Mice. In vivo efficacy of GSKJ4 is aided by its demonstrated ability to cross the blood-brain barrier and to permeate the brainstem.

Rapid advances in understanding DIPG biology and identifying new potential therapies have resulted from discovery of the DIPG H3.3 K27M mutation. New insights into the molecular mechanisms underlying DIPG tumorigenesis have revealed promising molecular therapeutic agents that alter histone methylation biology (such as GDKJ4 and MI-2) and form a foundation for clinical trials to improve DIPG outcomes in the near future.

Ray R. Zhang, BS
Kelli B. Pointer, BS
John S. Kuo, MD, PhD
University of Wisconsin
Madison, Wisconsin

REFERENCES

**Buzz Kill: Neuronal Activity Promotes Glioma Proliferation**

The impact of the tumor microenvironment on gliomagenesis and subsequent tumor growth is incompletely understood. Understanding the effect of surrounding cell subtypes on tumor and tumor-precursor cells is critical to understanding the mechanisms of glioma growth and is important to the search for
future therapeutic targets. Prior work has characterized the roles of the vascular niche and glial subtypes on glioma pathogenesis; however, less is known about neuronal-glioma interactions. On the basis of prior discoveries demonstrating the mitogenic influence of neuronal activity on normal neural precursor cells (NPCs) and oligodendroglial precursor cells (OPCs), along with a body of evidence implicating OPCs and NPCs as the putative cell of origin of high-grade gliomas (HGG), Venkatesh et al. explored the effect of neuronal activity on glioma proliferation.

In this study, recently published in Cell, the Monje laboratory at Stanford University induced neuronal activity in defined circuits within the premotor cortex with optogenetic control in a murine orthotopic xenograft model using pediatric HGG cells. As with normal NPCs and OPCs, the induction of neuronal activity induced proliferation of the implanted HGG cells, as measured by the Ki-67 index. Furthermore, the authors were able to demonstrate that persistent elevated neuronal activity, with repeated stimulation over 7 days, resulted in significantly increased tumor burden compared to control (P < 0.01; Figure). Surprisingly, the authors were able to demonstrate that a single stimulation was able to induce proliferation, pointing to a very robust effect. This finding could provide a possible mechanism of the rapid growth of these tumors often seen in patients after a period of quiescence.

Furthermore, using optogenetically stimulated murine cortical slices in culture, the authors demonstrated that the harvested conditioned medium was able to induce proliferation across multiple human-derived HGG cell lines. From this conditioned medium, using mass spectrometry, they identified the protein neuroligin-3 (Nlgn-3) as the likely mitogen that mitigated proliferation, and this protein existed in high concentrations in the conditioned medium. Indeed, this protein demonstrated a sufficient dose-dependent effect on HGG proliferation and necessity, with the depletion of the mitogenic effect of Nlgn-3 with selective binding of neurexin-1B. Lastly, using RNA sequencing and Western blot analysis, the authors found...
that neuron-activated release of NLgn-3 induced tumor proliferation through the phosphoinositide 3-kinase-mammalian target of rapamycin (mTOR) pathway. Interestingly, the cultured HGG lines demonstrated increased expression of NLGN-3 when exposed to conditioned medium, implicating a feed-forward mechanism of expression. This further explains the robust proliferation seen in the experimental setting and allows greater rationale of targeting this pathway therapeutically.

The clinical implications of this discovery were made clear with a query of The Cancer Genome Atlas. Although somatic mutations of NLGN3 were infrequent in pediatric and adult brain tumors, mRNA expression was found to be inversely correlated with overall patient survival in 429 cases, with a hazard ratio for death with high vs low expression of 1.31 (95% confidence interval, 1.05-1.63). The specificity of the aforementioned results was strengthened by examination of the role of Nlg-2, which did not demonstrate increased proliferation or survival differences.

The implication of these findings, that the inherent physiological function of the host organ could be taken advantage of by a primary tumor, has far-reaching impact. Neuronal activity can induce tumor proliferation via the mTOR pathway, whereas the mTOR pathway has been implicated in the pathogenesis of a number of human diseases. Thus, brain activity may promote tumor growth, which in turn may induce pathological brain activity in a vicious cycle. However, this discovery also may provide a targetable checkpoint in glioma proliferation and pathogenesis. It adds a missing element to the glioma microenvironmental landscape and allows a new avenue for exploring diagnostic and therapeutic possibilities. Although a sobering finding regarding this devastating diagnosis, the neuronal contribution to the pathogenesis of HGG is critical to characterize and dissect so that it can be translated to the clinical treatment of glioma patients.

**Sensitization of Glioblastoma Cells to Irradiation by Modulating the Glucose Metabolism**

The mainstay of glioblastoma multiforme (GBM) treatment remains maximal safe resection followed by adjuvant chemotherapy and radiation. However, one of the tremendous challenges in the treatment of GBM is tumor resistance to radiation treatment. It has previously been demonstrated that GBM cells with inadequate blood supply and intermittent hypoxia-promoted radiation resistance in glioblastoma multiforme. 1. Hypoxia-inducible factor-1α (HIF1α) is a key mediator of hypoxia-induced gene expression. 2. DCA could induce increased glycolysis and upregulation of hypoxia-inducible factor-1α and pyruvate dehydrogenase kinase 1 (PDH1), with these changes triggering higher glycolytic activity and radioreistance. 3. DCA has been shown to modify tumor metabolism and to cause cells to return to oxidative phosphorylation. Through reversal of this metabolic characteristic of GBM, the tumor could be specifically targeted for treatment or sensitized to radiation in a way that would not occur in the surrounding tissues already dependent on oxidative phosphorylation.

To test this hypothesis of metabolic targeting, Shen et al studied the ability of DCA to sensitize GBMs to radiation.

Specifically, these investigators set out to verify the hypothesis that radiation therapy (RT) promotes glycolytic metabolism and to test whether reversal of the glycolytic phenotype with DCA resensitizes GBMs to RT. In vitro experiments were first performed whereby U87 GBM cells were irradiated and evaluated for changes in gene expression of glycolytic genes, which proved to be upregulated. In particular, isozymes of PDH were upregulated from 1.26- to 3.38-fold, with Western blotting demonstrating a concomitant hypoxia-inducible factor-1α upregulation. They also found that cell lines treated with DCA and RT demonstrated a significant decrease in post-radiation glycolytic rate (P < .01). DCA itself induced a component of proliferation arrest in GBM cells and reduced mitochondrial reserve capacity, both features indicative of an ability to sensitize to RT. In combination, DCA and RT induced an increase in reactive oxygen species, a crucial mechanism by which RT causes cell death.

Finally, athymic nude mice were intracranially injected with 5 × 10^6 U87 GBM cells into the right basal ganglia. Mice were then randomly divided into 4 treatment groups: no treatment, radiation alone, DCA alone, and both radiation and DCA. Radiation was delivered to the whole brain with the use of a self-contained x-ray system on day 13 after tumor implantation at a dose of 20 Gy given over 10 fractions. Tumor cell proliferation was estimated by hematoxylin and eosin staining and by immunostaining for Ki-67. The combination of RT and DCA yielded statistically significant increases in median cell survival (P < .001) and reductions in tumor proliferation compared with no treatment or either RT or DCA alone.

DCA, a small molecule able to penetrate the blood-brain barrier, was able to modulate glucose metabolism, sensitize malignant cells to RT, and improve tumor treatment in vitro and in an in vivo mouse model. It is already approved as an orphan drug for the treatment of several congenital disorders of mitochondrial metabolism. Much more investigation is necessary to evaluate such metabolic targeting, but this work serves as a proof of principle that an agent such as DCA could enhance standard GBM treatment without harming surrounding tissues already primarily using oxidative phosphorylation.

**REFERENCES**


**A Novel Device for Direct Determination of Tumor Chemotherapeutic Sensitivity**

Cancer treatment is evolving from a "one-size-fits-all" paradigm to regimens tailored to each patient’s specific tumor. This treatment customization is often achieved...