Aleksi Sedo · Rolf Mentlein *Editors*

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Preface

Gliomas are fatal malignant diseases, but also represent excellent models for tumor research with the aim to eventually discover new and appropriate therapeutics against this and other types of cancer. Gliomas are characterized by unregulated growth, apoptosis-resistance, diffuse invasion, strongly increased angiogenesis, and immunosuppression—all hallmarks of other tumor entities, but here focused in a particularly pronounced manner. However, there are also characteristics unique to gliomas, in particular the exceptional brain microenvironment with specialized cells and extracellular matrix. All this results in biological properties of this tumor.

Recently published books dedicated to the glioma are either partially or entirely dedicated to its diagnostic and/or clinical aspects. This book focusses on various aspects of glioma cell biology. They are systematically covered in chapters written by experts in their field, first looking on the "inner space"—biology of the glioma cells themselves, then on their "joint venture"—bidirectional interactions among the microenvironment of the brain tumor, and finally on the experimental models available for glioma research.

The book starts with an overview of the cancer stem cell hypothesis and its implications for gliomas. As gliomas appear to be extremely heterogeneous tumors (the term *glioblastoma multiforme*—now often only glioblastoma—for the most common and malignant form implies this), they have become a paradigm for the tumor stem cell hypothesis (generation of tumor cells from the stem-like cells) versus classical opinions of a clonal origin of tumors (mutations in differentiated cells). The molecular mechanisms driving the malignant phenotype are exemplified in further chapters. Epigenetic changes and the role of microRNAs are summarized, followed by chapters on altered signal transduction mechanisms and the role of apoptosis and autophagy in glioma cells.

The following part is more centered around the complex interactions of glioma cells with the individual cellular partner populations and with the whole extracellular microenvironment of the brain. First, the various types of growth factors mediating autocrine as well as paracrine interactions between tumor and tumor stroma cells are reviewed. Then, the particular interactions between glioma and endothelial cells are highlighted (angiogenesis factors) followed by a chapter on the communications of glioma cells with the immune system. Thus, the three

abovementioned hallmarks of gliomas—dysregulated growth, strong vascularization, and immunosuppression—are illuminated on the molecular level. Next to communication factors, the particular cellular and extracellular components of the glioma and brain microenvironment are reviewed. Besides few other cell types, microglia cells/macrophages constitute the dominant stroma cells of this brain tumor. Therefore, the origin, special properties, and the bilateral communication of these cells with glioma cells are highlighted. Often neglected, the unique brain extracellular matrix and the adhesion molecules mediating its interaction with tumor cells are reviewed in two chapters. These are — together with the glioma-associated or produced proteases and motility factors — of exceptional importance for the understanding of the highly invasive character of gliomas. Indeed, the invasion of the brain tissue without clear margins is the reason for the still relatively poor outcome of surgical tumor resection.

Finally in the third part, the preclinical models are introduced, in which newly identified targets can be tested. Two chapters highlight the general methods and special constraints to investigate these fatal brain tumors in animal models.

Thus, the composition of this book follows the general concept: analysis of molecular alterations of malignant cells, viewing this under different hypotheses, explaining the special phenotype of a tumor in its cellular/extracellular milieu of the host organ, extracting putative therapeutic targets (that will be described in different chapters) from these perceptions, and applying these to the preclinical models to cure the patient.

We are aware that we could not review all aspects of glioma cell biology. Besides the fact that our space is limited, all of experts invited hadn't had the time to contribute. Our view on gliomas might of course be influenced by our own research concepts and topics. Additionally, as many of the authors declared, not all relevant works could be cited due to the space (or knowledge) limitations. We therefore apologize for this to all who do not see them adequately mentioned as well as for our mistakes (which surely do occur!).

Nonetheless, we hope that this book will be helpful and encouraging for researchers and physicians in understanding the various aspects of tumor biology, particularly concerning the brain, and this concise information will be another a step in the combat against these diseases.

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Part I

The Inner Space: Molecular Mechanisms Driving the Malignant Phenotype of Glioma Cells

Cancer Stem Cells and Glioblastoma

1

Petra Hamerlik

Abstract

Gliomas are tumors of astroglial origin and the World health Organization (WHO) classifies them based on histological criteria into four grades of ascending malignancy. *Glioblastoma multiforme* (GBM, WHO grade IV) is among the most lethal of human cancers with conventional therapy offering only palliation. GBM accounts for the most frequent type of primary brain tumors in Europe and the USA, comprising more than a half of all gliomas, with a 5-year survival of patients of no more than 5 %. Despite concerted efforts and advances in currently available therapies, the expected survival of GBM patients remains dismal. Highly infiltrative character renders complete surgical resection impossible and together with notoriously known radio- and chemoresistance accounts for high recurrence rates and mortality of nearly 95 %. Traditionally, the approach to cancer treatment has been to eradicate all of the cancerous cells to achieve "cure" and was based on the idea that the vast majority of cells have tumorigenic potential. One reason for the lack of clinical advances is the lack of understanding of the GBM biology in general and the cellular origin of this disease in particular. The cancer stem cell hypothesis postulates that cancers contain a subset of highly aggressive cells that propagate and maintain the tumors through unlimited self-renewal and potent tumorigenicity. Within GBM, a distinct population of $CD133^+$ cells has been documented to display stem cell properties in vitro, in particular self-renewal, unlimited proliferative potential, capacity for multi-lineage differentiation, and recapitulation of patient's phenotype upon orthotopic implantation in immunocompromized host. The investigation and study of cancer stem cells received enormous

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attention over the past decade, yet it's relevance to therapeutic resistance remains controversial. Although the cancer stem cell hypothesis may have multiple implications in therapeutic management of glioblastoma, as well as other brain tumor malignancies, caution must be exercised as targeting a rare population of tumorigenic cells without consideration of the largely heterogeneous tumor bulk comprised of proliferative cells may not change overall patient survival.

Keywords
Heterogeneity • Self-renewal • Therapeutic resistance • GBM • Microenvironment

Abbreviations

ABC	ATP-binding cassette
bFGF	Basic fibroblast growth factor
Bmi1	B lymphoma Mo-MLV insertion region 1 homolog
CSC	Cancer stem cell
DDR	DNA damage response
EGF	Epidermal growth factor
GBM	*Glioblastoma multiforme*
IR	Ionizing radiation
NSC	Neural stem cell
PTC1	Patched-1
RTK	Receptor tyrosine kinase
Shh	Sonic hedgehog
Smo	Smoothened
TCGA	The cancer genome atlas
TIC	Tissue-initiating cell
TMZ	Temozolomide
VEGF	Vascular endothelial cell growth factor
WHO	World Health Organization

1.1 Cancer Stem Cell Hypothesis

The adult human brain has been for many years thought to be a static, fully differentiated organ. Today, it is generally accepted that both neural stem cells (NCS) and glial progenitor cells in multiple regions of the adult brain persist throughout life. The self-renewing and multipotent neural stem cells (NSCs) have been isolated from subventricular zone, the lining of the lateral ventricles, the dentate gyrus and the hippocampus, as wells as the subcortical white matter (Doetsch et al. 1997; Fukuda et al. 2003; Gage 2000; Kim and Morshead 2003).

While NSCs comprise a relatively quiescent cell population, these cells have the potential to proliferate and migrate extensively, characterizing the adult brain as dynamic system with surprisingly high plasticity (Altman and Chorover 1963; Altman and Das 1965; Doetsch et al. 1999). NSCs have been associated with tissue repair after stroke and severe injuries, and have been suggested as tools for treatment of neurological disorders, such as Alzheimer's disease. In the light of these facts, cancer can be considered organ system with aberrant activation of developmental and wound response pathways (Rich 2008; Rich and Eyler 2008). Recent evidence suggests that within the heterogeneous tumor mass, there is a cell subpopulation with the unique capacity for sustained self-renewal and tumor propagation in vivo.

Historically, the approach to cancer treatment has been to eradicate all cancerous cells, where individual cells are equal in respect to their potential to proliferate, self-renew, and drive tumor growth. This notion, known as the stochastic or clonal evolution model (Fig. 1.1a) of tumorigenesis, proposes that a transformed single cell gains unlimited proliferative capacity (Chen et al. 2010; Li et al. 2007a; Shackleton et al. 2009). During early stages of tumorigenesis, a single or very few cells transform, where "pro-survival" mutations allow for clonal expansion of the "fittest" cells, resulting in a symbiotic coexistence of various subpopulations within the heterogeneous tumor mass. Importantly, during the lifetime of the tumor, any of the cancer cells can participate in tumor progression or develop resistance resulting in disease recurrence. This model has been challenged by the recently revived hierarchical model or the cancer stem cell hypothesis (Reya et al. 2001; Rich 2008; Sanai et al. 2005). The cancer stem cell hypothesis (Fig. 1.1b) postulates that there is a rare subpopulation of cancer cells with stem-like cell properties, including the ability to self-renew, that gives rise to multi-differentiated progeny and sustained proliferation. In contrast to stochastic model, the multipotent nature of these cells results in heterogeneity within tumor as a result of aberrant differentiation and epigenetic modification of the progeny, whereas the vast majority of progeny does not contribute to tumor growth and recurrence after therapeutic intervention.

This concept is not new, as already in 1855, Rudolph Virchow followed by Julius Cohnheim proposed (Rahman et al. 2011) that cancer develops from activation of dormant embryonic-tissue remnants. Their observations were based on the histological similarities (proliferation index and degree of differentiation) between fetal tissues and cancer. In the 1960s, ethically questionable experiments performed by Brunschwig, Southam, and Levin demonstrated a low frequency of tumor initiation when tumor cells harvested from patients with malignancy were injected subcutaneously into the same or different patients (Brunschwig et al. 1965). According to their results, tumors were formed only when more than 10^6 cells were injected. This and further reports showing (Bruce and Van Der Gaag 1963; Brunschwig et al. 1965) the clonogenic potential of lymphoma cells in vivo lead to hypothesis that tumor growth may be initiated and maintained by a minority of cancer cells, not the entire population. In 1994, John Dick and colleagues published their seminal findings that human acute myeloid leukemia is organized as a hierarchy that originates from a primitive hematopoietic cell and this report turned into a

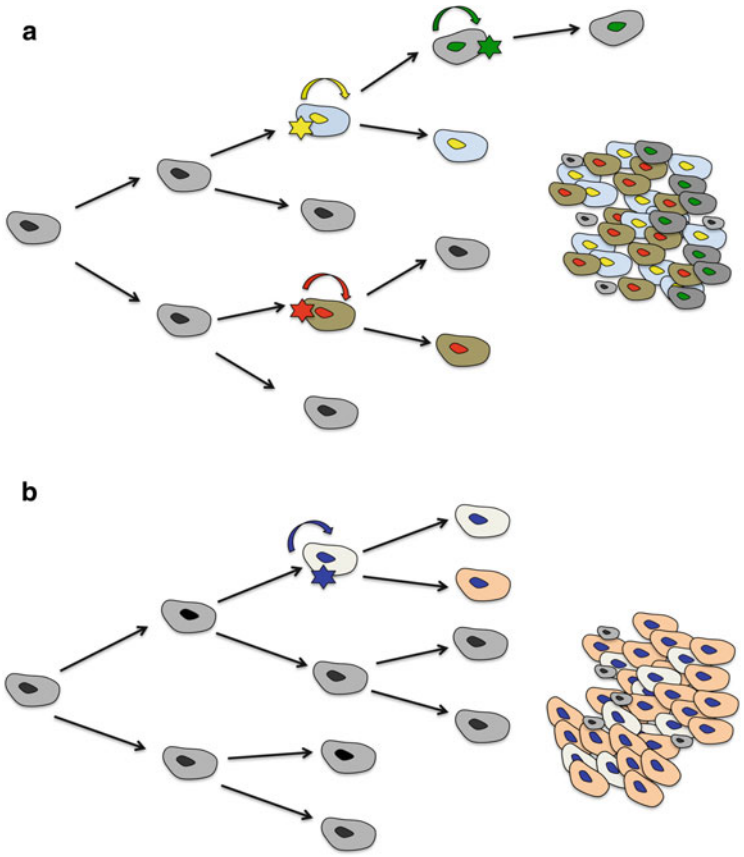

Fig. 1.1 Different models of tumorigenesis: stochastic or clonal evolution model (**a**) versus hierarchical model or the cancer stem cell hypothesis (**b**)

paradigm for later studies, which proposed the existence of a similar model for solid tumors (Lapidot et al. 1994). The cancer stem cells (CSCs) hypothesis and clonal evolution model do not contradict each other, instead highlight the importance of abnormal differentiation program in tumorigenesis, thereby suggesting a key role of cellular hierarchy in tumor evolution.

1.2 Evidence of CSCs in Gliomas

Traditionally, gliomas have been thought to originate from a parenchymal differentiated glial cell, which undergoes a series of genetic alternations accompanied by a dedifferentiation process (Jellinger 1978). The persistence of proliferative pool of NSCs and progenitor cells in the adult brain encouraged multiple investigators to evaluate these as putative cells of origin (Ligon

et al. 2007; Noble and Dietrich 2002; Pardal et al. 2003). Park et al. have already suggested the existence of CSCs in 1971 (Stopschinski et al. 2013). The concept of CSCs was first extended to brain tumors by Ignatova et al. (2002), who isolated clonogenic, neurosphere-forming stem-like cells from human GBM (Ignatova et al. 2002). GSCs isolated using neurosphere culture were subsequently shown to differentiate into multi-lineage progeny and formed tumors transplantation (Rahman et al. 2011). In 2003, studies by Singh et al. showed that cancer-initiating cells are enriched in the $CD133^+$ population and injection of only as many as 100 $CD133^+$ cells initiated tumor whereas $CD133^-$ could not, even when 10,000 cells were injected (Singh et al. 2003, 2004). These reports were followed by outburst of similar studies in GBM as well as other solid tumors (Rich and Eyler 2008; Strauss et al. 2012; Yan et al. 2013). GBM stem cells share many characteristics with NSCs, such as self-renewal, neurosphere formation, marker expression, multilineage differentiation, high motility, and localization in highly specialized "stem cell" niche (Gilbertson and Rich 2007; Kim and Morshead 2003; Reya et al. 2001). However, the term "stem cell" in gliomas refers to their function, not their origin. The true "cell of origin" has not yet been identified. The proper terminology remains unsettled, which limits our ability to effectively communicate the precise meaning of these labels and inform literature searches (Rich 2008), with most groups using the term cancer stem cells (CSCs), tumor-initiating/propagating cells (TICs), or cancer stem-like cells. Although controversial, the concept of cancer stem cell hypothesis recognizes the intra-tumoral heterogeneity and provides a novel framework to study tumor biology as wells as resistance of aggressive and genetically unstable cancer cells to current treatment in GBM.

1.3 Cancer Stem Cells: Identification and Quantitation

1.3.1 CSC in the Context Inter-/Intra-tumoral Heterogeneity

The defining features of CSCs in GBM are evolving and prospective functional enrichment approaches poses challenges. There are ongoing disputes across the field due to technical variance and lack of universal markers, suggesting that there is not a single marker to identify CSCs, probably due to inter- and intra-patient heterogeneity or lack of absolute fingerprints. The Cancer Genome Atlas initiative (TCGA) and GBM genome sequencing efforts resulted in integration of multidimensional genomic data and molecular classification of glioblastoma into proneural, classical, and mesenchymal subtypes that have potential implications for patient prognosis and therapeutic management (Phillips et al. 2006; Verhaak et al. 2010; Verhaak and Valk 2010). The evidence of inter-patient heterogeneity fueled numerous studies, some of them investigating the differences of CSCs pools in individual subtypes (Bhat et al. 2013; Mao et al. 2013).

The intra-tumoral heterogeneity is not only caused by the macroscopic localization of the tumor (i.e., brain stem versus brain hemispheres) (Schonberg et al. 2013; Stopschinski et al. 2013) but also by the microenvironmental factors (hypoxia,

acidosis) within the tumor as such. The multi-lineage differentiation capacity of CSCs into committed progenitors or terminally differentiated cells further enhances the degree of intra-tumoral heterogeneity, which may in turn contribute to the inter-patient variability and support the notion of molecular subclassification of glioblastoma (Huse et al. 2011; Lottaz et al. 2010; Mao et al. 2013; Verhaak et al. 2010). $CD133^{+}$ CSCs isolated from proneural (Joo et al. 2008; Lottaz et al. 2010) have shown to share similarities with $CD133^{-}$ cells in mesenchymal GBM subtype. Recently, Ichiro et al. reported the differential properties of CSCs isolated from proneural and mesenchymal subtypes of GBM in respect to their metabolic demands and resistance to ionizing radiation. Albeit these findings suggest that the heterogeneity of CSCs corresponds to the heterogeneity of GBM, where unfortunately a functional and experimental proof is still lacking (Huse et al. 2011).

1.3.2 Technical Aspects of CSC Identification and Isolation

The most popular way of identification and isolation of CSCs in GBM uses specific cell surface markers, such as CD133, CD15, CD44, and others or their combinations (Cheng et al. 2010; Hambardzumyan et al. 2008; Persano et al. 2013). Other approaches take advantage of the known overexpression of multidrug resistance genes (encoding pumps like ABCG2) responsible for exclusion of Hoechst 33342 dye and marking so-called side population which has been postulated to be enriched in CSCs. Novel and not as much utilized technique is the use of Aldefluor assay (based on the measurement of alcohol dehydrogenase 1 activity; ALDH1). Both "side population" and Aldefluor assay are imperfect, as they require substantial in vitro culture time (Rich 2008).

The first and most critical technical problem may occur already at the stage of tumor dissociation. The time post-surgery and the method of enzymatic tissue digestion dictate the recovery rates of most CSC-specific surface antigens and account for viability rates later affecting the engraftment efficiencies in immunocompromized hosts. Despite the progresses in defining proper cell culture media allowing for CSC maintenance, extensive passaging in vitro influences the metabolic and expression prolife, as well as genomic stability of cultured cells (Eyler and Rich 2008; Lee et al. 2006). The growth of derived CSCs in the mouse environment might be more complex and contribute to technical difficulties associated with their maintenance. Upon biopsy dissociation and derivation of a primary sphere culture, one practically strips off any cells and components of the supportive microenvironment, which can't be properly substituted and so only cells with minimal dependence on their "niche" survive and will be propagated in vitro or in vivo (Rahman et al. 2011). The frequency of CSCs is often quantitated based on the number of cells, which are tumorigenic, when transplanted into immunocompromized mice. Although this method has been considered as the most reliable, it seems that one may greatly underestimate the frequency of CSCs depending on the animal model used (nude mice vs. SCID mice or SCID with no residual immunity). Using NOD/SCID interleukin-2 receptor gamma chain null

mice, Quintana et al. (2008) found that 27 % of melanoma cells could form a tumor with a single cell transplant, suggesting that these cells are much more common, at least in some human tumors than anticipated (Quintana et al. 2008).

1.3.3 CSC Markers

1.3.3.1 CD133

CD133 is a penta-membrane glycoprotein (also known as Prominin-1) and was first discovered as a cell surface marker for hematopoietic stem cells (Miraglia 1997). Uchida et al. (2000) have described its expression in human fetal brain as a marker for neural stem cells (Uchida et al. 2000). Very little is known about the cellular function of CD133 (Fargeas et al. 2003). CD133 knockout mice manifest with a progressive photoreceptor degeneration resulting in vision loss (Zacchigna et al. 2009). Several groups have reported that CD133 is a marker of poor survival in astrocytomas (Beier et al. 2008; Hermansen et al. 2011; Joo et al. 2008; Mak et al. 2011; Zeppernick et al. 2008), with CD133+ cells localized to clusters near vascularized regions or as solitary cells invading non-neoplastic brain parenchyma. It has been demonstrated that the expression of CD133 is cell cycle-dependent and may be upregulated by hypoxia and acidosis (Beier et al. 2007; Jaksch et al. 2008). The biological function of CD133 in CSCs biology remains elusive; however, a recent report by Wei et al. (2013) implies its pro-survival role upstream from phosphotidylinositol 3-kinase (PI3K)/Akt kinase signaling (Wei et al. 2013).

1.3.3.2 CD15

CD15 is (also known as SSEA-1 and Lewis-X Antigen) a carbohydrate adhesion molecule associated with glycolipids and glycoproteins. First reports have shown its expression in NCS derived from human embryonic stem cells and embryonic neural stem cells (Barraud et al. 2007; Pruszak et al. 2007). This marker has been used as an alternative to CD133 in identifying GBM-derived CSCs and the frequency of expression varied from 2.4 to 70 % (Son et al. 2009). Later reports indicate that CD15 labels actively proliferate progenitor rather than cancer stem cells (Cheng et al. 2010).

1.3.3.3 CD44

The CD44 proteins form a ubiquitously expressed family of cell surface adhesion molecules involved in cell–cell and cell–matrix interactions (Ishimoto et al. 2010; Jin et al. 2006). CD44 is expressed in multiple tumors types as well as normal tissues where it functions in the regulation of cell proliferation, cell migration, transmission of survival signals, and other cell–cell and cell–matrix interactions. GBM tumor initiation was reported to be attenuated by targeting TGF-β and its receptor CD44 in GBM-derived CSCs localized in vascular niche in vivo (Anido et al. 2010). Another reports by Jijiwa et al. (2011) and Mao et al. (2013) propose CD44 as a complementary marker to CD133 for CSCs identification and isolation in mesenchymal GBM subtype (Jijiwa et al. 2011; Mao et al. 2013).

1.3.3.4 CSC Transcription Factors

Central to regulation of survival, maintenance, self-renewal, and transduction of extracellular signals from cellular microenvironment into CSCs are these transcription factors: Sox2, Oct4, Nanog, c-Myc, Olig2, and Bmi1 (Schonberg et al. 2013; Stopschinski et al. 2013; Yan et al. 2013; Zhou et al. 2009). Increased Oct4 expression correlates with the degree of malignancy in gliomas (Hambardzumyan et al. 2008; Schonberg et al. 2013) and its inhibition leads to decreased sphere formation and differentiation of CSCs (Ikushima et al. 2011). Sox2 and Nanog interact with Oct4 and so contribute to CSC tumor-initiating capacities. c-Myc has been for decades known as oncogene with high frequency of genomic as well as regulatory alternations contributing to cancer progression (Sheiness et al. 1978; Vennstrom et al. 1982). shRNA-mediated knockdown of c-Myc in CSCs lead to abrogation of tumor initiation in orthotopic GBM model, demonstrating the importance of c-Myc for CSCs tumorigenecity and maintenance (Wang et al. 2008). Olig2 has been long known as a basic helix-loop-helix (bHLH) transcription factor in CNS (Dimou et al. 2008) with functions in the oligodendroctye lineage as well as multipotential neuron/glia progenitor maintenance (Zhu et al. 2012). Of the $CD133^{+}$ subpopulation of GBM cells, nearly 98 % are positive for Olig2, which is crucial for their proliferation and cell cycle progression (Ligon et al. 2007). Bmi1 is a polycomb group protein (component of the Polycomb Repressive Complex 1) belonging to epigenetic silencers with crucial function during embryonic development (Acquati et al. 2013). Bmi1 has been found enriched in CSCs and is required for their self-renewal (Facchino et al. 2010) and its inhibition leads to radiosensitization of CSCs.

1.4 Pathways Regulating CSCs

1.4.1 Notch Signaling

Notch proteins include four transmembrane receptors, which mediate cell–cell communication as well as cellular proliferation, differentiation, and apoptosis (Schonberg et al. 2013; Wang et al. 2012). There are five ligands that bind Notch receptors: Delta-like 1, 3, 4 and Jagged-1, -2 (Ohishi et al. 2002; Schonberg et al. 2013). The activation of Notch requires sequential proteolytic cleavages by the γ-secretase complex to release its intracellular domain (NICD) and translocate it from membrane to nucleus (Cheng et al. 2010). Notch signaling promotes the proliferation of normal neural stem cells and is indispensable for maintenance of neural progenitors both in vitro and in vivo. Inhibiting Notch by a γ-secretase inhibitor (GSI-18) induces CSC differentiation and apoptosis (Fan et al. 2006) and sensitizes CSCs to radiation (Guo et al. 2009; Purow et al. 2005; Radtke and Raj 2003; Ronchini and Capobianco 2001). Additionally, in a K-Ras-induced murine gliomas model, Notch activates intermediate filament protein and stem cell marker, nestin, further supporting Notch role in maintaining the stem cell phenotype of GBM-derived CSCs (Shih and Holland 2006). Other Notch regulators

like Delta/Notch-like epidermal growth factor-related receptor (DNER) and the Notch ligand Delta-like 4 (DLL4) have also been reported to regulate GBM growth and progression (Li et al. 2007b; Sun et al. 2009).

1.4.2 Wnt/β-Catenin Signaling

The canonical Wnt signaling cascade is one of the key regulators in embryonic and adult stem cells. Wnt proteins bind to cell surface receptors of the Frizzled family and their activation leads to nuclear accumulation of nuclear β-catenin, which promotes transcription of multiple target genes including c-Myc and cyclin D1 (Pu et al. 2009; Tanaka et al. 2013). In brain, the Wnt pathway regulates development, in particular the proliferation and self-renewal of NSCs and progenitors cells in fetal ventricular zone, the postnatal subventricular zone, and hippocampus (Kalani et al. 2008; Nusse et al. 2008). Primarily, Wnt signaling and its alternations were linked to medulloblastoma, but recent reports indicate that Wnt/β-catenin pathway may be associated with GSCs maintenance and resistance to ionizing radiation (Chen et al. 2007; Woodward et al. 2007).

1.4.3 Sonic Hedgehog Signaling

Sonic hedgehog (Shh) is a key regulator of cell fate determination and proliferation of adult stem cell including neural stem cells. Upon binding of Shh to its associated transmembrane receptor Patched-1 (PTC1), membrane protein Smoothened (Smo) gets released and activates Gli transcription factors. Once Gli is activated, it translocates into nucleus, where it induces or represses the transcription of downstream genes, such as Wnt, IGF, and PDGFR-α, c-Myc, and cyclin D1 (Dietrich et al. 2008, 2010). Shh pathway abrogation has been reported to deplete CSCs in GBM and increase their radio-resistance (Clement et al. 2007).

1.4.4 Phosphotidylinositol 3-Kinase/Akt Signaling

Receptor tyrosine kinases (RTKs) transduce oncogenic signaling from growth factors, among others Epidermal Growth Factor (EGF) and basic Fibroblast Growth Factor (bFGF), two mitogens commonly used to propagate CSCs in vitro (Lee et al. 2006). Among the most studied and frequently mutated in GBM is the EGFR-mediated growth signaling through PI3K/Akt kinase (Rich 2008; Tanaka et al. 2013). Malignant gliomas, GBM in particular, frequently display EGFR amplifications and/or constitutive activation of EGFRvIII variant that result in elevated PI3K/Akt signaling. Transducing primary astrocytes with c-myc and Akt induces tumorigenicity and increases expression of several stem cell markers (Schonberg et al. 2013). Interestingly, CD133 was found to directly interact with p85 regulatory subunit of PI3K, where knockdown of CD133 resulted in decreased

PI3K/Akt signaling and ultimately reduced CSCs self-renewal and tumorigenicity (Wei et al. 2013). This finding is in concordance with previous studies, where inhibition of Akt disrupted CSC invasion potential, proliferation, and maintenance in vitro and in vivo, primarily by increasing the rates of apoptosis (Eyler et al. 2008; Gallia et al. 2009).

1.5 Radio- and Chemo-resistance of CSCs

Standard of care treatment in GBM currently involves the use of both ionizing radiation (IR) and DNA-alkylating agent temozolomide (TMZ). In GBM, DNA damage responses (DDR) were shown preferentially activated in the pool of $CD133^+$ CSCs when compared to their negative counterparts, possibly contributing to lower rates of apoptosis after IR. Moreover, IR treatment of mice bearing orthotopic GBM tumors resulted in enrichment of CSCs. CSC were shown to have higher metabolic activity (measured by ATP production) resulting in higher reactive oxygen species levels (ROS) and consequently higher level of oxidative damage to DNA (Venere et al. 2014). Intriguingly, reports on actual DNA repair efficiency of CSCs versus non-CSCs are discrepant, most probably due to technical issues accompanying CSC isolation and maintenance in vitro (McCord et al. 2009b; Ropolo et al. 2009). The cell surface adhesion protein and GSC marker, L1Cam (CD171), further enhances the DDR activation via direct regulation of NBS1 and ATM/Chk1/Chk2 pathway in response to IR-induced double strand DNA breaks (Cheng et al. 2011). The polycomb group protein, Bmi1, represents additional levels of DDR response regulation. Bmi1 contributes to radioresistance of CSCs by remodeling the chromatin structure, which leads to impairment of repair factor recruitment to damaged DNA (Facchino et al. 2010).

Chemotherapeutic management of GBM has undergone considerable changes in the last two decades. Since the late 1970s, alkylating substances such as nimustine (ACNU), carmustine (BCNU), and lomutine (CCNU) were the main choices (Beier et al. 2011). Introduction of TMZ as standard treatment in addition to radiotherapy and surgical resection improved both the overall survival and progression-free survival in patients with newly diagnosed GBM (Stupp et al. 2005). Compared to non-CSCs, CSCs exhibit significantly higher expression of O6-methylguanine-DNA-methyltransferase (MGMT), which makes them more resistant to TMZ treatment (Binello and Germano 2011). In addition to TMZ, GBM-derived CSCs are more resistant to several other chemotherapeutic agents, including carboplatin, paclitaxel, and etoposide (Capper et al. 2009; Liu et al. 2006).

Notably, methylations and other epigenetic modifications may also impair the effect of chemotherapeutics; for examples, CSCs have a hypermethylated caspase-8 promoter that renders them resistant to therapies utilizing the tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) pathway (Capper et al. 2009). The most straightforward mechanism that may be actively contributing to CSCs' chemoresistance is the overexpression of ABC (ATP-binding cassette) transporters (Bleau et al. 2009a, b). Moreover, their overexpression correlates with the levels of several stem-cell markers such as CD133, nestin (Yamamoto et al. 2009), or Notch-

1 and Nanog (Bourguignon et al. 2008; Patrawala et al. 2005). Ectopic expression of CD133 results in an upregulation of ABC transporter upon treatment of CSCs with anti-cancer drugs, camptothecin and doxorubicin (Angelastro and Lame 2010). Interestingly, a report by Venere et al. (2014) reported preferential sensitivity of CSCs (compared to non-CSCs) to olaparib—a potent small molecule inhibitor of the nuclear enzyme poly(ADP-ribose) polymerase (PARP) (Venere et al. 2014). It acts by binding to PARP, inhibiting PARP-mediated repair of single strand DNA breaks. Furthermore, inhibition of PARP sensitized CSCs to IR, albeit opening a new window for therapeutic intervention in glioblastoma.

1.6 Microenvironmental Regulation of CSC

Over 30 years ago, Schofield (1978) proposed the existence of unique spatially defined regions within each tumor, which were suspected to provide factors necessary to the survival and development of cells capable to regenerate tissues in adult organisms (Schofield 1978). A number of studies have clearly demonstrated that the so-called niche directs proliferation, differentiation of cells, and as such constitutes a key regulator of stem-cell fate (Blanpain and Fuchs 2006; Calvi et al. 2003; Fuchs et al. 2004). The key role played by the local microenvironment in the initiation and progression of tumors is becoming increasingly clear.

A series of recently published studies have shown that aberrant vascular stem cell niches, reminiscent of those observed in normal brain, exist in glioblastoma and other types of brain tumors and support CSCs (Gilbertson and Rich 2007). Calabrese et al. (2007) provided convincing data that brain tumors orchestrate vascular niches that maintain CSC pool and disruption of these ablates the fraction of self-renewing tumor cells ultimately leading the tumor growth arrest (Calabrese et al. 2007). Bao et al. showed that CSCs secrete high levels of pro-angiogenic cytokines, among the most abundant being Vascular Endothelial Growth Factor (VEGF)—a key factor in tumor angiogenesis (Bao et al. 2006). They have demonstrated that freshly resected CSCs, but not non-CSCs, human glioblastoma cells readily form highly vascular and hemorrhagic tumors in vivo (Bao et al. 2006). Furthermore, cultures enriched for CSCs induced higher levels of endothelial progenitor cell proliferation, recruitment, and mobilization compared to non-CSC cultures. When VEGF or stromal-derived factor 1 (SDF-1, CXCL12) is inhibited in CSCs, all aspects of angiogenesis were dramatically reduced (Bao et al. 2006; Folkins et al. 2009). Hamerlik et al. (2012) have reported autocrine VEGF/VEGFR2 signaling as an evasion tool of CSCs to anti-angiogenic therapy (Hamerlik et al. 2012; Knizetova et al. 2008). Knocking-down VEGFR2 not only decreased VEGF secretion, but it significantly decreased CSCs' viability, self-renewal potential, and tumorigenicity. Moreover, abrogation of VEGFR2 signaling sensitized GBM cells to IR (Hamerlik et al. 2012; Knizetova et al. 2008). Wang et al. (2010) and Ricci-Vitiani et al. (2010) proposed the neoplastic origin of tumor endothelium and CSC as a possible source to endothelial progenitors (Ricci-Vitiani et al. 2010; Wang et al. 2010). In contrast, Cheng et al. (2013) have shown CSCs to

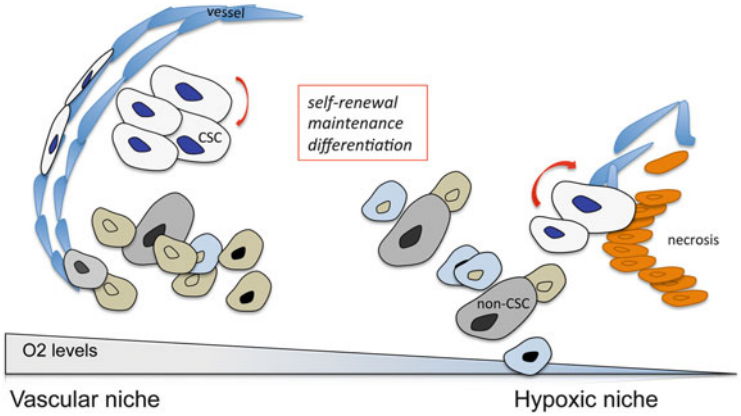

Fig. 1.2 The vascular and hypoxic niches in gliomas as drivers for cancer stem cell self-renewal, maintenance, and differentiation, see text

differentiate into pericytes supporting vessel function and so tumor growth (Cheng et al. 2013). Collectively, this data add yet another complexity to CSC–vascular niche interaction.

Hypoxia has been reported to play a crucial role in the maintenance and regulation of NSC, leading to a recognition of a "hypoxic nice" (Mazumdar et al. 2010; Roitbak et al. 2008). Evans et al. analyzed oxygenation of normal brain and gliomas (Evans et al. 2004, 2008). Their measurements showed that physiological oxygen levels in healthy brain range between 12.5 and 2.5 %, while GBM tumors showed mild to moderate/severe hypoxia with oxygen tensions ranging between 2.5 and 0.1 %. Several reports have established key regulatory functions of the hypoxic niche in CSC maintenance and survival (Bar 2011; Heddleston et al. 2009; Li et al. 2009a). Expression of several of stem cell markers (e.g., CD133, A2B5, Nestin, Oct-4, and Sox2) is upregulated (Li et al. 2009a, b; McCord et al. 2009a; Seidel et al. 2010), whereas the expression of differentiation markers (GFAP) is downregulated under hypoxia. Treatments that would efficiently disrupt aberrant tumor niche(s) would therefore prove active against glioblastoma (Gilbertson and Rich 2007).

Conclusion and Future Perspectives

The emergence of cancer stem cells and recent advances in our understanding of signaling pathway crucial to their self-renewal, survival, and tumorigenic potential have led to the development of novel targeted therapies, currently being evaluated in clinical trials (Sathornsumetee and Rich 2008; Schonberg et al. 2013; Tanaka et al. 2013). Phase I and phase II clinical trials using γ-secretase inhibitors to inhibit Notch signaling or an oral hedgehog antagonist (vismodegib) are ongoing (Tanaka et al. 2013). The current challenge is to develop experimental models encompassing the complexity of this highly heterogeneous malignancy (Fig. 1.2), including three-dimensional cyto-

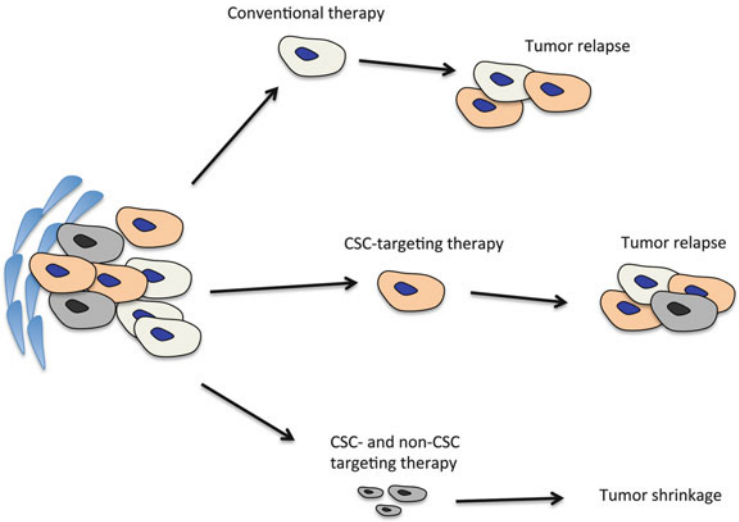

Fig. 1.3 Therapeutic implication of cancer stem cell hypothesis. Conventional therapies fail to target CSCs and often result in tumor relapse. CSC-targeting therapies delay significantly the tumor growth, but due to high plasticity of remaining tumor cells (hypoxia/acidosis-induced stemness), recurrence is inevitable. Treatment modalities combining the CSC-targeting and conventional approaches may be beneficial for successful therapeutic intervention in gliomas

architecture, vascular and hypoxic niches, and stromal component contribution, as targeting every single component may represent exciting new approach for cancer treatment.

Despite great advances in glioblastoma treatment modalities, the effects on patient survival are dismal. Although many compounds demonstrated strong efficacy in preclinical studies, very few of them showed similar effect in clinical trials, due to negligible antitumoral activity and/or severe side effects, which might be reflecting the intra- as well as inter-tumoral heterogeneity common to GBM. For this reason, a better understanding of CSC biology within the complex tumor microenvironment and its interplay with resistance to currently available therapies must be improved. Scientists advocating CSC hypothesis suggested CSC-directed therapy to be the most likely successful cancer treatment. Because of the high GBM cell plasticity, the capacity of non-CSCs to dedifferentiate into CSC, only targeting cancer stem cells would ultimately lead to disease recurrence (Eyler and Rich 2008; Persano et al. 2013; Rich 2008). Therefore, a combined treatment targeting both CSCs and their differentiated progeny is more likely to be efficient approach in glioblastoma management (Fig. 1.3).

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