Glioma cells transfected with mms6 enhance cellular iron oxide nanoparticles uptake and retention in vitro

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Introduction

Iron oxide nanoparticles (IONPs) and magnetic resonance (MR) reporter genes are tools used for cell labeling and tracking using MRI [1]. Because IONPs are metabolized and diluted with cell growth and division, the IONP-induced MR contrast may be lost over time. On the other hand, MRI reporter genes can generate MR contrast by accumulating iron in cells but their robustness to track cells may be limited if there is insufficient endogenous iron [2].

mms6 is a gene expressed in magnetotactic bacteria known to bind iron [3]. We have shown previously that glioma cells transfected with *mms6* produce a strong increase in transverse relaxivity *in vitro* and thus have the potential to be an MR reporter gene [4]. In this work, we hypothesized that expressing *mms6* with IONPs could enhance cellular IONPs uptake and retention.

Materials and Methods

Rat glioma cells (9L) 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.

Cellular labeling of IONPs: After 24 hours of incubation, both parental 9L and 9L4S cells were labeled with PEG coated IONPs with a core size of 10 nm (Ocean Nanotech, Springdale, AR) at concentrations of 0, 1, 2.5, 5, 10 and 20 μg Fe /mL for 24 hours.

Prussian blue staining: After incubation with IONPs for 24 hours, both 9L and 9L4S cells were stained using a Prussian blue staining kit (Ocean Nanotech) and examined under an Olympus BX51 inverted microscope.

Intracellular iron measurement: After incubation with IONPs for 24 hours, both 9L and 9L4S cells were collected, counted and measured. The colorimetric method described by Gupta et al. was used to measure iron concentrations [5]. Absorption measurements were read using a microplate reader (Promega, Madison, WI) at a wavelength of 490 nm.

Relaxivity measurement: Both 9L and 9L4S cells were labeled with different concentrations (0, 1, 2.5, 5 and 10 μg Fe /mL) of IONPs for 24 hours. The cells were then collected in a 1.5 ml eppendorf tube. After an hour of settling by gravity at room temperature, the transverse relaxation time (T2) of the cell pellets was measured using a 3T MR scanner (Siemens Medical Solutions, Malvern, PA) [TR: 2 sec, TE: 7.7 msec, echoes: 20].

Results and Discussion

As shown in Figure 1, Prussian blue staining showed significant intracellular iron differences between 9L and 9L4S cells, which had been cultured with a concentration of IONPs at 2.5 μg Fe/mL. Compared to the parental 9L cells, 9L4S cells showed more widespread blue staining, indicating higher intracellular iron uptake.

Relative to 9L cells, 9L4S cells showed a statistically significant ($p < 0.01$, n=3) change in R2: a 35.2% increase in R2 at 1 μg Fe/mL, a 26.7% increase in R2 at 2.5 μg Fe/mL, and a 12.5% increase in R2 at 5 μg Fe/mL (Fig 2 *left*).

The cellular uptake of iron was found to increase rapidly with increasing concentration of IONPs (Fig 2 *right*). The intracellular iron content in 9L4S cells was 2.2, 4.3, and 10.9 pg Fe/cell after 24 hours of incubation with 1, 2.5, and 5 μg Fe/mL of IONPs, respectively, all of which are significantly higher than the iron content of 9L cells ($p < 0.01$, n=3). This increase in intracellular iron is consistent with the increase in R2 observed when cells were incubated with IONPs. When cultured with a high concentration of IONPs (>10 μg Fe/mL), there was not a significant difference in intracellular iron uptake in 9L and 9L4S cells.

To determine whether *mms6* would increase the retention of iron in cells, intracellular iron was quantified at different passages after the incubation with IONPs. Both the 9L and 9L4S cells labeled with a high concentration of IONPs, 10 μg Fe/mL, were passaged three times successively, and the intracellular iron was measured after each passage. As shown in figure 3, no significant difference in intracellular iron between 9L and 9L4S was found at passage 0, however, 9L4S cells contained significantly more iron than 9L cells from passage 1 to passage 3 ($P < 0.01$, n=3).

Conclusion

Our results show that expression of *mms6* can potentially enhance cellular uptake and retention of IONPs. This capability of *mms6* may allow us to develop a mean for targeted uptake and retention of IONPs for MR molecular imaging.

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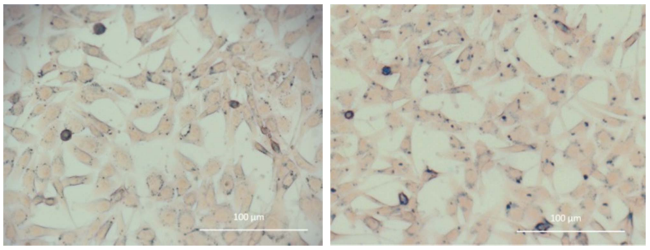

***Figure** 1. Histology of whole cells (9L cells, left; 9L4S cells, right) fixed and stained with Prussian blue after being cultured in media supplemented with IONPs for 24h. The color dark blue indicates the*

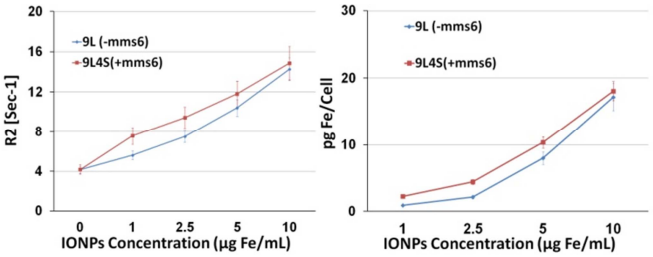

***Figure 2.** (left) R2 measurements of cell pellets after cells were labeled with different concentration of IONPs. (Right) Intracellular iron measurement of 9L and 9L4S cells after incubation with different concentration of IONPs. Error bars indicate ±S.E.M.*

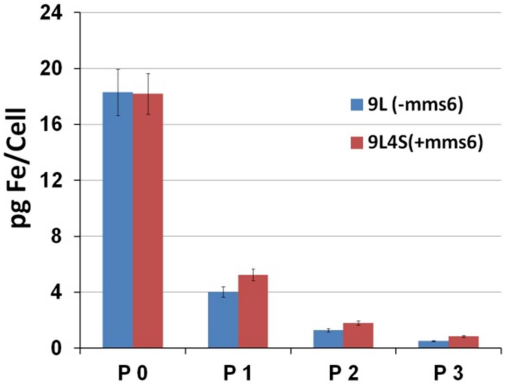

***Figure 3**. The remaining intracellular iron in 9L4S and parental 9L cells labeled with IONPs after various passages (P0 to P3).*

References

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