How neuronal activity regulates glioma cell proliferation

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Tumors are complex organs with numerous interactions among malignant and nontransformed cells of their microenvironment. Nonmalignant cells in the tumor microenvironment were recently shown to contribute to all hallmark capabilities of cancer cells and exert a tumor-promoting effect at all stages of carcinogenesis.¹

In a recent issue of *Cell*, Venkatesh et al² present a fascinating and novel mechanism through which high-grade gliomas (HGGs) hijack normal neuronal activity in their microenvironment for their own advantage. The authors elegantly demonstrate that neuronal firing in nearby neurons induces the production and secretion of a mitogen, neuroligin-3, and so increases proliferation and survival of HGGs.

A first clue to this mechanism of tumor growth came from previous work in the same laboratory,³ where Monje and colleagues showed that neuronal firing has a strong mitogenic effect on oligodendroglial precursor cells and earlier neural precursor cells—two cell types thought to give rise to gliomas.^{4–6} Their studies addressing normal development and tumor growth both used in vivo optogenetic activation of cortical neurons as an elegant way to induce action potentials with time- and location-specific precision. Specifically, this method genetically introduces light-sensitive excitatory channels (channelrhodopsin-2 or ChR2) into a defined population of neurons.⁷ When these neurons are exposed to blue light pulses by an implanted optical fiber, they respond with action potentials. Importantly, this method can be used to stimulate the brains of awake, moving animals.

Venkatesh et al put this method to work by crossing the ChR2 mouse onto an immunodeficient background. In this way they could use optogenetics to modulate neuronal activity adjacent to orthotopic xenotransplanted human glioma cells. Remarkably, glioma cells taken from a 15-year-old male with cortical glioblastoma multiforme (GBM) proliferated significantly better in the vicinity of light-activated cortical neurons, even after a single light stimulation. While this effect was initially small, mathematical modeling predicted a tumor increase of ~50% after 14 cell divisions. Indeed, repetitive light stimulation over the course of a week increased tumor burden by ~42% compared with orthotopic xenografts growing without additional neuronal activity in their microenvironment.

Monje and colleagues next investigated the mechanism of this effect by collecting conditioned media from optogenetically

stimulated cortical slices from ChR2 mice growing in vitro and exposing patient-derived HGG cultures to that media. Again, conditioned media from light-stimulated cortices led to increased proliferation of HGG cells, indicating an activity-regulated and secreted mitogen as the key player. This effect was seen in a wide variety of HGG cell cultures, including adult and pediatric GBM as well as pediatric diffuse intrinsic pontine gliomas.

In order to identify the secreted signal(s) responsible for this mitogenic effect, the authors utilized mass spectrometric analyses of the conditioned media from light-activated ChR2 and wild-type cortical slices. Two-dimensional gel electrophoresis was used to separate all secreted proteins, and each differentially expressed protein spot was identified by quantitative mass spectrometry. The primary candidate mitogen repeatedly and consistently identified was neuroligin-3, a member of the neuroligin synaptic protein family. This finding was validated by experiments in which recombinant neuroligin-3 was shown to promote proliferation of adult and pediatric HGG cell cultures. Interestingly, only the neuroligin-3 ectodomain was identified by mass spectrometric analysis, not the transmembrane and cytoplasmic domains of the protein, pointing to an enzymatically cleaved version of neuroligin-3 similar to the activity-regulated secretion mechanism already demonstrated for family member neuroligin-1.

In order to understand how neuroligin-3 increases proliferation of HGG cells, the authors performed RNA sequencing and western blot analysis on cultured HGG cells exposed to the activity-regulated mitogen. Pathway analysis revealed that members of the phosphatidylinositol-3 kinase (PI3K)–mammalian target of rapamycin (mTOR) pathway were activated in response to neuroligin-3. Intriguingly, expressions of neuroligin-3 mRNA and protein were also upregulated in HGG cells exposed to neuroligin-3 protein, suggesting a feed-forward mechanism in glioma cells. Together these findings link the neuroligin-3 response to the PI3K pathway, which is well known to be important in HGG growth (Figure 1).

The authors next analyzed the neuroligin-3 gene in The Cancer Genome Atlas database. While neuroligin-3 mutations are rather infrequent in adult and pediatric brain tumors, neuroligin-3 gene expression was found to be inversely correlated with overall patient survival in 429 cases of adult GBM—higher neuroligin-3 expression was correlated with a

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Fig. 1. Model demonstrating that neuronal activity induces neuroligin-3 secretion, leading to subsequent glioma cell proliferation through activation of PI3K/mTOR signaling.

5-months shorter lifespan than that of patients with lower neuroligin-3 expression. This finding was specific, as no association was found between family member neuroligin-2 expression and either patient survival or in vitro proliferation. Moreover, an extended analysis of neuroligin-3 in additional international databases revealed more frequent mutations across multiple cancer types, including thyroid, pancreatic, prostate, and gastric cancer.

Monje and colleagues not only elucidated a novel and unexpected role of neuronal firing in the brain tumor microenvironment but also revealed neuroligin-3 as a potential new therapeutic target in gliomas. Additional work is necessary to further delineate the mechanism leading to neuroligin-3 cleavage, how downstream PI3K signaling is activated, and whether the functions of tumor cell-derived and neuron-derived neuroligin-3 differ from one another. Another interesting aspect is the finding that neuroligin-3 was able to exert its mitogenic effect across multiple brain tumors of different locations and patient ages. It raises the possibilities that there might be additional mitogenic factors in the microenvironment and that some additional components might be region and age specific. Indeed, the authors identified brain-derived neurotrophic factor as also being secreted in response to neuronal firing and showed that this secreted factor is also able to increase HGG tumor growth.

This study has multiple important implications for patients. Clearly, one potential therapeutic strategy suggested by these studies is to design new therapies targeting the effects of neuroligin-3. Such approaches might be useful for diverse HGGs in combination with conventional chemo- and radiation therapy, or in combination with PI3K inhibitors. An intriguing question that arises from this demonstration of a link between neuronal activity and cancer growth is whether seizure activity in brain tumor patients per se actually increases glioma proliferation in patients. If so, more vigilant suppression of seizure in brain tumor patients would be indicated.

The study by Monje's group adds to a growing realization that cancers do not develop in isolation but grow in the context of a specific and active microenvironment. Therefore, effective treatments will need to target oncogenic mutations both in the context of the surrounding tumor cell and in the larger context of the adjacent normal and reactive cells.

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