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Using a magnetic field to redirect an oncolytic adenovirus complexed with iron oxide augments gene therapy efficacy

Joung-Woo Choi [a, 1], Ji Won Park [b, 1], Youjin Na [a], Soo-Jung Jung [a], June Kyu Hwang [a], Dongho Choi [c], Kyeong Geun Lee [c], Chae-Ok Yun [a, *]

[a] Department of Bioengineering, College of Engineering, Hanyang University, 222 Wangsinmi-ro, Seongdong-gu, Seoul, Republic of Korea
[b] Graduated School, Dept. of Polymer Science & Engineering, SungKyunKwan University, Suwon 440-746, Republic of Korea
[c] Department of Surgery, College of Medicine, Hanyang University, 222 Wangsinmi-ro, Seongdong-gu, Seoul, Republic of Korea

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ABSTRACT

Adenovirus (Ad) is a widely used vector for cancer gene therapy but its therapeutic efficacy is limited by low coxsackievirus and adenovirus receptor (CAR) expression in tumors and non-specifically targeted infection. Ad infectivity and specificity can be markedly improved by creating Ad-magnetic nanoparticles cluster complexes and directing their migration with an external magnetic field (MGF). We electrostatically complexed GFP-expressing, replication-incompetent Ad (dAd) with PEGylated and cross-linked iron oxide nanoparticles (PCION), generating dAd-PCION complexes. The dAd-PCION showed increased transduction efficiency, independent of CAR expression, in the absence or presence of an MGF. Cancer cell killing and intracellular oncolytic Ad (HmT)-PCION replication significantly increased with MGF exposure. Site-directed, magnetically-targeted delivery of the HmT-PCION elicited significantly greater therapeutic efficacy versus treatment with naked HmT or HmT-PCION without MGF in CAR-negative MCF7 tumors. Immunohistochemical tumor analysis showed increased oncolytic Ad replication in tumors following infection by HmT-PCION using an MGF. Whole-body bioluminescence imaging of tumor-bearing mice showed a 450-fold increased tumor-to-liver ratio for HmT-PCION with, versus without, MGF. These results demonstrate the feasibility and potential of external MGF-responsive PCION-coated oncolytic Ads as smart hybrid vectors for cancer gene therapy.

1. Introduction

Successful cancer gene therapy requires a delivery system that is nontoxic and can achieve efficient transduction and transgene expression *in vivo*. Adenovirus (Ad) serotype-5 has been widely used in clinical application due to its high titer ability, high transduction efficiency in both dividing and non-dividing cells, and absence of genomic integration-mediated mutagenesis. Several clinical trials have reported success using locally administered Ads for cancer gene therapy [1,2]. Ad-based cancer gene therapy continues to evolve with cancer cell-specific replicating oncolytic Ads that are far superior to their predecessors [3,4]. Oncolytic Ad has many advantages, including the ability to self-propagate, lyse infected cancer cells, and produce 1000 to 10,000 copies of progeny per infected cell, thus causing secondary infection of neighboring cancer cells in a tumor [5]. Therapeutic gene-inserted oncolytic Ads showed high gene-delivery efficiency and potent antitumor efficacy, *in vitro* and *in vivo* [6–8]. However, Ads are dependent on the coxsackie and adenovirus receptor (CAR) for target cell entry, limiting the clinical efficacy of Ad-mediated cancer gene therapy. Earlier studies have demonstrated that cells with low CAR expression show poor Ad infectivity [9,10]. Thus, overcoming the transduction efficiency of Ads in CAR-negative tumors is a crucial step in improving the therapeutic efficacy of oncolytic Ads.

Currently, hybrid vectors that combine the advantages of both viral and non-viral components have been explored [4,11]. One proposed strategy to overcome the limitations of Ads' CAR-dependence is to modify the Ad surface with a polymer that bypasses the need for CAR-mediated endocytosis [12,13]. Modifying Ads with cationic polymers [13–15] or lipids [16–18] enhances Ad-mediated gene delivery. However, these strategies do not address targeted tumor-specific Ad-mediated gene delivery because

* Corresponding author.
E-mail address: chaeok@hanyang.ac.kr (C.-O. Yun).
[1] These authors contributed equally to this study.

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injected polymer/lipid-modified Ads rapidly disseminate into surrounding non-target tissues [19,20].

Over the last decade, researchers have implemented and optimized a new physical gene delivery technology by using a magnetic field gradient and magnetic nanoparticles to improve efficient site-specific delivery of hybrid vectors [21–23]. Magnetic nanoparticles provide accelerated vector accumulation in target sites when directed with magnetic field-enforced delivery. This approach is customizable by adjusting particle size, surface charge density, and surface functionality with therapeutic drugs or genes, and results in enhanced cellular uptake and replication efficacy, and specific delivery to target tissues [19–21,23–25].

Coupling Ad viruses to polyethyleneimine (PEI)-coated superparamagnetic iron oxide (Fe_3O_4) nanoparticles improves gene transfection efficiency when these vectors are directed by an external magnetic field (MGF) [26]. Scherer et al. have demonstrated that PEI-coated super paramagnetic nanoparticles are highly beneficial to modification of Ad vector as they can enhance transfection efficiency and reduce viral/polymer dose requirement. Considering the point that the usage of 25 kDa PEI is limited *in vivo* due to substantial cytotoxicity, magnetofection with PEI-coated superparamagnetic iron oxide nanoparticle-coated Ad can provide a strong platform for efficacious and safe delivery of therapeutic genes. Moreover, the PEI coating on magnetic nanoparticles condenses anionic nucleotides such as plasmid DNA and siRNA into compact complexes, and facilitates their escape from endosomes via the proton sponge effect [27,28]. Gene delivery mediated by magnetic nanoparticles exhibits higher transfection efficiency compared to conventional polyplex transfection [20,21]. Park et al. reported clustered, magnetize, PEI-encapsulated, superparamagnetic Fe_3O_4 particles to enhance magnetization properties and sustain superparamagnetism, without exhibiting magnetic hysteresis. These particles induce faster sedimentation and greater accumulation within cells, and deliver drugs or genes rapidly under an MGF [29]. In addition, PEG-coated, cross-linked, iron oxide nanoparticles (PCIONs) have been shown to deliver plasmid DNA into mesenchymal stem cells efficiently in response to an exterior MGF [30].

This study is aimed to improve the therapeutic efficacy of oncolytic Ads *in vitro* and *in vivo* in combination with PCIONs. We show that magnetofection-mediated Ad-PCION cellular uptake was more efficient and rapid compared with naked Ad's CAR-mediated endocytosis, resulting in significantly elevated gene transfer efficiency. Further, PCION facilitated Ad cell entry without hindering the oncolytic Ad's inherent viral replication rate and cancer cell oncolysis, resulting in greater therapeutic efficacy against MCF7 tumor xenografts in mice. Importantly, site-specific magnetically-guided PCIONs overcame the potential risk of non-specific liver tropism inherent to conventional cationic polymers and naked Ads, showing a 450-fold increase in tumor-to-liver uptake ratio with, versus without magnetically-guided delivery.

2. Materials and methods

2.1. Cell culture and Ad preparation

All cancer cell lines (HeLa, U343, A549, SK-BR3, MCF7, and B16F10) were cultured in Dulbecco's modified Eagle medium (DMEM; Gibco-BRL, Grand Island, NY) supplemented with 10% fetal bovine serum (FBS; Gibco-BRL) at 37 °C in a humidified atmosphere containing 5% CO_2. A human cervical cancer cell line (HeLa), brain cancer cell line (U343), lung cancer cell line (A549), breast cancer cell lines (SK-BR3 and MCF7), and embryonic kidney cell line (HEK-293), and a mouse melanoma cancer cell line (B16F10) were purchased from the American Type Culture Collection (Manassas, VA). Magnetofection-mediated Ad gene delivery efficiency *in vitro* was examined using GFP-expressing replication-incompetent Ad (dAd) [12]. HmT is a firefly luciferase-expressing oncolytic Ad that replicates under the control of a cancer-specific modified TERT promoter and a hypoxia-responsive element. Ads were propagated in HEK-293 cells and purified by CsCl gradient centrifugation. Viral particles (VP) were enumerated using optical density measurements at 260 nm (OD_{260}), where 1 absorbency unit ($OD_{260} = 1$) equaled 1.1×10^{12} VP/ml. Purified viruses were stored at −80 °C until use.

2.2. Materials

Methoxy poly(ethylene glycol)-succinimidly-succinate (mPEG-SS, MW 2000) was purchased from Sunbio Inc. (Anyang, Korea). 1-Ethyl-3-(3-dimethlyaminopropyl)-carbodiimide hydrochloride was obtained from Tokyo Chemical Industry Co. (Tokyo, Japan). Poly-L-lysine hydrobromide (PLL, MW 25,000), hydrocaffeic acid, and rhodamine B isothiocyanate (MW 536.08) were obtained from Sigma Aldrich (St. Louis, MO). The Cell Counting Kit-8 (CCK-8) was purchased from Dojindo Molecular Technologies, Inc., (Rockville, MD). Dialysis membranes were obtained from Spectrum Laboratories, Inc. (Rancho Dominguez, CA). DMEM, RPMI 1640 medium, Dulbecco's phosphate-buffered saline (PBS), FBS, penicillin/streptomycin, and trypsin were from Gibco-BRL. Iron oxide nanoparticles sized below 10-nm diameter were supplied by the National Creative Research Initiative Center for Oxide Nanocrystalline Materials and School of Chemical and Biological Engineering at Seoul National University (Seoul, Korea).

2.3. Synthesis of PEGylated and cross-linked iron oxide nanoparticles (PCION)

Catechol-grafted PLL (PLL-DN) polymers were synthesized by chemical coupling of carboxylic acid group from hydrocaffeic acid to a primary amine group of PLL. EDC (65 mg, 350 mmol) dissolved in 2 mL of methanol was slowly reacted with 2 mL of DMF solution containing hydrocaffeic acid (55.2 mg, 350 mmol). The reactant was added into PLL (100 mg, 4 mmol) dispersed in 2 mL of methanol and then stirred at room temperature for 12 h. The polymer was dialyzed against HCl solution (pH 4) with a dialysis membrane (Mw cutoff of 3000) for 2 days, and then lyophilized. The chemical structure and the substitution degree of catechol groups were confirmed using a ^{1}H-NMR spectroscopy (Brucker DRX 400 spectrometer operating at 400 MHz).

The cross-linked iron oxide nanoparticle (CION) was synthesized by the oil-in-water (O/W) single emulsion and evaporation method. 1 mL of chloroform solution containing 2 mg of iron oxide nanoparticles coated with oleic acid was added to 20 mg of PLL-DN dispersed in 10 mL of deionized water. Concurrently, the mixture was performed for 5 min via a tip-type Branson sonifier with a duty cycle of 30 and output of 3 to apply oil-in-water (O/W) emulsion. After evaporating organic solvent under reduced pressure, the unbound PLL-DN polymer and remaining solvent were removed by ultrafiltration with Amicon® Ultra-4 centrifugal filter (Mw Cutoff of 100 kDa). For synthesis of PEGylated CION (PCION), 2 mg of the purified CION were reacted with 200 μg of mPEG-SS (Mw 2000) dissolved in 1 mL of deionized water, and then this solution was purified using Amicon® Ultra-4 centrifugal filter (Mw Cutoff of 100 kDa) to remove remaining mPEG-SS polymer. Moreover, the rhodamine labeled PCIONs were produced by conjugating 200 μg of PCIONs to 2 μg of rhodamine B isothiocyanate dispersed in 200 μL of DMSO. After reacting for 24 h, the final product was washed by several cycles of dispersion in deionized water and centrifugation.