Chimeric ferritin as a reporter for MRI

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Introduction

The iron storage protein ferritin is a heteropolymer composed of H and L subunits (1). The H subunit contains a ferroxidase site, whereas the L chain provides the nucleation centers for deposition of the ferrihydrite core (1). The subunit ratio H:L varies across different tissues and cell types (2). Recent reports used the paramagnetic ferritin core as a probeless MRI reporter (3, 4); however ferritin detection depends on the amount of stored iron and often has relatively low sensitivity. The iron loading capacity of ferritin increases with the fraction of H-chains in the protein shell and reaches a saturation point ~40% (5). Pathologic consequences of long-term ferritin H-chain overexpression have been reported in aging mice (6). Conversely, protection from oxidative stress has been shown in mice that conditionally overexpress ferritin H-chain, because of the iron-chelating properties of ferritin (7). These data underscore the importance of controlling the H and L subunit ratio for sensitive and nontoxic use of ferritin as an MR reporter.

In this study, we fixed the ferritin subunit stoichiometry by engineering chimeric molecules H*L and L*H by fusing the H and L subunits. We report chimeric shell size distribution, iron loading and NMR relaxation rates of these constructs expressed in human cell lines and in mouse brain. We show that one specific design, L*H, exhibits significantly stronger R2 effects than H*L and wild-type ferritin.

Materials and Methods

We constructed the ferritin chimeras by fusing the C-terminus of one subunit to the N-terminus of the other using a flexible linker containing a FLAG epitope. All of the ferritin genes were devoid of the native 5' untranslated region and included a Kozak sequence for increased expression in mammalian cells. We subcloned reporters into a pAd-X plasmid under a CMV promoter and generated recombinant adenovirus-5 (AdV) vectors. U2OS cells were transduced and incubated with 1mM ferric citrate in DMEM for 24 hours. We isolated the chimeric ferritins and investigated their size distribution using electron microscopy (EM) and dynamic light scattering (DLS). Cells were fixed in 4% paraformaldehyde. We measured the transverse relaxation rate (R2) of the cell pellet using a 500 MHz NMR spectrometer. We used a CPMG sequence, NEX=4, delay time of 10 sec and 14 echoes ranging from 10 ms to 250 ms. For the *in vivo* MRI we injected 5 µl L*H AdV inoculum unilaterally into the cortex and striatum of C57BL/6J male mice, age 4-6 weeks (n=6). The contralateral side was injected with the same vector expressing control gene LacZ. All imaging was performed on 11.7 T micro-imaging system. At 5 days post-injection, mice were imaged *in vivo*. We acquired spin echo images with TE/TR=15/1200ms and NEX=4. We used 9 contiguous slices covering the injection site with 1mm thickness, a 256x128 matrix and a 59x117 µm in-plane resolution. In addition we acquired gradient echo (GE) images, with TE/TR=7/500ms, and NEX=4 and the same slice parameters as above. Post-imaging we perfused the mice and cryosectioned the brains for immunohistochemistry (IHC) and hematoxylin and eosin (H&E). Using IHC and confocal microscopy, we verified transgene expression and its colocalization with neurons and glia using neurofilament and glial fibrillary acidic protein (GFAP) markers, respectively.

Results and Discussion

The transgene vector mediated expression of L*H resulted in significantly enhanced R2 relaxation rate in cell pellets, compared to control LacZ, wild-type ferritin and homopolymers (Fig. 1A). The EM and DLS measurement revealed that the L*H ferritin had a slightly larger shell size (data not shown). The R2 of the L*H chimera showed pronounced contrast *in vivo* at the site of vector inoculation (Fig. 1B). The H&E staining showed no apparent tissue toxicity at the site of vector inoculation (data not shown). In addition immunoreactivity showed colocalization of the L*H ferritin FLAG epitope predominantly with cell populations positive for GFAP. Our study shows that the single chain L*H chimera may potentially be an improved MRI reporter.

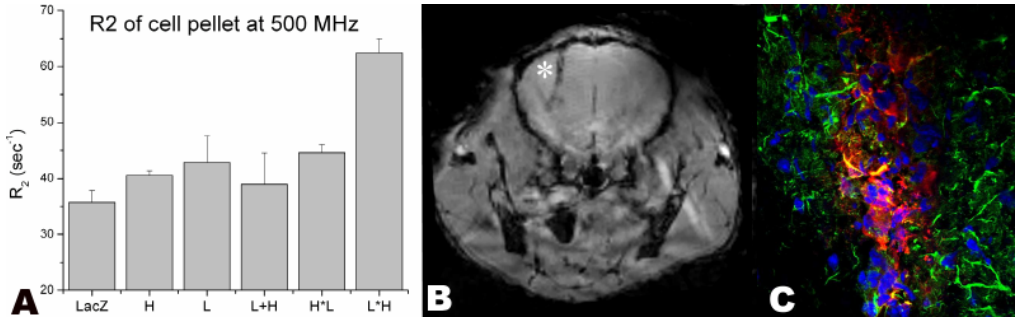

Fig. 1. (A) R_2 NMR measurements of cell pellets expressing the chimeras, homopolymers and wild type ferritin. (B) In vivo MRI of mouse brain inoculated with L*H on the left side (asterisk) and control LacZ on the right side. (C) IHC of mouse brain showing colocalization of the MRI reporter FLAG epitope (red) with GFAP (green), and Hoechst nuclear stain (blue).

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