Editorial

The role of glutamate transport and SLC7A11 expression in tumor-associate seizures and survival in patients with malignant gliomas

Joseph R. Keen[1], Sharon A. Swanger[2], Stephen F. Traynelis[2], Jeffrey J. Olson[1]

[1]Department of Neurosurgery, [2]Department of Pharmacology, Emory University, Atlanta, GA 30322, USA

Correspondence to: Joseph R. Keen, D.O. Department of Neurosurgery, Emory University School of Medicine, 1365-B Clifton Road, NE, Suite B2200, Atlanta, GA 30322, USA. Email: jkeen3@emory.edu.

Submitted Sep 01, 2016. Accepted for publication Sep 07, 2016.
doi: 10.21037/atm.2016.10.23
View this article at: http://dx.doi.org/10.21037/atm.2016.10.23

Treatment for glioblastoma (GBM) remains largely palliative despite maximal combined approaches (surgery, radiation, and chemotherapy) with a median survival of approximately 15 months due to relentless invasion and recurrence (1). Even with advances in drug delivery, molecular targeting, and immunomodulative therapy, prognosis is grave (2) and the clinical course is characterized by progressive, symptomatic decline secondary to edema, invasion of eloquent areas, and seizures. Up to 80% of patients will experience one seizure with approximately one-third developing recurrent seizures, which are either refractory to anti-epileptic drugs or lead to use of drugs that interfere with chemotherapeutic agents (3). Novel treatments and therapeutic targets are essential to not only extend survival but also to help improve quality of life once the diagnosis has been established.

One exciting new target, as elucidated by the recent report by Robert *et al.* (4), is the glutamate transport system. Multiple studies, including several by authors within this group, have implicated the excitatory neurotransmitter, glutamate, as a culprit involved in the production of a hostile peritumoral environment that can lead to tumor-associated seizures (TAS) (3,5,6) and invasion (7,8). Microdialysate glutamate levels within the tumor margins of patients with GBM have been found to be elevated by 100-fold (9). It is postulated that peritumoral glutamate release is an act of self-preservation by tumor cells, in which the cysteine/glutamate exchanger, System x_c^- (SXC), is upregulated to increase intracellular cysteine for the synthesis of glutathione, an antioxidant thought to support tumor growth and survival (4). While the SXC pathway appears to be the preferred cysteine-glutamate exchange pathway, the catalytic subunit of SXC, SLC7A11, is integral to the release of glutamate. Robert *et al.* systematically confirm that SXC-mediated glutamate release from malignant gliomas contributes to the peritumoral excitoxicity that leads to tissue destruction and TAS. Subsequently, the authors translated this finding into a small, prospective clinical study demonstrating that the FDA-approved SXC-inhibitor, sulfasalazine (SAS), decreased peritumoral glutamate levels in glioma patients over the short-term.

To test their hypothesis that glioma patients with seizures were more likely to have high SLC7A11 expression, and thus SXC activity, they compared biopsied tissue microarrays of GBM tissue and peritumoral brain matched from 41 patients. Two subgroups were unexpectedly discovered, one with low-SLC7A11 expression (46% with equal or lower expression compared to average peritumoral brain) and another with high expression (54% with elevated expression). Western blots from the same tissue showed a similar expression profile (6 of 11 patients with high expression *vs.* 5 of 11 with low expression), and when implanted intracranially into mice treated with the SXC-inhibitor, *(S)*-4-carboxyphenylglycine, glutamate release was selectively blocked in highly-expressing SLC7A11 GBM tissue but not in GBM tissue with low expression.

To establish that SXC-expressing gliomas produce peritumoral excitotoxicity, cortical neurons harvested from rats were cultured, loaded with the fluorescent Ca^{2+} indicator Fura-2, and then exposed to either SXC-expressing or non-expressing gliosphere-conditioned medium. Ratiometric Fura-2 imaging showed that neurons exposed to SXC-expressing glioma medium had more than double the increase in intracellular Ca^{2+} levels compared to those exposed to SXC non-expressing glioma medium. This rise in Ca^{2+} was effectively decreased when treated

with various glutamate receptor antagonists. Neuronal viability was significantly decreased when neurons were co-cultured with SXC-expressing gliomas using a Transwell system. This decrease in viability was prevented by SXC inhibitors and glutamate receptor antagonists. To assess the degree of excitotoxicity *in vivo*, SXC-expressing glioma cells were implanted intracranially into immunodeficient mice, which resulted in approximately 80% reduction in the number of neuronal nuclei (NeuN)-positive peritumoral neurons compared to contralateral brain or mice implanted with non-expressing tumor. The mean survival of mice implanted with SXC-expressing tissue was shortened to 20–22 days compared to 27–32 days for those implanted with SXC-non-expressing tumors. Histologically, SXC-expressing tumors had more poorly defined margins, greater peritumoral edema, and more cells migrating away from the tumor.

Next, to determine if increased glutamate release does, in fact, contribute to TAS, the electrophysiological properties of peritumoral neurons in mice implanted with either SXC-expressing or non-expressing tissue and shams, were assessed using whole-cell patch-clamp recordings. Layer II/III pyramidal neurons peritumoral to SXC-expressing gliomas showed more depolarized resting membrane potentials than shams and generated more action potentials at increasing current injections. When pharmacologically induced, seizures in SXC-expressing tumors not only occurred significantly more rapidly than SXC-non-expressing neurons and shams, but there was also a larger percentage of neurons with epileptiform activity. Continuous EEG up to 23 days showed 70–77% of SXC-expressing implanted mice developed tonic-clonic type seizures lasting 30 to 120 seconds, whereas non-SXC mice rarely had seizures. Not only do the findings within the Robert et al paper cogently support SXC as a major pathway for glutamate release in glioma-implanted mice, but they also establish that SLC7A11 expression is correlated with excitotoxicity and TAS.

To translate these findings to humans, patient survival data was extracted from the REMBRANDT database and stratified into groups based upon degree of SLC7A11 expression, revealing that those with reduced expression (≤66% mRNA compared to non-neoplastic brain tissue) lived 9 months longer than those with high expression (≥150% mRNA). And, finally, in a small randomized, prospective pilot study to assess whether SXC inhibition reduces peritumoral glutamate in humans, peritumoral glutamate levels were measured in nine patients with various grades of glioma (II to IV—3 each with oligodendroglioma, astrocytoma, GBM) using MR glutamate spectroscopy (MRS) after receiving oral SAS. Biopsies for each patient were then matched to the MRS data. For each glioma subtype, higher SCL7A11 expression correlated with larger decreases in glutamate after each SAS dose. However, results were tempered by a delayed "rebound" increase in glutamate release at 24 hours after pharmacological inhibition. The authors acknowledge the imperfection of SAS and advocate for continued development of more specific and bioavailable SXC inhibitors.

Though underpowered to establish clinical efficacy, the pilot study did establish the SXC pathway as an exciting target for attacking glioma self-preservation mechanisms, which could not only translate into extended survival but may also provide a means for reducing seizures and, thus, maximizing quality of life. Because glutamate is a final common mediator of neuronal excitotoxicity, the findings herein are potentially applicable to other similar pathological process such as traumatic brain injury, stroke, epilepsy, and other lower-grade brain tumors. And, although a recent study showed that SAS can potentiate the effects of temozolomide in a human glioma cell line and reduce glioma-related brain edema as measured by T2-weighted MRI in glioma-implanted rats (10), further prospective clinical studies are needed to establish whether SXC inhibitors can ultimately reduce excitotoxicity, tumor spread, and TAS in humans over the long-term.

In summary, this multi-faceted paper presents a wide range of experiments that together support a glutamate-related mechanism by which gliomas persist, grow, and cause seizures. The group also confirmed that glioma patients vary in terms of SXC-expression and that aggressiveness may be linked to glutamate release mechanisms, which may prove to be a promising target for the subset of SXC-expressers who appear to be more susceptible to symptomatic decline and shorter length of survival. And, though the long-term effects and efficacy of SXC inhibitors were not proven, pharmacologically altering glutamate release is a viable therapeutic option worthy of continued study and larger *in vivo* trials. The implication of the glutamatergic system also raises the possibility of additional adjunct therapeutic strategies that may prove useful in reducing seizure burden. Should the SXC inhibitors prove to have long-term efficacy, the possibility of non-invasively monitoring patient responsiveness with MRS would be particularly interesting and ideal as the technology of spectroscopic biomarker imaging continues

to evolve (11).

Acknowledgements

None.

Footnote

Provenance: This is a Guest Editorial commissioned by Section Editor Ning Ding, PhD (Department of Respiratory Medicine, the First Affiliated Hospital of Nanjing Medical University, Nanjing, China).
Conflicts of Interest: J. J. Olson is a consultant for the American Cancer Society. S. F. Traynelis is cofounder of NeurOp Inc., the Primary Investigator on a research grant to Emory University from Janssen, and a paid consultant for NeurOp, Pfizer, and J. R. Keen and S. A. Swanger have no conflicts of interest to declare.

Comment on: Robert SM, Buckingham SC, Campbell SL, *et al.* SLC7A11 expression is associated with seizures and predicts poor survival in patients with malignant glioma. Sci Transl Med 2015;7:289ra86.

References

1. Alifieris C, Trafalis DT. Glioblastoma multiforme: Pathogenesis and treatment. Pharmacol Ther 2015;152:63-82.
2. Wilson TA, Karajannis MA, Harter DH. Glioblastoma multiforme: State of the art and future therapeutics. Surg Neurol Int 2014;5:64.
3. Buckingham SC, Campbell SL, Haas BR, et al. Glutamate release by primary brain tumors induces epileptic activity. Nat Med 2011;17:1269-74.
4. Robert SM, Buckingham SC, Campbell SL, et al. SLC7A11 expression is associated with seizures and predicts poor survival in patients with malignant glioma. Sci Transl Med 2015;7:289ra86.
5. Buckingham SC, Robel S. Glutamate and tumor-associated epilepsy: glial cell dysfunction in the peritumoral environment. Neurochem Int 2013;63:696-701.
6. Yuen TI, Morokoff AP, Bjorksten A, et al. Glutamate is associated with a higher risk of seizures in patients with gliomas. Neurology 2012;79:883-9.
7. Sontheimer H. A role for glutamate in growth and invasion of primary brain tumors. J Neurochem 2008;105:287-95.
8. Panosyan EH, Lasky JL, Lin HJ, et al. Clinical aggressiveness of malignant gliomas is linked to augmented metabolism of amino acids. J Neurooncol 2016;128:57-66.
9. Marcus HJ, Carpenter KL, Price SJ, et al. In vivo assessment of high-grade glioma biochemistry using microdialysis: a study of energy-related molecules, growth factors and cytokines. J Neurooncol 2010;97:11-23.
10. Sehm T, Fan Z, Ghoochani A, et al. Sulfasalazine impacts on ferroptotic cell death and alleviates the tumor microenvironment and glioma-induced brain edema. Oncotarget 2016;7:36021-36033.
11. Cordova JS, Shu HK, Liang Z, et al. Whole-brain spectroscopic MRI biomarkers identify infiltrating margins in glioblastoma patients. Neuro Oncol 2016;18:1180-9.

Cite this article as: Keen JR, Swanger SA, Traynelis SF, Olson JJ. The role of glutamate transport and SLC7A11 expression in tumor-associate seizures and survival in patients with malignant gliomas. Ann Transl Med 2016;4(Suppl 1):S18. doi: 10.21037/atm.2016.10.23

