Galectins and neovascularization in central nervous system tumors

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Despite advances in diagnosis and treatment, the overall outcomes for patients with brain tumors remain unpredictable. New prognostic markers are still needed to identify high-risk patients for whom the standard treatment has poor outcomes and would thus be well suited for more aggressive therapies. Neovascularization has long been implicated as a salient feature of glioma progression. In fact, high-grade gliomas are among the most vascular of all solid tumors, and vascular proliferation is a pathological hallmark of glioblastomas. Galectins are known to play important roles in cancer biology, including cancer cell migration, tumor immune escape or tumor angiogenesis. Moreover, galectins were reported to be involved in glioma progression. Given the key role of angiogenesis in brain tumors, the expression of galectins in tumor-associated endothelial cells (EC) and the implication of galectins in angiogenesis, the present review will focus on the expression of galectins in ECs of normal brain and brain tumors.

Keywords: angiogenesis / brain tumors / endothelial cells / galectins / glioma

Introduction

Primary malignant brain tumors comprise a variety of lesions that differ in the type of cells they have grown from. The World Health Organization (WHO) has developed a classification (by cell type) and a grading system to standardize communication and treatment planning and to predict outcomes for these tumors (Table I) (Louis et al. 2007). Gliomas are the most common type of brain tumor and account for more than half of all primary brain tumors. Grade I lesions generally include tumors with low proliferative potential and the possibility of curing following surgical resection. Grade II tumors are generally infiltrative and are characterized by recurrence, despite low-level proliferative activity. Grade III tumors show histological evidence of malignancy, including nuclear atypia and mitotic activity. Grade IV is assigned to cytologically malignant, mitotically active, necrosis-prone neoplasms that are often associated with a fatal outcome. The WHO grade is one component of a combination of criteria (such as histopathological type, age, tumor location, multifocality, extent of surgical resection and molecular biomarkers) that are used to predict a response to therapy and outcome (Cairncross et al. 2006; Forshew et al. 2009; Sanson et al. 2009). However, the overall outcomes for patients with diffuse gliomas remain unpredictable. New prognostic markers are still needed to identify high-risk patients for whom the standard treatment has poor outcomes and would be well suited for more aggressive therapies (Maris et al. 2008; Rorive et al. 2010; Le Mercier et al. 2012). Neovascularization has long been implicated as a salient feature of glioma progression. In fact, high-grade gliomas are among the most vascular of all solid tumors, and vascular proliferation is a pathological hallmark of glioblastomas (GBMs) (Hardee and Zagzag 2012). The rationale for applying antiangiogenic strategies in malignant brain tumors includes (i) the high degree of neovascularization in high-grade gliomas; (ii) avoidance of problems related to crossing the blood–brain barrier, in contrast to certain chemotherapeutic agents and (iii) normalization of vascular networks leading to synergism with other therapeutic strategies (Hardee and Zagzag 2012). Early Phase II trials using bevacizumab, a humanized monoclonal antibody targeted against vascular endothelial growth factor (VEGF) in patients with recurrent GBM showed dramatic responses on imaging, leading to approval of this drug by the Food and Drug Administration in 2009 for the treatment of recurrent GBMs. However, controversies exist about the true clinical benefit of bevacizumab, given the relatively short duration of response in most patients and the highly infiltrative pattern of recurrence in GBMs treated by bevacizumab. Very recently, two randomized trials that address the clinical benefit of adding bevacizumab to the standard treatment (radiotherapy + temozolomide) for newly diagnosed GBMs were published (Chinot et al. 2014; Gilbert et al. 2014). Both trials showed a 3- to 4-month prolongation of progression-free survival with bevacizumab but no significant effect on overall survival. Moreover, the results of the trials diverge regarding the effects of bevacizumab on quality of life and performance status.

Blood vessels within tumors contain an abnormal endothelial cell (EC) physiology that provides a potential focus for...
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affecting vascular growth and viability. Therefore, the tumor vasculature has become an attractive target for antineoplastic therapies, and several novel agents that target components of the tumor vasculature are currently in clinical development (LoRusso et al. 2011). Effective EC-targeted therapy depends on the availability of molecules that distinguish tumor-associated ECs (TAECS) from normal ECs. Different studies have highlighted the diversity of ECs according to the organ or pathology (normal vs tumor) (St Croix et al. 2000; Chi et al. 2003; Luttun et al. 2004). In particular, this heterogeneity was observed regarding galectin-1 and -3 expression in ECs. We and others have observed an overexpression of galectin-1 or -3 in TAECS (Clausse et al. 1999; D’Haene et al. 2005, 2008; Thijsen et al. 2006, 2007; Jia et al. 2010; Croci et al. 2012). Galectins are known to play important roles in cancer biology, including cancer cell migration, tumor immune escape or tumor angiogenesis (Liu and Rabinovich 2005; Camby et al. 2006; Demetter et al. 2008; Le Mercier et al. 2010; Newlaczyl and Yu 2011), and endothelial galectins can be targeted for therapeutic applications (Rabinovich 2005; Thijsen et al. 2006). Moreover, galectin-1 and -3 are targets of myeloid malignancies. In some brain tumors, the differential expression of galectins in TAECS and the implication of galectins in angiogenesis, the present review focuses on the expression of galectins in ECs of normal brain and brain tumors.

Galectin-1

Galectin-1 expression in normal brain ECs

Stancic et al. (2011) observed a moderate level of galectin-1 expression in ECs of normal human white matter (Stancic et al. 2011). Moreover, in mouse models, galectin-1 is expressed in brain ECs (Lotan et al. 1994). Galectin-1 colocalizes with the EC-specific marker VE-cadherin in the blood vessels in the brain of zebrafish embryos, and injection of galectin-1 antisense nucleotides induced hemorrhages in the head and cerebral vascular defects (Thijsen et al. 2006). These results indicate that galectin-1 is important for the formation of a normal cerebral vascular network.

Galectin-1 expression in glioma-associated ECs

Using quantitative immunohistochemistry, Rorive et al. (2001) showed that expression of galectin-1 was significantly higher in the vessel walls of oligodendrogliomas than in those of astrocytomas. In contrast, the galectin-1 expression was lower in the vessel walls of ependymomas than in those from the two other glioma groups (Rorive et al. 2001). When the tumor grade was taken into account, the percentage of vessel wall area exhibiting galectin-1 expression increased from pilocytic astrocytomas (PAs; grade I) to diffuse astrocytomas (grades II–IV) (Camby et al. 2001).

Galectin-1 expression in gliomas and its impact on angiogenesis

Galectin-1 is a hypoxia-regulated protein (Le et al. 2005) that has been shown to play major roles in angiogenesis (Thijsen et al. 2006; Hsieh et al. 2008; D’Haene et al. 2013; Croci et al. 2014). Because galectins are present inside and outside of cells, their role on angiogenesis could be related to their expression by TAECS or to their secretion by tumor cells. The expression of galectin-1 in brain tumors was first studied by Yamaoka et al. (2000). In this study, the expression of galectin-1 mRNA was shown to correlate with increased malignancy in human astrocytic tumors ranging from low-grade astrocytomas to high-grade gliomas (Yamaoka et al. 2000). Two other studies using clinical samples have shown that the galectin-1 protein is expressed in all glioma types and that the level of galectin-1 expression was lower in PAs than in grades II–IV astrocytomas, as observed for their ECs (Camby et al. 2001; Rorive et al. 2001). In addition, serum levels of galectin-1 were higher in patients with high-grade gliomas compared with controls (Verschuere et al. 2013), which could reflect galectin-1 leakage from tumor cells or ECs into the blood, suggesting an extracellular role of galectin-1.

A murine glioma model supports a proangiogenic role for brain tumor-derived galectin-1 because silencing of galectin-1 in glioma cells delays in vivo tumor progression with a decrease in VEGF secretion and vascular density (Verschuere et al. 2014). Moreover, in vivo delivery of an anti-galectin-1 siRNA in an orthotopic glioma xenograft mouse model significantly decreased angiogenesis. Galectin-1 was shown to regulate the expression of oxygen-regulated protein 150, which, in turn, controls VEGF maturation (Le Mercier et al. 2008). Finally, it was reported that downregulating galectin-1 expression in Hs683 human glioma cells through targeted siRNA provokes a marked decrease in the expression of the brain-expressed X-linked gene (BEX2), a feature that impairs vasculogenic mimicry channel formation in vitro and angiogenesis in vivo (Le Mercier et al. 2009).

Galectin-1 expression in other brain tumor-associated ECs

Little is known about the expression of galectin-1 in other brain TAECS. We found only that all primary central nervous system lymphomas (PCNSL) showed no galectin-1 expression in ECs (D’Haene et al. 2008).

Galectin-3

Galectin-3 expression in normal brain ECs

Mouse brain ECs express galectin-3 (Lotan et al. 1994). In human normal brain, different authors showed that the blood vessel walls express galectin-3 (Bresalier et al. 1997; Gordower et al. 1999; Tews 2000; Neder et al. 2004; Borges et al. 2010; Paixao Becker et al. 2010).

Galectin-3 expression in glioma-associated ECs

Some conflicting results exist regarding the expression of galectin-3 in ECs of PAs. Neder et al. (2004) report that a majority of PAs (13/15) did no stain for galectin-3. The same group confirmed this result in two series of 31 and 21 patients with PAs (Borges et al. 2010; Paixao Becker et al. 2010). In contrast to the previous studies, Camby et al. (2001) found that galectin-3 expression was high in blood vessel walls in a series of 22 supratentorial PAs. This difference could be explained by tumor location. Indeed, Camby et al. studied supratentorial
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PAs, while the series of Borges, Neder or Paixao Becker largely consisted of infratentorial PAs (only four supratentorial PAs were included). Moreover, Park et al. (2008) reported a tendency for negative galectin-3 expression in proliferative ECs of PAs but did not specify the tumor locations. A recent study showed that a specific molecular fingerprinting distinguishes supratentorial PAs from those originating in the posterior fossa (Mascelli et al. 2013). This suggests that PAs, with similar pathological features but located at different sites, may be distinguishable on the basis of cancer genetics. Differences were also observed in regard to the extracellular matrix. Maris et al. (2008) demonstrated that tenascin-C expression in PAs varies also observed in regard to the extracellular matrix. Maris et al. (2008) demonstrated that tenascin-C expression in PAs varies.

Different authors observed that galectin-3 was expressed in ECs in most cases of grades II and III astrocytomas but decreased or were absent in the proliferative ECs of GBM (Gordower et al. 1999; Camby et al. 2001; Strik et al. 2001; Neder et al. 2004; Park et al. 2008). All together, these data suggest that galectin-3 expression is decreased in ECs of GBM, which is a tumor characterized by EC proliferation. However, Park et al. observed that proliferative ECs of small-cell GBM expressed galectin-3 (Park et al. 2008) and Tews (2000) observed that proliferative ECs of GBM expressed occasionally galectin-3.

In oligodendrogliomas, the galectin-3 expression in ECs is variable; however, different authors found lower expression in high-grade tumors (Deininger et al. 2002; Neder et al. 2004). Moreover, Deininger et al. (2002) found that patients with low endothelial galectin-3 expression in oligodendrogliomas (grades II–III) had significantly shorter time to progression and overall survival than patients with high endothelial galectin-3 expression.

Galectin-3 expression in gliomas and its impact on angiogenesis

Galectin-3 is also a hypoxia-regulated protein (Greijer et al. 2005; Zeng et al. 2007) that has been shown to play major roles in angiogenesis (Nangia-Makker et al. 2000; Markowska et al. 2010, 2011; Machado et al. 2014). Similar to galectin-1, the role of galectin-3 in angiogenesis could be related to its secretion by tumor cells rather than its expression by ECs. As observed for galectin-3 expression in ECs, conflicting results exist about galectin-3 expression in gliomas (Le Mercier et al. 2010). Some authors found that the expression of galectin-3 is positively correlated with tumor grade (Bresalier et al. 1997; Strik et al. 2001; Deininger et al. 2002), whereas others showed the opposite result (Gordower et al. 1999; Camby et al. 2001) or showed not clear correlation between galectin-3 expression and tumor grade (Tews 2000). Moreover, whereas Deininger et al. (2002) described that fewer galectin-3-positive oligodendroglioma cells were detected in grade II than in grade III oligodendrogliomas, Tews (2000) observed that oligodendroglioma cells were galectin-3 negative (in grades II and III).

Galectin-3 expression in ECs of other brain tumors

The majority of hemangioblastomas showed galectin-3 expression in ECs (Al-Salam et al. 2013).

In a previous study on PCNSLs, we identified 11 of 46 cases with endothelial galectin-3 expression, whereas a majority of cases showed no galectin-3 expression in lymphomatous or ECs. Of the 11 cases expressing endothelial galectin-3, 5 presented endothelial hyperplasia. However, no significant association was observed between these two endothelial-related features. Moreover, endothelial expression of galectin-3 was evidenced (by means of mono- and multivariate survival analyses) as a bad prognostic factor for immunocompetent PCNSL patients. In addition, a combination of endothelial hyperplasia and/or endothelial galectin-3 expression was shown as an independent prognostic factor for immunocompetent PCNSL patients treated with methotrexate-based chemotherapy (D’Haene et al. 2008).

Other galectins

Galectin-2, -8 and -9 expressions were observed in normal brain ECs (Saal et al. 2005; Stancic et al. 2011).

Little is known about the expression of other galectins in brain tumors. Using quantitative immunohistochemistry, Camby et al. showed that galectin-8 is diffusely expressed in the vessel walls of astrocytic tumors without difference between grades (Camby et al. 2001).

Discussion and conclusion

Regarding galectin expression in brain ECs (Table I), we can conclude the following: (i) galectin-1 and -3 are expressed in normal brain ECs; (ii) ECs of high-grade gliomas are characterized by overexpression of galectin-1 and decreased expression of galectin-3; however, there are some discrepancies for the latter (positivity for small-cell GBMs); and (iii) a great heterogeneity was observed across tumor types (with an opposite profile of EC galectin expression between PCNSLs and gliomas).

The overexpression of galectin-1 in ECs and glioma cells suggests a proangiogenic role of galectin-1 in glioma angiogenesis, as described for other tumors (Thijssen et al. 2006, 2010; Croci et al. 2012; Mathieu et al. 2012; Laderach et al. 2013; Huang et al. 2014). Galectin-1 could be a new target in treatment of gliomas. Moreover, it should be interesting to study galectin-1 expression in patients treated by bevacizumab. Indeed, galectin-1 has been reported as being upregulated in anti-VEGF refractory tumors and silencing tumor-derived galectin-1 converted refractory into anti-VEGF sensitive tumors (Croci et al. 2014).

In contrast, the conflicting results observed for galectin-3 expression (in ECs and tumor cells) do not enable a conclusion about the angiogenic role of this galectin in brain tumors. Moreover, the clinical significance of galectin-3 expression in ECs differs between tumor types, i.e., good prognostic factor for oligodendrogliomas (Deininger et al. 2002) and poor prognostic factor for PCNSLs (D’Haene et al. 2008).

In their study, Deininger et al. (2002) showed that fewer galectin-3-positive oligodendroglioma cells were detected in grade II than in grade III oligodendrogliomas. In contrast, significantly more galectin-3-positive ECs were detected in grade II than in grade III oligodendrogliomas. These findings suggest
that a balance might exist between ECs and tumor galectin-3 expression for the regulation of angiogenesis and that, depending of the tumor type and/or localization, the role of galectin-3 in angiogenesis might depend on its expression by ECs or secretion by tumor cells.

A better understanding of the role of galectin-3 in brain tumor angiogenesis requires more specific studies that should incorporate precise information about the cells expressing galectin-3 as well as its subcellular localization. Indeed, different studies have shown that the functions of the galectins are related to their localization, and opposite functions of the same galectin have been observed to depend on the galectin being extra- or intracellular and nuclear or cytosolic (van den Brule et al. 2000; Cali et al. 2004; Hsu et al. 2006). Moreover, the identification of galectin-3 ligands in ECs of brain and the functional consequences of these interactions may provide new information about the role(s) of galectin in brain tumors and angiogenesis.

Additionally, in vitro studies should also focus on the specificities of brain blood vessels. Indeed, the ECs lining the cerebral capillaries differ fundamentally from other vascular endothelia in their capacity to regulate the passage of molecules and cells to and from the neural parenchyma. The brain is protected from the free diffusion between blood plasma and the interstitium by a vascular blood–brain barrier (BBB) to maintain CSF homeostasis. The mature BBB consists of a complex cellular system in which capillaries are lined by a single EC that is connected to neighboring ECs by different junctions (tight junctions and non-occluding adhesions junctions) and are regularly covered by a high number of pericytes that are embedded in a basal membrane and by astrocytic endfeet (Liebner and Plate 2010). A disruption of the BBB is a hallmark of many CNS pathologies, including tumors.

Finally, because the role of galectins are not limited to angiogenesis (Cambry et al. 2006; Newlaczyl and Yu 2011), it could be hypothesized that the difference in the expression profiles of galectin-3 in ECs of glioma and PCNSL could be related to different roles played by this galectin in these two types of tumors (e.g., cell adhesion, immunomodulatory function and apoptosis).

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Conflict of interest statement

None declared.

Abbreviations

BBB, blood–brain barrier; EC, endothelial cell; GBMs, glioblastomas; PAs, pilocytic astrocytomas; PCNSL, primary central nervous system lymphomas; TAECs, tumor-associated ECs; VEGF, vascular endothelial growth factor; WHO, World Health Organization.

References


