



A Salt in the Buffer Zone: Potassium Dysregulation Drives Glioma Growth?

Glioma Epileptiform Activity and Progression Are Driven by IGSF3-Mediated Potassium Dysregulation

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Seizures are a frequent pathophysiological feature of malignant glioma. Recent studies implicate peritumoral synaptic dysregulation as a driver of brain hyperactivity and tumor progression; however, the molecular mechanisms that govern these phenomena remain elusive. Using scRNA-seq and intraoperative patient ECoG recordings, we show that tumors from seizure patients are enriched for gene signatures regulating synapse formation. Employing a human-to-mouse *in vivo* functionalization pipeline to screen these genes, we identify IGSF3 as a mediator of glioma progression and dysregulated neural circuitry that manifests as spreading depolarization (SD). Mechanistically, we discover that IGSF3 interacts with Kir4.1 to suppress potassium buffering and found that seizure patients exhibit reduced expression of potassium handlers in proliferating tumor cells. *In vivo* imaging reveals that dysregulated synaptic activity emanates from the tumor-neuron interface, which we confirm in patients. Our studies reveal that tumor progression and seizures are enabled by ion dyshomeostasis and identify SD as a driver of disease.

Commentary

Gliomas are the most common primary brain cancer, with a typically aggressive natural history, high morbidity, and poor survival outcomes. Glioblastoma, the most common glioma variant, has the lowest median survival of under 2 years.¹ A common and debilitating feature of all gliomas is glioma-related epilepsy (GRE), with seizures occurring in as many as 75% of patients with low-grade gliomas,¹ and which severely impact patient quality of life.

Within the emerging field of cancer neuroscience, new findings by Curry and colleagues² add to a growing consensus of a reciprocity between dysregulated peritumoral neural activity and tumor growth.³⁻⁵ Their data demonstrate a positive feedback loop that generates and reinforces the associated pathologies of GRE, in which gliomas alter the local microenvironment to skew peritumoral neural activity toward hyperexcitability, and subsequent neural hyperactivity further promotes tumor growth. The work reports a novel pathway by which gliomas may promote hyperexcitability through the upregulated expression of the membrane protein immunoglobulin superfamily 3 (IGSF3), modulating the capacity of its binding partner, the glial-specific inwardly rectifying potassium channel Kir4.1, to buffer potassium ions in peritumoral tissue.

The authors firstly identified differentially expressed gene sets from glioma patients with and without epilepsy. Cross-referencing identified candidate genes with existing publicly available human glioma expression datasets and highlighted IGSF3 as the sole candidate gene whose enriched expression was associated with worsened survival across glioma patients. Accordingly, IGSF3 expression was significantly increased in glioma tissue samples, with little evidence of significant IGSF3 expression in nontumor tissue.

To causally probe the effects of dysregulated IGSF3 expression, the authors developed a transgenic glioma-bearing mouse line with depleted or elevated IGSF3 (IGSF3-loss of function (LOF) or -gain of function (GOF), respectively). Single cell RNA sequencing of glioma cells demonstrated broad changes to the genetic landscape that accompany perturbations in IGSF3 expression, with gene ontology highlighting genes involved in cell proliferation, synaptic transmission, and glutamatergic and postsynaptic potentials.

These genetic changes corresponded with changes in the peritumoral synaptic milieu, with increased excitatory synapses observed in the local region of IGSF3-GOF tumors and reduced inhibitory synapses seen around IGSF3-LOF tumors. Importantly, the authors demonstrated that the alterations in synaptic density appear to be caused by changes in





astrocytic IGSF3 expression, with neurons forming significantly more excitatory synapses and exhibiting dendritic beading when cocultured with IGSF3-GOF astrocytes.

The authors subsequently investigated how IGSF3 overexpression heightens synaptogenesis. Using immunoprecipitation-mass spectrometry, they identified 17 binding partners of IGSF3, including Kir4.1, encoded by human gene *KCNJ10*. To assess whether IGSF3 mediates changes in Kir4.1 activity that manifest in a difference in the ability of peritumoral tissue to buffer potassium, the authors measured extracellular potassium concentration in tumor slices of IGSF3-WT, -LOF, and -GOF mice, showing a significant rise in extracellular potassium concentration only in IGSF3-GOF. Accordingly, genetic analyses identified significant downregulation of gene expression of a number of potassium handlers in proliferating tumor cells in both human and mouse glioma, including that of *KCNJ10/Kcnj10*.

Importantly, the authors demonstrated that while IGSF3-GOF animals showed reduced median survival relative to IGSF3-WT and IGSF3-LOF, Kir4.1 overexpression on the IGSF3-GOF line rescued median survival to baseline. This key finding causally implicates glial potassium buffering in IGSF3-mediated poor outcomes, identifying a novel pathway by which gliomas can bias nearby tissue toward hyperexcitable states, induce synaptic remodeling, and ultimately promote tumor growth. Conversely, overexpressing Kir4.1 in IGSF3-WT mice had no impact on survival, suggesting that this effect is specific for IGSF3-GOF glioblastomas. This pathway sits in context of other potential means by which gliomas may be ictogenic—for instance, via glutamate release,³ or GABAergic disinhibition following neuronal death and depleted KCC2 in persisting neurons.⁴

The authors also sought to identify electrophysiological features of IGSF3-mediated potassium dysregulation. Intracranial EEG recorded from tumor-bearing mice showed waves of spreading depolarization (SD) exclusively in IGSF3-GOF animals. Spreading depolarizations are commonly observed in epileptic brain tissue and have been proposed to be a self-regulating means of terminating seizure activity.⁶ Indeed, from human intraoperative electrocorticography, the authors reported SD-like events and seizure-like activity in electrodes closest to the leading edge of the tumor that resembled the electrophysiological signatures recorded from mice. However, SDs are also seen following ischaemic or traumatic damage to brain tissue.⁷ No matter the pathogenesis of SDs, they appear to be triggered by the increase of extracellular potassium to above a critical concentration,⁷ which could explain their emergence in neural tissue near the glioma, where potassium homeostasis is most strongly dysregulated.


Curiously, while a reduction in peritumoral interictal spiking was observed in IGSF3-LOF animals, indicating a reduction in hyperexcitability of tissue associated with IGSF3 depletion, no corresponding increase in interictal spikes was observed in IGSF3-GOF mice. It may be that potassium buffering is still impaired in IGSF3-wild type animals, leading to a


baseline hyperexcitability associated with regular tumor growth that IGSF3-GOF mutations did not exacerbate further—although upregulating Kir4.1 expression in IGSF3-WT animals did not show any improvement in median survival. A more detailed exploration of *in vivo* electrophysiology datasets, from both humans and mice, is required to address this apparent paradox.

In humans, GRE is associated with improved median survival in both high- and low-grade glioma patients.^{8,9} However, as increased neural activity is argued to drive tumor growth, it may be expected that individuals with evidence of epilepsy would have reduced survival. Indeed, in the murine model, IGSF3-GOF animals displayed both evidence of neural hyperactivity and worse outcomes. While improved outcomes in GRE patients are likely in part related to the observation that GRE is more commonly observed in lower grade gliomas than in high grade glioblastoma,⁸ with glioblastoma patients expiring before growth of the cancer into susceptible areas of cortex, this does not fully explain why the relationship between GRE and survival is opposite in this murine model to clinical data, and ultimately highlights the multifactorial genetic, metabolic, and systems neuroscientific contributors to the natural history of glioma.


These data are fascinating in their own right; however, it is important to note the translational potential that emerges from this work. Terminating the reciprocal positive feedback loop between peritumoral network hyperactivity and tumor growth, by targeting either the epilepsy or the tumor itself, may prove an effective intervention to improve the morbidity and mortality of glioma. Indeed, as has been previously suggested,¹⁰ control of GRE by use of appropriate anti-seizure medications (ASMs) may help to delay tumor progression, and several clinical trials are ongoing to assess ASM use in glioma (NCT03048084, NCT04497142, NCT02815410). For instance, some studies show valproic acid administered as an adjuvant therapy may improve overall survival, although this is debated and may be via direct anticancer properties of the drug rather than its anti-seizure effect.¹¹ Further work is needed to elucidate the role of anti-seizure therapy in glioma patients. Conversely, surgical management of the tumor frequently leads to seizure freedom.¹²


Targeting potassium handling in peritumoral tissue constitutes a further mechanism by which this reciprocity may also be disrupted. For instance, increasing potassium buffering by Kir4.1, either pharmacologically or through gene therapy, could restore physiological potassium concentrations in peritumoral tissue and prevent neural hyperexcitability, thereby delaying tumor growth. Currently this intervention would be restricted to gliomas with increased IGSF3 expression. Future research may elucidate the role of elevated potassium in general in epileptogenesis in glioma, with sight on the development of novel therapies that hold the potential to improve both the survival and the quality of life in these patient cohorts.

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