High resolution in vivo imaging of transgene expression

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Purpose

The goal of the current study was to determine whether transgene expression can be detected by NMR imaging in live animals.

Introduction

In vivo imaging of gene expression at high temporal and spatial resolutions has been a highly sought but unattained goal in the world of molecular imaging research in recent years. If such techniques were available, both endogenous (e.g. oncogene activation, loss of tumor supressor genes) and exogenous gene expression (e.g. gene therapy) would potentially be amenable to visualization. In addition, because most of our knowledge of gene expression has come from in vitro studies such as in situ hybridization, immunohistochemistry or molecular analyses (on mRNA or protein levels) which generally do not allow the study of time course or extensive spatial distribution of gene expression within a given tissue, in vivo imaging techniques are essential and complimentary to existing techniques. Furthermore, NMR is a good choice for in vivo imaging of gene expression as it has many advantages over isotope techniques including higher spatial resolution as well as the ability to extract physiologic and anatomic information simultaneously without administration of a probe.

Methods

Stably transfected 9L gliosarcoma tumors encoding for a non-downregulatable human transferrin receptor mutant (ETR+) were implanted in the left flanks of nude mice (n=20). Non-transfected, wild-type 9L tumors (ETR-) were implanted into the contralateral flanks to serve as an internal control. Probing of in vivo receptor expression was performed using an affinity ligand containing monocrystalline iron oxide particles conjugated with human transferrin (MION-tf). MR imaging was performed in live animals using a 1.5 T superconducting magnet (Signa 5.0) using a 5-inch surface coil. The imaging protocol consisted of coronal T1 weighted spin echo (SE 300/12), T2 weighted SE (SE 3000/variable TE) and gradient echo (GE 50 variable TE/variable flip angle) sequences after intravenous administration of MION-tf (3 mg). MR microscopy of mouse tumor specimens was performed at 7.1T with a 6-cm bore Oxford superconducting magnet equipped with actively shielded gradients capable of 85 Gauss/cm. A 12-mm solenoid coil was used. Images were acquired using a gradient-recalled acquisition-steady-state 3-D sequence. The raw data were reconstructed by Fourier transform and displayed as magnitude images.

Results

Significant differences in MR signal-to-noise ratios between ETR+ (1.7 +/- 0.2) and ETR- (9.1 +/- 1.4) were observed (Fig 1, $p<0.01$). These differences in MR signal intensity were most pronounced using T2 and T2* weighted imaging pulse sequences consistent with increased R2 upon cellular internalization (from 45mMsec^{-1} to 178 mMsec^{-1}). The imaging data are also consistent with biodistribution studies demonstrating a higher concentration of the probe in ETR+ (2.4% ID/g) compared to matched ETR- (0.8% ID/g) tumors in the same animal. MR microscopy at 7.1T confirmed the results from in vivo imaging studies demonstrating an overall lower mean signal intensity in ETR+ tumors compared to ETR- tumors (Fig 2).

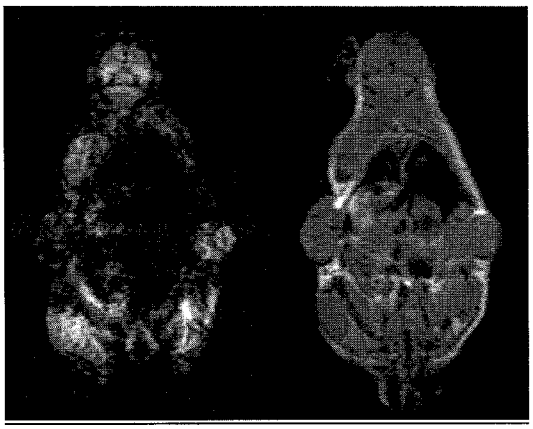

Fig 1. T1 (R-anatomy) and T2 (L)weighted pulse sequences. A clear difference in signal intensity is seen between the tumors on T2 weighted images (ETR+ tumor on right flank, ETR- tumor on left flank). The signal-to-noise ratio for the ETR- tumor was measured at 9.1; the signal-to-noise ratio for the ETR+ tumor was 1.7 for an overall difference between the tumors of 5.3.

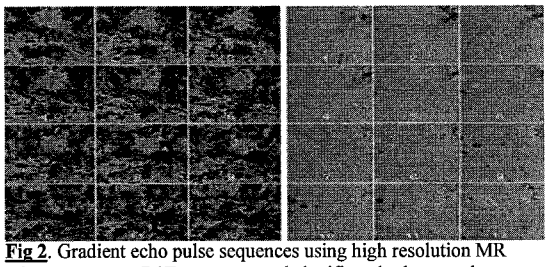

Fig 2. Gradient echo pulse sequences using high resolution MR microscopy on a 7.1T system reveal significantly decreased heterogenous signal within the hTfR+ tumor (left) with no significant change in signal seen within the hTfR- tumor (right).

Discussion

The current research represents the first direct evidence that transgene expression can indeed be visualized non-invasively b MR imaging in live small animals. The studies also demonstrate that relatively modest increases in receptor levels can cause dramatic changes in MR signals. As more and more cell surface proteins are shown to be upregulated in different human tumors (e.g. breast cancer) it is conceivable that specific superparamagnetic markers for each cell surface protein might be utilized instead. This method holds promise not only for high resolution mapping but also for in vivo imaging and repeated sampling. Additionally, the availability of a universal MR marker gene to image gene expression could be particularly important in monitoring gene therapy where exogenous genes are introduced to ameliorate a genetic defect or to add an additional gene function to cells.

References

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