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Dissecting Glioma Invasiveness in a 3D-Organotypic Model

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Abstract

Diffuse brain infiltration by tumor cells is a hallmark of glioma. Molecular mechanisms of brain invasion are poorly understood due to the difficulty to model this process in culture. A new study published in *Cell Reports* developed a three-dimensional organotypic model and identified interferon regulatory factor 3 (IRF3) as repressor of glioma invasion.

Gliomas are the most common malignant primary brain tumors in adults and comprise a heterogeneous group of tumors. Glioblastoma (GBM) is the most aggressive glioma subtype with a median survival below two years despite treatment with surgery, radiation and chemotherapy (1). Even gliomas of a lower histopathological grade, as defined by the World Health Organization (WHO), cannot be cured with current therapy. Multiple factors likely contribute to therapy resistance in glioma, including inadequate drug delivery across the blood-brain barrier, intratumoral heterogeneity in cancer signaling and metabolism, persistence of drug-resistant tumor cell populations, and the immune-suppressed state associated with the disease and its treatment.

A universal and particularly challenging aspect of glioma biology and therapy is diffuse brain infiltration by tumor cells along the perivascular space and white matter tracts. Brain infiltration is likely the net result of multiple molecular processes, including increased motility of glioma cells and tumor-related changes in the local microenvironment and extracellular matrix (ECM) (2). Previous studies have shown that the ability of glioma cells to invade the brain can be influenced by the distance of the tumor cell from blood vessels, the ECM composition and stiffness, hypoxia, and other effects such as ionizing radiation and chemotherapy (3). In terms of molecular signaling pathways, aberrant NF- κ B activation has been observed in GBM cell lines, associated with an invasive growth behavior (4). Moreover, overexpression of matrix-metalloproteinases (MMP) in glioma cells has been

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reported to enhance their ability to degrade the ECM and promote invasive tumor growth. Certain ECM components have also been shown to promote glioma invasiveness by providing ligands and ECM “anchors” for tumor cell migration (5). A molecular model of glioma invasiveness, however, has remained elusive thus far, largely due to the inability to dissect this process in an environment that recapitulates the complex architecture and cellular diversity of the human brain.

The study of glioma invasion has historically relied on widely used motility and invasion assays, such as the scratch assay or the Boyden/Transmembrane chamber assay. Recent advances in bioengineering and cancer biology provide opportunities to study tumor cell invasion within three-dimensional (3D) extracellular environments, including organoids, spheroid cultures embedded into chemically inert scaffolds, and primary cells grafted directly into biologically relevant matrix preparations (6). A new study by Pencheva et al. published in *Cell Reports*, describes a new method of quantifying glioma invasion in 3D and determining the contribution of specific signaling pathways in this process (7). In brief, the authors seeded patient-derived GBM cell lines – expressing green-fluorescent protein - onto the center of a 300µm-thick organotypic mouse brain slice and quantified their invasion into the brain tissue in 11 consecutive confocal imaging z-planes at 20µm increments, ultimately resulting in a 3D score for tumor cell invasion. As expected, the authors observed considerable variability in the ability of different GBM cell lines to invade normal brain structures. GBM cell lines harboring a transcriptional signature of the ‘mesenchymal’ GBM subtype – with poor outcome in patients (8)— were the most invasive. For one of the GBM cell lines, the authors further isolated subclones with increased invasive potential and compared their transcriptomes to parental, less invasive cells. The invasive subclones showed an enrichment of several ECM components, including multiple collagens, collagen-interacting proteins, and collagen-processing enzymes. These same genes were upregulated in GBM cell lines belonging to the more invasive mesenchymal subtype (7). The authors then identified interferon regulatory factor 3 (IRF3) as the transcriptional repressor of these “pro-invasive” genes and showed that IRF3 knock-down increased expression of all 17 ECM-signature genes. Since the serine/threonine protein kinase Casein Kinase II (CK2) had previously been identified as a negative regulator of IRF3 activation (9), the authors tested whether inhibition of CK2 with the ATP-competitive inhibitor CX4945 (10) could reduce glioma invasiveness via a mechanism that restored IRF3 activity. This was indeed the case in multiple models; moreover, CK2 inhibition was associated with downregulation of these ‘proinvasive’ ECM genes, increasing IRF3 recruitment to their promoters. Conversely, genetic silencing of IRF3 increased glioma invasiveness (7).

The study by the Bernards’ laboratory represents an important milestone in our understanding of glioma invasiveness. It describes a rigorous method that quantifies glioma invasion in a biologically relevant model and provides a blueprint for pharmacological and genetic screens to identify inhibitors of glioma invasion (Figure 1). Furthermore, the study identifies IRF3 as an endogenous suppressor of GBM invasion, proposing at least one pharmacological approach to augment IRF3 function through inhibition of CK2. Further studies are evidently warranted to determine whether the effects of CK2 inhibition are mediated exclusively through IRF3 or perhaps other previously reported functions of CK2 in glioma. Robust testing is also needed to dissect how the collagens identified in this study,

such as collagen I and collagen V, promote glioma invasiveness. The observation that CX4945 did not reduce tumor growth in the current study (7) supports the notion that glioma invasion and glioma proliferation are driven by different pathways (3). In an attempt to improve glioma patient outcomes, oncological research should place its efforts into revealing strategies and mechanisms that can simultaneously combine the blockade of glioma invasion and cell proliferation pathways. It will indeed be exciting to follow how these efforts do unfold in the future.

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Figure 1. 3D Organotypic Glioma Invasion Model

Patient-derived GBM cells are engineered to express green-fluorescent protein (GFP) and seeded onto a mouse brain slice. There, they can interact with the brain microenvironment, which is mainly constituted by neural and glial cells (in grey) and blood vessels (in dark red). An Invasion Index is calculated based on the area of invasion in a series of adjacent confocal microscopy images taken at different depths (z-stacks) through the mouse brain slice (7). This novel approach allows the study of both intrinsic pathways that affect at the same time, cell motility (e.g. NF-κB, TGF-β and the newly discovered IRF3), and brain microenvironment changes (mainly ECM-related, such as collagens and matrix

metalloproteinases (MMPs)) (3,7). Moreover, it may provide a blueprint for the evaluation of candidate drugs that could bear the potential to reduce glioma invasiveness (when comparing measurements of Invasion Index under different treatment conditions: gray dashed line versus solid green line).

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