can protect against recurrence of murine liver cancer after surgery intervention, whereas vectors not expressing the cytokine cannot [47]. IL-12-producing oncolytic HSV vectors can also better inhibit angiogenesis in the tumors compared to vectors without IL-12 gene transfer. These results show that combination of cytokine gene transfer and oncolytic HSV vectors can result in more optimal in vivo immune responses against tumor metastases and growth after administration [48].

It was shown in human clinical trials that injections into blood vessels or tumors with oncolytic HSV lead to tumor disease stabilization and regression [49–53]. The administration of vectors was well-tolerated. Oncolytic HSV viruses preferentially replicate and persist in the tumor site for a long time after administration, where antiviral immune response may provide an additive effect on the efficacy of oncolytic therapy on tumors [50,51,54]. Intratumoral injections of second-generation oncolytic HSV vectors carrying GM-CSF result in tumor regression in patients with various cancers, including breast, head and neck, melanoma, or colorectal cancer refractory to prior treatments [52,53]. HSV replicated in tumor sites, and injected as well as un-injected tumors both had inflammation and signs of necropsy, showing the complex, combined efficacy of immune responses and viral oncolysis on tumors [52,53].

As most humans are infected with HSV, pre-existing neutralizing antibodies could reduce the efficiency of intravenous or intratumoral delivery of HSV vectors. Thus, plasmapheresis may be needed to remove antibodies to allow efficient tumor transduction with HSV vectors.

4. Poxvirus, new castle virus, reovirus

Attenuated double-stranded DNA viruses from poxvirus family, such as vaccinia virus, and modified vaccinia virus Ankara strain (MVA), have been developed as oncolytic viruses. Vaccinia virus is more cytopathic and immunogenic than MVA, which result in greater T cell and B cell response to the viral antigens and carried transgene product [55,56]. The cellular receptors for poxviruses are not well-known, although some evidence suggest chemokine receptor for the myxoma virus and glycosaminoglycans, and lamins are involved [57,58]. Studies show that cellular permissiveness to viral infections are restricted at the intracellular signaling level such as through antiviral interferon and PKR signaling and growth signaling ERK1/ERK2 [57]. Both vaccinia viruses and MVAs have been deleted of multiple viral virulence genes in order to produce viruses that can replicate selectively in tumors by viruses of over-activation of cellular growth pathway such as EGFR-ras and loss of cellular interferon response [59–62]. Support for the oncolytic poxviruses has led to the phase I clinical trial where engineered vaccinia virus XJ-594 expressing GM-CSF showed partial responses in metastatic and primary liver cancers [63]. Clinical trials with modified MVA expressing tumor antigen 574 also showed some anti-tumor responses in phase II trials, but in a randomized phase III trial for renal carcinoma with 733 patients, there was no clear benefit of MVA administrations compared to placebo control [64,65].

New castle virus as well as reovirus, both RNA viruses, have also been explored as oncolytic viral vectors. These viruses were oncolytic due to their preferential replication in tumor cells from normal cells [66,67]. Cellular receptors for reoviruses include sialic acids and junction adhesion molecule (JAM), while new castle viruses use glycoproteins and sialic acids [68–71]. Both viruses replicate selectively in tumors due to the dysregulated cellular pathways, such as overactivated ras activation pathway or loss of anti-interferon response to support selective viral replications [66,67,72]. In addition, there are natural human diseases associated with wildtype virus infections that are less severe or considered benign. Phase I and phase II human clinical trials have supported the use of these viruses as oncolytic agents where they demonstrated safety as well as regression of disease burden in metastatic tumors [66,73,74]. Both immune responses generated by the oncolytic vectors and direct lysis of tumors contribute to clinical improvement. New modifications on the vector such as improving the sensitivity to type I interferon or expressing IL-2 for combined immune gene therapy have generated vectors with greater tumor selectivity or tumor-lysis ability [75,76].
5. Future Direction

Plasmapheresis may be required for repeated treatments with oncolytic vectors. New viral vectors for the treatment of metastasis and growth of cancers can be improved by enhanced tropism for tumors, novel anti-cancer targets and more stringent regulations on replication, and combination therapy with other anti-tumor agents, as shown in Fig. 2. The improvements in these areas would lead to safer viral vectors with greater antimetastasis efficacy. Also, it would be necessary for the clinicians to actively monitor serum inflammatory cytokines and viral loads due to active viral replications with oncolytic viruses.

5.1. Enhanced tropism for tumors

Modifications of viruses with greater specificity for tumor cell transduction and replication will lead to safer profile and fewer adverse events associated with applications of vectors. Integrins αvβ are expressed on a variety of cells, including ovarian and renal tumor cells. The incorporation of integrin-targeting peptide Arg-Gly-Asp (RGD) on Ad viral capsid enhances the oncologic capabilities of the replication competent Ad [77–80]. The modified capsid protein allows Ad to replicate in CAR receptor-deficient tumor cells and transduce renal and ovarian tumor cells that were previously poorly targeted by oncolytic Ad.

Pseudotyped alternative serotypes can also generate viral vectors with novel tropism for tumor cells [80]. Alternative human Ad serotypes 35 and 3 can be combined with human Ad serotype 5 genome, with selective expression of alternative serotype Ad knob involved in cellular receptor binding [chimeric virus production], to produce Ad with CAR-receptor-independent tropism of the serotype 3 or 35 [80,81]. Genetic combinations of viral capsids from alternative serotypes with common serotypes can also generate vectors with novel tropism. In mosaic virus production, alternative serotype capsid and human serotype 5 capsid are co-expressed in the vector-producing cell. Human Ad serotype 5 modified with serotype 3 capsid has been shown to be able to transduce and lyse breast cancer stem cells [82]. The use of chimpanzee Ad can circumvent the preexisting immunity to human Ad in humans to provide more efficient tissue transduction, albeit their tropisms for individual tumor types remain to be determined [23]. Chimpanzee Ad can also mediate CAR-independent tissue transduction [20].

Additional approaches to modify the viral tropism include the use of “bridging molecule” or “targeting antibody” to enhance transduction of tumors expressing the target recognized by the bridging molecule/antibody. Incorporation of Ad capsid with Fc binding motif of Staphylococcus protein A enables the virus to bind to monoclonal antibody targeting carcinoembryonic antigen (CEA) [80,83]. Ad virions can also be biotinylated to allow for binding with avidin or strepavidin-conjugated targeting antibodies [84]. For other viruses, such as herpes virus or MVA, the antibody-mediated receptor-retargeting would be feasible with genetic engineering on non-essential viral components. For replicating oncolytic viral vectors, it would be necessary to incorporate the targeting molecule/antibody in the viral genome to be synthesized in situ for continuous “targeted” viral replications. Better targeting would permit for more efficient and precise transduction in systemic administrations [80]. This antibody-mediated transduction system expands greatly the in vivo targets for viral vectors by bridging with the plethora of cancer cell-targeting antibodies that are already currently available for many cancers.

5.2. Novel anti-cancer targets and more stringent regulations

MicroRNAs, recently discovered non-coding RNAs, represent a new class of therapeutic targets from the traditional anti-tumor p53 or p21 gene. MicroRNAs can act as tumor suppressors or tumor oncogenes [85,86]. MicroRNAs can be directly incorporated into viral vectors for expression to inhibit tumor metastasis or growth. Systemic delivery of microRNA 34a (miR-34a) has been shown to lead to inhibition of clonal expansion of prostate cancer stem cell and inhibition of metastasis and growth of prostate cancers [87]. Viral vectors expressing the tumor suppressor microRNAs such as miR-34a would exert direct anti-tumor effect in addition to other carried transgenes such as immune genes IL-12 and GM-CSF or viral cytokopathy, miR-200 family (miR-200a, miR-200b, miR-200c, miR-141 and miR-429) and miR-205 have target sites on ZEB1, a transcriptional repressor of epithelial cell marker E-cadherin [88]. Loss of these microRNAs leads to epithelial-to-mesenchymal transition (EMT), a phenomenon enabling cancer cells to invade other tissues as well as resist apoptosis [89–92]. Loss of these microRNAs has been shown in metastatic breast cancer cells [88]. Expression of these microRNAs in cancer cells can suppress ZEB1 and EMT and restore cancer cell sensitivity to death inducing agent, such as 5-FU [92,93]. Inhibition of cellular targets in EMT could revert the aggressiveness of tumor progression and prohibit tumor cells from becoming undifferentiated like stem cells [6,7].

Alternatively, viral vectors can be customized to express anti-microRNA oligonucleotides important for tumor growth and metastasis. Inhibition of miR-10b (miR-10b) with oligonucleotides has been demonstrated to specifically inhibit breast cancer metastasis in mouse model [94]. Viral vectors expressing anti-microRNA transcripts such as miR-10b would be expected to inhibit tumor metastasis or growth. Anti-microRNAs oligonucleotides can be as short as the 19 bp target sequence of the microRNA, and thus can be applied broadly and simultaneously to many microRNA targets through overexpression.

Viral vector replication or carried transgene expression can also be further regulated for safety by RNA aside from promoter targeting. Let7-microRNA is expressed in many normal cells, but downregulated in lung carcinoma or colorectal cancer [86,95]. Incorporation of let7 microRNA complementary target sequence in viral transcripts has been shown to limit viral replication only in tumor cells [96]. mRNA 3′ UTR region can also be targeted to stabilize viral transcripts selectively in tumors through over-activated pathways such as ras pathway, in order to provide more restricted replications in tumor cells [97]. The incorporation of these RNA-based regulation systems with promoter targeting will further lead to safer and more tumor-selective viral vectors, which could reduce random adverse events due to off-target viral replications for patients.

5.3. Combination therapy

It has already been demonstrated that combination of radiotherapy or immune gene therapy with oncolytic vectors leads to greater reduction of tumor metastasis and growth [44,98,99]. Specific radiation or chemotherapy can be systemically explored with viral vectors to increase the anti-tumor effect with both agents [100]. The direct viral cytopathy with viral vectors often has synergistic effect on stimulating immune recognition of tumor cells as well as sensitizing tumors to previously refractory antitumor agents. It has been shown that viral lysis of tumor cells sensitizes tumor mass to chemotherapy agent cisplatin and reduces metastasis of ovarian cancer cells [101]. Coexpression of the ligand for extrinsic pathway of apoptosis, TRAIL, with oncolytic Ad could result in elimination of liver cancer cells that were not transduced by the viral vectors and that were of different genetic mutations targeted by the oncolytic vectors [102]. Agents promoting apoptosis such as TRAIL have shown efficacy in clinical therapies against cancers, and the combination of both viral vector-induced oncylasis and apoptosis-inducing agents would lead to enhanced anti-tumor efficacy.

Viral vectors could also be combined with cellular adoptive therapy for greater antitumor efficacy. Viruses could be used to infect anti-tumor T cells in vitro and adoptively transferred to patients. The infected anti-tumor T cells provide cell carriers that protect viruses from the neutralizing antibodies against the viral vectors that may be present in the systemic circulation [103]. It has been shown tumor-selective oncolytic vaccinia viruses loaded on the adoptively transferred anti-
tumor cytokine-induced killer cells (NK cells and T cells) were able to infect tumors in vivo and provide synergistic and enhanced anti-tumor efficacy in mice [104]. This approach would be benefited by the therapeutic advantages of both viral-based as well as T cell-based adoptive immunotherapy.

Cancer gene therapy holds great promise for treating cancers. By maximizing tumor cell transduction and minimizing side effect in normal cells, cancer gene therapy could provide tremendous therapeutic efficacy and safety for patients and outperform traditional cancer treatments. With further development on cancer biology research, vector engineering, and preclinical studies and clinical trials, more regulatory agency approved cancer gene therapy agents are sure to come.

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References


