The magnetosome membrane protein Mms6 produces MR contrast in vitro

X. Zhang[1], B. Robledo[1], S. Harris[1], and X. Hu[1]
[1]Biomedical Engineering, Georgia Institute of Technology and Emory University, Atlanta, Georgia, United States

Introduction

Magnetotactic bacteria are able to orient themselves along the Earth's magnetic field lines using specialized membrane-bound organelles called magnetosomes that contain iron oxide crystals. The genomes of these species are of great interest for molecular imaging as magnetosomes can also function as contrast agents for MR imaging [1]. It has been shown that the expression of a single gene present in magnetotactic bacteria, *magA*, may produce sufficient contrast for cellular imaging [2, 3]. Another protein, Mms6, is thought to initiate magnetite crystal nucleation within the magnetosome [4]. Mms6 has been found to be bound to bacterial magnetite [4] and to regulate crystal size and shape [5]. These biominerization functions may be especially important for image contrast applications where the magnetization and relaxivity changes induced by the reporter genes must be maximized. We hypothesize that once the Mms6 gene, *mms6,* is transfected and expressed in mammalian cells, it will function as a reporter gene producing iron oxide crystals that can be visualized with MRI.

Materials and Methods

AMB-1 *mms6* was cloned into the pBudCE4.1 vector (Invitrogen, Inc) and stably transfected into 293T cells using Lipofectamine™ 2000 (Invitrogen, Inc). Transfection of *mms6* was confirmed with RT-PCR, and expression of Mms6 was confirmed with a western blot analysis using mouse monoclonal antibodies. A single AMB-1 *mms6* positive clone (M1-3) and *mms6* negative control cells were selected for *in vitro* studies.

Iron staining assays were performed using a Prussian Blue Stain Kit (Eng. Scientific, Inc). Cells were incubated with and without 200 μM iron (ferric citrate) at 37 °C for 3 days, washed twice with phosphate buffered saline (PBS), and fixed with formaldehyde (2%). The cells were then treated with a staining solution containing a 1:1 mixture of 5% potassium ferrocyanide and 5% HCl acid for 30 min. After that the cells were treated with nuclear fast red for 10-15s, the cells were then examined under an Olympus IX71 epifluorescence microscope equipped with a digital color CCD camera.

For the imaging studies each cell line were plated in four 100 mm dishes. The cell culture media of the four plates was supplemented with ferric citrate such that the final concentration of iron in the media was 0, 100, 150, or 200 μM. The cells were then grown for three days to approximately 95% confluency. They were resuspended in 1 mL PBS and transferred into 1.5 mL microcentrifuge tubes. After an hour of settling by gravity at 4°C, the transverse relaxation time (T2) of the cell pellets was measured using a 3T MR scanner (Siemens Magnetom Trio) [MSME: TE = 20–400 ms in increments of 20 ms, TR = 1500 ms, FOV = 128 × 128 mm, NEX = 2, and a slice thickness = 1 mm].

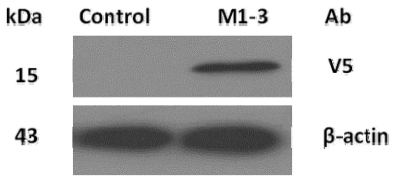

Figure 1 (left): Western blot showing the expression of Mms6 in 293T (control) and M1-3 cells. V5 is conjugated with Mms6.

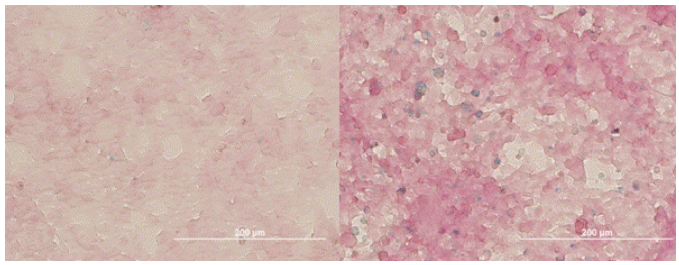

Figure 2 (above): Histology of whole cells stained with Prussian blue. 293T cells (mms6 negative, left) and M1-3 cell (mms6 positive, right) with 200 μM iron supplement. The color blue indicates the presence of iron. 20X magnification.

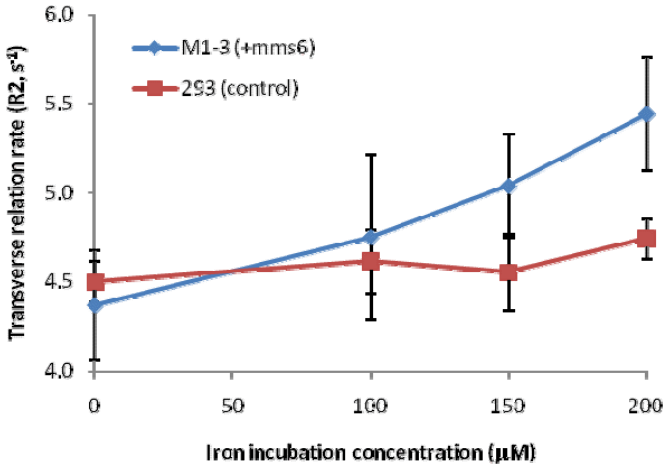

Figure 3 (above): R2 measurement of cell pellets after cells were cultured for 72 hours in varying concentrations of iron supplementation (n = 8).

Results and Discussion

Mms6 expression in the clonal population of M1-3 cells was confirmed by Western blot analysis (Fig 1). In contrast, the control 293T cells did not show any Mms6 expression. Following three days of incubation in iron supplemented media, whole cell histology showed greater iron staining in cells expressing Mms6 as compared to control cells, suggesting increased iron uptake by these cells (Fig 2).

In order to function as a reporter gene the key factor is the effect of the gene on MR contrast. To test this, M1-3 cells were incubated for three days with and without additional iron in the growth media. Cells expressing the gene showed a statistically significant ($p < 0.05$) increase in transverse relaxivity, R2, with increasing iron supplementation in the culture media which was not observed in the control cells (Fig 3). This difference in relaxivity may be due to the role of Mms6 in iron oxide crystal biominerization in the M1-3 cells.

Conclusion

In vitro cellular imaging suggests that *mms6* may function as an MR reporter gene. Given its reported biological function in the developing magnetosome, Mms6 may play an important independent or complementary role in molecular imaging.

Acknowledgements

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References

[1] Hartung A *et al. J Magn Magn Mater.* 2007, 311(1): 454-9. [2] Zurkiya O *et al. Magn Reson Med.* 2008, 59(6):1225-31 [3] Goldhawk DE *et al. Mol Imaging.* 2009, 8(3):129-39 [4] Arakaki A *et al. J Biol Chem*. 2003, 278(10):8745-50 [5] Amemiya Y *et al. Biomaterials*. 2007, 28(35): 5381-9.