In-vivo and *in-vitro* detection of pancreatic cancer using functionalized CuInSe/ZnS QDs with NIR emission

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Statement of Purpose: Cancer of the pancreas is the fourth leading cause of cancer death in the united states, and the survival rate amongst pancreatic cancer patients is extremely low, primarily due to the fact that a large fraction (about 80%) of tumors are metastatic at the time of diagnosis. Therefore, to improve survival of pancreatic cancer patients, there is an urgent need for detection at an early, and hence potentially curative, stage. The histologic progression from PanINs (Pancreatic Intraepithelial Neoplasia) to invasive and metastatic pancreatic cancer is associated with the sequential appearance of molecular abnormalities. These molecular abnormalities provide the basis for selection of targeting markers that could allow detection and identification of the stage of progression of pancreatic cancer. The ability to exploit these molecular abnormalities in early detection, and ultimately treatment, of pancreatic cancer requires a suitable delivery vehicle and its proved ability to detect pancreatic cancer *both in-vitro* and *in-vivo*. In this study, functionalized semiconductor quantum dots (QDs) with high quantum yield and emission of near infrared (NIR) light were synthesized and demonstrated to target marker proteins of pancreatic cancer cells and detect tumors of human pancreatic cancer in a xenografted mouse model. Both *in-vitro* and *in-vivo* results were quantitatively analyzed.

Methods: NIR CuInSe/ZnS core/shell QDs were synthesized by combining the precursors in trioctyl phosphine with co-ordinating ligands. The QDs were transferred into water by forming a monolayer of lipid (80:15:5, MHPC: DSPE-PEG2000: DSPE-PEG2000-NH_2). The reaction of the primary carboxylic acids on the antibody with the NH_2-termal groups on QDs was catalyzed by EDC and sulfo-NHS. In-vitro study were performed using four antibodies for biomarkers that are overexpressed in different stages of pancreatic cancer development; anti-PSCA, anti-CLDN4, anti-MUC5B, and anti-MSLN. Using these antibody conjugated QDs, the expression level of some important marker proteins were quantitatively evaluated on pancreatic cancer cell lines (MIA PaCa-2, Panc-1, and Capan-1) and a normal pancreas epithelial cell line (HPDE). Based on the results of the *in-vitro* studies subcutaneous xenografts were generated by inoculating 5 x 10^6 Panc-1 cells subcutaneously in the bilateral flanks of six-week old CD1a athymic mice. QD-aMSLN conjugates were injected into the tail vein, at concentrations from 300 pmol when the tumor size reached 0.5 - 0.8 cm^3. Fluorescence images were recorded at time points post-injection using the Xenogen IVIS 200 optical imaging system.

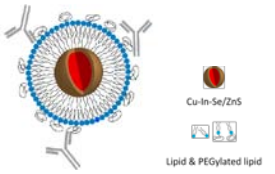

Fig. 1. Schematic structure of lipid coated NIR QDs with high QY for *in-vitro* and *in-vivo* detection of pancreatic cancer.

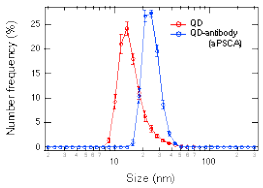

Fig. 2. Size distribution of lipid coated QDs and antibody-conjugated QDs measured by

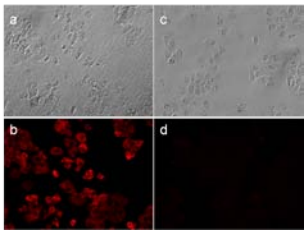

Fig. 3. *In-vitro* targeting of Panc-1 cancer cells with QD-aMSLN conjugates. (a) Phase contrast and (b) fluorescence images of Panc-1 cells incubated with QD-aMSLN. (c) Phase contrast and (d) fluorescence image of Panc-1 cells incubated with QDs with terminal OH groups (no antibody, negative control).

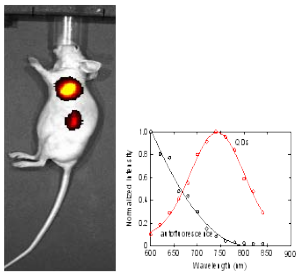

Fig. 4. *In-vivo* dectection test of CuInSe/ZnS QDs. (a) Whole body image from Xenogen IVIS at 740 nm and (b) comparison of QD emission with mouse auto-fluorescence.

Results: Fig. 1 shows a schematic structure of lipid-modified NIR QDs (CuInSe/ZnS) conjugated with anti-mesothelin antibody. After careful conjugation, the average QD diameter increases from about 15 nm to about 23 nm as shown in Fig. 2. Note the sharp size distribution and absence of aggregates, characteristic of successful conjugation. Fig. 3 shows phase contrast and fluorescence images of Panc-1 pancreatic cancer cells incubated with QDs functionalized with and without anti-mesothelin. The QD-Ab conjugates selectively target the pancreatic cancer cells since mesothelin is overexpressed in the majority of pancreatic cancers. The fluorescence is uniform over all of the cells. In contrast, QDs with no antibody (OH-terminated) do not target the pancreatic cancer cells. In Fig. 4 we have determined the appropriate concentration of QDs for effective imaging, at least for subcutaneous xenografts. This concentration results in a much higher signal than the background autofluorescence with a NIR emission wavelength at 740 nm.

Conclusions: In this study CuInSe/ZnS core/shell QDs functionalized with antibodies against pancreatic cancer biomarkers were successfully synthesized and characterized. It is demonstrated both in-vitro and in-vivo that functionalized QD-Ab conjugates with NIR emission can be used to target and indentify pancreatic cancer.