

Tumors established with glioma cells transfected with the gene *mms6* produce a strong increase in transverse relaxivity *in vivo*

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Introduction

MRI is an imaging modality that may be useful for *in vivo* studies of cancer cell proliferation, metastasis, and treatment [1]. In order to allow MRI, to visualize tumors, reporter genes have been developed to produce endogenous contrast which can be sustained as the cells grow and divide [2-4]. One such 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 previously found that transfecting glioma cells with *mms6* increased the cells' ability to uptake iron as well as increased their spin-spin relaxation rate (R2) *in vitro* [7], so we hypothesized that *mms6*-positive cells would uptake endogenous iron *in vivo* and thereby produce MR contrast *in vivo*.

Materials and Method

Rat glioma cells (9L, ATCC) 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 vivo* studies.

Tumor inoculation: Approximately 1×10^6 9L cells in 100 μ l of PBS were injected into the right flank of a nude rat (Charles River, Wilmington, MA). Approximately 1×10^6 9L4S cells in 100 μ l of PBS were injected into the left flank of the same nude rat.

Imaging: Two and three weeks after tumor inoculation, the rats were sedated with isoflurane. Spin-echo and gradient echo images of the flank tumors were measured using a 9.4T MR scanner (Bruker, Billerica, MA) [MSME, TR: 4 sec, TE: 9.3 msec, echoes: 12]. T2 and R2 were calculated from the MSME images using exponential fitting.

Prussian blue staining: Immediately after the last imaging session, the two flank tumors were excised and fixed in O.C.T. compound. The tissue was then sectioned into 0.8 μ m slices. Each tissue slice was then placed on a glass slide, fixed in acetone, and stained using a Prussian blue staining kit (Ocean NanoTech, Springdale, AR). The tissue slices were then examined under an Olympus BX51 inverted microscope.

Results

Previously, we found that *in vitro* 9L4S cells, after being incubated in medium containing an iron supplement, had both an increase in intracellular iron concentration and an increase in R2 relative to 9L cells incubated in the same conditions [7]. Specifically, 9L4S showed statistically significant ($p < 0.05$) changes in R2: a 57.1% increase in R2 at 3 T and a 124.3% increase in R2 at 9.4 T [7].

Two weeks after tumor inoculation, an MRI scan revealed that the tumors had formed in the flank of the rat (Fig 1, left). We found that the 9L4S tumor (*mms6*-positive) had a shorter T2 than the 9L tumor (*mms6*-negative) (Fig 1, right). Two weeks after tumor inoculation, the R2 value of the 9L4S tumor was 19.6% higher than the R2 value of the 9L tumor (Fig 2). Three weeks after tumor inoculation, the R2 value of the 9L4S tumor was 21.6% higher than the R2 value of the 9L tumor (Fig 2).

After three weeks of tumor growth, the Prussian blue staining of the 9L4S tumor tissue showed there was an increase in iron present in that tumor compared with the 9L tumor tissue (Fig 3).

Conclusion

Our results show that *mms6*-positive tumors produce MR contrast *in vivo*, likely due to increased intracellular iron accumulation. Contrast was produced despite the fact that the rat was not given any type of iron supplement. If the animal were to be given an iron supplement, it is possible that the MR contrast would be enhanced. Our results suggest that *mms6* may function as an MR reporter gene for cancer studies.

Acknowledgements

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References

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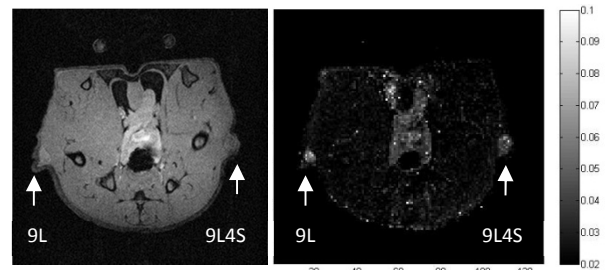


Figure 1. Magnetic resonance imaging of flank tumors in rats. Both fast low-angle shot (FLASH, left) and T2 map (right) images were acquired 20 days after tumors were established. Arrows indicate tumors. : -*mms6* 9L cells (left arrow) and +*mms6* 9L4S (right arrow). A majority of the *mms6*-positive tumor was hypointense on the T2 map.

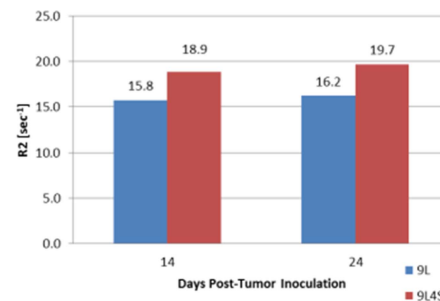


Figure 2. Graph of R2 measurements of flank tumors two and three weeks after tumors were established with 9L and 9L4S cells. Results were obtained at 9.4T.

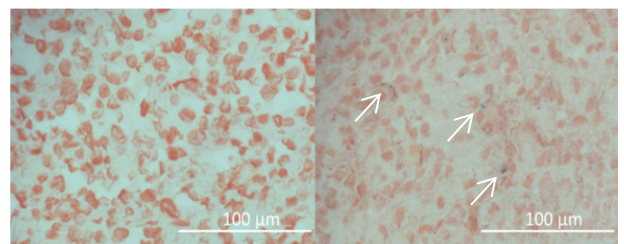


Figure 3. Histology of tumors excised from rat 24 days post-tumor inoculation. The tissue was fixed and stained with Prussian blue. Left: tumor established with 9L cells. Right: tumor established with 9L4S cells. Arrows: the color blue indicates the presence of iron.