Tumor-targeted imaging and delivery of siRNA

Z. Medarova[1], M. Kumar[1], and A. Moore[1]
[1]Molecular Imaging Lab, Athinoula A. Martinos Center for Biomedical Imaging, Department of Radiology, Massachusetts General Hospital, Charlestown, MA, United States

Background.

Since their discovery in 1998, small interfering RNAs (siRNAs) have emerged as a powerful new tool for post-transcriptional gene silencing. Since the initial studies demonstrating the feasibility of combined imaging and delivery of siRNA to tumors using iron oxide nanoparticles, there has been active interest in exploring different imaging and delivery platforms suitable for detection by a variety of modalities. Here, we describe the synthesis and testing of a tumor-targeted nanoparticle probe (MN-EPPT-siSurvivin) to specifically shuttle siRNA to tumor cells. The probe binds the tumor antigen uMUC-1, found on a range of adenocarcinomas, and useful as an imaging target.

Methods and Materials.

MN-EPPT-siSurvivin consists of superparamagnetic iron oxide nanoparticles (for magnetic resonance imaging), labeled with Cy5.5 dye (for near-infrared in vivo optical imaging), conjugated to peptides (EPPT) specifically targeting the tumor antigen uMUC-1, and to DY547-labeled synthetic siRNA targeting the tumor-specific anti-apoptotic gene birc5, which encodes Survivin. The siRNA was conjugated through a disulfide linker, which is degraded in the reducing intracellular environment, followed by dissociation of the siRNA from the nanoparticles. Probe uptake by human breast, colorectal, and pancreatic adenocarcinoma cells was demonstrated by flow cytometry. The feasibility of MRI and optical imaging of probe uptake by these cells was demonstrated in vitro in cell phantom studies. Silencing efficacy was assessed by quantitative RT-PCR.

Results.

Gel electrophoresis demonstrated dissociation of the siRNA from the nanoparticles under reducing conditions. Following a 48-hr incubation, 57.8+/-5.3, 97.9+/-0.8 and 80.7+/-2.5% of pancreatic, breast, and colorectal cells, respectively took up the probe (Fig. 1A). This resulted in a significant reduction in birc5 mRNA abundance, as measured by qRT-PCR ($p = 0.014$ for pancreatic; 0.004 for breast, and 0.012 for colorectal cancer cells). Quantitative MRI analysis of the T2 relaxation times of cell pellets (Fig. 1B) revealed that the breast adenocarcinoma cell line was associated with the highest degree of T2 shortening (representing the highest probe uptake), followed by the colorectal and pancreatic adenocarcinoma cell lines.

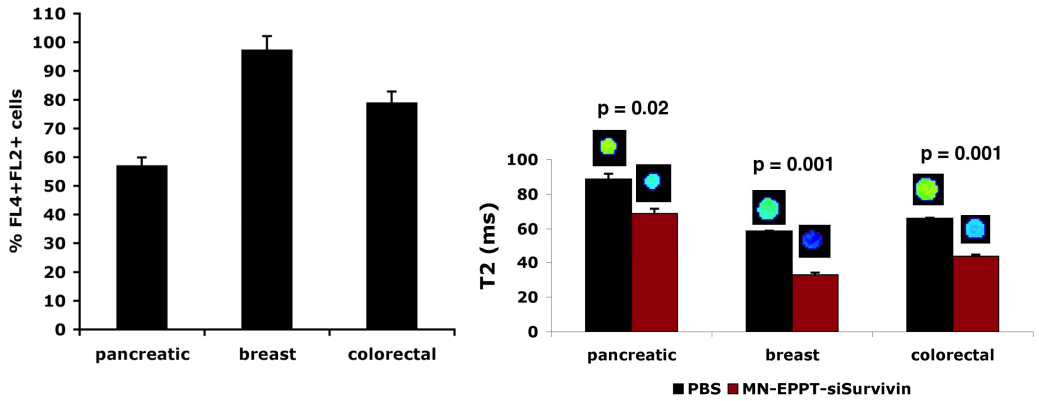

Figure 1 A. Flow cytometry of pancreatic, breast, and colorectal adenocarcinoma cells after incubation with MN-EPPT-siSurvivin. B. In vitro MRI of human pancreatic, breast, and colorectal adenocarcinoma cells incubated with MN-EPPT-siSurvivin. There was a significant shortening of the T2 relaxation times of the cells incubated with MN-EPPT-siSurvivin, relative to PBS-incubated controls, indicating that probe cellular uptake can be detected by MRI.

These results mirrored the relative uptake levels by the three cell lines, as measured by flow cytometry, indicating that indeed MRI can be used to quantitatively evaluate MN-EPPT-siSurvivin accumulation in the tumor cells.

Summary.

There is a lack of clinically-relevant non-invasive imaging strategies for the direct monitoring of siRNA delivery. Our approach is important in that it utilizes a single nanoparticle as the backbone for a combined imaging/therapy probe. This strategy permits the simultaneous tumor-specific delivery of siRNA to tumors and the imaging of the delivery process. The significance of this research agenda is defined by the potential to apply it to many human cancer studies, including basic tumor biology and therapy. Ultimately, the developed technology can be applicable in a clinical setting as related iron oxides are already in clinical use.