Monitoring Myelination by Transplanted Oligodendrocyte Precursors in Dysmyelinated Mice with MT and DT Imaging

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Introduction: Treatment of neurological diseases remains one of the major challenges of modern medicine. A new approach is the use of stem/progenitor cells to replace dead or defective endogenous cells and restore their functions. Patients suffering from multiple sclerosis, congenital dysmyelination and other myelin disorders can benefit from cell-based therapy using myelinating oligodendrocytes. We and others have shown that human glial precursor cells (hGRPs) myelinate extensively upon transplantation into neonatal dysmeylinated mouse brain (Walczak et al. 2010; Windrem et al. 2008). Non-invasive imaging of remyelination would be particularly valuable for longitudinal monitoring and optimization of cell-based therapies in patients. In the past, magnetization transfer imaging (MTI) and diffusion tensor imaging (DTI) have been used to interrogate myelination. In this study, we transplanted hGRPs into neonatal shiverer mice, a model of congenital dysmyelination characterized by a lack of proper, compact myelin, and investigated the sensitivity of MTI and DTI in for detecting myelination by transplanted hGRPs .

Methods:

Cell transplantation: On postnatal day 1, rag2$^{-/-}$ shi/shi mice were cryoanesthetized by brief submersion in ice. hGRP cells (Q Therapeutics®) suspended in Hanks' Balanced Salt Solution at a concentration of 1x10^5 cells/µl were loaded into a Hamilton syringe with attached 31G needle. The needle was lowered into the presumptive lateral ventricle of the brain and 4 µl of cell suspension was infused over the course of one minute. MRI: in vivo MRI was performed at 30, 60, and 90 days after cell implantation on a 9.4T spectrometer. Co-registered T2-weighted images (TE/TR = 40/2000 ms), M0 and MT images (TE/TR=9/1500 ms, offset frequency=5 kHz) were acquired from rag2-/- mice (n=4), shi/shi mice (n=5), and shi/shi mice receiving transplanted hGRP cells (n=4) with a resolution of 0.1x0.1x0.6 mm. DTI data (TE/TR=30/1000 ms, six directions, b=1000 mm^2/s) were acquired from the shi/shi mice with transplated hGRP cells with a resolution of 0.15 mm x 0.15 mm x 0.5 mm. Mean values of magnetization transfer ratio (MTR) and fractional anisotropy (FA) in the genu and splenium of the corpus callosum were obtained by manually placing regions of interest. Histological Analysis: Sixty days after transplantation mice were transcardially perfused with PBS followed by 4% paraformaldehyde. Brain tissue was cryopresereved and sectioned at 30µm and processed for immunohistochemistry. Anti-human nuclei antibody (mouse anti-HuNu, Chemicon) was used for detection of transplanted cells, and staining for myelin basic protein (rat anti-MBP, Serotec) was used to visualize myelination by transplanted cells, as endogenous mutant myelin is not reactive to this antibody.

Results:

Immunohistochemistry: Histological analysis revealed that transplanted cells survived well and were found bilaterally in the brain structures surrounding the lateral ventricles, including the corpus callosum, striatum, and hippocampus. There was a marked preference for cells to localize and migrate within the white matter (Fig. 1A). Two months after transplantation, some hGRPs stained positive for MBP with a characteristic myelin-like morphology (Fig. 1B). While there was some evidence for myelination it was much less pronounced than in wild type controls (Fig. 1C). MRI: Over the period of the experiment (90 days), MTR values in the corpus callosum of rag2$^{-/-}$ mice gradually increased. In both shiverer controls and transplanted shiverer mice, the MTR remained unchanged and at significantly lower level than the rag2$^{-/-}$ mice with no difference between the two shiverer groups (Figs. 1D-G). FA measurements in the caudal corpus callosum (splenium) demonstrated an upward trend in FA values over time (Figs .1H-K).

Conclusions: hGRPs myelinated the shiverer mouse with the onset of myelination at about 60 days after transplantation. Serial MRI indicates that fractional anisotropy measurements may be more sensitive than the magnetization transfer ratio for assessing early myelination.

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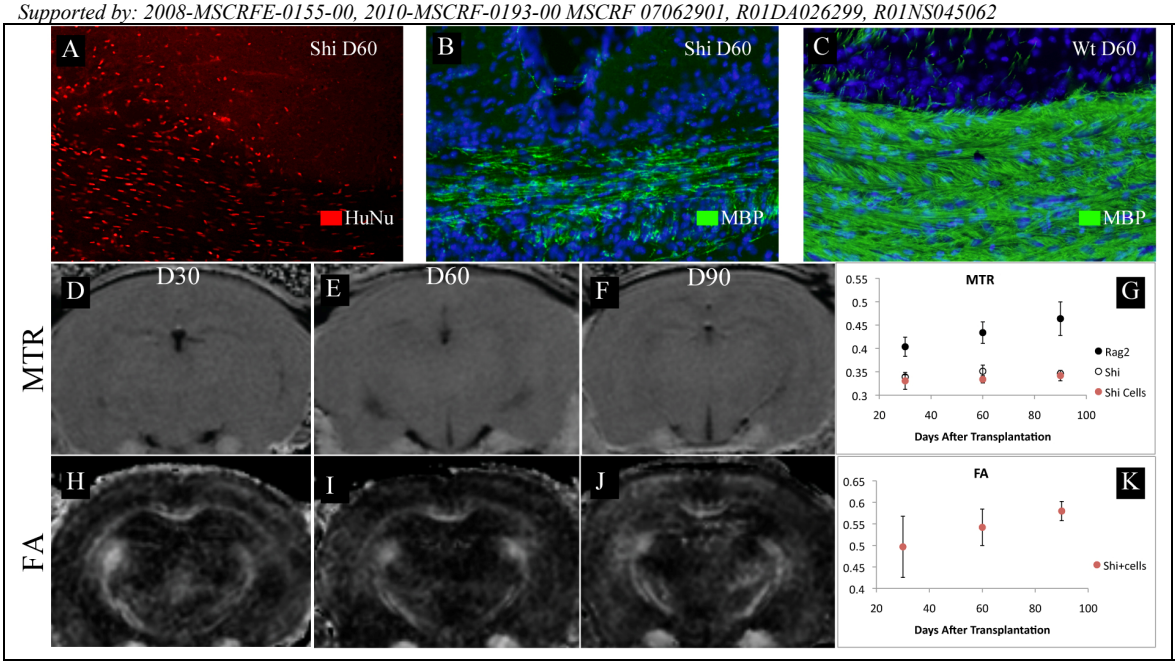

Figure 1:
(A) Anti-human nucleus (HuNu) staining (red) of brain tissue 60 days after transplantation. (B) transplanted shiverer mouse corpus callosum stained for myelin basic protein (MBP) (green). (C) Wild type mouse corpus callosum stained for myelin. (D-F) MTR images of transplanted shiverer mouse. (G) Graph representing MTR values for rag2, shiverer and transplanted shiverer mice. (H-J) DTI scans of transplanted mice. (K) graph representing FA values for posterior corpus callosum of transplanted mice.

References:

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Windrem MS, and others. 2008. Cell Stem Cell 2(6):553-65.