Biosynthesis of chimeric magnetoferritin, a novel MRI reporter gene

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Introduction Ferritin is one of the most extensively studied MRI reporter genes.[1] Such heightened interest in ferritin is due to its paramagnetic ferrihydrate core, which induces MRI contrast and can be applied to monitor gene expression in targeted cells. However, compared to iron in magnetite, iron in ferritin demonstrates lower relaxivity and thus lower sensitivity for detection by MRI. To date the largest gain in iron relaxivity was achieved chemically by replacing the native ferrihydrate core with a synthetic magnetite to form magneto-ferritin.[2] Biological synthesis of magnetite can be found in magnetotactic bacteria[3] that synthesize magnetite from iron oxide under microaerophilic conditions. Amongst the key proteins involved in this process, Mms6 controls the structure and growth of the magnetite crystal within the magnetosome membrane vesicle. We report here the generation of chimeric Ferritin-M6A protein, in which 12 amino acids (M6A peptide) of Mms6 protein were attached to the C-terminal of the inner surface of heavy (H) chain of HA-Ferritin[1] (Ferr). Our hypothesis is that M6A, freely rotating within inner core of ferritin, will interact with iron oxide and facilitate the conversion of ferrihydrate into magnetite and by this induce MRI contrast. **Methods** Rat C6 glioma cells were transfected with either HA-Ferr or HA-Ferr-M6A constructs cloned into the pEIRES vector and stably selected with puromycin. Cells were suspended in various densities in 1% agarose phantoms. MRI was acquired at Bruker Biospec 9.4 T scanner using Multi-slice multi-echo (MSME) pulse sequence with following parameters: nEchoes=60, TE/TR=8/3500 ms, FOV=4x4 cm, slices=7, coronal orientation, thickness=1mm, NA=2, 256x256). After MRI, cells were isolated and cellular iron content was determined via ICP-MS. R_2 maps were reconstructed on a pixel-wise basis. Subsequent *in vivo* studies were performed in order to evaluate the sensitivity of the HA-Ferr-M6A construct. CD1-nude female mice received subcutaneous injections of either HA-Ferr or HA-Ferr-M6A cells. Three weeks after inoculation MRI was acquired at Bruker Biospec 9.4 T scanner using MSME with: nEchoes=30, TE/TR=8/3000 ms, FOV=3x3 cm, nSlices=5-7, axial orientation, thickness=0.8mm, NA=2, 256x256). After MRI tumors were removed and analyzed by transmission electron microscopy (TEM) and Prussian blue histological staining. **Results and Discussion** *In vitro* experiments revealed enhanced R_2 relaxation in HA-Ferr-M6A compared to HA-Ferr phantoms (Fig. A). *In vivo* measurements demonstrate significantly enhanced R_2 relaxation rate in a central hypoxic region of HA-Ferr-M6A (Fig. B). TEM images revealed phospholipid vacuoles loaded with ferritin in the central parts of HA-Ferr-M6A tumors that were also observed in Prussian blue stained histological sections, especially in central hypoxic regions, of HA-Ferr-M6A tumors. In contrast, HA-Ferr tumors did not display similar vacuoles and demonstrated low amounts of iron in the peripheral region of the tumors (Fig. C). Here we report on a new HA-Ferr-M6A considerably enhanced r_2 relaxivity, cell iron loading and R_2 relaxation rate in the central hypoxic region of tumor, which represent a significant advance over existing ferritin reporter gene strategies.

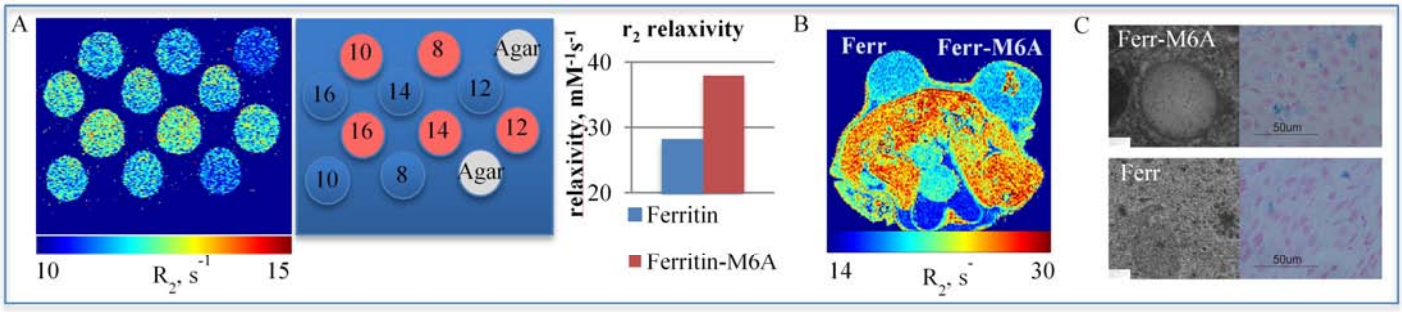

Figure. (A) R_2 map demonstrates enhanced R_2 relaxation rates that correspond to significant increase of r_2 relaxivity in HA-Ferr-M6A relative to HA-Ferr. Numbers indicate the number of cells $*10^6$. (B) R_2 maps of a single axial slice through the center of the both tumors HA-Ferr and HA-Ferr-M6A demonstrates significant increase in R_2 relaxation rate in the central hypoxic region of HA-Ferr-M6A. (C) TEM images and Prussian blue staining revealed elevated iron in HA-Ferritin-M6A relative to cells expressing HA-Ferritin.

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