PRIZE ESSAY

CATEGORY WINNER: MOLECULAR MEDICINE

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Humsa Venkatesh received her undergraduate degree from the University of California, Berkeley, and her Ph.D. from Stanford University. She is currently completing her postdoctoral fellowship at Stanford University. Her research combines principles of neuroscience and cancer biology to understand the electrical components of cancer pathophysiology and harness these malignant dependencies for therapeutic intervention.

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MOLECULAR MEDICINE

The neural regulation of cancer

Cancers hijack mechanisms of neural plasticity to promote malignant disease progression

By **Humsa S. Venkatesh**

Cancer has been called the "emperor of all maladies" (*1*). An estimated 40% of us will be diagnosed with it at some point in our lives (*2*). We have all witnessed the irreverent brutality of this disease that seems to know no bounds. Like weeds in a garden, cancers emerge suddenly and proliferate aggressively by co-opting vital systems necessary for survival. Although current treatment approaches have targeted the vascular and immune systems as well as cell-intrinsic growth mechanisms, the nervous system's role in cancer growth has been largely underrecognized. My research aims to combine principles of neuroscience and cancer biology to harness microenvironmental dependencies of tumors and disable hijacked mechanisms of neural plasticity.

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Cancers, at their core, survive by recapitulating mechanisms of normal development. Activity of the nervous system has been well established as a powerful modulator of neurodevelopment and plasticity. Electrical activity shapes brain organogenesis as well as the behavior of persistent populations of neural precursor cells (NPCs) in pediatric and adult brains (*3*). In the context of cancer, we hypothesized that this neurodevelopmental principle may similarly inform tumor progression.

Gliomas provide a compelling case for studying these microenvironmental cues because these cancers arise in striking spatiotemporal patterns. Given the robust influence of active neurons on NPC populations, many of which are candidate cells of origin for gliomas, we sought to uncover a relationship between neuronal activity and glioma growth.

UNDERSTANDING THE ROLE OF THE NERVOUS SYSTEM

In the Monje laboratory at Stanford University, we created a first-of-its-kind mouse model that enables both transplantation of patient-derived glioma cells and control of neuronal activity through optogenetic stimulation. This platform facilitated manipulation of the neuronal component of the tumor microenvironment and isolation of its direct effect on tumor biology. Using this model, we found that neuronal activity robustly promotes circuit-specific glioma growth and progression (*4*). These findings demonstrated, for the first time, the critical role of neurons in the brain tumor microenvironment.

To study the molecular mechanism mediating this effect, we developed an acute optogenetic slice culture model in combination with biochemical and proteomic assays to isolate a small list of specific activity-dependent secreted proteins. Soluble neuroligin-3 (NLGN3; a cleaved fragment of the full-length version normally involved in synaptic adhesion) was identified as a differentially secreted protein. It had the most robust effect on glioma proliferation through phosphatidylinositol 3-kinase (PI3K) pathway activation and, interestingly, induced an up-regulation of synapse-related genes in gliomas (*4*, *5*). Thus, soluble extracellular NLGN3, previously unknown to be secreted or involved in growth, was identified as a potent activity-dependent mitogen.

IDENTIFYING A THERAPEUTIC TARGET

Clinical analysis of hundreds of adult glioma patient samples revealed that higher *NLGN3* expression levels predicted a substantially worse prognosis, indicating an important role for NLGN3 in glioma pathogenesis. To verify this necessity, we examined multiple patient-derived pediatric and adult gliomas in mice genetically deficient of NLGN3. Strikingly, gliomas failed to grow in the absence of NLGN3, emphasizing its vital requirement in the tumor neuromicroenvironment and its promise as a therapeutic target (*5*). Our focus subsequently shifted to studying this unknown mechanism of NLGN3 secretion.

Using a series of genetic mouse models and protease inhibitor analyses, we identified ADAM10 (A disintegrin and metalloproteinase domain-containing protein 10) as the enzyme responsible for neuronal activity-dependent cleavage and secretion of NLGN3 from the membrane of neurons and oligodendrocyte precursor cells (OPCs) as well as from the glioma cells themselves. The enzyme, we surmised, was thus an attractive therapeutic target. When tumor-bearing

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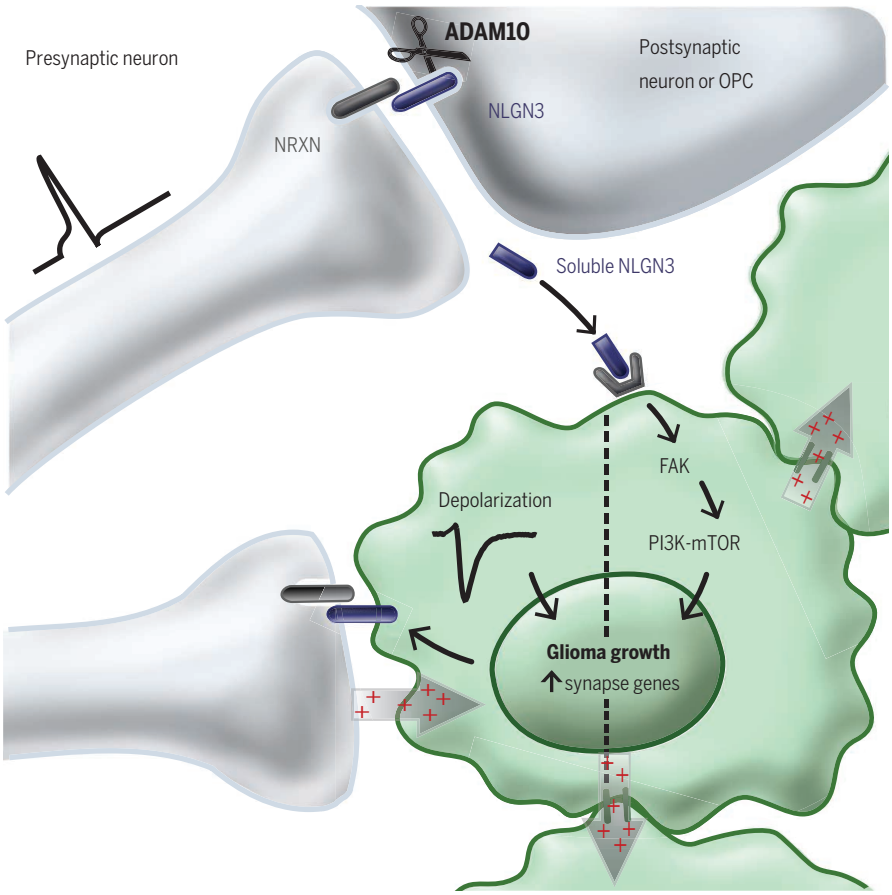

Neuronal activity drives cancer growth and progression through direct signaling mechanisms and the functional integration of glioma into neural circuitry. An important mechanism that mediates the neural regulation of brain cancer is activity-dependent cleavage and secretion of the synaptic adhesion molecule neuroligin-3 (NLGN3), which promotes glioma proliferation through the PI3K–mammalian target of rapamycin (mTOR) pathway and induces up-regulation of several synapse-related genes. NLGN3 is cleaved through the ADAM10 sheddase. ADAM10 inhibitors prevent the release of NLGN3 into the tumor microenvironment and robustly block cancer growth. Additional neuron-glioma interactions include electrical and synaptic integration of glioma into neural circuitry through direct electrochemical synaptic transmission. Synaptic activity depolarizes electrically coupled glioma cell networks, and this depolarization in turn induces proliferation. These findings suggest that neuron-glioma circuit dynamics are integral to tumor progression. [Figure adapted from (*5*)]

mice were treated with an ADAM10 inhibitor, glioma growth stagnated as it had in the genetically deficient NLGN3 models (*5*). These results formed the rationale for upcoming clinical trials for the treatment of both pediatric and adult high-grade glioma patients with an ADAM10 inhibitor.

ADDITIONAL NEURON-GLIOMA SYNAPTIC COMMUNICATION

The stark reduction in glioma growth after NLGN3 removal suggests that the protein plays a role more fundamental to glioma progression, well beyond its role as a mitogen. Given its function in the postsynaptic assembly in normal cells and its involvement in the up-regulation of synaptic genes in gliomas, we hypothesized that NLGN3 may be facilitating direct glioma engagement with neurons.

Neurons form functional synapses with OPCs, and many gliomas molecularly and phenotypically resemble these OPCs. Do malignant glioma cells, similar to their glial counterparts, form functional synapses with neurons to further hijack mechanisms of normal neural plasticity, we wondered?

To answer this question, we used single-cell transcriptomics and immuno-electron microscopy techniques to identify synaptic interactions between neurons and malignant cells. Whole-cell patch clamp recordings of xenografted glioma cells with simultaneous stimulation of afferent neurons subsequently revealed AMPA receptor–dependent postsynaptic currents in the glioma cells (*6*). Further, using real-time two-photon calcium imaging, we uncovered rapid depolarization and ion flow through gap-junction–connected tumor cell networks in response to neuronal stimulation. These data suggest that gliomas functionally integrate into electrically active neural circuits through neuron-to-glioma synapses and that the effects of neuron-glioma signaling are amplified throughout the tumor network by means of gap junction–mediated connections (*6*).

Membrane depolarization of normal neural precursor cells can regulate proliferation, differentiation, and survival. Thus, we hypothesized that depolarization similarly promotes glioma growth. Using in vivo optogenetic techniques to depolarize xenografted glioma cells, we found that glioma membrane depolarization robustly promoted proliferation, whereas pharmacologically or genetically blocking electrochemical signaling inhibited glioma xenograft growth and extended mouse survival (*6*).

Together, these results demonstrate, for the first time, that electrical circuit integration of glioma promotes its progression (see the figure).

A NOVEL CANCER TREATMENT STRATEGY

The study of the neural regulation of cancers is a burgeoning field that highlights the nervous system's central role in facilitating tumor progression. Unveiling other critical mechanistic details of the neurobiology of cancers will help us further understand how cancers exploit these powerful microenvironmental interactions. My work identifies activity-dependent mitogen secretion, synaptic neurotransmission, and gap junction–mediated electrical coupling as previously unknown mechanisms controlling glioma progression.

This demonstration of the critical role of neural elements in the microenvironment has been extended to prostate (*7*), pancreatic (*8*), skin (*9*), and gastric cancers (*10*, *11*), illustrating its expansive applicability. Taken together, we, and others, demonstrate that targeting neuron-cancer circuit dynamics represents a promising therapeutic intervention.

REFERENCES AND NOTES

1. S. Mukherjee, *The Emperor of All Maladies: A Biography of Cancer* (Scribner, 2010).
2. National Cancer Institute, data from 2013–2015; https://www.cancer.gov/about-cancer/understanding/statistics.
3. H. S. Venkatesh, M. Monje, *Trends in Cancer* **3**, 89 (2017).
4. H. S. Venkatesh *et al.*, *Cell* **161**, 803 (2015).
5. H. S. Venkatesh *et al.*, *Nature* **549**, 533 (2017).
6. H. S. Venkatesh *et al.*, *Nature* **573**, 539 (2019).
7. C. Magnon *et al.*, *Science* **341**, 1236361 (2013).
8. B. W. Renz *et al.*, *Cancer Cell* **33**, 75 (2018).
9. S. C. Peterson *et al.*, *Cell Stem Cell* **16**, 400 (2015).
10. C.-M. Zhao *et al.*, *Sci. Transl. Med.* **6**, 250ra115 (2014).
11. Y. Hayakawa *et al.*, *Cancer Cell* **31**, 21 (2017).

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