NEURAL REGENERATION RESEARCH
November 2017, Volume 12, Issue 11
www.nrronline.org

REVIEW

Research on human glioma stem cells in China

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How to cite this article: *Zhao YD, Zhang QB, Chen H, Fei XF, Shen YT, Ji XY, Ma JW, Wang AD, Dong J, Lan Q, Huang Q (2017) Research on human glioma stem cells in China. Neural Regen Res 12(11):1918-1926.*
Funding: *This study was supported by the National Natural Science Foundation of China, No. 81172400, 81101909, 81272793, 81302180, 81302196, 81472739.*

Abstract

Research on human glioma stem cells began early in the 21st century and since then has become a rapidly growing research field with the number of publications increasing year by year. The research conducted by our diverse group of investigators focused primarily on cell culture techniques, molecular regulation, signaling pathways, cancer treatment, the stem cell microenvironment and the cellular origin and function of glioma stem cells. In particular, we put forward our view that there are inverse or forward transformations among neural stem cells, glial cells and glioma stem cells in glioma tissues under certain conditions. Based on the background of the progress of international research on human glioma stem cells, we aim to share our progress and current findings of human glioma stem cell research in China with colleagues around the world.

Key Words: *nerve regeneration; glioma stem cells; China; cell culture; molecular characteristics; cellular origin; cell function; microenvironment; molecular targeted therapy; chemotherapy; radiotherapy; neural regeneration*

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doi: *10.4103/1673-5374.219055*

Accepted: *2017-07-31*

Introduction

Research on human glioma stem cells (GSCs), like other human cancer stem cells (CSCs), occupies a central role in the development, diagnosis, and treatment of cancers. Currently, GSCs are considered to be a small percentage of the G_0-arrested cells located in the glioma niche. GSCs serve as the seed cells for tumorigenesis and metastasis within the tumor microenvironment (Adorno-Cruz et al., 2015).

The existence of CSCs was proposed by the Cancer Center of Michigan University approximately 150 years ago. More recently, Lapidot et al. (1994) identified acute myeloid leukemia-initiating cells *via* transplantation in severe combined immune deficiency mice. Confirmation of the existence of CSCs was first demonstrated by Reya et al. (2001). The relationship between malignant brain tumors and stem cells was suggested in the 1980s by Rosenblum et al. (1983), but 20 years later, Hemmati et al. (2003) identified neural stem-like cells in human glioma cell lines *in vitro*. Singh et al. (2003) isolated GSCs from pediatric medulloblastoma and astrocytoma. The following year, they reported that the injection of as few as a hundred $CD133^+$ cells was enough to produce a tumor, whereas the injection of a hundred thousand $CD133^-$ cells resulted in engraftment but no tumor formation (Singh et al., 2004b). Subsequently, Singh et al. (2004a) concluded that $CD133^+$ cells that also express the neural stem cell (NSC) marker, nestin, but not differentiated neural lineage markers, represent only a minority fraction of the entire brain tumor cell population that exclusively generate clonal tumor spheres in suspension culture and exhibit an increased self-renewal capacity. Subsequently, the study of GSCs has become the focus of research, mainly looking at cell culture techniques, molecular regulation, signaling pathways, cancer treatment, stem cell microenvironment and the cellular origin and function of GSCs.

Literature Analysis

To date (as of DEC, 2016), Chinese scholars have published approximately 28% (256/908) of the research papers worldwide (**Figure 1**). In this review, we have collected and analyzed articles from various Chinese universities and Chinese research institutions published in journals included in the Science Citation Index (SCI) system. In addition, we pay special attention to the progress of Chinese research on GSCs, while reviewing the overall global status of GSC research.

Research on human GSCs in China began when $CD133^+$ cells and side population cells excluding Hoechst 33342 from the human glioma cell line SHG-44 were first cloned in 2004 and 2005 (Huang, 2004; Wang et al., 2005). In 2006, we reported differences between the differentiation profiles of GSCs and NSCs (Zhang et al., 2006) in which the results of the Chinese research community was not in complete agreement with those of Singh et al. (2004a). Our results showed that these GSCs became $CD133^-$ after being induced to differentiate by sodium valproate. However, some of these cells may dedifferentiate into floating tumor spheres with a $CD133^+$ phenotype, differing from NSCs, which can be terminally differentiated. Research on human GSCs by other Chinese groups began in 2008 at Southwest Hospital of the

Third Military Medical University of PLA, where Yu et al. (2008) harvested GSCs from the U87 glioma cell line. The top 14 Chinese universities publishing research on GSCs are shown in **Figure 2**.

GSC Culture and Molecular Characteristics of GSCs

The general method for culturing GSCs involves cultivating glioma cells or tissues after enzymatic dissociation with basic fibroblast growth factor, leukemia inhibitory factor, and epidermal growth factor in a serum-free medium (Zhou et al., 2012). The floating tumor spheres are then collected and examined for stemness. Finally, purified GSCs are harvested using immunomagnetic beads or a flow cytometer for $CD133^+$ cells. Several other revised methods have been applied including the isolation of GSCs from loose, irregular clone spheres (Cao et al., 2013). Alternatively, GSCs can be harvested *via* the passage and purification of $CD133^+$ cells directly from tumor spheres, without using immunomagnetic beads or a flow cytometer (Qiu et al., 2012b). Various other methods have been used to culture GSCs (Pollard et al., 2009; Kievit et al., 2014).

Several questions remain after harvesting GSCs: How do we identify and differentiate these cells from others? Are there some specific markers for GSCs? There are still no true specific markers for them, although CD133 is the marker that is used by most scholars. However, CD133 is also regarded as a marker protein for NSCs (Uchida et al., 2000) and CSCs of other types of cancers, and the existence of $CD133^-$ GSCs has also been demonstrated. There are some sub-markers that have been used for the identification of GSCs, such as CD15, neuronal cell adhesion molecule L1 (L1CAM), CD90, B7-homologue 4 and 1 (B7-H4/1), CXC chemokine receptor 4 (CXCR4), and A2B5, stage-specific embryonic antigen 1. Of these, the CD15 is a type of adhesion molecule, and it was reported that the tumorigenicity of $CD15^+$ medulloblastoma cells was even higher than that of $CD133^+$ cells. (Read et al., 2009; Ward et al., 2009). L1CAM is also adhesion molecule, and it is required for maintaining the growth and survival of $CD133^+$ glioma cells both *in vitro* and *in vivo* (Bao et al., 2008). B7-H4 is a member of B7 family that negatively regulates T cell-mediated immunity. However, studies showed that B7-H4 was preferentially expressed in GSCs (Yao et al., 2008). CXCR4 is a cell surface molecule expressed in a certain subset of glioma cells with enhanced tumorigenicity. Zheng et al. considered that $CXCR4^+$ subsets of glioma cells met the standard of "cancer stem cell" (Zheng et al., 2011). A2B5 is predominantly expressed in embryonic and neonatal neural tissue, and it is also considered as a marker for immature glial-committed precursors. However, some studies claimed that A2B5 was a possible marker of GSCs by comparing the different tumorigenicity of $CD133^{+/-}$ glioma cells and $A2B5^{+/-}$ cells (Ogden et al., 2008).

The differentiation trend appears to be more important than the so-called specific protein markers in the identification of GSCs. The definition of GSC follows the theory of NSCs, namely that GSCs are identified based on the presence of cells expressing markers for neurons, astrocytes, and microglia, in differentiated cell populations. This outlines the importance of surface markers for the identification of GSCs.

Side population cells have also been used by some researchers to identify CSCs on the basis of high expression of the ATP binding transporter G superfamily-2 protein on the surface of stem cells. ATP binding transporter G superfamily-2 can pump the Hoechst 33342 fluorochrome out of the cytoplasm, which facilitates the screening of side population cells with flow cytometry. The similar characteristics between side population cells and CSCs suggest they could be identical. However, the proportion of side population cells among SHG44 human glioma cells is approximately 29.1%, while that of $CD133^+$ cells is only about 2.3% (Wang et al., 2005). This implies an evident significant difference between them. Each of the methods for CD133 immunophenotyping, side population flow assays and neurosphere counting have their own uses and limitations.

Many of the substances that regulate GSCs are positive regulators, including pyruvate kinase isozymes M2 (which phosphorylates histone H3), the CDC20-anaphase promoting complex/sex determining region-box 2 signaling axis, cyclin-dependent kinase 7-MYCN, Beta1, 4-galactosyltransferas, vascular endothelial growth factor-vascular endothelial growth factor receptor, nuclear related factor 2 (Nrf2) (Zhu et al., 2013, 2014b), H3K4me3 and H3K27me3, topoisomerase II alpha, Bcl-2, S100A9 (Chen et al., 2013), reactive oxygen species (Yuan et al., 2015) and Zinc finger protein 217. Other signaling pathways show negative correlations with the activities of CSCs, such as Fas/FasL-L. GSCs can be induced to proliferate with 2% sevoflurane *in vitro* with the up-regulation of CD133, vascular endothelial growth factor, hypoxia-inducible factor-1, and hypoxia-inducible factor-2 (Shi et al., 2015b).

There are gene mutations related to CSCs. For example, in GSC-SU2 (Zhao et al., 2009), there are many mutations of amino acid residues (from the 8^{th} to 14^{th} amino acid (AA), 238^{th} AA, and 398^{th} AA) in the peptide chain of phosphate and tensin homologue (PTEN) deleted on chromosome ten. These mutated regions are involved in membrane interactions, particularly those involving the phospholipid phosphatidylinositol bisphosphate, and in maintaining the protein stability of PTEN. Therefore, these mutations not only lead to the rapid degradation of PTEN, but also inhibit the cellular function of PTEN to down-regulate PI3K signaling. Isocitrate dehydrogenase 1 mutation and dependent promoter methylation of O6-methylguanine-DNA methyltransferase (MGMT) were reported as predictive biomarkers for glioma patients (Wick et al., 2013).

In fact, the formation of GSCs results from not only one or two specific molecular mechanisms, but many other signalingpathways as well. CSCs are thought to be derived from the adult stem cells (ASCs) of the corresponding tissues or organs in which many of the pathways share a common molecular basis for both these cell types. Only molecules that are upregulated or downregulated may be different in ASCs,

for tissue repair and regeneration, from CSC tumorigenesis.

Moreover, the Notch signaling pathway contributes to the maintenance of GSCs and NSCs and promotes the re-newal of GSCs (Hu et al., 2014). It has been reported that neurotensin signaling may maintain the stemness of GSCs through the activation of the interleukin-8/CXCR 1/signal transducer and activator of transcription 3 (IL-8/CXCR1/STAT3) signaling pathway (Zhou et al., 2014). Aurora A kinase could control GSC self-renewal through beta chain protein/Wnt signaling (Zhou et al., 2014). Activation of the Akt/phosphatidylinositol-3 kinases (Akt/PI3K) pathway through interaction with CD133-p85 promotes GSC oncogenicity (Wei et al., 2013). The stemness and radiation resistance of GSCs is maintained through regulation of the mi-croRNA-153/ Nrf-2/ GPx1 pathway by reactive oxygen species (Yang et al., 2015). GSCs enhance the migration and proliferation of endothelial cells through the Hedge-hog pathway (Zhou et al., 2014). Gong et al. (2015) reported that FoxM1 promotes the self-renewal and tumorigenicity of GSCs by driving a feed-forward STAT3-activation signaling loop.

These findings are limited to the origin of CSCs as ASCs, a view that is still controversial. The epithelial-to-mesenchymal transition of CSCs and normal stem cells from their corresponding normal tissue involve similar stem cell programs. However, they differ significantly in terms of their paralogous epithelial-to-mesenchymal transition-inducing transcription factors Slug and Snail programs (Ye et al., 2015).

It has become increasingly clear that miRNAs and GSCs are connected. Studies have shown that miRNAs, such as miR-125b (Wan et al., 2012, 2014), miR-123b, miR 20a (Wang et al., 2015d), and miR-210 (Yang et al., 2014a), play a positive regulatory role in GSC invasion and proliferation. Other miRNAs, such as miR 181b, miR-134a, miR-21 (Shang et al., 2015), miR-124, miR-186 (Zheng et al., 2015), and miR-145 (Shi et al., 2014), negatively regulate GSC proliferation. In addition, miR-330 negatively regulates the expression of SH3GL2 in GSCs, which promotes the oncogenic progression of GSCs through activating the ERK and PI3K/AKT signaling pathways (Yao et al., 2014b), and miR-152 plays a tumor suppressor role in GSCs (Yao et al., 2015a).

Cellular Origin and Function of CSCs

There is still no consensus on the cellular origin of CSCs. There are four main theories. (1) Cloning evolution: tumors represent a molecular disease in which gene mutations within originating cells will be passed onto future daughter cells, where further gene mutations may occur. Thus, the accumulation of many genes in descendant cells leads to carcinogenesis and malignant development. (2) The CSC theory, which was first put forward by Hamburger and Salmon (1977) and later improved by Bonnet and Dick (1997) indicated that cancer originates from a single ASC. Cancer is also induced and driven by carcinogenic agents that activate the necessary pathways and related genes for ASC proliferation and differentiation. Therefore, CSCs are also referred to ASC-like cells (*e.g.*, GSCs are also known as NSC-like cells). (3) The balance theory: there is considerable plasticity between non-CSCs (NCSCs) and CSCs. NCSCs can reacquire the phenotype of CSCs under certain environmental conditions, and that bidirectional conversion occurs between them (Marjanovic et al., 2013). In 2006, we found that differentiated glioma cells might dedifferentiate into GSCs when we compared the differentiation profiles between NSCs and GSCs. Zheng et al. (2007) found that most C6 cells were cancer stem cells. As $CD133^-$ C6 cells also possessed clonogenic, self-renewal, and tumorigenic capacities, this may indicate the reversion of $CD133^-$ cells to $CD133^+$ cells. (4) The precancerous stem cell (pCSCs) theory: Gao (2008) believed that pCSCs are at an early stage of development of CSCs, similar to the traditional theory about tumor formation; *i.e.*, a similar histological development process from cell proliferation, metaplasia, and precancerous lesions to cancerous tissue. This process is regulated by Piwi like RNA-mediated gene silencing 2, and, while pCSCs can continue their malignant evolution, they can also revert back to a benign state different from that of CSCs.

The function of CSCs has been elucidated. The known characteristics include (1) self-renewal, which refers to the mitosis of CSCs and includes two types: symmetric mitosis and asymmetric mitosis. The former produces two CSCs, while the latter produces one CSC and one NCSC, followed by downstream differentiation. This is similar for ASCs, which produce a new stem cell to replace the old one. Further investigation is required to determine whether the self-renewal of GSCs and ASCs is truly identical. The self-renewal of ASCs only occurs in the stem cell niche, while the self-renewal of CSCs may occur outside of their niche or be completed by differentiated NCSCs after homing (Clarke and Fuller, 2006). (2) High tumorigenicity. It has also been reported that only one hundred $CD133^+$ GSCs produce tumors in non-obese diabetic/severe combined immune deficiency mice *in vivo*, whereas even 100,000 $CD133^-$ NCSCs do not lead to tumor formation in the same period of time (Singh et al., 2004b). (3) Greater invasiveness. Qiu et al. (2012a) reported that GSCs are more invasive than their differentiated progeny cells *in vitro*. Invasive cells exhibit higher tumorigenicity *in vivo*, and Akt activity is significantly increased in invasive cells compared with normal cells in the corresponding tumor mass. The molecules involved in invasion include Toll-like receptors and matrix metalloproteinases-9 (Wang et al., 2015b), TGF-β1, ADAM17, and IL-6. (4) Radiation resistance. Bao et al. (2006) believe that the increase of the proportion of $CD133^+$ cells in the glioma cell population after radiation therapy is due to the radiation resistance of GSCs, which are preferentially preserved. The molecules associated with radioresistance include Wnt/β-catenin, Notch, JAK/STAT and PI3k-mTOR, checkpoint kinase 1, CD133 and MGMT. (5) Chemotherapy resistance. Hu et al. (2012) reported that the chemoresistance of human GSCs is correlated with a low level of Tap73. Furthermore, down-regulation of autophagy in GSCs contributes to the strong ability of GSCs to resist temozolomide. High expression of ATP binding transporter G superfamily-2 in SU2-GSCs results in resistance to ACNU (Huang et al., 2008;

Jin et al., 2009). It has been reported that GSCs adapt to reduce their glucose dependence and this is associated with radio-chemoresistance (Ye et al., 2013). High expression of MGMT has been reported to contribute to temozolomide resistance in GSCs (Qiu et al., 2014). However, interferon-α/β may enhance the sensitization of MGMT-positive GSCs to temozolomide by suppressing NF-κB activity (Shen et al., 2015a). (6) Angiogenesis. Tumor-derived endothelial cells originating from GSCs were detected in tumor tissues from a p53 (+/–) homozygous mouse model bearing GBM. Both Li et al. (2013) and our group (Zhao et al., 2010a) have reported the trans-differentiation of GSCs into vascular endothelial-like cells *in vitro* and we demonstrated a novel mechanism for angiogenesis that GSCs contribute to the neovascularization of glioma *via* transdifferentiation *in vivo* (Dong et al., 2011; Sun et al., 2015). However, Cheng et al. (2013) reported that GSCs contribute to vascular pericytes. In addition, it has been demonstrated that GSCs have the potential to show vascular mimicry and trans-differentiate into vascular endothelial cells in different conditions (Mao et al., 2013a, b; Yao et al., 2013).

Microenvironment of GSCs

Reciprocal causation occurs when GSCs rebuild the tumor microenvironment, and the tumor microenvironment influences the phenotype of GSCs. Zhang et al. (2013b) cultivated U251 glioma cells under various culture conditions and harvested the GSC-like cells of different phenotypes (U251-Adh, U251-SC-Sph and U251-SC-Adh). These cells also showed distinct growth patterns and self-renewal capacities. The chemokines secreted by GSCs promote the migration of NSCs to GSCs. NSC co-culture with GSCs may also induce the differentiation of GSCs *in vitro*, and reduce their stemness. NSCs injected into the cerebral hemisphere, *in vivo*, migrate towards the GSCs in the tumor, reducing their malignancy (Zhang et al., 2014c). We reported that GSCs play an important role in the vascular remodeling of transplanted tumors (Zhao et al., 2010b). However, all the above studies were completed *in vitro* or in the so-called tumor microenvironment *in vivo*.

The microenvironment of GSCs is where the GSCs are anchored, also known as the stem cell niche. Different stem cell niches may exhibit different structures, however, all stem cells including GSCs generally present unique niches (Fuchs et al., 2004). A niche exists along micro-blood vessels in cancerous tissue, acting as an umbrella to maintain stem cell self-renewal and prevent differentiation. The structure of niches includes niche cells, soluble factors from niche cells, and extracellular matrix (Lin, 2002).

A niche is composed of cells such as vascular endothelial cells, peripheral blood cells, and astrocytes. The niche cells produce cadherin and integrin molecules that mediate the adherence of ASCs and some other cell types in and around the niche (Lin, 1998; Song and Xie, 2002; Song et al., 2002; Zhang et al., 2003; Arai et al., 2005). Only the ASCs anchoring in the niche maintain quiescent conditions, whereas cells outside the niche enter the differentiation process (Zhang et al., 2003). The ASC niche serves two functions: to maintain ASC self-renewal, and to supply our bodies with different types of cells *via* multi-directional differentiation.

In the tumor microenvironment, there are tumor stroma in addition to the CSC niche. The stroma of a glioma contains myeloid-derived suppressor cells, including tumor-associated macrophages, dendritic cells, and secreted cytokines. GSCs can recruit macrophages and microglia in brain glioma cells to promote tumor cell growth (Shi et al., 2015c). We also observed malignant transformation of macrophages and microglia induced by GSCs (Chen et al., 2015; Dai et al., 2015; Wang et al., 2015a).

Treatment Strategies Targeting GSCs

Approximately three years ago, Cho et al. (2013) summarized and introduced five methods of targeting CSCs. These included new chemotherapy drugs, radiation-sensitizing agents, cell immunotherapy, induced differentiation, and gene therapy. The advances in this research have led to the following therapies.

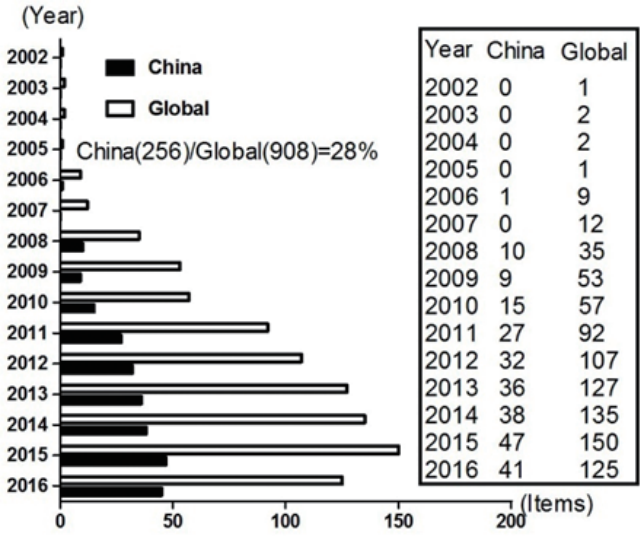
Molecular targeted therapy

Treatment aimed at GSCs should be focused on specific molecular targets. Promising results have been demonstrated for some molecules, *e.g.*, Knock-down of target genes of L1CAM, miR-101 (Yao et al., 2015b), and miR-152 (Ma et al., 2014); upregulation of Cx43; inhibition of Alox-5 with dl-nordihydroguaiaretic acid (Nordy); expression of an exogenous Endo-Angio fusion gene [VAE] (Zhu et al., 2011; Zhang et al., 2014a); use of the nuclear factor-κB inhibitor SN50 (Zhang et al., 2014b); endothelial-monocyte activating polypeptide-II (Liu et al., 2014); down-regulation of TGF-β2 with temozolomide; an attenuating the expression of ID1 *via* TGM2 inhibition (Fu et al., 2013). However, the problem of whether the specific target molecules of CSCs/GSCs have been blocked remains unclear. Targeted molecular therapy will continue to be inconclusive until a truly specific stem cell marker protein is found.

Smart nanomedicines

Smart nanomedicine refers to nanomedicines showing tumor-targeting properties and controlled release for use in tracing and combination therapy *in vivo*. Such nanoparticles can respond sensitively to stimuli including temperature, pH, the redox environment (Glutathione), ionic strength, and electromagnetic fields. Moreover, the size, shape, and structure of nanoparticles can be changed according to the treatment, delivery and release of the drug to the target GSCs/CSCs. However, it is not easy to fully meet the above requirements. Current research generally only meets a subset of these requirements. For example, nanoparticles conjugated with a CD133 monoclonal antibody can be used to treat $CD133^+$ GSCs and transplanted tumors when combined with infrared laser irradiation.

The metallofullerenol nanomaterial Gd@C82(OH)22 possesses intrinsic inhibitory activity against triple-negative breast cancer cells, while remaining relatively non-toxic to normal mammary epithelial cells (Liu et al., 2015). The de-



Year	China	Global
2002	0	1
2003	0	2
2004	0	2
2005	0	1
2006	1	9
2007	0	12
2008	10	35
2009	9	53
2010	15	57
2011	27	92
2012	32	107
2013	36	127
2014	38	135
2015	47	150
2016	41	125

Figure 1 Number of articles about human glioma stem cells published in China and worldwide is rising year by year.

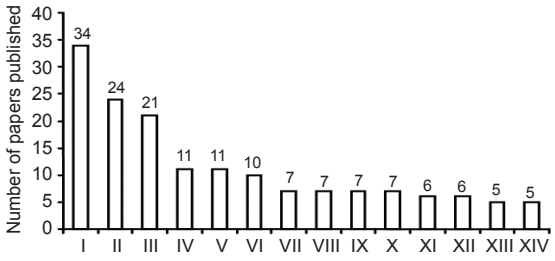


Figure 2 Ranking of the top 14 universities in China that have published ≥ 5 original articles on human glioma stem cells.
I: Soochow University; II: The Third Military Medical University; III: China Medical University; IV: The Fourth Military Medical University; V: Fudan University; VI: Jiangsu University; VII: Sun Yat-sen University; VIII: Southern Medical University; IX: Huazhong University of Science and Technology; X: Harbin Medical University; XI: Nanjing Medical University; XII: Jilin University; XIII: Shanghai Jiao Tong University; XIV: Anhui Medical University.

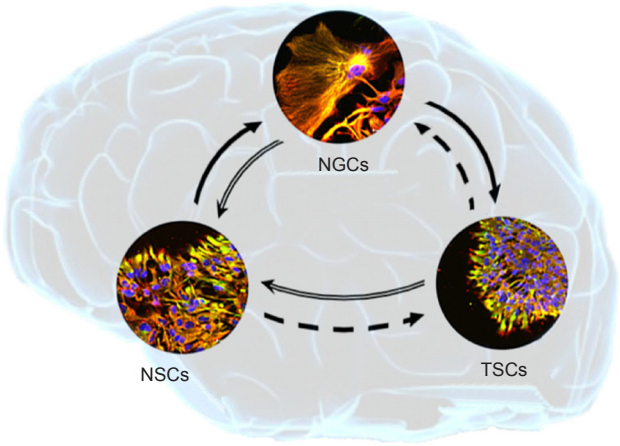


Figure 3 Schematic diagram showing the transformation between NGCs, NSCs and TSCs in the brains of glioma patients.
The solid arrow indicates that the transformation of NSCs to NGCs and the transformation of NGCs to TSCs have been well established. The dotted arrow indicates that the transformation of TSCs to NGCs and the transformation of NSCs to TSCs have received support from some research results, but currently this support is not sufficient. The hollow arrow indicates that a few reports have provided support for the transformation of NGCs to NSCs and the transformation of TSCs to NSCs, but research in this field is meagre. NGCs: Normal glial cells; NSCs: neural stem cells; TSCs: tumor stem cells.

livery of epirubicin by nanodiamonds is a highly effective nanomedicine-based approach for overcoming chemoresistance in hepatic CSCs (Wang et al., 2014). A transferrin-modified graphene oxide used as a glioma-targeted drug exhibited significantly improved therapeutic efficacy for glioma both *in vitro* and *in vivo* (Liu et al., 2013). Silica nanorattle-doxorubicin-anchored mesenchymal stem cells used for tumor-tropic therapy show the potential to be developed as a robust and generalizable method for targeted tumor therapy, with a high efficiency and low systematic toxicity.

Chemotherapy

Some of the effective drugs targeting CSCs/GSCs include curcumin (Zhuang et al., 2012; Shi et al., 2015a), rapamycin, temozolomide (Zhitao et al., 2015), the topoisomerase I inhibitors shikonin and topotecan (Zhang et al., 2013a), TRAIL and paclitaxel, Nordy (Yang et al., 2014b), wheat germ agglutinin and tamoxifen (Li et al., 2014), TRF2 (Bai et al., 2014), metformin and temozolomide (Yu et al., 2015), Cisplatin, the glycolytic inhibitor 3-BrOP and carmustine (Yuan et al., 2013), Korean herbal recipe MSC500 (Yao et al., 2014a), and suberoylanilide hydroxamic acid (Chiao et al., 2013). There are also some medicines not designed to attack cancer that are effective in the treatment of CSCs/GSCs, such as metformin and gemcitabine (Chai et al., 2015) and nicardipine (Jin et al., 2009; Lou and Zhao, 2015).

Although many drugs have been designed to target GSCs, none have shown an effect on GSCs at G_0 phase within the niche. In fact, the niche does not exist *ex vivo*, and even in research *in vivo*, most authors were not able to detect changes in GSCs in the niche. Reactions of the micro-vascular density are only changes in the vascular endothelium, and not in the niche and, particularly not in the GSCs. Therefore, the improved curative effects of most of the drugs mentioned above are not strictly through the targeting of CSCs/GSCs.

Radiotherapy

Sun et al. (2012, 2013) first reported that boron neutron capture therapy induces cell cycle arrest and cell apoptosis of glioma stem/progenitor cells *in vitro*. Inhibition of the PI3K/mTOR pathway with NVP-BEZ235 may enhance the radiosensitivity of human glioma stem cells *in vitro* (Wang et al., 2013), and it has been reported that induction of autophagy promotes the radiosensitivity of glioma-initiating cells. These authors also found that knockdown of the DNA-dependent protein kinase catalytic subunit could radiosensitize glioma-initiating cells by inducing autophagy. Inhibition of

Notch signaling can enhance the radiosensitivity of malignant stromal cells induced by glioma stem/progenitor cells or GSCs themselves (Shen et al., 2015b).

Summary and Prospects

Research on GSCs began more than 10 years ago and 908 articles on this topic have already been published. However, fully understanding the theory and mechanisms of GSCs remains a great challenge in considering the occurrence, development, prevention and treatment of glioma. Only when the GSCs are fully understood can glioma be overcome. Therefore, the focus of the study of glioma should still be placed on GSCs.

The number of relevant papers published in China addressing GSCs continues to show an increasing trend year by year, as in other countries around the world (**Figures 1** and **2**). However, what is more urgently needed is innovative research. The question is where to start? We believe that more in-depth research associated with induced pluripotent stem cells may be one of many directions to consider.

In 2010, we published in China's cancer forum (Huang and Du, 2010) the hypothesis of transformation among normal glial cells (NGCs), neural stem cells (NSCs) and tumor stem cells (TSCs) in the tumor microenvironment where the concept of induced pluripotent stem cells was implied (**Figure 3**). First, can NGCs be translated into induced pluripotent stem-like NSCs (one of the two hollow arrows in the figure)? The transfection of neuronal differentiation-related gene Ngn2 or Dlx1 into astrocytes resulted in a subsequent transformation of astrocytes into functional neurons (Heinrich et al., 2010). Also, when reactive glial cells and fibroblasts were transfected with NeuroD1, it resulted in the reprogramming of both types of cells into functional neurons (Pang et al., 2011; Guo et al., 2014).

Second, can TSCs be transformed into NSCs? Introduction of Ngn2 in GSCs induces massive cell death, proliferation arrest and a drastic reduction of neurosphere formation. Moreover, the few surviving cells adopt a typical neuronal morphology, and some generated action potentials (Guichet et al., 2013).

The third question (dotted arrow in the figure) is whether TSCs can be translated into NGCs? Dimethylformamide and hexamethylene bisacetamide and all-trans retinoic acid, have been used to induce the differentiation of glioma cells or GSCs. Zhu et al. (2014a) induced the differentiation of GSCs by knocking down the expression of Nrf2. All of these results showed that GSCs differentiated in a benign direction after induction; however, none of these cells reached a terminal differentiation stage. The fourth question is whether NSCs can be transformed into TSCs, as mentioned in the "Cellular origin of GSCs/CSCs" section. It can be concluded that tumorigenesis originates from a single ASC, triggered and driven by carcinogenic factors, which can be considered to be ASCs with abnormal phenotypes. Therefore, scholars generally refer to CSCs as ASC-like cells (*e.g.*, GSCs are known as NSC-like cells).

The two solid arrows in **Figure 3** represent traditional theories. However, tumor stromal cells, which are normal cells, may be transformed into cancer cells under malignant pressure from the tumor microenvironment. In gliomas, we have proven that macrophages, oligodendrocytes, and fibroblasts in the microenvironment of SU3-GSCs can transform into cancer cells *in vivo* (Chen et al., 2015; Dai et al., 2015; Wang et al., 2015a). These progressions indicate that the three types of cells can be transformed into each other under specific microenvironmental conditions, as indicated by our hypothesis. However, the so-called specific microenvironmental conditions currently refer to the corresponding experimental conditions, and the situation in spontaneous human cancer remains unknown, which is a task to be addressed in the future.

Animal models are often used to understand certain processes and mechanisms that occur within the human body. According to our studies (Shen et al., 2015c; Wang et al., 2015c), nude mice transfected with GFP are generally an ideal model for research on the relationship between the tumor microenvironment and tumorigenesis/tumor development, because cells from the host microenvironment all show high expression of GFP, a "natural" tracer molecule.

In short, the future research on human GSCs should be expanded to examine the mutual influence between GSCs and tumor microenvironment, and to explore the key regulatory factors for the potential forward and reverse transformations among NSCs, NGCs and TSCs in glioma tissue.

Summary of work done by the contributors: *Our team has been engaged in the research of glioma for a long time, and it has been more than 30 years since the establishment of the first human glioma cell line SHG-44 in China by us. In recent years, along with the international research hotspots, we focus on the research of glioma stem cells (GSC). And we are the first team in China to publish GSC research articles in journals e.g. Cell Res and BMC cancer, where we first present glioma cells could reverse differentiate into GSC, and GSC from recurrent glioma tumor tissues are more invasive and aggressive than that of primary glioma. These articles now have been cited for more than 150 times.*
Author contributions: *YDZ, QBZ, HC and XFF drafted the paper and took part in the literature reviews. YTS, XYJ, JWM, and ADW were in charge of literature reviews. JD, QL, and QH were responsible for the design of this paper. All authors approved the final version of this paper.*
Conflicts of interest: *None declared.*
Plagiarism check: *Checked twice by iThenticate.*
Peer review: *Externally peer reviewed.*

Open peer reviewer: *Paul Lu, University of California, USA.*

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Copyedited by Yu J, Li CH, Qiu Y, Song LP, Zhao M