7T-MRI of Transferrin Receptor and Ferritin Gene Expression in a Mouse Neural Stem Cell Line

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Introduction and Background.

The possibility of utilizing endogenous iron as an MRI contrast agent has emerged with the development of high magnetic field imaging due to its increased sensitivity to the paramagnetic ion. By manipulating the genes involved in normal iron regulation, causing a cell to increase uptake and stable storage of iron, it has been proposed that these genes could be used as an MRI reporter of associated gene expression. In previous literature, the transferrin receptor [1] and ferritin [2] have individually been overexpressed in *in vivo* systems and were found to lead to either insufficient iron accumulation for MRI visualization or cellular toxicity. In this study, we attempted to boost iron accumulation to an MRI detectable level by over-expression of both the transferrin receptor and ferritin proteins in an *in vitro* system.

Materials and Methods.

Cell lines: The C17 mouse cerebellar progenitor cell line [3] was transfected with a construct consisting of the human transferrin receptor (hTfR) and the human ferritin H chain (hFTH) genes connected by an internal ribosomal entry site (IRES) in a pZeo-SV2(+) vector (Invitrogen) [4]. The construct was transfected by electroporation and zeocin-resistant subclones were selected. Based on RT-PCR confirmation of expression of both genes, subclone #12 was selected for further analysis.

Transferrin/Iron supplementation and sample preparation: The C17 control and #12 subclone were grown in standard growth medium (DMEM with 10% FBS, 5% Horse serum, 200mM L-glutamine, 100mM sodium pyruvate) or in standard medium supplemented with human holo-transferrin (1mg/mL) and iron citrate (1mM) for 48 hours prior to imaging and histology. For imaging, the cells were washed to remove unbound iron, pelleted by low rpm centrifugation into glass NMR tubes and mounted in an imaging phantom. For histology, cells were washed and fixed in 4% PFA for 10 minutes prior to PPB staining and neutral red counterstain.

MRI and data analysis: Imaging was performed on a SMIS console interfaced to a 7T horizontal bore magnet with 250mT/m actively shielded gradients (Magnex) using a quadrature birdcage coil (ID=35mm, L=46mm) and the following parameters: T1 (3DGE, Mx: 512x256x256, FOV: 80x40x40, TR/TE= 50/5ms, FA=65º), T2 (2DSE, Mx: 512x512, FOV: 40x40, TR/TE= 2000/50ms, slice thickness= 250μM), and T2* (3DGE, Mx and FOV as in T1, TR/TE=50/15ms, FA=20º). The mean signal intensity was obtained by manual selection of ROIs corresponding to each cell pellet in a cross sectional image and SMIS system measurement.

Results.

Iron staining shows grossly increased iron accumulation (blue precipitate) in the #12 subclone when grown in supplemented conditions versus C17 control cells (Fig.1a). T1 images showed no signal difference between cell types with or without supplementation (data not shown). However, both T2 and T2*-weighted images showed increased signal loss in the #12 subclone with supplementation versus the supplemented C17 control (Fig. 1b, c). T2 mean signal intensity decreased by a mean of 70% in supplemented #12 cell pellets versus 58% in C17 controls with supplementation (Fig. 2a). T2* mean signal intensity decreased in the #12 subclone by 61% with iron supplementation while C17 control signal decreased by 39% with supplementation (Fig 2b). Initial relaxometry on similar fixed samples showed a decrease in T2 with iron supplementation in the #12 subclone of 63% (from 129ms to 48ms) versus 45% in C17 controls (from 125ms to 68ms).

Conclusions.

These results indicate that cells overexpressing transferrin receptor and ferritin proteins in combination can accumulate sufficient iron for visualization by MRI, showing the potential of this approach for controlling cell-specific MR contrast.

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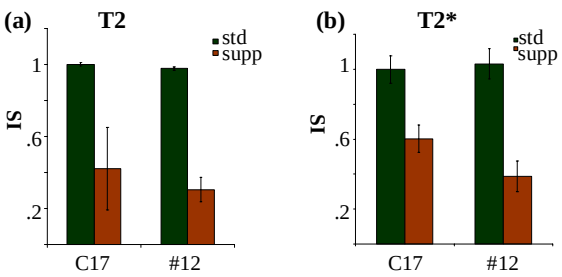

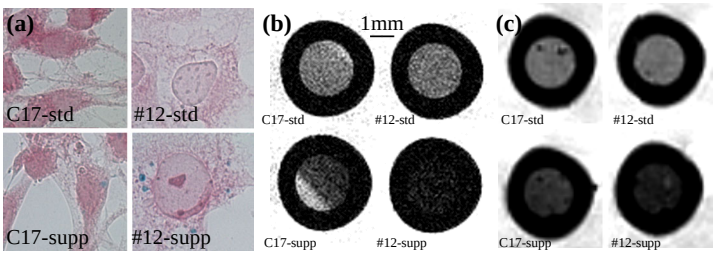

Figure 1(above): (a) C17 Control cells and the #12 hTfR/ hFTH-expressing subclone grown in standard ("std") or supplemented ("supp") conditions with PPB stain for iron and neutral red counterstain. (b)T2 MRI and (c) T2* MRI of cell pellets.

Figure 2 (left): (a) Normalized T2 and (b) T2* signal intensity (SI) measurements for C17 cells and the #12 subclone, in standard growth conditions ("std"-green bars) and in iron rich conditions ("supp," orange bars).