Ferritin-N6A as an improved reporter gene for MRI

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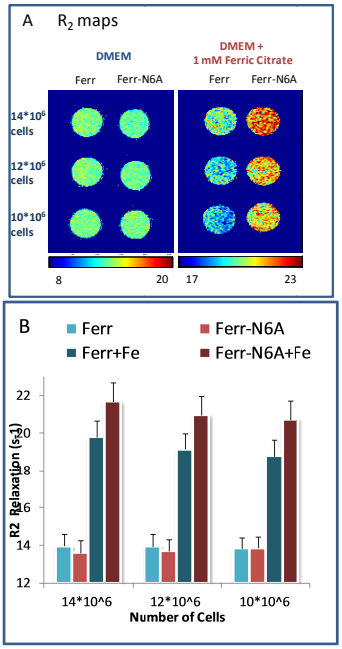
Introduction In nature, many organisms have evolved proteins that participate in production of iron oxides in forms of paramagnetic ferrihydrate (Fe_2O_3*nH_2O) and ferromagnetic magnetite (Fe_3O_4). For instance, ferrihydrate is found in a core of ferritin, the main iron storage intracellular protein. Due to this paramagnetic core, ferritin has recently proposed as MRI reporter gene.[1-3] However, ferritin has low R_2 relaxivity and thus provides relatively low sensitivity. One way to increase its sensitivity is to convert the ferrihydrate in the protein core into magnetite as has been done chemically, to form magneto-ferritin.[4,5] Magnetite can also be generated biologically. Magnetotactic bacteria, which synthesize intracellular magnetic nanoparticles comprising iron oxide, serve as an incredible example of such process. Recent molecular studies indicated some of the key proteins which involved in formation of magnetite. Mms6 is one of these important proteins. It functions in the regulation of crystal surfaces to control the magnetite crystal morphology during crystal growth in magnetotactic bacterial cells.

Purpose In this study, we have designed a recombinant fusion protein where N6A peptide (13 amino acids from Mms6 protein) is attached to the C-terminal of the inner surface of ferritin to generate ferritin-N6A (Ferr-N6A).This construct was designed to facilitate conversion of ferrihydrate into magnetite and by this induce MRI contrast.

Methods Rat C6 glioma cells were transfected with HA-ferritin/HA-ferritin-N6A pEIRES vector and stably selected with puromycin. Ferritin expression levels were monitored by Western blot analysis. For *in vitro* MRI measurements, the cells were cultured in DMEM. The experiment was performed with or without an addition of ferric citrate (1 mM for 48 hr). The cells were trypsinized and washed with PBS to remove excess of iron. A different number of live cells were imaged in the test tubes on top of agarose layer. MRI was acquired at 9.4 T using a multi-spin multi echo (MSME) pulse sequence with 30 echoes (TE = 10ms, TR = 3s, FOV = 4x4 cm, thickness = 1mm, matrix = 256x256). R_2 maps were reconstructed on a pixel-wise basis.

Results and Discussion Recombinant fusion ferritin-N6A was designed in order to enhance cell contrast for non-invasive MRI measurements. *Figure A* exhibits R_2 maps of cells in different concentrations with and without iron supplementation. Significant differences in contrast between cells cultured with iron supplementation in the medium were observed. However, R_2 map which provides quantitative information on contrast changes did not reveal significant changes between cells with different constructs in unsupplemented medium. Thus, fusion of ferritin with N6A peptide was proven to result in more effective R_2 contrast agent in vivo. Such considerable enhancement of sensitivity opens new biological applications of magneto-ferritin (Ferr-N6A) as reporter for MRI.



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