Cellular MRI contrast via Co-expression of Transferrin Receptor and Ferritin

A. E. Deans[1], Y. Z. Wadghiri[1], L. M. Bernas[2], X. Yu[1], B. K. Rutt[2], D. H. Turnbull[1]

[1]New York University School of Medicine, New York, New York, United States, [2]University of Western Ontario, London, Ontario, Canada

Introduction and Background. With the increasing prevalence of high field MRI instrumentation, there has been growing interest in the development and use of genetic methods to achieve iron-based cellular contrast. Previous efforts have included over-expression of the transferrin receptor (TfR) [1,2], the main mediator of cellular iron uptake, and the ferritin (FT) subunits (H and L) [3,4,5], which compose the cellular iron storage protein complex. In this study, we have investigated co-expression of the *TfR* and *FTH* genes to induce cellular contrast in a biological system, including a comparison of the effects on R2 and R2* relaxivities at 7T and 1.5T. The results indicate that dual expression of proteins at different critical points of the iron metabolism pathway may improve cellular contrast without compromising cell viability.

Experimental Design and Results. A transgene construct including the coding sequence for the human *TfR* gene and the coding sequence for the human ferritin H-chain (*FTH)* gene connected by an internal ribosomal entry site was subcloned into the pZeoSV2(+) plasmid (Invitrogen). This transgene was stably transfected by electroporation [6] into an immortalized mouse neural stem cell line, C17 [7]. The C17-12 subclone, selected based on RT-PCR detection of *TfR* and *FTH* mRNA, was observed to have increased TfR-mediated uptake of fluorescent transferrin, and increased FT protein levels on western blot. C17 cells and C17-12 cells were grown in control medium (denoted C17c and C17-12c) [7] or in supplemented medium (C17s and C17-12s) including human holo-transferrin (1mg/mL) and iron citrate (1mM) for 48 hours. Increased iron accumulation, measured by atomic absorption (AAS), was observed in C17-12s cells compared with C17s cells (Fig.1a) with no concurrent evidence of cell toxicity (data not shown). R2 and R2* measurements were made on fixed cell pellets at 7T and 1.5T. 7T R2 and R2* measurements were made, respectively, with a 2D spin echo sequence with varying echo times and a 2D multi-slice multi-gradient echo sequence. (Other parameters: R2: TR=6000ms, TE=7-100ms, matrix(mx)=64x64, FOV=25mm x 25mm, slice thickness=500μm; R2*: TR=2000ms, TE=7-35.5ms, echo spacing= 7.1ms (5 echoes), flip angle (FA)=90° mx=256x256, FOV=25mm x 25mm, slice thickness=250μm). 1.5T R2 and R2 * measurements were made using a 3D fast spin echo sequence with varying echo times, and a fast SPGR sequence with varying gradient echo times. (Other parameters: R2: TR=4500ms, TE=33-500ms, mx=100x100, FOV=20mm x 20mm, slice thickness=600μm; R2*: TR=100ms, TE=5.2-40ms, mx, FOV, slice thickness as for R2, FA=15°). R2 and R2* measurements of C17-12s cells were significantly increased compared to all other cell types at 7T (Fig. 1b and c). Relaxometry on the same samples at 1.5T also demonstrated increased R2 and R2*, though the increase in R2* did not reach significance. The percent increase in R2 and R2* values between C17s and C17-12s cells at 1.5T and 7T shows the expected increase in contrast effect at high field strength. C17s and C17-12s cells transplanted into mouse brain show contrast with surrounding tissue on *ex vivo* (Fig. 2a, b) and *in vivo* (Fig. 2c, d) T2*-weighted MRI (parameters: 3DGE, TR=50ms, TE=15ms, FA=20°, mx=256x256x256, FOV=25.6mm x 25.6mm x 25.6mm). R2* measurements from the cortex (ctx), striatum (str), and injection sites (is) made at 7T show increased R2* in transplanted transgenic cells versus control (Fig. 2e).

Conclusions. Our results demonstrate that cells which co-express transgenic *TfR* and *FTH* have increased iron accumulation with no observed cellular toxicity. The differential accumulation of iron resulted in a significant increase in transverse relaxivities R2 and R2*, with increased effect at higher field strength. Supplemented transgenic cells also demonstrated excellent contrast on T2*-weighted brain images after transplantation. Together, these results indicate that co-expression of *TfR* and *FTH* shows promise as a genetic method of establishing cellular contrast.

Acknowledgments.

We would like to thank Dr. Alexandra Joyner for her advice in cloning the gene construct, as well as Tim Blenkinsop and Frada Berenshteyn who helped develop the C17 subclones. This research was supported by NIH grants NS38461 and HL78665.

References

[1] Koretsky A *et al. Proc ISMRM.* **5471** (1996).
[2] Weissleder R *et al. Nat Med.* **6**: 351-355 (2000).
[3] Grabill C *et al. Br Res.* **971**: 95-106 (2003).
[4] Genove G *et al. Nat Med.***11**(4): 450-4 (2005).
[5] Cohen B *et al. Neoplasia* **7**(2): 109-17 (2005).
[6] Liu A *et al. Mech Dev.* **75**: 107-115 (1998).
[7] Ryder EF *et al. J. Neurobio.* **21**: 356-375 (1990).

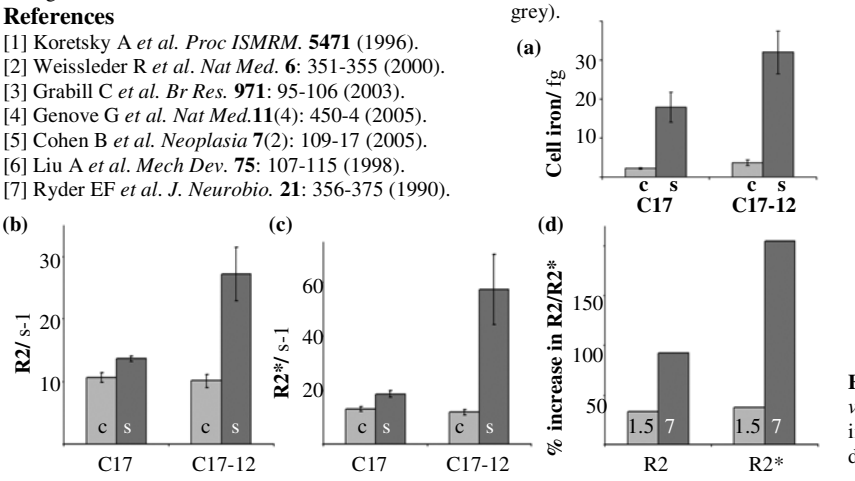

Fig. 1 (a) Cell AAS iron measurements normalized to cell number. (b) R2 values at 7T. (c) R2* values at 7T. (d) Percent increase in relaxivity due to transgene expression (C17s vs C17-12s) at 1.5T (light grey) and 7T (dark grey).

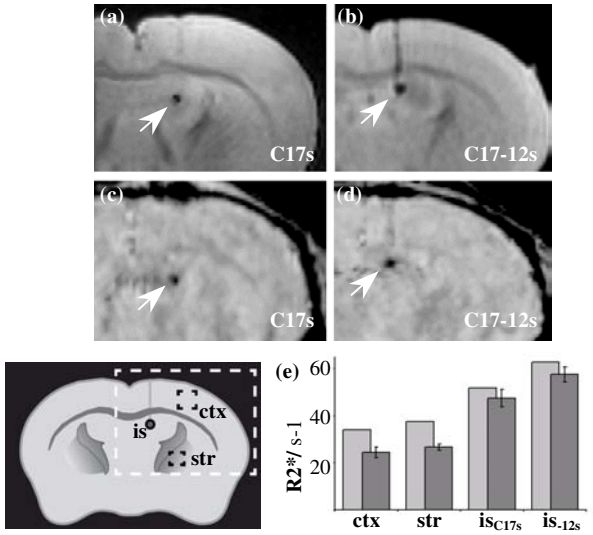

Fig. 2 T2*-weighted MRI of transplanted brains *ex vivo* (a, b) and *in vivo* (c, d). (e) R2* values from cortex (ctx), striatum (str) and the injection site (is) from *ex vivo* (dark bars) and *in vivo* (light bars) data (ROIs as shown in schematic).