

Glioma cells transfected with the gene *mms6* produce a strong increase in transverse relaxivity *in vitro*

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Introduction

Capable of capturing non-invasive, deep tissue images with high spatial resolution, MRI is an ideal imaging modality for *in vivo* studies of cancer cell proliferation, metastasization, and treatment [1]. However, cancer cells must generate MR contrast in order to be localized. While cells can be labeled with a contrast agent prior to implantation, an alternative approach is to develop reporter genes that produce endogenous contrast which can be sustained as the cells grow and divide [2-4].

A gene, *mms6*, originally identified in magnetotactic bacteria, expresses a protein that is thought to initiate magnetite crystal nucleation within specialized membrane-bound organelles called magnetosomes [5]. The same protein was also found to regulate iron-oxide crystal size and shape [6]. We hypothesized that transfecting glioma cells with *mms6* would lead to magnetite formation and MR contrast.

Materials and Methods

Rat glioma cells (9L), which are *mms6* negative, were transfected with AMB-1 *mms6*. Expression of *mms6* was confirmed with RT-PCR and Western blot analysis. A single *mms6* positive clone (9L4S) was selected for *in vitro* studies.

Prussian blue staining: Cells were incubated with and without 200 μM iron (ferric citrate) for 3 days, washed twice with PBS, and fixed with 2% formaldehyde. The cells were then treated with a 1:1 mixture of 5% potassium ferrocyanide and 5% HCl acid for 30 minutes. The cells were treated with nuclear fast red for 10-15 seconds and then examined under an Olympus IX71 inverted microscope.

Electron microscopy: Cells were cultured with 200 μM iron for 3 days, and then fixed with 2.5% glutaraldehyde in 0.1 M cacodylate buffer followed by 1% OsO_4 in the same buffer. Cells were dehydrated and embedded in Eponate resin. Ultrathin sections were observed on a Hitachi H-7500 transmission electron microscope (TEM) without counterstaining.

Intracellular iron measurement: Cells were incubated with and without 200 μM iron for 3 days. At the Chemical Analysis Laboratory at UGA, the cells were subjected to the dry ash method, to freeze drying, and to ICP-Mass Spectrometry.

Imaging: Cells were incubated with media supplemented with iron such that the added concentration of iron in the media was 0 or 200 μM . After three days, the cells were collected in a 1.5 ml tube. After an hour of settling by gravity at 4°C, the transverse relaxation time (T_2) of the cell pellets was measured using a 3T MR scanner (Siemens Magnetom Trio) [TR: 2 sec, TE: 10 msec, echoes: 20] and 9.4T MR scanner (Bruker) [TR: 4 sec., TE: 8.6 msec, echoes: 16].

Results and Discussion

Following three days of incubation in iron supplemented media, whole cell histology showed greater iron staining in *mms6* positive cells (9L4S) compared to control cells (9L), suggesting increased iron uptake by these cells (Fig 1). ICP-Mass Spectrometry showed an 8.5 fold increase in intracellular iron in 9L cells that had been cultured in media supplemented with iron as compared to 9L cells cultured with no iron supplement. Under the same culture conditions, 9L4S showed a 24.7 fold increase in intracellular iron (Fig 1), 2.8 times more iron storage than the control cells.

In order to investigate the location and structure of the intracellular iron, electron micrographs were acquired. Unlike in the 9L cells, particles can be found throughout the 9L4S cytoplasm, both within and outside of membrane-enclosed structures (Fig 2).

To determine whether the higher density of nanoparticles observed in the electron micrographs would enhance MR contrast, the transverse relaxivity (R_2) was measured. The percent increase in R_2 between cells cultured without an iron supplement and the same cells cultured with an iron supplement was calculated. 9L4S showed statistically significant ($p < 0.05$) changes in R_2 : a 57.1% increase in R_2 at 3 T and a 124.3% increase in R_2 at 9.4 T (Fig 3). This increase in R_2 with iron supplementation was not observed in 9L cells, which produced only a 7.8% and 18.6% increase in R_2 at 3 T and 9.4 T, respectively (Fig 3). We then calculated the change in R_2 between 9L cells and 9L4S cells, both of which had been cultured in media supplemented with iron. Relative to 9L cells, 9L4S cells showed a 46.6% and 90.6% increase in R_2 at 3 T and 9.4 T, respectively.

Conclusion

Our results show that *mms6* positive cancer cells produce significant MR contrast, possibly due to increased intracellular iron accumulation and magnetite formation. Our results suggest that *mms6* may function as an MR reporter gene for cancer studies.

Acknowledgements

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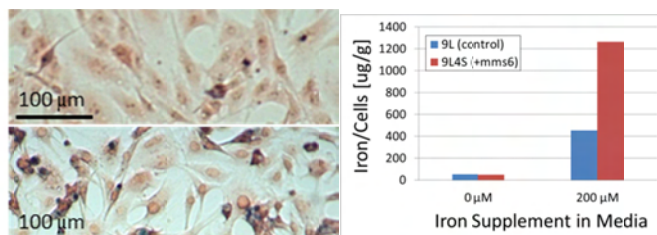


Figure 1. (left) Histology of whole cells (9L cells, top left; 9L4S cells, bottom left) fixed and stained with Prussian blue after being cultured in media supplemented with iron. The color blue indicates the presence of iron. (right) Graph of the intracellular iron measurements of 9L and 9L4S cells after incubation with and without iron.

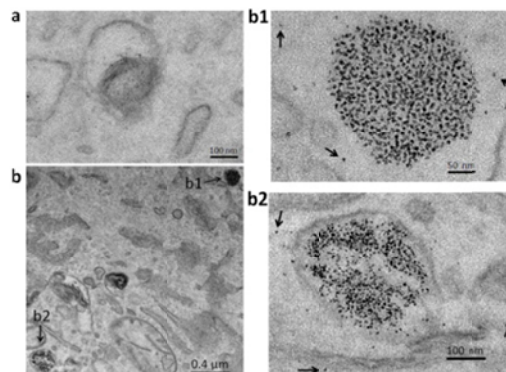


Figure 2. TEM images of 9L cells (-*mms6*; a) and 9L4S cells (+*mms6*; b, b1, and b2) after three days of incubation with 200 μM iron. In 9L4S cells, nanoparticles can be found within (b2) and outside (b1) membrane enclosed vesicles. Arrows in b1 and b2 indicate particles in cytoplasm.

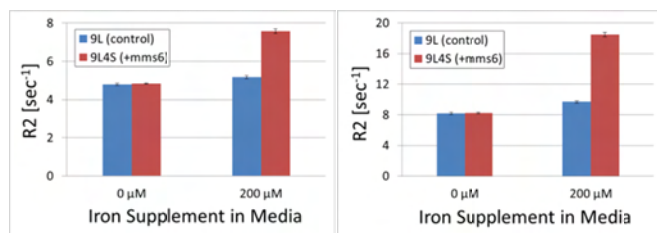


Figure 3. R_2 measurements of cell pellets after cells were grown for 72 hours in media supplemented with or without iron. Results were obtained at 3T (left) and 9.4T (right). $N = 24$ (left) and $N = 6$ (right). Error bars indicate \pm S.E.M.

References

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