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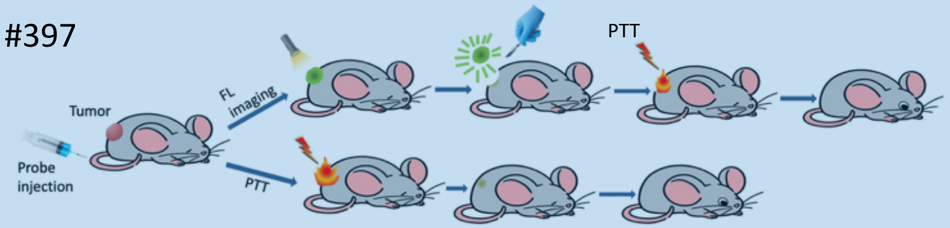

PSMA-Targeted Novel Dual-functional Agent for Fluorescence Imaging-Guided Surgery and Photothermal Therapy for Prostate Cancer

Lifang Zhang, Ethan Walker, Sriprada Chavali, Arpan Mahanty, Sophia Liu, Selena Liao, Xinning Wang, James Basilion.
Case Western Reserve University

Introduction: According to American Cancer Society's estimates, there will be 299,010 new cases of prostate cancer in 2024, and about 35,250 deaths from prostate cancer. Surgical removal of the tumor has been the main treatment for localized prostate cancer, but it shows a more than 60% recurrence rate after radical prostatectomy, mainly due to the difficulty in visualizing areas of prostate cancer invasion. The imprecise surgery commonly results in positive surgical margins and damage or removal of muscles and nerves surrounding the prostate. Recently, fluorescent imaging guided surgery (FIGS) emerges and attracts extensive attention helping surgeons identify anatomic landmarks and surgical planes. Meanwhile, a noninvasive therapeutic method, photothermal therapy (PTT), has gained momentum for cancer treatment. PTT is a technique that converts light energy to heat energy to induce protein denaturation and cell membrane damage, leading to rapid cell death[1]. Thus, searching for dual-functional fluorescent reagents that can improve the visualization of tumor margins and simultaneously eliminate residual tumor tissue has become a new research hotspot.

Methods: PSMA-1-ICG was synthesized using our highly specific, negatively charged, urea-based PSMA ligand PSMA-1 [3]. To verify if PSMA-1-ICG binds selectively to PSMA, a binding assay of PSMA-1-ICG was performed in PSMA positive PC3pip cells and PSMA negative PC3flu cells. To detect the in vivo uptake of PSMA-1-ICG, Nu/Nu mice were inoculated with both PC3pip and PC3flu cells. PSMA-1-ICG (100 nmol/kg body weight) was administered via tail vein injection and fluorescent images were taken by IVIS spectrum at different time points. To verify whether PSMA-1-ICG generated heat under the light stimulation, PSMA-1-ICG solution in PBS and PC3pip cells pre-incubated with PSMA-1-ICG were illuminated with 808 nm light and the temperature of the solution or cell pellets was measured. PTT was also performed on mice with a PC3pip tumor at 48 h post injection of PSMA-1-ICG.

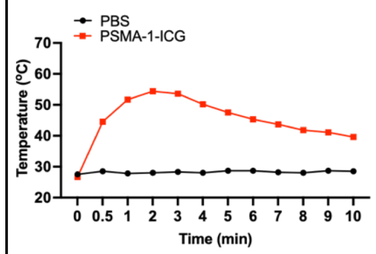

Figure 3. PSMA-1-ICG in solution can be irradiated and generate heat

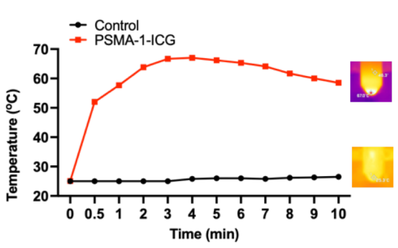

Figure 4A. Irradiation of PC3pip cells containing PSMA-1-ICG effectively generates heat

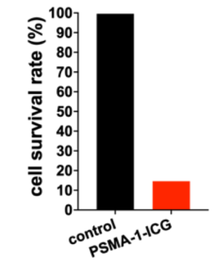

Figure 4B. PTT effectively kills PC3pip cells containing PSMA-1-ICG

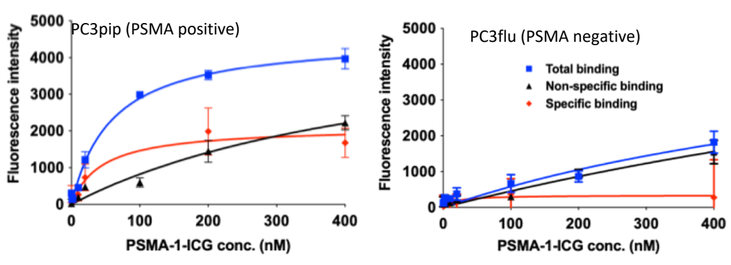

Figure 1A. PSMA-1-ICG binds selectively to PSMA positive cells

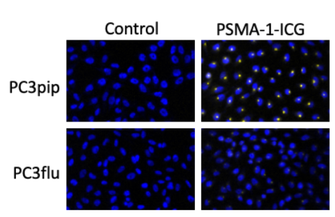

Figure 1B. PSMA-1-ICG is selectively taken up by PSMA positive cells

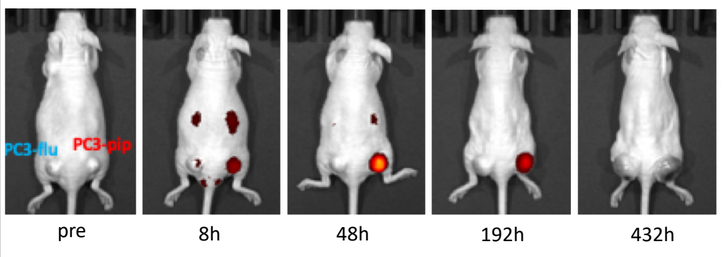

Figure 2A. Selective accumulation of PSMA-1-ICG in PSMA positive PC3pip tumors.

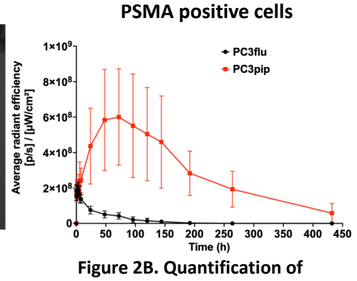

Figure 2B. Quantification of fluorescence signal on tumors

Results: In the in vitro binding assay, PSMA-1-ICG only showed specific binding to PC3pip cells with equilibrium dissociation constant (K_D) of 15.3 nM; no specific binding was observed in PC3flu cells (Fig. 1). In in vivo fluorescence imaging studies PSMA-1-ICG selectively accumulated in PC3pip tumors, reached a peak at 72h, then slowly decreased (Fig. 2). At peak time, the signal in PC3pip tumors was about 5-fold higher than that in PC3flu tumors. In a PSMA-1-ICG heat generation assay, it was found that upon light irradiation, PSMA-1-ICG solution was able to effectively generate heat (Fig. 3). Heat generation was also observed in PC3pip cells treated with PSMA-1-ICG with highest temperature reaching 67°C. Consequently, PTT on PC3pip cells resulted in 85% cell death (Fig. 4). In vivo PTT studies on mice receiving PSMA-1-ICG showed that PSMA-1-ICG effectively generated heat in PC3pip tumors when irradiated with 800 nm light (Fig. 5).

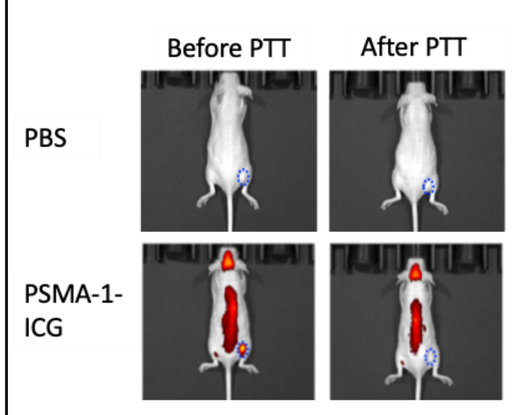

Figure 5A. PTT on PC3pip tumors bleaches PSMA-1-ICG fluorescence signal on the tumor

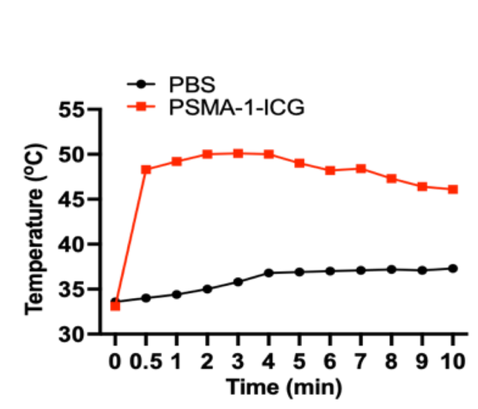

Figure 5B. PTT effectively increases temperature on PC3pip tumors containing PSMA-1-ICG

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Goal: ICG is an FDA approved near-infrared (NIR) reagent used for fluorescence-based intraoperative imaging with minimal toxicity [2]. ICG also has a potential application for photothermal therapy. This study aims to find a dual-functional fluorescent reagent which can selectively bind to prostate cancer biomarker PSMA and can be effectively used in FIGS and PTT.

Conclusion: PSMA-1-ICG showed selective binding to PSMA positive PC3pip cells and tumor tissue, which makes it a good probe for fluorescence image guided surgery for localized prostate cancer. In vitro and in vivo study showed PSMA-1-ICG can generated heat under light stimulation. The highest temperature can reach 50°C in PSMA-1-ICG containing PC3pip tumor and 67°C in in vitro assay, which indicated its cell killing capability by PTT. The approach here will provide a new theranostic approach for the treatment of prostate cancer. Further studies of PSMA-1-ICG application in FIGS and PTT are ongoing.

Reference

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