

## TIM2: A Reporter Gene for T<sub>1</sub> and T<sub>2</sub> weighted Magnetic Resonance Imaging

Stephen Patrick<sup>1</sup>, Tiago B. Rodrigues<sup>1,2</sup>, Scott K. Lyons<sup>2</sup>, Mikko I. Kettunen<sup>1,2</sup>, and Kevin M. Brindle<sup>1,2</sup>

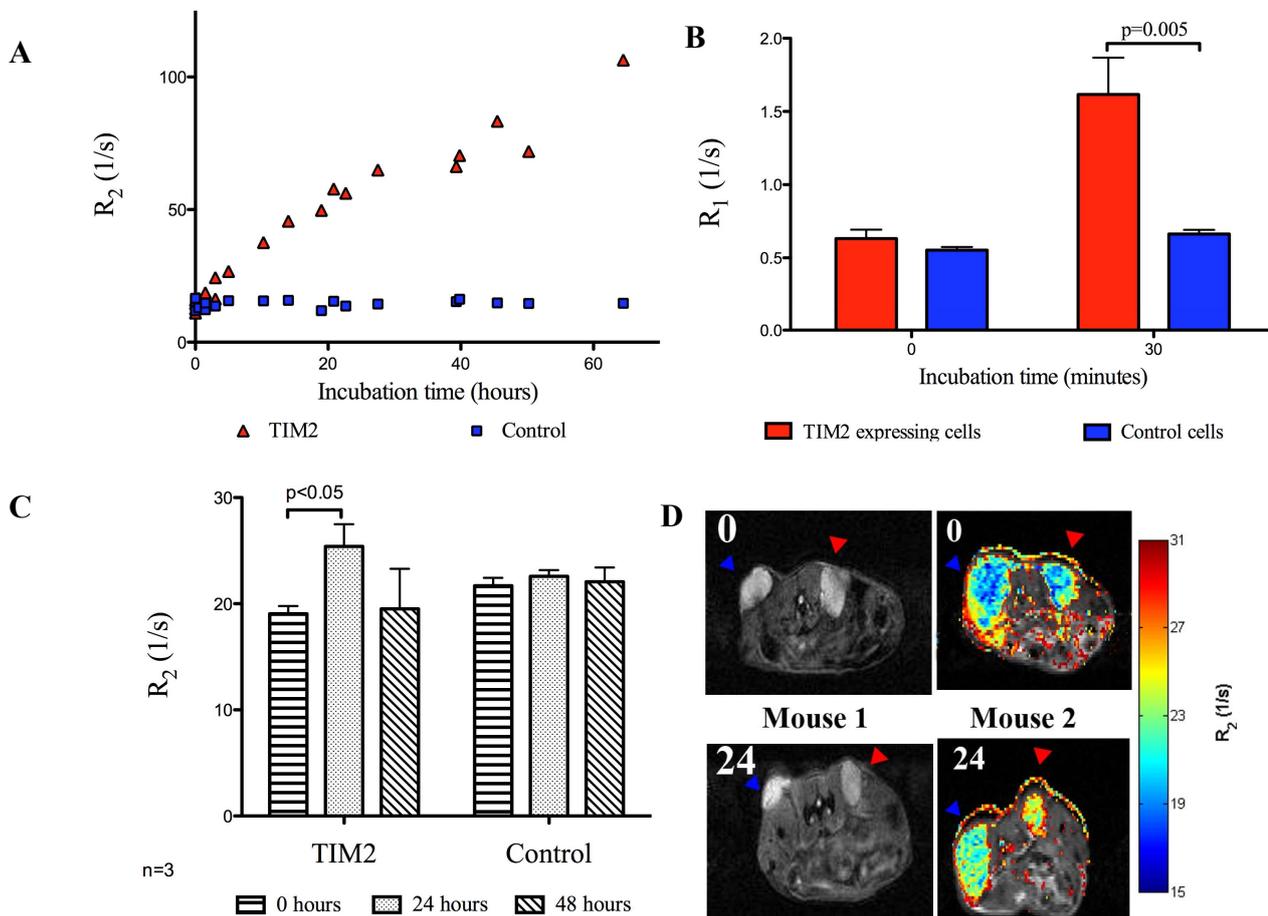
<sup>1</sup>Department of Biochemistry, University of Cambridge, Cambridge, United Kingdom, <sup>2</sup>Cancer Research UK, Cambridge, United Kingdom

**Introduction:** Reporter genes are valuable tools for the visualisation of gene expression in organisms and cells. There are several effective optical reporter genes, which can be detected using fluorescence and bioluminescence imaging modalities, but few good reporter genes for magnetic resonance imaging (MRI) (Gilad *et al.* 2008). MRI has the advantages over these other imaging modalities of much better tissue penetration, and provides higher spatial resolution and anatomical detail *in vivo*. Current MRI reporter genes have not found widespread use due to the relatively small changes in contrast obtained and therefore there is a need to develop new and better reporters.

The aim of this project was to develop TIM2 as a reporter gene for MRI. TIM2 is a mouse derived receptor protein that binds ferritin and facilitates its endocytosis. Ferritin is a spherical polymeric iron storage protein, with the capacity to hold thousands of iron atoms in its internal cavity in a super-paramagnetic core. When present in tissue, ferritin will produce negative contrast in T<sub>2</sub> weighted <sup>1</sup>H MR images. When the iron from the ferritin cavity is removed and replaced with manganese, it can shorten T<sub>1</sub> relaxation times, producing positive contrast in T<sub>1</sub> weighted images (Kalman *et al.* 2010). The ferritin gene has been used previously as a reporter gene for MRI, where the ferritin synthesised intracellularly has been detected through enhanced T<sub>2</sub> relaxation at relatively high field strengths (Cohen *et al.* 2005, Genove *et al.* 2005).

**Methods and results:** We transduced HEK 293T cells with a lentiviral vector encoding TIM2 and the red fluorescent protein mStrawberry. Expression of these genes was driven by a constitutively active PGK promoter and their co-expression by linking them with a 2A sequence. Virally transduced cells incubated in growth media with added ferritin showed a large increase in transverse relaxation times (R<sub>2</sub>) (Figure A). When TIM2 expressing cells were incubated with manganese-loaded apoferritin, R<sub>1</sub> increased significantly above that of control cells within 30 minutes (Figure B). Preliminary studies have confirmed the ability of TIM2 expression to produce T<sub>2</sub> contrast *in vivo*. Tumors were obtained by implanting TIM2-expressing HEK 293T cells into the right flank of SCID mice, with non-transduced cells implanted into the left flank as a control. Following intravenous administration of ferritin, we measured a significant decrease in the T<sub>2</sub> of TIM2 expressing tumors after 24 hours (n=3, p=0.02), but not in control tumors (n=3) (Figures C, D). MRI was performed at 7T in a horizontal bore magnet (Agilent). There was no effect of TIM2 expression on either cell growth or viability, either in normal media or in the presence of ferritin. Trypan blue dye exclusion assays showed a 12% decrease in viability of TIM2 expressing cells following overnight incubation with manganese-loaded apoferritin. This loss of viability was not observed in untransduced cells or in cells incubated with iron-loaded ferritin.

**Conclusion:** These results suggest that TIM2 has the potential to make an effective reporter gene for MRI, providing T<sub>1</sub> and T<sub>2</sub> contrast enhancement at a high magnetic field strength.



(A) R<sub>2</sub> of TIM2 expressing cells increases following incubation in growth media containing 75 nM ferritin. (R<sup>2</sup> = 0.9465). TR=2000ms, TE = 10ms, Data matrix 256x128, Scan time ~ 4 minutes, slice = 2mm

(B) R<sub>1</sub> of TIM2 expressing cells increases following 30 minute incubation in growth media containing 75 nM manganese-apoferritin, while control cells show no change in relaxation. After incubation, cells were washed, trypsinised, and pelleted, before being imaged at 7T. Error bars show S.E.M. TR= 4.22ms, TE = 1.96ms, Flip angle = 90°. Scan time ~14 minutes.

(C) TIM2 expressing tumors show a significant increase in R<sub>2</sub> following ferritin injection. n=3, error bars show S.E.M.

(D) T<sub>2</sub> weighted images, with and without an overlaid colour-scale R<sub>2</sub> heatmap, from two representative mice before and 24 hours after iron-loaded ferritin administration. SCID mice were implanted with HEK 293T cells in the left flank, and HEK 293T cells that had previously been transduced with a TIM2 encoding lentivirus in the right flank. After tumor formation mice were imaged at 7T (the control tumors are arrowed in blue, TIM2 in red). The TIM2 expressing tumors show negative contrast and shorter T<sub>2</sub> in comparison to the control tumors 24 hours after intravenous ferritin administration (0.2mL, 56mg/mL).

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### References

Gilad *et al.* 2008. *J. Nucl. Med.* 49: 1905–1908 / Kálmán *et al.* 2010. *Angew. Chem. Int. Ed.* 49: 612–615 / Cohen *et al.* 2005. *Neoplasia*, 7, 109–117 / Genove *et al.* 2005. *Nat. Med.* 11: 450–454.