Multimodal Image-Guided Enzyme/Prodrug Cancer Therapy

C. Li[1], P. T. Winnard Jr[1], T. Takagi[1], D. Artemov[1], and Z. M. Bhujwalla[1]

[1]JHU ICMIC Program, The Russell H. Morgan Department of Radiology and Radiological Science, The Johns Hopkins University School of Medicine, Baltimore, MD, United States

Introduction: Enzyme/prodrug strategy is an actively developing area for cancer therapy. A tumor targeted drug-activating enzyme is delivered and after the clearance of the unbound enzyme from circulation and normal tissues, a nontoxic prodrug, which is a substrate of the enzyme, is administered. The prodrug is converted to the anticancer drug by the enzyme in the tumor, while normal tissues lacking the enzyme are spared from toxicity. However, determining the optimal time-window for prodrug injection is critical since injections that are "too early" or "too later" may lead to normal tissue toxicity or low therapeutic efficiency. Non-invasively imaging the delivery of a drug-activating enzyme would be ideal to optimize the timing of prodrug delivery. The focus of our work is therefore to design, synthesis, characterize and test prototype novel multimodal imaging guided enzyme/prodrug cancer therapy of a human breast cancer xenograft.

Method and Experiment: Cytosine deaminase converts the nontoxic prodrug 5-fluorocytosine (5FC) to the anti-cancer drug 5-fluorouracil (5FU). Additionally, the conversion of 5FC to 5FU can be detected noninvasively *in vivo* with ^{19}F MR spectroscopy.[1] In this work, bacterial cytosine deaminase (bCD) was chosen as the therapeutic enzyme due to its high enzymatic and thermodynamic stability.[2] Poly-$_L$-lysine (PLL) (M_r = 5.6 kD) was selected as a carrier of the imaging reporters because of its extended conformation, which can facilitate efficient extravasation of the conjugate into the tumor interstitium.[3] PLL was functionalized with Gd^{3+}-DOTA and rhodamine, to dynamically monitor the distribution of bCD by either MRI or optical imaging. Rhodamine can also track the enzyme in excised tissue. Biotins grafted on PLL allow the rapid clearance of the conjugate from circulation by using avidin chase without affecting the extravasated material.[4] The resulting bCD-PLL conjugate (MW > 300 kDa, Figure 1) is expected to extravasate into the tumor interstitium, but not the normal tissues due to the high permeability of tumor vasculature.

Results and Discussion: The molecular weight and hydrodynamic radius of bCD-PLL were determined as 435 kD and 9.4 nm respectively. The longitudinal proton relaxivity of bCD-PLL was 8.6 $mM^{-1}s^{-1}/Gd^{3+}$ ion (14 conjugated Gd^{3+}-DOTA per PLL) at 4.7 Tesla, and emission was centered at 590 nm. Kinetic studies with 5-fluorocytosine as substrate revealed that bCD-PLL had K_m = 3.7 mM, k_{cat} = 71 s^{-1}, comparable to the values of bCD, 3.9 mM and 74 s^{-1} respectively. Moreover, bCD-PLL demonstrated lower cytotoxicity and higher enzymatic stability than bCD *in vitro*. Fluorescence microscopy showed that bCD-PLL can be internalized into human breast cancer MDA-MB-231 cells efficiently *in vitro* after 30 min treatment. *In vivo* MRI demonstrated that bCD-PLL can extravasate into the interstitium of MDA-MB-231 tumor xenografts in SCID mice after injection for 1.5 h (Figure 2). Efficient conversion of 5FC to 5FU at the tumor site was also validated by *ex vivo* HPLC studies of tumor homogenates. Significantly, the 5FU concentration in liver decreased much faster than that in the tumor when the interval between the enzyme and prodrug injections increased from 3h to 12 h, suggesting that 12 h after the injection of therapeutic enzyme is a good time-window for prodrug administration. Immunofluorescence confocal microscopy images of mouse liver sections demonstrated that Kupffer cells stained by the macrophage antibody CD68 colocalized well with the injected bCD-PLL, which indicates that bCD-PLL can be degradated in the liver. H&E staining of the sections of the livers and kidneys of the mouse that were treated with bCD-PLL/5FC for 5 days did not show any obvious necrosis, which suggest that bCD-PLL/5FC therapy does not induce acute systemic toxicity.

Conclusion: In summary, we have developed a novel cancer therapeutic enzyme labelled with multimodal imaging reporters demonstrating high relaxivity, low cytotoxicity, improved enzymatic specificity to prodrug, efficient cell uptake, and high enzymatic stability in serum as well as in breast cancer cell culture. Preliminary *ex vivo* and *in vivo* experiments demonstrated that the image guided prodrug administration is possible by dynamically monitoring the enzyme levels in tumor and normal tissue. Further *in vivo* studies of this imaging guided enzyme/prodrug cancer therapeutic strategy, including the monitoring of the prodrug conversion in tumor xenograft by ^{19}F MRS, are currently underway.

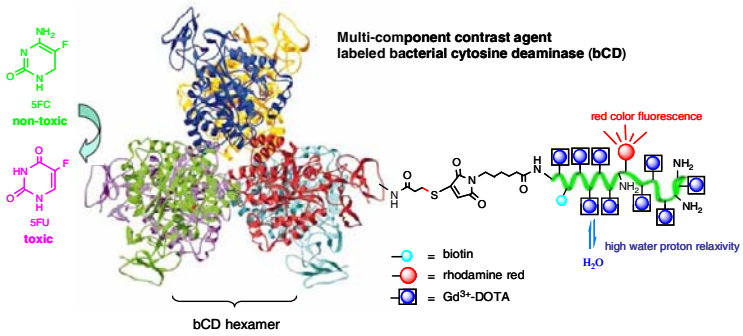

Figure 1. Schematic structure of bCD-PLL conjugate.

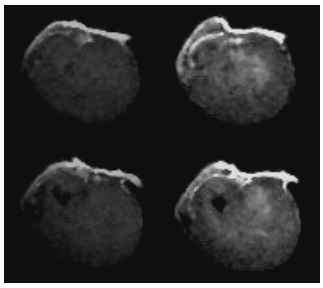

Figure 2 T1-weighted MR images (TE=10ms, TR=500ms) from two slices (st=1mm) of a human breast MDA-MB-231 tumor xenograft before and 1.5 h after the injection of bCD-PLL (10 mg/mouse).

References

(1) Bhujwalla et al *Cancer Res.* **1998**, *58*, 4075–4078; (2) Mahan et al. *Protein Eng. Des. & Sel.* **2004**, *17*, 625–633; (3) Uzgiris. *Invest. Radiol.* **2004**, *39*, 131–137; (4) Dafni et al. *Magn. Reson. Med.* **2003**, *50*, 904–914.