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Review

A compendium of adenovirus genetic modifications for enhanced replication, oncolysis, and tumor immunosurveillance in cancer therapy

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ABSTRACT

In this review, we specifically focus on genetic modifications of oncolytic adenovirus 5 (Ad5)-based vectors that enhance replication, oncolysis/spread, and virus-mediated tumor immunosurveillance. The finding of negative regulation of minor core protein V by SUMOylation led to the identification of amino acid residues, which when mutated increase adenovirus replication and progeny yield. Suppression of Dicer and/or RNAi pathway with shRNA or p19 tomato bushy stunt protein also results in significant enhancement of adenovirus replication and progeny yield. Truncation mutations in E3-19K or i-leader sequence or overexpression of adenovirus death protein (ADP) potently increase adenovirus progeny release and spread without affecting virus yield. Moreover, E3-19K protein, which was found to inhibit both major histocompatibility complex I (MHCI) and MHC-I chain-related A and B proteins (MICA/MICB) expression on the cell surface, protecting infected cells from T-lymphocyte and natural killer (NK) cell attack, may be tailored to selectively target only MHCI or MICA/MICB, or to lose the ability to downregulate both. At last, E3-19K protein may be exploited to deliver tumor-associated epitopes directly to the endoplasmic reticulum for loading MHCI in the transporter associated with antigen processing (TAP)-deregulated cells.

1. Introduction

Human adenovirus 5 (Ad5 together with Ad2 belong to the species C of the family *Adenoviridae*) is a non-enveloped virus with stable double-stranded linear DNA genome (≈36 kilobase pairs in length), which shows a low rate of spontaneous mutagenesis (1.3×10^{-7} per base per cell infection cycle) (Risso-Ballester et al., 2016). In the infected cells, the Ad5 genome persists episomally that prevents from adverse insertional mutagenesis inherent to the DNA integrating viruses (Lee et al., 2017). Ad5 can be armed with several therapeutic transgenes, produced and enriched to high titers (1×10^{11}–1×10^{13} viral particles/ml) (Dobbins et al., 2015; Lee et al., 2017). Ad5-based vectors are well-clinically tested and safe, and have been used in about a quarter of all gene therapy clinical trials (Appaiahgari and Vrati, 2015; Lee et al., 2017). In permissive tumor cells, oncolytic Ad5 infection cycle results in cell lysis (oncolysis) and release of multiple progeny virions, which are able to infect adjacent cells (Barry et al., 2011). In cancer clinical trials, the therapeutic efficacy of oncolytic Ad5-based vectors have been intensively explored (Pesonen et al., 2011; Rosewell Shaw and Suzuki, 2016; Toth and Wold, 2010; Ulasov et al., 2014).

Abbreviations: Ad, adenovirus; ADP, adenovirus death protein; CAR, coxsackievirus and adenovirus receptor; KIR, killer inhibitory receptor; MHCI/HLA, major histocompatibility complex class I; MICA/MICB, MHC-I chain-related A and B proteins; MLP, major late promoter; MTT, tetrazolium dye; NK, natural killer cells; NKT, natural killer T cells; PKR, double-stranded RNA-activated kinase; RISC, RNA-induced silencing complex; SUMO, small ubiquitin-like modifier; TAP, transporter associated with antigen processing; VA RNA, virus-associated RNA

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Table 1
A compendium of adenovirus genetic modifications for enhanced replication/progeny yield, oncolysis/spread, and tumor immunosurveillance in cancer therapy.

Target	Modification	Main effects	References
Protein V	K7R, K23R, K24R, and K162R	Increased replication and progeny yield	(Freudenberger et al., 2017)
miRNA pathway	P19 expression	Increased replication, progeny yield, and oncolytic activity	(Rauschhuber et al., 2013)
Dicer protein	Downregulation by shRNA	Increased replication, progeny yield, and oncolytic activity	(Machitani et al., 2017, 2016)
E3-19K protein	C11S, C28S, C22S or C83S	Increased expression of MHCI and MICA/B on the cell surface	(McSharry et al., 2008; Sester and Burgert, 1994)
	Y93G	Abolished the interaction with MHCI	(Fu and Bouvier, 2011)
	W52A, M87A or W96A	Selectively increased expression of MHCI on the cell surface	(Sester et al., 2010)
	T14A or M82A	Selectively increased expression of MICA/B on the cell surface	(Sester et al., 2010)
	Insertion of a furin-cleavable linkers and exogenous epitope(s) after the N-terminal signal sequence	Increased immunogenicity and antitumor cytotoxic T cell responses in a TAP-independent manner	(Rodríguez-García et al., 2015)
	C-terminal truncation, ORF shift: SRRSFIEEKKMP 149–160 → KQTQLYTer	Relocalization of E3-19K protein to the cell surface; enhanced virus release and spread	(Gros et al., 2008)
I-leader protein	C-terminal truncation: Q108Ter	Enhanced virus release, spread, and oncolytic activity	(Subramanian et al., 2006)
	C-terminal truncation: Q125Ter		(van den Hengel et al., 2011; Yan et al., 2003)
	C-terminal truncation: E133Ter		(Puig-Saus et al., 2014, 2012)
Adenovirus death protein (ADP/E3-11.6K)	(Over)expression	Enhanced virus release, spread, and oncolytic activity	(Doronin et al., 2000; Gros and Guedan, 2010; Kim et al., 2003; Yun et al., 2005; Zou et al., 2004)

During last two decades, the research community's efforts have been directed at the design of conditionally-replicating adenoviral vectors (replicating mainly in tumor but not normal cells) largely based on serotype 5 with modified tropism (utilization for cell entry of other receptors than its primary native coxsackievirus and adenovirus receptor, CAR), the increased transductional, replicative and lytic efficiency, enhanced intratumoral spread and immune-stimulating properties to overcome the intratumoral radio/chemotherapeutic resistance, phenotypic and genetic heterogeneity and markedly immunosuppressive microenvironment. The genetic and non-genetic approaches to modify Ad5 tropism, increase the transduction efficiency, restrict replication selectively to tumor cells and overcome preexisting antibody-mediated immunity are discussed elsewhere (Alonso-Padilla et al., 2016; Coughlan et al., 2010; Panek et al., 2017; Sonabend et al., 2006; Stepanenko and Chekhonin, 2018; Yoon et al., 2016; Zhang and Ehrhardt, 2017). In this review, we specifically focus on genetic modifications of Ad-based vectors, which were found to enhance replication/progeny yield, oncolysis/spread, and virus-mediated tumor immunosurveillance (Table 1).

2. The genetic modifications for enhanced adenovirus replication

2.1. Protein V

SUMOylation is a reversible post-translational protein modification, which plays essential roles in many cellular functions. Ad5 modulates the host SUMOylation system. The adenoviral E1B-55K and E4-ORF3 proteins are small ubiquitin-like modifier (SUMO) E3 ligases, inducing SUMOylation of cellular proteins by conjugating SUMO to lysine residues of substrate proteins. Additionally, adenoviral E1A protein associates with the SUMO machinery, while E1B-55K protein itself is a SUMOylation target (reviewed in (Sohn and Hearing, 2016). Recently, Freudenberger et al. revealed that minor core protein V, which binds the viral DNA and protein VI and is involved in the assembly of infectious virions, linking the nucleoprotein core to the capsid (Pérez-Vargas et al., 2014), is also SUMOylated at multiple amino acid residues. Replacing the SUMOylation lysine residues K7, K23, K24, and K162 by arginine residues reduced accumulation of core protein V at the host nucleoli, where wild type protein largely resides. More importantly, these four point mutations increased virus replication and progeny yield. Different viral mRNAs and proteins were expressed/produced earlier and/or more abundantly, and only the amount of mutated core protein V was reduced. It seems that virus internalization into the cells might have been also enhanced, since more viral DNA was isolated from the mutant virus-infected cells already at 1 h post infection (Freudenberger et al., 2017).

2.2. P19 protein

The tomato bushy stunt virus, a member of the *Tombusvirus* genus in the *Tombusviridae* family, encodes p19 protein, which is a suppressor of RNA silencing and functions to sequester small RNA duplexes, impairing their load onto a RNA-induced silencing complex (RISC consists of Dicer, the Argonaute proteins (Ago), TAR RNA-binding protein (TRBP) and protein activator of PKR (PACT) (Danielson and Pezacki, 2013; Scholthof, 2006). P19 protein suppresses RNAi pathway in diverse systems, which are not a host for tomato bushy stunt virus, including human, insect, *Arabidopsis thaliana*, *Caenorhabditis elegans*, and *Drosophila* cells (Scholthof, 2006). P19 protein exhibits size-specific and sequence-independent interaction with its small RNA ligands, binding with high affinity to duplexes 20–22 nucleotides long (Danielson and Pezacki, 2013). Rauschhuber et al. inserted the *P19* cDNA into the Ad5 genome under the control of the major late promoter (MLP) by connecting it to the fiber gene sequence via a spacer and internal ribosomal entry site (IRES) sequence. The RNAi suppressor p19 significantly enhanced Ad5 replication, viral mRNA and protein expression, substantially improved production of recombinant adenoviral particles and enhanced killing of tumor cells (Rauschhuber et al., 2013). Additionally, HEK293 cells (reviewed in (Stepanenko and Dmitrenko, 2015a) stably transfected with a plasmid harboring the *P19*-encoding sequence produced increased yields of recombinant Ad vectors (Rauschhuber et al., 2013). These results suggest that the cellular miRNAs contribute to anti-Ad defence. It is worth noting that although Ad5 infection is accompanied by global deregulation in miRNA expression, only a few miRNAs were confirmed to potently suppress (e.g., hsa-miR-27) or increase (e.g., hsa-miR-26b) Ad replication (Piedade and Azevedo-Pereira, 2017).

2.3. Dicer protein

Ad5 express two non-coding virus-associated RNAs, VA RNAI and VA RNAII, which are essential for efficient virus replication. During