MRI reporter gene providing contrast based on chemical exchange saturation transfer (CEST)

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Introduction

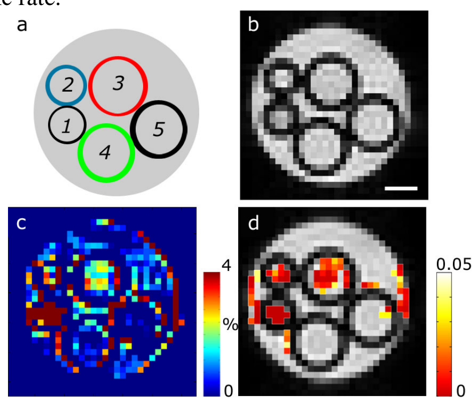
MR reporter genes have the potential to provide information on the biodistribution and viability of transfected cells *in vivo*. Previous reporter genes relied on the administration of (super)paramagnetic substrate [1, 2] or on cellular accumulation of iron [3]. Chemical exchange saturation transfer (CEST) is a new, alternative contrast mechanism, in which saturated contrast-agent protons are transferred to water [4]. Poly-l-Lysine (PLL) has been shown to be an effective CEST agent in the micromolar range [5]. We report the design, cloning and expression in mammalian cells of an artificial CEST reporter gene encoding for a Lysine Rich Protein (LRP, 32 kDa). This new gene provides cellular MR contrast that is independent of cell division and is visible only following a specific frequency-selective saturation pulse, and therefore does not interfere with other MRI contrast mechanisms.

Materials and Methods

Eight synthetic oligonucleotides (84 base pairs each) were cloned into a bicistronic expression vector (pIRES2-EGFP, Clontech). Rat glioma (9L) cells were transfected with the vector encoding to the LRP gene (200 Lysine residues) or with an empty vector containing only EGFP. Stable cell populations were selected with 0.6 mg/ml of G418. LRP expression was assayed using a custom-designed polyclonal antibody (Proteintech). Viability, proliferation, and metabolic rate were assayed using an MTS assay (Promega). From both cell populations, cytoplasmic proteins were extracted (using M-PER® Reagent; PIERCE), and dialyzed twice against 1 mM PBS. Protein concentrations were determined using the Bradford assay. Both cell extract samples were diluted to final concentrations of 845 or 818µg/ml in 10 mM PBS in two independent experiments. A protease inhibitor cocktail was added. MR experiments were performed on a 11.7T Bruker spectrometer at 310 ºK. Frequency-dependent water saturation spectra (Z-spectra) were obtained with a saturation power of 2.34 µT for 4 sec, and a TR/TE of 10000/2 msec. Spin echo images were acquired with a TR/TE of 9000/25msec, a saturation power of 1.64 µT for 4 sec and resolution of 0.156 x 0.156 x1 mm. Maps of change in signal intensity (SI) were generated 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 frequency of $\Delta\omega=\pm3.6$ ppm from the water ^1H frequency. The + 3.6 ppm frequency corresponds to the broad amide proton resonance of PLL, the –3.6 ppm frequency is for reference. Pixel-by-pixel t-test maps were generated as described previously [6].

Results and Discussion

The CEST effect is highly dependent on the exchange rate of amide protons of the protein, which in turn is determined by the protein amino acid sequence and structure. Previous findings [5] indicated that PLL has a favorable amino acid sequence for providing CEST contrast, with an amide proton exchange rate of about 150 s^{-1} under physiological conditions. This is well above the endogenous protein CEST exchange rate measured in vivo (28 s^{-1}) [7]. Thus, a gene encoding to LRP was designed, cloned, and expressed as validated by EGFP expression and LRP-immunostaining. LRP expression was found not to affect cell proliferation and metabolic rate.



An MR phantom was created to image the contrast gained from the Lysine Rich Protein. A 5mm NMR tube filled with PBS contained 5 capillaries with the following content (panel **a**): PLL 30kDa 10µM (1) PLL 30kDa 100µM (2), protein extract from 9L cells transfected with the vector encoding to LRP (3), protein extract from control cells transfected with empty vector (4) or PBS only (5). Panel **b** displays the image acquired with saturation pulse at $\Delta\omega$=-3.6 ppm from the water ^1H peak, giving no MR contrast, (scale bar 1 mm). However, the map of change in SI (panel **c**) reveals that the protein extract from cells expressing LRP showed an average change in SI of 1.33% (P value = 0.00064) while the protein extract from control cells showed no effect (-0.13%; P value = 0.65). Moreover, in pixel by pixel T-test maps (comparing the set of images saturated at $\Delta\omega=\pm3.6$ ppm), the pixels in the capillary containing the Lysine Rich Protein showed a significant change in MR signal, which was not detected in the control capillaries (Panel **d**; color coded pixels represent P values < 0.05).

Conclusion

The artificial gene encoding to LRP provides a detectable MR contrast that can be switched "on and off" repeatedly by applying a frequency-selective irradiation pulse. This gene has the potential to be used for tracking cells in vivo without the need for an exogenous (metal) substrate. Moreover, the MR contrast is produced only by live cells, and will not be diluted during (rapid) cell proliferation.

References

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