# Artificial reporter gene providing MRI contrast in vivo based on chemical exchange.

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## Introduction

MRI is a powerful tool for tracking the biodistribution of labeled cells *in vivo*. However, all existing labeling techniques involve loading cells with contrast agents, therefore the contrast decreases with every cell division. In order to maintain a constant level of labeling, reporter genes have been used. In those systems transgene cells are overexpressing specific proteins that create a detectable contrast, with addition of exogenous substrate [1, 2] or without [3, 4]. However, those MRI reporters rely on the presence of (super)paramagnetic substances. As such, they all use water relaxation to gain contrast, making it impossible to distinguish differentially labeled target cells. Chemical Exchange Saturation Transfer (CEST) [5, 6] is a new and alternative contrast mechanism, that has the potential for imaging multiple targets simultaneously by magnetic tagging of different exchangeable protons and subsequently detecting changes in the water proton signal. Previously, we described the cloning and expression of an artificial lysine rich protein (LRP) in 9L glioma cells [7], which provided contrast sufficient to distinguish between cell extracts of expressing and non-expressing cells. The current study demonstrates that LRP can be used as an endogenous switchable marker for specific MR detection of xenografted brain tumors *in vivo*.

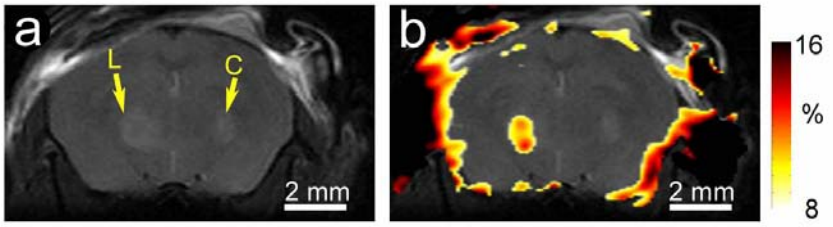
## Materials and Methods

Intracranial 9L tumors were generated by stereotaxic inoculation of $5x10^4$ cells into the striatum of NOD-SCID male mice. MR imaging was performed 6 days after cell transplantation. Mice were anesthetized by isoflurane inhalation (1-2%) and immobilized in a vertical custom-made probe, equipped with a transmitter-receiver coil. MR experiments were performed using a vertical 11.7T Bruker spectrometer equipped with 25 mm imaging probes. For mice anatomical imaging a RARE TR/TE=1000/10 ms sequence and for CEST imaging a spin echo sequences of TR/TE=9000/6.3 ms were used. Eight images were acquired alternately with a saturation power of 4.68μT for 4000ms at $\Delta\omega$ ±3.758 ppm from the water protons frequency. Total acquisition time =38.4 min, FOV=1.05-1.8x1.8cm with outer volume suppression, slice thickness=1mm, and matrix=32x32 pixels. Images were zero-filled to 64x64 and 128x128. Maps of change in signal intensity (SI) were generated pixel by pixel from $[[SI^{-\Delta\omega} - SI^{+\Delta\omega}]/SI^{-\Delta\omega}]x100$, where $SI^{-\Delta\omega}$ and $SI^{+\Delta\omega}$ are the average SI of 4 images acquired with saturation at $\Delta\omega$ ±3.758 ppm from the water protons frequency, respectively. SNR was 50:1.

## Results and Discussion

In order to evaluate the potential of the LRP as an MR reporter gene, LRP/EGFP-expressing (L) and EGFP-expressing (C, control) tumors were inoculated in the contralateral hemispheres of mouse brains (panel a). In maps of the change in SI between irradiating at $\Delta\omega$ = ±3.758 ppm, the LRP expressing tumors (L) could be differentiated from control (C) tumors and the adjacent brain tissue (panel b). In this panel, the contrast surrounding the brain is due to magnetic field inhomogeneity. The average change in SI for LRP tumors was 8.2±3.2% above the brain baseline relative to 3.5±3.3% in the control tumors. This significant difference (6 mice, t-test, 2-tailed, unpaired, P=0.03) indicates that LRP can be used as a potential genetic reporter for tracking cells.

LRP is a prototype of MR reporter gene based on CEST, in which contrast can be generated only when a frequency selective pulse is applied. In the case of the LRP the pulse is at the amide proton frequency, however, different reporters with different exchangeable protons such as amine and guanidyl protons can be designed and further used for labeling multiple targets *in vivo*.




## Conclusion

This work is a first demonstration that xenografted mammalian brain tumor cells expressing artificial Lysine-Rich Protein (LRP) provide MR contrast *in vivo* that can be switched "on and off" by applying radiofrequency pulses at the amide proton frequency. This new concept offers a general approach for constructing a family of endogenous reporter genes suitable to detect, simultaneously, multiple cell populations by MRI.

## References

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