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Enhanced anti-tumor efficacy and safety profile of tumor microenvironment-responsive oncolytic adenovirus nanocomplex by systemic administration

Joung-Woo Choi [a], Kasala Dayananda [a], Soo-Jung Jung [a], Soo-Hwan Lee [a], Donghyun Kim [b], Jun Hu [c], You Han Bae [c,d,*], Chae-Ok Yun [a,*]

[a] Department of Bioengineering, College of Engineering, Hanyang University, 222 Wangsimni-ro, Seongdong-gu, Seoul 133-791, Republic of Korea
[b] School of Electrical and Electronic Engineering, Yonsei Institute of Medical Instruments Technology, Seoul 120-749, Republic of Korea
[c] Department of Pharmaceutics and Pharmaceutical Chemistry, The University of Utah, 30 S 2000 E, Room 2972, Salt Lake City, UT 84112, USA
[d] Utah-Inha Drug Delivery Systems (DDS) and Advanced Therapeutics Research Center, 7-50 Songdo-dong, Yeonsu-gu, Incheon 406-840, Republic of Korea

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ABSTRACT

Oncolytic adenovirus (Ad) holds great promise as a potential gene therapy for cancer. However, intravenously administered Ad may encounter difficulties due to unfavorable host responses, non-specific interactions, and the heterogeneity of the tumor cell population. As an approach to combine the advantages of oncolytic Ad and synthetic polymers and to address the associated difficulties, Ad was physically complexed with a pH-sensitive block copolymer, methoxy poly(ethylene glycol)-*b*-poly(L-histidine) (mPEG-*b*-pHis). The *in vitro* transduction efficiency at an acidic extracellular pH was remarkably enhanced in cancer cells when treated with the Ad expressing green fluorescent protein (GFP) coated with mPEG-*b*-pHis (c-dE1/GFP) as compared to that of naked Ad (n-dE1/GFP). Time-lapse total internal reflection fluorescence microscopic imaging revealed a significantly enhanced cellular uptake rate of c-dE1/GFP at acidic tumor pH when compared with that at neutral pH or naked cognate Ad (n-dE1/GFP). In addition, c-dE1/GFP remained relatively stable in human serum-containing media, and considerably reduced both the innate and adaptive immune response against Ad. Moreover, the therapeutic efficacy and survival benefit of mPEG-*b*-pHis-complexed oncolytic Ad (c-H5mT/Luc) by systemic treatment was significantly enhanced compared to that with naked oncolytic Ad (n-H5mT/Luc) in both coxsackie and adenovirus receptor-positive and -negative tumors. Whole-body bioluminescence imaging showed 7.3-fold higher luciferase expression at the tumor site and 23.0-fold less luciferase expression in liver tissue for c-H5mT/Luc relative to that for naked oncolytic Ad (n-H5mT/Luc). Considering the heterogeneity of tumor tissue, these results are important for guiding the development of more potent and specific treatment of devastating metastatic cancers using this viral system.

Statement of significance

Although adenoviral systems have shown considerable promise and undergone extensive evaluation attempts to specifically target Ad vectors to cancer cells have met limited success. This shortcoming is due to the strong immune response stimulated by Ad and the hepatotoxicity of the viral particles. To overcome restricted vector issues, we generated Ad/mPEG-*b*-pHis for tumor microenvironment-targeting hybrid vector systems, an oncolytic Ad coated with a pH-responsive polymer, mPEG-*b*-pHis. The Ad/mPEG-*b*-pHis exhibited pH-dependent transduction efficiency and cancer-cell killing effects. Moreover, systemic administration of oncolytic Ad/mPEG-*b*-pHis led to marked suppression of tumor growth and tumor-specific viral replication. Ad successfully avoided the innate and adaptive immune responses and liver accumulation with the help of mPEG-*b*-pHis on its surface.
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1. Introduction

Despite extremely limited success, oncolytic adenovirus (Ad) has entered into a significant number of clinical trials after being

* Corresponding authors at: Department of Pharmaceutics and Pharmaceutical Chemistry, University of Utah, Skaggs Research Building, 30 S 2000 E, Room 2972, Salt Lake City 84112, USA (Y.H. Bae).
E-mail addresses: you.bae@utah.edu (Y.H. Bae), chaeok@hanyang.ac.kr (C.-O. Yun).

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investigated in small animals as a potential treatment for tumors [1–5]. The administration route for most trials is intra-tumoral if the exact locations, sizes, and shapes of tumors are known and accessible for direct injection into multiple locations within a tumor mass [6–8]. Systemic administration of nanoparticles is generally more favorable and is justified by the hyper-permeability of the tumor vasculature, although only a limited fraction of an injected dose actually reaches the tumor site after a long time in circulation [9]. However, systemic administration of viral particles is limited due to fast clearance from neutralizing antibodies, uptake by reticuloendothelial system organs, potential immune responses, and hepatic toxicity [10–12].

Coxsackie and adenovirus receptor (CAR)-dependent internalization of Ad serotype 5 is another hurdle for systemic administration of oncolytic Ad [13]. CAR expression is often ablated or downregulated in cancer cells which hampers Ad's internalization, resulting in limited therapeutic efficacy. Additionally, CAR is overexpressed in the liver, thus systemically administered oncolytic Ad can be nonspecifically trafficked into the liver, causing hepatotoxicity. Furthermore, naked Ad is highly immunogenic, resulting in the onset of host's antiviral innate immune response and production of anti-Ad neutralizing antibodies that annuls therapeutic efficacy when administered repeatedly. Therefore, successful and clinically relevant Ad-mediated cancer gene therapy requires overcoming of CAR-dependency and immunogenicity of naked Ad before Ads can be systemically administered to treat disseminated and metastatic cancers [14].

As efforts for formulating systemically injectable Ad, a variety of experimental approaches have been devised, ranging from a physical coating to covalent modification of the Ad surface with hydrophilic polymers along with targeting molecules [15–25]. This often compromises the beneficial properties of cancer gene therapy, such as tropism to specific target organs, transfection, and replication in cancer cells. For example, poly(ethylene glycol) (PEG) grafting endows reduced visibility to existing antibodies and opsonins for absorption, leading to longer time in circulation, but compromising the tropism to target organs or cells [26–28]. Further, the tethering of targeting molecules to the end of PEG may endow cell specificity, as demonstrated for numerous non-viral drug carriers.

Potential reasons for suboptimal clinical performance of targeted nano-systems may include unforeseen side effects, suboptimal accumulation at the tumor site after systemic circulation, poor intratumoral penetration and distribution, and tumor heterogeneity. All of these aspects are serious barriers to the ability of Ad or any nanomedicine to reach and interact with individual malignant cells within a tumor. After meeting individual cells, Ad and nanocarriers perform designed or anticipated biological functions at the cellular and subcellular levels with less effects on healthy cells, tissues, and organs. Any single barrier or hurdle can be a serious bottleneck in determining the overall success of these delivery systems as therapies; however, most reported experimental approaches do not fully consider all of the required properties.

Herein, we report a simple physical coating of the ionizable amphiphilic block copolymer, mPEG-*b*-pHis, under mild conditions to coat the surface of Ad. We examine enhanced infection efficiency and cancer cell killing of oncolytic Ad under a hypoxic and low-pH condition, which is a hallmark of the tumor microenvironment. During systemic administration, Ad coated with mPEG-*b*-pHis is hypothesized to present a PEG surface outside of the Ad/mPEG-*b*-pHis complex when in circulation. In contrast, Ad coated with mPEG-*b*-pHis is believed to become cationic at the tumor site, provoking non-specific interactions with cancer cells for cellular uptake and becoming un-coated in acidic endo-lysosomal compartments. The ability of this polymer to switch from a neutral PEG surface to a cationic and then uncoated surface is due to the decrease in pH from the blood (pH 7.4) to the extracellular pH of the tumor (6.0–7.0) to the endosomal and lysosomal pH (4.0–6.5).

2. Materials and methods

2.1. Cell lines and adenoviruses

The following cell lines were purchased from the American Type Culture Collection (ATCC, Manassas, VA): A549, a non-small cell lung carcinoma cell line, U343, a brain glioma cell line, and MCF7, a breast carcinoma cell line. All cell lines were cultured in Dulbecco's Modified Eagle's Medium (DMEM; Gibco-BRL, Grand Island, NY) with 10% fetal bovine serum (FBS; Gibco-BRL) and penicillin/streptomycin (Gibco-BRL) at 37 °C incubated with 5% CO_2. The dE1/GFP virus is an E1-deleted, non-replicating Ad expressing green fluorescent protein (GFP) under the control of the cytomegalovirus promoter. H5mT/Luc is a luciferase-expressing oncolytic Ad that replicates under the control of a cancer-specific modified TERT promoter [29,30]. Ads were propagated in 293 cells and purified by CsCl gradient centrifugation. The number of viral particles (VP) was calculated from the optical density measurement at 260 nm (OD260), at which 1 absorbency unit is equivalent to 1.1×10^{12} viral particles per mL.

2.2. Block copolymer of mPEG-b-PHis

The α-methoxy-ω-amino poly(ethylene glycol) (mPEG-NH_2; molecular weight (MW) = 2000) and poly(L-histidine) (PHis; MW = 3700) were synthesized and characterized as previously described [31]. The mPEG-*b*-PHis was dissolved in DMSO (DMSO final concentration; 10%) to solubilize the polymer for complexation with Ad.

2.3. Size and zeta-potentials

For the complexation of Ad particles with mPEG-*b*-PHis polymers, mPEG-*b*-PHis polymers (50 μl) were added drop-wise to the solution of Ad particles (Ad in 450 μl of PBS), and then mixed by inversion or tapping in a tube diluted to total volume of 500 μl. The Ad/polymer was allowed to complex at room temperature for 30 min through electrostatic interaction. The average sizes and zeta-potentials of n-dE1/GFP and c-dE1/GFP were measured with a Zetasizer 3000HS (Malvern Instruments, Inc., Worcestershire, UK) with a He–Ne laser beam (633 nm, fixed scattering angle of 90°) at room temperature. After the formation of complexes, PBS (pH 7.4) was added to a final volume of 1 mL. The obtained sizes and potential values are presented as the average values from three measurements.

2.4. Transmission electron microscopy (TEM) imaging

The surface morphologies of n-dE1/GFP and cAd-dE1/GFP were observed by TEM (JEM-2000EXll, JEPL; Nikon, Tokyo, Japan). The n-dE1/GFP and c-Ad-dE1/GFP (4×10^6 molar ratio) were placed on glow-discharged, collodion/carbon-coated, 400-mesh copper grids (TED PELLA, Inc., Redding, CA). The solution was wicked off with filter paper and replaced with 1% aqueous uranyl acetate for 30 s. After removal of this solution, the grid was allowed to dry, and images were visualized using TEM at 200 kV (JEM-2000EX, JEOL, Japan).

2.5. Transduction efficiency assay

Transduction efficiency of n-dE1/GFP or c-dE1/GFP was assessed by measuring GFP expression in CAR-positive (U343 and

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