PSMA-Specific Theranostic Nanoplexes for Combination Gene and Prodrug Therapy of Prostate Cancer

Zhihang Chen[1], Marie-France Penet[1], Balaji Krishnamachary[1], Sangeeta Ray Banerjeee[1], Martin G. Pomper[1], and Zaver M. Bhujwalla[1]
[1]*Russell H. Morgan Department of Radiology and Radiological Science, Johns Hopkins University School of Medicine, Baltimore, MD, United States*

Introduction: We previously developed a prostrate-specific membrane antigen (PSMA)-based polyethyleneimine (PEI) platform to deliver a prodrug enzyme and small interfering RNA (siRNA) silencing the choline kinase gene for theranostic imaging [1]. To determine the feasibility of inducing gene expression for theranostic imaging, here we delivered the tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) cDNA gene and the prodrug enzyme bacterial cytosine deaminase (bCD) therapies using this PSMA-specific theranostic PEI nanoplex. PSMA is a type II integral membrane protein with abundant expression on the surface of castrate-resistant prostate carcinoma. TRAIL has been reported to specifically kill malignant cells specifically but to be relatively nontoxic to normal cells. Our PSMA-targeted nanoplex carries a near-infrared fluorescent probe for optical detection. The GFP-TRAIL cDNA expresses a GFP-TRAIL fusion protein. The TRAIL protein damages malignant cells but is relatively nontoxic to normal cells. The green fluorescence of GFP can help evaluate cDNA expression with optical imaging. The nanoplex also carries the prodrug enzyme bCD that converts the nontoxic prodrug 5-fluorocytosine (5-FC) to the classical chemotherapy drug 5-fluorouracil (5-FU) that can be detected by ^{19}F MRS.

Methods: Our prototype nanoplex was synthesized by conjugating three components: (i) the prodrug-activating enzyme bCD, (ii) the cDNA (pGFP-TRAIL) delivery vector: PEI (polyethyleneimine)-PEG (polythethyeneglycol) co-grafted-polymer that carries a near-infrared fluorescent probe Cy5.5 for optical imaging and, (iii) the linker poly-L-lysine (PLL) between the PEI and bCD. These three components were covalently conjugated and TRAIL cDNA was associated with the PEI-PEG co-grafted polymer through electrostatic binding. For PSMA targeting, we used a low molecular weight urea-based PSMA targeting compound.
Cell and tumor imaging studies with PSMA targeted nanoplexes were performed with PC-3 human prostate cancer cells and tumors genetically engineered to overexpress PSMA (PC3-PIP). Non-PSMA expressing PC3-FLU cells and tumors were used as controls. Cell imaging was performed with a Zeiss LSM510-Meta single-point laser scanning confocal microscope. Fluorescence imaging of tumors was performed *in vivo* and *ex vivo* with a Li-Cor Spectrum system. ^{19}F MRS of tumors was performed with a Bruker horizontal bore 9.4T MR spectrometer using a home-built RF resonator.

Results and Discussion: Higher GFP expression level in PC3-PIP cells indicated more accumulation and incorporation of the cDNA delivered by the nanoplex in cells expressing PSMA (Figure 1A). Images obtained with PIP and FLU tumors demonstrated increased uptake in the PSMA overexpressing PIP tumor compared to the non-PSMA expressing FLU tumor (Figure 1B). Real time PCR mRNA expression detected higher expression of TRAIL and EGFP in PIP compared to FLU tumors for cDNA coding for pEGFP-U6 or pEGFP-TRAIL (Figure 1C). The prodrug enzyme bCD converted the prodrug 5-FC to 5-FU at 24 h as shown in Figure 1D. Inhibition of PIP tumor growth was demonstrated in Figure 1E.

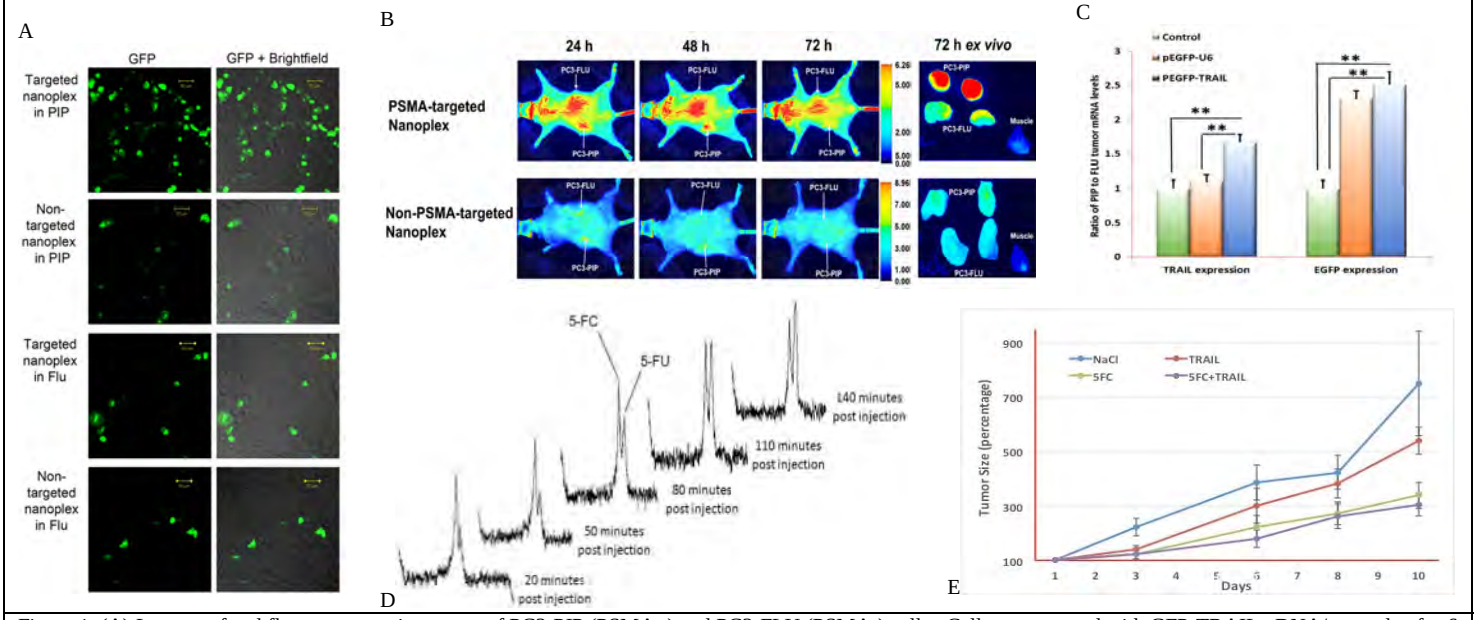

Figure 1. (A) Laser confocal fluorescence microscopy of PC3-PIP (PSMA+) and PC3-FLU (PSMA-) cells. Cells were treated with GFP-TRAIL pDNA/ nanoplex for 6 h (concentration of pDNA: 2 µg/mL; N/P ratio: 50). (B) *In vivo and ex vivo* optical imaging of SCID mouse bearing PIP and FLU tumor. Mouse was injected *i.v.* with nanoplex (150 mg/kg in 0.2 mL saline). (C) Real time PCR mRNA expression studies of PC3-PIP and FLU tumors with different treatments. Values represent Mean ± SD. Tumor tissue was collected at 24 hours after the mice were injected with nanoplex carrying cDNA of pEGFP-U6 or pEGFP-TRAIL (dosage of cDNA: 3.2 mg/kg; N/P ratio: 50; **, P<0.01). (D) *In vivo* ^{19}FMR spectra acquired from a PC3-PIP tumor at 24 h after *i.v.* injection of the nanoplex (100 mg/kg) carrying bCD. 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. (E) Inhibition of PIP tumor growth (values represent median±SD (n =3), following a combined *i.v.* and *i.p.* injection of 5-FC (450 mg/kg) at 24 h after *i.v.* injection of the nanoplex (100 mg/kg)).

These data demonstrate the feasibility of PSMA targeted expression of a gene of interest using cDNA delivery in tumors. The targeted nanoplex that we have developed, and which carries imaging reporters together with cDNA and a prodrug enzyme, will be useful for theranostic imaging of metastatic prostate cancer. The specificity of this platform technology can be expanded to different cancer subtypes and therapeutic targets with the goal of increasing the efficacy, safety, and efficiency of chemo- or radiation therapies.

Acknowledgements: This work was supported by NIH P50 CA103175, U54 CA151838 and R01 CA134675. We thank Drs Sridhar Nimmagadda, Dmitri Artemov, Cong Li, and Ms Mrudula Pullambhatla, for expert technical assistance and useful discussions.

References: 1. Chen, Z. *et al.*, *ACS Nano*, 2012. 2. Li, C. *et al.*, *ACS Nano*, 2010.