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Putting Glioblastoma in its Place: IRF3 Inhibits Invasion

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Abstract

With an unsurpassed capacity for invasion into normal brain tissue, glioblastoma multiforme is the most lethal primary brain tumor. New research suggests that altering a subset of extracellular matrix factors, including interferon regulatory factor 3 (IRF3) and casein kinase 2 (CK2), may decrease the migratory potential of these aggressive tumors.

The most common primary malignant brain tumor, glioblastoma multiforme (GBM), WHO grade IV astrocytoma, harbors a dismal prognosis with a median survival of less than 15 months from diagnosis [1, 2]. Despite surgical resection, radiotherapy, and concomitant temozolomide treatment, GBM is capable of evading a battery of clinical interventions, earning its hallmark as the most lethal malignant brain tumor. With marked proliferative capacity combined with significant migratory potential, GBM cells seem to mimic embryonic glial propagation, yet, via aberrant cues in abnormal temporal and physiological contexts. How GBM cells can hijack host cell properties once restricted to the developing nervous system has not been well defined. Additionally, while research on the molecular and genetic underpinnings of breast cancer, lung cancer, melanoma, chronic myeloid leukemia (CML), among others, has garnered victories for advancing treatment options extensive characterization of genomic and transcriptomic alterations in GBM cells, has not proven to be as clinically fruitful.

With a wealth of data surrounding methylation status, copy number alterations, and mutational burden, what makes GBM's molecular kryptonite so elusive to basic, translational, and clinical researchers? In part, large-scale analyses reveal frequent genetic instability ranging from whole chromosomal alterations, to focal events and epigenetic changes. Despite a shared histopathological WHO grade, these tumors collectively constitute a vastly heterogeneous disease [2–5]. While histopathological features are used to primarily

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stratify diffuse gliomas from their lower grade counterparts, hierarchical subdivision of those with CpG island methylator phenotype ((CIMP) isocitrate dehydrogenase (IDH) mutant) and non-CIMP (IDH wild-type) appear to be better predictors of survival across all grades. Within non-CIMP GBM, expression based molecular subclasses have been discovered beyond initial WHO classification criteria and highlight increasingly diverse tumor cell populations within this already heterogeneous group of tumors [3, 6].

Advances in big data visualization and large-scale genomic analyses are becoming progressively more important for uncovering candidate biomarkers and predicting clinical outcomes. As such, characterization of the behavioral properties of GBM cells along with an in-depth understanding of what renders them so resistant to intervention are paramount to conquering this devastating disease. In addition to an enhanced capacity for radio- and chemoresistance, GBM cells harbor a remarkable capacity for invasion, limiting surgical resection to the tumor bulk, while sparing the diffuse ‘wanderers’ infiltrating the parenchyma. Crawling through surrounding neural tissue with tentacle-like projections, these cells appear to reinstate several migratory properties reminiscent of primitive neural stem cells. To make their way through the parenchyma, invading GBM cells exhibit features typically associated with the glial migration of the developing nervous system. Since the 1930s, glioma cells have been known to migrate away from the tumor mass, snaking their way through surrounding normal tissue [7]; however, the mechanism explaining the invasion of surrounding parenchyma is not fully understood.

While several decades of *in vitro* research have uncovered various transcription factors and signaling pathways enriched in migratory GBM cells (such as nuclear factor kappa-light-chain-enhancer of activated B cells (NFκB), transforming growth factor beta (TGFβ), mitogen-activated protein kinase (MAPK) pathways) [2, 5], new research published in *Cell Reports* extends on previous findings; Pencheva et al. have specifically focused on factors that can regulate human GBM cell invasion of using a 3D *ex vivo* murine glioblastoma slice culture model. The use of an *ex vivo* organotypic slice culture model allows for the exploration of tumor cell invasion through brain tissue, facilitating the interaction within an extracellular environment, which is not achievable using *in vitro* 2D cultures or matrigel migration assays [8].

Indeed, work by Bernards’ laboratory uncovers a novel network of extracellular matrix (ECM) factors that are significantly upregulated in a highly invasive subset of patient-derived GBM cell lines. The authors identify 71 upregulated genes in invasive tumor cells compared to minimally invasive controls and find that close to 25% of all upregulated genes (17/71) involve ECM components. These consist of multiple collagen members including both subunits of collagen I (*COL1A1* and *COL1A2*) and collagen V (*COL5A1* and *COL5A2*), collagen-interacting proteins, and collagen processing enzymes. Exogenous addition of these collagens to collagen-deficient glioma neural stem-like cells (GNS) increased their capacity for brain invasion in the 3D slice cultures [8]. Moreover, analysis of three separate glioma patient cohorts demonstrated that expression of these collagen subunits significantly correlated with glioma stages and overall survival [8].

The authors identified interferon regulatory factor 3 (IRF3) as a transcriptional repressor of pro-invasive ECM factors by demonstrating that depletion of IRF3 led to robust upregulation of ECM target transcript levels, whereas increased IRF3 -- via exogenous addition or constitutive activation— led to repressed ECM target gene expression, along with significantly reduced GBM cell invasive capacity . As previous work identified casein kinase 2 (CK2) as a negative regulator of IRF3 activation [9], the authors demonstrate that CK2 inhibition can activate noncanonical IRF3-dependent transcriptional repression of ECM target genes across multiple human GBM cell lines [8]. Furthermore, CK2 inhibition suppressed brain slice invasion in a dose-dependent manner for cell lines that were representative of mesenchymal, classical, and proneural GBM tumor subtypes. Consequently, this highlighted CK2 inhibition (using CK2 inhibitors CX4945 and 4,5,6,7-Tetrabromobenzotriazole [TBB]) as a potential target across multiple GBM subtypes, leading to reduced expression of IRF3 target ECM genes [8]. An orthotopic glioma mouse model also showed that mice treated with CX4945 retained more confined GBM brain lesions with distinct borders compared to highly infiltrative and diffusely scattered control tumors from untreated mice. The overall tumor volume and proliferation index remained unchanged, which suggests that the effects of CK2 inhibition on suppressing GBM cell invasion may be decoupled from cell survival/proliferation [8]. In several elegantly-performed experiments across multiple systems, the study shows that ECM components are necessary and sufficient to alter the migratory properties of GBM cells. Future work will benefit from exploring the potential pleiotropic effects of CK2 inhibition aiming to confirm that these observed effects are indeed specifically due to IRF3 activation and noncanonical IRF3 ECM-mediated signaling, and not a result of interference with other CK2 targets.

This new line of research suggests that highly infiltrative GBM cells are faced with an increasing need to create their own scaffold matrix in order to migrate through the surrounding parenchyma, which typically lacks rigid ECM structures. According to this study, selectively invasive GBM cells might do so via up-regulation of various ECM genes, including collagens, and pharmacological inhibition of CK2 may in turn suppress upregulation of these genes by acting on IRF3.

This line of work highlights several interesting aspects of glioma biology, namely, the importance of examining ECM factors and collagens in tumor cell migration and exploring the potential reinstatement of other neurodevelopmental factors during GBM invasion. The ECM, and collagens in particular, are well known to have substantial roles in the developing nervous system, and together with trophic factors, can influence not only migration, but also survival, proliferation, and differentiation of immature neural cells [10]. Unlike invasive GBM, the developing nervous system turns off these pro-proliferative and pro-migratory cues at tightly regulated junctures. Could CK2 and IRF3 also be involved in helping to determine when these cells halt their migration and become integrated within more permanent surroundings during development? Consequently, from basic oncology and developmental neuroscience perspectives, further exploration into the effects of ECM components and trophic factors at various stages of the migratory process will be of great interest, especially because these components may harbor clinical relevance surrounding the relentless invasiveness of GBM.

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Figure 1. CK2 Inhibition Decreases GBM Cell Migration via IRF3-Mediated Repression of Extracellular Matrix Genes

Transcription of genes involving extracellular matrix (ECM) components such as collagen members (*COL1A1*, *COL1A2*), collagen-interacting proteins, and collagen processing enzymes are upregulated in invasive human glioblastoma (GBM) cells. Recent findings suggest that inhibition of casein kinase 2 (CK2) can activate noncanonical interferon regulatory factor 3 (IRF3)-dependent transcriptional repression of ECM genes leading to suppression of GBM invasion [8]. Inhibition of CK2 allows for phosphorylation of TANK binding kinase 1 (TBK1), which subsequently leads to phosphorylation (P) of IRF3, downstream repression of ECM genes and decreased invasiveness of GBM cells.