Editorial

Tenascin-C a novel regulator of brain tumor-initiating cells (BTIC) in glioma acts through NOTCH

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Glioblastoma is the most common and malignant adult brain tumor. Standard treatment consists of temozolomide (TMZ), surgery and radiotherapy (RT) (1). However, despite multimodal treatment, the average survival of patients with glioma is between 9–12 months after initial diagnosis, and tumor recurrence is almost 100% (2). The main characteristics of glioma are: high rates of cell proliferation; highly invasive and infiltrative across the surrounding vessels, myelinated fibres and areas of necrosis and highly angiogenenic. Glioblastoma is composed by heterogeneity cells; some cells have a genetic and cellular heterogeneity characteristic in human tumor. It is important to identify these subpopulations of tumor cells with stem-cell like characteristics (3) because these cells are related with treatment resistant and glioma recurrence (4). Understanding these cells characteristics and their metabolism are important to design new treatments against glioma.

Sarkar *et al*., now report on a novel connection between the secreted protein Tenascin-C on self-renewal of glioma stem cells by acting through an integrin/NOTCH signaling pathway (5).

In glioma, different studies have observed the presence of subpopulations of cells that are highly tumor initiating with multi-potential capacity and characteristics of neural stem cells, also called brain tumor-initiating cells (BTIC). These cells are responsible for tumor growth and progression, and express markers of neural stem cells like CD133 and Nestin. CD133 is a cell surface marker common to stem cells and progenitor cells in the hematopoietic system and central nervous system (6). Nestin is an intermediary filament expressed in immature neuronal cells. BTIC have significant therapeutic implications as they have been demonstrated to be tumor initiating, correlate with tumor grade and relapse (7), and are therapy-resistant (8). Clonogenic survival after cancer treatment is predictive for local tumor control and therefore BTIC must be eliminated to achieve durable responses and cure. Mechanisms to explain this intrinsic resistance include efficient repair of DNA damage, cell cycle redistribution, accelerated repopulation, and tolerance to low oxygen concentration (9). BTIC are responsible for tumor recurrence after surgical resection and usually found in hypoxic areas and leading edge of the tumor, surrounding normal brain parenchyma (10).

Tenascin-C (TNC) is a hexameric glycoprotein expressed by astrocytes, neurons and endothelium during embryogenesis, and is found in the extracellular matrix and influences cell migration, inhibits focal contact formation, promotes angiogenesis and, in some tissues acts as a cell survival factor (11). Each TNC subunit consists of an N-terminal tenascin assembly domain, 14.5 epidermal growth factor (EGF)-like repeats, a variable number of fibronectin type III-like repeats (FN III) and a C-terminal fibrinogen-like domain. TNC can bind to different cell receptors, mostly EGFR and integrins. TNC is overexpressed and correlated with tumor malignancy observed in many cancers types and associated with glioma outcome (12).

The NOTCH pathway is a short-range communication pathway between receptor and ligand-expressing cells and a master regulator of cell fate in development and adult tissue homeostasis by regulating stem cell renewal and differentiation (13). NOTCH signaling is implicated in resistance to chemotherapy (14), radiotherapy (15) and

targeted agents (16). In addition, and consistent with its broad role in normal stem cell function, NOTCH is implicated in cancer stem cells (17). In normal brain, NOTCH is expressed in immature glial cells, maintains stem cells in an undifferentiated state and responsible for stem cell turnover (6,18). Notch is overexpressed in glioma, and its signaling is related with tumorigenesis and resistance to radiotherapy (19).

Recently Sarkar *et al*., reported that the secreted extracellular matrix glycoprotein Tenascin-C regulates the proliferation of BTIC in malignant glioma in a NOTCH dependent manner. TNC and BTIC were found co-localized and overexpressed at the invasive front of glioma biopsies. TNC is produced by BTIC and essential for proliferation and spheroid formation *in vitro*. Moreover, TNC upregulates gene expression of NOTCH ligand, Jagged1, in BTIC which is correlated with stem cell proliferation and poor prognosis in malignant glioma. TNC knock down, reduced Jagged1 expression which was essential for the ability of TNC was incapable to promote BTIC growth. TNC also enhanced the generation of the γ-secretase cleaved and active fragment from NOTCH receptors; NICD (NOTCH intracellular domain) 1 and 2 in BTIC's. Conversely blockade of NICD formation using γ-secretase inhibitors diminished BTIC cell number and sphere formation, showing that NOTCH receptor signaling is mediating BTIC survival through TNC. This work extends earlier studies by Sivasankaran *et al*., who demonstrated that TNC is a direct transcriptional target of NOTCH/NICD (20).

Sarkar *et al*., now show that integrin α2β1 might be a direct link between TNC and NOTCH signaling in BTIC. Integrin α2β1 receptors have been strongly implicated in tumor invasion, angiogenesis and resistance to chemotherapy and radiotherapy (21). They show that α2β1 receptors directly bind TNC and by blocking α2β1 with neutralizing antibodies or with siRNA completely abrogates the ability of TNC to promote self- renewal and viability of BTIC demonstrating the importance of α2β1.

While this is a very interesting study several questions remain. How does TNC regulate Jagged1 expression, is it through NOTCH and which of the four NOTCH receptors? In this way TNC would provide a positive feedback loop. While TNC is essential for BTIC growth *in vitro* such studies may not always predictive for *in vivo* growth so it will be interesting to study TNC role *in vivo* tumor growth as well. While their intracranial orthotopic models are consistent with a role for TNC at the leading edge, the role of TNC in these orthotopic tumors was not specifically addressed and would be informative. Can tumors form in the absence of TNC are they less infiltrative? What would the effect be of integrin or NOTCH blockade? Furthermore it is clear that BTIC comprise a heterogeneous group of self-renewing cells characterized by marker expression (e.g., CD133) and driver mutations all with potent tumor initiating capacity but different phenotypic outcomes (22). Another question is whether TNC is involved in all glioma subtypes (23)? Using public datasets Sarkar *et al*., illustrate that Jagged1 and NOTCH1 expression are higher in GBM than in normal brain. Keeping in mind that expression does not mean NOTCH activity, their findings are in contrast to other studies were in some subtypes NOTCH expression predicts a good prognosis and acts as a tumor suppressor (22)? It will be important to study the involvement of TNC regulation in these NOTCH-deficient glioma's as well and to clarify the relationship between TNC/NOTCH in larger datasets comprising both IDH/wt and IDH/mutant glioma. Other studies have shown that targeting NOTCH using γ-secretase inhibitors are not effective in blocking BTIC (19) but that blocking of RBP-Jκ, the common mediator of the NOTCH transcriptional response efficiently blocks BTIC growth through a NOTCH independent mechanism (24). If these BTIC still produce TNC is not known.

Failures in clinical trials often drive new biological insights that help us explain why drugs fail. In the case of NOTCH, antibodies or small molecule inhibitors have shown limited efficacy (25). It is clear that while targeting NOTCH is still a potential therapeutic target the complexities of its context dependent regulation in normal tissues and heterogeneous tumors are incompletely understood. What is needed are predictive signatures that will identify subsets of human brain tumors that may respond favorably to targeting NOTCH in BTIC. The novel connection between TNC-NOTCH and integrin axis maybe further exploited to target a subclass of gliomas regardless of their prognosis. When combined with radiation or chemotherapy NOTCH/TNC targeting may enhance treatment efficacy by specifically sensitizing BTIC (15,19). Clearly more preclinical work must be done to assert a predictive or therapeutic role for TNC in glioma.

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