Mammalian expression of a CEST reporter gene based on human protamine-1

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Target Audience: Researchers and clinicians who are interested in molecular and cellular MRI, particularly in non-invasive monitoring of gene expression, cell therapy, and transplantation, as well as pre-clinical drug screening with advanced MRI-based techniques.

Purpose: We have previously demonstrated that the arginine-rich protein human protamine-1 (hPRM1) gene can be used as a reporter gene in *E. coli* since it provides a higher chemical exchange saturation transfer (CEST) contrast than non-expressing cells[1]. The purpose of this study was to optimize the transgene expression in mammalian cells and image the cells in three-dimensional culture. Our goal was to capitalize on the biocompatibility of a human protein to enhance the CEST contrast while increasing cellular tolerance and avoiding an immune response.

Methods: CEST experiments were performed on an 11.7T Bruker Avance system, as previously described[2]. A modified RARE (TR/TE=6000/9.4 ms), including a magnetization transfer module (B_1=4.7 μT/4000 ms), was used to acquire CEST-weighted images. The absolute water resonant frequency shift was measured using a modified WASSR method, with the same parameters as in CEST imaging, except for TR=1.5 sec and a saturation pulse of B_1 =0.5 μT/250 ms, which was used for B_0 correction for each voxel using MatLab. MTR asymmetry (MTR_{asym})=100×($S_{-\Delta\omega}$ – $S_{+\Delta\omega}$)/S_0 was computed at different offsets, Δω. Human Embryonic Kidney cells (HEK293), were engineered to express the gene encoding to *hPRM1 (NM_002761*; 293^{hPRM1}). Non-expressing wild type cells (293^{wt}) were used as controls. The cells we lysed or encapsulated in alginate-based microcapsules using Ba^{2+} ions as the gelating cation[3].

Results: To examine the feasibility of detecting hPRM1 in mammalian cells using CEST, we constructed a lentivirus that encodes the hPRM1 under the cytomegalovirus (CMV) promoter and transduced human embryonic kidney (HEK293) cells (**Fig. 1a**). A significantly higher CEST contrast was observed from the lysate containing the recombinant protamine. As can be seen in the MTR_{asym} plots (**Fig. 1b**), higher MTR_{asym} values were obtained for the 293^{hPRM1} lysate compared to the lysate from wild type cells. All the MTR_{asym} values from 1.3 ppm to 3.1 (19 points) showed a significant difference ($p<0.05$, student's t-test, unpaired two-tailed). At 3.6 ppm, the 293^{hPRM1} MTR_{asym} was still higher than the 293^{wt}; however, the p value was 0.098. This is likely due to the high variability. The MTR_{asym} maps (**Fig. 1c**), obtained following saturation at the guanidyl (1.3 ppm) and amide (3.6 ppm) resonance frequencies, demonstrated that indeed a higher CEST contrast was observed for the 293^{hPRM1}.

To demonstrate the ability to image hPRM1 expression in live cells using CEST, we used a three-dimensional culture of encapsulated 293^{wt} and 293^{hPRM1} cells. As expected, the CEST contrast from encapsulated live cells that express hPRM1 was higher than the contrast from non-expressing wild type cells. This observation was found for saturation at both 1.3 ppm and 3.6 ppm offsets from the water resonance frequency (**Fig. 2**).

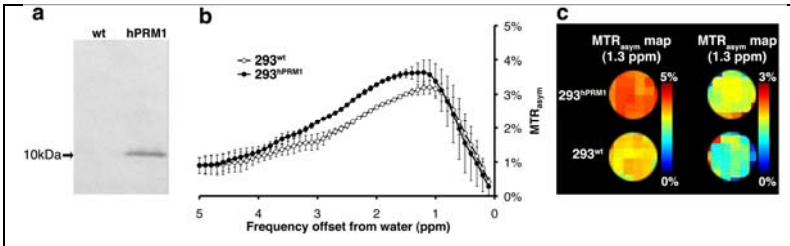

Fig 1. a) Western blot showing hPRM1 expression. b) MTR_{asym} plots (n=3 for each cell type). c) Representative MTR_{asym} maps obtained at 1.3 ppm and 3.6 ppm frequency offsets.

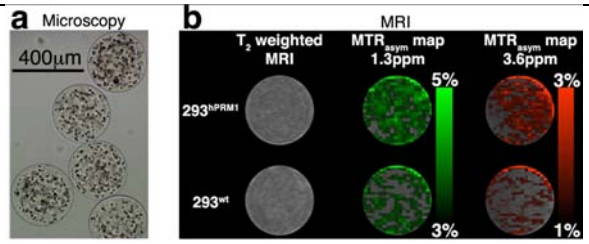

Fig. 2. (a) Bright-field microscopic images of the microcapsules. (b) T2-weighted images and overlaid MTR_{asym} maps of microcapsules containing cells obtained at 1.3 ppm and 3.6 ppm frequency offsets.

Discussion: We have previously demonstrated the feasblity of using lysine- and arginine-rich synthetic genes as CEST based reporters[4,5]. In addition, we demonstrated that hPRM1 over-expression could be detected in prokaryotic cells using CEST[1]. In this study, we have established that the human protamine could function as a CEST-based reporter gene in mammalian cells. We have demonstrated one of the many potential applications for hPRM1 in an immuno-isolation model of transplanted cells. In this approach, cells are surrounded with thin alginate membranes that are permeable to soluble factors (e.g., insulin and metabolites), but are impermeable to native antibodies. This approach provides a means to reduce or avoid immunosuppressive therapy altogether. Although several methods have been successfully developed to monitor encapsulated cell transplantation[3,6], a non-invasive method to visualize the viability and function of the transplant at the molecular level remains an unmet need. Thus, developing a reporter gene, such as hPRM1, could be a most valuable tool.

***Conclusion*:** We have developed a novel MRI reporter gene based on human protamine-1 and demonstrated its expression and detection in eukaryotic cells, as well as in three-dimensional cell culture.

***References*:** 1. Bar-Shir, A., *et al.* Genetic Engineering of Human Protamine-1 for use as MRI Reporter Gene Based on Proton Exchange. in *Proceedings 19th Scientific Meeting, International Society for Magnetic Resonance in Medicine* 1725 (Montreal, Canada, 2011). 2. Liu, G., Gilad, A.A., Bulte, J.W., van Zijl, P.C. & McMahon, M.T. *Contrast Media Mol Imaging* **5**, 162-170 (2010). 3. Barnett, B.P., *et al. Nat Protoc* **6**, 1142-1151 (2011). 4. Airan, R.D., *et al. Magn Reson Med* (2012). 5. Gilad, A.A., *et al. Nat Biotechnol* **25**, 217-219 (2007). 6. Arifin, D.R., *et al. Radiology* **260**, 790-798 (2011). Supported by NS065284, NS045062, EB015032 and MSCRFF-0103-00.