Transcription MRI Contrast Probe Enables the Detection of Different Cerebral Messenger RNA Levels

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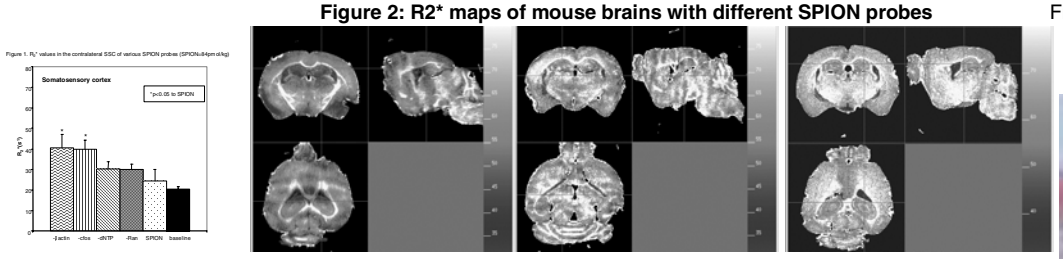
Introduction Few existing molecular assays can detect gene transcription except on biopsy and autopsy samples. We have developed techniques for visualizing gene transcription on live animals using MRI by labeling a biotinylated and phosphorothioate-modified oligodeoxynucleotide (sODN) with NeutrAvidin (NA)-tagged superparamagnetic iron oxide nanoparticles (SPION). We infused male C57black6 mice intracerebroventricularly (ICV) with one of these SPION conjugates: (1) SPION-cfos and SPION-βactin, which are targeting conjugates containing sODN of 26 bases with sequence complementary to c-fos and β-actin mRNA, respectively. (2) SPION-Ran, SPION-dNTP or SPION as controls which contains either randomized sequence (Ran), a single deoxynucleotide triphosphate (dNTP) or no conjugation to SPION, respectively. SPION-NA has one biotin binding site. Studies in molecular biology have shown that, in the resting state, the number of cerebral c-fos transcript is lower than β-actin transcript. We reported here the potential of these customized SPION conjugates to differentiate the levels of gene expression in the brain using MRI.

Methods SPION and s-ODN were conjugated via the Avidin-biotin linkage. Male C57black6 mice were anesthetized using katamine (80 mg/kg, i.p.) and xylazine (16 mg/kg, i.p.). Animals were infused with the contrast probe (SPION =190 pmol/kg) via ICV route (LR:-1; AP:-0.2; DV:-3 mm, bregma). One to three days after, we acquired MR images in live animals (with pure O_2 plus 2% halothane [800 ml/min flow rate]) using a 9.4Tesla magnet. We acquired R_2* maps using serial GEFI sequences (TR/TE=500/3, 4, 6, 8, 10ms, FOV=1.5cm, 128×128, α=30). Region of interest was placed in the somatosensory cortex contralateral to the infusion hemisphere. Averaged R2* values were compared in both anterior and posterior to the infusion site among different animal groups that received either SPION-cfos, SPION-βactin (targeting) or one of the control (non-targeting) probes. The brain samples were then collected for validations using *ex vivo* MR microscopy (14T, R2* maps: 3D Multi-GE, TR/TE=50/4, 7, 13, 18ms, FOV=1.28cm, 256×256×128, α=30; T2* weighted images: 3D FLASH, TR/TE=50/18ms, FOV=1.28cm, 256×256×128, α=20) and histological confirmations of iron and sODN uptakes.

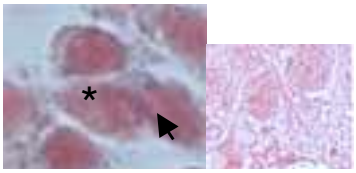
Results We observed comparable R2* enhancement in the animal groups infused with targeting probes from in vivo MRI. We observed significant, regional R2* elevation due to increased concentration of SPION-cfos and SPION-βactin in the somatosensory cortex ($p<0.05$) of mouse brains one day after ICV infusion, as compared to animals infused with the control probes (Figure 1). From ex vivo MR microscopy study, we observed that, while subtle localized R2* enhancement was present in baseline brain (no infusion, Figure 2A), there is noticeable elevation in the 3D R2* maps obtained from the brain infused with either SPION-cfos (Figure 2B) or SPION-βactin (Figure 2C). Regions of enhanced R2* include cortex, striatum, hippocampus and cerebellum. Outlines of the neuronal formation in the pyramidal cell layer and Dente Gyrus were more clearly seen in the brain infused with SPION-βactin (* in Figure 2C). These observations are consistent with the results from histological iron staining showing that SPION was taken up in brains infused with SPION-cfos, but not in brain infused with SPION alone (Figure 3A and 3B).

Figure 2: R2* maps of mouse brains with different SPION probes



2A. Baseline 2B. SPION -cfos 2C. SPION -βactin

Figure 3: Iron staining for SPION uptake



A. SPION-cfos B. SPION

Conclusions This ODN-based contrast probes exhibit the ability to document mRNA transcripts using *in vivo* and *ex vivo* MRI. This may prove a useful tool to monitor gene expression in space and time of animal disease model systems. It ofers a new minimally invasive molecular approach to directly assess mRNA transcription in the brain of live animals. (NSR01045845, P41RR14075 & the MIND Inst).