Combined imaging and delivery of siRNA to pancreatic islets

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Background. A powerful new approach for the directed regulation of gene expression utilizes the phenomenon of RNA interference. RNA interference is an innate cellular mechanism for post-transcriptional gene silencing, which is triggered by the delivery of double-stranded RNA molecules to the cell cytoplasm. Here, we establish the feasibility of a novel technology centered around multifunctional magnetic nanocarriers (MN-NIRF-siRNA), which concurrently deliver siRNA to intact pancreatic islets and can be detected by magnetic resonance (MRI) and optical imaging. **Methods and Materials.** MN-NIRF-siRNA consists of superparamagnetic iron oxide nanoparticles (for magnetic resonance imaging), labeled with Cy5.5 dye (for near-infrared optical imaging), and conjugated to a DY547-labeled synthetic siRNA duplex targeting the gene for Enhanced Green Fluorescent Protein (egfp). Pancreatic islets were isolated from Tg(GFPU)5Nagy/J mice, transgenic for egfp. Islets were incubated for 72 hrs with MN-NIRF-siEGFP (50 µg/ml Fe, ~ 39 µg/ml siRNA). Following incubation, the islets were pelleted and imaged by MRI using the following parameters: 9.4T, T2 weighted spin echo (SE) pulse sequences- TR/TE = 3000/8, 16, 24, 32, 40, 48, 56, 64ms, FoV = 3.2x3.2 cm2, matrix size 128 x 128, resolution 250 x 250 µm2 and slice thickness = 0.5 mm. Following the MR imaging session, the islets were imaged by in vivo optical imaging in the bright-field channel, Cy5.5 channel (NIRF), DY547 channel (siRNA) and GFP channel (EGFP). Finally levels of egfp message were measured by quantitative RT-PCR.

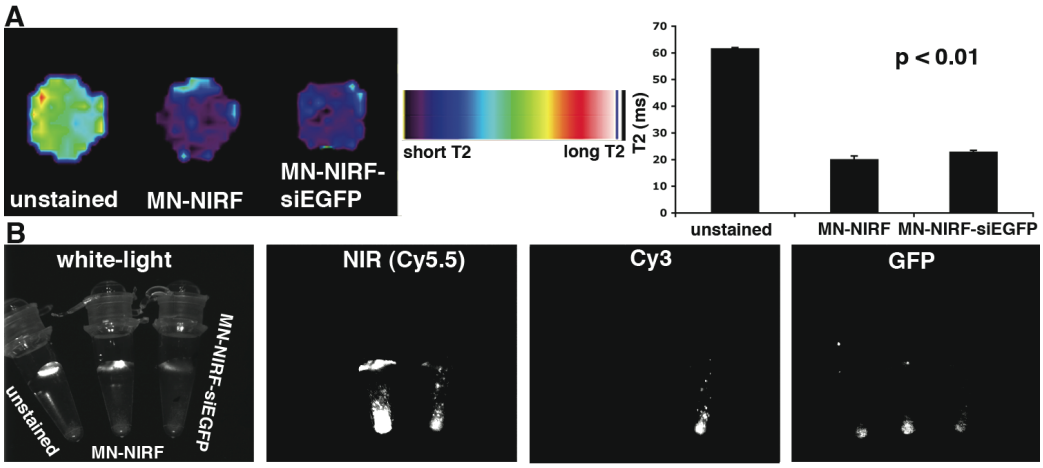

Figure 1 Magnetic resonance (A) and optical imaging (B) of EGFP-expressing pancreatic islets. A. MRI revealed a drop in T2 relaxation times in the presence of the MN-NIRF or MN-NIRF-siEGFP label ($p < 0.01$, $n = 3$). B. NIRF optical imaging permitted the detection of the Cy5.5 label on MN-NIRF and MN-NIRF-siEGFP in islets, following in vitro incubation. Optical imaging in the red channel allowed the direct detection of the DY547-labeled siRNA in islets incubated with MN-NIRF-siEGFP. Optical imaging in the green channel suggested a relative reduction of EGFP fluorescence in islets incubated with MN-NIRF-siEGFP.

Results. MRI revealed a significant ($p < 0.01$) drop in the T2 relaxation times of the islets after incubation with MN-NIRF or MN-NIRF-siEGFP compared to unstained islets (buffer), indicating that we can obtain a semi-quantitative estimate of the accumulation of the probe in islets by MRI (Fig 1A). NIRF optical imaging confirmed the MRI findings. There was a bright near-infrared signal, representative of probe accumulation, in the islets incubated with MN-NIRF and MN-NIRF-siEGFP but not in unstained islets (Fig. 1B). A direct indicator of siRNA accumulation in the islets was obtained by optical imaging in the red channel. Since the siRNA molecule is labeled with the red dye DY547, fluorescence in the red channel could be detected only in the islets incubated with MN-NIRF-siEGFP (Fig. 1B). Finally, to evaluate the extent of egfp silencing, we also imaged the islets by optical imaging in the GFP channel. There was a noticeable reduction in the level of green fluorescence in islets incubated with MN-NIRF-siEGFP compared to controls, indicating effective silencing (Fig. 1B). Quantitative RT-PCR confirmed the successful silencing of the *egfp* message. There was a significant 72±27% ($p < 0.01$) reduction in target mRNA transcript levels in islets treated with MN-NIRF-siEGFP compared to control islets, incubated with MN-NIRF.

Summary.

Our studies establish the feasibility of nanoparticle-based image-tagged siRNA delivery to pancreatic islets, using a novel multifunctional probe, which, in addition to its capability to deliver gene therapy in the form of siRNA, can also serve as an imaging contrast agent capable of detecting and following the fate of the probe in pancreatic islets. Our approach can be used to answer specific questions about islet biology in intact pancreatic islets. Our findings also lay the groundwork for future studies, in which genes implicated in islet graft loss can be targeted pre-transplant in order to improve graft outcome.