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Synergistic antitumor effect mediated by a paclitaxel-conjugated polymeric micelle-coated oncolytic adenovirus

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ABSTRACT

Combination treatment consisting of oncolytic adenovirus (Ad) and paclitaxel (PTX) is a promising strategy to achieve synergistic antitumor effect. However, a co-administration approach is subject to inherent limitations due to the poor solubility of PTX and chemoresistance of tumor cells. In order to overcome these limitations, an oncolytic Ad expressing a p53 variant (oAd-vp53) that is resistant to p53 inactivation in the tumor microenvironment was complexed with PEGylated and PTX-conjugated polymeric micelle (APP). This approach generated an oAd-vp53/APP complex (176.4 nm in diameter) that could concurrently deliver both oncolytic Ad and the nanoparticulate drug APP to tumors. APP-complexed replication-incompetent Ad (dAd/APP) exhibited 12-fold higher transduction efficiency than naked dAd in coxsackie adenovirus receptor (CAR)-negative cancer cells. This increased efficiency was attributed to more efficient cellular internalization mediated by charge interactions between APP and anionic cell membranes. Furthermore, oAd-vp53/APP elicited synergistically higher cancer cell killing than naked oAd-vp53, APP, or oAd-vp53 in combination with PTX (oAd-vp53 + PTX); this synergistic effect was shown to be due to superior induction of apoptosis and viral replication. Importantly, oAd-vp53/APP induced more potent and synergistic antitumor effect through both local and systemic administration by enhancing replication of oncolytic Ad and induction of apoptosis in tumor tissue. Further, the APP coating on the surface of Ad markedly attenuated the host immune response against Ad and decreased hepatic sequestration, resulting in minimal hepatotoxicity and a good safety profile. These attributes enabled oAd-vp53/APP to elicit potent antitumor effect over multiple treatment cycles. Altogether, we demonstrate that concurrent delivery of oncolytic Ad and APP as a single nanocomplex is a promising strategy for achieving synergistic antitumor effect.

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1. Introduction

Over the past two decades, oncolytic adenovirus (Ad) has become a promising modality to deliver and express therapeutic genes for the treatment of various diseases [1,2]. However, further advancement in oncolytic Ad-mediated gene therapy requires innovative strategies to overcome challenges faced in clinical settings [3]. For efficient oncolytic Ad-mediated cancer gene therapy, oncolytic Ad must achieve higher therapeutic potency and be systemically administrable to treat disseminated metastases. However, successful systemic delivery of Ad must overcome significant hurdles due to the native tropism and immunogenicity of Ad, which induce nonspecific trafficking to normal tissues (e.g. the liver) and rapid clearance, respectively. In order to bypass Ad-mediated hepatotoxicity and the undesirable host immune response, various genetic and chemical engineering strategies have been extensively studied [2,4–6]. Genetic engineering of Ad tropism requires extensive modification of the Ad capsid to incorporate a new targeting moiety, which is a laborious process that often generates noninfectious or dysfunctional virus. In contrast, chemical engineering is simple and straightforward, since nanomaterials can be easily complexed with Ad through chemical conjugation or electrostatic interaction.

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Various chemocytotoxic drugs, including paclitaxel (PTX), are currently utilized as first-line of treatment for a wide spectrum of cancers from different tissue origins [7,8]. Despite the potent therapeutic efficacy of PTX, it has low solubility and is severely toxic to healthy organs and hematological systems due to its lack of cancer specificity. Furthermore, poor penetration of PTX into tumor tissue has also been reported [9,10]. To overcome these limitations, polymeric micelles or nanoparticles (e.g. albumin, silica, gold, and lipid) have been utilized as PTX delivery vehicles to increase solubility and provide protection against the host environment, leading to an enhanced half-life of PTX in the blood and intratumoral accumulation through passive targeting [11]. Nam et al. recently developed a cationic polymeric micelle consisting of a PEGylated PTX-conjugated arginine-grafted bioreducible polymer (APP) and showed that this micelle could selectively release PTX within the reductive conditions of the cytoplasm and induce more potent cancer cell killing efficacy than unmodified PTX [12].

We have previously demonstrated that the combination of oncolytic Ad and cytotoxic chemotherapeutics elicits synergistic antitumor effect [13]. PTX is one of the most promising candidate chemotherapeutics for combination with oncolytic Ad [14,15]. Multiple mechanisms have been proposed to explain this synergy, including increased cellular internalization through elevated expression of the coxsackie adenovirus receptor (CAR) and integrins and enhanced viral spread through enhanced formation of apoptotic bodies [15–17]. Furthermore, an oncolytic Ad expressing proapoptotic genes (e.g. TNF-related apoptosis-inducing ligand (TRAIL), p53 upregulated modulator of apoptosis (PUMA), and p53) was reported to chemosensitize cancer cells toward cytotoxic chemotherapeutics [18–20]. In the present study, we have identified oncolytic Ad expressing the proapoptotic gene p53VPΔ30 (oAd-vp53) [21], which drives the expression of an N-terminal and C-terminal region-deleted p53 variant fused with the herpes simplex virus VP16 transactivation domain that is capable of escaping p53 inhibition in the tumor microenvironment, as a suitable candidate for complexation with APP. We demonstrate that concurrent delivery of PTX and oAd-vp53 using cationic polymeric micelles, through either local or systemic administration, can induce synergistic antitumor effect through a multimodal process that augmented cellular uptake, immunoescape, and production of oncolytic Ad.

2. Materials and methods

2.1. Cell culture

The following human cancer cell lines were purchased from the American Type Culture Collection (ATCC, Manassas, VA): MCF-7 and SKBR-3 (human breast cancer), MDAH 2774 (human ovarian cancer), H460 (human lung cancer), WRL-64 (human normal hepatocyte), RAW 264.7 (murine macrophage) and HDF (human dermal fibroblasts). MDAH 2774 cells were maintained in RPMI-1640 (Gibco BRL, Grand Island, NY). The other cell lines were cultured in high glucose Dulbecco's Modified Eagle's Medium (DMEM, Gibco BRL) containing 10% fetal bovine serum (FBS, Gibco BRL) in an incubator at 37 °C with 5% CO_2.

2.2. Preparation of Ads

The transduction efficiency of Ad was assessed using a green fluorescence protein (GFP)-expressing replication-incompetent Ad (dE1/GFP; hereafter denoted as dAd). The construction of dAd was previously described [22]. The construction of oncolytic Ad expressing a p53 variant (p53VPΔ30) (Ad-mΔ19/p53VPΔ30; denoted as oAd-vp53) was also previously described [21]. The p53VPΔ30 variant consisted of wild-type p53 harboring deletions of the N-terminal and C-terminal regions and the transcriptional activation domain of herpes simplex virus VP16 protein. Replication-incompetent dAd and replication-competent oAd-vp53 were propagated in HEK293 and A549 cells, respectively, and purified by CsCl gradient centrifugation. Ad viral particle (VP) number was calculated by measuring the optical density at 260 nm (OD_{260}); an absorbance value of 1 was considered to be equivalent to 1.1×10^{12} VP/mL. Purified viruses were stored at −80 °C until use.

2.3. Complexation of Ad with APP or ABP

dAd/APP complexes were generated through electrostatic interaction between negatively charged capsid of dAd (1×10^{10} VP) and cationic APP (APP:dAd molar ratio of 1:1.75×10^4, 8.75×10^4, 3.5×10^5, and 8.75×10^5) in PBS buffer (pH 7.4) at room temperature. Briefly, a solution containing Ad and APP was mixed gently with a pipet and then incubated at room temperature for 30 min prior to further use. The same process was used for the complexation of oAd-vp53 with APP, thereby generating oAd-vp53/APP complexes. For the generation of control nanocomplex combining arginine-grafted bioreducible polymer (ABP) [23] and Ad [either fluorescein isothiocyanate (FITC)-conjugated and replication-incompetent Ad (dAd-FITC) or oAd-vp53], 3.5×10^5 polymer:Ad ratio was used and complexed through electrostatic interaction as described above, generating dAd-FITC/ABP and oAd-vp53/ABP, respectively.

2.4. Gel retardation assay

To evaluate the encapsulation efficiency of APP-complexed Ad, various APP-coated Ad of different molar ratios were prepared (Section 2.3). Subsequently, the complexes formed at various molar ratios were treated with virus lysis buffer and incubated at 56 °C for 30 min. Following incubation, each sample was loaded onto a 0.8% (w/v) agarose gel in 1X TAE buffer (10.0 mM Tris/HCl, 1% (v/v) acetic acid, and 1.0 mM EDTA) containing ethidium bromide and electrophoresed at 120 V for 30 min in the same buffer. Finally, viral DNA bands were visualized using a ChemiDoc gel documentation system (Syngene, Cambridge, UK).

2.5. Characterization of naked Ad and Ad/APP

The average sizes and zeta potentials of the dAd and dAd/APP complexes were measured using a Zetasizer Nano ZS (Malvern Instrument, Inc., Worcestershire, UK) with a He–Ne laser beam (633 nm, fixed scattering angle of 90°) at 25 °C. Briefly, dAd/APP complexes formed at various molar ratios (Section 2.3) were prepared in 1 mL of PBS buffer and measured with the Zetasizer Nano ZS (Malvern Instrument, Inc.). The sizes and zeta potential values of the dAd/APP complexes are presented as mean ± SD of three measurements.

2.6. Transmission electron microscopy imaging

Transmission electron microscopy (TEM) imaging of naked dAd and dAd/APP complexes (3.5×10^5 polymer:Ad molar ratio) was carried out by incubating each sample on TEM copper grid for 30 min at room temperature, and morphologies were subsequently characterized by TEM (JEM-2000EXll, JEPL; Nikon, Tokyo, Japan) at 200 kV.

2.7. Transduction efficiency of Ad/APP

To determine the transduction efficiency of dAd/APP, CAR-

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