A New MRI Reporter Gene: Mn Binding Protein MntR Produces T1 Weighted Cellular Contrast

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Introduction and Significance: Direct imaging of gene expression has been a goal of the MRI contrast agent field for nearly a decade. We have taken a new approach to genetic MR contrast by using paramagnetic Manganese (Mn) which is accumulated in the chelating protein MntR. Free Mn is already well established as a contrast agent when used in conjunction with T1 weighted imaging methods in Mn enhanced MRI (MEMRI) **(1)**. MntR binds Mn with nanomolar affinity while still keeping the Mn atoms in close proximity to solvent Hydrogens (H) **(2)**, similar to the chelating domains of synthetic chemical contrast agents.

Results and Experimental Design: Because MntR is a bacterial protein the original sequence did not express at detectable levels in eukaryotic cell culture (not shown). MntR was synthesized with a mammalian codon bias and an N terminal Myc tag.The new gene was then cloned into a mammalian expression vector with an IRES-eGFP to assay transfection efficiency via eGFP expression. HEK293T cells were transfected with the MntR IRES-eGFP plasmid using a chemical transfection reagent. After 36 hours the media was supplemented with 10µM Mn for 2 hours. Expression of MntR was confirmed by a Western blot against the integrated Myc tag (not shown). Cells were assayed for viability and for accumulation of Reactive Oxygen Species (ROS) to show no deleterious effects from MntR expression or Mn supplementation. **(Fig 1a,b)**. The live cells were washed with PBS before being freed from the plate and fixed in 4% paraformaldehyde. Fixed cells were gently pelleted into 100µL NMR tubes and imaged using a 3D gradient echo T1 weighted sequence previously used for MEMRI **(3)** (3DGE: TR=5ms TR=20ms, FA=65°) ROI analysis was performed to determine differences in MRI signal intensity between cell types**(Fig. 2a,b)**. Contrast was defined as the difference between sample and background over the background. MntR expressing cells showed a clear contrast compared to untransfected cells. Repeated experiments gave an average of 30% contrast with a 6% standard deviation.

Conclusions: MntR clearly functions as an genetically expressed contrast agent with physiological levels of Mn. Our ultimate goal is to determine the feasibility of using MntR as an MRI reporter for *in vivo* imaging, for example in transgenic mice expressing MntR selectively. Towards this goal, several technical issues must be addressed. Firstly, the pulse sequence being used to image MntR must be optimized. T1 relaxometry will determine the optimal pulse sequence parameters for MntR and could yield substantially greater contrast. Second, some correlation between expression levels of MntR and MR signal must be established. To address this issue, cell lines stably expressing MntR are being made. With stable expression in cell culture the epitope tagged protein levels can be assayed and compared to MR signal to determine MntR's range of detectability. Solving these issues will give us a greater understanding of MntR's properties as a contrast agent and are critical preliminary steps before moving on to *in vivo* expression and imaging.

References:

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3.Yu X, et. al. (2005) *Nature Neuroscience* 7:961-8.

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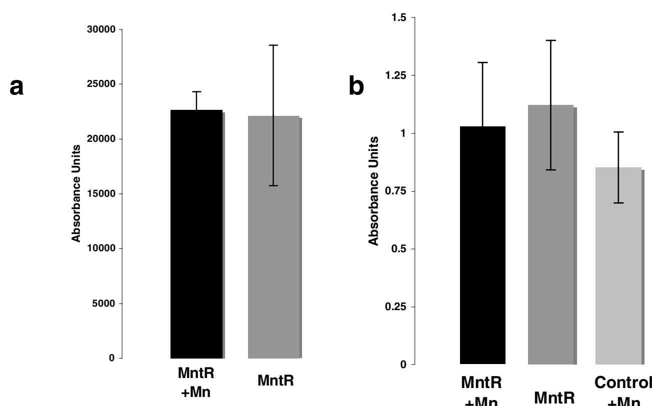

Fig. 1: a) Assay for ROS in MntR expressing cells with and without 10µM Mn. **b)** Viability assay for MntR expressing cells with and without 10µM Mn as well as control cells with Mn. (n = 12 samples)

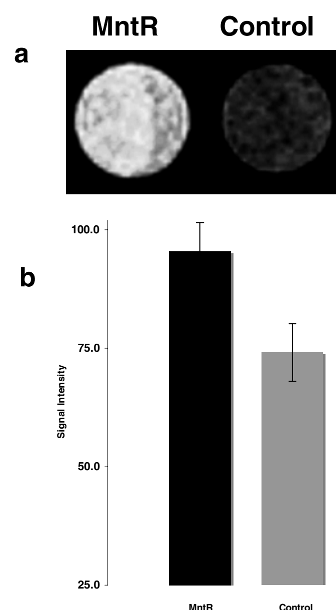

Figure 2: a)T1 weighted image of pellets of MntR expressing cells and control cells both supplemented with 10µM Mn. **b)** Average signal intensity from n = 7 MR experiments.