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Targeting of metastasis-promoting tumor-associated fibroblasts and modulation of pancreatic tumor-associated stroma with a carboxymethylcellulose-docetaxel nanoparticle

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ABSTRACT

Pancreatic ductal adenocarcinomas are characterized by the desmoplastic reaction, a dense fibrous stroma that has been shown to be supportive of tumor cell growth, invasion, and metastasis, and has been associated with resistance to chemotherapy and reduced patient survival. Here, we investigated targeted depletion of stroma for pancreatic cancer therapy via taxane nanoparticles. Cellax-DTX polymer is a conjugate of docetaxel (DTX), polyethylene glycol (PEG), and acetylated carboxymethylcellulose, a construct which condenses into well-defined 120 nm particles in an aqueous solution, and is suitable for intravenous injection. We examined Cellax-DTX treatment effects in highly stromal primary patient-derived pancreatic cancer xenografts and in a metastatic PAN02 mouse model of pancreatic cancer, focusing on specific cellular interactions in the stroma, pancreatic tumor growth and metastasis. Greater than 90% of Cellax-DTX particles accumulate in smooth muscle actin (SMA) positive cancer-associated fibroblasts which results in long-term depletion of this stromal cell population, an effect not observed with Nab-paclitaxel (Nab-PTX). The reduction in stromal density leads to a >10-fold increase in tumor perfusion, reduced tumor weight and a reduction in metastasis. Consentingly, Cellax-DTX treatment increased survival when compared to treatment with gemcitabine or Nab-PTX in a metastatic PAN02 mouse model. Cellax-DTX nanoparticles interact with the tumor-associated stroma, selectively interacting with and depleting SMA positive cells and macrophage, effects of which are associated with significant changes in tumor progression and metastasis.

1. Introduction

The desmoplastic response, a dense accumulation of fibrous and cellular stroma, is a prominent feature of pancreatic tumors. Stromal components, such as fibroblasts, endothelial cells, inflammatory cells and extracellular matrix, provide a tumor microenvironment that promotes tumor progression, metastasis and treatment resistance [1,2]. Cancer-associated fibroblasts (CAFs) represent the majority of non-cancer cells within the tumor and are characterized by the expression of smooth-muscle actin (SMA). Rather than being bystanders, CAFs closely interact with tumor cells and actively promote tumor growth through the secretion of proteins that activate signaling pathways involved in proliferation and metastasis [1]. In the context of therapy, stroma is understood to significantly impair drug delivery: the contractile phenotype of CAFs, inadequate vascularization, high extracellular matrix (ECM) density, and high interstitial fluid pressure (IFP) impair the transport of chemotherapeutics to tumor cells [3–5]. Tumor-associated stroma is widely recognized as a target for therapy, both to normalize the microenvironment, and to reduce drug delivery barriers [3–9].

In contrast to other solid tumors, tumor-associated stroma represents the most prominent component of pancreatic tumors, and therefore, stroma has become a focus of pancreatic cancer therapeutic development. Approaches to stromal control have included modulation of matrix [5], targeting of VEGF-driven angiogenesis [10], and suppression of the hedgehog pathway [9], but clinical success has been limited, as human tumors tend to become resistant to drugs that block these pathways [3]. Recently, Von Hoff et al. [11] reported that gemcitabine

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plus Nab-PTX (Abraxane) significantly prolongs survival compared to gemcitabine alone [12]. Nab-PTX is a complex of paclitaxel with human albumin, and is understood to interact with SPARC protein, which acts to concentrate the drug in the tumor compartment [13–15]. Pancreatic tumors display high SPARC expression, consistent with highly stromal tumors [16], and it has been demonstrated that Nab-PTX depletes pancreatic tumor stromal content, a reduction correlated to increased uptake of gemcitabine [11]. These findings support the idea that the stroma represents a clinically relevant target in pancreatic cancers.

Cellax-DTX is a conjugate of PEGylated carboxymethylcellulose and docetaxel: this macromolecule condenses into well-defined 120 nm nanoparticles in saline, and is stable in serum, against dilution, and in storage at 4 °C. It has been demonstrated that these particles are long circulating compared to native DTX and Nab-PTX, and exhibit a 5 and 10-fold increase in tumor accumulation, respectively [17–19]. We recently reported that Cellax-DTX depletes SMA positive stroma in breast cancer models, with a significant reduction in metastatic potential [20]. In that study, the model was established by injecting tumor cell lines into the mammary fatpad, which did not exhibit the true anatomical structure of a tumor microenvironment presented in humans [21]. This previous study only reported significant association of Cellax with CAFs, but did not systemically examine the uptake of Cellax nanoparticles in other major cell populations of the tumor microenvironment, nor did prior work determine the pharmacodynamic impact on these cell types. These mechanistic studies are required to conclude the target of Cellax in solid tumors. In the current study, we aimed at further delineating the biofate of Cellax nanoparticles in a relevant tumor microenvironment, identifying the target of Cellax through a pharmacodynamic mechanistic study and validate its stromal modulating effect in another tumor type (i.e. stroma-rich pancreatic tumor). We employed the human derived pancreatic xenograft models, which displayed a relevant tumor microenvironment with the true anatomical structure reported with human tumors [22], to study Cellax uptake and compare the pharmacodynamics of Cellax and Nab-PTX in the pancreatic tumor microenvironment. Nab-PTX was included as a control in this study, as it is a nanoparticle formulation of taxane that was recently approved for pancreatic tumor therapy, and appears to exhibit a microenvironment modulating activity.

2. Materials and methods

2.1. Reagents and reference drugs

Carboxymethylcellulose (CMC) sodium salt 30000-P was purchased from CPKelco (Atlanta, GA, USA). Docetaxel (DTX) was purchased from LC Laboratories (Woburn, MA, USA). Polyethylene glycol methyl ether (mPEG-OH, MW = 2000), N-hydroxysuccinimide (NHS), 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide HCl (EDC.HCl), and 4-dimethylaminopyridine (DMAP) were purchased from Sigma Aldrich (Oakville, ON, CA). DiI (1,10-dioctadecyl-3,3,3030-tetramethylindocarbocyanine perchlorate, D-307) was purchased from Invitrogen (Burlington, ON, CA). Gemcitabine was purchased from BetaPharma (Branford, CT, USA). Abraxane (Nab-PTX) was purchased from the University Health Network (UHN) pharmacy. Tissue-Tek® OCT™ compound was purchased from VWR (Mississauga, ON, CA).

2.2. Preparation of Cellax-DTX nanoparticles

The Cellax-DTX polymer consists of an acetylated carboxymethylcellulose backbone coupled to DTX and PEG via ester linkages: the synthesis and characterization are previously described [17–19,23]. Cellax-DTX nanoparticles were prepared by a nanoprecipitation technique as previously described [17–19,23], yielding sterile 120 nm particles in saline. Particle size was measured on a Malvern NanoZS (Malvern Instruments, Malvern, UK) instrument. To determine the concentration of DTX in nanoparticle suspension, Cellax-DTX particle solutions (100 μL) were lyophilized, dissolved in deuterated dimethylsulfoxide (^{d}DMSO, 900 μL) containing 2-methyl-5-nitrobenzoic acid (1 mg/mL) internal standard (IS), and were analyzed by ^{1}H NMR. DTX concentration was calculated using a DTX/IS response factor generated using a DTX (1 mg/mL) solution containing the IS. Cellax-DTX particles containing DiI (Cellax-DTX-DiI) were prepared and characterized as reported previously: all Cellax-DTX-DiI nanoparticle suspensions were adjusted to 300 μg DiI/mL for intravenous administration (200 μL) for tissue distribution analyses [19].

2.3. Cell culture

The mouse PAN02 pancreatic cancer cell line was obtained from American Type Culture Collection (ATCC). Cells were maintained in Dulbecco's Modified Eagle's Medium (DMEM, Invitrogen, Burlington, ON, CA) supplemented with 10% fetal bovine serum at 37 °C and 5% CO_2 for a maximum of 10 passages.

2.4. Mouse models of pancreatic cancer

The OCIP19 and OCIP23 primary pancreatic xenografts were established from patient pancreatectomy samples in accordance to institutional guidelines for human and animal research, as previously described [24]. Briefly, tumor fragments were surgically implanted on top of the pancreas of 4–5 week old male SCID. Treatments were initiated when tumors were 5 mm in diameter.

For experiments involving orthotopic implantation of the PAN02 cell line, female C57/BL6 mice (Jackson Laboratories, Bar Harbour, ME, USA) were anesthetized, a left lateral laparotomy (subcostal 4 mm incision into the peritoneal cavity) was performed, and the spleen and pancreas were exposed and mobilized. PAN02 cells (50 μL, 2.0×10^7 cells/mL) were injected into the pancreas, the spleen and pancreas were placed back within the abdominal cavity, and the muscle and skin layers were closed with sutures.

All animal use protocols were approved by the Animal Care Committee of the University Health Network (AUP 786 and 2402). Maximum tolerated doses (MTDs) of Cellax-DTX, DTX, gemcitabine, and Nab-PTX were established in previous reports [17,19].

2.4.1. OCIP19 models

Pharmacodynamic study (1 dose):, five mice per treatment group were treated intravenously with saline, Nab-PTX (50 mg PTX/kg), or Cellax-DTX (170 mg DTX/kg), and were sacrificed at 1, 3 and 6 d post-treatment. At each timepoint, tumors were fixed in buffered formalin for histological analysis. A subset of mice (n = 5) in this experiment were treated with Cellax-DTX-DiI (fluorescent particles), and were sacrificed 24 h after treatment: tumors were frozen in OCT for cryosectioning and histology analysis.

Efficacy study (3 dose): primary human pancreatic xenografts (OCIP19 and OCIP23) were divided into fragments and sutured to the surface of the pancreas in male SCID mice [24]. When tumors were palpable, mice were treated intravenously (iv) with saline, Nab-PTX (50 mg PTX/kg), or Cellax-DTX (170 mg DTX/kg) q1w × 3. Weight and mouse health were monitored throughout the course of therapy. Two weeks following the 3rd treatment, mice were injected with FITC-lectin (Sigma Aldrich, Oakville, ON, CA L0401, 0.05 mg in saline), and sacrificed 4 h later. On necropsy, tumors were weighed and fixed for histology analysis, and mice were examined for metastatic presentation in the peritoneum, diaphragm, liver, hepatic portal, spleen, and mesentarium.

2.4.2. PAN02 models

1 dose sc model: 2 wks after inoculation with PAN02 cells, mice were treated with a single dose IV with therapies at MTD: DTX (40 mg/kg), Nab-PTX (170 mg PTX/kg), and Cellax-DTX (170 mg DTX/kg). Six