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 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 [11F]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 [111In]DOTA for SPECT or [64Cu]DOTA for MR and a fluorescent probe (Cy5.5 or rhodamine) and, (iii) the siRNA delivery vector: PEI (polyethyleneimine)-PEG (polyethylene glycol) 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)-heptanoyl]-pentyl]-ureido)-pentanedic 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.

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