Tumor-specific expression and detection of a CEST reporter gene

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Target Audience: Researchers and clinicians interested in MR molecular imaging of cancer, CEST, and/or those who are developing MR-based reporter genes.

Purpose: Genetically encoded reporters with exchangeable protons can be used to study gene expression with MRI. The purpose of this study was to develop a new MRI-based tool that allows the detection of malignant tissue with enhanced specificity using the exquisite spatial resolution of MRI. By capitalizing on our previously published, genetically encoded CEST reporter, the lysine-rich protein (LRP) (1), and our expertise in tumor-specific imaging, we sought to develop a tumor-specific MRI reporter system.

Methods: We cloned two mammalian gene expression vectors that express LRP under the control of the progression elevated gene-3 promoter (PEG-Prom) (2, 3) to enable constitutive and tumor-specific expression of LRP, respectively. Using these vectors we established stable cell lines of 9L rat glioma $9L^{PEG\text{-}LRP}$. We generated a murine model of glioma by injecting these two cell lines intracranially to NOD/SCID/IL2rγ^{null} (NSG) mice. Seven days after cell injection, CEST MRI experiments were performed using an 11.7 T MR scanner (Biospec, Bruker), as previously described (4, 5), except for the following changes: CEST images were acquired with a modified RARE pulse sequence (TR/TE=5000/9.4 ms, B1= 3.6 μT/3000 ms). The mean Z-spectra were calculated from an ROI for each sample after B_0 correction of each voxel. $MTR_{asym} = (S^{-\Delta\omega}-S^{+\Delta\omega})/S^0 \times 100$ was computed. To reduce effects from endogenous CEST contrast, the normalized MTR_{asym} values, i.e., ΔMTR_{asym}, were calculated for each region of interest (ROI) by dividing the MTR_{asym} value of the ROI by the MTR_{asym} value obtained from normal, non-malignant brain tissue.

Results and Discussion: $9L^{PEG\text{-}LRP}$ cells showed increased CEST contrast compared with 9L cells *in vitro* (data not shown). $9L^{PEG\text{-}LRP}$ cells were capable of providing tumors in the brains of NSG mice, with a similar growth rate to tumors derived from wild type 9L cells (Fig.1 a). An increase in CEST contrast was clearly visible in tumors derived from $9L^{PEG\text{-}LRP}$ cells, as shown in the representative images (Fig. 1 b). The average CEST contrast, i.e., ΔMTR_{asym} value, was significantly higher (P<0.05, n=8) for tumors derived from cells expressing LRP than from the wild type 9L tumor at 3.5 ppm (Fig. 1 c). These results demonstrate that a CEST reporter gene can be expressed *in vivo* in a tumor-specific manner and can be used as a tumor-specific biomarker for MRI applications. Because of the ubiquity of MR imaging in clinical practice, reagents of this class can be used to translate molecular-genetic imaging rapidly.

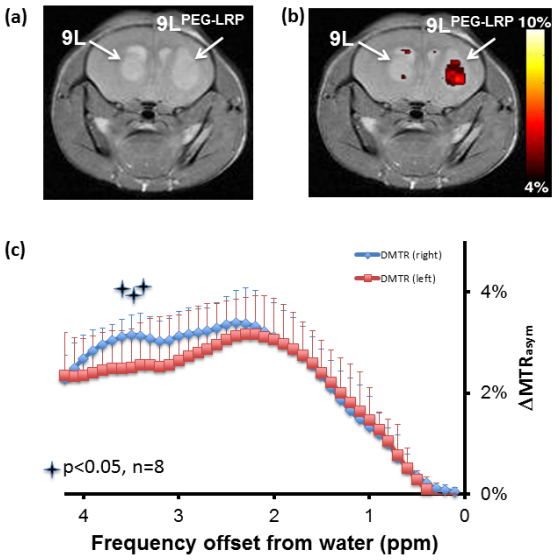

Figure 1. PEG-Prom driven LRP exhibited CEST contrast in mice brain tumor model. Representative images of T2-weighted (a) and CEST images superimposed on T2-weighted images (b). Left hemisphere has 9L tumors and right hemisphere has $9L^{PEG\text{-}LRP}$ (a, b). Temporal changes in the ΔMTR_{asym} values (mean ± s.d.; 8 mice) of each tumor type (c).

Conclusions: The PEG-Prom:LRP system can be used as a cancer-specific, molecular-genetic imaging reporter system *in vivo*.

References:

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