Mannose Overexpression as a New CEST MRI Biomarker for Highly Aggressive Glioblastoma Stem Cells Shifting to a Mesenchymal Phenotype

Behnaz Ghaemi[1,2], Hernando Lopez-Bertoni[3,4], Shreyas Kuddannaya[1,2], John J. Laterra[3,4], Guanshu Liu[1,5], Jeff W.M. Bulte[1-3,5]

[1]Russell H. Morgan Department of Radiology and Radiological Science, Division of MR Research, [2]Cellular Imaging Section and Vascular Biology Program, Institute for Cell Engineering, [3]Department of Oncology, [4]Department of Neurology, the Johns Hopkins University School of Medicine, Baltimore, Maryland, USA. [5]F.M. Kirby Research Center for Functional Brain Imaging, Kennedy Krieger Inc., Baltimore, Maryland, USA.

INTRODUCTION:

Glioblastoma is one of the most aggressive cancers known to men. Non-invasive assessment of aggressiveness is crucial for treatment planning, but current MRI protocols lack specificity. Amide proton transfer CEST MRI can grade diffuse gliomas, but not GBM aggression levels. GBM invasiveness arises from a shift from a pro-neural to mesenchymal phenotype. Based on a report that mannose-weighted (MANw) CEST MRI can detect unlabeled mesenchymal stem cells (MSCs) overexpressing mannose [1], we investigated if mesenchymal cancer stem cells can be detected “label-free” in a similar fashion.

METHODS:

Low aggressive GBM1a and highly aggressive M1123 cells were used throughout. Mannose expression was assessed using fluorescein-labeled galanthus nivalis lectin (GNL-FITC, specific for mannose) staining, and the mesenchymal cellular phenotype by anti-CD44 immunostaining. MANw CEST MRI was conducted using a Bruker 11.7T vertical bore spectrometer. For in vivo tumor models, 2×10^5 M1123 and GBM1a spheres were injected into the striatum of NSG mouse brain. In vivo T2-w and MANw CEST MRI was performed 1, 8 and 16 days after injection. Tumor and brain ROIs were manually drawn based on T2-w images. For M1123 cells, the mannose-binding lectins LMAN1 and LMAN2 were knocked down using liposomal transfection with LMAN 1/2 siRNA, and LMAN1/2 expression was quantified with qRT-PCR

RESULTS:

Low mannose expression was seen for both 2D cell cultures, but 3D M1123 spheres contained more mannose compared to GBM1a. In vitro MANw CEST MRI showed the highest CEST signal for the M1123 3D spheres. T2-w MRI showed M1123 cells growing much faster than GBM1a invading across the entire hemisphere on day 16. On day 1, a distinct MANw CEST MRI signal was observed for M1123, but not for GBM1a. Eight and 16-day post-injection follow-up revealed a continuous pronounced MANw CEST signal only for M1123, which correlated with anti-mannose staining. The MANw CEST signal of M1123 was significantly higher (>1.8-fold) than GBM1a and host brain for all time points. Anti-CD44 immunostaining revealed an abundance of MSCs in M1123, but none in GBM1a. Silencing LMAN1/2 in M1223 cells resulted in a 4-fold reduction of LMAN1/2 and mannose expression, which was accompanied by a 10% reduction in MANw CEST MRI signal.

DISCUSSION:

It appears feasible to apply MANw CEST MRI to assess GBM aggressiveness by virtue of their innate mannose overexpression. This advancement may decrease the time interval between the initial diagnosis anda personalized treatment plan, increasing patient survival.

CONCLUSION:

We have introduced a new molecular MRI approach to distinguish GBM aggressiveness without the need of injecting exogenous contrast agents. Since brain tumor patients already undergo routine MRI, its clinical implementation could be immediate without obtaining further regulatory approval.

ACKNOWLEDGMENTS:

This study was funded by NIH R01 EB030376.

REFERENCES:

1. Yuan, Y, *et al*. Nat Biomed Eng 2022;6:658-666.