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Bio-inspired polymer envelopes around adenoviral vectors to reduce immunogenicity and improve *in vivo* kinetics

Gerui Fan [1], Minmin Fan [1], Qin Wang, Jiayu Jiang, Yu Wan, Tao Gong, Zhirong Zhang, Xun Sun *

Key Laboratory of Drug Targeting and Novel Drug Delivery Systems, Ministry of Education, West China School of Pharmacy, Sichuan University, Chengdu, Sichuan 610041, PR China

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ABSTRACT

Adenoviral vectors have attracted substantial interest for systemic tumor gene therapy, but further work is needed to reduce their immunogenicity and alter their biodistribution before they can be used in the clinic. Here we describe a bio-inspired, cleavable PEGylated β-cyclodextrin-polyethyleneimine conjugate (CDPCP) that spontaneously coats adenovirus in solution. This cleavable PEG coating reduces the innate and adaptive immunogenicity of adenovirus particles, as well as improves their biodistribution away from the liver and into the tumor. Insertion of a matrix metalloproteinase substrate sequence into the conjugate allows PEG cleavage at the tumor site, simultaneously reducing liver biodistribution and increasing transgene expression in tumors, thereby avoiding the "PEG dilemma". Cationic β-cyclodextrin–PEI not only provides electrostatic attraction to promote envelope attachment to the viral capsid, but it also improves vector internalization and transduction after PEG cleavage. These results suggest that CDPCP may help expand the use of adenoviral vectors in cancer gene therapy.

Statement of significance

The synthesized β-cyclodextrin–PEI-MMP-cleavable-PEG polymer (CDPCP), held great potential for gene therapy when applied for adenovirus coating. The β-cyclodextrin–PEI provided a powerful electrostatic attraction to attach the whole polymer onto the viral capsid, while the MMPs-cleavable PEG reduced innate and adaptive immunogenicity and improved the biodistribution of adenovirus vectors due to the tumor-specific enzyme triggered PEG cleavage. More importantly, an ingenious cooperation between the two components could solve the PEG dilemma. The CDPCP/Ad complexes exhibited a comprehensive and valued profile to be a candidate vector for future tumor gene therapy, we believe the current investigation on this kind of biomaterial may be of particular interest to the readership of Acta biomaterialia.

1. Introduction

After studies into gene therapy began in the 1980s, adenovirus became an obvious candidate gene vector because of high transgene expression efficiency, non-integration to the host genome, ability to infect dividing and non-dividing cells and feasibility of making high-titer stocks [1]. However, using adenoviral vectors in some gene therapy trials led to unacceptable severe adverse events including toxicity and complications [1,2], which has limited the therapeutic use of adenovirus vectors [3].

Soon after injection, adenovirus vectors appear to provoke innate immune responses involving macrophages and dendritic cells [4,5]. Much of the remaining virus is then inactivated quickly and specifically by preexisting neutralizing antibodies [6]. The few virus particles that survive these responses enter the circulation and often associate with blood coagulation factors IX and X [7,8], leading to adenovirus accumulation in the liver and concomitant hepatotoxicity [9].

In an effort to reduce the immunogenicity of adenovirus vectors and improve the kinetics of gene delivery, researchers have modified the vectors with specially designed conjugates or coatings/envelopes based on polyethylene glycol (PEG), poly-N-(2-hydroxy propyl)methacrylamide (pHPMA), poly(ethylenimine) (PEI), poly (L-lysine) (PLL), and chitosan [10–15]. PEGylating adenovirus has been shown to prolong blood clearance, reduce liver biodistribution as well as weaken antibody-mediated neutralization and innate immune responses [10]. The kinetics of adenovirus-mediated gene delivery depends on the density of PEGylation [16,17], which was not high in most previous studies because

* Corresponding author at: West China School of Pharmacy, Sichuan University, No. 17, Section 3, Southern Renmin Road, Chengdu 610041, PR China.
E-mail address: xunsun22@gmail.com (X. Sun).
[1] These authors contributed equally to this work.

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PEG chains were attached only to exposed lysines on the adenovirus surface [18–22]. While PEGylation slows adenovirus biodistribution and interactions with blood factors, it also severely reduces adenovirus transduction efficiency in coxsackie-adenovirus-receptor (CAR)-positive or -negative cells through non-specific steric hindrance [23,24]. The simultaneous ability of PEGylation to reduce liver biodistribution and lower transduction efficiency in tumors is called the "PEG dilemma" [25].

To solve this dilemma, researchers have investigated bio-inspired cleavable PEGylation, in which endogenous factors cleave the PEG chain off the vector after it has travelled through the circulation. Matrix metalloproteinases (MMPs) are an attractive choice of endogenous cleavage factor because they are not expressed in normal cells but are highly expressed in malignant tumors. MMPs participate in cancer proliferation, migration, differentiation, and angiogenesis [26].

Cationic polymers such as PEI and PLL can complement the function of PEGylation by increasing adenovirus transduction efficiency [12,27]. These polymers carry highly positive, monodisperse charges, allowing them to coat the anionic adenovirus particles, thereby increasing the entropy of the system and making the ζ-potential positive. This ζ-positivity usually improves transduction efficiency but also increases cytotoxicity, as observed when coating adenovirus with high-molecular-weight PEI [28]. In an attempt to reduce this cytotoxicity, our group has experimented with cross-linking low-molecular-weight PEI with diethylene glycol [29], and another group has coated adenovirus vector with a conjugate of 600-Da PEI–β-cyclodextrin-folic acid (H1) [30]. These approaches have not solved problems associated with adenovirus vector in the circulation, including fast clearance, severe liver accumulation and immunogenicity.

To avoid these problems, we adopted an adenovirus modification strategy that combines PEI with MMP-cleavable PEGylation. Here we describe the synthesis and characterization of a β-cyclo dextrin–PEI-MMP-cleavable-PEG polymer (CDPCP), and we examine the immunogenicity, toxicity, pharmacokinetics, and transduction efficiency of a CDPCP-modified adenovirus vector (Fig. 1) *in vitro* and *in vivo*.

2. Materials and methods

2.1. Materials and animals

Polyethylenimine (PEI, branched, MW 2 and 25 kDa), β-cyclodextrin (β-CD, MW 1135 Da), N-succinimidyl-3-(2-pyridyl dithiol) propionate (SPDP), 1,1′-carbonyldiimidazole (CDI), gelatin, polyacrylamide, and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenylte trazolium bromide (MTT) were obtained from Sigma–Aldrich (St. Louis, MO, USA). PEG5k-MMP-2 cleavable peptide (GPLGIAGQC) and PEG5k-MMP-2 uncleavable peptide (IPGQGALGC) were custom-made by Apeptides (Shanghai, China). Cesium chloride was purchased from Amersco (Solon, Ohio, USA). Human matrix metalloproteinase-2 (hMMP-2) was purchased from Sino Biological (Beijing, China). All other reagents were of analytical purity and were used as received.

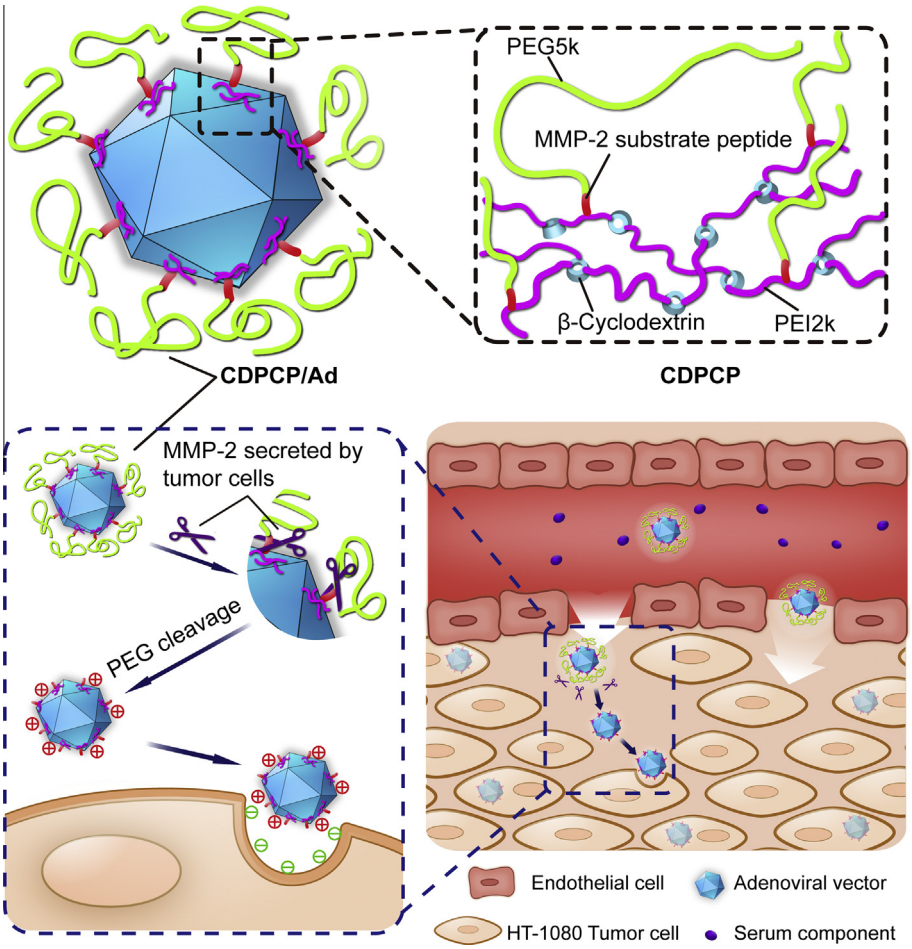

Fig. 1. Schematic diagrams of the complex between adenovirus and enzymatically cleavable PEG-PEI-β-cyclodextrin (CDPCP), as well as the fate of the complex in tumor tissue.

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