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Synaptic communication in brain cancer

Michelle Monje, MD PhD

Department of Neurology and Neurological Sciences, Stanford University

Abstract

Increasing evidence indicates that the nervous system plays a critical role in cancer progression. This is particularly true in cancers that occur within the central nervous system. Communication between neurons and cancer cells is a fundamental component of brain cancer pathophysiology, both for primary gliomas and for brain metastases. Neuronal activity drives growth of glial malignancies through secreted growth factors and through direct electrochemical synaptic communication. Reciprocally, brain cancers influence neuronal function, increasing neuronal activity and modulating the function of the circuits into which the cancer cells structurally and electrically integrate. Advancing understanding of neuron-cancer interactions will elucidate new therapeutic strategies for these presently lethal brain cancers.

Cancers frequently subvert mechanisms of development and plasticity. The nervous system is a master regulator of organogenesis, homeostasis, regeneration and plasticity in a broad range of tissues. Concordantly, a pivotal role is emerging for the nervous system in the initiation and progression of numerous cancers (1). This principle is especially true in the brain, where activity shapes neurodevelopment and parallel mechanisms of neuronal activity-dependent brain cancer growth are coming to light. Here we will consider recent advances in understanding the neuroscience of brain cancers, open questions relevant to the activity-regulated development of both normal and malignant circuitry, and emerging therapeutic targets.

Primary brain cancers are thought to originate from neural precursor cell populations. Depending on the cancer, the cellular origin may be a multipotent neural stem cell, lineage-restricted neuronal precursor cell or lineage-restricted glial precursor cell. The various molecular and clinical subtypes of high-grade gliomas are thought to arise from precursor cells along the differentiation trajectory from less differentiated neural stem cells to more lineage-committed oligodendroglial precursor cells (pre-OPCs or OPCs). Neuronal activity drives proliferation of OPCs and pre-OPCs throughout life (2). In the healthy brain, this activity-regulated OPC proliferation results in new oligodendrocyte production and plasticity of myelination that supports adaptive neurological functions such as learning and memory (2–5). Activity-regulated secretion of brain-derived neurotrophic factor (BDNF) is a required mechanistic component of myelin plasticity in cortical projection neurons (4). Neuronal BDNF signaling to the TrkB receptor on OPCs stimulates the expected pro-

Correspondence should be addressed to: Michelle Monje MD PhD, 265 Campus Drive, G3035, Stanford, CA 94305, Phone: 650 721-5750, mmonje@stanford.edu.

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proliferative MAPK-ERK signaling cascade (4), and also increases the response of OPCs to glutamate (6). Bona fide synapses exist between neurons and OPCs (7, 8), and while the role of such axon-glial synapses remain incompletely understood, it is hypothesized that synaptic communication between neurons and OPCs may contribute to activity-regulated plasticity of myelin.

Parallel to the effect on healthy glial precursor cells, neuronal activity similarly drives proliferation of malignant glioma cells. Studies leveraging modern neuroscience tools to control neuronal action potentials in patient-derived orthotopic xenograft models of high-grade glioma have demonstrated a circuit-specific effect of cortical projection neuronal activity on glioma proliferation and growth (9). Cortical projection neurons are chiefly glutamatergic, i.e. communicate through use of the primary excitatory neurotransmitter in the central nervous system, glutamate. High-grade gliomas represent a group of clinically and molecularly diverse cancers, but the proliferative response to glutamatergic neuronal activity is evident in each major molecular subtype of pediatric and adult high-grade gliomas, from glioblastoma to diffuse intrinsic pontine glioma (9). While the effect on glioma growth is clear, the influence of glutamatergic neuronal activity on glioma initiation is not yet known.

Activity-regulated secreted factors contribute to the growth-promoting influence of cortical projection neurons, and two key paracrine signaling mechanisms between neurons and malignant glioma cells have been identified: BDNF and neuroligin-3 (NLGN3). Mechanistically parallel to its role in healthy neuron-glial interactions, BDNF promotes activity-dependent glioma proliferation (9). Neuroligin-3 has emerged as an unexpectedly robust activity-regulated paracrine factor promoting glioma growth. Neuroligin-3 is a post-synaptic adhesion molecule composed of a short C-terminal transmembrane domain and a large N-terminal ectodomain; neuronal activity-regulated function of the protease ADAM10 cleaves neuroligin-3 at the membrane and releases the N-terminal ectodomain into the glioma microenvironment (Figure 1) (10). Neuroligin-3 is shed from post-synaptic cells, both post-synaptic neurons and post-synaptic OPCs (10). Binding of neuroligin-3 to glioma cells, through an as-of-yet unidentified receptor, stimulates upstream focal adhesion kinase (FAK) activity and downstream RAS, SRC and PI3K-mTOR signaling pathways, accounting for its robust mitogenic function (10). Neuroligin-3 binding also triggers feed-forward upregulation of neuroligin-3 expression in malignant glioma cells; glioma expression of neuroligin-3 appears to be strictly regulated by this feed-forward mechanism, with low-to-none expression by glioma cells in the absence of neuroligin-3 exposure (9). Glioma expression of neuroligin-3 is thus an indicator of neuroligin-3 exposure - and indirectly of neuronal activity levels - in the tumor microenvironment. Indicating an important role in glioma progression, tumor *NLGN3* expression levels inversely correlate with patient survival in adults with high-grade glioma (9).

Even more unexpected than a mitogenic function of a synaptic adhesion molecule is an apparent requirement for neuroligin-3 to enable glioma progression. To test the relative contribution of neuroligin-3 to glioma progression, patient-derived gliomas were orthotopically xenografted into the brains of wildtype or *Nlgn3* knockout mice. Surprisingly, patient-derived glioma xenografts failed to grow in the absence of neuroligin-3 in the brain

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microenvironment. This unexpected dependency on microenvironmental neuroligin-3 was found in patient-derived xenograft models of diffuse intrinsic pontine glioma (DIPG), pediatric glioblastoma and IDH WT adult glioblastoma, but did not extend to a patient-derived model of breast cancer brain metastasis. Together, these findings suggest that neuroligin-3 dependency is conserved across high-grade glioma subtypes, but does not extend to all brain cancers (10). A clinical trial of ADAM10 inhibition to target neuron-glioma interactions has recently opened for children with malignant gliomas (NCT04295759).

Neuroligin-3 is only one of several cell-intrinsic and microenvironmental mechanisms promoting glioma cell proliferation, so what could explain this stark dependency? In an effort to begin to understand what role neuroligin-3 may play that is fundamental to glioma progression, transcriptomic changes elicited by neuroligin-3 exposure were examined. Together with the feed-forward effect of neuroligin-3 on its own expression in glioma, a number of other synapse-related genes were found to be upregulated in response to neuroligin-3 signaling, including genes encoding glutamate receptor subunits and other synapse-related structural proteins (10). Examination of the single cell transcriptomes from the major classes of high-grade glioma, H3K27M-mutant diffuse midline gliomas, IDH-WT and IDH-mutant adult glioblastoma, revealed robust expression of synapse-related genes in the subpopulation of malignant cells most resembling OPCs (Figure 1)(11). These transcriptomic observations sparked the hypotheses that, like the axon-glial synapses between neurons and healthy OPCs, axon-glioma synapses may form between neurons and malignant glioma cells and that neuroligin-3 may promote such malignant synaptogenesis.

Electron microscopy of primary tissue samples and patient-derived xenograft models unambiguously demonstrate synaptic structures between neurons and glioma cells (11, 12). Co-culture of patient-derived glioma cells with neurons from WT or *Nlgn3* knockout mice supports the hypothesis that neuroligin-3 promotes structural synapse formation (11). Whole cell patch clamp electrophysiology in patient-derived orthotopic xenograft models of high-grade glioma demonstrate excitatory post-synaptic currents in a subpopulation of glioma cells evoked by neuronal action potentials. The neuron-to-glioma synapses described to date are mediated by calcium-permeable AMPA receptors (a subtype of glutamate receptor)(11, 12). Whether additional types of synapses mediated by other neurotransmitter receptors exist remains to be determined. A second electrophysiological response to neuronal activity was observed within a distinct subpopulation of malignant cells for each tumor model examined. This second type of neuronal activity-evoked glioma current was longer in duration than the classically synaptic currents discussed above, and the duration and amplitude of these prolonged currents scaled with the field potential, meaning that more neurons firing in the microenvironment results in longer, larger currents. These prolonged currents, reminiscent of currents observed in healthy astrocytes, are evoked by increased extracellular potassium concentration caused by neuronal action potentials (11). Glioma cells exhibiting these evoked prolonged currents also exhibit coupling to one another through gap junctions, which serves to amplify the potassium-evoked current (11). The glioma cell network thus integrates structurally and electrically into neural circuitry. Disrupting neuron-to-glioma synaptic communication or gap junctional coupling impairs glioma growth in preclinical models (11), suggesting possible therapeutic targets.

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Glioma is thus an electrically active tissue in which neuron-to-glioma synapses and glioma-to-glioma gap junctional coupling promote synchronous membrane depolarizations and consequent calcium transients throughout networks of cancer cells (11, 12). That glioma cells exhibit at least two distinct mechanisms of membrane depolarization suggests an important role for tumor growth reminiscent of the functions that membrane voltage changes play in neurodevelopment. In the developing brain, neural precursor cell membrane depolarization influences cell proliferation, differentiation or survival through incompletely understood voltage-regulated mechanisms. Depolarization-induced calcium transients occur in neural stem cell populations in the prenatal brain during corticogenesis and cortical neuron cell fate specification is regulated by membrane potential. The importance of precisely regulated electrical signaling during brain development is underscored by observations that mutations in voltage-gated ion channel or neurotransmitter receptor genes whose expression is enriched during prenatal brain development result in striking cortical maldevelopment (13). To test the functional consequences of membrane depolarization in glioma, patient-derived glioma cells were engineered to express the blue light-sensitive cation channel ChR2 and cortical xenografts were optogenetically depolarized. As predicted, glioma membrane depolarization robustly promotes malignant cell proliferation (11). The intracellular events triggered by changes to membrane potential that regulate glioma proliferation remain to be determined and represent an area of intense investigation.

As glutamatergic neuronal activity promotes glioma progression, both through secreted growth factors and through electrical signaling, it is not surprising that gliomas actively promote neuronal excitability. Glioma cells increase neuronal excitability (11, 14–18) through numerous mechanisms, including non-synaptic secretion of glutamate via glutamate-cysteine exchangers (14), glioma-associated loss of inhibitory interneurons (18), glioma-associated alterations in the response of glutamatergic neurons to the inhibitory neurotransmitter GABA (18), and glioma secretion of synaptogenic factors (16) such as glypican-3 (17). Increased neuronal synapses and consequent increased neuronal excitability appears to be driven by a distinct subpopulation of glioma cells (16). Fascinatingly, different variants of mutations in the same oncogene (*PI3CA*) differentially regulate the release of synaptogenic proteins, suggesting selective regulation of this malignant cellular subpopulation (17). The increase in glutamatergic neuronal activity that results from glioma-induced neuronal hyperexcitability promotes glioma growth and contributes to glioma-associated seizures. Whether glioma-induced synaptogenesis extends beyond neuronal excitability to cause meaningful neural circuit remodeling remains to be determined. Furthermore, how such putative remodeling may contribute to neurological dysfunction in brain tumor patients also requires exploration.

Given the heterogenous effect of neurotransmitter-mediated and electrical signaling in neural precursor cell populations, either promoting differentiation or proliferation depending on cell identity and state, the effect of neuronal activity cannot be predicted and must be empirically determined for each brain cancer and each neuronal type. The majority of work to date has focused on the effects of glutamatergic neurons in gliomas. The effects of diverse neuronal subtypes on glioma progression are emerging. In adult glioblastoma, dopaminergic signaling appears to promote tumor progression (19), while GABA-ergic signaling may inhibit adult glioblastoma growth (20). The effects of other types of neurons (e.g

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serotonergic, cholinergic etc), and the potentially heterogenous responses of distinct glioma subtypes to diverse neuronal signals requires future study. Furthermore, the effect of neuronal activity on other primary brain cancers, such as those arising from neuronal-lineage precursor cells like medulloblastoma, remains to be demonstrated.

A role for neuronal regulation in secondary brain cancers is beginning to come to light. Breast cancer cells metastatic to brain increase expression of the NMDA subtype of glutamate receptor and extend a malignant cell process around glutamatergic synapses, much like the perisynaptic process extended by normal astrocytes. In this perisynaptic position, glutamate released at the neuronal synapse stimulates NMDA receptors on the perisynaptic breast cancer cell, and this drives progression of the breast cancer brain metastasis (21). Future work will elucidate whether other metastatic cancer types engage in synaptic or perisynaptic structures in the brain.

Much remains to be learned, and collaboration between cancer biologists and neuroscientists is urgently needed. Mechanisms of neuron-malignant cell circuit assembly must first be defined if we hope to therapeutically disrupt this integration of brain cancer into functional neural circuits. The role that astrocytes, microglia, endothelia and other stromal brain cells may play in malignant circuit assembly, how neuron-malignant circuit dynamics evolve over the course of a cancer, and the putative mechanisms and consequences of malignant synaptic ‘plasticity’ each require exploration. The relevant ion channels, neurotransmitter receptors and downstream signaling components mediating brain cancer electrophysiology need to be identified and therapeutic targets prioritized. As the neuroscience of brain cancers comes to light, therapies aimed at neurophysiological targets may prove complementary to more traditional cytotoxic therapies and may improve outcomes for those suffering from these presently intractable brain cancers.

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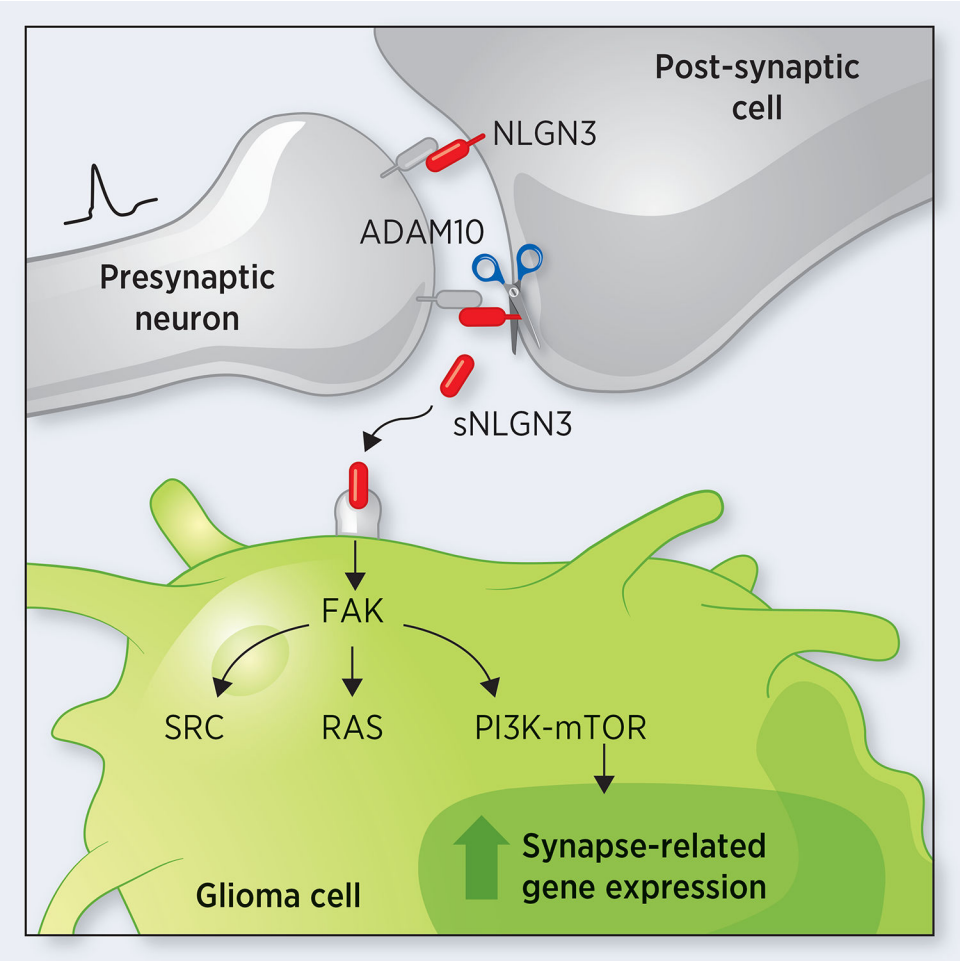

Figure 1. Neuronal activity-regulated neuroligin-3 shedding drives glioma progression

Neuronal activity regulates the ADAM10-mediated cleavage and release of neuroligin-3 (red) from post-synaptic neurons and post-synaptic OPCs. The ectodomain of neuroligin-3 is shed (s-NLGN3) into the glioma microenvironment, where it binds to an as-of-yet unidentified binding partner on the glioma cell (green). NLGN3 binding activates focal adhesion kinase (FAK) and downstream SRC, RAS and PI3K-mTOR signaling pathways. NLGN3 binding also induces feed-forward expression of *NLGN3* expression in a PI3K-mTOR-dependent manner. Together with upregulating its own expression in the glioma cell, NLGN3 increases expression of a number of synapse-related genes and promotes neuron-glioma synapse formation. Illustration by Sigrid Knemeyer at SciStories.