## MntR, an MRI reporter provides cellular T1 contrast without Mn supplementation.

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**Introduction and Significance:** Previously, we introduced the bacterial Mn-binding protein, MntR, as a novel MRI reporter for T1 weighted (T1w) imaging **(1)**. Paramagnetic Mn is already well established as a contrast agent when used in conjunction with T1w imaging methods in Mn-enhanced MRI (MEMRI) **(2)**. In our current studies we have moved MntR closer to *in vivo* expression by creating stably expressing cell line with lower levels of protein expression compared to transiently expressing cells, comparable to levels achievable in a transgenic animal. These cell lines have significant T1w contrast without any additional supplementation of Mn, indicating MntR's potential use as a reporter of transgene expression.

**Experimental Design:** MntR-IRES-eGFP was cloned into a retroviral vector containing an internal CMV promoter and a neomycin resistance gene. The retroviral plasmid was pseudotyped to a viral coat protein VSV-G to create a viral particle that is replication defective but paninfectious with high efficiency. Cells infected by the virus express MntR and eGFP while being resistant to the antibiotic neomycin (or G418). Mouse fibroblast (NIH3T3) cells were exposed to the virus and cultured under G418 selection to eliminate uninfected cells. Individual subclones were then screened based on eGFP expression. Expression of MntR was confirmed by a Western blot against an integrated Myc tag. Relative expression levels of a subset of cell lines were quantified with immunofluorescence using a digital IR fluorescence scanner **(Fig.1)**.

For MRI analysis, 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 (3DGE: TE=3.7ms TR=50ms, FA=45º) ROI analysis was performed to determine differences in MRI signal intensity between cell types **(Fig.1)**. Contrast was defined as the difference in MR signal intensity (SI) of a sample and a control over the control SI

**Results and Discussion:** MntR functions as a genetically expressed contrast agent even at low levels of Mn and moderate expression levels of stable cells. Cell lines that expressed MntR gave up to 23% contrast compared to non expressing cells, with no supplementation of Mn **(Fig.2)**. However, there was no obvious correlation between expression levels and contrast between cell lines **(Fig.1)**. These data suggest a threshold level of MntR expression is needed for significant contrast, but there may be other limiting factors such as intracellular Mn concentration. To better resolve the expression/signal threshold of MntR, future cell lines and transgenics will include a tetracycline controlled promoter for tunable MntR expression.

These results provide motivation to test MntR *in vivo*. The generation of an MntR carrying retrovirus used to make these cell lines now allows us to express our MRI reporter in the brain of a mouse embryo by injecting viral particles. This will serve as an important intermediary step as we move towards *in vivo* expression in transgenic mice.

**References:**

1: Bartelle B. Deans A.E., Turnbull D.H. Proc ISMRM 15:859,2007
2: Aoki I, et. al. (2004) *NeuroImage* 22; 1046-1059.

This research was supported by NIH grant NS038461

**Fig. 1: Three MntR expressing cell lines show increasing T1w signal enhancement compared to MntR expression level, assessed by immunofluorescence (IF).**

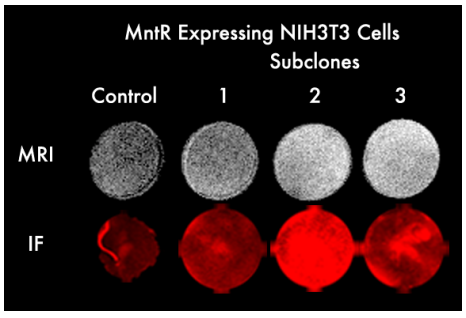


**Figure 2: Quantitative measurement of T1w contrast of cell lines from Fig.1.**