Mesenchymal/proangiogenic factor YKL-40 related to glioblastomas and its relationship with the subventricular zone

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Abstract
Glioblastoma is the most common primary brain tumor. Despite multimodality therapy with aggressive microsurgical resection and adjuvant chemotherapy and radiotherapy, the median survival is below 15 months. Glioblastomas are heterogeneous tumors with high resistance to most chemotherapeutic drugs. According to reliable evidence, YKL-40, one of the best investigated chitinase-like protein, may facilitate invasion, migration and angiogenesis, and could be also responsible for temozolomide resistance in glioblastoma, thus conferring a dismal prognosis. Previous studies have demonstrated that glioblastoma stem cells give rise to endothelial cells through an YKL-40 influence. Such factor is closely related to the subventricular zone. This review focuses on the most recent theories involving the possible relationship between topographic gliomagenesis related to the subventricular zone and YKL-40.

Key words: glioblastoma, subventricular zone, YKL-40, glioblastoma stem cells.

Introduction
Glioblastoma (GB) is the most common primary malignant brain tumor in adults [6,86], accounting for more than 45% of primary malignant brain tumors. Glioblastoma has an incidence that increases with age and peaks between 75 and 84 years old, being more common in white males, according to the most recent Central Brain Tumor Registry of the United States (CBTRUS) statistical report. The median survival for patients diagnosed with GB using the current standard of care is only 12 to 15 months [8,52,77,81] despite multimodality treatment with aggressive microsurgical resection, combined radiation and chemotherapy, and adjuvant chemotherapy [81]. GB cells are diffusely infiltrative and motile; consequently, GB renders them incurable by surgery alone [24,78,79]. Thus, a novel therapeutic approach is urgently needed to control recurrence and overcome resistance to treatment.

Over the last few decades, it has become clear that GBs are characterized by an extreme degree of phenotypic, cellular, genetic, epigenetic, and radiological heterogeneity, as implied by the older term “multiforme” [37,73], which challenges our ability
to understand or treat it. There have been several studies that seek to determine which factors can be prognostic or predictive to impact on overall survival and progression free survival [3,19,33,41,46,53,55].

Although currently more research is needed, biomarkers must be taken into account when deciding which treatment modality is most appropriate for the individual patient. We review the evidence and theories involving the possible relationship between topographic gliomagenesis related to the subventricular zone (SVZ) and YKL-40, in an attempt to reveal either cellular mechanisms or molecular factors associated with ubiquitous GB stem-like cells (GSCs) support and motility. The following aspects are summarized: subventricular zone, mesenchymal factor YKL-40, radial glia and the perivascular tumor cells.

Levels of circulating, subgranular or subventricular YKL-40 (chitinase 3-like 1 or cartilage glycoprotein-39), have the potential to be used in the optimization of glioblastoma therapies. Elevated serum levels of YKL-40 were found in 55-75% of patients with GB with shorter OS [6,7,29,31].

One of the most recent and attractive evidence included YKL-40 as the most predictive and prognostic marker in patients with GB [15,87], and it has been shown directly associated with tumor radioreistance, invasiveness, migration, recurrence, chromosome 10 loss [6,29,54], hypoxia-induced mesenchymal transition [36], and poor clinical outcome prognosis. Recently, Akiyama et al. using a TMZ-resistant (TMZ-R) U87 GB cell in vitro and in vivo identified that YKL-40 could be also responsible for temozolomide resistance in GB and suggested that therapies targeting YKL-40 may be potentially beneficial in GB treatment [2].

**Glioblastoma stem cells and YKL-40**

Glioblastoma is composed of cancer cells and surrounding stromal cells with diverse genetic/epigenetic backgrounds. Increasing evidence suggests that the tumorigenic process in GB is apparently initiated and maintained by a rare and special subpopulation of slow-cycling clonogenic cells referred to as GSCs [28,62,66,67]. It has been assumed that overall survival heterogeneity [33,45,46] in patients with GB might be related to GSCs variability and brain microenvironment [58]. Presently, it is not clear what the origin of GSCs is, but presumably it may arise from SVZ stem cells. Based on in vivo evidence, GSCs are responsible for tumor growth, recurrence, and resistance to therapies [15,48,59] and endowed with unregulated self-renewal, robust proliferative potential, high motility, diversity of progeny association with blood vessels and white matter tracts, multi-lineage differentiation capacities, invasiveness [22,63], and relatively resistant to radio- and chemotherapies [56,80], which express markers of both undifferentiated and differentiated cells [73], with a similar behavior to neural stem cells (NSCs) [5], which are present during the early development of the brain [7]. Nevertheless, the specific intrinsic factors that govern such characteristics are not well understood [27,66,83].

Mounting evidence shows that GSCs are largely dependent on distinctive and specialized vascular, perivascular or perinecrotic microenvironment called “niche” [11,30,63,64,66,68,76]. Furthermore, some investigators observe that GSCs give rise to endothelial cells (ECs)60, as shown in Figure 1, and induce changes in vascular niches, characterized by the sprouting of new blood vessels, consisting of an abundant, leaky and highly disorganized “glomeruloid” vascular network through the cooperative secretion of pro-angiogenic factors [1,61,67], such as VEGF, IL-8 and YKL-40 [2,29,56], highly different in patients with the same tumor [42,56,60,82]. YKL-40, also known as chitinase-like protein 1 or human cartilage glycoprotein-39 [6], is a highly conserved glycoprotein that belongs to the glycosyl hydrolase family 18 with no chitinolytic activity [7,84], included as a mesenchymal marker overexpressed in GB and postulated as one of the most promising predictive serum markers since it was found to have elevated levels in the serum of patients with GB [6,29,31,32,35].

So far, YKL-40 has been found to induce tight interplay between the membrane receptors syndecan-1 and an adjacent membrane-associated protein integrin αvβ5 [21] on endothelial cells [70,71], and triggered a signaling cascade through pFAK [8,61] to MAP kinase ERK-1 and ERK-2 by regulating VEGF expression and inducing angiogenesis as an independent angiogenic factor under hypoxic conditions.

However, these vascular formations usually lack basement membrane and pericyte coverage. In addition, recent studies [1,44,66] support that vasculogenesis [34,56] by GSCs may occur directly via
differentiation of cells that participate in vasculogenesis, tumor growth, or indirectly via cytokines and chemokines production stimulated by hypoxia, which are known to activate endothelial cells. Interestingly, through a three dimensional reconstruction, Calabrese et al. demonstrated that brain GSCs are preferentially located in close contact to tumor microvasculature and that endothelial cells release trophic factors that maintain these cells in a self-renewing and undifferentiated state [12].

As a well-recognized component of the tumor microenvironment, intratumoral low oxygen concentration upregulates the expression of multiple factors such as hypoxia-inducible factors (HIFs), members of a subfamily of basic helix-loop helix transcription factor that regulates different aspects of cell biogenesis such as metabolism, migration, proliferation, differentiation, apoptosis, angiogenesis, resistance to chemotherapy, and stem cell maintenance [13,14,28,44,50]. Importantly, recent findings indicate that GSCs are the origin of tumor recurrence in glioblastoma [13,62,83]. Indeed, it has been demonstrated through a genetically-engineered mouse model that after arrest of tumor cell proliferation with temozolomide, the first cell population to undergo proliferation and lead to tumor regrowth is the nestin-positive (a marker also for neural stem cells) GSC population [13].

Growing evidence indicates that nuclear accumulation of HIF results in transcriptional activation of the vascular endothelial growth factor (VEGF) whose pathway is modulated by reactive oxygen species (ROS), and demonstrating VEGF downregulation following HIF1a gene deletion and that HIF1/2 determined VEGF levels [4,28,44]. Francescone et al. identified that YKL-40 (CHI3L1) closely upregulates VEGF expression, and YKL-40-induced tumor vasculogenesis is at least partially dependent on VEGF [21].

**Adult stem cells, human subventricular zone and YKL-40 expression in glioblastoma**

In the adult human brain, astrocytes are the largest glial population, and provide structural, metabolic, and trophic support for neurons. Astrocytes can also support proliferation of adult NSCs lining the SVZ. Adult neurogenesis is a lifetime process, which has been isolated from two specific neurogenic regions: the dentate gyrus of the hippocampus, and the subventricular zone of the lateral ventricles. In both regions, NSCs are identified as a subpopulation of astrocytes that are able to produce undifferentiated neuronal and glial precursors [13,18,40,67].

The adult SVZ, most pronounced in the dorso-lateral wall of the lateral ventricle, is the main

![Fig. 1. Model depicting the glioblastoma stem-like cells (GSCs) transdifferentiation into Ecs. YKL-40 acts as an angiogenic factor to trigger tumor vascular development. B) Magnetic resonance image of a glioblastoma tumor, and (A) tissue sample from the same tumor illustrating tumor microenvironment.](image-url)
source of new neurons in the adult brain, and contains a subset of astrocytes which behave as stem cells both in vivo and in vitro [18,25,30,43,45,68], and derive from radial glia (RG) cells [17,39]. In fact, RG cells also act as NSCs and source of neurogenesis, and probably give rise to astrocytes in the cerebral cortex [17,23,64,74]. In the adult human brain, the cellular composition and cytoarchitecture of the SVZ is organized into four distinct layers: layer I is found adjacent to the lateral ventricle, and represents a single layer of multi-ciliated ependymal cells; layer II, also known as a hypocellular layer [64], consisting of a diffuse network of a large number of astrocytic, ependymal and neuronal processes, but a few cell bodies; layer III, a strip of astrocytic bodies, and externally, layer IV, adjacent to the brain parenchyma, we find a transition zone composed of many myelin tracts and neuronal bodies (Fig. 2) [25,26,30,38,57].

Interestingly, NSCs, identified as a subpopulation of astrocytes called B1 astrocytes, give rise to actively proliferating transit amplifying progenitors (type C cells), which in turn differentiate into neuroblasts (type A cells) that differentiate into interneurons and eventually migrate toward the olfactory bulb (OB) circuitry, via the rostral migratory stream (RMS), preferentially located in the ventral anterior SVZ of the adult human brain (Fig. 3). In the adult human brain, there are a small number of migratory neuroblasts in the SVZ and RMS. Nevertheless, so far there has been no consensus about the exact mechanisms underlying such neural migration toward OB in the adult human SVZ, and also whether there is an RMS [16,57,65]. Although the cytoarchitecture of the adult human SVZ have been characterized, a transcriptional analysis has not been fully established and understood. Interestingly, a recent transcriptional analysis [51] distinguished human SVZ astrocytes from parenchymal astrocytes based on gene expression, suggesting that SVZ astrocytes (type B) maintain the stemness in the adult human brain. Alternatively, it was found that in vitro CSCs have a tropism toward normal vasculature.

A putative source of glioma cells is the SVZ, the largest area of neurogenesis in the adult human brain. NSCs line the lateral ventricles in the SVZ, and recruitment of these progenitor cells may play a role in the aggressive behavior encountered in GB. In animal studies, the SVZ demonstrated increased susceptibility to tumorigenesis compared with cortical regions. Experiments and clinical findings provide evidence that neuronal progenitor cells in the SVZ with a high migratory potential are involved in the aggressive GB subtype. Recently, the SVZ has been identified as the source cells of malignant gliomas [55,63-66,68].
Based on cancer stem cell theory, and images of GB, SVZ was classified according to one of these categories: type I – tumor in which the contrast-enhancing lesion contacts both the SVZ and the cortex; type II – tumor contacts the SVZ but not the cortex; type III – tumor contacts the cortex but not the SVZ, and type IV – tumor contacts neither the SVZ nor the cortex [46]. Regarding the multifocal and/or multicentric GBs there are many theories, but supported by few studies [46,69], showing an association with group I given the findings consistent with high migratory and invasiveness of cells, according to Willis’ theory. Although relevant data suggest that GSCs may be important in gliomagenesis originating from SVZ, recent research has differed from this argument, hypothesizing through a combination of clinical observations and mathematical modeling that GBs may arise from cells distributed throughout the white matter and not limited to the region of the SVZ [9].

In a novel and interesting study on the YKL-40 expression in developing human embryonic and fetal tissues conducted by Bjornbak [7], YKL-40 was found to be associated with tissues undergoing morphogenetic changes. In this research, YKL-40 was found significantly marked in GB, as compared with normal human brain SVZ. By using immunohistochemical, double-labeling immunofluorescence and mRNA analysis through brain development (from 6th to 21st week post-conception), the authors pointed out that YKL-40 may be implicated in controlling angiogenesis and access of peripheral cells to the forebrain.

On the cellular lever, Bjornbak also suggested that YKL-40 plays a role in the developing brain barriers as well as is possibly involved in the differentiation of a particular astrocytic lineage. Consistent with our previous findings [55], in this study there was a decreased YKL-40 immunoreactivity in proximity to the cortex and ECs of the pia mater were not positive for YKL-40. Additionally, YKL-40 immunoreactivity was found also to be produced by the choroid plexus epithelium and secreted into the ventricular system and either detected in both neuroepithelial cells and radial glial end feet. Strikingly, Antonelly et al. [3] reported on 22 children with GBs who underwent tumor resection and immunohistochemistry was performed on tumor tissue for YKL-40 immunoexpression, showing less expression and better OS. However, such disagreement with recent data may be due to a small sample size, as stated by the authors.

Recent studies with gene expression profiles have established that cells expressing increased mesenchymal properties have a tendency to display self-renewal capacity. Interestingly, mesenchymal signature genes such as YKL-40 (shown in the early stage of development and probably related to neural stem cells) and oncostatin M – which belongs to interleukin 6 group of cytokines – receptor are associated with highly invasive feature and worse prognosis in GB patients [49,72].

Current data provide several useful insights [47,71]. First, YKL-40-positive cells may be responsible for the aggressive and invasive pattern seen in GSC. What are the properties of the microenvironment that permit widespread invasion? The previous phenomenon probably could be mediated by NSCs cues or by direct response of YKL-40 over ECs. Since most GBs tumors occur late in life and recently it was suggested a relationship between YKL-40 and SVZ, we can estimate that GBs could be initiated either by unknown trigger factors over preformed pathways. As regards YKL-40 immunoexpression, is there a special association between SVZ regionalization and GB formation? Under this topic, further studies in large series are needed to evaluate how YKL-40 measurements and pathways change throughout life. Furthermore, currently we are conducting a study that seeks to determine the factors causing tumor YKL-40 overexpression and whether such factors are expressed in both SVZ in close relationship to GB and in SVZ without a close relationship with GB in the same brain patients.

The role of YKL-40, glial cells and perivascular scaffold in migration and invasion

The existence of possible anatomical scaffolds allowing motility and migration of neuronal precursors toward the olfactory bulb along the vessels was first suggested and reported by Bovetti et al. [11] based on experimental analysis of olfactory bulb (OB) in rodents. Ontogenically, RG cells have been described as the first glia to appear, developing probably from the neuroepithelial youngest cells [17]. Although RG cells maintain a close contact with the SVZ in humans throughout adulthood, its apical processes are shortened by a probable retractable
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Conclusions

Glioblastoma with SVZ infiltration showed decreased PFS and OS rates, probably due to GSCs and its aggressive mesenchymal growth pattern. YKL-40 seems to play a key role in the motility and migrating patterns of GSCs and their transdifferentiation into ECs. These findings may be associated with the localization of GSCs in the SVZ and the occurrence of a more invasive and migratory GB subtype. Furthermore, GSCs can be the source for inter-tumoral heterogeneity with an impact on clinical outcome.

Although tumor development is highly dependent of several molecular factors, angiogenesis may be the key to developing novel therapeutic agents able to inhibit molecular pathways responsible for GB.

Disclosure

Authors report no conflict of interest.

References


