Gliogenesis in Live Animals using Targeted MRI: Detecting neural progenitor cells in vivo

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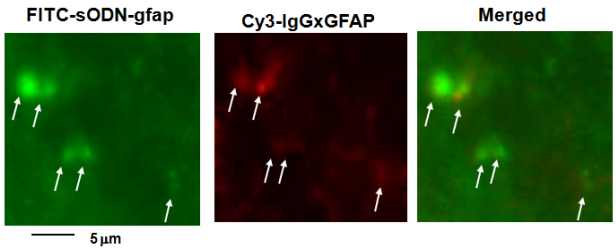
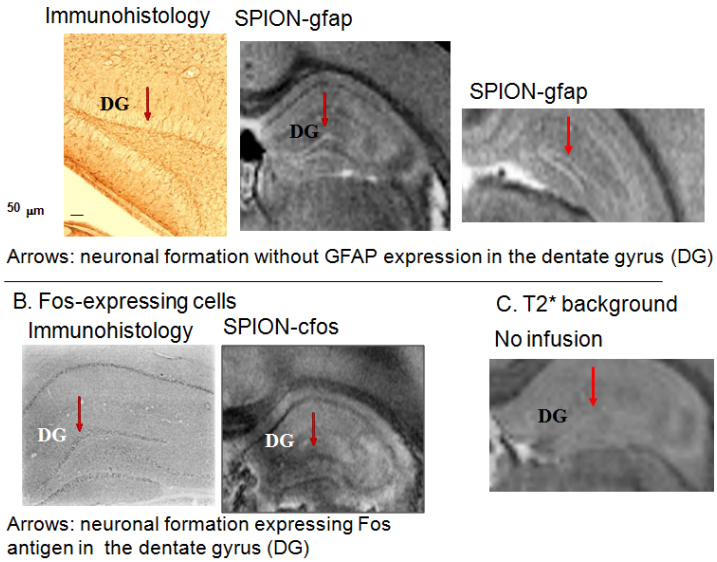
Introduction Gliogenesis, revascularization (angiogenesis), and neurogenesis are three major events thought to contribute to brain repair. The interaction of these three processes after global cerebral ischemia (GCI) in the brain is not totally understood. Gene expression at the transcription level during brain repair that follows GCI may be related to metabolic response and plasticity, and all involve the presence of neural progenitor cells (NPC); therefore, understanding the changes that occur throughout the repair process will aid translation of gene targeting for therapies. However, detection of de novo NPC in the brain is not routinely performed clinically because current techniques rely on the use of biopsy or autopsy samples. The biopsy procedure to obtain brain tissue severely limits the utility of these methods because they remove the same cells that we wish to save in vivo, and often clearly precludes longitudinal therapeutic evaluation. To overcome these problems we developed an alternative method that uses molecular probes for magnetic resonance imaging (MRI); this novel method provides a powerful and less invasive means of in vivo detection of gene action in brain cells. Here we focused on providing data for the specificity of gene targeted MR-visible probes.

Methods Superparamagnetic iron oxide nanoparticles (SPION, a T2 susceptibility MR contrast agent, BIOPAL Inc) were activated to contain NeutrAvidin (NA) (*1*). SPION-NA was linked to biotin-labeled phosphorothioate-modified antisense oligodeoxynucleotides (sODN) before use. SPION-sODN or fluorescein isothiocyanate (FITC)-sODN (40 μg Fe or 120 pmol DNA per kg) targeting endogenous gene transcripts of cfos in neurons or glial fibrillary acidic protein (GFAP) in astroglia was delivered to the cerebrospinal fluid in cerebral ventricles of C57black6 mouse by cortical puncture (intracerebroventricular, icv, delivery). Two controls were included: no infusion (baseline) and SPION-fos infusion for non astroglia. MRI was acquired for SPION-gfap uptake in live brains in 9.4T magnet at 7 hours after delivery and post mortem brains were obtained for histopathology of FITC-sODN-gfap uptake. For MR detection of uptake specificity, three dimensional T2* weighted MRI (Gradient Echo, TR/TE = 50/21 ms, 50X50X100 μm^3, NA=24, α=20) was acquired in post mortem brains using a 14T magnet (*2*).

1. Specificity: Transfection by FITC-sODN-gfap to GFAP⁺ cells



2. Targeting Specificity of MR-visible probes



Results and Conclusion We show here specificities of targeted MRI. By detecting differential retention, we would demonstrate specificity of SPION-sODN uptake. Cells that retained FITC-sODN-gfap (green) overlap with cells expressing GFAP antigen (red, Fig 1). Our hypothesis was that we would detect specific signal reduction by SPION-sODN in neuronal formation of the dentate gyrus (DG). We tested this hypothesis using SPION-gfap and SPION-fos using *ex vivo* MR microscopy (Figure 2). T2*-weighted MRI acquired at 14T system showed signal reduction in all brain regions of mice received SPION-gfap (panel 2A) or SPION-cfos (panel 2B), compared to baseline T2* MRI (panel 2C). The differences among these mice are in the neuronal formation of the DG (arrows) where differential expression of GFAP (Fig 2A) and Fos antigen (Fig 2B) are cleared observed. MRI shows null signal in the DG after SPION-gfap. We concluded that sODN-gfap was retained in astroglia and that T2 signal reduction by SPION-gfap exhibited specific gene expression profiles in mouse brains. The specificity is consistent with the results showing targeting specificity is determined by the sequence in the sODN (*3*). Cell typing in genetically similar C57black mice is an advantage over gene knockout mutants whereby we would not have to compare null signal in genetically variant strains of mice. [Supported by NIH (R21NS057556, R21DA024235, RO1DA026108), NCRR (P41RR14075).]

References

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