Theranostic Imaging of Metastatic Disease

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Introduction: The ideal therapy would target cancer cells while sparing normal tissue. With prodrug therapy, where a drug-activating enzyme is delivered to the tumor followed by the administration of a non-toxic prodrug administered systemically, side effects can be minimized. The ability to image the delivery of the prodrug enzyme can be exploited to time prodrug administration to minimize damage to normal tissue. We previously synthesized a prototype agent consisting of the prodrug enzyme bacterial cytosine deaminase (bCD) labeled with multimodal MR and optical imaging reporters [1]. CD converts a non-toxic prodrug 5-fluorocytosine (5-FC) to 5-fluorouracil (5-FU). Here we have developed a prototype targeted nanoplex that delivers a prodrug enzyme together with multiple siRNA for theranostic imaging of metastatic disease. Prostate-specific membrane antigen (PSMA) is a type II integral membrane protein that has abundant expression on the surface of prostate carcinomas, particularly in androgen-independent, advanced and metastatic disease [2-3]. We have therefore incorporated a low molecular weight PSMA binding agent in the nanoplex to target the nanoplex to prostate cancer cells [4]. Since choline kinase (Chk) is significantly upregulated in aggressive cancer cells we have used siRNA against Chk in the nanoplex.

Methods: Our prototype nanoplexes are 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 [Gd3+]DOTA for MR and a near-infrared fluorescent probe Cy5.5 and, (iii) the siRNA delivery vector: PEI (polyethyleneimine)-PEG (polyethyleneglycol) co-grafted-polymer. These three compartments are covalently conjugated and siRNA-Chk is associated with the PEI-PEG co-grafted polymer through electrostatic affiliation. For the targeted nanoplexes, a low molecular weight urea-based PSMA targeting moiety (2-(3-[1-carboxy-5-[[2,5-dioxo-pyrrolidin-1-yl]oxy]carbonyl]-heptanoylaminio]-pentyl)-ureido)-pentanedioic acid (MW 572.56) is used for conjugating NHS-PEG-NHS (MW ~3000) to PEI. Imaging studies (optical and MR) with nontargeted nanoplexes were performed with MDA-MB-231 breast cancer xenografts in SCID mice. 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 (FLU) were used as controls. Additional studies were also performed with PSMA expressing LNCaP human prostate cancer xenografts. 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 Xenogen IVIS Spectrum system. SPECT/CT images were acquired on a Gamma Medica X-SPECT scanner.

Results and Discussion: Initial studies with the [Gd3+]DOTA labeled nontargeted nanoplex carrying the prodrug enzyme and Chk siRNA established the ability of the nanoplex to effectively downregulate Chk and total choline and convert the nontoxic prodrug 5-FC to 5-FU (Figure 1). The addition of a low molecular weight PSMA targeting moiety allowed specific targeting of the nanoplex to PSMA expressing prostate cancer cells in vivo as shown with SPECT imaging in Figure 2 and MRI in Figure 3. It was possible to achieve a T1 value of 32 ms in the injection solution of the [Gd3+]DOTA labeled PSMA targeted nanoplex (concentration of 15 mg/ml).

Figure 1. Non-invasive detection of nanoplex delivery, prodrug conversion and total choline down-regulation in tumors in vivo MR. (A) Representative high resolution T1-weighted MR images (upper panel) and quantitative T1 maps (lower panel) of a tumor (400 mm³) pre- and post-injection of nanoplex (300 mg/kg, i.v.). (B) Time-dependent Mean T1 values of the tumors (n = 4) pre- and post-injection of the nanoplex. Inset panel shows the T1 variation within the first 60 min of injection. (C) [1H] MRS demonstrated efficient conversion of prodrug 5-FC to 5-FU and its metabolite F-Nucl by nanoplex localized in the tumor. (D) Representative total choline maps and color coded total choline maps overlaid on corresponding T1-weighted images of a tumor before and at 24 and 48 h after nanoplex injection (300 mg/kg, i.v.).

Figure 2. SPECT imaging of SCID mouse bearing PIP (PSMA+ve) and FLU (PSMA-ve) tumor. Mouse was injected i.v. with 1.4 mCi of [111In]DOTA labeled PSMA targeted nanoplex (150 mg/kg in 0.2 ml). SPECT images were acquired in 64 projections at 30 sec/projection. CT images were acquired in 512 projections to allow coregistration. Decay corrected volume rendered SPECT/CT images at 48 h, and 72 h demonstrate specific accumulation in PSMA expressing PIP tumor. Transaxial slices show accumulation of radioactivity in PIP tumor at 48 and 72 h.

Figure 3. Representative quantitative T1 maps from a single slice (left) of a PSMA expressing LNCaP tumor following administration of PSMA targeted nanoplex labeled with [Gd3+]DOTA, demonstrating feasibility of detection with MRI (injection dose: 150 mg/kg). The bar graph (right) shows changes in T1 averaged over the four slices of the tumor.

The nanoplexes being developed have the ability to deliver siRNA. 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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