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Terminating the criminal collaboration in pancreatic cancer: Nanoparticle-based synergistic therapy for overcoming fibroblast-induced drug resistance

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ABSTRACT

Pancreatic ductal adenocarcinoma (PDAC) is a lethal cancer with a dismal overall prognosis mainly unchanged over the past decades. PDAC is generally refractory to conventional treatments, and thus novel therapies are urgently needed. Recently, accumulating evidence has indicated that human pancreatic stellate cells (PSCs) facilitate PDAC development and drug resistance through paracrine activation of hedgehog pathway. Here, we report that smart SN38 (active metabolite of irinotecan) polymeric prodrug-based nanoparticles effectively encapsulate the commercial hedgehog pathway inhibitor GDC-0449 for co-delivery. More intriguingly, we obtained size-tunable nanoparticles with increased GDC-0449 loading efficiency by simply extending the chain length of the hydrophobic SN38 block. To better evaluate the efficacy and investigate the synergistic mechanisms, we immortalized human PSCs and established fibroblast-containing models *in vitro* and *in vivo*. In PSCs, BxPC-3 cells and MIA PaCa-2 cells, GDC-0449 suppressed the co-culture induced up-regulations of the two drug resistance contributors: sonic hedgehog transcription factor glioma-associated protein1 (GLI-1) and UGT1A glucuronosyltransferase. Importantly, the nanoparticle-mediated co-delivery system exhibited potent antitumor efficacy with enhanced apoptosis and reduced collagen, α-SMA and GLI-1 expression in tumor tissues. These findings reveal a potential strategy to utilize nanoparticle-mediated drug co-delivery platform as an effective combination therapy for fibroblast-enriched PDAC.

1. Introduction

Pancreatic ductal adenocarcinoma (PDAC) is one of the most lethal human malignancies with an overall 5-year survival rate below 7% [1]. Despite extensive efforts, the efficacy of chemotherapy and targeted therapies has remained virtually unchanged for many decades. Previous research mainly focused on pancreatic cancer cells, while the tumor microenvironment, particularly the influence of stromal components on tumor progression, was widely neglected [2]. Recently, tumor activated pancreatic stellate cells (PSCs), also known as cancer associated fibroblasts (CAFs) in PDAC, have attracted increasing attentions as accumulating evidence indicates the crosstalk between PSCs and pancreatic cancer cells facilitates tumor growth, metastasis and drug resistance [3–7].

Aberrant activation of the sonic hedgehog (SHH) signaling has been observed widely in pancreatic cancer and paracrine SHH signaling plays an important role in the communication between tumor and stromal cells [8–10]. SHH ligands derived from tumor cells could bind to the Patched protein in stromal cells and relieve the inhibition of transducer protein Smoothened (SMO). The accumulation of activated SMO mediates downstream signal transduction includes the dissociation of GLI-1 transcription

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factors, triggering synthesis of ECM proteins, such as collagen and hyaluronic acid [11]. The downstream target genes of GLI-1 include those upregulating cell growth, drug resistance and epithelia-mesenchymal transition (EMT) [12,13]. Therefore, SHH blockage seems a good promising way to prevent drug resistance, increase intratumoral drug diffusion and inhibit the invasion and metastases of pancreatic cancer [14–16]. GDC-0449 (vismodegib) is an FDA (U.S. Food and Drug Administration) approved hedgehog inhibitor for the treatment of basal-cell carcinoma. Several combination treatments comprising GDC-0449 are now in clinical trials for pancreatic cancer (NCT01088815, NCT00878163).

In the past two decades, increasing therapeutic nanoparticles have been approved for cancer therapy [17,18] due to the advantages of nanomedicine, such as the enhanced permeability and retention (EPR) effect [19]. Importantly, nanoparticles loaded with different therapeutic molecules could offer a platform for effective combination therapy, which is widely used in the clinic for complex cancers involving multiple pathways. Compared to combination therapy of small-molecule drugs, nanoparticle-mediated co-delivery provides improved and normalized pharmacokinetics and biodistribution of loaded drugs that have disparate pharmacological behaviors, resulting in improved efficacy and reduced toxicity [20,21]. For instance, CPX-351 is a liposomal nanoparticle of cytarabine and daunorubicin encapsulated at an optimized 5:1 M ratio. Significantly improved overall survival (9.56 vs. 5.95 months) but without any increase in the frequency of adverse events was reported in a recent phase III clinical trial of CPX-351 versus traditional 7 + 3 chemotherapy in older patients with secondary acute myeloid leukemia (AML). FDA has granted a breakthrough therapy designation to CPX-351 [22,23].

The nanoliposomal formulation of Irinotecan (ONIVYDE) in combination with fluorouracil (5FU) and leucovorin (LV) has been approved to treat patients with advanced pancreatic cancer. SN38 (7-ethyl-10-hydroxy camptothecin) is the active metabolite of irinotecan and 1000 times more active. However, SN38 cannot be used directly because of its poor solubility in water and pharmaceutically approved solvents [24]. In this study, we first demonstrate that GDC-0449 reverses co-culture-induced SN38 resistance through downregulation of GLI-1 and UGT1A. Then, we construct amphiphilic SN38 prodrug polymers PEG_{5K}—$P(HEMASN38)_x$ with size-tunable and hydrophobic SN38 inner cores, where GDC-0449 is encapsulated. Increased GDC-0449 loading efficiency and reduced particle size are obtained by simply extending the SN38 block chain length. Finally, the nanoparticle-mediated co-delivery system exhibits robust antitumor efficacy with enhanced apoptosis and reduced collagen and GLI-1 expression in tumor tissues (Fig. 1). These results highlight a potential strategy to improve therapeutic efficacy in pancreatic cancer *via* modulating the fibroblast-enriched microenvironment using nanoparticle-mediated co-delivery system.

2. Materials and methods

2.1. Materials, cell lines and animals

7-Ethyl-10-hydroxycamptothecin (SN38) was purchased from Xi'an Xindifu Science and Technology Co. (Xi'an, China). GDC-0449 was obtained from Southeast Pharmaceuticals Co. (Suzhou, China). Mono-4-[2-(methacryloyloxy)ethoxy]-4-oxobutanoic acid and PMDETA were purchased from Sigma-Aldrich, China. All reactions were performed under argon atmosphere. All solvents were dried over calcium chloride and redistilled before use. The human pancreatic adenocarcinoma cell lines BxPC-3, Panc-1 and Miapaca-2 were purchased from Cell Bank of Type Culture Collection of Chinese Academy of Sciences (Shanghai, China). All cell lines above were initially obtained several years ago without additional authentication. Primary human pancreatic stellate cells were kindly provided by Professor Yi Miao from the First Affiliated Hospital of Nanjing Medical University with additional authentication. BALB/C female nude mice, ICR mice and Sprague-Dawley rats were obtained from the Institute of Medicine, Zhejiang province and housed in sterile cages with a standard condition. All works performed on animals were approved by the Animal Care and Use Committee of Zhejiang University in accordance with the Chinese guidelines for the care and use of laboratory animals.

2.2. Characterization of PEG_{5K}—$P(HEMASN38)_x$ polymers

PEG_{5K}—$P(HEMASN38)_x$ polymers with different block lengths were prepared *via* Atom Transfer Radical Polymerization (ATRP) as described previously [25]. To characterize the chemical compositions of synthesized copolymers, ^1H nuclear magnetic resonance (NMR) spectra were obtained using a Bruker Avance DRX-400 spectrometer (Bruker BioSpin Corporation, Billerica, MA) with dimethyl sulfoxide-d6 (DMSO-d_6) as solvent. Molecular weight distribution and polydispersity index of obtained copolymers were measured by gel permeation chromatography performed on a Wyatt GPC/SEC-MALS (Wyatt technology Corporation, Santa Barbara, CA) system equipped with a DAWN® HELEOS® II 18-angle static light scattering detector and an Optilab® T-rEX™ refractive index detector, a 50 × 8.0 mm MZ-GEL SD*plus* 100 Å 10 μM GPC-PRECOLUMN, a 300 × 8.0 mm MZ-GEL SD*plus* 10E4 Å 10 μM and a 300 × 8.0 mm MZ-GEL SD*plus* 100 Å 10 μM at 50 °C. Data were recorded and processed using ASTRA v6.0 (Wyatt Technology Corporation). Polystyrene was used as the standard material for calibration and DMF was used as the mobile phase at a flow rate of 0.80 mL/min.

2.3. Preparation and characterization of PEG_{5K}—$P(HEMASN38)_x$ nanoparticles

The PEG_{5K}—$P(HEMASN38)_x$ nanoparticles encapsulating GDC-0449 were prepared by the outgrowth method. In brief, PEG_{5K}—$P(HEMASN38)_{9K}$ (5 mg) and GDC-0449 (2 mg) were dissolved in 2 mL DMSO and then 5 mL water was added into the solution in a dropwise manner under stirring. The resultant solution was dialyzed against 3 × 2 L water (Mw = 3500 Da), filtered with 0.22 μm membrane and concentrated to 1 mL. The size distributions of nanoparticles were measured by dynamic light scattering using a Zetasizer Nano-ZS (Malvern Instruments Co., UK). The transmission electron microscope (TEM) observation of nanoparticles was performed by the outgrowth method. In brief, the nanoparticles were diluted in water at a concentration of about 0.05 mg/mL (equal concentration of SN38). One drop of the solution was dropped onto a copper grid covered with a nitrocellulose membrane. The copper grid was lyophilized and stained with phosphotungstic acid. Samples were observed with a JEOL 1230 electron microscope.

2.4. Determination of GDC-0449 and SN38 by HPLC

The concentrations of GDC-0449 and SN38 were measured by the HPLC system which consists of a 1525 binary HPLC Pump, a 2475 multi-λ Fluorescence Detector and a 2998 Photodiode Array Detector (Waters Co., Singapore). An XBridge™ C18 (4.6 × 250 mm, 5 μm) column was used in this HPLC system. The Waters Breeze 2 System was used for data acquisition and analysis. The