Recent advances in oncolytic adenovirus therapies for cancer

Amanda Rosewell Shaw[1,2] and Masataka Suzuki[1,2]

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Oncolytic adenoviruses (Onc.Ads) selectively replicate in and lyse cancer cells and are therefore commonly used vectors in clinical trials for cancer gene therapy. Building upon the well-characterized adenoviral natural tropism, genetic modification of Onc.Ad can enhance/regulate their transduction and replication within specific cancer cell types. However, Onc.Ad-mediated tumor cell lysis cannot fully eliminate tumors. The hostile tumor microenvironment provides many barriers to efficient oncolytic virotherapy, as tumors develop structure and immune-evasion mechanisms in order to grow and ultimately spread. For these reasons, Onc.Ads modified to deliver structural or immune modulatory molecules (Armed Onc.Ads) have been developed to overcome the physical and immunological barriers of solid tumors. The combination of oncolysis with tumor microenvironment modulation/destruction may provide a promising platform for Ad-based cancer gene therapy.

Addresses
[1] Department of Medicine, Baylor College of Medicine, Houston, TX, USA
[2] Center for Cell and Gene Therapy, Baylor College of Medicine, Texas Children's Hospital, Houston Methodist Hospital, Houston, TX, USA

Corresponding author: Suzuki, Masataka (suzuki@bcm.tmc.edu)

Introduction

Oncolytic viruses (OVs) are promising cancer gene therapy agents, as they have the unique ability to selectively replicate in malignant cells, causing cancer cell lysis and inflammation, which in turn can stimulate host immune responses to cancer cells [1]. OV-induced necrosis results in the release of damage-associated molecular patterns (DAMPs), which stimulate tumor-infiltrating antigen presenting cells (e.g. dendritic cells) and subsequent adaptive immune responses [2]. However, solid tumors are complex, heterogeneous structures that impede OV-dependent oncolysis. OVs can be modified to increase their lytic potency by delivering modulatory molecule(s) ('Armed' OVs) that target the structure of the tumor microenvironment, thereby destroying malignant cells and also cells providing support for the growing tumor. Additionally, OVs can be armed with immunostimulatory molecules to further increase the development of anti-tumor immune responses. Recent clinical studies have demonstrated that Armed OVs such as herpes simplex virus (T-VEC), poxvirus (Pexa-vec), and adenovirus (ONCOS-102) can mediate clinical responses with few severe side effects [3]. The US FDA recently approved T-VEC expressing granulocyte macrophage colony-stimulating factor (GM-CSF) to treat melanoma [4]. Treatment of advanced melanoma with T-VEC was safe and resulted in a 10.8% complete response rate, significantly higher than systemic administration of GM-CSF alone [5••]. Thus, oncolytic virotherapy represents a new class of promising cancer immunotherapy agents. In this review, we will specifically discuss the application of adenoviral-based oncolytic viruses.

Specific transduction and replication of oncolytic adenovirus in cancer cells

Oncolytic adenoviruses (Onc.Ads) have long been studied and tested in patients with malignancies without severe side effects and several clinical trials are ongoing (Table 1) [3]. For effective oncolytic activity, Onc.Ads must specifically infect and efficiently replicate within cancer cells; however, many cancer cells do not express or downregulate the coxsackie and adenovirus receptor (CAR), resulting in decreased transduction of serotype 5 Ad (Ad5), which is commonly used for Ad-based vectors [6]. Therefore, Ad5 fibers, the capsid moiety responsible for virus–cell surface receptor interaction, have been modified to increase their transduction to cancer cells. An RGD-motif inserted into the fiber knob increases viral interaction with integrins, which are highly expressed on some cancer cells, including prostate [7] and ovarian cancers [8]. Ad5 fiber has also been replaced by other serotype fibers to redirect to different receptors. For instance, serotype 35 fibers bind CD46 [9], which is upregulated in breast and colorectal cancers, among others [10], while serotype 3 utilizes desmoglein 2, a component of the epithelial cell adhesion structure known to be overexpressed in multiple epithelial malignancies [11]. These fiber modifications have been utilized in both preclinical studies and clinical trials to enhance Onc.Ad transduction to malignant cells.

Table 1

Recently completed and current clinical trials using oncolytic adenoviruses

OncAd	Modification	Transgene	Target	Administration	Phase	ClinicalTrials. gov identifier
ICOVIR-5	E2F-E1A Δ24 RGD		Solid tumors	Mesenchymal stem cell delivery IV	I,II	NCT0184461
LOAd703	5/3 Δ24	CD40L & 4-1BBL	Pancreatic cancer	IT	I/IIa	NCT02705196
Ad5-yCD/ mutTKSR39rep-hIL12	E1B-55K	CD/TK hIL12	Prostate cancer	Intraprostatic	I	NCT02555397
ONCOS-102 w/ cyclophosphamide	5/3 Δ24	GM-CSF	Advanced neoplasms	IV	I	NCT01598129
VCN-01 w/or w/o Abraxane® and Gemcitabine	DM-1-E2F-E1A Δ24 RGD	Hyaluronidase	Advanced solid tumors	IV	I	NCT02045602
VCN-01 w/Abraxane® and Gemcitabine	DM-1-E2F-E1A Δ24 RGD	Hyaluronidase	Advanced pancreatic cancer	IV	I	NCT02045589
CG0070	E2F-E1A	GM-CSF	Bladder cancer	IV	III	NCT02365818
CG0070	E2F-E1A	GM-CSF	Bladder cancer	IV	II/III	NCT01438112
Colo-Ad1	Ad11p/Ad3		Colon, NSCLC, bladder, renal cancer	IT, IV	I	NCT02053220
DNX-2401 w/ Temozolomide	E1A Δ24 RGD		Glioblastoma multiforme	IT	I	NCT01956734
DNX-2401 w/IFNγ	E1A Δ24 RGD		Brain tumors	IT	I	NCT02197169
Ad5-yCD/ mutTKSR39rep-ADP w/IMRT	E1B-55K	CD/TK	Prostate carcinoma	Intraprostatic	II/III	NCT00583492
OBP-301	hTERT		Hepatocellular carcinoma	IT	I/II	NCT02293850

Abbreviations: CD, cytosine deaminase; GM-CSF, granulocyte macrophage colony-stimulating factor; IFNγ, interferon γ; IMRT, intensity-modulated radiation therapy; IT, intratumoral; IV, intravenous; NSCLC, non-small cell lung cancer; TK, tyrosine kinase; w/, with; w/o, without.

Onc.Ads have been genetically modified to allow for selective replication in cancer cells with abnormal protein expression patterns compared to normal cells. Adenovirus replication is initiated by E1A, the first adenoviral transcription unit. E1A gene products are responsible for dissociation of the retinoblastoma (Rb)/E2F complex, resulting in free transcription factor E2F activation of the remaining early transcription units, E1B, E2, E3, and E4 genes [12]. A commonly used Ad5-based Onc.Ad contains a 24 bp mutation in the E1A gene (E1AΔ24), which disrupts the retinoblastoma (Rb) binding domain and releases free E2F from Rb/E2F complex, resulting in an E1AΔ24 protein that cannot promote virus replication without free E2F [13]. Cancer cells, on the other hand, typically have high levels of free E2F resulting in the preferential replication of Onc.AdΔ24 in cancer cells. The Onc.Ads ICOVIR-5, -7, and -15 were developed to take advantage of excessive free E2F by regulating E1A transcription *via* the insertion E2F binding sites or E2F promoters [14], and these ICOVIRs still harbor E1AΔ24 as an additional safety switch. Onc.Ads have also been generated by replacing the native E1A promoter with a tissue-specific or cancer cell-specific promoter such as the tCCN1 promoter, which is active in prostate cancer [15•]. Although E1 gene regulation has improved cancer cell specific lysis and the safety of Onc.Ads *in vitro* and in preclinical xenograft models, human solid tumors are more complex, as discussed below.

Physical barriers to oncolytic adenovirus dissemination within solid tumors

Solid tumors are heterogeneous structures made up of malignant cells that recruit normal cells such as immune cells, fibroblasts, and endothelial cells to promote tumor growth (Figure 1). Additionally, the presence of dense stromal tissue and high amounts of extracellular matrix (ECM) results in high fluid pressure in tumors, which inhibits the ability of Onc.Ads to spread throughout the tumor, thus limiting their effectiveness. Therefore, to enhance viral spread Armed Onc.Ads have been generated to target the ECM and angiogenesis. Onc.Ads expressing molecules such as relaxin [16] and hyaluronidase [17••] to disrupt the ECM have shown promising preclinical results. Onc.Ad expressing relaxin, known to upregulate matrix metalloproteinases (MMPs), successfully increased viral spread in multiple tumor models [18]. VCN-01, an Onc.Ad armed with hyaluronidase, was able to decrease the presence of hyaluronic acid, a component of the ECM, and prolong survival in two orthotopic murine glioma models [17••]. Contrastingly, Onc.Ads have also been armed with inhibitors of metalloproteinases (TIMPs), as these molecules inhibit the degradation of the ECM to affect tumor cell proliferation, migration, and angiogenesis. An Onc.Ad expressing TIMP2 had increased viral replication in primary ovarian tumor tissue; however, viral distribution was not evaluated [19]. Lucas *et al*. recently reported that a modification