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A multi-functional polymeric carrier for simultaneous positron emission tomography imaging and combination therapy

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ABSTRACT

Multifunctional nanoplatforms offering simultaneous imaging and therapeutic functions have been recognized as a highly promising strategy for personalized nanomedicine. In this work, we synthesized a farnesylthiosalicylate (FTS, a nontoxic Ras antagonist) based triblock copolymer POEG-*b*-PVBA-*b*-PFTS (POVF) composed of a poly(oligo(ethylene glycol) methacrylate) (POEG) hydrophilic block, a poly(FTS) hydrophobic block, and a poly(4-vinylbenzyl azide) (PVBA) middle block. The POVF polymer itself was active in inhibiting the tumor growth in vitro and in vivo. Besides, it could serve as a carrier to effectively encapsulate paclitaxel (PTX) to form stable PTX/POVF mixed micelles with a diameter around 100 nm. Meanwhile, POVF polymer provides the active azide group for incorporating a positron emission tomography (PET) imaging modality via a facile strategy based on metal-free click chemistry. This nanocarrier system could not only be used for co-delivery of PTX and FTS, but also for PET imaging guided drug delivery. In the 4T1.2 tumor bearing mice, PET imaging showed rapid uptake and slow clearance of radiolabeled PTX/POVF nanomicelles in the tumor tissues. In addition, the FTS-based multi-functional nanocarrier was able to inhibit tumor growth effectively, and the co-delivery of PTX by the carrier further improved the therapeutic effect.

Statement of Significance

Due to the intrinsic heterogeneity of cancer and variability in individual patient response, personalized nanomedicine based on multi-functional carriers that integrate the functionalities of combination therapy and imaging guidance is highly demanded. Here we developed a multi-functional nanocarrier based on triblock copolymer POEG-b-PVBA-b-PFTS (POVF), which could not only be used for co-delivery of anticancer drugs PTX and Ras inhibitor FTS, but also for PET imaging guided drug delivery. The POVF carrier itself was active in inhibiting the tumor growth in vitro and in vivo. Besides, it was effective in formulating PTX with high drug loading capacity, which further enhanced the tumor inhibition effect. Meanwhile, we developed a simple and universal approach to incorporate a PET radioisotope (Zr-89 and Cu-64) into the azide-containing PTX/POVF micelles via metal-free click chemistry in aqueous solution. The radiolabeled PTX/POVF micelles exhibited excellent serum stability, rapid tumor uptake and slow clearance, which validated the feasibility of the PET image-guided delivery of PTX/POVF micelles.

1. Introduction

Over the years, a variety of nano drug delivery systems, including polymers, micelles, liposomes, dendrimers and inorganic nanoparticles have been developed to improve the therapeutic efficacy of the chemotherapeutic drugs and decrease their systemic toxicities [1–5]. Although many formulations have demonstrated

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promising outcomes in preclinical studies for cancer treatment, clinical translations of these nanoformulations are still at a slow pace. One of the major hurdles is that different patients in clinic may respond differently to a given formulation with respect to its bioavailability at tumor tissues [6]. Thus, personalized nanomedicine aiming to individualize nanotherapeutic treatment based on in vitro and in vivo disease- and patient-specific information is highly demanded [7,8]. A promising strategy in the personalized nanomedicine is to incorporate both noninvasive imaging and therapeutic functions into a single formulation, which can provide insights on the individual patient response to therapy and help to adjust the follow-up treatment plans [9–11]. In addition, it may also aid in preselecting the patient groups who benefit maximally from a particular treatment [12].

Polymeric nanoparticles with advantages of small size, flexible structure, and ease of functionalization have attracted increasing attention as dual-functional carriers for simultaneous imaging and drug delivery [13–16]. However, most of these systems only include one single drug which may not meet the clinical need because of the intrinsic heterogeneity of cancer. To achieve better therapeutic outcomes, there is still a need for the construction of multifunctional carriers that can integrate the functionalities of imaging guidance and combination therapy.

As prodrug carriers, amphiphilic polymers attached with hydrophobic drugs via covalent bonds offer an attractive feature for co-delivery of two or more drugs [17–19]. The conjugated multiple hydrophobic drug molecules with proper structures could endow the polymeric carriers with the capabilities to efficiently load other drugs through hydrophobic interaction and π-π stacking effect. The conjugated drug in the carriers can be chosen such that it could counteract the side effects caused by the loaded drug and/or promote synergistic effect with the loaded drug [20,21].

S-trans, trans-farnesylthiosalicylic acid (FTS) is a synthetic farnesylcysteine mimetic that acts as a potent and especially nontoxic Ras antagonist [22]. Constitutively active Ras caused by mutation in the Ras family of proto-oncogenes is present in one-third of human cancers [23,24]. The activated form of Ras constitutively activates its downstream effectors, contributing to cell transformation [25]. FTS inhibits excessively activated Ras proteins, resulting in the inhibition of Ras-dependent tumor growth [26,27]. FTS causes significant reduction of Ras levels in a wide array of established cancer models and inhibition of tumor growth in animals with no adverse toxicity [28]. In addition to its antitumor activity by itself, FTS can sensitize tumors to other treatments such as chemotherapy and radiation therapy [29]. However, the efficacy of FTS is limited by its poor water solubility and limited oral bioavailability. To improve the delivery efficacy of FTS, we have recently developed a series of FTS-based prodrug carriers that consist of a PEG hydrophilic segment and various FTS-based hydrophobic domains [30,31]. The carriers could inhibit the tumor growth by themselves. More importantly, they could self-assemble to form micelles that are effective in formulating a number of hydrophobic agents, including paclitaxel (PTX), doxorubicin (DOX) and curcumin, for combination therapy.

In this work, we developed a multi-functional FTS-based carrier system by combining a co-delivery function and a positron emission tomography (PET) imaging modality together, which facilitated direct assessment of the biodistribution and efficiency of in vivo delivery. PET imaging is a clinically used noninvasive imaging technique that facilitates quantitative analysis of pharmacokinetics and biodistribution due to its high sensitivity and unlimited penetration depth [32,33]. It is known that high radiolabeling specific activity is important for nanoparticle-based PET imaging system to obtain high-quality images at low dose of radioactivity [34]. In order to tag FTS-based system with PET radioisotopes efficiently, we synthesized a POEG-*b*-PVBA-*b*-PFTS (POVF) tri-block copolymers composed of a poly(oligo(ethylene glycol) methacrylate) (POEG) hydrophilic block, a poly(FTS) hydrophobic block, and a poly(4-vinylbenzyl azide) (PVBA) middle block via reversible addition fragmentation chain transfer (RAFT) polymerization. As shown in Scheme 1, the triblock polymers could form micelles with multiple FTS moieties in the hydrophobic core, which is beneficial for co-loading hydrophobic PTX via π-π stacking effect and hydrophobic interaction. The POEG hydrophilic shell can stabilize the micelles and protect the loaded drug from premature release in the circulation system [35]. The azide groups in the intermediate layer of micelles allow further reaction with isotope-bearing compounds with alkyne functional groups for radiolabeling via metal-free click chemistry [36,37]. The resulting radiolabeled PTX/POVF micelles were used for PET imaging of mice bearing 4T1.2 tumor xenografts to monitor the biodistribution in a real-time manner. Additionally, in vitro and in vivo therapeutic efficacies of this multi-functional system were evaluated.

2. Materials and methods

2.1. Materials

4-Vinylbenzyl azide (VBA-monomer) was synthesized by reaction of sodium azide with 4-vinylbenzylchloride according to the literature [38,39]. FTS-monomer and POEG macroCTA were synthesized as previously reported [31]. 2,2′-Azobis (2-methylpropionitrile) (AIBN) was recrystallized in anhydrous ethanol before use. Paclitaxel was purchased from AK Scientific Inc. (CA, USA). Trypsin-EDTA solution, 3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) and Dulbecco's Modified Eagle's Medium (DMEM) were all bought from Sigma-Aldrich (MO, USA). Fetal bovine serum (FBS) was purchased from Invitrogen (NY, USA). Copper-64 was obtained from Washington University (St. Louis, MO) and University of Wisconsin (Madison, WI). Zirconium-89 was obtained from Washington University (St. Louis, MO). Luna C-18 HPLC columns were from Phenomenex (Torrance, CA, USA).

2.2. Characterization

^{1}H NMR spectrum (400.0 MHz) was recorded on a Varian 400 FT-NMR spectrometer with $CDCl_3$ as the solvent. Molecular weights (M_n and M_w) and molecular weight distributions (M_w/M_n) of the synthesized polymers were determined by gel permeation chromatography (GPC) equipped with a Waters 2414 refractive index detector. THF was used as the eluent with a flowing rate of 1.0 mL/min at 35 °C. A series of polystyrene standards with narrow molecular weight distribution were applied for calibration. HPLC and FPLC were performed on a Waters 1525 Binary HPLC pump (Milford, MA) with a Waters 2489 UV/visible detector and a model 106 Bioscan radioactivity detector for the analysis of either ^{64}Cu or ^{89}Zr labeled conjugates using either a two-components buffer (0.1 v% TFA in de-ionized water + 0.1 v% TFA in acetonitrile) or PBS as the eluting phase for HPLC and FPLC respectively. PET/CT data were acquired using an Inveon Preclinical Imaging Station (Siemens Medical Solutions).

2.3. Synthesis of POEG-b-PVBA polymers

AIBN (1 mg, 0.0062 mmol), POEG macroCTA (233 mg, 0.031 mmol), VBA-monomer (59 mg, 0.372 mmol) and 2 mL dried tetrahydrofuran (THF) were added in a Schlenk tube, and deoxygenated by three freeze-pump-thawing cycles. The mixture was stirred at 70 °C under N_2 protection for 3 h, and then the reaction was stopped by immersing the tube into liquid nitrogen. The reac-

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