Ferritin as a tissue specific MRI reporter of inducible gene expression in transgenic mice

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Introduction:

Ferritin, the iron storage protein serves to protect cells, by sequestration of free iron, from the toxic effects of reactive oxygen species generated in the presence of iron and oxygen. Ferritin generates unique and particularly large R_2 relaxivity at low iron loading, and thus we previously proposed that overexpression of ferritin and redistribution of intracellular iron would lead to detectable MR contrast (1). Using C6 glioma cells that were engineered to express EGFP and the heavy chain of ferritin in a tetracycline inducible manner, we recently demonstrated that ferritin can be used as an endogenous MRI reporter of gene expression in vitro and in vivo (2). Similarly, Genove et al reported that infection of cells for expression of the light and heavy ferritin resulted in MRI detectable contrast (3). Here we report the generation of novel transgenic mice that over-express HA-tagged ferritin and EGFP in a tissue specific and tetracycline inducible manner. These mice were used for demonstration of the MR visibility and physiological impact of liver selective induced expression of the heavy chain of ferritin.

Materials and methods:

Generation of Tet:EGFP-HAferritin trangenic mice: The bidirectional expression cassette containing both HAferritin and EGFP was isolated from the pBI-EGFP-HAferritin expression vector (Clontech Laboratories,Inc) and injected into fertilized oocytes of Cb6F1 mice. Founder Tet:EGFP-HAferritin mice were identified by screening tail biopsy specimens for the presence of both HAferritin and EGFP cDNA by PCR and were selected for expression of the transgene products by crossing them with mice expressing the tetracycline transactivator (tTA) systemically. Homozygous Tet:EGFP-HAferritin mice were crossed with heterozygous tTA:LAP (liver activator protein) mice expressing tTA in liver hepatocytes (4). Double transgenic offsprings are expected to show induced expression of the transgene in the liver upon withdrawal of tetracycline while single transgenic Tet:EGFP-HAferritin siblings were used as negative controls.

MRI studies: horizontal 4.7T Biospec Bruker spectrometer was used. R_2 relaxation was measured using multi echo spin echo sequence with 8 echo times (TR= 2000 ms, TE 11-88 ms, 2 averages, FOV 7X7 cm, slice thickness 1 mm, matrix 128 X 128, SW=50,000Hz). R_2 maps were generated by pixel-by-pixel non-linear single exponential fit using Matlab. Analysis of R_2 according to regions of interest (ROI) was also applied. Following MRI analysis, livers were retrieved for evaluation of EGFP under fluorescence microscopy and for histological evaluation.

Results:

The double transgenic Tet:EGFP-HAferritinXtTA:LAP mice, the single transgenic siblings and the homozygous Tet:EGFP-HAferritin mice were fertile, developed normally and showed no gross phenotype. Two experimental protocols were used for MRI experiments. Constitutive transgene expression was studied in mice (n=5,6, double/single transgenic respectively), which were raised without tetracycline in the water (the transgene is always expressed in LAP expressing liver cells). MRI derived R_2 maps showed a significant change in liver relaxation relative to single transgenic siblings (that do not express the transgene). Surprisingly R_2 was lower in the double transgenic ferritin-expressing mice (Figure 1). Induced expression of the transgene was studied in mice (n=9,8, double/single transgenic respectively), which were raised with tetracycline in the water from mating (transgene expression is suppressed). MRI was applied for dynamic follow up of the induction of transgene expression upon tetracycline withdrawal. Here again, ferritin expression in the liver was associated with time dependent reduction in R_2 relaxation. Transgene expression was confirmed by liver specific EGFP fluorescence that was observed only in the double transgenic mice. Histological sections revealed prevalent intracellular vacuoles in hepatocytes of the double transgenic mice but not in single transgenic siblings. These could explain the unexpected ferritin induced shift in R_2 relaxation.

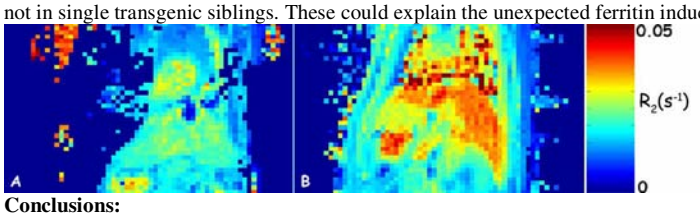

Figure 1: R_2 maps of Tet:EGFP-HAferritinXtTA:LAP double transgenic mice (age 2 months) with constitutive transgene expression (no tetracycline). A: Ferritin over-expressing double transgenic mice, B: matched single transgenic sibling.

Conclusions:

Transgenic Tet:EGFP-HAferritin mice were generated, carrying the MRI/optical imaging reporter genes HA-ferritin and EGFP but not tTA, which is needed for inducing expression. Tissue specific expression was achieved by crossing these mice with mice expressing tTA in the liver. Timed activation of transgene expression was controlled in the double transgenic offspring using tetracycline. Surprisingly, liver expression of h-ferritin resulted in reduced R_2 relaxation. A similar change in R_2 was previously reported for ferritin iron overload in IRP-2 knockout mice (5). The ability to detect significant transgene induced MRI contrast changes in the liver demonstrates the feasibility of using ferritin for MRI detection of gene expression in transgenic mice. The Tet:EGFP-HAferritin mice are valuable for studying the impact of tissue specific perturbation of iron metabolism by induced expression of h-ferritin, and for tissue specific tagging of cells for tracking by MRI. Generality of the use of these MRI reporter mice will be tested by mating with mice expressing tTA in other organs.

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