Quantitative Analysis of Pancreatic Cancer Cell Lines using Functionalized QDs

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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.[1] 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.[2] 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 quantitative imaging analysis. In this study, functionalized semiconductor quantum dots (QDs) were synthesized and used to target marker proteins of pancreatic cancer and the fluorescence images were quantitatively analyzed.

Methods: Monodisperse CdSe/ZnS core/shell QDs were synthesized using methods published elsewhere.[3] The QDs were functionalized with mercaptoundecanoic acid (MUA, SH-$(CH_2)_{11}$-COOH) and transferred to water. Then the reaction of the primary amines on the antibody with the COOH-terminated QDs was catalyzed by EDC and sulfo-NHS. In this study we used four antibodies for proteins 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). As control experiments OH-terminated QDs (no antibody) incubated with pancreatic cancer cell lines and a normal pancreas epithelial cell line (HPDE) were also tested. All immunofluorescence images were taken and analyzed under the same conditions.

Results: Fig. 1 shows particle size distributions for MUA-modified QDs (QD-COOH) and QDs functionalized with mesothelin (QD-MSLN). After careful conjugation, the average QD diameter increases from about 7 nm to about 12 nm. Note that the shape of the distribution is unchanged, characteristic of successful conjugation. Fig. 2 shows absorbance and PL spectra for QD-aMSLN conjugates in PBS. The PL peak at 620 nm has a FWHM of 24 nm, characteristic of monodisperse QDs. 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.

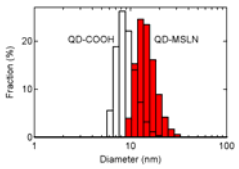

Fig. 1. Size distributions for un-functionalized QDs and QD-aMSLN conjugates in water.

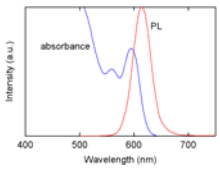

Fig. 2. Absorbance and PL spectra for QD-aMSLN conjugates in PBS.

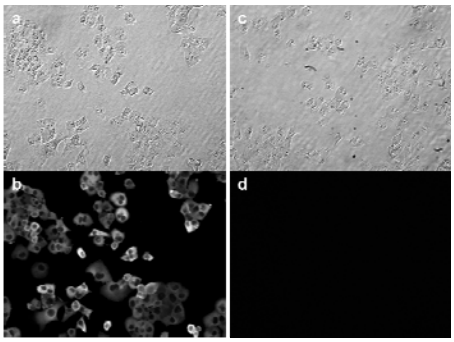

Fig. 3. Selective targeting of pancreatic cancer cells with QD-Ab conjugates. (a) Phase contrast and (b) fluorescence images of Panc-1 cells incubated with QDs conjugated with anti-mesothelin (QD-aMSLN). (c) Phase contrast and (d) fluorescence image of Panc-1 cells incubated with QDs conjugated with terminal OH groups (no Ab, negative control).

	Expression Level			
Stage:	PanIN 1 +		PanIN 2 +	Metastasis +
Marker:	PSCA	MUC5B	CLDN4	MSLN
Cell Line				
Capan-1	****	***	***	****
Panc-1	0	**	**	***
MIA PaCa-2	0	0	*	*/**
HPDE	*	*	0	0

Table 1. Expression level for marker proteins in specific cell lines used in this study. 0 - no expression; * - low level; **- moderate level; ***- high level; ****- very high level expression.

Conclusions: In this study CdSe/ZnS core/shell QDs functionalized with different antibodies were successfully synthesized and characterized. It is demonstrated that QD-Ab conjugates can be used to target and identify the stage of development of pancreatic cancer. Immuno-fluorescence images were acquired and quantitatively analyzed using NIS-elements allowing us to make quantitative comparisons between different cell lines and different antibodies as summarized in Table 1.

References:

1. The American Cancer Society, http://www.cancer.org.
2. A. Maitra et al. *Mod Pathol* 2003**,** 16(9), 902-912.
3. J. Park et al. *J Phys Chem C*, 2008, in press.