Multi-contrast MRI of Myelination after Transplantation of Human Glial-Restricted Progenitor Cells in a Dysmyelinated Mouse Model

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Target audience: Physicians and neuroscientists interested in stem cell-based therapies for myelin disorders.

Purpose: A wide range of neurological disorders result in loss or dysfunction of myelin. Recent reports and our previous data indicate that the transplantation of glial-restricted progenitor cells (GRPs) may be an effective approach to restore brain function in patients who suffer from myelin loss. Primary fetal-derived human GRPs have a high potential to proliferate, migrate, and remyelinate axons after transplantation to the brains of dysmyelinated shiverer mice. Application of immunodeficient animals as graft recipients facilitates testing of both, mismatched allografts and human cell xenografts without the interference of immune rejection. The goal of this study was to assess the utility and efficacy of non-invasive MR imaging for evaluation of myelination by human GRPs.

Methods: Transplantation and validation of therapeutic effect: Human GRPs (Q Therapeutics, Inc) were transplanted into the brain of myelin-deficient and immunodeficient shiverer rag2$^{-/-}$ mice. Cells were injected bilaterally (2x2µl; 4x10^5 cells) into the lateral ventricles of neonatal (P1-3) mice. The mice transplanted with human GRPs (n=30) were monitored serially with MR imaging (T_2-w, magnetization transfer imaging (MTI), diffusion tensor imaging (DTI)) for more than 600 days. The grafted mice were studied in comparison to non-transplanted shiverer and wild type controls, also monitored serially by MRI for up to 600 days using a Bruker horizontal bore 11.7 T scanner. Individual animals were sacrificed at 360, 440, and >500 days after transplantation for assessment of grafted cells with immunohistochemistry. Imaging parameters: *In vivo* 3D DTI was performed with TE/TR = 25/500 ms, 6 diffusion directions; b=3000 s/mm^2; FOV = 16 mm x 16 mm x 17.5 mm, a matrix size of 128 x 128 x 70. Multi-slice T2-w images were acquired with TE/TR = 60/3800 ms, RARE-factor = 8, four signal averages, field of view (FOV) = 15 mm x 15 mm, in-plane resolution of 0.08 mm x 0.08 mm. *In vivo* MT-MRI scan was conducted using a modified RARE (TR/effective TE = 6000/4ms, RARE factor=8, two slices, slice thickness=1mm, FOV=14x14 mm^2, matrix size=96 x 96, resolution=0.156 x 0.156 mm2, and 2 averages) including a MT module. A BP shape RF pulse was used to acquire MT weighted images at 12 offsets ranging from 1 to 12.5 KHz (2 to 25 ppm). The saturation time (tsat) was 3 s and radiofrequency field strength (B1) was 2.7 µT. The M0 images were acquired using the exact same pulse sequence except B1=0. MTR was calculated by 1-M_S(-5KHz)/M0. The quantitative MT parameters were calculated as previously reported by Ramani et al., 2002.

Results: The life-span of engrafted shiverers with human GRPs was dramatically extended with 50% of mice surviving over 570 days and 14% surviving over 650 days reaching nearly normal life-span of a laboratory mouse. Myelin Basic Protein and human Glial Fibrillary Acidic Protein staining at 420 days revealed extensive engraftment, migration over the entire neuroaxis and robust differentiation towards myelinating oligodendrocytes and astrocytes (Figure 1). At the same time point MRI data for the white matter continued to appear hyperintense in T2, with low MTR and FA suggesting no myelination. After 450 days post transplantation, the MRI scans for human GRP grafted shiverers showed patterns that was consistent with myelination including hypointensive T2, increased MTR, and evaluated bounded proton fraction (*f*) from MTI in corpus callosum. *In vivo* DTI showed no significant change in radial diffusivity and fractional anisotropy over the screened time points. MTR map and *f* map showed a strong contrast for transplanted shiverer mice similar to wild type controls (Figure 2).

Discussion: Transplantation of human GRPs revealed high therapeutic potential of these cells including extensive migration, myelination, and most importantly, extended survival of animals. Robust graft-derived myelination was confirmed with immunohistochemistry. Multi-contrast *in vivo* MRI provides a non-invasive approach to evaluate the degree of myelination from different aspects.

Conclusion: We have shown that human GRPs have a strong therapeutic potential to repair myelin disorders. Our optimized MRI protocols (T2-w and MTI) can hopefully be used as imaging tools to non-invasively monitor myelination-based therapies in patients.

Reference: Ramani, A., Dalton, C., Miller, D.H., Tofts, P.S. & Barker, G.J. Precise estimate of fundamental in-vivo MT parameters in human brain in clinically feasible times. Magnetic resonance imaging 20, 721-731 (2002).

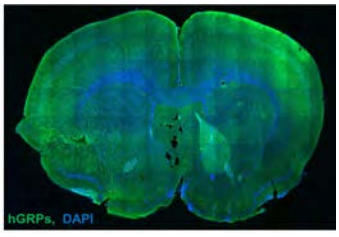

Figure 1: Representative image of engraftment of hGRPs in shiverer mouse brain. IHC staining was performed for human nuclei and counterstained with DAPI.

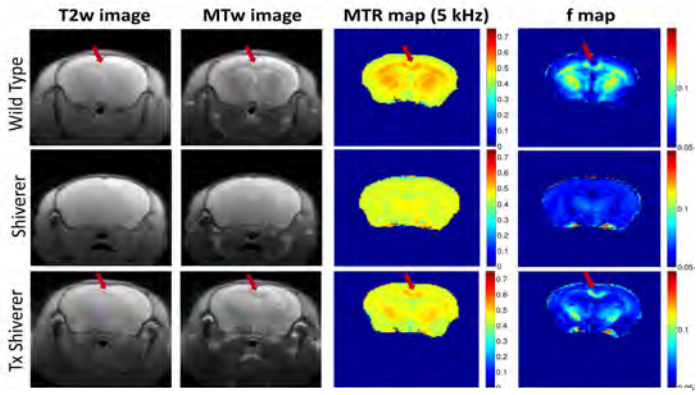

Figure 2: Representative T2w, MTw images, MTR map and fmap for wild type, non- transplanted shiverer and hGRP engrafted shiverer mice 650 days post transplantation.