Profiling Amino Acid Transporters at the Blood-Brain Tumor Barrier

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Introduction

Surgical resection remains the first line treatment for both low-grade gliomas (LGG) and high-grade gliomas (HGG) and maximizing safe resection decreases chances of recurrence. Fluorescent image-guided surgery (FIGS) that utilizes fluorescence dyes, such as indocyanine green (ICG) and 5-aminolevulinic acid (5-ALA), have been successful in improving maximal resection rate in HGGs.[a] However, the uptake of ICG and 5-ALA relies on a disrupted blood-brain tumor barrier (BBTB), which has limited their utility in LGGs and some HGGs that have intact BBTBs. Moreover, large clinical studies have also confirmed the need to improve distinguishing between healthy brain tissue and LGGs.[b]

To address this need to improve resection rates and progression-free survival, our project this summer focused on identifying targets for second-generation fluorescent imaging agents that will define brain tumor margins regardless of a permeable or intact BBTB. It has been observed that amino acid transport is increased significantly in gliomas and could serve as a target for fluorescent agents. Previous studies have utilized amino acid-based positron emission tomography (AA-PET) and used tracers such as ^{11}C-methionine (MET), ^{18}F-fluoroethyltyrosine (FET) and ^{18}F-fluciclovine to help characterize LGGs. In these tumors, the uptake of MET, FET, and ^{18}F-fluciclovine seemed to be regulated by amino acid transporters LAT1/2 (SLC7A5/7A8) and ASCT2 (SLC1A5), respectively. Based on these studies, we optimized and validated the immunostaining conditions which will be used to investigate the expression profiles of SLC transporters such as LAT1, LAT2, and ASCT2 at the BBTB relative to known tumor markers in normal brain-, low-grade, and high-grade gliomas to better understand their relative expression levels and identify the most suitable target for second-generation, amino acid-targeted fluorescent imaging agents.

Hypothesis

Amino acid transporters are differentially expressed at the BBTB compared to blood-brain barrier (BBB) in normal brain tissue and can be targeted by fluorescent imaging agents to define brain tumors and tumor margins intraoperatively.

Methodology

Formalin-fixed paraffin embedded human low grade glioma tissues (acquired under IRB #844736) were deparaffinized, rehydrated, antigens were retrieved using heat-induced epitope retrieval, 3% H_2O_2 was used to deactivate endogenous peroxidases, and sections were blocked with blocking buffer. We then focused on optimizing and validating immunostaining conditions for each antibody. Serial dilutions for each primary antibody were then used to determine the concentration range that provided the best contrast under microscopic imaging. Depending on the initial results, serial dilutions were again performed within the range to determine the optimal dilutions. All stained tissues were observed under the microscope at 10x and 20x magnification.

Results

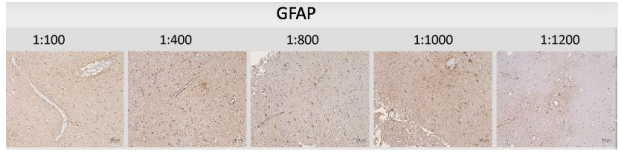

Figure 1: Human LGG tissue show staining for antibody GFAP at dilutions 1:100, 1:400, 1:800, 1:1000, and 1:1200. Staining is expressed most clearly at 1:800 (middle) and 1:1200 at endothelial

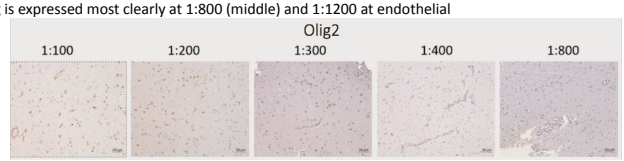

Figure 2: Human LGG tissue show staining for antibody OLIG2 at dilutions 1:100, 1:200, 1:300, 1:400, and 1:800. Nuclear staining is most clearly at 1:200.

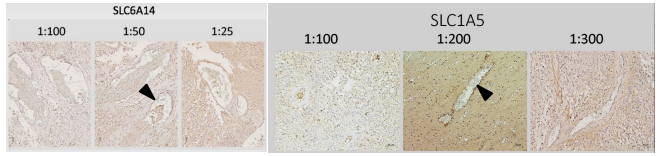

Figure 3+4: Human LGG tissue show staining for antibody SLC6A14 (left) at dilutions 1:100, 1:50 and 1:25. Endothelial staining is expressed clearly at 1:50 (arrowhead). Human LGG tissue show staining for antibody SLC1A5 (right) at dilutions 1:100, 1:200, and 1:300. Staining is expressed most clearly at 1:200 (middle) at endothelium.

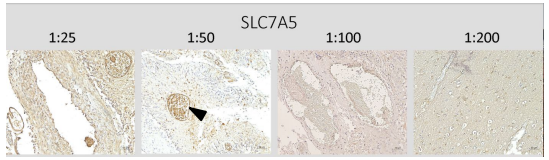

Figure 5: Human LGG tissue show staining for antibody SLC7A5 at dilutions 1:25, 1:50, 1:100 and 1:200. Staining is expressed most clearly at 1:50 at endothelial

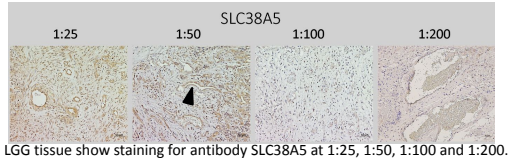

Figure 6: Human LGG tissue show staining for antibody SLC38A5 at 1:25, 1:50, 1:100 and 1:200. Staining is expressed most clearly at 1:25 (left) and 1:50 at endothelium.

Table 1: Optimal Dilutions Summary for antibodies

Target	Cat#	Vendor	Vendor Conditions	Optimized Conditions
GFAP	AB5804	Sigma	1:1000	1:800
OLIG2	AV31464	Sigma	N/A	1:200
SLC1A5	SAB2108565	Sigma	1:250	1:200
SLC6A14	HPA003193	Sigma	1:50	1:50
SLC7A5	SAB2900089	Sigma	1:50	1:50
SLC38A5	HPA047411	Sigma	1:200	1:50

Discussion

We validated and optimized immunostaining conditions on LGG tissues and confirmed most of the vendor provided antibody dilutions. However, since we did not have access to normal brain tissue, we could not assess overexpression of our SLC targets in LGG relative to normal brain tissues.

Conclusion

We optimized the immunostaining conditions for SLC transporters involved in amino acid uptake as well as reference tumor markers (GFAP and Olig2). All SLCs were expressed at the BBTB and could potentially be viable targets for new fluorescent dyes.

Future Direction

The optimized immunostaining conditions will be used to perform multiplexed immunohistochemistry (mIHC) using the co-detection by indexing (CODEX) to simultaneously assess SLC expression and reference tumor and vasculature markers in normal brain- ,LGG- and HGG-tissues to identify targetable SLC proteins for intraoperative fluorescent dye development to improve surgical outcome

References

[a]Stummer W, Pichlmeier U, Meinel T, et al. Fluorescence-guided surgery with 5-aminolevulinic acid for resection of malignant glioma: a randomised controlled multicentre phase III trial. *Lancet Oncol*. 2006.

[b]Capelle L, Fontaine D, Mandonnet E, et al. Spontaneous and therapeutic prognostic factors in adult hemispheric World Health Organization Grade II gliomas: a series of 1097 cases: clinical article. *J Neurosurg*. 2013