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# Effects of nanoparticle coatings on the activity of oncolytic adenovirus–magnetic nanoparticle complexes

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ABSTRACT

Limitations to adenovirus infectivity can be overcome by association with magnetic nanoparticles and enforced infection by magnetic field influence. Here we examined three core-shell-type iron oxide magnetic nanoparticles differing in their surface coatings, particle sizes and magnetic properties for their ability to enhance the oncolytic potency of adenovirus Ad520 and to stabilize it against the inhibitory effects of serum or a neutralizing antibody. It was found that the physicochemical properties of magnetic nanoparticles are critical determinants of the properties which govern the oncolytic productivities of their complexes with Ad520. Although high serum concentration during infection or a neutralizing antibody had strong inhibitory influence on the uptake or oncolytic productivity of the naked virus, one particle type was identified which conferred high protection against both inhibitory factors while enhancing the oncolytic productivity of the internalized virus. This particle type equipped with a silica coating and adsorbed polyethylenimine, displaying a high magnetic moment and high saturation magnetization, mediated a 50% reduction of tumor growth rate versus control upon intratumoral injection of its complex with Ad520 and magnetic field influence, whereas Ad520 alone was inefficient. The correlations between physical properties of the magnetic particles or virus complexes and oncolytic potency are described herein.



## 1. Introduction

Multidrug resistance (MDR) is a major obstacle in cancer chemotherapy. One of the underlying mechanisms of MDR is the cellular over expression of P-glycoprotein, which acts as an efflux pump for various anticancer drugs, thereby decreasing their efficacy [1]. Recently, a viable approach has emerged that uses oncolytic viruses to specifically kill tumor cells [2]. In particular, replication-selective adenoviruses have been designed to selectively replicate in and lyse human tumor cells and then spread the virus particles to adjacent tumor cells while sparing normal cells [3]. The coxsackie and adenovirus receptor (CAR) is a common cellular receptor for adenoviral attachment and infection [4]. The potential infectivity and antitumor capacity of an oncolytic adenovirus can be limited by the lack or low expression of CAR in tumor cells [5,6], whereas expression of the receptors in non-targeted cells can result in increased toxicity [7]. These limitations can be overcome by targeting adenoviral infection via a CAR-independent pathway using genetic modifications of the adenoviral capsid protein, targeting ligands or a chemical modification of the virus. However, these strategies are not sufficient for rapid infection of the cells at the target site, as the delivery process itself is diffusion-limited [8,9]. Magnetofection is a powerful method for gene delivery that uses an association of viral or non-viral gene vectors with magnetic nanoparticles (MNPs) and the application of a gradient magnetic field for site-specific and efficient delivery of the vector. Upon magnetofection, the full vector dose is rapidly sedimented at the target cells, resulting in enhanced cellular uptake of the vector and considerably improved efficacy of delivery [8–13].

Core-shell-type MNPs with a core of magnetite ($Fe_3O_4$) or maghemite ($\gamma$-$Fe_2O_3$), which encompass most magnetic iron oxides, are of interest for biomedical applications, e.g., cell separation, drug/gene delivery, magnetic resonance imaging (MRI) and hyperthermia, because of their biocompatibility and relative non-toxicity when equipped with appropriate coatings [14–16]. For association with nucleic acids and/or viral particles, coatings

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comprised of cationic polymers such as polyethylenimine are particularly useful [12,17–19]. The nature of the surface coatings and their thickness plays an important role in the biodistribution and kinetics of biodegradation [14,20]. Under the same conditions, the efficacy of MNPs for enhancing gene delivery is different depending on their surface coatings [19,21,22]. In addition, multifunctional magnetic nanoparticles are a highly active area of current research. Different surface modification strategies that are used to enhance the biocompatibility of these nanoparticles for biomedical applications have been described [23].

In our recent study [13], we have formulated complexes of oncolytic adenovirus Ad520 and positively charged PEI-Mag2 MNPs with a coating comprising of 25-kDa branched polyethylenimine and fluorinated surfactant that considerably enhanced the potency of the virus both *in vitro* and *in vivo*. The present study aimed to prepare complexes that further improve the oncolytic potency of the virus *in vitro* and *in vivo*. Another two candidates of particles that were very efficient in enhancing lentiviral transduction of primary cells [24] were selected; polybrene-modified nanoparticles (PB-Mag1) and silica-modified particles decorated with 25-kDa branched polyethylenimine (SO-Mag2) and compared with PEI-Mag2 MNPs. Thus, all three types of MNPs were used for preparing the magnetic adenovirus complexes. The oncolytic adenovirus used in this work was E1A-mutant adenovirus Ad520, which was designed to selectively replicate within MDR human cancer cells expressing nuclear transcription factor Y-box binding protein 1 (YB-1) [25]. The oncolytic adenovirus and its magnetic complexes were evaluated for their physicochemical characteristics, non-specific cytotoxicity, virus internalization and *in vitro* oncolytic effect in human CAR-deficient and MDR cancer cells under different infection conditions; they were also evaluated for *in vivo* tumor growth inhibition in mice bearing tumor xenografts.

## 2. Materials and methods

### 2.1. Materials

Leibovitz's L-15 medium was purchased from Bio Whittaker (Wokingham, UK), OptiMEM® I from Invitrogen (California, USA), Anti-Ad5 polyclonal antibody from Abcam (Cambridge, UK), and Matrigel™ basement membrane matrix from BD Biosciences (California, USA). Dulbecco's modified Eagle's medium (DMEM), L-glutamine, penicillin, streptomycin, phosphate-buffered saline-Dulbecco (PBS), minimal essential vitamins, and 0.25% trypsin/0.02% EDTA solutions were obtained from Biochrom AG (Berlin, Germany). Sodium $^{125}$iodide in NaOH was purchased from Amersham Biosciences (New South Wales, Australia), fetal calf serum (FCS) from PAN-Biotech (Aidenbach, Germany), Triton X-100 from AppliChem (Darmstadt, Germany), and D-luciferin and coelenterazine were from Synchem OHG (Felsberg, Germany). All other chemicals were of analytical grade and used without further purification (Sigma–Aldrich, Steinheim, Germany). Tissue culture plates and flasks were from Techno Plastic Products (Trasadingen, Switzerland). A 96-magnet plate (OZ Biosciences, Marseille, France) generated a permanent magnetic field with a field strength and gradient of 70–250 mT and 50–130 T/m, respectively, in the cell layer area.

### 2.2. Cell culture

The MDR human pancreatic carcinoma cell line EPP85-181RDB stably expressing either firefly luciferase or *Renilla* luciferase, further referred to herein as 181RDB-fLuc or 181RDB-RLuc, respectively, was used for *in vitro* experiments. The cells were grown in Leibovitz's L-15 medium supplemented with 10% FCS, 2 mM L-glutamine, 6.25 mg/L fetuin, 2.5 mg/L transferrin, 0.5 g/L glucose, 1.1 g/L $NaHCO_3$, 1% minimal essential vitamins, 100 units/ml penicillin, and 100 μg/ml streptomycin (further referred to as cell culture medium) and treated with daunorubicin (250 ng/ml) every 2 weeks to ensure P-glycoprotein expression [26]. Human embryonic kidney cells (293 cells) were cultured in DMEM containing 10% FCS and 2 mM L-glutamine. All cells were grown at 37 °C in a humidified atmosphere of 5% $CO_2$.

### 2.3. Adenovirus preparation

The E1A-mutant oncolytic adenovirus dl520, referred to as Ad520, and the E1-minus replication-defective adenovirus encoding firefly luciferase (Ad-Luc) under the control of a cytomegalovirus (CMV) promoter were propagated in 293 cells and purified by double cesium chloride gradient centrifugation. Virus titers expressed as physical virus particles (VP) and plaque-forming units (pfu) per ml were determined by measuring the absorbance at 260 nm after the virus was disassembled in 0.1% sodium dodecyl sulfate [27] and by end-point dilution assays on the 293 cells, respectively. The preparation yielded 134 VP per pfu. Aliquots of adenoviruses were stored at −80 °C.

### 2.4. Synthesis and characterization of core-shell-type magnetite nanoparticles

Core-shell-type iron oxide MNPs were synthesized by precipitation of Fe(II)/Fe(III) hydroxide from an aqueous solution of a mixture of Fe(II) and Fe(III) salts, followed by transformation into magnetite in an oxygen-free atmosphere with spontaneous adsorption or condensation of shell components as described elsewhere [19,28,29]. To modify the surface of the magnetite nanoparticles, the fluorinated surfactant ZONYL FSA (lithium 3-[2-(perfluoroalkyl)ethylthio]propionate) was combined with 25-kDa branched polyethylenimine (PEI-$25_{Br}$) for the PEI-Mag2 nanoparticles, and the fluorinated surfactant ZONYL FSE (ammonium bis[2-(perfluoroalkyl)ethyl]phosphate) was combined with the cationic polymer polybrene (PB) for the PB-Mag1 nanoparticles. For SO-Mag2 nanoparticles, the surface coating was achieved by condensation of tetraethyl orthosilicate (TEOS) and 3-(trihydroxysilyl)propylmethylphosphonate (THPMP), followed by secondary surface decoration with PEI-$25_{Br}$ as previously described [28]. The resulting coated magnetic nanoparticle suspensions were dialyzed against double-distilled water ($ddH_2O$) to remove unbound coating components and then sterilized using $^{60}$Co gamma irradiation at a dosage of 25 kGy [4]. The particle stock concentrations in terms of dry weight and iron content were determined as described previously [19].

The average crystallite size $\langle d \rangle$ of the cores was calculated from the X-ray diffraction (XRD) data using the Scherer formula [30]. The mean hydrodynamic diameter ($D_h$), polydispersity index (PI) and zeta potential ($\xi$) of the MNPs suspended in $ddH_2O$ were measured by the dynamic light scattering (DLS) technique using a Malvern 3000 HS Zetasizer (UK). The saturation magnetization per unit of iron weight ($M_s$) was measured at 298 K using a vibrating sample magnetometer (Oxford Instruments Ltd.).

### 2.5. Adenovirus association and magnetic sedimentation with magnetic nanoparticles and stability of the complexes in the presence of FCS

Adenovirus Ad520 was labeled with radioactive $^{125}$iodide as recently described [13]. The stock of the labeled virus containing $2.08 \times 10^{10}$ VP/ml with a radioactivity of 1160 kBq/ml (determined using a Wallac 1480 Wizard 3 automatic gamma counter, Finland) was used to quantify virus association with the MNPs. To form magnetic virus complexes at MNP-to-virus ratios of 0–40 fg of Fe/VP for binding studies, 20 μl of a 2-fold serial dilution of the MNPs in $ddH_2O$ (0–6 μg of Fe) was mixed with 268 μl of $^{125}$I-labeled Ad520 suspended in serum-free OptiMEM or in PBS in a U-bottom 96-well plate at a virus concentration of $5.4 \times 10^8$ VP/ml for assembly of the complexes and then incubated for 20 min at room temperature (RT). Eighty microliters of the mixture were transferred to the wells of a new U-bottom 96-well plate containing 80 μl of OptiMEM, PBS, or FCS, followed by gently mixing and incubation for 20 min. All samples were prepared in triplicate. The U-bottom plate was positioned on a 96-magnet plate for 1 h, the radioactivity was measured in 100 μl of each supernatant using a gamma counter and the percentage of adenovirus particles associated and magnetically sedimented with MNPs was calculated as described in ref. [13].

### 2.6. Magnetic adenovirus complexes

#### 2.6.1. Complex preparation

Oncolytic adenovirus Ad520 diluted in 450 μl of OptiMEM and 50 μl of 2-fold serial dilutions of MNPs in $ddH_2O$ containing 0.7–10.7 μg of Fe were mixed, resulting in compositions of 2.5–40 fg of Fe/VP and a virus concentration of $5.4 \times 10^8$ VP/ml, and kept at RT for 20 min to allow complex assembly. To investigate the effect of the medium (OptiMEM or PBS) and virus concentration on the physicochemical characteristics of the Ad520-SO-Mag2 complexes at a MNP-to-VP ratio of 10 fg of Fe/VP, $1.62 \times 10^9$ or $3.6 \times 10^9$ VP were mixed in OptiMEM or PBS with 16.2 or 36 μg of Fe SO-Mag2 nanoparticles in a total volume of 3 or 1.5 ml, respectively, resulting in a virus concentration of $5.4 \times 10^8$ or $2.4 \times 10^9$ VP/ml when assembling the complexes. All complexes were freshly prepared before characterization.

#### 2.6.2. Size, polydispersity index and zeta potential

The resulting complexes were studied by measuring their mean hydrodynamic diameters ($D_h$) and polydispersity indices (PI) using DLS methods and their zeta potentials ($\xi$) using laser Doppler micro-electrophoresis. For zeta potential measurements, 500 μl of the complexes were diluted in 1.5 ml of OptiMEM, PBS or cell culture medium containing 10% FCS.

#### 2.6.3. Sedimentation stability and magnetic responsiveness

The sedimentation stability and magnetic responsiveness of the complexes were evaluated by measuring the time course of the turbidity of complex suspensions using a Beckman DU 640 spectrophotometer with no magnetic field applied and with the application of inhomogeneous magnetic fields, respectively, as described previously [31,32].