Tumor-antigen Targeted Imaging of Pancreatic Adenocarcinoma

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Background

Previously, we have described the synthesis and testing of a novel dual-modality targeted contrast agent (CLIO-EPPT) for tumor imaging based on specificity for the underglycosylated mucin-1 tumor antigen (uMUC-1) (1). CLIO-EPPT consists of crosslinked superparamagnetic iron oxide nanoparticles (CLIO) for MR imaging, modified with Cy5.5 dye (for optical near-infrared imaging, NIRF), and has peptides (EPPT), specifically recognizing uMUC-1, attached to the nanoparticle's dextran coat.

In our earlier studies, we illustrated the applicability of CLIO-EPPT for tumor detection and delineation in *subcutaneous* (1) and *orthotopic* (2) mouse models. In addition, we demonstrated the feasibility of using the dual-modality MR/NIRF imaging approach for the accurate and efficient evaluation of change in tumor volume over time, as a measure of chemotherapeutic success in a pancreatic cancer orthotopic model. An important question that remains unanswered, however, deals with the possibility of extracting not only anatomic but also molecular information about tumor response to therapy, using our tumor-antigen targeted probe. With these new studies, we have begun to address this issue. First, we compared the accumulation of CLIO-EPPT in orthotopic pancreatic tumors to a scrambled control nanoparticle (CLIO-SCR). This would allow us to obtain more accurate information about the contribution of tumor-antigen specific vs. nonspecific effects to probe uptake by tumors. In addition, we have demonstrated that the chemotherapeutic regimen employed by us did not lead to impairment of probe uptake by tumors, as assessed by MR and NIRF imaging, and that this observation was in agreement with the lack of uMUC-1 antigen downregulation, following treatment.

Materials and Methods

In vitro studies: Differential uptake of CLIO-EPPT vs. CLIO-SCR by CAPAN-2 pancreatic adenocarcinoma cells was confirmed by fluorescence microscopy. Briefly, cells were incubated with probe at 37°C for 2 hrs and analyzed in the bright-field channel for cell identification, the green channel for detection of the FITC label on EPPT peptides, and in the NIR channel for detection of the Cy5.5 label on CLIO particles.

Imaging: *In vivo* MR imaging was performed on NOD.SCID mice bearing orthotopically-injected uMUC-1-positive CAPAN-2 pancreatic adenocarcinoma tumors before and 24 hours after i.v. injection of CLIO-EPPT or CLIO-SCR. For T2 map construction, imaging parameters were as follows: TR/TE = 3000/8, 16, 24, 32, 40, 48, 56, 64. FOV = 40x40mm, matrix size 128x 128, slice thickness = 0.5mm. NIRF imaging on the same animals was performed immediately after each MRI session.

Treatment: The treatment protocol involved i.p. injection of 30mg/kg of the pyrimidine analogue 5-fluorouracil (5-FU) once daily for five days beginning 14 d following tumor implantation. 5-FU is a clinically approved therapeutic agent for pancreatic cancer, which exerts its antitumor effects by inhibiting DNA/RNA synthesis.

Real-Time Quantitative RT-PCR: CAPAN-2 pancreatic adenocarcinoma cells were incubated with 5mg/ml of 5-FU for 48 hrs as previously described (17). Total RNA was extracted from treated and non-treated cells, using the Rneasy Mini kit, according to the manufacturer's protocol (Qiagen Inc., Valencia, CA). Relative levels of uMUC-1 mRNA in 5-FU treated and non-treated CAPAN-2 cells were determined by real-time quantitative RT-PCR (TaqMan protocol). TaqMan analysis was performed using an ABI Prism 7700 sequence detection system (PE Applied Biosystems, Foster City, CA). The PCR primers and TaqMan probe specific for MUC-1 mRNA were designed using Primer express software 1.5. Primer and Probe sequences were as follows:

Forward primer, 5'-ACAGGTTCTGGTCATGCAAGC-3' (nucleotides 64-84 in the 5' non-repetitive region);

Reverse primer, 5'-CTCACAGCATTCTTCTCAGTAGAGCT-3' (nucleotides 139-164 in the 5' non-repetitive region);

TaqMan Probe, 5'-FAM-TGGAGAAAAGGAGACTTCGGCTACCCAGA-TAMRA-3' (nucleotides 96-124 in the 5' non-repetitive region).

Eukaryotic 18S rRNA TaqMan PDAR Endogenous Control reagent mix (PE Applied Biosystems, Foster City, CA) was used to amplify 18S rRNA as an internal control, according to the manufacturer's protocol.

Results

CAPAN-2 pancreatic adenocarcinoma cells demonstrated negligible uptake of CLIO-SCR following in vitro incubation with the probe. By contrast, bright fluorescence in the green and near-infrared channels indicated significant accumulation of our targeted contrast agent, CLIO-EPPT.

In order to establish differential probe uptake in vivo, we constructed pre- and post-contrast T2 maps of tumor-bearing animals injected either with CLIO-EPPT or CLIO-SCR. Analysis of T2 maps revealed a 47% average T2 reduction in pancreatic adenocarcinomas following CLIO-EPPT administration ($p \leq 0.01$). No reduction in T2 associated with the tumors was evident following injection of CLIO-SCR. The specific accumulation of CLIO-EPPT in these tumors was confirmed by NIRF imaging. In mice injected with CLIO-EPPT, a high-intensity fluorescence signal was associated with the tumor implanted in the pancreas (3078.2±246.4 RFU). Background fluorescence, as defined by the raw fluorescence associated with an area immediately adjacent to the tumor ROI, was significantly lower (1722.4±154 RFU, $p = 0.006$. In vivo optical imaging of mice injected with CLIO-SCR revealed only background fluorescence within the tumor.

T2 analysis of 5-fluorouracil treated and non-treated tumors revealed no significant difference in relaxivity between the two groups. This observation was confirmed by in vivo NIRF imaging, which demonstrated no change in near-infrared fluorescence intensity, following treatment. The sustained uptake of CLIO-EPPT in the presence of treatment with 5-FU was consistent with the lack of uMUC-1 antigen downregulation by CAPAN-2 cells following exposure to the drug, as demonstrated by real-time quantitative RT-PCR ($p = 0.397$).

Summary

To our knowledge, this study represents one of the first reports of tumor antigen-specific imaging of orthotopically implanted pancreatic carcinomas. It attests to the feasibility of applying a targeted strategy for multimodal tumor imaging. The core value of this investigation lies in the fact that it describes a truly molecular imaging approach, which is an early step towards achieving the goal of in vivo molecular profiling of tumors.

References

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