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Minireview

Glioblastoma cells: A heterogeneous and fatal tumor interacting with the parenchyma

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ABSTRACT

Glioblastomas (GBMs) are considered to be one of the deadliest human cancers, characterized by a high proliferative rate, aggressive invasiveness and insensitivity to radio- and chemotherapy, as well as a short patient survival period. Moreover, GBMs are among the most vascularized and invasive cancers in humans. Angiogenesis in GBMs is correlated with the grade of malignancy and is inversely correlated with patient survival. One of the first steps in tumor invasions is migration. GBM cells have the ability to infiltrate and disrupt physical barriers such as basement membranes, extracellular matrix and cell junctions. The invasion process includes the overexpression of several members of a super-family of zinc-based proteinases, the Metzincin, in particular a sub-group, metalloproteinases. Another interesting aspect is that, inside the GBM tissue, there are up to 30% of microglia or macrophages. However, little is known about the immune performance and interactions of the microglia with GBMs. These singular properties of GBMs will be described here. A sub-population of cells with stem-like properties may be the source of tumors since, apparently, GBM stem cells (GSCs) are highly resistant to current cancer treatments. These cancer therapies, while killing the majority of tumor cells, ultimately fail in GBM treatment because they do not eliminate GSCs, which survive to regenerate new tumors. Finally, GBM patient prognostic has shown little improvement in decades. In this context, we will discuss how the membrane-acting toxins called cytolysins can be a potential new tool for GBM treatment.

Contents

Introduction

Glioblastoma (GBM) is the most common primary malignant glioma in adults and is characterized by a high mortality rate. Clinically, gliomas are divided into four grades and the most aggressive of these, grade IV astrocytoma or GBM, is also the most common in humans (Kleihues and Cavanee, 2000).The average survival of GBM patients from the time of diagnosis is less than a

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year. Standard treatment comprises resection of the majority of the tumor mass, followed by chemotherapy and radiotherapy (Kanu et al., 2009; McCarthy, 2006; Minniti et al., 2009). However, this kind of tumor is usually highly invasive, making it extremely difficult to treat by total surgical resection or radiotherapy, which contributes to frequent recurrences and a very poor prognosis. Few anticancer drugs can modify the rapid tumor growth, and none is ultimately efficient. Therefore, most patients develop tumor recurrences or progressions after this combination of treatments (Kumar et al., 2008; Yang and Aghi, 2009). Transforming GBM into a treatable entity will require new paradigms in cancer biology and the understanding of the mechanisms underlying GBM invasion, treatment resistance and recurrence. Like most solid tumors, GBMs consist of heterogeneous cancer cells (Faria et al., 2006), as well as competent to recruit vasculature, inflammatory cells and interact with stromal elements (Hanahan and Weinberg, 2000). In this report, we will approach the interaction of GBMs with their rich tumor microenvironment. In this context, we will discuss GBM capability to interact with other cell types, to grow and invade other brain regions rapidly, as well as the possibility of using new drugs in GBM treatment and the relevance of glioblastoma stem cells (GSCs) regarding tumor progression.

Glioblastoma vascularization

The growth of solid tumors is limited to the emergence of new blood vessels (Folkman, 1972; Greene, 1961). In 1986, Dvorak classified solid tumors as wounds that do not heal, based on their requirement of the surrounding stroma to grow beyond a minimal diameter size, and also, with regard to their capability to induce a massive and continuous angiogenesis.

GBMs are among the most vascularized tumors in humans (Plate and Risau, 1995; Takano et al., 2010). In this type of tumor, malignity is often followed by endothelium proliferation (Daumas-Duport et al., 1988) and angiogenesis is correlated with aggressiveness, grade of malignancy and inversely correlated with patient survival. Indeed, the high microvessel density can be used as a prognostic postoperative indicator for patients with GBMs. Analyses from 93 sectioned formalin fixed paraffin embedded glioma samples specifically immunostained for von Willebrand factor showed a direct correlation between patients with shorter survival and higher microvessel counts, although the typical histopathological heterogeneity in GBMs could induce incorrect results (Leon et al., 1996). However, these proliferative GBM vessels exhibit abnormal morphology. Feigin et al. (1958) observed hyperplastic endothelial cells with neoplastic properties, forming a sarcomatous tissue intermingled with the pre-existing GBM mass. The endothelial cells possessed fairly large, plump, elongated or ovoid, vesicular, moderately chromatic nuclei, evidencing a high degree of variability and ultrastructural disorganization of the wall of small blood vessels (Nystrom, 1959). Morphological and phenotypical differences were observed in these vessels, such as variable diameters, permeability, tortuosity, heterogeneous distribution and an irregular basal lamina (Bart et al., 2000; Vajkoczy and Menger, 2004), including low VE-cadherin expression (Charalambous et al., 2006) and the absence of the tight junction proteins claudin-1 and -5 (Rascher et al., 2002). Moreover, endothelial cells from GBM express α-smooth muscle actin and exhibit a high proliferation and migratory capacity, and are also more resistant to apoptosis when compared to normal endothelial cells (Bian et al., 2006; Charalambous et al., 2006). All these features mentioned above result in sub-functional newly formed vessels (Bart et al., 2000; Vajkoczy and Menger, 2004) as summarized in Fig. 1. This abnormal vasculature is associated with thrombi and, consequently, with adjacent necrotic areas (Pietsch and Wiestler, 1997).

One possible explanation for this chaotic vascular organization is the overexpression of the VEGF (vascular endothelial growth factor) and poor pericyte recruitment (Bergers and Benjamin, 2003). The

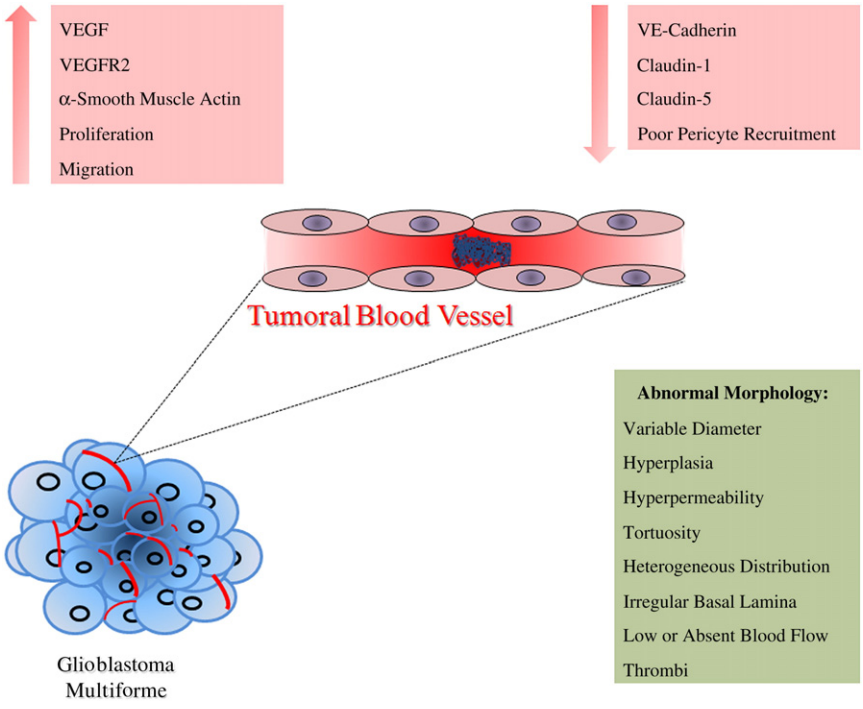

Fig. 1. Abnormal vascularization in Glioblastomas (GBM). Endothelial cells, that nourish GBMs, present morphological and molecular aberrations. Overexpression of the VEGF and its receptor, VEGFR2, can induce VE-cadherin cytoplasmic domain phosphorylation, which disrupts cell–cell contact, which then contributes to vessel hypermeability. Moreover, low levels of VE-cadherin or even the absence of the cytoplasmic tight junction proteins, claudin-1 and claudin-5, may equally result in the formation of leaky vessels. Pericytes are involved in mature blood vessel establishment, and, in GBMs, their poor recruitment can explain the immature blood vessel morphology. When compared to normal endothelial brain cells, GBM endothelial cells are more proliferative and migratory, prerequisites for angiogenesis. Finally, the angioarchitecture in GBMs is disorganized and subfunctional. GBM endothelial cells present common features such as: variable diameter, hiperplasia, hyperpermeability, tortuosity, heterogeneous distribution, irregular basal lamina, low or absent flow and thrombi.

VEGF is one of the most studied molecules concerning angiogenesis and vessel permeability. In GBMs, the VEGF acts mainly as a hypoxia-inducible angiogenic factor. *In situ* GBM analyses show that the VEGF production is specifically induced in a subset of tumoral cells in the immediate proximity of necrotic/hypoxic areas (Shweiki et al., 1992). The VEGF recognizes and binds to its receptor, the VEGFR2 (flk-1) on the endothelial cell surface, leading to subsequent phosphorylation of the cytoplasmic domain of VE-cadherin. Phosphorylated cadherins undo their homotypical interactions and then disconnect endothelial cells from each other. This cell–cell adhesion disruption results in augmented endothelial cell migration and an increase in vessel permeability (Esser et al., 1998).

It has been proposed that GBM angiogenesis begins with GBM vessel cooption. In murine models, the prolonged contact between tumor and vessels has been shown to lead to the disruption of normal endothelial layers (Zagzag et al., 2000). This subsequent hypoxic microenvironment would then be responsible for the induction of VEGF expression, which in turn would stimulate angiogenesis.

Since GBMs are mainly vascular tumors, one approach involves treatments directed to the inhibition of angiogenic mechanisms. Recently, the vessel normalization treatment, which targets the VEGF and/or VEGF receptors, has been proposed as an alternative for GBM treatment. After vessel normalization, the vascular structure and function is improved, with local hypoxia and vessel permeability decreasing and benefiting survival (Batchelor et al., 2007; Chae et al., 2010; Sorensen et al., 2009). However, this anti-angiogenic treatment seems to have its limitations. Although the reduction of cerebral edema and intracranial pressure in GBM patients are confirmed, the tumor apparently continues to grow even after being clinically silent (Verhoeff et al., 2009).

Our knowledge regarding the molecular events guiding GBM sustained angiogenesis has greatly increased in the last 40 years. However, it is clear that basic research is essential for better clinical results in GBM patients.

Glioblastoma invasion: molecular mechanisms

One of the first steps in tumor invasion is migration (Egeblad and Werb, 2002). Tumor cells move by extending their leading edges, following directed locomotion upon cellular contraction and rear release (Wolf and Friedl, 2006). GBMs exhibit characteristic migrating cells from the main tumor mass towards the neighboring normal tissue. The malignant cells have the ability to infiltrate and degrade physical barriers, such as basement membranes, extracellular matrix (ECM), and cell junctions.

The invasion process includes the overexpression of several members of a super family of zinc-based proteinases, the Metzincin, of which metalloproteinases (MMPs) are a part of. In particular, gelatinases MMP-2 and MMP-9 are expressed by glioma cells in human brain-tissue samples. However, it has been reported that it is the endothelial cells forming the connective framework for solid tumors that express MMP-2 and MMP-9, which, in turn, could be confiscated by malignant cell receptors (Forsyth et al., 1999). Moreover, active MMP-2 modulates glioma cell migration (Deryugina et al., 1997). In GBMs, another important subfamily within the Metzincin are the ADAMs (metalloproteinases with a disintegrin domain) and the related ADAMTSs, which have additional thrombospondin domains, (Held-Feindt et al., 2006). The tissue inhibitors of metalloproteinases (TIMP-1, -2, -3, and 4) in contrast, modulate the MMPs proteolytic activity by forming complexes with these endopeptidases. The addition of TIMP-2 and specific antibodies against MMP-9 has reduced the invasion of glioma cells *in vitro* (Rao et al., 1994; VanMeter et al., 2001).

Once secreted into the ECM, the MMPs can be activated even by other active MMPs or serine proteases, by a process called the "cysteine switch", that removes or modifies the propeptide domain and ruptures the coordination bond formed between a catalytic zinc ion and a cysteine prodomain, thus changing the enzyme conformation to an active proteolytic form (Sternlicht and Werb, 2001). These proteases selectively degrade the ECM components and also establish and maintain the surrounding microenvironment, facilitating tumor-cell survival (Rao, 2003). They are involved in shedding activities and the cleavage of ECM molecules and transmembrane proteins. MMP activity, therefore, comprises the solubilization of cytokine, growth factor, receptor and adhesion molecule ectodomains, thus placing them in pivotal positions in relation to the extracellular regulation of cellular signaling (Murphy, 2008). In fact, as observed by Kessenbrock et al. (2010), MMPs can modulate processes that control cell growth, invasion, metastatic niches, inflammation, cell survival, adipogenesis or angiogenesis and may even act in a nonproteolytic manner.

Efforts to elucidate the molecular mechanisms responsible for the invasion of glioma cells have confirmed not only MMP activity but also integrin participation (Goldbrunner et al., 1998; Teodorczyk and Martin-Villalba, 2010). It has been shown that $alpha_v$–$beta_3$ integrin can directly bind to active MMP-2, thereby concentrating this protease on the tumor cell surface (Brooks et al., 1996), and that this interaction may be crucial for cell invasive behavior (Rupp et al., 2008). As suggested by Uhm et al. (1999), the $alpha_V$–$beta_3$ integrin serves as a physical link between the tumor cells and the ECM for cell locomotion, also providing the tumor cells with the ability to concentrate and regulate protease function, thus modulating the infiltrative capacity of malignant cells.

Another molecule directly involved with integrins and MMPs in glioma cell invasiveness is the Transforming Growth Factor-β (TGF-β) (Platten et al., 2000). Before reaching the membrane receptors and activating the canonical and noncanonical signaling pathways, the human inactive forms of the TGF-β1, TGF-β2 and TGF-β3 molecules are released into the ECM. The TGF-β molecule is composed of a dimeric active part, a dimeric latency-associated protein (LAP), and a latent-TGF-β-binding protein (LTBP) bound to the ECM (Annes et al., 2003). Active TGF-β is derived from the inactive proprotein through the proteolytic conversion by furin or other proteinases, such as MMP-9 (Yu and Stamenkovic, 2000). Wick et al. (2001) reported the up-regulation of MMP-2 in the presence of increasing concentrations of exogenous TGF-β2 in GBMs. It has been shown that MMP-2 and MMP-9 expression can be modulated by TGF-β1 and TGF-β2, as well as by other growth factors, in GBM cell lines (Rooprai et al., 2000). In conclusion, the process of GBM cell invasion and dispersion can be modulated by the TGF-β ability to regulate integrins and MMP expression.

Microglia x glioblastoma interaction

In the tumoral microenvironment, the idea that GBMs contain particularly high levels of infiltrated microglia leads to the hypothesis that the microglia should present anti-tumoral activity. However, reports have suggested that the microglia in GBM sites provide an immunosuppressive environment, contributing to tumor progression (Daginakatte et al., 2008; Graeber et al., 2002; Kostianovsky et al., 2008). According to these studies, the accumulation of microglia in GBMs is the result of the local production of chemo-attractant factors produced by GBM cells, as the monocyte chemotactic protein-1 - MCP-1 (Platten et al., 2003; Prat et al., 2000). Microglial growth factors such as colony stimulating factors and the hepatocyte growth factor/scatter factor (HGF/SF) are also secreted by glioma and stimulate microglial proliferation at the lesion site (Alterman and Stanley, 1994; Badie et al., 1999). Furthermore, glioma cells also produce anti-inflammatory cytokines, such as interleukin-6 (IL-6) and TGF-β2. In particular, TGF-β2 inhibits the proliferation and secretion of pro-inflammatory cytokines by microglia (Hao et al., 2002; Parney et al., 2000). On the other hand, microglia are a source of MMPs. Their release

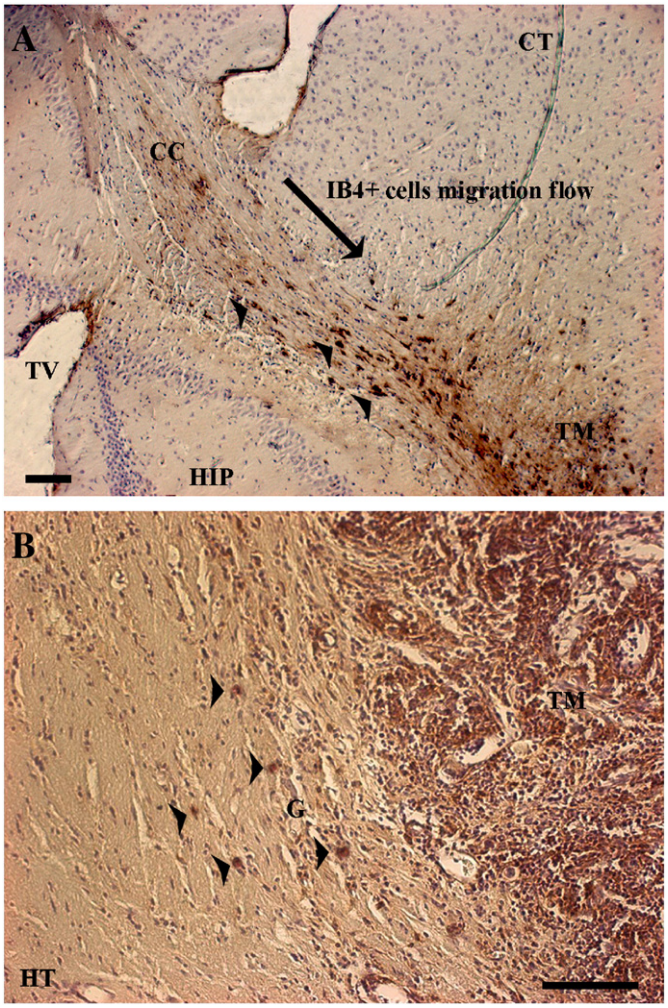

Fig. 2. Microglial cells migrate, through the corpus callosum, from the contralateral hemisphere to the ipsilateral hemisphere, where the GBM was xenotransplanted. The tumor was produced from GBM cells injected into the caudate putamen of mouse brains as previously described (Zhao et al., 2008). In this study, we used the human tumor cell line GBM95, established in our lab (Faria et al., 2006). After 15 days, the brains were perfused with fixative 4% paraformaldehyde, cut into slices and the microglial cells were stained with isolectin B4 (IB4, arrowheads). A: corpus callosum; B: ipsilateral hemisphere, note strong staining for IB4 in tumor mass. CTX = cortex, CC = corpus callosum, HIP = hippocampus, G = gliosis, HT = healthy tissue, TV = third ventricle, and TM = tumor mass. Bar: 100 μm.

at the lesion site facilitates the proliferation and migration of tumor cells and increases the progression and invasion of tumors in the brain parenchyma (Rao et al., 2003). In this sense, Markovic et al. (2009), using *in vivo* and *in vitro* mouse models, have demonstrated that Membrane-Type-1 MMP (MT1-MMP) is up-regulated in glioma-associated microglia, but not in the GBM cells themselves. Glioma-released factors trigger the expression and activation of MT1-MMP via microglial toll-like receptors and the p38 mitogen-activated protein kinase (MAPK) pathway. Consequently, microglial MT1-MMP, in turn, activates glioma-derived pro-MMP-2 and promotes glioma expansion (Markovic et al., 2009). The microglia also secretes factors that promote tumor proliferation, including the epidermal growth factor (EGF) and the VEGF (Lafuente et al., 1999; Tsai et al., 1995). The enzyme cyclooxygenase (COX)-2 is found abundantly in microglia isolated from GBMs, which increases the production of prostaglandin E2 (PGE2) and contributes to the development of cerebral edema in GBMs (Badie et al., 2003). In another inflammatory scenario, as infection by parasite (Rozenfeld et al., 2003) or by bilirubin-induced reactive microglia (Silva et al., 2010), for instance, the microglia response is also COX-2/PGE2 production and this could suggest a general microglia response during inflammation. Furthermore, a study using oncolytic virotherapy showed that, when macrophages/microglia are depleted in brain slices *ex vivo*, the intratumoral oncolytic viral titer increases 10-fold (Fulci et al., 2007), suggesting that phagocytosis mediated by these infiltrating macrophages directly affects viral clearance from the tumor. According to these studies, microglia have a key role both in the progression and invasion of GBMs.

Microglia may rapidly recruit, invade and infect GBM sites to participate in the destiny of glioblastomas as well as illustrated in Fig. 2. This Figure shows microglial cells crossing the corpus callosum from a contralateral hemisphere to invade a glioblastoma in an ipsilateral hemisphere. At first, infiltrated microglia acts by defending the brain parenchyma from the tumor (Galarneau et al., 2007; Synowitz et al., 2006); however the consequences of microglial recruitment depend not only on the microglia but also on the interaction of these cells with the microenvironment, where other cell components, including glioma cells, have an important role. According to Ghosh and Chaudhuri (2010), microglial pro-glioma action might just be a passive mode of assistance used by neoplastic cells into the central nervous system (CNS). Microglia, the CNS defense cells, when activated by gliomas, secrete a range of factors, such as MMPs, to degrade the ECM and arrive faster at the lesion site. However, glioma cells utilize this strategy in their own favor, to invade and expand into the brain parenchyma. Thus, microglia intend to fight against glioma, but lose control of the situation, favoring tumor progression (Ghosh and Chaudhuri, 2010). A better understanding regarding the activities of these CNS defense cells is essential in order to establish effective strategies to combat malignant gliomas (Ghosh and Chaudhuri, 2010; Kostianovsky et al., 2008; Yang et al., 2010).

Pore-forming proteins: a potential new class of chemotherapeutic drugs for GBM treatment

Despite the progress in cancer treatment and the consequent improvement in survival rates, only modest advancements in the treatment of GBMs have occurred in the last decades. To overcome this picture, new therapeutic strategies against the highly proliferative activity of GBM cells have been studied and new molecules acting against tumoral proliferation have been researched. Despite the use of flavonoids and other natural toxins, an unexpected molecule, the Stress-inducible protein 1 (STI1), also referred as hop (Hsp70/Hsp90 organizing protein) plays a role in the glioblastoma proliferation. It is a 66 kDa protein first identified in yeast and originally described as a cochaperone that binds to both Hsp70 and Hsp90, and regulates their activities (Chen and Smith, 1998; Nicolet and Craig, 1989; Song and Masison, 2005). We demonstrated that STI1 is produced and delivered by normal astrocytes (Lima et al., 2007) and glioblastoma cells. In the culture medium this molecule is competent to induce the proliferation of human glioblastoma, but not efficient to proliferate normal astrocytes and human breast cancer cells and this effect is mediated by the MAPK and PI3K signaling pathways (Erlich et al., 2007).

We have also tried a flavonoid against glioblastoma proliferation. Isoquercitrin, a flavonoid isolated from the aerial parts of *Hyptis fasciculata*, decreased GBM proliferation up to 90% without inducing apoptosis of the tumoral cells in culture, modulating the control of the cell cycle. The β-catenin-mediated signaling may be involved on this antiproliferative activity of Isoquercitrin (Amado et al., 2009). Similar results were obtained using curcumin, a curcuminoid derived from the rhizome of *Curcuma longa* (Senft et al., 2010). Another class of natural compounds, the cytolysins, do not need to be internalized to produce its cytotoxic effects (Parker and Feil, 2005). These proteins, produced by a large number of organisms, form pores in biological

Table 1
Equinatoxin-II (EqTx-II) increases chemotherapeutic drugs-induced cytotoxicity against U87 GBM cell line.

Chemotherapeutic drug	IC_{30} (μmol/l)	+ EqTx-II	
		IC_{30}	Ratio
–	–	8.5 μg/ml	–
Cytosine arabinoside	8.4	0.5 μmol/l	17
Doxorubicin	7.7	0.8 μmol/l	9.6
Vincristine	0.3	0.001 μmol/l	300

IC_{30} values were determined by linear regression from individual experiments using GraphPad software (GraphPad software Inc., San Diego, California, USA).
Ratio = IC_{30} from the chemotherapeutic drug/IC_{30} from the combination of chemotherapeutic drug + EqTx-II.

membranes, resulting in cell death (Parker and Feil, 2005). Despite being cytotoxic against many types of cancer cells per se, cytolysins could also be conjugated to other drugs to selectively kill cancer cells or improve the local delivery of chemotherapeutics.

We have studied the anti-proliferative action of two sea anemone cytolysins, toxin Bc2 (isolated from the Brazilian sea anemone *Bunodosoma caissarum*) and equinatoxin-II (EqTx-II a gift from Anderluh, G.; isolated from *Actinia equina*) against GBM cell lines in culture. Toxin Bc2 and EqTx-II decreased cell viability and increased lactate dehydrogenase (LDH) release in a concentration-dependent manner (Soletti et al., 2008, 2010a). The pre-treatment with mitogen-activated/extracellular regulated kinase (MEK1), protein kinase C (PKC) or Ca^{2+}/calmodulin-dependent kinase II (CaMKII) inhibitors blocked the toxic effects of toxin Bc2 and EqTx-II (Soletti et al., 2010a). Swollen, dead or dying cells were negative for TUNEL-staining, suggesting that sea anemona cytolysins induced necrotic-like cell death by activating those intracellular signaling pathways (Soletti et al., 2010a). These results are comparable to those obtained using gomesin, a pore-forming peptide from hemocytes of the spider *Acanthoscurria gomesiana,* that induced cytotoxicity in human neuroblastoma cells through MAPK/ERK, phosphoinositide 3-kinase (PI3K) and PKC signaling pathways (Soletti et al., 2010b).

Since cancer cells show changes in membrane lipid composition (Lavie and Liscovitch, 2000) and pore-forming cytolysins require high levels of sphingomyelin or cholesterol for membrane binding and permeabilization (Alegre-Cebollada et al., 2006), toxin Bc2 (1 μg/ml) and EqTx-II (10 μg/ml) were significantly more toxic to GBM cells, when compared to astrocytes in culture (Soletti et al., 2008).

Moreover, non-cytotoxic concentrations of EqTx-II (0.3 mg/ml) were able to enhance the cytotoxicity induced by the classic anticancer agents cytosine arabinoside, doxorubicin, and vincristine on glioblastoma cell lines, as summarized in Table 1. Vincristine, a microtubule disturbing agent, showed a high potential in killing GBM cells (up to 300-fold) with increased number of apoptotic and disturbed mitotic-like figures, which is a characteristic of vincristine treated cultures. Since sea anemone cytolysins form oligomeric transmembrane pores with 1–2 nm of diameter (Anderluh et al., 2003), it is possible to increase cell permeability to small molecules up to vincristine size (Soletti et al., 2008).

Therefore, both in high concentrations and in non-cytotoxic concentrations, as well as in combination with classic chemotherapeutic drugs, pore-forming proteins are a potential new drug class for treating GBMs.

Glioblastomas and their heterogeneity

In the late nineteenth century, scientists suggested that an exceptional population of cells with stem-like properties might be the source of tumors (Cohnheim, 1867, 1875; Durante, 1874). Although frequently present in small numbers, cancer stem cells (CSC) have the ability to originate tumors when xenotransplanted into animals, whereas the remaining non-CSC tumor mass most often cannot (Jordan et al., 2006; Wicha et al., 2006). Moreover, it has been found that GSCs have a high tumorigenic potential and a low proliferation rate and present some phenotypical similarities with normal stem cells, such as the CD133 gene expression and other genes commonly expressed in neural stem cells (Tirino et al., 2008). Furthermore, it has been reported that multidrug resistance-associated proteins, as well as P-glycoprotein, conferring multidrug resistance proteins (MDR) are currently associated with glioblastoma malignance. In fact, multidrug resistance in gliomas is the major challenge in clinical settings. We investigated the expression of P-glycoprotein (Pgp) and multidrug resistance-related protein 1 (MRP1) in 50 gliomas using immunohistochemistry (de Faria et al., 2008). Compared to Pgp, MRP1 positivity was observed in highest percentage of glioblastoma than other lower grade of gliomas. In the other hand, gliomas grade II exhibited a more important expression of Pgp if compared to grades III and IV (glioblastoma). These results suggest that the difference between the histological grade gliomas regarding MRP1 and Pgp expression must have implications in the choice of chemotherapeutic protocols (de Faria et al., 2008). However, looking to cancer stem-like cells obtained from human glioblastoma, defined as tumorospheres, was reported higher expression of these proteins when compared with primary adherent cells derived from the same tumor (Shervington and Lu, 2008).

Particularly, the higher expression of multidrug resistance-associated proteins 1 and 3 was observed when compared with primary adherent cells derived from the same tumor (Salmaggi et al., 2006). Taken these data together it seems possible to hypothesize that MDR proteins family with complementary studies would improve our prospects for developing effective glioma treatments.

The malignant glioma resistance to radiation and chemotherapy, evokes the aggressive study of the molecular mechanisms underlying cancer cell survival and expansion (Dean et al., 2005; Diehn et al., 2009; Eyler and Rich, 2008). The importance of targeting CSC derives

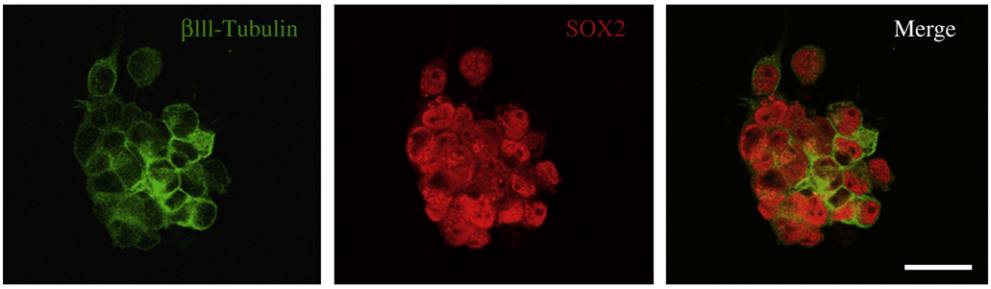

Fig. 3. GBM stem cells (GSCs) express βIII-tubulin and SOX2. Immunocytochemistry analysis of βIII-tubulin (neuronal marker) and SOX2 (stem cell marker) expression in a cancer stem cell line derived from primary cultures of adult human glioblastoma. Images were observed in an Axioplan 2 epifluorescence microscope (Zeiss, Göttingen, Germany). Bar: 10 μm.

from several observations showing that CSC, in addition to having increased tumor-seeding potential, are resistant to a variety of chemotherapy drugs and radiation treatment. This suggests that, as chemo- and radiation therapies fail to completely eradicate the disease, the residual cancer cells will be highly enriched for cells that persist in a CSC state. Therefore, these considerations indicate that, to be effective in the long-term, cancer therapies should include agents that target CSC to prevent the regrowth of neoplastic cell populations.

The development of cancer therapeutic strategies is restricted by the fact that most potential treatments perturb organ function by themselves, exhibiting negative side effects, which currently preclude its clinical application. So far, no effective pharmacological approach to selectively eliminate gCSC has been developed for practical use in clinical settings.

Although the origin of GSCs has not yet been unveiled, these cells have been described by several groups (Galli et al., 2004; Singh et al., 2004; Patru et al., 2010). GSCs are functionally defined by their self-renewal potential that can be confirmed by serial neurosphere (Fig. 3) formation assay and tumor propagation by intracranial xenotransplantation. Besides, GSCs have been shown to differentiate into astrocytes, oligodendrocytes and neurons (Galli et al., 2004; Singh et al., 2004;), as well as modulating immune responses (Wei et al., 2010), dispersing into new locations (Hoelzinger et al., 2007; Inoue et al., 2010) and supporting tumor neovasculature through angiogenesis promotion (Bao et al., 2006; Weller, 2010). We have differentiated stem cells from a human glioblastoma, isolated by neurosurgery from patients, into astrocytes, oligodendrocytes and neuron using oncospheres and soluble factors added to the cultures. Interestingly, some of these GSC show double staining, to GFAP and Neurofilament which could suggest a profil of a described malignant glioneuronal tumor (MGNT). This tumoral entity might arise from mutated neural stem cells in combination with aberrant environmental signals (Varlet et al., 2004; Patru et al., 2010).

All data above show that tumoral stem cells (TSC) are point-out as a new necessary target to anti-cancer therapy and the elucidation of TSC properties needs of more research to walk against the tumor.

Conclusions and the future

GBMs are the most malignant tumors with an astrocytic lineage. Despite a substantial increase in cancer research, which has led to the development of treatments for some solid human cancers, no therapies have been effective in treating these tumors. The potential of cytolisins as new chemotherapeutic drugs discussed in the present paper may be a way to fight GBMs. Our intention was to bring into debate some relevant properties of this kind of tumor, such as the presence of GSCs and their possible involvement in tumor growth. The resistance to chemotherapy and the processes of angiogenesis and invasiveness developed by the tumors may be due to these GSCs, as well as the GBM ability to induce microglia cooperation in tumor progression. Deregulation of signal transduction, which accounts for aberrant responses to distinct soluble factors, is also a common feature of these tumors, and modulation of signaling pathways has become an option for targeted therapies (Sebolt-Leopold and Herrera, 2004). There is an urgent need to research treatments that could control the growth and invasion of GBMs in the cerebral parenchyma. A better understanding about the properties of GBMs and the interactions of this type of tumor with its microenvironment is essential to establish effective strategies in the combat of malignant gliomas.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

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