Ferritin as novel MR-reporter for molecular imaging of gene expression

B. Cohen[1], H. Dafni[1], G. Meir[1], M. Neeman[1]
[1]Department of Biological Regulation, The Weizmann Institute of Science, Rehovot, Israel

Introduction

One of the challenges for molecular MR imaging is the development of a reporter gene that would allow non-invasive mapping of activation and suppression of genes during biological processes, thereby providing unique ability to follow and correlate dynamic processes. Advances in molecular biology and cellular biochemistry are providing new opportunities for developing new molecular imaging probes. Here we developed a novel reporter for MRI utilizing the H-chain of the murine ferritin, an iron storage molecule. Iron load and accumulation of ferritin increased relaxation rate in vitro and in vivo [1-2]. As a reporter, ferritin can directly alter MR contrast, in its expression site, in analogy to fluorescent proteins in optical imaging.

Methods

Molecular Biology: The murine ferritin H chain cDNA with a HA (influenza hemagglutinin) tag and a Kozak sequence at the N-terminus was generated by RT-PCR and cloned into the pBI-EGFP expression vector. The retroviral gene delivery and expression system RevTet-Off-IN was used to establish C6 cells expressing the murine HA-ferritin H-chain regulation of tetracycline (C6-TET-HA-ferritin cells). C6-TET-HA-ferritin cells grown in the presence or absence of tetracycline (1mg/ml) were injected subcutaneously ($1x10^6$/mice; n=5 in each group). Tetracycline and sucrose (or sucrose only) were added to the drinking water of the mice.

MRI experiments:

MRI data was acquired on a 4.7T horizontal bore spectrometer. R_1 and R_2 relaxation rates were determined from progressive saturation and multi-echo spin-echo data respectively. Data was analyzed by single exponential non linear best fit.

In vitro: $2.5x10^5$ cells were suspended in 0.2 ml agarose in 96 well plate. The plate was imaged using a bird-cage coil.

In vivo: Female CD1-nude mice were studied by MRI, 4 weeks after subcutaneous inoculation of tumor cells in the hind limb. Mice were anesthetized using intraperitoneal injection of 75 mg/kg ketamine and 3 mg/kg xylazine and placed with their tumor-bearing limb stretched above an actively RF decoupled 1.5 cm surface detection coil. A bird-cage coil was used for excitation.

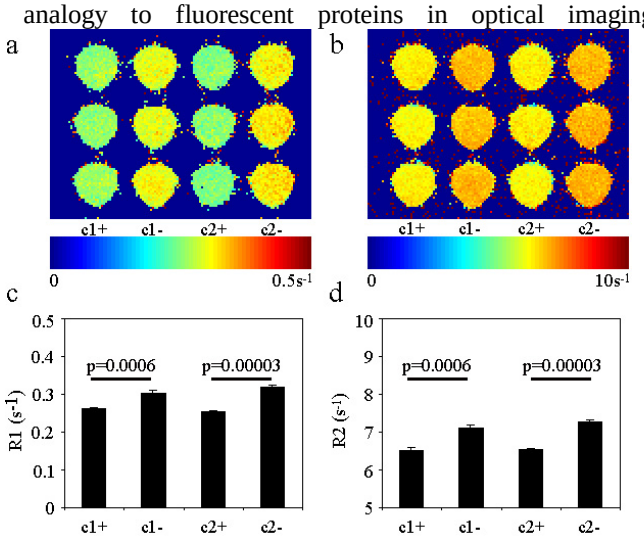

Figure 1. Relaxation rates of C6-TET-HA-ferritin cells with tetracycline-regulated ferritin expression. Relaxation maps R_1 (a) and R_2 (b) were derived for cell samples suspended in agarose and placed in a 96 well plate. Two different clones (c1, c2) are shown incubated either with (+) or without (-) tetracycline (n=3 for each clone). Averaged values of R1 (c) and R2 (d) ± standard deviation for each triplicate. P-values were derived by unpaired 2 tail t-test analysis, comparing relaxation rate with and without tetracycline.

Results

A number of clones stably expressing HA tagged ferritin and EGFP under tetracycline regulation were isolated. Expression of the EGFP protein as well as the HA tag as revealed by fluorescence microscopy, and by Western blot, respectively, served to validate the regulation of expression by tetracycline. Clones showing the highest expression level in the absence of tetracycline and most effective shut down of expression in the presence of tetracycline were selected for further analysis by MRI.

Significant contrast changes associated with activation of the transgene expression were detected by MRI both in vitro and in vivo and were consistent with differences observed for HA tag and for EGFP fluorescence. In vitro experiments included mapping of R1 and R2 relaxation in a multi well plate containing cells incubated in the presence or absence of tetracycline. Changes in contrast with and without tetracycline were highly significant for both R1 and R2 ($p<0.0006$ for two clones, n=3 of each clone; Figure 1). In vivo, the effects observed were smaller, and tetracycline-dependent changes in relaxivity were significant only for R2 ($p=0.04$). The lower effects of the transgene in vivo can be attributed to expression of the transgene by only a fraction of the tumor cells (possibly due to loss of expression or due to infiltrating stroma cells), as was detected by GFP fluorescence as well as by staining of histological sections for GFP and for the HA Tag.

Conclusions

We showed here the use of ferritin as a novel endogenous reporter for detection of gene expression by MRI. The sensitivity provided by ferritin was not as high as that provided by some of the reporters that rely on administration of exogenous contrast materials. Nevertheless, changes in relaxivity in response to selective induction of the expression of the transgene were detectable both in vivo and in vitro. The use of MR reporter gene that is genetically expressed and generates contrast in the absence of exogenous substrates opens new possibilities for non-invasive analysis of the regulation of gene expression in tumor models and transgenic animals.

References: 1. Gottesfeld, Z. and Neeman, M. Ferritin effect on the transverse relaxation of water: NMR microscopy at 9.4 T, Magn Reson Med. 35: 514-20., 1996. **2.** Bartzokis, G. and Tishler, T. A. MRI evaluation of basal ganglia ferritin iron and neurotoxicity in Alzheimer's and Huntingon's disease, Cell Mol Biol. 46: 821-33., 2000.

Acknowledgement: This work was supported by a grant from the Israel Science Foundation.