Divalent Metal Transporter, DMT1: A Novel MRI Reporter

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Motivation and Background

The development of effective genetic reporters for MRI has long been an elusive goal. Based on the observed correlation between expression of the divalent metal transporter DMT1 and contrast-enhanced regions of the mouse brain on T1-weighted manganese (Mn)-enhanced MRI (MEMRI) images, DMT1 was investigated as a candidate T1-based MRI reporter. We characterized DMT1-related contrast by expressing the protein in a cell culture mode and examined MR contrast created by ectopic DMT1 expression *in vivo*, using electroporation.

DMT1 Generates T1 Contrast in cells and *in vivo*

Using retroviruses we created an HEK cell line that overexpressed DMT1 by approximately 300% (+DMT1) and a line that expressed a shRNA which knocked down DMT1 expression to 40%(-DMT1) **(a).** The 2 cell lines showed T1 contrast compared to control cells supplemented with 100µM $MnCl_2$ for 1hr and washed before imaging (2DGE: TE=3.1ms; TR=50ms; Flip angle=45º) **(b).** Saturation recovery based relaxometry showed a small but significant reduction in R1 (=1/T1) for -DMT1 cells, with obvious and linear gains in R1 for +DMT1 cells above that of a $MnCl^2$ phantom (shown in red), with a maximal 6.7 fold gain in R1 compared to HEK cells at 300µM Mn **(c).**

Based on these data, postnatal day (P)0 neonatal mice were electroporated with DMT1-IRES-eGFP to co-express both DMT1 and enhanced Green Fluorescent Protein (eGFP). At P2, neonates were screened for eGFP with a fluorescence dissection microscope (Leica) and positive mice were imaged with MEMRI at P5, after which the brains were dissected and cut into 1mm coronal slices to analyze cortical eGFP expression **(d)**. For MEMRI, the mother mouse was administered an intraperitoneal injection of $MnCl_2$ (40mg/kg body weight), and T1-weighted MRI (3DGE: TE=3.1ms; TR=50ms; Flip angle=45º; 150 µm isotropic resolution) were acquired 8h after injection of $MnCl_2$, and Mn uptake in the neonatal brain via lactation. MEMRI revealed cortical enhancement on the eGFP-expressing side of the electroporated mice **(e)**. Immunohistochemistry for DMT1 revealed a clear correlation in DMT1 expression and Mn-enhanced T1 contrast **(f,g)**.

Discussion and Conclusion

DMT1 expression was clearly correlated with R1 in MEMRI both in cell culture and *in vivo*. These data provide optimism that DMT1 may be developed as an effective T1 MRI reporter, enabling *in vivo* imaging of gene expression patterns and long-term cell labeling and tracking in transfected or transgenic animals.

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