Cancer stem cells - tumor-initiating cells in most tumors - generally are characterized as the population of cells within a tumor that have the ability for self-renewal and aberrant differentiation, initiate tumorigenesis, migrate, and regenerate a phenocopy of the original patient's tumor when administrated into immuno-compromised animals *in vivo* (for a review see Soltysova et al., 2005). Cancer stem cells share many of the properties of normal stem cells and can originate from mutated stem cells and/or from somatic cell that converted to cells similar to stem cells (Fig.1). These include resistance to toxic drugs through the expression of several ABC transporters, an active DNA repair capacity, resistance to apoptosis, and lack of relative quiescent cell stages.

Cancer stem cells in glioblastoma are designated by different names. Glioblastoma stem cells, glioblastoma stem-like cells, glioma cancer stem cells and glioma-initiating cells are regarded as synonyms. Generally it is agreed that these cells are tumor initiating and involved in tumor progression (Altaner, 2008b). In this article I will call them glioma cancer stem cells (GCSC). Biological properties of glioblastoma cells, like resistance to chemotherapy and radiotherapy, their infiltrative nature, proliferative behavior, and progressive character, have strongly supported the suspicion that glioblastomas contain GCSC. Brain tumor mass is formed by dividing tumor cell clones, by GCSC and other stroma forming cells.
Glioma cancer stem cells and their role in therapy

There are several reasons why conventional therapies for malignant glioblastomas are not curative. High-grade brain tumors exhibit a devastating malignant progression, characterized by widespread invasion throughout the brain. The tumor invasion is facilitated by normal brain parenchymal cells, which secrete ligands that can stimulate receptors on invasive glioma cells. Glioblastoma cells produce factors, like growth factors, cytokines and chemokines, which support the growth and the infiltrating character of glioblastoma cells in paracrine and autocrine fashion (Liu, 2011). Tso et al., (2006) observed that high-grade glioblastomas express cellular and molecular markers that are associated with mesenchymal stem cells. It is not known whether glioblastoma cells are derived from transformed stem cells or that activated genes in glioma cells elicit mesenchymal properties of cancer cells. To the heterogeneity of glioblastoma multiforme cells contribute mesenchymal stem cells that home in the tumor mass, and together with other cells make up the tumor stroma. Secreted factors from all cells present in the tumor mass are able to diffuse through the peritumoral stroma, thereby influencing parenchymal cells that surround the tumor mass and simultaneously with hypoxic conditions are creating a permissive microenvironment for malignant progression (Hoelzinger et al., 2007; Oka et al., 2007a). In addition, the immunosuppressive character of MSCs might protect the tumor cells from attack by immune cells. The most serious reason for resistance of glioblastoma cells is very likely the existence of cancer stem cells - glioma cancer stem cells.

Glioblastoma multiforme (GBM) has a polyclonal character being composed from clones of dividing tumor cells and a few tumor initiating cells. Conventional therapies like radiotherapy and cytotoxic chemotherapy kill dividing cells, but slowly dividing cancer stem cells remain unaffected. These therapies select for the more aggressive GCSC, leading later to frequent tumor relapse with tumors resistant to further conventional therapy (Fig.2).

**Identification of cancer stem cells in brain tumors**

The first identification of human GCSCs that had the capacity to initiate tumors in vivo, using the xenograft assay approach, was reported by Singh et al., 2003 and 2004. A brain tumor fraction of CD133+ cells at a low dose (100 live cells) produced a tumor that was serially transplantable in NOD/SCID mouse brains and was a phenocopy of the patient's original tumor. A dose of $10^5$ live CD133- cells similarly injected did not cause a tumor. Similar findings were reported for adult glioblastomas (Yuan et al., 2004) and several childhood brain tumors (Hemmati et al., 2003). CD133 (Prominin-1) is a transmembrane glycoprotein consisting of five membrane domains: two N-glycosylated extracellular loops, two cytoplasmic loops, a cytoplasmic C-terminal and an extracellular N-terminal (Yin et al., 1997; Miraglia et al., 1997). Further characterization of GCSCs led to conclusions that they are stem-like cells. They express high levels of stem cell genes involved in self-renewal and genes that regulate neural stem proliferation and differentiation commitment, such as Sox2, Notch, Bmi1, Sonic hedgehog, Musashi-1, CD133, endothelin 3 (Hemmati et al., 2003; Chen et al., 2010; Tso et al., 2006; Liu et al., 2011). Obviously, key mechanisms that control the activity of normal neural progenitors are altered in brain tumors. Recently, using tissue microarrays, CD90 was identified as a marker for GCSCs in primary human high-grade gliomas. Expression levels of CD90 were in good correlation with WHO grades, in high-grade gliomas, and were...
significantly higher than in low-grade gliomas. Interestingly, CD90 and CD133 markers were co-expressed in GBM, where 100% of the CD133+ cells were also positive for CD90, but only a small part of CD90+ cells were positive for CD133, indicating that the CD133+ stem-like cells are a subpopulation of CD90+ cells in GBMs in vivo (He et al., 2011). Careful histochemical analysis of CD133(+) cells revealed that different cell phenotypes exist according to their in situ localization. CD133(+) niches contain stem-like cells with a lower proliferation index than CD133(+) single cells, which have an endothelial differentiation profile, suggesting a role in angiogenesis (Christensen et al., 2011). There are some doubts that CD133 is a universal marker for brain tumor stem cells. The biological function of the CD133 protein is unknown, and there is experimental evidence for the existence of both CD133+ve and CD133-ve populations in tumor initiating cells (Beier et al., 2007; Wang et al., 2008). The biological role of CD133+ progenitor cells seems rather wide and promiscuous. For instance, Barcelos et al., 2009 reported that CD133+ progenitor cells promoted the healing of ischemic ulcers by stimulating angiogenesis and activating the Wnt pathway.

Glioblastoma stem cells and the vascular niche

Cell microenvironment, the so-called stem cell niche, plays an important role in maintenance of stem cells (Lathia et al., 2011). Vascular endothelial growth factor promotes the proliferation of vascular endothelial cells and the neurogenesis of neural stem cells. From experiments with GCSCs, derived from glioblastoma infected with retrovirus expressing VEGF (Oka et al., 2007b; Gilbertson and Rich, 2007), it seems likely that glioblastoma stem cells are dependent on cues from aberrant vascular niches that mimic the normal neural stem cell niche. Mao et al., 2011 examined the role of the ZNF217 oncogene that is frequently amplified in many kinds of tumors. It is associated with aggressive tumor behavior and poor clinical prognosis. They found that the ZNF217 gene was amplified in 32% and over expressed in 71.2% of GBMs. Glioblastomas harbor multiple cell types, some with stem cell-like properties. Interaction of these tumor cells with tumor-associated parenchymal cells, such as vascular cells, microglia, peripheral immune cells, and neural precursor cells, also play a vital role in controlling the course of pathology. Microglial cells, which can contribute up to 30% of a brain tumor mass, play a role in glioblastoma cell invasiveness. Mutual interaction of all these cells, and trophic factors secreted by them, influence tumor behavior. The multiple interactions between parenchymal and tumor cells were reviewed by Charles et al. (2011).

Studies on mechanisms of tumor vasculogenesis in glioblastoma revealed that newly formed vessels might be of GCSCs origin. It was shown that a variable number (range 20-90%, mean 60.7%) of endothelial cells in glioblastoma carry the same genomic alterations as tumor cells, indicating that a significant portion of the vascular endothelium has a neoplastic origin. The connection between neural stem cells and the endothelial compartment seems to be critical in glioblastoma, where GCSCs closely interact with the vascular niche and promote angiogenesis through the release of vascular endothelial growth factor (VEGF) and stromal-derived factor 1. The neoplastic origin of glioblastoma capillaries is supported by experiments in immunocompromised mice with subcutaneous xenografts induced by inoculation of GCSCs. The vessels in tumor xenografts were found to be primarily composed of human endothelial cells (Ricci et al., 2010).

Role of hypoxia in GBM

Hypoxia or the so called "hypoxic niche" plays a crucial role in controlling the GCSC molecular and phenotypic profile. GCSCs may be maintained in vivo in a hypoxic niche. Therefore, targeting the hypoxic pathways therapeutically might be a promising additional therapy (for a review see Barr, 2011). Hypoxic environment of the GBM contributes to creation of a specialized hypoxic niche where GCSCs reside. The hypoxic niche regulates tumorigenic capacity primarily through the hypoxia-inducible factors HIF1α or HIF2α, reflected in the level of ZNF217 gene overexpression. The activity of protein phosphatase 2A (PP2A), a regulator of cell cycle in human GBM, is induced by hypoxia. It was found that patients with higher PP2A activity had significantly worse survivals compared to patients with low levels. PP2A appears to reduce the metabolic demand of hypoxic GCSCs and enhances tumor cell survival (Hofstetter et al., 2012 ). Persano et al. 2011 proposed the theory of the three-concentric layer model for GBM mass. According to this model, GBM stem cells reside preferentially within the hypoxic core of the tumor mass, while more differentiated cells are mainly localized along the peripheral and vascularized part of the tumor. Clinical implications arising from the three layer model of GCSC distribution in GBM recommend to neurosurgeons the complete removal of the central region of tumor in order to reduce the residual GCSC population. Unfortunately, GCSCs, even in very low numbers, are present in the more peripheral regions of the tumor, the likely reason for tumor recurrence, even after total removal of the primary tumor mass. Hypoxia inducible transcription factors (HIF1α and HIF2α) play an important role in expression of CD133 protein on stem cells. Li et al., 2009 found that HIF2α is highly expressed in CD133-positive cells, whereas HIF1α is expressed in CD133-negative cells. Furthermore, a significant association between HIF2α-transcription levels and poor patient prognosis has been demonstrated in glioblastoma. Griguer et al., 2008 have
shown that the established glioblastoma cell line U251MG, previously recognized to be CD133-negative, developed CD133-positivity with decreasing oxygen tensions.

**Tumor microenvironment and GCSCs**

Radiotherapy is the most effective non-surgical therapy for GBM patients. However, patients succumb due to tumor recurrence within a year. It was shown that treatment with ionizing radiation enriched the glioblastoma cell population with GCSCs positive for activated stem cell-associated pathways such as β-catenin (ABC), Sox2 and Wnt. (Kim et al., 2012). The molecular mechanism of radiation resistance of GBM is mediated by selection of subpopulation of GCSCs with activated stem cells genes in addition; radioresistance of GBM is not simply an intrinsic characteristic of glioma stem cells but a result of interactions between these cells and microenvironmental factors (Mannino and Chalmers, 2011). Recently Jamal and colleagues, 2012 experimentally proved that the brain microenvironment preferentially enhances the radioresistance of CD133(+) glioblastoma stem cells. They compared the response of CD133(+) glioblastoma stem cells with non-stem cell populations to irradiation under in vitro and intracerebral growth conditions. Radioresponse of CD133(+) glioblastoma stem cells and non-stem cells did not differ under in vitro growth conditions, while CD133(+) cells were radioresistant under orthotopic growth conditions. These findings are consistent with the suspected role for GCSCs as a determinant of GBM radioresistance. Obviously, the brain microenvironment is responsible for radioresistance of glioblastoma cells (Jamal et al., 2012).

There is evidence suggesting that activation of NOTCH signaling is required for propagation of GCSCs. One important function of endothelial cells in glioblastoma multiforme is to create a niche that helps promoting self-renewal of GCSCs. Zhu et al., 2011 clarified the molecular mechanism whereby endothelial cells provide the source of NOTCH ligands. They found that NOTCH ligands were expressed in endothelial cells adjacent to NESTIN and NOTCH receptor-positive cancer cells in primary GBMs. Therefore, targeting both GCSCs and their niche may provide a novel strategy to deplete GCSCs and improve GBM treatment. Burkhardt et al., 2012 hypothesized that intra-arterial chemotherapy might selectively target GCSCs residing in the perivascular stem cell niche.

**Therapy targeted to brain tumor initiating cells**

It is believed, that in most cancers therapy targeted to cancer stem cells might be curative. However, multiple evidence has recently indicated that GCSCs may not represent a restricted and infrequent GBM component; rather, they might constitute most cells within the tumor bulk (Mazzoleni and Galli, 2012; Kondo et al., 2004; Zheng et al., 2007; Zhou et al., 2009). Development of novel chemotherapeutic agents targeted to glioblastoma stem cells is of great interest. It is likely that all agents molecularly targeted to GCSCs will have cytostatic, but not cytotoxic inhibitory effects. Obviously, the combination of targeted cytostatic effects together with toxic effects to GCSCs and to the vascular niche would be necessary to achieve the elimination of those highly resistant non-diving cells. Some examples of inhibitors mainly acting on pathways important for GCSCs survival have been mentioned. Sai et al. 2012 found that a novel small molecule inhibitor of the JAK2/STAT3 pathway, WP1193, induced cell-cycle arrest and apoptosis in glioblastoma stem-like cells. WP1193 significantly decreased the proliferation of established glioma cell lines in vitro and inhibited the growth of glioma in vivo. Zhuang et al., 2012 reported that curcumin, a natural compound with low toxicity to normal cells, significantly induced differentiation of GCSCs in vitro and in vivo by inducing autophagy.

The Notch1-mediated signaling pathway has a central role in the maintenance of neural stem cells and contributes to growth and progression of glioblastomas. Fassl et al., 2011 demonstrated that the Notch1 receptor promotes survival of glioblastoma cells by regulation of the anti-apoptotic Mcl-1 protein. They have shown that inhibition of the Notch1 pathway overcomes apoptosis resistance and sensitizes glioblastoma cells to apoptosis induced by ionizing radiation. Similar observations were reported by Wang et al., 2010. Inhibition of Sonic hedgehog and Notch pathways enhances sensitivity of CD133+ glioma stem cells to temozolomide therapy (Ulasov et al., 2011). Glioblastoma cells grown in neural stem cell medium, supplemented with epidermal growth factor and basic fibroblast growth factor form spheroids are regarded as GCSCs. Ledur et al., 2012 reported that human U87 glioma form spheroids expressing the markers of glioma cancer stem cells CD133, Oct-4, and Nanog. However, messenger RNAs for several purinergic receptors were differently expressed in spheroids when compared to a cell monolayer not containing spheroids. Treatment of human gliomas U87 or U343, as well as rat C6 gliomas, with 100 µM ATP reduced the number of tumor spheroids in a dose-dependent manner. ATP also reduced the expression of Nanog, CD133 and Oct-4 showing that the purinergic system affects GCSC biology.

To block brain tumor stem cell self renewal and promote differentiation particularly if terminally differentiated cell types can be generated, such as neurons, may be another useful strategy for glioblastoma treatment (for a review see Dirks, 2010). Piccirillo and Vescovi, 2006 have shown that promotion of bone morphogenic protein 4 signaling can
enhance GCSCs differentiation and attenuate tumorigenic phenotype. Thus the differentiation therapy being successful in treatment of acute promyelocytic leukemia might be another approach worth to be studied.

Glioblastomas are highly vascular tumors. Therefore, it was expected that agents targeting angiogenesis may have efficacy. Recent preclinical and clinical investigations have revealed that agents targeting angiogenesis did not fulfill these expectations. Reasons for failure and new strategies based on molecular mechanisms of tumor vessel formation have been discussed by Tokano, 2012. Using patient derived specimens of glioblastoma, a subpopulation of GCSCs was identified being enriched for CD44(high)/Id1(high) cells, which tend to be located in a perivascular niche. It was found that growth of the CD44(high)/Id1(high) cells was inhibited by TGF-β inhibitors. Repression of DNA-binding protein (Id)-1 and -3 transcription factors decreased the GCSCs population that might inhibit the capacity of cells to initiate tumors (Anido et al., 2010).

**Stem cell based gene therapy**

Statistic data of cancer patient survival revealed that the most successful cancer therapy is surgery when applied early. Elimination of both dividing tumor cells and cancer stem cells before they can spread could provide a cure. This is usually not possible with high-grade brain tumors. At present, the standard therapy for GBM patients, consisting of tumor debulking, followed by radiotherapy and chemotherapy, is not a curative approach. There is increasing evidence that cytotoxic therapies select for more aggressive GTSCs. Recently developed tumor targeting therapy, driven by mesenchymal (stromal) stem cells (MSCs) possessing tumor tropic properties, brought hope for a novel therapeutic modality (Studeny et al., 2002; Studeny et al., 2004; Kucerova et al., 2007; Kucerova et al., 2008; Kucerova et al., 2010; Cavarretta et al., 2010; for a review see Altaner, 2008a). Prodrug cancer gene therapy mediated by MSCs transduced with yeast CD::UPRT might be one of several treatments with potential for curative therapy of high-grade brain tumors (Altanerova et al., 2012). The stem cell driven cytosine deaminase/5-FC system represents an attractive tool for activating the prodrug directly within the tumor mass, resulting in high local 5-FU concentrations without systemic toxicity. Expression of the yeast CD::UPRT fused gene (Kievit et al., 2000) and formation of 5-FC cause inhibition of both DNA and RNA synthesis, consequently leading to death of dividing and non-dividing cells, thus attacking GCSCs. In addition, mesenchymal stem cells transduced with cytosine deaminase induce the expression of pro-apoptotic genes in tumor cells (Cihova et al., 2011). Mesenchymal stem cells have many attributes that support their use as a tumor specific therapeutic vehicle in clinical practice. It has been shown that MSCs share some characteristics with pericytes (Bexell et al., 2009; Bexell et al., 2010). This property might facilitate the migration of MSCs to highly vascularised glioblastomas. MSCs lack major histocompatibility complex MHC-II and show only minimal MHC-I expression (LeBlanc, 2003; Koppula et al., 2009; Griffin et al., 2010). Thanks to their non immuno genetic character, allogeneic MSCs can substitute for autologous stem cells. We and others (Danks et al., 2007; Nakamura et al., 2004; Nakamizo et al., 2005) are encouraged by the results of stem cell driven enzyme prodrug therapy experiments to treat glioblastoma multiforme, a tumor with fatal prognosis. Our experiments took the advantage of the fact that human AT-MSCs are not immunogenic in treatment of rat glioblastoma C6 growing intracerebroventricularly. The cell population of C6 rat glioblastoma has been shown to be composed primarily of cancer stem cells. Therapeutic experiments were designed to simulate scenarios for future clinical applications for high-grade glioblastoma therapy by direct injections of therapeutic stem cells into the tumor. The results revealed that genetically modified therapeutic stem cells labeled with super paramagnetic iron nanoparticles still have the tumor tropism when injected to a distant intracranial site and effectively inhibited glioblastoma growth after 5-FC therapy (Altanerova et al., 2012). Intratumoral administration of therapeutic stem cells improved the survival in a cell dose-dependent manner. Furthermore, the repeated administration of therapeutic cells and continuous intracerebroventricular delivery of 5-FC led to an increased number of animals being completely cured.

**Conclusions and remarks**

Glioblastoma multiforme is a very complex tumor, containing the glioma tumor stem cells possessing properties fully in agreement with the name for the tumor. GTSCs are able to survive standard glioblastoma therapy. There are very likely numerous variants changing their character and gene expression influenced by the tumor microenvironment. It would be very difficult to find therapeutically efficient molecularly targeted drugs because of the promiscuous character of these cells. It is believed that the tumor tropic character of mesenchymal stem cells or neural stem cells, engineered to express suicide genes, might be therapeutic modality that could bring some progress for survival of GBM patients.

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