Theranostic Imaging of Metastatic Prostate Cancer

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Introduction: Our purpose is to develop theranostic imaging of metastatic prostate cancer (PCa) using a nanoplex platform that can ultimately be developed, modified, and applied for different cancers, different receptors, different pathways, and in combination with other treatments. Prostate specific membrane antigen (PSMA) is expressed on the membrane of androgen-independent metastatic PCa. Our PSMA-targeted nanoplex carries a radiolabel for detection, siRNA to downregulate a specific pathway, and a prodrug enzyme that synthesizes a cytotoxic drug locally from a systemically administered nontoxic drug at the nanoplex site. Each component of the nanoplex is carefully selected to allow us to evaluate each of its aspects *i.e.* image-guided delivery of nanoplex, siRNA-mediated downregulation, and conversion of prodrug to cytotoxic drug by the prodrug enzyme, with noninvasive imaging. We selected the prodrug enzyme bacterial cytosine deaminase (bCD) since it converts a non-toxic prodrug 5-fluorocytosine (5-FC) to 5-fluorouracil (5-FU) that can be detected by 19F MRS. Because changes in choline metabolism can be easily detected clinically with magnetic resonance spectroscopic imaging (MRSI) and with [^{11}C]choline PET imaging, and because choline kinase (Chk) is an important target in cancer, we have initially focused on using siRNA to downregulate choline kinase (Chk-siRNA).

Methods: Our prototype nanoplex is synthesized by conjugating three compartments: (i) the prodrug-activating enzyme bCD, (ii) the multimodal imaging reporter carrier poly-L-lysine (PLL) that carries [^{111}In]DOTA for SPECT or [Gd^{3+}]DOTA for MR and a fluorescent probe (Cy5.5 or rhodamine) and, (iii) the siRNA delivery vector: PEI (polyethyleneimine)-PEG (polythethyleneglycol) co-grafted-polymer [1]. These three compartments are covalently conjugated and siRNA-Chk is associated with the PEI-PEG co-grafted polymer through electrostatic affiliation. For PSMA-targeting, a low molecular weight urea-based PSMA targeting moiety (2-(3-[1-carboxy-5-[7-(2,5-dioxo-pyrrolidin-1-yloxycarbonyl)-heptanoylamino]-pentyl]-ureido)-pentanedioic acid (MW 572.56) [2] is used for conjugating NHS-PEG-NHS (MW ~3000) to PEI. Imaging studies with PSMA-targeted nanoplexes were performed with PC-3 human prostate cancer xenografts genetically engineered to overexpress PSMA (PC-3 Pip) in SCID mice. Non-PSMA-expressing PC-3 xenografts (PC-3 Flu) were used as controls. MR experiments were performed with a Bruker horizontal bore 9.4T animal MR scanner using a home-built RF resonator. Fluorescence imaging was performed *in vivo* with a Xenogen IVIS Spectrum system. SPECT/CT images were acquired on a Gamma Medica X-SPECT scanner.

Results and Discussion: Images obtained with Pip and Flu tumors in Figure 1A demonstrate increased uptake in the PSMA-overexpressing Pip tumor compared to the non-PSMA-expressing Flu tumor. In separate studies we performed optical imaging of the nanoplex in tissue slices without or with PSMA blocking in mice with Pip and Flu tumors. Increased uptake in the Pip tumor compared to Flu was observed without blocking, which was reduced with blocking (Figure 1B). Corresponding quantitative information is shown in the bar graph in Figure 1B. Administration of the theranostic nanoplex in mice bearing PC-3 Pip tumors resulted in a significant decrease of total choline (tCho) within 24 to 48 h as shown in Figure 2. The prodrug enzyme bCD converted the prodrug 5-FC to 5-FU at 24 h and at 48 h as shown in Figure 3.

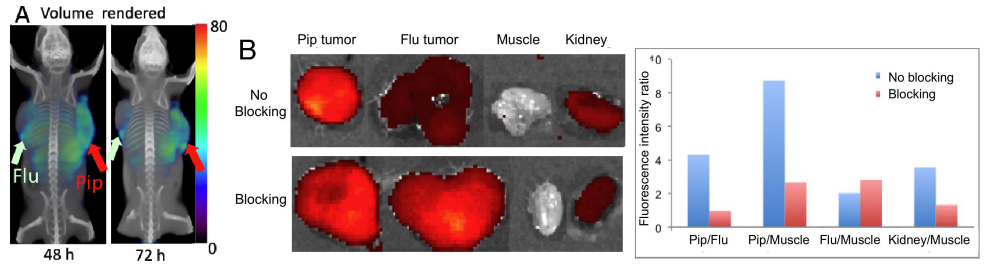

Figure 1A. SPECT imaging of SCID mouse bearing Pip (PSMA+ve) and Flu (PSMA-ve) tumor. Mouse was injected *i.v.* with 1.4 mCi of ^{111}In labeled PSMA-targeted nanoplex (150 mg/kg in 0.2 ml). SPECT images were acquired in 64 projections at 30 sec/projection. Following tomography, CT images were acquired in 512 projections to allow coregistration. Volume-rendered images were created using Amira image processing software. Decay-corrected volume-rendered SPECT/CT images at 48 h and 72 h demonstrate high liver uptake and specific accumulation in PSMA expressing Pip tumor.

Figure 1B. Nanoplex concentration in Pip and Flu tumors **without** (top panel) and **with blocking** (bottom panel). For the blocking studies100 µg of anti-PSMA mouse monoclonal antibody (Clone GCP-05, Abcam) were injected *i.v.* in a PC3-Pip and PC3-Flu tumor bearing mouse. Five hours after injection, 1.5 mg of nanoplex (75 mg/kg) were injected *i.v.* in the same mouse. Mice were sacrificed 48 h after nanoplex injection. Tumors, muscle and kidney were excised and imaged on the Xenogen Spectrum system to detect rhodamine present in the nanoplex. Images are scaled differently for unblocked and blocked tissues.

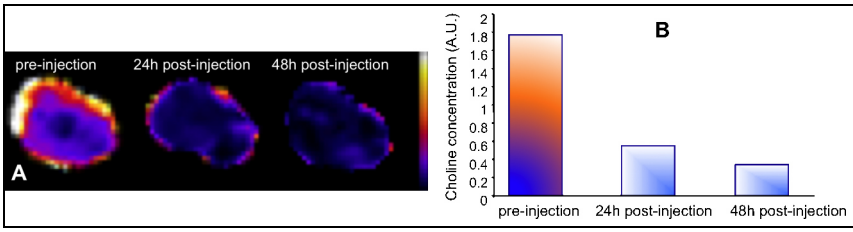

Figure 2. *In vivo* tCho maps from 2D CSI data sets acquired from a PC3-Pip tumor (~ 400 mm^3) before, 24h, and 48h after *i.v.* injection of the PSMA-targeted nanoplex (150 mg/kg) carrying bCD and Chk-siRNA. **B.** tCho concentration calculated in arbitrary units before, 24h, and 48h after injection of nanoplex. Parameters used were echo time (TE)=120ms, repetition time (TR)=1000ms, 4 scans per phase encode step. CSI spectra were acquired at 9.4T with an in-plane spatial resolution of 1 mm x 1 mm from a 4 mm-thick slice.

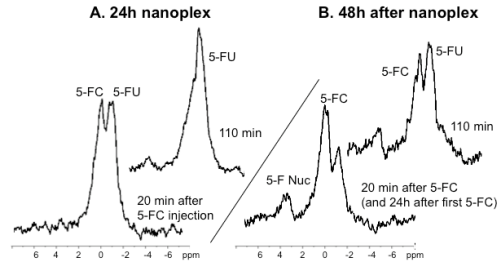

Figure 3. *In vivo* ^{19}F MR spectra acquired from a PC3-Pip tumor (~400 mm^3) at **(A)** 24 h and **(B)** 48 h after *i.v.* injection of the PSMA-targeted nanoplex (150 mg/kg) carrying bCD and Chk-siRNA. Spectra were acquired after a combined *i.v.* and *i.p.* injection of 5-FC (450 mg/kg), on a Bruker Biospec 9.4 T spectrometer using a 1 cm solenoid coil tunable to ^{1}H and ^{19}F frequency. Following shimming on the water proton signal, serial nonselective ^{19}F MR spectra were acquired with a repetition time of 0.8 s, number of scans, 2,000; spectral width, 10 KHz.

The nanoplexes being developed have the ability to deliver multiple siRNAs. The strategies developed here can be extended, in the future, to down-regulate multi-drug resistance pathways, or repair enzymes to increase the efficiency of chemo- or radiation therapy.

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References: 1. Li, C. *et al., ACS Nano, 2010*; 2. Mease, R., et al., *Clin. Cancer Res.,* 2008.