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RAPID COMMUNICATION

Surface charge critically affects tumor penetration and therapeutic efficacy of cancer nanomedicines

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Summary Physiochemical properties of nanomedicines determine their *in vivo* fate and ultimate therapeutic efficacy. Establishing correlations between nanoparticle properties and their physiological response is vitally important for nanomedicine design and optimization. To date, the correlation between surface charge, a fundamental property of a nanomedicine, and its therapeutic efficacy remains poorly understood. Here, we systematically investigated the influence of surface charge on the pharmacokinetics, tumor accumulation, penetration, and antitumor efficacy of nanoparticles constructed from PEG-*b*-PLA, loaded with docetaxel, and tuned by various lipids to yield three groups of ~100 nm nanoparticles with positive, neutral or negative charge. Our results indicate that cationic PEGylated nanoparticles, although slightly inferior in blood circulation time and tumor accumulation, outperform their anionic or neutral counterparts in inhibiting tumor growth in five different tumor models. Docetaxel-loaded

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cationic nanoparticles significantly suppressed tumor growth with an inhibition ratio of ~90%, compared with the ~60% achieved by their anionic or neutral counterparts. Further studies reveal that better tumor penetration and 2.5-fold higher cellular uptake of cationic PEGylated nanoparticles is responsible for their superior treatment efficacy. This fundamental study provides a foundation for engineering the next generation of nano-delivery systems for *in vivo* applications.

Introduction

Physiochemical properties of cancer nanomedicines such as size, shape, and surface chemistry play a critical role in their behavior in complex *in vivo* physiological environments, which ultimately determine their antitumor effect [1—3]. Establishing defined correlations between the basic physiochemical properties of nanomedicines and their *in vivo* fate, especially the therapeutic effect, is particularly instructive for improving the therapeutic outcomes and guiding the design of nano-delivery systems. In the past decade, numerous studies have investigated the influence of particle size on the interactions of nanoparticles (NPs) with biological systems [4—6]. In contrast, the influence of surface charge remains poorly understood. *In vitro*, surface charge decides the extent of cellular uptake, distribution in subcellular compartments, and permeability in multicellular spheroids [7—12]. *In vivo*, NPs with neutral and negative surface charges reduce the adsorption of serum proteins, resulting in longer circulation half-lives [13]. McDonald and colleagues have showed that positively charged liposomes exhibited higher binding and internalization by angiogenic endothelial cells in tumors than in normal vasculature [14]. Despite these advances, how surface charge affects polymeric nanoparticle transport in the tumor interstitium *in vivo*, along with how it correlates with the ultimate antitumor activity has not been elucidated.

In this study, we prepared a series of PEGylated lipid-associated polymeric NPs with variable surface charges and examined their *in vivo* performance. The NPs were prepared by assembling poly(ethylene glycol)-*block*-poly(D,L-lactide) (PEG-*b*-PLA) copolymers with varying lipid components to modulate surface charge (Fig. 1A). We systematically investigated the influence of surface charge on the pharmacokinetics, tumor accumulation, penetration, and therapeutic effect of these PEGylated NPs. Our results reveal that cationic PEGylated nanoparticles, although slightly inferior in blood circulation and tumor accumulation, are more effective in inhibiting tumor growth than their anionic or neutral counterparts in a variety of tumor models. Further studies demonstrate that cationic PEGylated NPs have improved penetration in the tumor interstitium, which ultimately enhances the intratumor drug availability and leads to the superior treatment efficacy.

Materials and methods

Materials

PEG-*b*-PLA (poly(ethylene glycol)-*block*-poly(D,L-lactide)) and BHEM-Chol (*N*,*N*-Bis(2-hydroxyethyl)-*N*-methyl-*N*-(2-cholesteryloxycarbonyl aminoethyl) ammonium bromide) were synthesized according to the literature [15]. DOTAP (1,2-dioleoyl-3-trimethylammonium-propane chloride), EPC (1,2-dioleoyl-*sn*-glycero-3-ethylphosphocholine chloride), DSPG (1,2-distearoyl-*sn*-glycero-3-phospho-(1′-rac-glycerol) sodium), and DOPE (1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine) were purchased from Avanti Polar Lipid Inc (Alabaster, AL), and used as received. Rhodamine B (RhoB)-labeled poly(ε-caprolactone) (PCL_{RhoB}) was synthesized as previously reported [16]. Docetaxel and epirubicin were purchased from Sigma—Aldrich (St Louis, MO).

Cells and animals

The human breast cancer cell line MDA-MB-231, human prostate cancer cell line PC3, human pancreatic adenocarcinoma cell line BX-PC3, murine colon adenocarcinoma cell line CT26 and murine melanoma cell line B16 were purchased from the American Type Culture Collection (Manassas, VA). Cells were maintained in Dulbecco's modified Eagle's medium (DMEM, Gibco BRL, Eggenstein, Germany) or DMEM- Ham's F-12 medium (DMEM/F12, Gibco BRL) supplemented with 10% Fetal bovine serum (FBS) at 37 °C in a 5% CO_2 atmosphere. HUVECs were purchased from Life technologies (Carlsbad, CA) and cultured with HUVEC proliferating culture technologies. The MDA-MB-231 cell line with stably green fluorescent protein (GFP) expression was obtained by transfection with a retrovirus according to a standard protocol as described previously [17].

Balb/c nude mouse, ICR and C57BL/6 mice were obtained from Beijing HFK Bioscience Co., Ltd. All animals received care in compliance with the guidelines outlined in the Guide for the Care and Use of Laboratory Animals. The procedures were approved by the University of Science and Technology of the Chinese Animal Care and Use Committee.

Preparation of drug-loaded or RhoB-labeled nanoparticles (NPs)

PEG-*b*-PLA (10.0 mg), drug (docetaxel or epirubicin, 1.0 mg) with or without lipid (0.1 mg) in ethyl acetate (200 μL, Fisher Scientific, Pittsburgh, PA) was emulsified by sonication (VC-130, Sonics & Materials, Inc., Newtown, CT) (80 W for 2 min) over an ice bath in 1 mL of ultra-purified deionized water (Millipore, Bedford, MA) to form an oil-in-water emulsion. Ethyl acetate was removed using a rotary evaporator at room temperature under a vacuum of 13 mbar. The resulting mixture was centrifuged at 2000 rpm for 20 min to remove potential aggregates. RhoB-labeled NPs were prepared similarly by replacing the drug with 3 wt% PCL_{RhoB}.