Combined in vivo imaging and delivery of siRNA to tumors

Z. Medarova[1], W. Pham[1], C. Farrar[1], V. Petkova[2], and A. Moore[1]

[1]Athinoula A. Martinos Center for Biomedical Imaging, Massachusetts General Hospital, Charlestown, MA, United States, [2]Beth Israel Deaconess Medical Center, Boston, Massachusetts, United States

Background

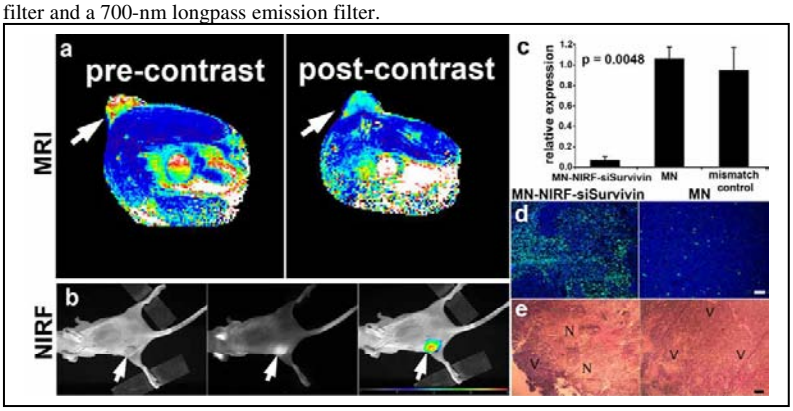
RNA interference (RNAi) emerges as one of the most promising platforms for therapeutic product development. Its broad applicability, superior efficiency, and exquisite specificity could potentially be harnessed to develop a powerful novel treatment paradigm with global relevance to any disease amenable to manipulation at the level of gene expression. The most significant obstacle to the advancement of siRNA-based therapies, however, is the delivery of the siRNA molecule to the tissue of interest. The development of clinically relevant imaging paradigms for the noninvasive assessment of siRNA delivery to tissues of interest is of paramount importance both for the conception and optimization of experimental treatment strategies. Recently, we demonstrated the feasibility of multi-modal magnetic resonance/optical imaging of siRNA delivery to tumors. In addition, we were able to follow the silencing process by in vivo optical imaging. Here we describe the application of this novel dual-purpose nanoparticle paradigm (1) for the simultaneous noninvasive imaging and delivery of synthetic siRNAs to tumors in a therapeutic scenario.

Methods and Materials

Probe: The probe (MN-NIRF-siSurvivin) consists of magnetic nanoparticles (for magnetic resonance imaging), labeled with Cy5.5 dye (for near-infrared in vivo optical imaging), and conjugated to a synthetic siRNA duplex targeting the anti-apoptotic gene survivin, which is specifically upregulated in tumors. In addition, it is modified with myristoylated polyarginine peptides (MPAP) serving as a membrane translocation module (2).

Treatment: Mice bearing subcutaneous human colorectal carcinoma tumors were injected intravenously with MN-NIRF-siSurvivin (10mg/kg Fe, 440 nmoles/kg siRNA) twice a week for 16 days beginning ~2 weeks after tumor implantation, once tumors had reached 0.5 cm in diameter. Magnetic resonance and optical imaging were performed before the beginning of treatment and afterwards, 24-h after each probe injection.

Imaging: MRI was performed using a 9.4T GE magnet with a Bruker Biospin Avance console equipped with ParaVision 3.0.1 software. The following imaging protocol was used: TR/TE = 3000/8, 16, 24, 32, 40, 48, 56, 64ms, FoV = 3.2x3.2 cm^2, matrix size 128 x 128, resolution 250 x 250 μm^2 and slice thickness = 0.5 mm. Image reconstruction and analysis were performed using Marevisi 3.5 software (Institute for Biodiagnostics, National Research Council, Canada). T2 relaxation times were determined by T2 map analysis of regions of interest drawn around the respective tissue. Tumor volumes were determined by placing a region-of-interest (ROI) manually over the entire tumor and counting the total number of voxels in each slice of the tumor ROI, adding up the total number of tumor ROI voxels across slices, and multiplying by voxel volume (0.03 mm^3). In vivo near-infrared optical imaging was performed immediately after each MR imaging session. Animals were placed into a whole-mouse imaging system (Imaging Station IS2000MM, Eastman Kodak Company, New Haven, CT), and imaged using a 630-nm optical bandpass excitation filter and a 700-nm longpass emission filter.



Ex vivo analysis: Survivin silencing in tumors was assessed by qRT-PCR using survivin-specific primers and probe. Levels of apoptosis in treated and control tumors were analyzed by terminal deoxynucleotidyl transferase–mediated dUTP nick end-labeling (TUNEL) assay. Tumor histopathology was analyzed by hematoxylin and eosin (H&E) staining.

Results

We showed that the delivery of the probe could be monitored in vivo by MRI and optical imaging (Fig. 1). On in vivo MR images, there was a significant drop in tumor-associated T2 relaxation times ($p = 0.003$), following MN-NIRF-siSurvivin delivery (Fig. 1a). The accumulation of MN-NIRF-siSurvivin in tumors was confirmed by in vivo NIRF imaging, where a bright fluorescence signal associated with the tumor was observed (Fig. 1b). No NIRF signal was detected in the tumor prior to administration of the probe (data not shown). In addition, we were able to demonstrate successful downregulation of the target gene, survivin, in tumors. Forty-eight hours after i.v. administration of MN-NIRF-siSurvivin, survivin mRNA levels were reduced by 83±2%, compared to those in mice treated with mismatch control ($p < 0.01$, Fig. 1c). This effect was accompanied by a noticeable increase in tumor-associated levels of apoptosis. Areas characterized by a high density of apoptotic nuclei (Fig. 1d) and considerable necrosis (Fig. 1e) were clearly identifiable in tumors treated with the probe. Our preliminary results in mice suggested that there might be a difference in tumor growth rates between mice treated with MN-NIRF-siSurvivin and animals injected with unmodified parental MN nanoparticles (Fig. 1e), which indicates the therapeutic potential of this probe. However, we expect this effect to be even more pronounced if siRNA treatment is combined with conventional chemotherapeutics. These studies are currently under way in our laboratory.

Summary

We believe that this new approach could lead to a significant progress in the clinical cancer intervention by utilizing RNA interference as a highly specific and efficient therapeutic modality. The concurrent imaging and delivery of therapy represents a unique and valuable research paradigm, since it permits the assessment of therapeutic agent bioavailability as well as the evaluation of the therapy outcome.

References

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