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Mini-review

Tumor vasculature and glioma stem cells: Contributions to glioma progression

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ABSTRACT

Glioblastoma multiforme (GBM), the most malignant of brain tumors, is characterized by extensive vascularization and a high degree of invasion. The current standard of care is not very effective, resulting in tumor recurrence with patients rarely surviving over 2 years. This tumor recurrence is attributed to the presence of chemo and radiation resistant glioma stem cells (GSCs). These cells are associated with vascular niches which regulate GSC self-renewal and survival. Recent studies suggest that while blood vessels support glioma stem cells, these tumor cells in turn may regulate and contribute to the tumor vasculature by transdifferentiating into endothelial cells directly or through the secretion of regulatory growth factors such as vascular endothelial growth factor (VEGF) and hepatoma derived growth factor (HDGF). The relationship between the tumor vasculature and the glioma stem cells is the subject of this review.

Introduction

Glioblastoma multiforme (GBM) is a highly malignant primary brain tumor with a median survival time of approximately 14 months from time of initial diagnosis [1]. Despite the current procedures of surgery, radiation and chemotherapy regimens, tumors usually recur. At this point there are few treatment options; and the subsequent prognosis is poor. GBM is characterized as a highly angiogenic tumor; and this extensive blood vessel growth is critical for tumor progression and invasion [2]. The vasculature is associated with GBM thereby reducing hypoxia, and is generally required for tumor survival [2]. There is a subpopulation of tumor cells, glioma stem cells (GSCs), thought to be responsible for GBM recurrence [3].

Glioma stem cells have properties similar to normal neural stem cells (NSCs), in that these cells have infinite self-renewal and multi-potential differentiation capacity [3]. Moreover, GSCs can initiate highly invasive tumors based on in vivo evidence [4]. There is compelling evidence that GSCs are resistant to a variety of chemotherapeutic agents, including temozolomide (TMZ), the standard chemotherapeutic agent for the treatment of GBM, enabling these cells to survive therapy and thereby become the basis for disease recurrence [5–7]. Therefore any novel strategy that targets glioma stem cells would be valuable for treating recurrent GBM.

Studies have shown that GSCs are found in close association with vascular niches [8,9]. Endothelial cells produce a variety of growth factors that stimulate GSC self-renewal and tumorigenicity [10–12]. Recent studies show that GSCs may transdifferentiate into endothelial cells or pericytes, creating their own vascular niches [13–16]. GSCs also produce a variety of cytokines and chemokines, some of which are known to activate endothelial cells [17–19]. This suggests that GSCs may in turn regulate the tumor vasculature and subsequently the extent of tumor angiogenesis. This review focuses on the role of GSCs in regulating tumor angiogenesis in GBM.

Tumor vasculature

The vasculature is a highly dynamic, tissue specific organ. It plays a critical role in transport of oxygen and nutrients, removal of cellular waste and regulation of blood flow. The normal vasculature is organized as a hierarchy of arteries, arterioles, capillaries, venules and veins. In capillaries, the blood vessels are lined with a thin layer of tightly packed endothelial cells (ECs) surrounded by pericytes; in larger vessels, arterioles and arteries, endothelial cells are associated with smooth muscle cells [20]. The basement membrane, secreted by ECs, is the extracellular matrix responsible for structural support, stability and anti-coagulation status [20]. Brain ECs are unique in that these cells are directly associated with astrocytes and express tight junction proteins, resulting in the formation of the blood–brain barrier [21,22]. Astrocytes and growth factors produced by the brain microenvironment regulate the integrity of the blood–brain barrier [21].

The formation of new blood vessels is a normal process and occurs by several mechanisms. Vasculogenesis, which is predominant during organogenesis and common throughout fetal development, is the formation of new blood vessels from migrating endothelial progenitor

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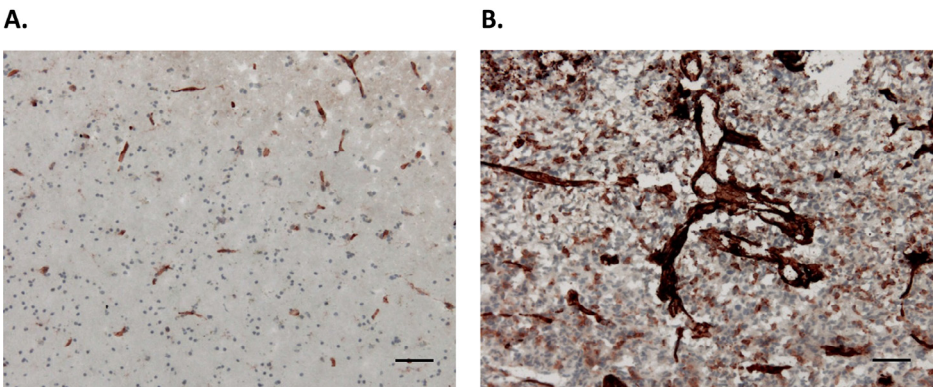

Fig. 1. Vasculature of normal human brain tissue and human glioblastoma multiforme (GBM) tissue specimens. Cryostat sections of human brain tissues were stained with anti-CD31, endothelial cell marker. (A) Blood vessels in normal tissue and (B) GBM tissues exhibited a red precipitate. Hematoxylin provided the contrasting nuclear staining. Scale bar: 0.1 mm. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

cells, usually bone marrow-derived cells [23]. Angiogenesis, new vessel formation from adjacent pre-existing vessels, is common in wound healing, and involves the proliferation of ECs and vessel formation as an extension of already existing vessels [24]. Intussusception is the formation of multiple vessels from the reorganization of existing vessels [25]. The normal vasculature is usually quiescent with only 0.01% endothelial cells dividing [26]. This quiescence is facilitated in part by the balance between pro-angiogenic factors, such as vascular endothelial growth factor (VEGF) and anti-angiogenic factors, such as thrombospondin (TSP-1) [27]. Thus the regulation of the levels of growth factors is key for the activation of blood vessel formation.

In sharp contrast to normal blood vessels, the tumor vasculature, particularly in GBM, is highly proliferative resulting in abnormal blood vessel structures [22,26]. These tumor blood vessels lack the organization and regulation found in normal vessels. Morphologically, tumor vessels are tortuous, exhibiting dead ends leading to hypoxic regions (Fig. 1). Furthermore these tumor blood vessels exhibit shunting from arterioles to venules thereby causing unstable blood vessel structures often resulting in hemorrhaging [22]. Tumor EC overexpress VEGF receptors, therefore an environment of high VEGF will cause increased endothelial cell proliferation, migration and blood vessel permeability [22]. Permeability changes are associated with increased edema routinely observed in GBM [22]. The tumor vasculature is also characterized as demonstrating a compromised blood–brain barrier [22]. Activated tumor endothelial cells also overexpress specific integrins which enhance the capacity of immune cells to bind to the tumor vasculature and thereby infiltrate into the tumor tissue [26]. Integrins specifically $\alpha 6\beta 1$ also play a cyto-protective role for endothelial cells by increasing expression of anti-apoptotic proteins such as cFLIP and inducing the pro-survival arm of the TNFα pathway [28]. The initiation of angiogenesis in tumors is thought to be activated by the resulting hypoxia due to the high density of tumor cells [26]. Hypoxia stimulates the expression of the transcription factor, HIF-1α, which triggers the production of VEGF, among other pro-angiogenic growth factors [26]. Thus the tumor vasculature is responsive to the microenvironment. Blood vessels can also develop by the seeding of bone marrow (BM) endothelial progenitor cells in the tumor [2]. Tumor cells produce stromal cell-derived factor 1 (SDF-1) causing the migration of endothelial cells to the tumor site. This mechanism of vasculogenesis is especially important during recurrent disease to allow tumor cells to continue to grow after radiation-induced damage to the vasculature [29]. In addition, vessel cooption – organization and migration of tumor cells along existing blood vessels and vascular mimicry – vascular channels lined by tumor cells are two other mechanisms unique to tumor vascularization [2]. Recent data reveal that a component of the tumor vasculature may also be derived from the transdifferentiation of the tumor initiating glioma stem cells into endothelial cells [13,14]. GSC cells expressing CD133, a common marker for stem cells, were found to differentiate into functional endothelial cells capable of tube formation and low density lipoprotein (LDL) uptake under appropriate in vitro culture conditions [13,14]. These data will be discussed later.

In our approach to understanding the interactions between the tumor vasculature and the tumor environment, we isolated tumor endothelial cells from glioma tissue and characterized these cells. We found that the tumor-associated brain endothelial cells exhibited distinct morphological and functional differences compared to normal brain endothelial cells [30,31]. Tumor-associated brain ECs are large, flat, veil-like cells as compared to normal brain ECs, which are small and plump in appearance. Tumor-associated brain ECs have lower proliferation rates, increased migration and enhanced invasion properties [30]. These tumor-associated brain ECs produce high amounts of pro-angiogenic growth factors such as VEGF, IL-8 and ET-1 [30]. A subpopulation of the tumor-associated brain EC also expressed α-smooth muscle actin, a marker of pericytes [30].

Other studies using laser dissection microscopy and microarray analysis have identified 95 differentially expressed genes between glioma-associated tumor vessels and vessels from non-tumor specimens [32]. Immunohistochemical (IHC) analysis of human GBM sections further validated increased expression of angiopoietin 2 (ANGPT2), endothelial cell specific molecule 1 (ESM1), CD93, EGF latrophilin and seven transmembrane domain containing protein 1 (ELTD1) and Filamin A interacting protein 1-like (FILIP1L) in glioma vessels compared to non-tumor vessels. Gliomas have elevated VEGFA and TGFB2 signaling and these growth factors were found to regulate expression of ANGPT2, CD93, ELTD1 and ESM1 in human dermal microvascular endothelial cells (HDMEC) in vitro [32]. Another study using serial analysis of gene expression (SAGE) of three tumor-associated brain EC and two non-tumor EC cultures showed a higher expression of genes involved in regulation of extracellular matrix architecture and cellular signaling such as matrix metalloproteinase 14 (MMP14) and integrin αv [33]. Laser capture microdissection, microarray analysis and immunohistochemistry identified insulin like growth factor binding protein 7 (IGFBP7) as a protein selectively expressed by GBM EC [34]. IGFBP7 is a glycoprotein and interacts with extracellular matrix components such as collagen IV but its role in the tumor vasculature remains to be elucidated. Furthermore, tumor-derived brain EC showed lower expression of genes involved in suppressing tumor growth and angiogenesis [33].

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