Mitochondrial ferritin with cytoplasmic localization as an MRI reporter in olfactory sensory neurons

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Introduction

The iron storage protein, ferritin, has gained interest as an MRI reporter in molecular imaging. Upon cellular expression, ferritin forms a superparamagnetic iron core *in situ* and generates hypointensity in T2*-weighted images (1, 2). Optimization of ferritin contrast efficacy is feasible using molecular biotechnology tools, and recently, an engineered ferritin chimera with improved iron loading and contrast has been reported (3). Mitochondrial ferritin (mtFt) is an intronless gene with 80% similarity to the heavy chain ferritin (HFt) (4). The iron storage capacity of mtFt is the same or greater than HFt. In addition, mtFt is more stable, resisting guanidine and heat denaturation compared to HFt, and thus potentially a more robust reporter molecule (5). When mtFt is expressed as a transgene in a cell line, it loads more iron than HFt directed to the mitochondria (6). Here, we report the design of a modified mtFt with localization to the cell cytoplasm (cyto mtFt) that displays high iron loading efficiency. We use this new MRI reporter to image gene expression in native mouse olfactory sensory neurons. Overall, cyto mtFt appears to represent an incremental improvement in nucleic acid-based MRI reporter technology.

Materials and Methods

We cloned human mtFt from peripheral blood and deleted the mitochondria localization sequence. We then inserted this truncated gene into replication-defective type-5 adenovirus (AdV) under a CMV promoter. We determined the iron content of U2OS cells expressing the wild type mtFt, the cyto mtFt, HFt and LacZ control using inductively coupled plasma atomic emission spectroscopy with Perkin Elmer Optima 3000DV spectrometer. All animal experiments were approved by the CMU Institutional Animal Care and Use Committee. Adult female C57BL/6J mice, 5-7 weeks old, were anesthetized using an intraperitoneal cocktail of ketamine and xylazine and placed in supine position. Animals (n=12) received intranasal instillation of 10 µl AdV expressing cyto mtFt ($2x10^{11}$ pfu/m). The contralateral control side received GFP AdV ($1x10^{11}$ pfu/ml). The mice remained in the supine position for at least 15 minutes post-administration to aid in absorption to the olfactory epithelium. After 48-72 hours, both nares received 10 µl of 10 mM ferric citrate with 2 mg/ml holotransferrin. At 48-72 hours after the iron supplementation *in vivo* MRI was performed at 7 T using a 3D gradient-echo (GRE) sequence with TE/TR=7/100 ms and 117 µm isotropic voxels. After imaging, animals were perfused transcardially, fixed and imaged *ex vivo* at 11.7 T using a 3D GRE sequence with TE/TR=7/90 ms and 60 µm isotropic voxels. Immunohistochemistry was performed on the fixed olfactory epithelium tissues using rabbit polyclonal antibody to mtFt and sheep polyclonal antibody to olfactory marker protein (OMP). Secondary antibodies were goat anti-rabbit Alexa Fluor 594 and donkey anti-sheep Alexa Fluor 488.

Results and Discussion

The truncated cyto mtFt protein remained in the cytoplasm and loaded more iron than the wild type mtFt and HFt (data not shown). The images found on Figures 1a and 1b display pronounced hypointensity at the naris site inoculated with the reporter cyto mtFt (white arrows). The contralateral side (asterisk) transduced with GFP shows normal intensity. The immunohistochemistry shown on Figure 1c confirms the GFP expression (left insert), and also displays colocalization of the MRI reporter with olfactory sensory neuron marker (Figure 1c, right insert). The successful imaging of cyto mtFt in the olfactory epithelium has potential applications in the study of molecular mechanisms of olfaction as well as monitoring the success of intranasal gene delivery.

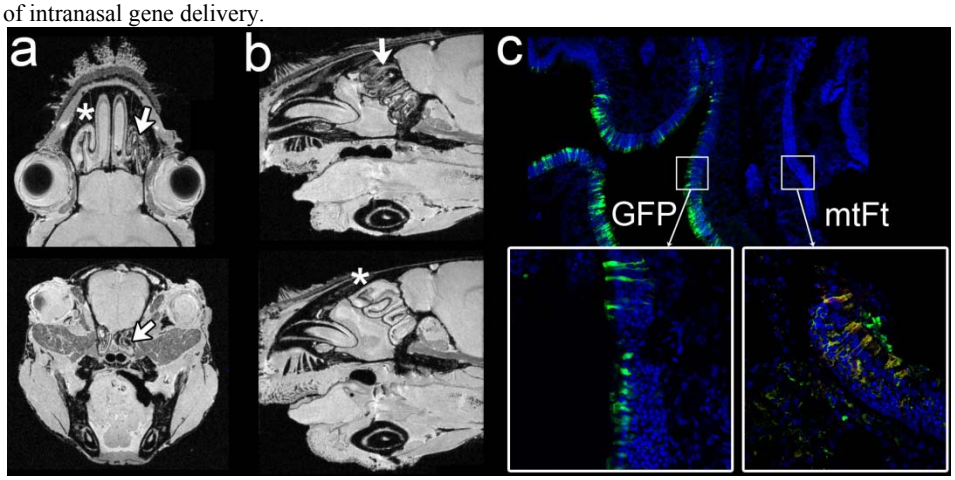

Figure 1. (a) Coronal (top) and axial (bottom) T_2*-weighted images of mouse brain. The white arrows point at darker regions of the olfactory epithelium expressing the cyto mtFt reporter. The asterisk at the contralateral side indicates normal intensity regions inoculated with the control reporter GFP **(b)** Sagittal images showing the extent of the contrast change at the site of the MRI reporter expression (top) and GFP control side (bottom). **(c)** Immunohistochemistry of axial sections across the mouse naris. The left side and the higher magnification insert displays cells expressing GFP with sensory neuron morphology. The insert on the right shows colocalization of mtFt (red) and olfactory marker protein (green). Cell nuclei are stained with Hoechst dye (blue).

Acknowledgments

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