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# Targeting Glioma Stem Cells: Path Leading to the Cure

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## 1. Introduction

In the United States, 22020 new cases of cancer in central nervous system (CNS) are expected to occur in 2010 (CBTRUS 2010). Although the incidence of cancers in CNS is much lower than that of malignancies in other organs such as lung, breast and colorectal cancers, CNS cancers are the second lethal cancer for males younger than 40 years (Jemal et al. 2010). In addition, with the unconstraint growth, brain cancers can often involve eloquent area. As a result, the neurological and psychological deficits may severely damage the health-related quality of life (QOL) in patients with brain cancers. Improvement of QOL and the prognosis of brain cancers is the goal of both physicians and basic investigators.

Glioblastoma multiforme (GBM) is the most frequent primary brain cancer, accounting for 17% of all primary tumours in CNS. In the past five decades, despite the advances in the fields of neurosurgery, radiotherapy and pharmaceutics, the prognosis of patients with GBM remains dismal, with a 5-year survival of only 9.8%(Stupp et al. 2009). The nature of extensive proliferation, diffuse infiltration and resistance to conventional treatments makes the chance to cure GBM slim. Exploration of mechanisms underlying therapeutic resistance of GBM and developing novel strategies against GBM are of urgent necessity.

The emergence of brain tumour stem cell (BTSC) theory is a great breakthrough in the field of neuro-oncology. BTSC theory assumes that brain tumour is a hierarchy of cancer cells maintained by a small population of cells sharing characteristics of normal embryonic and somatic stem cells. BTSC theory is confirmed by the isolation of BTSCs from established brain tumour cell lines and freshly surgical samples. Accumulated evidence suggests that BTSCs are responsible for the initiation, progression, recurrence and treatment resistance. Therefore, BTSCs are promising therapeutic targets. In this chapter, we aim to summarize advances in BTSC biology with the focus on the treatment strategies against BTSCs.

## 2. Identification of BTSCs

The hypothesis that tumours arise from cancer stem cells (CSCs) is not new. The histological similarity between cancers and embryonic tissues was observed by Rudolph Virchow more than one century ago (Huntly and Gilliland 2005). The heterogeneity of cancer and only a small cancer population capable of forming clones *in vitro* and reconstituting tumour *in vivo* have also been wildly recognized for decades. However, because of the limitation of

knowledge and technology in molecular biology, CSCs have not been confirmed in leukemia and solid tumours including brain tumours until late 1990's (Clevers 2011).

### 2.1 Isolation and characterization of BTSCs

In 2002, based on the finding that the expression of markers specific for undifferentiated neural cells was detected in malignant glial tumours, Ignatova and coworkers assumed the existence of cells sharing features of normal neural stem cells (NSCs) in gliomas (Ignatova et al. 2002). By using *in vitro* serum-free NSC culture system supplemented with epidermal growth factor (EGF) and fibroblast growth factor (FGF), the authors successfully isolated NSC-like cells from human glioma tissues. These cells are capable of forming clones morphologically resembling those generated by NSCs. And populations from the tumour-derived clones demonstrate positive for NSC marker nestin, differentiated neuronal marker β-III tubulin or glial lineage-specific marker glial fibrillary acidic protein (GFAP). Singh and coworkers confirmed the existence of BTSCs and did further feature analysis (Singh et al. 2004). Their investigation demonstrated that CD133-positive tumour cells in gliomas possess obvious stem cell characterization of extensive proliferation, self-renewal and lineage-restricted differentiation that recapitulates the original tumour phenotype. In addition, the CD133-positive tumour cells show a strong ability to form tumours in xenograft models. One hundred CD133-positive glioma cells efficiently initiate tumours in immunocompromised mice while 100,000 CD133-negative glioma cells fail to establish tumours. The BTSC model appears to be more robust in preclinical research than traditional established cell lines kept in serum-containing medium, because the former has been shown to mirror both the phenotype and genotype of their parental tumours (Lee et al. 2006). With transcriptome analysis, Fine's group found that GBM cells maintained in serum-free NSC media have gene expression profiles similar to NSCs and harbour all the genetic aberrations detected in primary tumours. Genetic stability maintains even after repeated passage. Moreover, these NSC-like glioma cells demonstrate a high tumourigenic potential and establish tumours with extensive infiltration into normal brain *in vivo*, which is frequently observed in human GBMs. By contrast, GBM cells under standard *in vitro* conditions with serum-containing media, undergo dramatic *de novo* genomic rearrangement over time, with a divergence from genotype of parental GBMs but displaying resemblance to genomic features with commonly used glioma cell lines. A majority of these cells lose the ability to initiate tumours in murine models. Even for a small subset of glioma cells that retain tumourigenic potential, the xenografts they generate phenotypically differ from their parental human GBMs. Collectively, BTSCs are defined as a small population in brain tumours, with the ability to maintain the BTSC pool by self-renewal and with the ability to extensively proliferate into differentiated non-tumourigenic brain tumour cell phenotypes that reconstitute the cellular heterogeneity of the parental brain tumours.

### 2.2 Identification of BTSCs

#### 2.2.1 CD133

Human CD133, also known as prominin-1, is a pentaspan transmembrane glycoprotein localized in membrane protrusions, which contains 865 amino acids with a nonglycosylated molecular weight of 97 kDa. The predicted structure of CD133 composes an 85-amino acid N-terminal extracellular domain, five transmembrane domains with two large extracellular loops containing eight potential N-linked glycosylation sites, and a 50-amino acid cytoplasmic tail. It was first identified as a hematopoietic stem cell antigen (Miraglia et al. 1997).

Although CD133 mRNA is found to be strongly expressed in multiple organs including adult kidney, trachea and digestive tract, glycosylation immunoreactivity of CD133 appears to be restricted to undifferentiated cell types and reduce during differentiation (Florek et al. 2005; Corbeil et al. 2000). Subsequently, CD133 has been demonstrated to be successfully used as a marker to define and purify stem and progenitor cell populations in various organs such as fetal liver, kidney, prostate and brain (Rountree et al. 2007; Angelotti et al. 2010 ; Vander Griend et al. 2008 ; Barraud et al. 2007).

Because the resemblance of cancer stem cells to normal stem cells, CD133 is also widely employed as a marker to identify and isolate cancer stem cells in multiple solid tumours such as prostate cancer, colon cancer and melanoma (Collins et al. 2005, O'Brien et al. 2007; Monzani et al. 2007). In addition, the clinical relevance of CD133 expression with the prognosis of brain tumour patients and the association of CD133 immunoreactivity with BTSCs are well documented. The proportion of CD133-postive cells in WHO grade 2 and 3 gliomas is found to be an independent risk factor for tumour recurrence and time to malignant progression (Zeppernick et al. 2008). Consistently, the presence of CD133 has been demonstrated to be correlated with a decreased survival in patients with high-grade oligodendroglial tumours (Beier et al. 2008). Preclinical studies demonstrated that the CD133-positive BTSCs are capable of extensive self-renewal and recapitulation of original tumours. Moreover, ectopic overexpression of CD133 in rat C6 glioma cells significantly enhances the chemoresistance of tumour cells to camptothecin and doxorubicin. Tumour cells with increased expression of CD133 appear to be more reluctant to undergo apoptosis after the treatment of cytotoxic agents, which is demonstrated as a result of elevated efflux of drugs by up-regulating ATP-binding cassette transporters ABCB1 (Angelastro and Lame 2010). The studies aforementioned suggest CD133 as a *bona fide* marker for BTSCs. However, the emergence of conflicting evidences indicates that CD133 is not necessarily required for BTSC phenotype. The existence of CD133-negative BTSCs is confirmed as well. It has been shown that a hierarchy of self-renewing BTSC types exists in GBMs. Both CD133-positive and CD133-negative glioma cells in individual GBMs exhibit self-renewal *in vitro* and initiate highly aggressive tumours *in vivo*. Notably, CD133-negative glioma cells can even give rise to CD133-postive cells (Chen et al. 2010). Moreover, CD133 expression has been proposed as an indicator of bioenergetic stress rather than to be obligatorily related with BTSC phenotype in human gliomas. Reduced oxygen levels have been shown to increase the CD133 expression in gliomas (Bar et al. 2010). Mitochondrial dysfunction can also up-regulate CD133 expression that is inversely correlated with changes in mitochondrial membrane potential. Genetic depletion of mitochondrial DNA results in a remarkable increase of CD133 expression, which can be reversed by re-introducing parental mitochondria (Griguer et al. 2008).

To reconcile the discrepancies in the CD133 studies, several hypotheses are proposed. First, the limitations of current antibodies against epitopes of CD133 can lead to inconsistent results. AC133 and AC141 mAb, reported to bind the extracellular glycosylation epitope of CD133, are the most commonly used antibodies to identify and purify BTSCs. However, little is known about the molecular nature of the epitopes recognized by these two antibodies. Although AC133 and AC141 mAbs are sometimes used interchangeably, discordant results about the expression of the AC133 and AC141 epitopes were observed in other cancer stem cell types (Green et al. 1999). Additionally, the presence of alternatively spliced CD133 isoforms is another complicating factor. Transcription of CD133 can be started at five tissue-restricted promoters, producing several alternatively spliced transcripts,

among which there are possible spliced isoforms without AC133 and AC141 epitopes. In this case, some BTSCs are actually CD133 false negative. Secondly, BTSC phenotypes that are independent of CD133 status may exist. Several lines of evidence support this hypothesis. With genetic approach, BTSC population depleted of CD133 was generated. BTSCs lacking CD133 expression can proliferate as tumour-spheres *in vitro* and produce gliomas when transplanted into mouse brain, indicating the dispensable role of CD133 in BTSC maintenance and gliomagenesis (Nishide et al. 2009). In addition, transcriptional profiles demonstrated a distinct gene expression in CD133-positive BTSCs compared to CD133-negative BTSCs. CD133-positive BTSCs, growing as neurospheres, display "proneural" transcriptional profiles with a resemblance to fetal neural stem cells, while CD133-negative BTSCs with a semiadherent/adherent growth pattern show "mesenchymal" signature genes similar to adult neural stem cells. The molecular heterogeneity of CD133-positive and –negative BTSCs implied the different origins of these two BTSC populations (Lottaz et al. 2010).

### 2.2.2 Side population technique

Side population (SP) cells are referred to a small population of cells which are capable of excluding vital dye Hoechst 33342 and featured in flow cytometry plots as the "side" of the bulk of positively labeled cells. The exclusion of Hoechst 33342 by SP cells has been suggested as a result of the cells' ability to pump out drugs mediated by ABCG2, an ATP-binding cassette (ABC) transporter (Scharenberg, Harkey, and Torok-Storb 2002). SP cells were first identified in mouse bone marrow, with the enrichment of stem cell properties. The observations that SP cells share stem cell features have been extended in other normal and tumour tissues. In glioma, SP cells demonstrate characteristics of pluripotency *in vitro* and high tumourigenicity *in vivo*. Kondo and coworkers successfully isolated SP cells from the rat glioma cell line C6, which only account for 0.4% of the cells maintained in serum-containing medium. C6 SP cells can form neurospheres in culture free of serum and produce SP as well as non-SP cells *in vitro*. Moreover, C6 SP cells are capable to initiate tumours containing multiple lineages such as neurons and glia when transplanted into various tissues in nude mice (Kondo, Setoguchi, and Taga 2004). In addition, SP cells have also been associated with the resistance to anti-cancer treatment. When exposed to temozolomide (TMZ) at DNA-damaging dose, SP cells purified from astrocytoma cell lines demonstrate a profound resistance to the cell arresting effects of the drug and maintain a robust proliferative capacity, compared with non-SP cells. The percentage of SP cells increases as high as 8-fold (Chua et al. 2008). Consistently in a platelet-derived growth factor (PDGF)-induced glioma mouse model, SP cells are shown to be less sensitive to TMZ relative to non-SP cells. Moreover, the *in vivo* aggressiveness of SP cells increases greatly after treatment with TMZ. Mechanistically, an increased level of O6-methylguanine-DNA methyltransferase (MGMT) in SP cells contributes to the chemoresistance to TMZ (Bleau et al. 2009).

Although SP technique is effective in enriching BTSCs, there is disagreement regarding the connection between SP and BTSC phenotypes. As mentioned above, only a small fraction of C6 glioma cells (0.4%), namely, SP cells, fulfill the criteria of BTSCs. However in a study using clonal and population analyses, Zheng and coworkers revealed that most single C6 cells have similar clone formation ability *in vitro* and tumourigenic potential *in vivo*, suggesting that most of C6 glioma cells are BTSCs. Furthermore, Hoechst 33342 is shown cytotoxic and can impair the clonogenicity and proliferation of individual C6 cells. The authors therefore postulated that Hoechst 33342 labeling and sorting can deprive non-SP cells of their stemness (Zheng et al. 2007). In addition, Srivastava and coworkers

demonstrated that both the SP and non-SP fraction in medulloblastoma can produce neurospheres and regenerate both fractions. The toxicity of Hoechst 33342 to medulloblastoma cells, especially non-SP cells, is also confirmed in this study and suggested as the reason for the biological differences between SP and non-SP fractions (Srivastava and Nalbantoglu 2008).

Collectively, CD133 and SP technique are successful in the identification and purification of BTSCs. However, these two approaches are unlikely to be enough to define all BTSC phenotypes because of their intrinsic limitations and the complexity of BTSCs. As a result, cautions should be exerted in interpreting results generated with these two methods. The development of novel approaches to enrich BTSCs is also critical.

### 2.3 Maintenance and expansion of BTSCs *in vitro*

The establishment of models in which BTSCs can be properly propagated is critical for the studies of BTSCs. To date, neurosphere culture is the most commonly used protocol to expand BTSCs. According to this protocol which was first developed by Reynolds and Weiss for neural stem cell studies, BTSCs are cultured in serum-free medium supplemented with growth factors, for example, EGF and FGF. Under this condition, BTSCs grow as floating aggregates with extensive self-renewal (Reynolds and Weiss 1992; Galli et al. 2004). When transferred to medium with serum, these cells differentiate and express neuron and glia markers. Notably, BTSCs maintained as neurospheres are highly tumourigenic and initiate tumours with phenotypical and genetical resemblance to their origins. Neurosphere culture successfully maintains the BTSC phenotype and is therefore widely used to explore key issues in BTSC biology. However, several studies call into question neurosphere culture as a reliable method to expand and analyze BTSCs. First, neurosphere culture protocol has a low efficiency to establish BTSC lines (Laks et al. 2009). Secondly, the majority of cells in neurospheres are more differentiated and/or dying progeny (Martens, Tropepe, and van Der Kooy 2000). Thirdly, the aggregating growth manner of BTSCs and the fuse between neurospheres make the evaluation of the efficacy of treatment difficult (Woolard and Fine 2009). In order to overcome the limitations of neurosphere culture, novel methodological approaches have been investigated, among which the adherent culture developed by Pollard and coworkers is of great promise (Pollard et al. 2009). With this adherent culture, tumour cells from surgical glioma tissues are maintained and passaged in laminin-coated flask with neural stem cell medium. BTSC phenotypes are retained under this condition, harbouring genetic aberrations consistent with parental tumours. The efficiency of successful establishment of BTSC lines is 100% for adherent culture but is only around 30% for neurosphere culture. In addition, the adherent population composes more true BTSCs compared with neurosphere culture, because of less differentiation and apoptosis. Furthermore, the adherent monolayer growth pattern of BTSCs is more suitable for chemical screens. Therefore, adherent culture appears to be superior in the maintenance of BTSCs.

### 2.4 Animal models for BTSCs

Although the *in vitro* culture protocol offers a convenient and less expensive method to maintain BTSCs, it has apparent limitations. Most of all, the *in vitro* culture is unable to establish a specific and interactive microenvironment as BTSCs have in brain tumour tissues (Sanai, Alvarez-Buylla, and Berger 2005). As a result, BTSCs may undergo genotypic and/or

phenotypic changes so as to adapt to the *in vitro* environment, which potentially weakens the clinical relevance of BTSCs, especially when to test the efficacy of novel chemotherapeutic drugs and small molecule inhibitors.

In order to overcome the drawbacks of the *in vitro* culture systems, different categories of *in vivo* animal models were developed to maintain BTSCs and were employed to dissect the molecular events in BTSCs. Transgenic murine model is one of them. In this model, mouse lines genetically engineered with specific genetic alterations frequently observed in human brain tumours were created. Notably, with the advances in genetic techniques, the gain and loss of one or multiple genes can be conditionally manipulated in a tissue- and/or time-specific manner. For instance, tet-regulated or CRE-inducible alleles of genes can allow for the control of the timing, duration, and tissue compartment of gene expression or inactivation (Glaser, Anastassiadis, and Stewart 2005). The spontaneous brain tumours generated from these transgenic murine models are appropriate resources of BTSCs. In a *S100β-verbB;Trp53* transgenic murine model that develops spontaneous gliomas, the existence of BTSCs was confirmed, which were enriched in the side-population cells and characterized with self-renewal, multipotentiality and enhanced tumour-initiating capacity (Harris et al. 2008). Similarly, Ward and coworkers harvested BTSCs from medulloblastomas arising from genetically engineered *Patched-1*-deficient mice. These BTSCs displayed a neural precursor phenotype and were capable to generate medulloblastomas following allogeneic orthotopic transplantation (Ward et al. 2009).

Besides, transgenic murine models provide valuable information on fundamental and mechanistic facets of BTSC biology. Until now, amounting evidences supports the role of BTSCs in the tumourigenesis of brain tumours. But questions are coming with the BTSC theory. Among them, where BTSCs originate is the most intriguing one. Three hypotheses on the origin of BTSCs are proposed: (1) mature astrocytes dedifferentiate to have stem cell-like properties, (2) committed progenitors acquire mutations that endow them with unstrained "stemness", (3) neural stem cells become tumourigenic as a result of the chaotic regulation in mitosis and differentiation. Physiologically relevant transgenic murine models that spontaneously generate gliomas provide an ideal and powerful approach to tackle this question. Studies with somatic cell mouse models demonstrated that the loss function of pivotal tumour suppressor genes render early cortical astrocytes susceptible to oncogenic transformation and dedifferentiate to initiate gliomas, suggesting the possibility of the reprogrammed astrocytes as the precursors of BTSCs (Uhrbom et al. 2005, ; Xiao et al. 2005). Committed progenitors have also been shown to be able to derive BTSCs. Overexpression of oncogenic $HRas^{L61}$ in *p53*-deficient oligodendrocyte precursor cells (OPCs) can result in the generation of BTSCs in mice (Hide et al. 2011). By using RAS/tv-a mouse model, Lindberg and coworkers specifically transferred PDGF-B into OPCs and successfully induced gliomas *in vivo*. The introduction of PDGF-B resulted in the expression of SOX2, OLIG2 and NG2 in tumour cells, implying a slight dedifferentiation of the targeted OPCs (Lindberg et al. 2009).

Because of the similarities that BTSCs share with neural stem cells, it is logical to assume that BTSCs may derive from neural stem cells. Clinical and genetic studies provide clues that some GBMs may arise from the subventricular zone where neural stem cells originate. Wang and coworkers employed a transgenic murine model with an in-frame *p53* deletion mutation specially targeted into the nervous system to investigate the role of neural stem cells in *p53*-mediated gliomagenesis (Wang et al. 2009). This study revealed that *p53* deficiency provides no significant growth advantage to adult brain cells, but can induce accumulation of cooperative oncogenic alterations in neural stem cells in subventricular

zone and subsequently result in glioma formation in experimental animals, which links BTSCs with neural stem cells. In addition, neural stem cells derived from mice with concomitant specific deletion of *p53* and *Pten* in the central nervous system have an enhanced self-renewal and impaired differentiation and initiated acute-onset infiltrative high-grade gliomas (Zheng et al. 2008). The oncogenic transformation of neural stem cells is demonstrated to be driven by the up-regulation of Myc protein.

As mentioned above, transgenic murine models shed light on the initiation of BTSCs and play an important role in the studies to analyze the functionality of specific genes and cooperative gene networks in BTSCs. But there are ongoing concerns about transgenic murine models: (1) targeting selected genes in transgenic these models are not likely to fully replicate the clinical and biological heterogeneities of brain tumours, (2) the mouse-derived brain tumours may biologically divert from human counterparts because of species difference.

Xenograft model is another option to investigate BTSCs. In this model, human glioma tissue or cells that enrich BTSCs are implanted heterotypically (in nonautochthonous site) or orthotopically (in the original site) in immunodeficient animals. Although heterotypical subcutaneous xenograft model is widely used to investigate brain tumours, subcutaneous implantation of GBM cells has been shown to generate tumours with less clinical relevance because organ-specific environment plays a vital role in glioma behaviour (Antunes et al. 2000). Therefore, orthotopic intracranial xenograft models appear to be superior in the preclinical studies of brain tumours and BTSCs. Orthotopic xenograft models of multiple brain tumour types such as GBM, medulloblastoma and ependymoma have been successfully established. The tumours generated from these models bear histopathological resemblance to their origins and BTSCs can be isolated from the orthotopic models (Fei et al. 2010 ; Shu et al. 2008,; Yu et al. 2010). Because of the advantage to faithfully recapitulate the biological phenotypes of original patient tumours and stably preserve BTSC pool, the orthotopic model is an optional approach to facilitate biological studies of BTSCs. In addition, novel therapeutic strategies such as telomerase angtagonist, oncolytic picornavirus and Akt inhibitors have been pre-clinically tested in orthotopic xenograft models and yield promising outcomes by preferentially targeting BTSCs (Marian et al. 2010; Yu et al. 2011; Eyler et al. 2008).

## 3. BTSC, the prime culprit for treatment resistance

The identification of BTSCs provides insight into the therapeutic resistance of brain tumours and lead to a reassessment of current treatment against brain tumours. Most of current therapies have the power of mass destruction against the non-BTSC population but fail to precisely strike BTSCs.

### 3.1 Radioresistance

Radiotherapy is a mainstay in the management of GBMs and eliminates glioma cells mainly through inducing a DNA double-strand break. Although effective, radiotherapy can seldom eradicate all the glioma cells and the recurrence of GBM after radiotherapy seems inevitable. A growing body of evidence supports that BTSCs play a pivotal role in the resistance of radiotherapy. With magnetic resonance (MR) imaging and L-[methyl-11C] methionine positron emission tomography (MET-PET) scanning, Taumura and coworkers demonstrated that most of malignant gliomas treated with Gamma Knife surgery (GKS) plus external

beam radiation (EBRT) recur even with initially well responding. Malignant glioma cells are found within the areas exposed to irradiation. In histological sections after GKS plus EBRT, CD133-positive tumour cells markedly accumulate while are infrequent in primary sections obtained before irradiation. These CD133-positive glioma cells are postulated as BTSCs and are capable of surviving high dose irradiation (Tamura et al. 2010).

The radioresistance of BTSCs may stem from an increase in DNA repair. After irradiation, CD133-positive BTSCs are enriched because of their capability to preferentially activate the DNA damage checkpoint and repair radiation-induced DNA damage more efficiently than those CD133-negative non-BTSCs. The radioresistance of CD133-positive BTSCs can be reversed with a specific inhibitor of Chk1 and Chk2 cell cycle checkpoint kinases (Bao et al. 2006). In addition, autophagy has been shown to contribute to the radioresistance of BTSCs. Autophagy is a process of self-cannibalization whereby cells maintain homeostasis and survive under stress via lysosomal degradation of cytoplasmic proteins and organelles. In a study carried out by Lomonaco and coworkers, the expression of autophagy-related proteins, such as LC3, ATG5 and ATG12, are found higher in CD133-positive BTSCs compared with CD133-negative cells. Gamma-radiation induces a larger degree of autophagy in CD133-positive BTSCs. Autophagy protects cancer cells from radiation damage by decreasing cytoplasmic acidification, by providing catabolites required for DNA repair and by removing toxic substances. Inhibition of autophagy can significantly decrease the viability of BTSCs in response to γ-radiation (Lomonaco et al. 2009). The mechanism underlying the induction of autophagy in BTSCs by γ-radiation is not elucidated. But recent studies suggested that the activation of ataxia-telangiectasia-mutated (ATM), a checkpoint protein, following radiation, can lead to an inhibition of mTOR pathway through the phosphorylation of LKB-1, which may initiate the process of autophagy. The role and the mechanism of autophagy in the radioresistance of BTSCs still need further investigation.

More recently, the microenvironment that BTSCs reside in has been found to contribute to the radioresistance of GBM (Jamal et al. 2010). When intracranially transplanted, BTSCs demonstrate a less susceptible to irradiation and have a faster repair of radiation-induced DNA damage reflected by the dispersal of γH2AX foci, compared with their counterpart grown *in vitro*. Microarray analysis revealed that genes involved in reactive oxygen species (ROS) scavenging and antioxidant response are significantly influenced in BTSCs in xenografts, which appears to subsequently render BTSCs to be more resistant to radiotherapy *in vivo*.

### 3.2 Chemoresistance

GBMs are notorious for their insensitivity to chemotherapeutic agents. Recently, BTSCs have been demonstrated to be involved in the chemoresistance of GBMs. *In vitro*, BTSCs isolated and cultured from surgical GBM specimens display marked resistance to cytotoxic drugs including TMZ, cisplatin, epotoside and vincristine. BTSCs can recover and proliferate quickly following treatment with antineoplastic agents (Eramo et al. 2006). Moreover, glioma cells isolated from multiple tumour areas have different sensitivity to TMZ. More committed glioma cells are found to distribute along the peripheral area and to undergo apoptosis in response to TMZ, while more immature cells with CD133 positivity localized in the inner core of GBMs and are more insensitive to TMZ (Pistollato et al. 2010). Gene expression analysis of GBM patients treated with concomitant chemotherapy with TMZ revealed that an expression gene set comprising CD133 is associated with treatment-resistance and predicts poor survival in this group of patients. Intriguingly, the gene set

includes a cluster that is reminiscent of a self-renewal gene signature identified in murine MLL-AF9-induced leukemic stem cells derived from committed progenitors, which suggests the relevance of a stem-like cell phenotype in the treatment resistance of GBMs (Murat et al. 2008). Pallini and coworkers conducted a prospective investigation to explore the prognostic potential of *in vitro* BTSC analysis and the presence of CD133-positive cells in 44 consecutive GBM patients treated with concurrent chemoradiation followed by TMZ. Fourteen GBMs which can generate BTSC *in vitro* have a less favourable prognosis, with a median overall survival of 8 months compared with 14 months among GBMs without generation of BTSCs. In addition, the presence of more than 2% CD133-positive cells in GBM lesions is associated with an early progression. The median progression-free survival is 10 months for GBM patients with less than 2% CD133-positive cells while it was only 5 months for those with more than 2% CD133-positive cells (Pallini et al. 2008). Similarly, the prognostic value of the presence of BTSCs and CD133 expression is also shown in high-grade oligodendroglial tumours treated with chemotherapy. Patients with tumours neither containing BTSCs nor showing CD133 expression have a favourable clinical outcome. In this study, the possibility of presence of CD133 vascular progenitor cells is excluded with immunohistochemical double staining of CD133 and the panvascular marker CD31. Weakly double-positive cells for CD133 and CD31 are only detected in one of 20 tumours investigated, which indicates that the CD133-positive cells are derived from tumours instead of vasculatures (Beier et al. 2008).

BTSCS have been found able to modulate expression of multidrug resistance related genes to reverse the cytotoxic effect of chemotherapeutic agents. Liu and coworkers demonstrated that CD133-posive BTSCs are significantly resistant to conventional chemotherapeutic agents including TMZ, carboplatin, paclitaxel and etoposide when compared to autologous CD133-negative GBM cells (Liu et al. 2006). Further real-time PCR analysis revealed that CD133-positive BTSCs have an enhanced gene expression of multi-drug resistance and DNA mismatch repair, as well as genes inhibiting apoptotic cascade. For instance, ATP-binding cassette sub-family G member 2 (ABCG2), a gene accounting for chemoresistance of multiple cancer types, is increased 6.5 times in BTSCs than in CD133-negative cells. Of note, the expression of MGMT, a pivotal DNA repair enzyme that confers resistance to alkylating agents, in CD133-positive cells, is found 32.4 times as high as that in non-BTSCs. In addition, anti-apoptotic genes, including FLIP, BCL-2 and BCL-XL, are also found markedly up-regulated while the expression of pro-apoptotic gene BAX is repressed in CD133-positive BTSCs. Hussein and coworkers established a panel of pediatric BTSC cell lines and analyzed their sensitivity to etoposide (Hussein et al. 2011). BTSCs are found to be enriched when they are maintained as neurospheres in serum-free medium supplemented with EGF and FGF compared to as monolayers in serum-containing FBS. The clonogenic survival analysis demonstrated that neurosphere-derived cells are significantly more resistant to etoposide. Two ATP-binding cassette multidrug transporters, ABCB1 and ABCC1, are enriched in CD133-positive BTSCs and are further increased by etoposide treatment.

### 3.3 Resistance of BTSCs to immunotherapy

Immunotherapy represents a promising strategy for the treatment of cancers in addition to conventional therapeutic approaches. The virtue of immunotherapy is that it mobilizes the patient's immune system to specifically recognize and eradicate cancer cells while spares normal cells at the same time. The researches of immunotherapy against gliomas were once hindered by the perception that the brain is an immune privileged organ, basing on several

evidences. For example, the existence of blood-brain barrier (BBB) separates brain parenchyma from systemic circulation, which was assumed to hamper the entrance of immune effector cells into the brain (Pachter, de Vries, and Fabry 2003). In addition, the paucity of major histocompatibility complex (MHC) expression on brain cells, and the lack of organized lymphoid tissue and lymphatic drainage also suggest the difficulty to initiate effective immune responses (Read et al. 2003; Walker, Calzascia, and Dietrich 2002). However, several studies threw doubt on the assumption of the complete immunological silence in CNS. The findings in CNS autoimmune diseases including multiple sclerosis indicate the capability of activated T lymphocytes to traverse the BBB (De Simone et al. 1995). In addition, BBB in patients with infection and tumour appears to be compromised (Avison et al. 2004; Davies 2002). Moreover, MHC antigens are found to be up-regulated at sites of brain injury, degenerative disease and tumour (Yang et al. 2006). All the observations mentioned above lead to intense interest in the application of immuotherapy against gliomas. However, until now, only limited clinical efficacy has been observed. Recent studies indicated that BTSCs employ various mechanisms of immune evasion. The generation of a successful classic specific immune response requires antigen uptake by antigen-presenting cells (APC) and subsequent activation of immune effector cells by APCs. Simply, APCs ingest antigenic peptides of target cells and then prime $CD8^+$ (cytotoxic T cells, CTLs) for MHC class I and $CD4^+$ (T-helper cells) for MHC class II, which will take effect to kill target cells (Sikorski and Lesniak 2005). BTSCs are found to express lower level of MHC than their differentiated counterpart in FBS medium. Since MHC I plays a critical role in the immune response, the altered MHC I expression shelters BTSCs from the recognition and subsequent lysis by CTLs (Di Tomaso et al. 2010). In addition to escaping immune response, BTSCs can profoundly influence the function of immune system components. BTSCs but not differentiated glioma cells are able to inhibit allogeneic T-cell proliferation. With enzyme-linked immunosorbent assay, Wei and coworkers found that BTSCs produce multiple immunosuppressive cytokines including transforming growth factor -β1 (TGF-β1) and prostaglandin E2 (PGE2) (Wei et al. 2010). TGF-β is a family comprising a large number of structurally related polypeptide growth factors and has been reported to be involved in immune response (Kirkbride and Blobe 2003). TGF-β inhibits the maturation and antigen presentation of APCs. As well, TGF-β has been found to inhibit T cell proliferation and activation. Moreover, TGF-β can impair CTL function through inhibiting the synthesis of cytotoxic molecules including FasL, IFN-γ and perforin. As for PGE2, it is a product of arachidonic acid metabolism, which is produced at sites of inflammation or tissue injure (Wang and Dubois 2006). PGE2 can suppress T cell activation and inhibit the anti-tumour activity of NK cells. Besides, PGE2 are shown to inhibit immune response through down-regulating the production of Th1 cytokines (IFN-γ and TNFα) and up-regulating Th2 cytokines (IL-4 and IL-10). PGE2 is also reported to enhance the suppressive activity of regulatory T cells. In addition, BTSCs are found to trigger T cell apoptosis mediated by co-stimulatory molecule B7-H1 and soluble Galectin-3. These findings suggested that BTSCs are able to escape immunotherapy through diminishing signals for immune recognition and inducing profound immune suppression.

## 4. BTSCs, promising therapeutic target

Although much effort has been made to aggressively treat malignant brain tumours, the prognosis is far from satisfactory. An accumulation of recent studies suggested the pivotal

role of BTSCs in the treatment resistance to conventional therapies. The current treatment strategies appear to effectively reduce the bulk of brain tumour cells but spare BTSCs. The improvement in the understanding of mechanisms underlying BTSC regulation provides insight into the development of novel treatment specifically targeting key signalling pathway and microenvironment of BTSCs, which appears to be a promising path leading to the cure of malignant brain tumours.

### 4.1 PI3K/Akt signaling

PI3K/Akt signaling is one of the most important and widely investigated pathways in brain tumours. As a major intracellular effector, PI3K transduces membrane-based activation from various sources, including G proteins such as Ras and receptor tyrosine kinases such as EGFR. Activation of PI3K/Akt pathway is implicated to regulate cellular proliferation, metabolism, differentiation and survival (Engelman, Luo, and Cantley 2006). Dysfunction of PI3K/Akt pathway is frequently observed in gliomas and suggested to mediate the gliomagenesis, progression and the resistance to therapy, which can be resulted from persistent activating signals from cell membrane (i. e. EGFR mutations), gain-of-function mutations in PI3K components (i. e. PIK3CA) and loss of tumour suppressor (i. e. PTEN deletion). In fact, PI3K-activating mutations are detected in almost all patients with GBMs (Comprehensive genomic characterization defines human glioblastoma genes and core pathways 2008, ; Parsons et al. 2008). Recently, PI3K/Akt pathway has been reported to be essential for the survival and treatment resistance of BTSCs. An animal model study demonstrated that the activation of PI3K/Akt pathway due to the loss of PTEN in gliomas greatly increases the SP cells and renders them to be more resistant to multiple chemotherapeutic agents including TMZ (Bleau et al. 2009). In addition, activated PI3K/Akt pathway protects BTSCs in their niche from radiation-induced apoptosis through the induction of p53-dependent cell cycle arrest (Hambardzumyan et al. 2008). Based on the critical role in BTSC biology, activated PI3K/Akt pathway can be a promising target against BTSCs. Eyler and coworkers revealed that BTSCs isolated from primary GBMs are more sensitive to Akt inhibition. Treatment with an Akt inhibitor resulted in a more remarkable reduction of BTSCs compared with non-BTSCs, which is associated with a preferential induction of apoptosis and a suppression of neurosphere formation. Furthermore, inhibition of Akt activity impairs the tumourigenicity of BTSCs and significantly prolongs the survival of mice bearing glioma xenografts initiated by BTSCs (Eyler et al. 2008). In another study performed by Sunayama and coworkers, dual blockade of PI3K and mTOR has a profound effect on BTSCs. Exposure to LY294002 combined with NVP-BEZ235 disrupts the self-renewal and triggers differentiation in BTSCs, with an inhibition of neurosphere formation and tumour initiation, as well as an up-regulation of neuronal differentiation marker, βIII-tubulin (Sunayama et al. 2010).

### 4.2 STAT3

Signal Transducer and Activator of Transcription 3 (STAT3) is a potentially attractive therapeutic target for BTSCs based on its dual role in regulation of stem cell functions and oncogenesis. STAT3 belongs to a family of latent transcription factors that are activated by multiple extracellular and intracellular signals including the Janus kinase (JAK) family and receptor tyrosine kinases (RTKs) (Darnell 1997). After phosphorylation of a tyrosine residue in the transactivation domain, activated STAT proteins dimerize, translocate into the nucleus and modulate gene expression by binding to specific DNA-response elements in the

promoter of target genes, including genes associated with cell survival, cell cycle regulation, immune response and differentiation, such as c-myc, cyclin D1, Bcl-2, VEGF and HIF1α. STAT3 is a crucial component in the regulation of embryonic development and the maintenance of multiple normal stem cell types, with mouse models demonstrating that STAT3-deficiency leads to lethality at E6.5 (Takeda et al. 1997). In neural development, STAT3 is necessary for the maintenance and self-renewal of neural stem cells and precursors (Yoshimatsu et al. 2006). In addition, STAT3 is an oncogene that induces transformation through increased expression of target genes that promote cell growth and inhibit apoptosis in multiple cell types. Constitutive activation of STAT3 is observed in a variety of tumours including glioma (Rahaman et al. 2002). STAT3 is found to be a master regulator of mesenchymal transformation in gliomas and correlate with poor prognosis of glioma patients. Elimination of STAT3 and another transcriptional factor C/EBPbeta leads to the collapse of the mesenchymal signature and reduces tumour aggressiveness (Carro et al. 2010). STAT3 has been identified as an important maintenance factor for BTSCs. Under undifferentiated condition, BTSCs have a constitutively high level of phosphorylated STAT3. Genetic knockdown of STAT3 leads to a significant inhibition of proliferation and neurosphere formation of BTSCs. Of note, even a transient inhibition of STAT3 is sufficient to result in irreversible growth arrest and diminishment of neurosphere formation, implying the critical role of STAT3 in the maintenance of BTSC self-renewal (Sherry et al. 2009). In addition, an inhibition of STAT3 synergizes the anti-cancer effect of TMZ against BTSCs and impairs the tumourigenic capacity of BTSCs *in vivo* (Villalva et al. 2011; Li et al. 2010).

### 4.3 Differentiation induction in BTSCs

Normal neural stem cells are characterized with their multipotency to differentiate into neurons, astrocytes and oligodendrocytes. BTSCs, although aberrantly, can also differentiate into tumour progenies with multi-lineage morphologies and markers. These differentiated cells constitute the bulk of brain tumours but lose the capacity of unlimited proliferation *in vitro* and the tumourigenicity *in vivo*. Therefore, exhaustion of BTSC pool by the induction of differentiation appears to be a practical therapeutic strategy. In fact, differentiation-inducing agents have already been used in the management of brain tumours before the surge of BTSC theory. The clinical activity of retinoids, known as powerful modulators of cellular differentiation and proliferation, was evaluated in patients with recurrent malignant brain tumours. In a Phase II study performed by Yung and coworkers, high-dose 13-cis-retinoic acid (CRA) is found to be effective against malignant gliomas with acceptable toxicity. CRA appears to work as a cytostatic rather than a cytolytic agent. The mechanism underlying the antitumour activity of CRA was not clearly known at that time (Yung et al. 1996). But now, under the BTSC scenario, this effect of CRA is attributed to its ability to induce differentiation in BTSCs. Exposure to all-trans-retinoic acid (ATRA) decreases CD133 level and results in an augmented expression of lineage markers in BTSCs. The disruption of angiogenesis and impairment of motility are also observed in BTSCs after ATRA treatment. In addition, the differentiation induced by ATRA can sensitize BTSCs to chemotherapy and irradiation by increasing cell death and apoptotic susceptibility (Campos et al. 2010).

Key components in several signalling pathways have been identified to be involved in the differentiation induction of BTSCs. Bone morphogenetic proteins (BMPs) belong to the transforming growth factor-β superfamily and play an important role in promoting differentiation in embryonic stem cells and neural stem cells. BMPs are also assessed for the

differentiation induction in BTSCs. Piccirillo and coworkers demonstrated that non-cytotoxic BMP4 starts the differentiation program in BTSCs, resulting in a depletion of BTSC pool by 50% and an induction of morphological transition into more committed tumour cells. BTSCs transiently pre-treated with BMP4 fail to initiate large invasive and highly vascular tumour masses *in vivo* but only generate small lesion with low capacity of proliferation and infiltration. In addition, intracranial delivery of BMP4 simultaneously or 10 days after the injection of BTSCs into mouse brain retards tumour growth and prolongs the survival of tumour-bearing mice (Piccirillo et al., 2006). This study presents differentiation induced by BMPs as a promising strategy to target BTSCs. However, subsequent investigations demonstrated that not all BTSCs are readily responsive to BMPs at all time. Induction of differentiation in BTSCs by BMPs can be blocked by intrinsic and extrinsic factors. The response of BTSCs to BMPs depends on the expression and normal function of BMP receptors. In a subset of BTSCs, the expression of BMP receptor 1B (BMPR1B) is lost due to epigenetic silencing by an EZH2-dependent mechanism (Lee et al. 2008). In addition, the differentiative effects of BMPs on BTSCs can be regulated by oxygen tension. A hypoxic condition renders BTSCs to be more resistant to BMP-induced differentiation, which is found to be mediated by HIF1α (Pistollato et al. 2009).

Notch, a modulator of neural stem cell fate, has also been demonstrated to play a key role in the differentiation of BTSCs. Notch proteins are cell surface receptors that mediate cell-cell communication. After binding with transmembrane ligands from adjacent cells, Notch releases its intracellular domain cleaved by γ-secretase. The intracellular domain then translocates into the nucleus and functions as a transcription factor to regulate target gene expression. In the embryonic central nervous system, Notch signalling maintains a pool of undifferentiated neural stem cells by promoting the proliferation while inhibiting their differentiation into neurons. The dysregulation of Notch signalling pathway has been reported in a variety of neoplasms, including breast cancer, leukemia, and melanoma. In brain tumours, increased expression of Notch-related genes and elevated Notch activities are also found. Gene expression array demonstrated that Notch ligand Jagged-1, Notch 3 and the downstream target of Notch (HES1 and HES2) are overexpressed in a majority of primary GBMs relative to non-neoplastic brain tissues (Kanamori et al. 2007). Purow and coworkers performed a detailed functional study on Notch signalling pathway in gliomas. In this study, the presence of Notch-1 was found in glioma tissues and six glioma cell lines. Down-regulation of Notch-1 with siRNA leads to decreased cell proliferation, cell cycle arrest and apoptosis in glioma cells. Knock-down of Notch-1 significantly prolongs the survival of mice orthotopically transplanted with glioma cells (Purow et al. 2005). Moreover, Notch signalling is also linked to the maintenance and differentiation of BTSCs. When investigating GBM BTSCs, Fan and coworkers found that BTSCs derived from GBMs are sensitive to Notch signalling blockade by γ-secretase inhibitors (GSIs). GSIs selectively reduce the proliferation of Nestin- or CD133-positive BTSCs, which results in an inhibition of neurosphere clonogenicity *in vitro* and tumourigenicity *in vivo* (Fan et al. 2010). In another study carried by the same group, Notch blockade is found to preferentially target BTSCs in medulloblastomas. GSI exposure reduces the CD133-positive cell fraction by almost 5-fold and totally depletes the side population cells. The viable cells after the GSI treatment have an increased expression of two neuronal markers, Tuj1 and GABRA6. These better-differentiated tumour cells can continue to proliferate but fail to establish soft-agar colonies or tumour xenografts (Fan et al. 2006). These studies indicate that Notch blockade can induce differentiation in BTSC and can be potentially useful in the treatment of malignant brain tumours.

### 4.4 BTSC niche

It has been recognized that stem cells are not randomly distributed throughout the organ where they remain. In fact, stem cells reside in niche, a specific microenvironment consisted of both cellular and acellular components, which plays an important role in regulating the function of stem cells (Burness and Sipkins 2010). In the brain, neural stem cells (NSC) are concentrated in the subgranular zone of the hippocampus and the subventricular zone of the lateral ventricle, with close proximity to blood vessels (Quinones-Hinojosa et al. 2007). The contact and communication of niche and NSCs are critical in maintaining the quiescence and driving the proliferation and differentiation of NSCs. BTSCs are proposed to origin from the oncogenic transformation of NSCs and appear to occupy similar niches. The subventricular zone has long been proposed as the source of glioma, based on the observation that many gliomas either grow around the ventricle or are contiguous with the subventricular zone (Glantz et al. 2009). In clinical studies, bordering lateral ventricle by gliomas has been associated with decreased survival. Chaichana and coworkers analyzed the relationship of the tumour location and the survival of GBM patients (Chaichana et al. 2008). The authors found that GBM patients with a radiological contrast-enhancing lesion bordering the lateral ventricles (LV CEL) have a more unfavourable prognosis compared to patients with non-LV CEL and therefore postulated that the region of lateral ventricles may have a microenvironment more conducive for potent tumour cells, perhaps BTSCs, to proliferate and/or infiltrate. This area also has been shown to have an increased propensity to form glioma in animal studies. Marumoto and coworkers successfully induced intracranial high-grade gliomas in adult immunocompetent mice by injecting Cre-loxP-controlled lentiviral vetors expressing oncogenes (Marumoto et al. 2009). Of note, more than 75% of mice show tumour formation when viral vectors are injected in neurogenic areas such as the subventricular zone and hippocampus, while there is little tumour formation in the cortex. Moreover, transplantation of these brain tumour cells into naïve recipient mouse brain results in the formation of GBM-like tumours containing BTSCs. This study suggested the susceptibility of cells in neurogenic areas to oncogenic stimuli and the possible origin of BTSCs in these areas.

Malignant gliomas are characterized as highly vascular tumours. The presence of microvascular proliferating structures is recognized as a pathological criterion in diagnosis of high-grade gliomas. The density of microvascular proliferation has been demonstrated to correlate with the prognosis of glioma patients (Wong et al. 2009). Accumulating evidence suggested that vasculatures in glioma are more than pipelines to supply nutrients and oxygen, they can function as niches, interacting with brain tumours and regulating BTSC behaviour (Gilbertson and Rich 2007). A dynamic analysis revealed that gliomas cells extensively infiltrate the brain parenchyma through migrating along blood vessels. Remarkably, the majority of glioma cells divide around vascular branch points, indicating that the microenvironment provides cues to trigger mitosis (Farin et al. 2006). Calabrese and coworkers did the first thorough investigation on the perivascular niche for BTSCs. With 3-dimensional (3D) imaging, the authors clearly demonstrated that Nestin-positive BTSCs directly associate with tumour capillaries in glioma specimens. Coculture with primary human endothelial cells (PHECs) maintains the self-renewing and undifferentiated status of BTSCs. Moreover, PHECs are found to robustly expand the proliferation of BTSCs and promote tumour growth *in vivo*. It is suggested that vascular endothelial cells may support BTSCs by secreting soluble factors similarly as they do for neural stem cells (Calabrese et al. 2007).

Because BTSCs have intimate relationship with their microenvironment, targeting the niche seems to be a desirable therapeutic strategy to eradicate BTSCs. Increasing the dosage of irradiation to BTSC niche has been shown to yield significant benefits for patients with high-grade gliomas. Evers and coworkers performed a retrospective analysis to investigate the effect of the radiation to the periventricular niche on the prognosis of 55 adult patients with malignant glioma (Evers et al. 2010). The progression-free survival of patients whose bilateral subventricular zone (SVZ) received greater than the median SVZ dose (43 Gy) is 15.0 months, which is much longer than that (7.2 months) of patients who received less than the median dose. Besides, disruption of the vascular structures that BTSCs depend on is also a promising option. Preclinically, anti-angiogenic agents are shown to specifically act on BTSCs (Calabrese et al. 2007). Erlotinib and Bevacizumab are successful in inhibiting the self-renewasl of BTSCs and suppressing the tumour growth in glioma-bearing mice, implying the efficacy of the disruption of BTSC niche. Similarly, Williams and coworkers revealed that creation of a barrier between BTSCs and endothelial cells can decrease the number of BTSCs in glioma xenografts (Williams et al. 2010). The authors treated the orthotopic glioma models with IFN-β. Although IFN-β is not directly toxic to BTSCs *in vitro*, treatment with IFN-β leads to tumour growth arrest *in vivo* by affecting the BTSC niche. IFN-β increases perivascular cells investing vasculature, resulting in the disruption of the communication between BTSCs and endothelial cells. Clinically, bevacizumab, a monoclonal antibody targeted against VEGF, and cediranib, a small molecule inhibitor of VEGF show some success. After a systematic review and survival-gain analysis, Xu and coworkers found that combination of bevacizumab and irinotecan largely improves response rates and has a possible moderate effect on overall survival in patients with recurrent adult high-grade glioma (Xu et al. 2010). A Phase II clinical trial performed by Batchelor and coworkers demonstrated that treatment for recurrent GBM with Cediranib monotherapy results in encouraging proportions of radiographic response and 6-month progression-free survival (Batchelor et al. 2010). In addition to depletion of the tumour blood supply, the clinical efficacy of anti-angiogenic drugs may be partly attributed to the disruption of the perivascular niche of BTSCs (Gilbertson and Rich 2007).

## 5. Future directions and challenges

The emergence and evolution of BTSC concept broaden our horizon in the understanding of brain tumour biology. The roles of BTSCs in the progression and treatment resistance of brain tumours lead us to have a reassessment of the current treatments. Key signalling pathways identified in BTSCs provide clues to develop novel therapeutics, which may pave the road to the cure of malignant brain tumours. However, this road can be winding and bumpy. There are still challenges we should realize. First, BTSCs are heterogeneous and the markers as well as approaches to purify BTSCs currently employed are unlikely to identify all BTSC phenotypes. New markers need to be explored and a combined use of a set of various markers and/or purification techniques appears to be more effective in the identification of different BTSC population. Secondly, many signalling pathways pivotal to the maintenance and self-renewal of BTSCs are also necessary for normal stem cells. Approaches aiming at these signalling pathways may do harm to normal stem cells. Therefore, critical signalling pathways specific in BTSCs are superior targets. Thirdly, the stemness of BTSCs is not unchangeable, which can be tuned in order to adapt tumour cells to the changes in microenvironment. Therefore, BTSCs appear to be running targets instead

of stationary ones. The therapeutic strategies against BTSCs should be continuously modulated. Finally, the microenvironment contributes significantly to the phenotype and plasticity of BTSCs. The effective treatment in future should focus on not only BTSCs *per se* but also the niches BTSCs reside in.

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**Cancer Stem Cells - The Cutting Edge**

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Over the last thirty years, the foremost inspiration for research on metastasis, cancer recurrence, and increased resistance to chemo- and radiotherapy has been the notion of cancer stem cells.The twenty-eight chapters assembled in Cancer Stem Cells - The Cutting Edge summarize the work of cancer researchers and oncologists at leading universities and hospitals around the world on every aspect of cancer stem cells, from theory and models to specific applications (glioma), from laboratory research on signal pathways to clinical trials of bio-therapies using a host of devices, from solutions to laboratory problems to speculation on cancersâ€™ stem cellsâ€™ evolution. Cancer stem cells may or may not be a subset of slowly dividing cancer cells that both disseminate cancers and defy oncotoxic drugs and radiation directed at rapidly dividing bulk cancer cells, but research on cancer stem cells has paid dividends for cancer prevention, detection, targeted treatment, and improved prognosis.

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