Cancer gene therapy with oncolytic adenoviruses

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Summary

Metastatic cancer remains difficult to treat effectively and treatments are in most cases not curative despite significant side effects. Novel, targeted approaches such as gene therapy hold promise for the treatment of various tumor types. Among the most promising cancer gene therapy approaches are oncolytic adenoviruses, which are able to infect, replicate in and lyse tumor cells. Recent data from clinical trials with these vectors have shown that they are safe. However, antitumor efficacy needs to be improved to make oncolytic adenoviruses a viable treatment alternative for cancer patients. This review focuses on targeting strategies to improve tumor cell transduction and cancer cell selective replication. Strategies to improve antitumor efficacy by arming the virus with therapeutic transgenes are also discussed. Furthermore, an overview of the most important clinical approaches with oncolytic adenoviruses is given.

Key words: cancer gene therapy, clinical trials, oncolytic adenovirus

Introduction

Cancer is a global life-threatening disease with an estimated 10.9 million new cases and 6.7 million deaths worldwide in 2002 [1]. Thus, it is the second most common cause of death in developed countries and the fourth most common worldwide. Researchers around the world are investigating new approaches in an effort to treat cancer patients more effectively, and this has led to many promising preliminary results. However, in most cases cancer remains incurable, especially when metastatic. One of the most promising novel treatment approaches is cancer gene therapy which can be divided into different categories:

- Missing or altered genes are replaced with their healthy counterparts. Because some missing or altered genes (e.g. p53) are involved in carcinogenesis and tumor growth, replacing them with their intact copies can be used to treat cancer.
- Insertion of genes into cancer cells that make them more susceptible to chemotherapy, radiotherapy or other treatments.
- Introduction of suicide genes into cancer cells. A pro-drug is then given to the patient, which is converted into a toxic drug by the pro-drug converting enzyme that is produced by the suicide gene. The toxic drug then kills the cancer cells containing the suicide gene and tumor cells surrounding them by the bystander effect.
- Gene therapy to improve the patient’s immune response to cancer.
- Inhibition of tumor angiogenesis with gene therapy approaches. This deprives the tumor of sufficient blood supply and therefore inhibits cancer development.
- Oncolytic viruses, such as adenoviruses, vaccinia virus, herpes simplex virus or measles virus.

Adenoviruses

Adenoviruses have been extensively characterized since their initial description in the early 1950s [2]. They are generally not considered to be highly pathogenic because adenoviruses are mostly associated with
self-contained respiratory infections, epidemic conjunctivitis and infantile gastroenteritis [3]. Adenoviruses are divided into 51 different serotypes [4] all of which are nonenveloped viruses with an icosahedral capsid mostly made of the proteins hexon, penton base and knobbed fiber [5]. The linear double-stranded DNA genome is about 36 kb in size and consists of early (E1-E4) and late genes (L1-L5) which are transcribed before and after virus DNA replication respectively [5]. The major advantages of adenoviruses for use in gene therapy are: 1) a reasonable understanding and characterization of adenovirus biology; 2) relatively low pathogenicity in humans; 3) ability to infect both dividing and quiescent cells; 4) capacity to accommodate a large genetic payload; 5) low risk for insertional mutagenesis due to infrequent integration into the host cell genome; and 6) relatively simple genetic manipulation of the virus genome and easy high titer production [6].

Replication deficient adenovirus serotype 5 has been widely used as gene transfer vehicle in basic research as well as in gene therapy approaches for various diseases including cancer. However, adenoviral vectors coding for therapeutic transgenes have only had limited success in clinical trials for cancer, which is probably due to inefficient tumor penetration. Therefore, strategies to enhance transduction to cancer cells have been developed. Moreover, to improve tumor penetration, replicating oncolytic adenoviruses have been studied and to further enhance their antitumor efficacy, oncolytic adenoviruses have been armed with therapeutic proteins. In an effort to reduce potential side effects, transcriptional targeting methods have been used to achieve tumor cell selective gene expression and/or replication.

**Transductional targeting**

Adenoviruses efficiently transduce a wide range of epithelial tissues. Virus tropism *in vitro* is mainly determined by recognition of the primary receptor, which is the coxsackie-adenovirus- receptor (CAR) for the widely used serotype 5 adenovirus [7]. Since efficient gene transfer is the basis for successful cancer gene therapy, low CAR expression on tumor cells may be a major challenge [8]. To overcome CAR deficiency, adenoviruses can be transductationally retargeted by adapter-molecule based approaches or genetic manipulation of the virus capsid. Recent data, however, have suggested that *in vivo* tropism may be determined by completely different aspects, such as coagulation factors FIX and FX [9-11]. However, mouse data remain to be correlated to humans.

Adapter-molecule based targeting is based on a molecule that crosslinks the adenovirus particle with an alternative cell surface receptor. This targeting approach therefore represents a two-component system, which is a potential drawback in the context of clinical application. The stability of such two-component systems in humans is not well known and the effects the adapter molecule itself has in organisms should be studied first. A one-component system, such as genetic capsid modifications, which is more stable, might therefore be more convenient.

So far, three different genetic capsid manipulation strategies for retargeting adenoviruses have been developed: the so-called “fiber-pseudotyping”, ligand incorporation into the fiber knob and “dé-knobbing” of the fiber coupled with ligand addition [12].

Fiber-pseudotyping was first accomplished by Krasnykh et al. who replaced the knob of a serotype 5 adenovirus with a serotype 3 knob (Figure 1) [13]. The adenovirus 3 receptor is still disputed but CD46 [14], CD80 and CD86 [15] as well as an additional unknown receptor X [16] and heparan sulfate proteoglycans [17] were shown to be involved in cell entry. 5/3 chimera viruses have displayed significantly enhanced transduction to tumor cells *in vitro* and *in vivo* in many types of cancer [18-21].

Other studies have revealed the feasibility of manipulating the C-terminus and the HI-loop within the fiber (Figure 1). Wickham et al. [22] added a polylsine tail to the C-terminus to mediate adenovirus binding to heparan sulfate proteoglycans (HSPGs), which are highly expressed on cancer cells [23]. Adenoviruses with 7 lysine residues at the C-terminus

![Figure 1. Adenovirus capsid modifications. Adenovirus based on serotype 5 with genetically engineered capsid modifications in comparison with the wild type knob: 5/3 serotype chimerism, RGD motif in HI-loop and polylsine chain at C-terminus.](image-url)
(pK7) have demonstrated improved transduction to cancer cells [20,21,24,25]. Another promising location for incorporation of targeting moieties is the HI-loop of the knob which is exposed towards the outside and can tolerate up to 100 amino acids [26]. Dmitriev et al. [27] inserted an arginine-glycine-aspartic acid (RGD) motif into the HI-loop, which resulted in enhanced infectivity of various cancer cell types [18,20,21,28]. An asparagine-glycine-arginine (NGR) motif incorporated in the HI-loop also demonstrated improved adenovirus transduction to cancer cells [29]. Wu et al. combined the polylysine tail at the C-terminus modification with RGD motif in the HI-loop incorporation and achieved increased transduction to CAR deficient cells [30].

Also other locations for incorporation of targeting moieties have been explored. Vigne et al. incorporated an RGD motif into the hexon monomer protein, achieving enhanced gene delivery to CAR deficient cells [31]. Furthermore, replacing the RGD motif of the penton base with receptor specific peptide motifs can target the adenovirus to different kinds of cancer tissue [32]. Also the pIX location was found to be useful for incorporating peptide motifs for retargeting or imaging purposes [33,34].

**Transcriptional targeting**

To achieve cancer cell selective adenoviral gene expression, transcriptional targeting can be employed. For cancer gene therapy purposes, tumor specific promoters can be used to control the expression of genes coding for peptides with antitumor activity. A vast number of tumor specific promoters have been used for cancer gene therapy [35,36]. Notable examples include the carcinoembryonic antigen (CEA) promoter for gastric and lung cancer [37,38], cyclooxygenase 2 (COX-2) promoter for gastric, pancreatic and ovarian cancer [39-42], and hypoxia response elements (HREs) for kidney cancer [43,44].

Most researchers have used tissue specific promoters in first generation adenoviral vectors to express certain transgenes. However, this kind of transcriptional targeting can also be applied to replicating adenoviruses, where the genes necessary for replication are placed under the control of the tissue-specific promoters. Replication and oncolytic effect should then be restricted to tumor tissue, thereby reducing possible toxicity. In these transcriptional targeting approaches the E1A gene cassette of oncolytic adenoviruses was placed under the control of various tissue-specific promoters, such as E2F [45], CXCR4 [46], hTERT [47] or HREs [44,48,49].

**Targeted conditionally replicating adenoviruses for cancer therapy**

Solid tumor masses are large and complex and therefore difficult to efficiently transduce with first generation replication-deficient viruses. Thus, oncolytic viruses may be more useful since they have the advantage of multiplying themselves inside the tumor and are therefore able to spread more efficiently and subsequently transduce more cancer cells. Transduction of cancer cells as the first step in the adenovirus life cycle will finally lead to cell lysis resulting in antitumor efficacy. However, administering wild type adenoviruses to human cancer patients might not be optimal because of possible uncontrollable replication in healthy tissue which could lead to severe toxicity. Therefore, transcriptional and/or transcriptional targeting methods have to be employed. Moreover, replication can be restricted to tumor cells by deleting adenoviral genes that are necessary for replication in normal cells but not in cancer cells. Viruses that have been rendered tumor-specific in this way are called “conditionally replicating adenoviruses” or oncolytic adenoviruses.

The first published oncolytic adenovirus was named dl1520 and is nowadays better known as ONYX-015, has two mutations in the E1B gene, which codes for the E1B-55kD protein [50]. p53 is one of the major tumor suppressor proteins and is activated upon virus infection causing cell cycle arrest or apoptosis. To avoid this cellular shutdown, adenoviruses have evolved countermeasures in the form of the expression of E1B-55kD. This protein binds and inactivates p53, leading to induction of S-phase-like state which is required for viral replication [3]. It was proposed and later disputed that deletion of E1B-55kD will render the virus unable to replicate in normal cells since p53 will initiate cell cycle arrest or apoptosis [50,51]. However, since most tumor cells have a defective p53 pathway no cellular shutdown occurs and the virus will be able to replicate [50]. It turned out that some tumor cells fail to support replication of E1B-55kD deleted viruses. One reason might be that the E1B-55kD protein is also responsible for preventing host mRNA nuclear export and therefore the E1B mutant viruses might fail to initiate host protein shutoff [52].

Another strategy to create oncolytic adenoviruses is to delete 24 bps in the constant region 2 of the E1A. The resulting E1A protein is not able to inactivate the function of the tumor suppressor/cell cycle regulator Rb anymore. The result is similar to that caused by dl1520 described in the previous paragraph: replication is attenuated in normal cells, however, in cancer cells with defective Rb pathways (practically
all human tumors) [53] replication is unhampered (Figure 2). Viruses featuring this 24 bp deletion were shown to be selective for cancer cells without losing their oncolytic potential [54,55].

To maximize cancer selective replication and minimize side effects, the described targeting methods can be combined. Guse et al. and Bauerschmitz et al. showed in a triple targeting approach that a conditionally replicating adenovirus, which in addition is transcriptionally and transcriptionally targeted, exhibited increased tumor cell selectivity while retaining oncolytic potency [42,44].

In theory, a targeted oncolytic adenovirus would selectively replicate in the tumor cells of a cancer patient until all of them are lysed. It could spread through blood vessels, find metastases, replicate in them and lyse them as well. Subsequently, the virus would not find any cells that allow replication anymore and would therefore be cleared from the system.

**Arming approaches for enhanced antitumor efficacy**

Targeted oncolytic adenoviruses have been shown to be safe in many preclinical models as well as in human clinical trials. However, treatment with virus alone has only rarely led to significant responses in patients with advanced cancers. This might be due to the complexity of large human tumors featuring stromal barriers as well as necrotic, hyperbaric, acidic and hypoxic regions, which are difficult to penetrate by oncolytic adenoviruses [56,57]. Furthermore, ambitious approaches to restrict replication through over-stringent methods might have resulted in “overly safe” adenoviruses that no longer have sufficient oncolytic potency to act as effective anticancer agents.

The efficacy of oncolytic adenoviruses can be enhanced by arming them with transgenes coding for therapeutic proteins. The advantage of this approach is that the expressed therapeutic protein has a different tumor cell killing mechanism from the oncolytic virus itself. Therefore, a wider range of cancer cell populations can be affected which might improve the overall antitumor efficacy.

Pro-drug converting enzymes, also known as “suicide genes”, have been used in several studies as therapeutic transgenes [58]. One of the most famous suicide genes is herpes simplex thymidine kinase (HSV-TK), which converts the non-toxic drug ganciclovir (GCV) into a cytotoxic metabolite. The active metabolite can spread into surrounding cells causing the so-called cytotoxic bystander effect. Some studies have shown that GCV enhances the antitumor

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**Figure 2.** Mechanism for cancer cell selective replication. Wild type adenovirus is able to replicate in normal cells since Rb is inhibited by the E1A protein (A). In a cancer cell with non-functional Rb replication occurs as well (B). An oncolytic adenovirus with a 24 bp deletion in E1A is unable to inhibit Rb and therefore no replication in normal cells occurs (C). However, an oncolytic adenovirus can replicate in cancer cells with mutated Rb (D).
efficacy of HSV-TK armed oncolytic adenoviruses [59,60]. However, others have reported that activated GCV might inhibit virus replication and therefore does not augment antitumor efficacy [61,62]. It may be that HSV-TK/GCV augments the efficacy of oncolytic virus only in the context of inefficient oncolysis [60]. Cytosine deaminase (CD) is a suicide gene that converts 5-fluorocytosine into a toxic metabolite. Oncolytic adenoviruses armed with CD have shown improved antitumor efficacy in several cancer models [63,64]. Oncolytic adenoviruses with both HSV-TK and CD combined with radiotherapy have also shown promising results in preclinical models [65,66] and in clinical trials [67,68].

Another promising approach is to arm oncolytic adenoviruses with antiangiogenic molecules since tumors are often highly vascularized and antiangiogenic therapies have demonstrated efficacy in cancer therapy [69]. Several groups have demonstrated improved antitumor efficacy with oncolytic adenoviruses featuring different antiangiogenic transgenes that target VEGF [44,70,71]. However, it has also been shown that combining antiangiogenic therapy with oncolytic adenoviruses can be counterproductive in certain animal models [72].

Another promising approach is to induce antitumor immunity by arming oncolytic adenoviruses with interleukins [73,74]. Furthermore, oncolytic adenoviruses expressing p53 have shown enhanced anticancer activity [75,76].

Clinical trials with oncolytic adenoviruses

ONYX-015 was the first targeted oncolytic adenovirus used in a phase I study resulting in 14% regression rate in head and neck cancer patients [77]. In combination with cisplatin and 5-FU, ONYX-015 resulted in tumor regression in 65% of the patients in a phase II trial [78]. A randomized phase III trial with H101 (an oncolytic adenovirus closely related to ONYX-015) in combination with chemotherapy was performed in 2004 in China reporting 79% response rate in the combination group vs. 40% in the chemotherapy-only group [79,80]. The Chinese regulatory agencies subsequently granted market approval for H101 to be used in combination with chemotherapy for the treatment of head and neck cancers, making H101 the first approved oncolytic virus product. Other selected clinical trials with oncolytic adenoviruses are listed in Table 1.

Also, oncolytic adenoviruses have been administered to cancer patients in compassionate use schemes with promising results in terms of safety and efficacy [81-84].

In summary, the available clinical data with oncolytic adenoviruses suggest excellent safety and thousands of patients have been treated without reported treatment-associated mortality. However, antitumor efficacy greatly varied depending on used virus, treated tumor type and administration route. Although some patients obtain tremendous benefit from treatment with available viruses [84,85], there is room for improvement with regard to the efficacy of the treatment in the context of the average patient.

Future prospects

Although the clinical data obtained thus far has suggested that oncolytic adenoviruses are safe for treatment of human cancer, it would be worthwhile to further enhance their antitumor efficacy. Efficient tumor transduction and penetration remain the major issues. Therefore, infectivity enhancement and other targeting strategies have to be further improved to ensure efficient cancer cell transduction and tumor penetration. Moreover, to increase the antitumor efficacy, various therapeutic transgenes for arming the virus are being investigated. With increasing understanding of tumor immunology it becomes apparent that modulating the host immune system to attack cancer cells might be an effective therapy approach. Thus, oncolytic adenoviruses have been armed with immunostimulatory proteins such as CD40L or GM-CSF, which have shown promising results in preclinical and clinical studies [86-88]. However, much work remains to be done to optimize these approaches.

Conclusions

Cancer remains a challenging disease to treat and is still often incurable in advanced stages. Therefore, novel and more efficient therapies have to be developed. Oncolytic adenoviruses have shown excellent safety and promising results in clinical trials with various tumor types. However, the antitumor efficacy of oncolytic adenoviruses alone has not been sufficient in all patients and therefore extensive research is being conducted towards improving infectivity, tumor penetration and oncolytic potency. Over the last years, many promising oncolytic adenovirus constructs have been developed which resulted in excellent data in preclinical models. It will be important and interesting to test these agents in clinical trials.
## Table 1. List of selected clinical trials with oncolytic adenoviruses

<table>
<thead>
<tr>
<th>Virus/treatment agents</th>
<th>Genetic modification</th>
<th>Phase</th>
<th>Administration route</th>
<th>Max. dose</th>
<th>Cancer type</th>
<th>Responses/ total number of patients</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>ONYX-015</td>
<td>E1B-55kD deletion</td>
<td>I</td>
<td>i.t.</td>
<td>1×10^{11} pfu</td>
<td>SCCHN</td>
<td>2/22</td>
<td>77</td>
</tr>
<tr>
<td>ONYX-015</td>
<td>E1B-55kD deletion</td>
<td>I</td>
<td>i.v.</td>
<td>2×10^{13} vp</td>
<td>Cancer metastatic to the lung</td>
<td>0/10</td>
<td>90</td>
</tr>
<tr>
<td>ONYX-015</td>
<td>E1B-55kD deletion</td>
<td>I</td>
<td>i.p.</td>
<td>1×10^{11} pfu/d on 5 days</td>
<td>Ovarian cancer</td>
<td>0/16</td>
<td>91</td>
</tr>
<tr>
<td>ONYX-015</td>
<td>E1B-55kD deletion</td>
<td>I</td>
<td>i.v., i.t.</td>
<td>3×10^{11} pfu</td>
<td>HCC</td>
<td>1/5</td>
<td>92</td>
</tr>
<tr>
<td>ONYX-015 + 5-FU + etarcept</td>
<td>E1B-55kD deletion</td>
<td>I</td>
<td>i.t.</td>
<td>1×10^{10} pfu</td>
<td>Glioma</td>
<td>3/24</td>
<td>94</td>
</tr>
<tr>
<td>CV706</td>
<td>PSA promoter controlling E1A</td>
<td>I</td>
<td>i.t.</td>
<td>1×10^{13} vp</td>
<td>Prostate cancer</td>
<td>5/20</td>
<td>96</td>
</tr>
<tr>
<td>Ad5-CD/TKrep + GCV/5-FU + radiation</td>
<td>E1B-55kD deletion</td>
<td>I</td>
<td>i.t.</td>
<td>1×10^{12} vp</td>
<td>Prostate cancer</td>
<td>15/15</td>
<td>67</td>
</tr>
<tr>
<td>ONYX-015 + 5-FU</td>
<td>E1B-55kD deletion</td>
<td>I-II</td>
<td>i.t., i.ha., i.v.</td>
<td>3×10^{11} pfu</td>
<td>HCC and colorectal cancer metastatic to the liver</td>
<td>3/16</td>
<td>97</td>
</tr>
<tr>
<td>ONYX-015</td>
<td>E1B-55kD deletion</td>
<td>II</td>
<td>i.t.</td>
<td>2×10^{11} vp on 10 days</td>
<td>SCCHN</td>
<td>5/40</td>
<td>98</td>
</tr>
<tr>
<td>ONYX-015 + cisplatin+5-FU</td>
<td>E1B-55kD deletion</td>
<td>II</td>
<td>i.t.</td>
<td>1×10^{10} vp/d on 5 days</td>
<td>SCCHN</td>
<td>19/37</td>
<td>78</td>
</tr>
<tr>
<td>ONYX-015 + gemcitabine</td>
<td>E1B-55kD deletion</td>
<td>I-II</td>
<td>i.t.</td>
<td>2×10^{11} vp/wk; 8 cycles</td>
<td>Pancreatic cancer</td>
<td>2/21</td>
<td>99</td>
</tr>
<tr>
<td>ONYX-015</td>
<td>E1B-55kD deletion</td>
<td>II</td>
<td>i.v.</td>
<td>2×10^{12} vp every 2 weeks</td>
<td>Metastatic colorectal cancer</td>
<td>0/18</td>
<td>100</td>
</tr>
<tr>
<td>H101+cisplatin/ adriamycin + 5-FU</td>
<td>E1B-55kD deletion</td>
<td>III</td>
<td>i.t.</td>
<td>1.5×10^{12} vp/d on 5 days</td>
<td>SCCHN</td>
<td>71/160</td>
<td>79</td>
</tr>
<tr>
<td>ONYX-015 + MAP + chemotherapy</td>
<td>E1B-55kD deletion</td>
<td>I-II</td>
<td>i.t.</td>
<td>5×10^{10} pfu</td>
<td>Sarcoma</td>
<td>1/6</td>
<td>101</td>
</tr>
</tbody>
</table>

CD: cytosine deaminase, HSV-TK: herpes simplex virus thymidine kinase, i.t.: intratumoral, i.v.: intravenous, i.p.: intraperitoneal, i.ha.: intrahepatic artery, 5-FU: 5-fluorouracil, MAP: mitomycin C + doxorubicin + cisplatin, pfu: plaque forming units, SCCHN: squamous cell carcinoma of the head and neck, vp: virus particles, HCC: hepatocellular carcinoma.

## References

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