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Review

Cancer stem cell-vascular endothelial cell interactions in glioblastoma

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ABSTRACT

Glioblastoma (GBM), a higher grade glial tumor, is highly aggressive, therapy resistant and often shows poor patient prognosis due to frequent recurrence. These features of GBM are attributed to presence of a significantly smaller proportion of glioma stem cells (GSCs) that are endowed with self-renewal ability, multi-potent nature and show resistance to therapy in patients. GSCs preferably take shelter close to tumor vasculature due to paracrine need of soluble factors secreted by endothelial cells (ECs) of vasculature. The physical proximity of GSCs to ECs creates a localized perivascular niche where mutual GSC-EC interactions regulate GSC stemness, migration, therapy resistance, and cellular kinetics during tumor growth. Together, perivascular niche presents a therapeutically targetable tumor structure for clinical management of GBM. Thus, understanding cellular and non-cellular components in perivascular niche is vital for designing *in vitro* and *in vivo* GBM tumor models. Here, we discuss the components and structure of tumor vascular niche and its impact on tumor progression.

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1. Introduction

Glioblastoma (GBM) is an aggressive tumor of central nervous system with overall median survival of 14 months. The high heterogeneity due to functionally diverse cell types, chaotic leaky

vasculature, and infiltrative nature of tumor cells contributes to poor prognosis in patients [1]. As a standard therapy regime, GBM patients undergo surgical de-bulking of tumor which is followed by chemotherapy (Temozolomide or TMZ) along with radiation therapy. Despite aggressive treatment 5 years patient survival in GBM is below 5% due to rapid recurrence [2]. The causative agent for recurrence in GBM is due to a rare subset of tumor cell clones known as Glioma Stem Cells (GSCs) [3]. GSCs are undifferentiated, multipotent, and self-renewing cell types which are functionally characterized either via neurosphere formation assay *in vitro* [4] or by limiting dilution assay for tumorigenicity *in vivo* Similar to adult

Abbreviations: GSCs, Glioma Stem Cells; PN, Perivascular Niche; ECs, Endothelial Cells.

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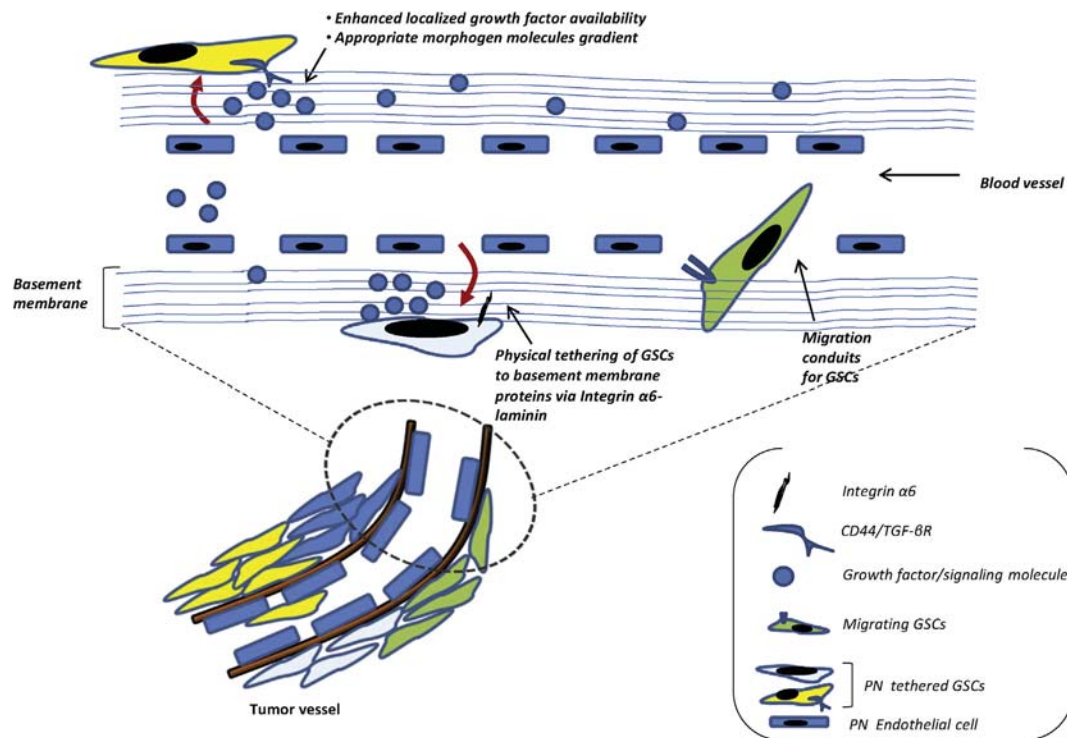


Fig. 1. Perivascular niche as GSC-EC interaction hub. Phenotypically diverse GSC populations anchor, migrate, and/or engage in active signaling within endothelial niche micro environment.

stem cells that reside in specialized anatomical milieu, GSCs are dependent on micro-environmental cues for maintaining their phenotype and therefore, prefer to associate with auxiliary microenvironment for their functionality and phenotype maintenance [5].

Recent reports indicate that GSCs physically locate around blood vessels for physiological or pathological purposes. Typically, blood vessel endothelial cells create anatomical compartmentalization of tumor regions known as perivascular niche (PN) [5–7]. PN is a functional unit of cell–cell interaction which maintains self renewal, therapy resistance and growth properties of GSCs [8,9]. Interestingly, PN requirement is elaborated by multiple studies demonstrating that GSCs undergo trans-differentiation and give rise to functional ECs [10]. Similarly, trans-differentiation of GSCs into ECs is a contributing factor in resistance to anti-angiogenic therapy in glioblastoma [11]. Several studies are indicative that treatment failure in GBM is due to 1) inherent tumor cell heterogeneity 2) presence of GSCs and PN like structures, and 3) invading glioma cells which occupy surrounding brain parenchyma.

2. Perivascular niche-components and soluble factors

Cell–cell communication is vital not only for sensing the surrounding microenvironment but also for cell fate determination. In the chaotic cellular landscape of GBM it is pivotal for GSCs to maintain their undifferentiated state. Predominantly, both physical association and release of soluble factors via cell–cell interactions are augmented when interacting cells are in close proximity. For GSCs, PN offers both. It helps in GSCs to adhere to vascular structures where they can physically interact with ECs (Fig. 1). Subsequently, PN allows access to increased localized concentrations of soluble factors secreted by ECs [12,13], (Fig. 1). Interestingly, Calabrese et al. showed that physical proximity of GSCs to ECs is a key driver of tumor growth [5]. Using a mouse xenograft glioma model,

they show that hierarchically organized mouse glioma tumors decrease in size upon ablation of blood vessels. Thus, self-renewal is represented as a function of number of endothelial cells in the tumor. To functionally maintain self-renewal and stemness GSCs require several autocrine and paracrine factors (e.g. soluble, non-soluble) [14]. Many such factors are transcription factors, micro-RNAs, or chromatin associated proteins and acts as cell autonomous drivers of GSC cell fate by functioning in an autocrine manner [15]. However, other soluble factors such as ligands, cellular enzymes and small hormone messengers are provided by ECs of PN and function as paracrine modulators [13,16,17]. Due to the fact that GSCs are key determinant of GBM aggressiveness and PN is a positive regulator of GSC functionality, many groups have recently explored the underlying molecular mechanisms intersecting what occurs inside PN compartment.

2.1. Notch pathway

Notch signaling is one of the most studied stemness pathway and appears to maintain self-renewal of GSCs [18,19]. GSCs preferentially express Notch receptors Notch1 and Notch2 and show activation of Notch signaling as evident by expression of target gene *Hes5* [20]. Paracrine activation of GSC Notch receptors occurs via two key Notch ligands Delta-like 4 (DLL4) and Jagged-1 that are expressed on endothelial cells. In a mouse xenograft study, it was found that when GSCs and ECs were co transplanted, EC specific knock-down of Notch ligands caused decrease in tumor progression [21]. Similarly, DLL4 ligand is strongly expressed in tumor endothelial cells and this promotes angiogenesis in an autocrine manner [22]. Evidently, PN architecture is mutually beneficial to both GSCs as well as ECs as GSC promotes angiogenesis by allowing proliferation of endothelial cells [23]. PN, therefore, becomes hub for convergence of multiple cellular phenotypes (e.g. self renewal maintenance, angiogenesis, cell proliferation).

Table 1

Autocrine and paracrine interaction between PN GSC-EC and involvement of multiple signaling pathways.

Soluble GSC self renewal factors	Expression pattern		Shown in perivascular niche
	Tumor endothelial cell	GSC	
TGF-beta pathway			
CD44/Id1	No	Yes	Yes
Nitric Oxide	Yes	No	Yes
Cytokine pathway			
Ligand- (IL-6)	Yes	No	Yes
Receptors- CXCR1, CXCR2	No	Yes	
Notch pathway			
Ligands-(JAGG1, Dll4)	Yes	No	Yes
Receptors- Notch1, Notch2	No	Yes	
Osteopontin	(PN specific localization)		Yes
ECM signaling			
Receptor- Integrin $\alpha 6$	No	Yes	Yes
Ligand- Laminin	–	–	
Sonic Hedgehog pathway			
Ligand- SHH	Yes	No	Yes/No

2.2. TGF-beta pathway

TGF beta signaling is implicated in embryonic stem cell self-renewal [24,25]. However, due to multiple novel downstream targets in cancer stem cells, the TGF- β pathway is involved in maintaining cellular plasticity, Epithelial Mesenchymal Transition (EMT), and therapy resistance [26]. TGF beta signaling promotes self-renewal, inhibits differentiation and enhances oncogenic potential of GSCs. This occurs through Smad induced JAK-STAT pathway along with TGF-beta-Sox4-Sox2 driven signaling cascade [26,27]. Further, convergence of TGF-beta and PDGFR signaling in aggressive gliomas contributes to enhanced proliferation and oncogenic potential of glioma stem cells [26]. In particular, perivascular TGF-beta signaling is shown as therapy response predictor in GBM. Anido et al. showed that TGF pathway inhibition targets inhibitors of DNA-binding protein (Id)-1, and -3 in perivascular GSCs [28]. Moreover, targeting a GSC subpopulation of CD44^{high}/Id1^{high} cells derived from GBM patient inhibited tumor re-occurrence in mouse xenograft model indicating a synergy between self-renewal and angiogenesis. Surprisingly, even though perivascular ECs regulate GSC self-renewal, the role of TGF-beta ligand in ECs that vitally comprise PN microenvironment is not yet known.

2.3. Nitric oxide (NO) pathway

Nitric oxide (NO) is one of the dual function molecules that promote neural stem cell proliferation in a dose dependent manner [29]. NO inhibition regulates stem and progenitor number in hematopoietic compartment [30]. Using a genetically engineered mouse model of PDGF driven glioma, Charles et al. showed that GSC phenotype in perivascular niche is maintained by endothelial nitric oxide (eNOS) [13]. Their study showed that eNOS induced paracrine signaling in GSCs activates Notch pathway, increases neurosphere formation, induces *in vivo* tumorigenicity, and thereby enhances stem cell phenotype. In addition, functional studies revealed that eNOS inhibition suppressed tumor formation resulting in increased survival of tumor bearing mice. In another report, convergence of PDGF-NOTCH-NO axis drives perivascular promotion of GSC phenotype and angiogenesis simultaneously [31]. PDGF treatment up-regulates stemness genes in patient derived neurosphere lines via NO mediated inhibitor of differentiation-4 (ID4). In this multi-lateral signaling study, PDGF treatment of glioma cells and endothelial cells with ID4 caused JAGGED1 induction by inhibiting microRNA-129. Together, the study shows simultaneous up

regulation of stemness markers in GSCs and angiogenic phenotype in endothelial cells, respectively. Importantly, even though *in vivo* co-existence of GSCs-ECs is not incorporated in the experimental design, co-convergence of multiple pathways proposes a much more complex perivascular niche.

2.4. Other pathways

Other pathways of GSC self-renewal include sonic hedgehog (SHH), Wnt and integrin signaling [32,33]. SHH-Gli1 pathway promotes GSC self-renewal and glioma growth. Although, tumor endothelial cells specific SHH expression in genetically engineered PDGF mouse tumors is known [32], paracrine activation of SHH-Gli1 in GSCs is unexplored. Similarly, Wnt pathway is a stem cell specific activator, however, its role in GBM perivascular niche is not well-established. Additionally, extracellular matrix (ECM) signaling in tumor microenvironment regulates multiple tumor cell functions. In particular, ECM protein laminin regulates perivascular anchorage of GSCs and self renewal through its cognate receptor integrin $\alpha 6$ on GSCs [12]. Therefore, Integrin $\alpha 6$ may assist in generation of perivascular niche like structures in the tumor (Fig. 1).

3. Niche interaction model(s)-scope and pitfalls

The study of perivascular niche is relatively advanced in multiple tumors due to its importance in understanding cancer stem cell biology and tumor pathology. In GBM, since GSCs are not amenable to inhibition by any known drugs and constitute therapy resistant cell types, it is imperative to know how they achieve and maintain such a state by locating themselves in PN microenvironment. This will be most imperative for designing effective tumor growth models and estimating therapy outcomes. To, extrapolate GSC microenvironment of PN under *in vitro* conditions, *non-physical* *in vitro* GSC-EC co-culture in a trans-well insert is often used. Other options comprise use of human umbilical vein endothelial cells (HUVEC) or other immortalized EC lines. Our search for existing literature on PN related studies revealed that transwell insert remained candidate choice for co-growing tumor cells with endothelial cells for understanding molecular pathways in PN conditions. As for any cell-cell interaction induced effects to occur, not only expression but also a defined concentration of respective signaling molecule is important for phenotype generation. In PN, GSC-EC interactions occurs in a spatial-temporal manner in which specified amount of signaling effectors, along with physical tethering is required [33]. Since most of the GSC regulators (e.g. TGF-

beta, Wnt, Notch, NO) belong to morphogen signaling cascades [34–36], they may exert differential effects according to existing gradient. Similarly, in the highly heterogeneous tumor microenvironment optimum concentration of these molecules in PN microenvironment is essential for obtaining a specific phenotype. However, un-availability of a relevant co-culture system in addition to non-availability of established tumor endothelial lines hinders the development of a refined, physical heterotypic GSC-EC interaction platform (Table 1).

4. Targeting niche-therapeutic opportunities

PN is shown to provide radio-resistance to CSC in a medulloblastoma mouse model [9]. Here, Hambardzumyan et al. showed that enhanced Akt/PI3K activity in CSCs along with transient cell cycle arrest increased their survival in PN microenvironment. Moreover, Akt pathway inhibition occurred during radiation induced apoptosis in CSCs. Similarly, PN specific Osteopontin-CD44 signaling axis radio-resistance in GSCs [16]. As during radiotherapy, co-irradiation of GSCs and ECs occur in patient tumors. It was seen that ECs up-regulated JAGGED1 expression upon radiation [37]. This is not only suggestive of enhanced GSC-EC interaction in PN during radiation, but also indicates Notch1 as therapeutic target. Notch inhibition either alone or synergistically with radiotherapy is shown to kill GSCs [18,20].

5. Conclusion

PN is a vital assembly point of two clinically important tumor cell types- GSCs and ECs. Even after 10 years of GSC identification, there is very little improvement in patient survival either due to lack to GSC specific therapies or limitations GSC biology under micro environmental conditions. GSCs generate GBM heterogeneity, promote angiogenesis, invade normal brain regions along tumor vasculature and escape therapy [38–40]. All these therapy impeding tumor features occur due to interactions with the surrounding milieu. Hence, cellular, molecular, and functional characterization of PN is crucial as it serves as a region of go-or-grow regulator point for GSC phenotype maintenance. These studies may help in determination of novel therapeutic opportunities for GBM patients. It is interesting to study how ECs of PN are different from ECs of non PN tumor vasculature. Also, during therapy, multiple signaling pathways related to cell survival, apoptosis, migration, cell cycle are activated and it would be important to study how they incorporate and maintain diversity in dynamic pool of GSCs under PN microenvironment [41–43]. Interestingly, most of these studies related to PN analyze GSC phenotype maintenance; however, regulation of PN ECs is not well studied. Lastly, as PN is preferred region where GSCs reside, identification of PN labeling molecules for visualizing local niches may allow *niche painting* for both therapeutic PN targeting and surgical removal of PN specific regions.

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Transparency document

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