

# The Role of Cancer Stem Cells in Glioblastoma Treatment: New Strategies for Targeted Therapy

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**Abstract.** Glioblastoma (GBM) is a highly aggressive and recurrent brain tumor for which traditional treatments often yield limited success. According to recent research, cancer stem cells (CSCs) are essential for the development, spread, and resistance to treatment of GBM. This review systematically explores the critical roles of cancer stem cells (CSCs) in glioblastoma (GBM), especially through the regulation of Notch, Wnt, and Hh signaling pathways. The maintenance of CSC properties is primarily dependent on the Notch, Wnt, and Hedgehog (Hh) signaling pathways. This review explores the mechanisms of these pathways in GBM, discusses multi-targeted combination therapies, and highlights emerging strategies and drugs for targeted therapy. The results indicate that targeting Notch, Wnt, and Hh pathways can effectively suppress CSC proliferation and invasiveness in GBM, significantly delaying tumor recurrence. Future research should further explore the clinical potential of these pathway inhibitors and develop more specific targeted therapies to improve GBM treatment outcomes and patient prognosis.

**Keywords:** Glioblastoma; cancer stem cells; Wnt/beta-catenin signaling pathway; Notch signaling pathway.

## 1. Introduction

As one of the most prevalent and deadly primary brain tumors, glioblastoma (GBM) poses a significant challenge to neuro-oncology. Due to its high heterogeneity, invasiveness, and resistance to conventional treatments, the median survival time for GBM patients is often less than 15 months. Although treatments like chemotherapy can temporarily alleviate symptoms, most patients experience rapid tumor recurrence. According to recent research, cancer stem cells (CSCs) are essential for the development, progression, along with development of drug resistance in GBM. This finding opens up new avenues for targeted treatment approaches.

CSCs are tumor cells with stem cell-like properties, including self-renewal and multi-potent differentiation. These cells are thought to be capable of initiating tumors and driving cancer progression, and their presence in the circulatory system can lead to distant metastasis [1]. CSCs maintain their stemness and proliferative capabilities through various signaling pathways, with the Notch [2], Wnt/beta-catenin [3], and Hedgehog (Hh) [4] pathways being particularly significant. These pathways are critical to the biological characteristics of CSCs, making them prime goals for the course of treatment.

Current researches focusing on the Notch pathway have suggested that inhibiting this pathway significantly reduces the self-renewal capacity of CSCs, thereby decreasing tumor invasiveness and recurrence rates. Similarly, abnormal activation of the Wnt pathway has been closely associated with GBM development, and drugs targeting this pathway have demonstrated promising anti-tumor effects in preclinical studies. Additionally, the Hh pathway plays a vital role in maintaining CSCs in GBM, and its inhibition has been shown to markedly suppress tumor growth.

This review aims to systematically analyze the role of CSCs in GBM treatment by exploring novel therapeutic strategies targeting these key pathways to overcome the limitations of conventional treatments. Specifically, this review will discuss the mechanisms by which these pathways contribute

to the biology of CSCs, evaluate current therapeutic approaches targeting these pathways, and assess their potential for clinical application. The review will also cover the progress of targeted therapy research, including gene editing technology, monoclonal antibodies, and small molecule inhibitors.

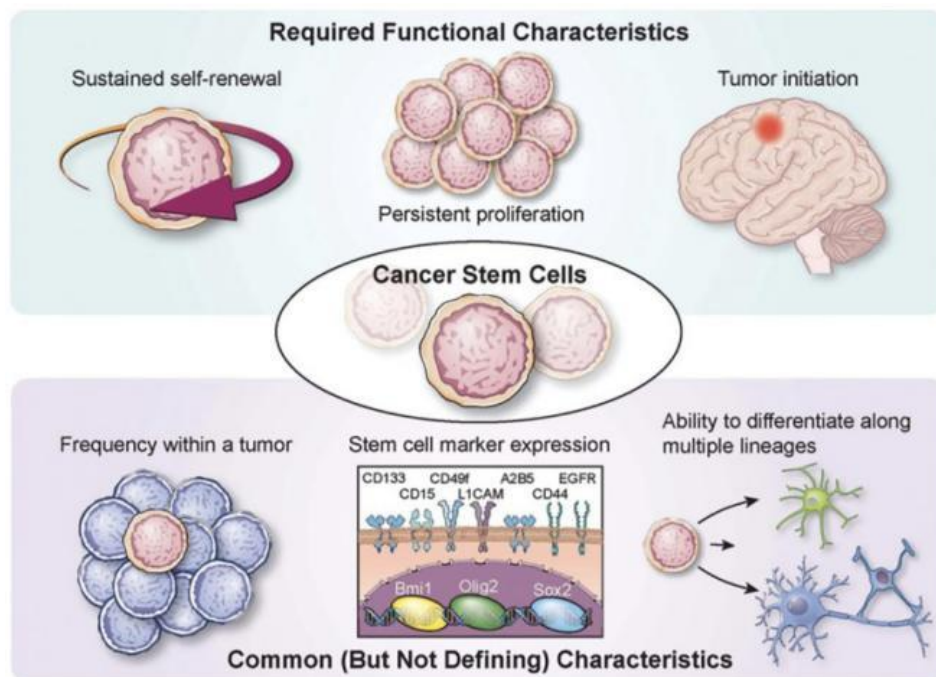
In summary, by comprehensively analyzing and evaluating the role of CSCs and their signaling pathways in GBM, in order to give GBM patients fresh hope and prospects, this review aims to provide an analytical framework and useful guidelines for the creation of more efficient and individualized treatment strategies.

## 2. CSCs and Their Drug Resistance

CSCs are known as quiescent and multipotent tumor cells with self-renewal abilities. Initially found in hematologic malignancies, they were later found in solid tumors. The significance that CSCs play in the tumor's resistance to both chemotherapy and radiation and in tumor recurrence caught the attention of numerous researchers. The involvement of CSCs in GBM is now the subject of numerous investigations.

GBM is a common adult primary brain tumor, initially presenting with nonspecific signs and symptoms like headaches, changes in personality, nausea, and stroke-like symptoms, which often rapidly worsen and may progress to altered consciousness. Due to its harsh approach and unsatisfactory therapy response, GBM has a poor prognosis.

The characteristic CSCs in GBM possess three functional traits: self-renewal, proliferation, and tumor initiation. Their common features include lower magnetic resonance frequency, expression of stem cell markers, and differentiation potential (Fig. 1).



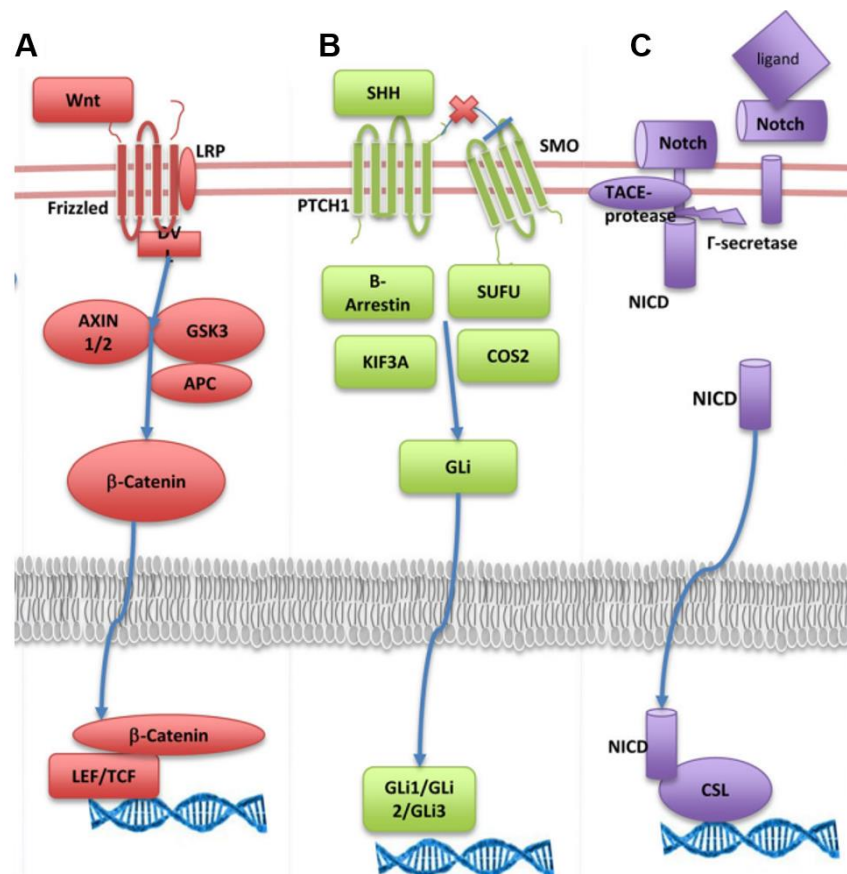
**Fig. 1** Functional Characteristics Required for CSCs in GBM [5]

Drug resistance presents a significant obstacle to the treatment of cancer since cancer cells have the ability to create defense mechanisms against the damaging effects of therapeutic substances. This can result in the creation of fitter and more active clones, which is eventually associated with an undesirable prognosis. Resistance can be intrinsic, but they can be acquired as well. These are complex events with multiple contributing factors, some of which are connected to CSC adaptability and the epithelial-mesenchymal transition (EMT). Among these factors, CSCs are a primary source of resistance; CSC-mediated resistance results from the interplay of intrinsic and extrinsic factors [6].

In recent years, increasing research has shown that CSCs regulate tumor growth, progression, and especially drug resistance through various signaling pathways. This section will focus on three key pathways: the Notch pathway, Wnt/beta-catenin pathway, and Hh pathway.

### 3. Wnt/beta-catenin Pathway and Its Targeted Therapy

The Wnt/beta-catenin pathway is a remarkably conserved pathway responsible for regulating embryonic development [7] and is also partially responsible for stem cell maintenance and directional cell movement (Fig. 2-A).



**Fig. 2** Schematic representation of the Wnt/beta-catenin (A), Hedgehog (Hh) (B) and Notch (C) pathways in CSCs. The double line above represents the cell membrane, and the lipid bilayer below represents the nuclear membrane [6].

The Wnt signaling pathway plays a crucial role in the development and evolution of CSCs. Aberrant activation of Wnt signaling is associated with a number of cancers, including GBM. Previous research has demonstrated that fibroblasts release many substances, including hepatocyte growth factor (HGF), which have the ability to stimulate the Wnt/beta-catenin pathway and promote the proliferation of CSCs in both in vivo and in vitro settings.

In terms of treatment, traditional multimodal strategies did a poor job in preventing GBM from recurring and improving the survival rates of patients. This is largely because GBM's CSCs present significant challenges in treatment, despite the effectiveness of these strategies in other cancers. The limited number of therapies aimed at preventing CSC renewal and promoting tumorigenesis in GBM impacts treatment outcomes; targeting GBM CSCs is challenging due to their similarities to normal neural stem cells. An in vivo study focused on the hypoxia pathway in GBM CSCs, finding that xenografting S100 $\beta$ -v-erbB/p53 $^{-/-}$ GSCs into mice and treating with Brefeldin A and EHT-1864 increased survival and reduced cell renewal [8].

Additionally, small molecule inhibitors like XAV939 have been shown to significantly inhibit the proliferation and invasion of GBM cells, with a notable suppressive effect on CSCs [9]. Furthermore,

monoclonal antibodies targeting the Wnt signaling pathway, such as OMP-18R5, have shown promising preclinical effects [10], suggesting potential for clinical application.

#### **4. Hh Pathway and Its Targeted Therapy**

The Hh pathway plays a critical role in embryonic development, as it is responsible for the formation of structural components such as the neural tube, lungs, skin and axial skeleton. Hh ligand binding releases its inhibition of the Patched (PTCH) receptor, activating Smoothed (SMO). This activation triggers a signaling cascade, leading to GLI transcription factor activation and nuclear localization, and by stimulating the activation of Hh target genes, a significant portion of which have a role in cellular proliferation, survival, and angiogenesis (Fig. 2-B).

The Hh signaling pathway is associated with the determination of cell fate, self-renewal, and regulation of CSCs in different types of malignancies, such as GBM. The facilitation of Hh signaling plays a role in the development of treatment resistance, along with the promotion of tumor regrowth following chemo-radiation, hence presenting a promising avenue for enhancing radiotherapy efficacy.

Significant progress has been made in targeting the Hh pathway for cancer treatment. Various Hh pathway inhibitors have been developed, such as Vismodegib and Sonidegib, which significantly inhibit GBM cell proliferation and migration, with a pronounced effect on CSCs [11]. Additionally, monoclonal antibodies targeting the Hedgehog signaling pathway, such as MEDI-5304 [12], have shown promising preclinical results, indicating potential for clinical application.

#### **5. Notch Pathway and Its Targeted Therapy**

The Notch pathway is widely recognized as critical to CSCs in various types of tumors. This pathway is triggered when ligands bind to Notch receptors, triggering the release of the Notch intracellular domain (NICD) through cleavage by ADAM family proteases and gamma-secretase. NICD then translocates to the nucleus, where it interacts with CSL transcription factors. The process involves the transition of the compound from a restrictive state to an activating state for Notch target genes (Fig. 2-C). The maintenance of CSCs' stemness, improvement of treatment resistance, and promotion of hypoxic conditions all depend on Notch activation. It has been observed that the Notch pathway is vital for CSC survival and self-renewal and plays a critical role in regular stem development, differentiation and death [7].

Research indicates that a number of glioma cell lines and primary human gliomas have high expression levels of Notch1, a member of the Notch receptor family, and its ligands [13]. Using siRNA to target Notch1 and its ligands can weaken GBM cell proliferation and growth [14]. Additionally, using gamma-secretase inhibitors (GSIs) to block the pathway can reduce the proliferation of CSCs in GBM [15].

Targeted therapy against the Notch pathway has become a significant focus in GBM research. Recent studies indicate that inhibiting this pathway can markedly decrease CSC self-renewal capabilities, thereby reducing tumor invasiveness and recurrence rates. Currently, Notch pathway inhibitors, such as GSIs, have shown promising anti-tumor effects in preclinical models. One of the most widely utilized GSIs, DAPT, suppresses the growth of GBM by blocking Notch pathway via changing receptor expression. [16]. When treated with DAPT, GBM neurospheres exhibiting elevated Notch activity show more marked phenotypic alterations and are more sensitive to the drug [17]. Targeting Notch1 with DAPT in GBM slows cell growth, causes apoptosis, and lowers associated expression [18].

#### **6. Emerging Targeted Therapy Strategies and Novel Drug Approaches**

Beyond the targeted therapies for the three pathways, new treatment methods have been developed, including gene editing technologies, immunotherapies, and nanomedicine. For example, the

CRISPR/Cas9 gene editing technique has been employed to target and knock out key genes associated with CSCs, thereby inhibiting tumor growth and progression [19]. Additionally, CAR-T cell therapy and PD-1/PD-L1 immune checkpoint inhibitors have been applied to target CSCs in GBM, showing promising anti-tumor effects.

In terms of novel drug strategies, nanomedicine, owing to its unique physicochemical properties, offers the potential for targeted drug delivery, enhancing treatment efficacy and safety. For instance, using nanoparticle carriers to deliver pathway-targeted drugs to tumor sites can significantly increase drug concentration and efficacy while reducing side effects [20].

## 7. Conclusion

This review systematically examines the critical role of CSCs in GBM, particularly through the regulation of the Notch, Wnt, and Hh signaling pathways. These pathways are not only crucial for CSC self-renewal and differentiation but also closely linked to GBM's invasiveness, recurrence, and treatment resistance. Targeted therapies against these pathways have shown promising preclinical results, offering new therapeutic avenues. Inhibition of these pathways can significantly reduce CSC numbers, thereby decreasing GBM growth and invasiveness. Additionally, emerging treatment approaches, such as gene editing technologies, immunotherapies, and nanomedicines, provide new hope for GBM therapy.

Based on the methods discussed in this review, future studies should focus on developing more specific and effective targeted therapies to minimize side effects on normal cells. For instance, by further investigating specific CSC markers, more targeted monoclonal antibodies and small molecule inhibitors can be developed.

A single targeted therapy may have limitations in addressing the heterogeneity and complexity of GBM. Therefore, future research should emphasize the combined application of multiple therapeutic strategies. For example, GSIs like DAPT can inhibit the Notch pathway and reduce CSC numbers. However, using GSIs alone may be insufficient to eliminate CSCs completely, so combining them with DNA damage repair inhibitors, PARP inhibitors for instance, could enhance therapeutic efficacy. This combined strategy can not only inhibit CSC self-renewal but also increase tumor cell sensitivity to chemotherapy, improving overall treatment outcomes.

Despite the challenges, novel therapies targeting CSCs and their signaling pathways hold great promise for GBM treatment. Future research should further explore the potential of these strategies, aiming to develop more effective and personalized therapies, bringing more hope and opportunities to GBM patients.

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