Editorial

Stem Cell INVESTIGATION

The STAT3 and hypoxia pathways converge on Vasorin to promote stemness and glioblastoma tumorigenesis through Notch1 stabilization

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Gliomas are the most common primary intracranial neoplasms in adults and a leading cause of cancer-related morbidity and mortality in the United States (1). Grade I gliomas are considered the least malignant brain tumors, and grade IV or GBM the most aggressive and deadliest, which accounts for nearly 75% of all gliomas. Surgical resection of GBM remains the primary treatment modality with present adjuvant chemotherapy and radiation therapy only providing slight improvement in the disease course and outcome (2). The overall median time for GBM recurrence after surgery is 7 months, and its 5-year overall prognosis is dismal (<10% survival) and has remained unchanged for decades (1). Although gene expression profiles of GBM samples in The Cancer Genome Atlas (TCGA) database identified four general molecular subtypes (3), single cell analysis shows that multiple molecular subtypes exist within a GBM tumor and gene expression profiles can even vary dramatically across individual cells within the tumor (4), illustrating that individual GBM tumors are highly heterogenous.

The highly aggressive nature of GBM as well as its heterogeneity at the cellular level have been attributed to a subpopulation of glioma stem-like cells (GSCs), also called glioma-initiating cells (GICs) or brain tumor-initiating cells (BTICs) (5). GSCs share several features of neural stem cells including the expression of nestin and Sox2 (6), the ability to migrate within the brain (7), and the capacity to self-renew and undergo differentiation (8). The high tumor-initiating capacity of GSCs and therapeutic resistance is believed to drive tumorigenesis and tumor recurrence after therapy (9). GSCs reportedly reside in a hypoxic niche that supports their stem-like state (10), through the activation of the hypoxia-inducible factor (HIF) pathway that promotes expression of GSC maintenance factors by tumor and stromal cells (11). Notch pathway activation emerges as an essential molecular event to promote the GSC phenotype in the hypoxic microenvironment (11). However, the molecular mechanism underlying HIF-driven Notch activation in GSC is not fully delineated.

Dysregulation of the epidermal growth factor receptor (EFGR) is found in ~50% of GBM patient samples analyzed (12). Various oncogenic signaling pathways, including the EGFR pathway, contribute to GBM progression by converging on the important STAT3 molecular hub. STAT3 is activated through its phosphorylation by a wide variety of cytokines and growth factors, and STAT3 regulates various cellular processes critical in GBM tumorigenesis, including proliferation, invasion, and migration (13). High STAT3 activation is found in GBM and actively participates in GBM tumor formation and progression (14). STAT3 serine and tyrosine phosphorylation, which is markedly upregulated in GSCs, has been shown to be critical for GSC proliferation *in vitro* and GBM tumor formation in immunocompromised mice (15,16). Moreover, STAT3 induced the expression of various genes in the Notch pathway (17). The Notch pathway plays an important role in stem cell fate determination, survival,

proliferation and maintenance (18).

Notch signaling activation is initiated by the binding of the transmembrane ligands on one cell to Notch receptors present on an adjacent cell, resulting in the proteolytic release of the Notch intracellular domains (NICDs) that functions as transcription factors (19). In human, four Notch receptors (Notch 1-4), five ligands (Jag1, Jag2, DLL1, DLL3 and DLL4), and multiple effector molecules (Hes1-6, Hey1, Hey2 and HeyL) have been identified (19). In normal tissues, Notch ligands are generally produced by differentiated cells to modulate fate choice of adjacent Notch-expressing cells (20). Notch is abnormally activated in many cancers including GBM through multiple mechanisms, including increased secretion of Notch ligands by tumor and stromal cells (17,21), enhanced proteolytic cleavage of Notch intracellular domain by the ADAM (a disintegrin and metalloprotease) and γ-secretase families of proteases (22), and elevated expression of Notch proteins (17).

Following up on their previous finding that Vasorin was induced by hypoxia and overexpressed in GBM (23), Man and colleagues (24) show that Vasorin gene expression is induced in GSCs by a HIF/STAT3-dependent pathway. Most interestingly, Vasorin functions as a competitive inhibitor of Numb to inhibit Notch turnover, and thereby augmenting Notch signaling under hypoxic conditions. To initially assess the role of Vasorin in glioma, the TCGA glioma database was queried and it was found that Vasorin expression was elevated in GBM as compared to low-grade gliomas. An increase in Vasorin expression was detected by immunohistochemistry of higher-grade glioma tissue and compared to low-grade gliomas, which suggests that Vasorin is increased in more aggressive tumors. In addition, Vasorin was found to be co-expressed with various hypoxic and stem-cell markers, indicating that Vasorin is expressed in a putative stem-cell niche in GBM. Vasorin expression was also found to correlate with multiple hypoxia response genes in the TCGA glioma database. To further study the hypoxic regulation of Vasorin, GSCs that carried an EGFP reporter under the control of hypoxic responsive element were injected orthotopically in immunocompromised mice. In the orthotopic tumor xenografts, Vasorin staining co-localized with EGFP as well as stem cell and hypoxic markers, providing further support for Vasorin being expressed in the GSC population within the hypoxic niche. To assess the role of individual HIF proteins in regulating Vasorin, HIF1 and HIF2 were silenced using specific sh-RNA sequences. Under hypoxic conditions silencing HIF1, but not HIF2, reduced Vasorin protein and mRNA levels. Moreover, knockdown of the STAT3 transcription factor, which is constitutively activated in GSCs, also decreased both Vasorin protein and mRNA levels. Furthermore, both STAT3 and HIF1 were found to bind to the Vasorin promoter as assessed by chromatin immunoprecipitation. These results are of particular interest because STAT3 was previously found to form a complex with HIF1, but not HIF2, to drive a unique set of target genes to drive tumorigenesis under hypoxic conditions in cancer cell lines (25). Therefore, constitutive STAT3 activation may be a prerequisite for Vasorin induction in GSCs.

To further characterize its function, Vasorin expression was silenced in GSC lines by shRNA. Vasorin silenced GSCs were found to have impaired tumorsphere formation, reduced GSC viability and proliferation in vitro, and reduced formation of orthotopic xenografts in animal models. Thus, these studies provide strong evidence that Vasorin is needed to maintain the GSC population and promote GBM tumorigenesis. To determine the mechanism that underlies the role of Vasorin in GSC, Notch proteins were identified as Vasorin binding partners after analysis of Vasorin immunoprecipitates by mass spectrometry. The interaction of Notch 1 with Vasorin was validated in several different GSC lines, and silencing Vasorin in GSCs was found to reduce Notch1 levels as well as the levels of a several Notch downstream targets (Hey-1 and Hes-1). Somewhat surprising was the finding that Vasorin stabilized membranous Notch1 by inhibiting its lysosomal degradation in part by competing with Numb (an inhibitor of Notch signaling that regulates membranous Notch expression) for Notch binding to suppress Notch1 degradation. In addition, expression of the Notch intracellular domain restored GSC self-renewal in Vasorin-silenced GSCs and enhances their tumorigenic potential.

Taken together the findings of Ma and colleagues identify a novel HIF/STAT3 pathway to target Vasorin in GSCs that appears to play a critical role in GSC stemness. Furthermore, these studies suggest that inhibiting Vasorin in combination with Notch inhibitors such as γ-secretase inhibitors may be a novel strategy to treat GBM, which is a deadly cancer with few therapeutic options.

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Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

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