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Review Article

# SOX2 in Glioblastoma: Regulation Mechanisms and A Potential Therapeutic Target

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## Abstract

Glioblastoma Multiforme (GBM) is the most common malignant brain tumor in adults. Today it is considered the primary treatment with the Stupp method, which combines radiotherapy and chemotherapy (temozolomide) after surgery, which has a moderate success rate and causes tumor recurrence. Despite improved overall survival rates, this cancer still lacks an effective, reliable, and less toxic treatment option. One of the characteristics that a tumor cell must possess in order to survive therapeutic treatment and grow tumor is the ability to self-renew. Therefore, it is important to understand the molecular mechanisms that regulate this process. The sex-determining region Y (SRY)-Box 2 (SOX2) is a transcription factor whose activity is associated with maintaining the undifferentiated state of cancer stem cells in various tissues, including the brain. Several studies have found elevated SOX2 levels in biopsies from GBM patients, with higher levels being associated with poor outcomes. Therefore, SOX2 silencing could represent a new therapeutic approach in the combat of cancer and brain tumors in particular. In this Review, we focus on the role of SOX2 in GBM tumorigenesis and discuss recent advances in SOX2 inhibition as a promising therapeutic strategy for GBM.

**Keywords:** Glioblastoma; Glioblastoma Stem Cells; Signaling Pathway; SOX2; Therapeutic Treatment

## Introduction

Glioblastoma (GBM) is the most common primary malignant tumor of the Central Nervous System (CNS), comprising about 45% of all gliomas [1]. It is characterized by its aggressive nature, high degree of malignancy, and poor prognosis [1,2]. According to the 2016 World Health Organization (WHO) Classification of Tumors of the CNS, glioblastoma is considered a grade IV glioma and is categorized into isocitrate dehydrogenase (IDH)-wildtype and IDH-mutant subtypes. The IDH-mutant subtype is associated with better prognosis and is more commonly found in long-term survivors, whereas the IDH-wildtype subtype is more aggressive and has a poorer prognosis [3]. The standard treatment for newly diagnosed glioblastoma involves surgical resection followed by adjuvant radiotherapy. The addition of temozolomide, a chemotherapy drug, to radiotherapy has been shown to improve survival in patients with glioblastoma [4]. However, despite this multimodal treatment, glioblastoma still has a low survival rate, with median survival limited to 16 to 19 months and the 5-year survival rate being less than 10% [4-6].

A key factor contributing to therapy resistance of glioblastoma is the presence of Cancer Stem Cells (CSCs) dominating the heterogenous cellular hierarchy of the tumor tissue. CSCs phenotypically and functionally resemble normal stem cells and contribute to the tumor's growth and invasion [7]. These cells are characterized by their capacity of (1) self-renewal, (2) multipotency, and (3) the ability to initiate tumors in animal models [8]. CSCs have been shown to evade the immune system

[9], and are believed to play a significant role in glioblastoma's invasiveness [10], radioresistance [11], chemoresistance [12], and recurrence [13].

Sex-determining region Y (SRY)-box 2 (SOX2) is a member of the SOXB1 subclass of transcription factors involved in embryonic development, particularly in the developing CNS [14], and it plays a pivotal role in maintaining stemness properties of different stem cell populations in the adult. SOX2 is crucial for the stemness of CSCs, and its silencing causes CSCs to stop proliferation and lose tumorigenicity in immunodeficient mice [15,16]. Moreover, it has been shown that SOX2 is overexpressed in glioblastoma patients. Schmitz et al. first reported the elevation of SOX2 expression in 90% of human biopsies at the mRNA and protein levels [17]. Another study reported overexpression and promoter hypomethylation of SOX2 gene in most patient samples, along with gene amplification in some samples, compared to normal cell lines [18]. Furthermore, a positive correlation has been identified between SOX2 expression and malignancy grade in gliomas, with higher SOX2 expression indicating the hypercellular and hyperproliferative areas of glioblastoma [19]. Nevertheless, the analysis of all glioblastoma tumors present in the Cancer Genome Atlas (TCGA) and in-house patient databases revealed that about half of the tumors can be mathematically classified into high miR-21/low SOX2 (Class A) or low miR-21/high SOX2 (Class B) subtypes, with the latter exhibiting longer overall survival than the former [20]. This evidence makes SOX2 a potential molecular candidate to be targeted for the treatment of glioblastoma.

In the present article, we provide a synopsis of the roles played by SOX2 in CSCs in the context of glioblastoma. Moreover, we comprehensively review the evidence that support targeting SOX2 as a potential therapeutic alternative for the treatment of glioblastoma.

## Role of SOX2 in GBM

Several studies have identified SOX2 overexpression in GBM patient samples. For the first time in 2007, an increase was found in 90% of human biopsies examined at the mRNA and protein levels [17]. Analysis of GBM case data generated as part of The Cancer Genome Atlas project showed that there is Sox2 overexpression (86%), Sox2 gene amplification (8.5%) and Sox2 promoter hypomethylation (100%), suggesting an importance of this factor in malignant GBM phenotype [18]. Importantly, high SOX2 levels were associated with tumor aggressiveness and worse prognosis [21]. Moreover, SOX2 shows and enrichment in the undifferentiated glioblastoma stem cells (GSCs) and plays a central role in the control of their pluripotency which may provide valuable opportunities to disrupt cellular phenotypes associated with cancer stem cell survival and proliferation [22]. A comprehensive characterization of the SOX2 response program identified 4,883 SOX2-binding regions in the GBM cancer genome, 105 SOX2-regulated microRNAs (miRNAs) using next-generation sequencings and 489 genes whose expression changed in response to SOX2 regulation [23]. A study done by Bradshaw et al. suggest the possibility of the presence of different isoforms or modified versions of SOX2 in different GBM tumors and that a particular isoform present may affect tumor growth and tumor aggressiveness in specific ways. The almost ubiquitous abundance of SOX2 in GBM indicates that this marker is a putative marker for progenitor cells in GBM samples. This would confirm the putative existence of the stem cell hierarchy in GBM [24]. SOX2 is a master gene involved in the self-renewal of several stem cells, particularly neural stem cells. Silencing of SOX2 show proliferation cessation and lose their tumorigenic effects. SOX2 is therefore also fundamental to maintain the self-renewal ability of neural stem cells once they have acquired neoplastic properties. These observations support a hierarchical model of brain cancer controlled by SOX2 [25]. Furthermore, SOX2 silencing leads to a reduction in migration and invasive capacity [18], while increasing senescence and causing cell cycle arrest in G0/G1 [26]. The effect of SOX2 on glioblastoma cells was confirmed by overexpression studies. Indeed, ectopic upregulation of SOX2 increases invasive and migratory capacity [18] as well as cell proliferation and self-renewal activity in conventional glioblastoma cell lines [26]. Moreover, intratumor heterogeneity is a hallmark of GBM, which contains a mixture of cell populations with different GBM subtype gene expression signatures. SOX2 was found to confer the gene expression signature of the proneural glioma subtype, whereas SFRP2, an antagonist of SOX2, is able to induce the signature of the mesenchymal glioma subtype. SFRP2 represses SOX2 via the signaling axis SFRP2/non-canonical-WNT/KLF4/PDGFR/phospho-AKT/SOX2. Proneural cells were converted to a mesenchymal glioma gene expression signature, confirming an involvement of SOX2 in GBM cancer cell plasticity and tumor progression [27]. These works raise the idea to target SOX2 or to find its upstream regulators and downstream targetable genes as a strategy to eliminate GSCs and subsequently the tumor thus offering a very attractive therapeutic avenue for cancer treatment.

## Role of SOX2 as a Stemness Factor for CSCs

GBM is initiated by the conversion of neural stem cells (NSCs) into GSCs. Similarly, glial progenitor cells are able to stimulate tumor development after malignant transformation of normal progenitor cells. Astrocytes, neurons, oligodendrocytes and ependymal cells also have the potential to trigger carcinogenesis [28]. The stem cell-like properties of GSCs can also be developed during transformation and differentiated non-stem cancer cells can dedifferentiate into cancer stem cells. The most common cause of death in GBM patients is tumor recurrence, which is attributed to

refractory GSCs in and around the primary tumor. Ample evidence shows that cancer stem cells (CSCs), or cancer-initiating cells (CICs), play important roles in several cancer types, including GBM [29]. Interestingly, in GBM and other malignancies, CSC enrichment can occur both by increasing the symmetric self-renewal rate of CSCs and by reprogramming non-CSC cells, resulting in phenotypic plasticity in the tumor population [30]. The concept of non-CSC to CSC dedifferentiation has increased the complexity of understanding tumor heterogeneity, the possible mechanism of therapeutic relapse, resistance to cancer therapies, and concerns about developing therapeutic strategies. It is hypothesized that the main features of GSCs are mainly closely related to the expression of pluripotency gene SOX2 [31]. SOX2 is thought to be the most highly enriched gene among stemness signature in CD133+ GBM cells. SOX2 overexpression consistently increased stem cell efficacy in GBM cell lines. SOX2 plays an important role in genetic plasticity and contributes to drug resistance, the development and maintenance of GSCs. A putative link was identified between CD133 and SOX2 revealed a new regulatory axis: the CD133/SOX2-mediated axis, where Sox2 is one of the downstream targets of CD133 that promotes self-renewal in GSCs [32]. In addition, Sox2 regulates the expression of key genes and signaling pathways involved in GBM tumors in stem and differentiated cells and preserve plasticity for bidirectional conversion between the two states, which has important clinical implications. Therefore, SOX2 may be critical in maintaining developmental plasticity during glial tumor progression, regulating dedifferentiation and acquisition of GSC properties [33]. In addition, SOX2 sustains neuroglial stem cell proliferation and inhibits neuronal fate commitment. It can be considered a marker for differentiated glial tumors and not a specific marker for brain tumor stem cells BTSCs, including those with astroglial, oligodendrocyte and ependymal lineages, regardless of pathologic grade. Although SOX2 is an established marker of neuroglial stem cells. In addition, it was recently found that brain tumors contain stem cells that resemble normal neuroglial stem cells in many aspects. This study suggests that SOX2 may be a tumor marker for glial lines rather than a universal marker for brain tumor stem cells, as its expression pattern was found to be consistent with differentiation pathways [18].

GSCs have the capacity to efficiently carry out the oncogenic functions suggests their endowment with special mechanisms for escaping innate and adaptive anti-tumor immunity [34]. The molecular mechanisms that GSCs employ to evade antitumor immunity are largely unknown. Combined transcriptional profiling and functional studies of GSCs showed that Oct4 and SOX2 jointly induce an immunosuppressive tumor microenvironment. These immunosuppressive responses to Oct4/SOX2 co-expression are driven by a BRD4/H3k27Ac-dependent immunosuppressive transcriptome composed of multiple checkpoint immunosuppressive molecules, cytokines, and chemokines compatible with the oncogenic immunosuppressive tumor microenvironment. These show for the first time that Oct4 and SOX2 can stimulate GBM growth by inducing adaptive and innate immunosuppressive effects, as well as their ability to induce GBM cells to express stem-like tumor-propagating phenotypes [35]. For the first time, it is demonstrated that SOX2 expression was found to be dynamic and bidirectional based on microenvironmental stimuli. Because GSCs are the most chemo-resistant cells in the GBM tumor mass, SOX2 is more highly expressed in human GBM specimens than in lower-grade gliomas, suggesting a direct association with the poor prognosis of GBM patients. For the first time it has been shown that the plasticity of the GBM stem cell state is not static but dynamic and depends on the tumor microenvironment [36].

## Regulation and Expression of SOX2

As a critical transcription factor with vital biological functions, SOX2 levels require precise regulation. In fact, SOX2 mRNA and protein are regulated at the transcriptional, post-transcriptional, and post-translational levels. The finely tuned SOX2 then regulates multiple signal transduction pathways involved in many physiological and pathological processes when dysregulated [37,38]. Understanding how SOX2 levels are carefully controlled in cancer cells will provide new insights into the fundamental biology of these tumors [39].

## At the Transcriptional Level

The mammalian SOX2 gene is transcriptionally regulated during various developmental stages by multiple distal enhancers. However, only a few enhancers have been identified as functionally active in mammalian cells and further investigation is needed to clearly define which regulatory enhancers of the SOX2 gene are active in specific cellular situations [40]. In addition, thousands of genes linked by long-range interactions with defined distal enhancers that are epigenetically associated with SOX2, were identified, and many of these genes, including genes important for neurodevelopment, were downregulated after Sox2 ablation. Thus, SOX2 is a master regulator of gene expression through enhancer network connections in NSCs. Sox2-dependent mechanisms are involved in the long-term maintenance of NSC self-renewal. In conclusion, this demonstrates the important role of SOX2 in controlling gene expression at the linked promoter level. Although, the loss of Sox2 decreases the expression of about 1,000 genes [41]. Ali et al. underscore the importance of careful regulation of the expression and function of SOX2 in brain tumor cells. The results suggest that overexpression of SOX2 defines a significant stress on brain tumor cells and that small changes in SOX2 expression significantly alter their fate [39].

A study integrates the SOX2-controlled coding and non-coding transcriptome into GSCs for the first time and illustrates

a complex scenario in which SOX2 plays an important role in the regulation of various molecules and signaling pathways in GBM. Microarray data identified a total of 2,048 coding transcripts with differential expression and 261 non-coding transcripts, suggesting that SOX2 acts primarily as a transcriptional activator, thus providing insight into understanding the possible roles of SOX2 in GBM. They also defined and classified a series of noncoding transcripts whose expression is regulated by SOX2 in GSCs [42]. Understanding how SOX2 is regulated in cancer cells is important for tackling tumorigenesis. The SOX2 regulatory region 2 (SRR2) lies downstream of the SOX2 coding region and controls SOX2 expression in embryonic and adult stem cells and in GBM, thus maintaining high levels of SOX2 expression. Deletion of SRR2 halts tumorigenic activity of SOX2 in cancer cells. In conclusion, this study defines the requirements of this regulatory region for SOX2-induced neoplastic activities [43]. Co-expression of FOXG1and SOX2, two transcriptional regulators act in complementary roles to drive unconstrained self-renewal in GSCs through transcriptional control of core cell cycle regulators and epigenetic targets. Results underscore the growing body of evidence in demonstrating the critical role of neurodevelopmental Transcription factors in promoting unrestrained self-renewal in GBM [44].

Increased SOX2 expression is induced by the transcription factor Forkhead Box M1 (FoxM1), which is an oncogenic regulator involved in GBM clonogenic growth, stem cell maintenance, and radioresistance. These data, together with the direct binding of FoxM1 to the SOX2 promoter region in GBM cells, suggest that FoxM1 regulates the stemness of primary GBM cells via SOX2, which is an important downstream regulator of FoxM1 signaling in GBM [45]. This is consistent with another evidence that forkhead box O proteins (FoxO1 and FoxO3) are critical for stemness maintenance and cell survival in GSCs by upregulating the expression of SOX2 [46]. Also, SOX2 expression is activated by the tripartite motif containing 24 (TRIM24; also known as Transcription Intermediary Factor [TIF] 1 alpha) to regulate GBM stemness and invasion in vitro and in vivo. It seems unlikely that this regulation depends on EGFR/STAT3/ID1 signaling in GSCs [47]. In addition, the myeloid transcription factor Elf-like factor-1 (MEF, also known as ELF4) is highly expressed in GBM and contributes to gliomagenesis by promoting stem cell properties through direct activation of SOX2 expression, is identified as a target directed downstream of MEF and may be responsible for its ability to modulate stem-like properties [48].

Interesting findings manifest that SOX2 controls the expression of the SOX family proteins SOX1 and SOX18, and that SOX2 downregulates brain expressed X-linked 1 and 2 (BEX1 and 2), two genes with tumor suppressive activity in GBM [49]. In addition to the aforementioned data, forkhead box O (FOXO1) regulates OCT4 and SOX2 through direct linkage to their promoters, thus promoting pluripotency and preventing differentiation [50]. Otherwise, SOX2 expression also undergoes negative regulation at the transcriptional level. The dual-specificity tyrosine phosphorylation-regulated kinase 1A, DYRK1A, plays a significant role in stem cell regulation with CDK5 kinase. The CDK5-CREB-SOX2 pathway, which is essential for the activation of self-renewal programs, is attenuated by DYRK1A in GSC. This is a significant discovery since it sheds new light on the maintenance and survival of GSC [51]. Moreover, Methylation of the SOX2 promoter by DNA methyltransferase (DNMT) suppresses SOX2 transcription, and this SOX2 hypermethylation appears to be a critical epigenetic event leading to SOX2 silencing in various human cancers, including lung cancer [52], esophageal cancer [53] and endometrial cancer [54]. However, loss of SOX2 in these tumor subsets is associated with poorer clinical features and poor prognosis [54], which is an attractive topic for future research.

## At the Post-Transcriptional Level

miRNAs are post-transcriptional modulators of gene expression that play an important role in many developmental processes. A growing list of miRNAs that regulate SOX2 expression at the post-transcriptional level has been identified. MicroRNA-145 (miR-145), which is tightly upregulated during differentiation, directly target SOX2 upon binding its 3′-UTR region. Another miRNA, miR-425-5p, has emerged as an important and robust candidate regulated by SOX2 in GSC, and a GBM stem cell survival factor that positively correlates with SOX2 expression and interfere with GSC neurosphere formation, cell survival and cell cycle progression [55]. SOX2 is a new clinically important target of microRNA-21 (miR-21) in GBM. Using the miR-21-Sox2 regulatory axis, which classifies approximately 50% of patients into two subtypes with distinct molecular, radiological, and pathological features: high miR-21/low Sox2 (Class subtype A) or low miR-21/high Sox2 (Class subtype B). This classification reflects phenotypically and molecularly distinct traits and is not covered by existing classifications. Patients with class B tumors had longer overall survival than patients with class A tumors. Stratification of patients based on the miR-21-Sox2 axis also revealed a class C tumors from the third group had high miR-21 levels and high Sox2 levels, or low miR-21 levels and low Sox2. Class C tumors were intermediate between class A and class B tumors, not only in terms of the MiR-21-Sox2 ratio, but also in terms of biological characteristics such as patient survival [56]. Increased miR-145 expression inhibits SCs self-renewal and CSC-like properties [57], expression of pluripotency genes, and induces lineage-limited differentiation. This uncovers a direct relationship between key reprogramming factors and miR-145, revealing a dual negative feedback loop [23] involving OCT4, SOX2 and

KLF4 that are directly post-transcriptionally controlled. In contrast to the decline in pluripotency factors during differentiation, miR-145 levels in human embryonic stem cells (hESCs) were relatively low and increased during differentiation. The function of miR-145 demonstrated that miR-145 is both necessary and sufficient to modulate differentiation progression via the OCT4/SOX2 pathway [58]. In addition, microRNA-145 promotes chemosensitivity of GSCs to demethoxycurcumin (DMC) which has been previously affirmed to inhibit GSCs proliferation and induce apoptosis by targeting the SOX2-Wnt/β-catenin axis [59]. A recent study manipulating the impact of Oct4 and SOX2 on stem-like phenotype in GBM, identified miR-486-5p, a previously unknown miRNA regulating GBM neurospheres, as SOX2-regulated oncomiRs that inhibit tumor suppressor genes PTEN and FoxO1 and regulate GSCs. These results define a previously unrecognized and therapeutically targeted axis SOX2:miR-486-5p-dependent regulation of tumor suppressor genes that are feedback-regulated to monitor GSCs survival and stemness [60]. Moreover, miR-130b [61], miR-340-5p [62] and miR-30c [63], all negatively regulate the expression of SOX2 and thus act as tumor suppressors in GBM. Using junctional adhesion molecule A (JAM-A), an adhesion protein specific for CSCs, the authors attempted to identify miRNA regulatory loops that connect niche adhesion molecules to broader network signals identified miR-145 as a negative regulator of JAM-A-mediated CSC maintenance. The latter binds directly to the 3'UTR region of the JAM-A message, thus attenuating self-renewal. This miR-145 signaling system extends beyond JAM-A to key pluripotency factors, including SOX2. These data are consistent with previous results manifesting miR-145 as a regulator of SOX2 [58]. Also, an association between miR-145 levels and patient survival was reported, with patients with lower miR-145 levels having lower median survival. Taken together, this indicates CSCs possess specific mechanisms for the maintenance of cell adhesion molecules and self-renewal genes, including the miR-145/JAM-A/SOX2 axis that controls CCS maintenance [64].

Besides, several studies focus on understanding how transcription factor reprogramming drives the tumor stem-like phenotype through DNA methylation-dependent regulation of miRNAs. A study of Lopez-Bertoni et al. identified miR-148a that induce Oct4/Sox2 to regulate tumor cell phenotype and tumor proliferative capacity through a mechanism involving transactivation and methylation events of the DNMT promoter [65]. A study describes a novel transcription loop involving DNA methylation, regulation of miRNA expression, and HMGA1-dependent chromatin remodeling at the Sox2 promoter to regulate the GSC phenotype. It presents new evidence that miR-296-5p is repressed in a DNMT-dependent manner and identify the miR-296-5p:HMGA1:Sox2 axis as a novel regulator of the tumor propagating capacity GSCs [66]. Furthermore, SOX2 was first shown to inhibit TET2 demethylase (the Ten-Eleven family of enzymes) that switch 5-methylcytosine (5mC) to 5-hydroxymethylcytosine (5hmC) where loss of 5hmC correlates with poor prognosis, and reduce 5hmC in GSCs, thus contributing to the hypermethylated GSC phenotype. MiR-10b-5p, an onco-miR that is SOX2-induced, directly targets TET2, mediates onco-methylation, GSC induction and GBM malignancy [67]. Conversely, HMGA2 promotes GBM propagation and demonstrate poor survival outcome by enhancing SOX2 expression through an IL-6/miR142-3p/Sox2 feedback-loop-dependent regulation of GBM malignancy [68].

In addition to the cross-talk of SOX2-miRNAs regulation, there is also cross-regulation between SOX2 and long non-coding RNAs (lncRNAs), a class of non-protein-coding RNAs composed of more than 200 nucleotides. Structurally, SOX2 interacts directly with high-affinity with lncRNAs via its HMG-DNA-binding domain [69]. A better understanding of the molecular mechanism involved in the lncRNA-miRNA-mRNA network underlying GBM development is helpful in the treatment of GBM. LncRNAs are often act as to sponge miRNAs, and these sponged miRNAs have their own genetic targets that they inhibit. This relationship results in an inverse relationship between lncRNA and sponged miRNAs, but a direct correlation between lncRNA and sponged miRNA targets due to de-repression of target genes when miRNAs are sponged. The NEAT1 lncRNA has been suggested to be a tumor promoter in a number of malignancies including GBM. The lncRNA NEAT1, which is upregulated in GBM, downregulated the expression of miR-132, which is inhibited in GBM. NEAT1 promoted the development of GBM by indirectly promoting SOX2 expression through repression of miR-132 [70]. Another lncRNA, the Myocardial Infarction-Associated Transcript (MIAT), sponges miRNAs, including let-7a-5p and miR-29b-3p, thereby upregulate SOX2 expression. Thus, MIAT controls brain tumor cell proliferation, migration and metastasis by regulating the Sox2/let-7a-5p/miR-29b-3p axis [70]. Furthermore, silencing of MIAT downregulated the expression of stem factors, followed by upregulation of their downstream miRNAs, let-7a-5p and miR-29b-3p [71]. This is consistent with SNHG6, a lncRNA that supports GBM progression by upregulating SOX2 [72]. Circular RNAs (CircRNAs) are a class of noncoding RNAs with stable loop contractures (Shen et al, 2019) [73]. Previous studies have shown that circRNAs primarily serve as competitive endogenous RNAs to regulate the pathogenesis of various cancers including GBM [74]. circABCC3 that is upregulated in GBM, controls SOX2 expression through absorbing miR-770-5p. In vivo assays showed that inactivation of circABCC3 prevented the development of GBM and angiogenesis through targeting miR-770-5p/SOX2 axis upon regulation of the PI3K/AKT pathway [75].

## At the Post-Translational Level

Post-translational modification of SOX2 through phosphorylation, SUMOylation, methylation, acetylation, poly (ADP)-ribosylation (PARPylation), O-glycosylation, and ubiquitylation is another type of regulatory mechanism that mainly affects SOX2 activity. Fan et al. stated that DNA-PK, a kinase preferentially expressed in GSCs, is an upstream regulator of SOX2 and plays an essential role in maintaining GSC by precise regulation of SOX2 protein stability. This kinase binds SOX2, phosphorylates S251, and inhibits the binding of SOX2 to its ubiquitin ligase WWP2 E3 [76]. SOX2 is regulated by PLK1, a serine/threonine kinase that is upregulated in GBM cells. Biological and pharmacological inhibition of this kinase leads to marked growth inhibition accompanied by apoptosis and loss of SOX2 expression in GBM cells, thereby impairing the ability of GSCs to self-renew and form tumorspheres [77].

In the niche of SOX2-enriched and therapy-resistant organoids of GBM patients, WDR5 is identified as an essential epigenetic regulator for this population. WDR5 is a component of the WRAD complex that promotes SET1 family-mediated Lys4 methylation of histone H3 (H3K4me), which is associated with upregulation of transcription of genes involved in CSC-relevant oncogenic pathways such as SOX2. Targeting WDR5 offers an alternative approach to directly inhibit central stem transcription factors (such as SOX2) that regulate CSCs [78]. N6-methyladenosine (m6A), one of several mRNA modifications catalyzed by methyltransferase type 3 (METTL3), affects various events in RNA metabolism. The authors report for the first time the involvement of METTL3 in the maintenance of GSC and radioresistance. They propose stabilization of SOX2-modified m6A mRNA, causing METTL3 to retain a stemlike phenotype, and identified SOX2 as the bonafide target of METTL3 m6A in GSC maintenance, confirming that SOX2 is an essential mediator of METTL3function [79].

Oct4, also known as POU5F1, is a transcription factor involved in stem cell pluripotency. Oct4A palmitoylation induces the formation of the Oct4A-SOx4 complex and activates the activator region of SOX2 gene to maintain positive-loop efficiency of the CSC stemness [80]. Oc4A palmitoylation mediated by the acetyltransferase ZDHHC17 induces formation of the Oct4A-SOX4 complex and activates the enhancer region of SOX2 gene to keep stemness of CSC in positive loop. In CSCs, SOX2 is largely absent from the transcription factor complex of the SOX2 enhancer region. Instead, Sox4 forms a transcription complex with Oct4A, which activates the SOX2 enhancer region [41]. These results suggest that upregulation of Sox4 increases SOX2 expression in GSCs, which is regulated by a self-perpetuating circuit in neural progenitor cells and is relatively independent, thereby preserving the stem cell properties of GSCs and increasing the carcinogenic activity of glioblastoma [80].

Regulation of SOX2 by proteasomes has previously been shown to be crucial for GSC maintenance and function [81]. It was reported that the fine regulation of SOX2 protein turnover is a common trait of primary patient-derived GSCs, highlighting this phenomenon as a potential common therapeutic target. They identify TRIM26 E3 ubiquitin ligase as a key regulator of SOX2 proteasome degradation in GSCs and show that TRIM26, regardless of its catalytic activity, inhibits WWP2, a SOX2-driven E3 ubiquitin ligase in GSCs, thereby protecting SOX2 from polyubiquitination and degradation of the proteasome by manipulating the counter-regulatory ubiquitin ligases TRIM26 and WWP2 E3, they consistently show that modulation of SOX2 protein stability has a significant impact on GSC self-renewal in vitro and carcinogenicity in vivo in mouse models [82]. The E3 ubiquitin ligase anaphase promoter (APC) complex works in concert with the CDC20 coactivator to promote mitosis [83]. GSC invasiveness and self-renewal are regulated by CDC20-APC in a way distinct from its role in cell cycle control. SOX2 is identified as a CDC20-interacting protein and show that CDC20-APC regulates the human GSC stemness via SOX2.These results highlight that maintenance of human GSC function is mediated by a key role in CDC20-APC/SOX2 signaling axis and suggest that targeting this pathway in GBM may disrupt GSC status. Downstream from CDC20, regulation of SOX2 expression appears to occur at two levels that are not necessarily mutually exclusive: binding of CDC20 to SOX2 and CDC20-APC control SOX2 protein stability. Binding of SOX2 to CDC20 appears to be important for SOX2 to control the invasiveness of GSCs [84]. It is possible that CDC20 binding enhances SOX2 function, possibly through recruitment of CDC20-APC-dependent transcriptional activators [84,85].

Moreover, Phosphorylation is the most common type of post-translational modification of SOX2. It is known that several serine and threonine residues on SOX2 are phosphorylated in cultured cells (Malak et al, 2015; Ouyang et al, 2015) [86,87]. The tyrosine kinase c-Met contributes to the dynamic regulation of GSCs through SC-dependent mechanisms. C-Met signaling has been shown to contribute to GBM growth and recurrence by regulating the expression of transcription factors known to induce stem-like properties in differentiated cells such as SOX2 (Li et al, 2011) [88]. Another kinase, the Protein Kinase R-like ER Kinase (PERK) pathway contributes to ER stress-induced cytotoxicity in GBM neurospheres and has identified a non-canonical PERK-dependent mechanism that regulates GSC self-renewal and differentiation, including post-transcriptional regulation of SOX2 expression by a yet unknown Mechanism (Penaranda-Fajardo et al, 2019) [89].

## SOX2 Cross-Talk with Multiple Signaling Pathways

Four major signaling pathways are involved in SOX2 expression, including TGF-β, SHH, Wnt, EGFR, and FGFR. All of these signaling pathways are irregularly activated in GBM, resulting in tumor maintenance, at least in part, through SOX2 overexpression. Thus, manipulating these pathways would directly and indirectly affect all the downstream molecules and collateral pathways that interact with them. Autocrine TGF-β signaling induces SOX2 expression and plays an essential role in stemness maintenance of glioblastoma-initiating cells. Inhibition of TGF-β signaling reduces the carcinogenicity of GSCs by suppressing the activity of SOX2 [16]. Also, it is showed that SOX4 is an important mediator of TGF-β-induced SOX2 expression. This suggests that TGF-β-SOX4-SOX2 pathway is essential for maintaining the stemness of GBM-initiating cells [16]. Sonic hedgehog (Shh) is a secreted signaling protein that plays several critical roles in cerebellar and CNS development [90]. The downstream Shh signaling factors GLI1/2 upregulate SOX2 expression by binding to its proximal promoter, thus contributing to self-renewal and tumorigenesis [91]. Conversely, Shh itself is the target of SOX2 in neural stem cells. This regulation of Shh by SOX2 is important for the maintenance of neural stem cells [90]. A study by Lee et al. reported that SOX2 stimulates the expression of KLHDC8A, which belongs to the large family of kelch proteins, that in turn promotes ciliogenesis (assembly of primary cilia) to activate hedgehog signaling [92]. Mechanistic studies have shown that SOX2 acts upstream of SOX9. Results indicate that SOX2-SOX9 is the oncogenic axis that regulates stem cell properties and chemoresistance via a feedback loop between them. Signaling cascade inhibitors for Shh (cyclopamine) and mTOR (rapamycin) significantly decreased SOX2 activity and SOX9 by 40% up to 80% reduced. This shows that pharmacological silencing of SOX2 is possible through inhibitors of these signaling pathways [93].

The Wnt/β-catenin signaling pathway plays a critical role in development and tumorigenesis of GBM [94]. Multiple evidence has shown that SOX2 works in concert with the Wnt/β-catenin signaling pathway to determine cell lineages during normal tissue development. Wnt activation is a molecular mechanism that confers GBM radioresistance. The population of cells expressing high levels of active β-catenin and SOX2 continued to increase after radiotherapy, suggesting the interesting possibility that the β-catenin+/Sox2+ population function as GSC-like cells and that invasiveness and radioresistance are common Wnt signaling phenotypes [95].

FGFR and EGFR are members of the membrane-expressed receptor tyrosine kinase family that binds to its ligands (EGF protein family members) to elicit mitogenic effects in target cells, thereby participating in various vital physiological processes such as cell proliferation, differentiation, migration and survival [96]. The FGFR signaling pathway regulates SOX2 expression via two major signaling cascades: (i) MEK/ERK and (ii) PI3K/AKT/mTOR, two GBM-activated signaling pathways whose repression leads to inhibition of self-renewal ability of GSCs and tumorigenesis [97]. Extensive molecular research has identified the (PI3K)/Akt signaling cascade as one of the most frequently altered signaling pathways in GBM. EphA2 is overexpressed in GBM and is associated with a poorer prognosis. It is reported that the EphA2-Akt-SOX2 signaling axis as the driving mechanism underlying GSCs intracranial invasion by maintaining the stem cell properties of GSCs through increased SOX2 expression [98]. A study showed that anoctamin-1 (ANO1), a Ca2+-activated Cl channel, interacts with EGFRvIII, significantly affects self-renewal activity and invasion of GSCs in vitro, and is involved in growth and tumor invasion in vivo by regulating the expression of EGFRvIII and SOX2, which confers stem cell properties on GBM cells [99]. In addition, PARK7, a PD-related protein, has been shown to play a key role in maintaining stemness and radiation resistance in GSCs by stabilizing the EGFRvIII-SOX2 signaling axis [100].

GBM resides in a hypoxic microenvironment and undergoes malignant progression regulated by hypoxia-inducible factors (HIFs) that inhibit apoptosis and promote proliferation (Wang, Zhao, et al, 2021) [101]. HIF1α and HIF2α regulate Sox2 expression as upstream genes, and are regulated by the PI3K/AKT-mTOR pathway as downstream genes [102]. That is, EGF induces HIF1α expression via the EGFR-PI3K/AKT mTOR pathway and thereby regulates GBM Kimgrowth via a SOX2-mediated positive feedback mechanism, thereby promoting resistance to GBM therapy and providing a novel cancer development model and treatment strategy for GBM. Analysis using the CGGA database showed that HIF1α and HIF2α were positively correlated with SOX2 in GBM [101]. HIF2α demonstrates a novel signaling mechanism in the regulation of invasive and stem like traits through increased expression of Pan Mena and Mena INV following this HIF-2α-mediated increase in SOX-2 [103]. Moreover, SOX2 is also regulated by EGFRvIII, a common mutant in GBM that leads to activation of pro-oncogenic signaling in GBM. Indeed, EGFRvIII expression is positively correlated with SOX2 expression and is associated with an increase in self-renewal capacity and tumor initiating ability [104]. This relationship has been shown to be mediated by the EGFRvIII-STAT3 PEDF notch axis [105]. Several studies have shown that GSCs exhibit high Notch activity, which helps inhibit differentiation and maintenance of stem cell-like properties, thus contributing to their resistance to conventional treatments. There is increasing evidence that demonstrates the

existence of a biologically active interaction between adipokine-leptin and Notch signaling pathway that increases the concentration of stem cell markers such as SOX2. It was shown for the first time that leptin promotes the proliferation, migration and deformation of GBM cells by activating the Notch signal [106].

## SOX2 and Drug Resistance in GSCs and GBM

The development of drug resistance in cancer is closely associated with a poorer clinical prognosis and represents an enormous challenge that severely limits the effectiveness of current anticancer therapies. A growing body of data has shown that upregulation of SOX2 in cancer cells is often associated with anticancer drug resistance [107]. There are a variety of different mechanisms that contribute to SOX2-induced therapy resistance. An important mechanism is that SOX2 expression in cancer cells is related to CSC status, which is defined as a subpopulation of cells within a tumor that have the capacity for indefinite self-renewal, differentiation into different cell types, resistance to cancer therapies, and to tumor recurrence [108,109]. Therapy surviving tumor cells capable of tumor reproduction after removal of therapeutic pressure exhibit organized networks of vessel-like structures formed by tumor cells expressing CD133 or OCT4/SOX2. Therefore, it needs to be investigated whether a therapeutic strategy that specifically targets GSCs in the GBM model can completely eradicate a GBM tumor. It is showed that even after prolonged treatment against GSC replication, a pool of resistant tumor cells is selected and tumors can proliferate when relieved of therapeutic pressure. Results suggest reasons for the failure of some therapeutic strategies: Some cytotoxic therapies targeting tumor-initiating cell replication can significantly reduce tumor burden but do not eradicate tumors, instead inducing the formation of vascular-like structures causing tumor recapitulation [110].

A study by Gong et al. provides direct evidence that CSCs are closely associated with chemoresistance and shows that TMZ chemotherapy induces cell development, resulting in complex heterogeneity and enrichment of GSCs. They also identified a particularly advantageous CSC niche in avoiding TMZ responses. The molecular signature of this subset included upregulated expression of canonical neural stem cell markers, including SOX2 [111]. GBM is able to convert between stem and nonstem states by assessing the expression of SOX2 and cell communication proteins such as connexins. It has been shown that non-GSC and GSC can undergo sequential rounds of gain and loss of stem properties, demonstrating a bidirectional pattern of cellular plasticity that accompanies changes in connexins expression thus reprograming their state of differentiation and contributes to the development of chemotherapy resistance mechanisms. It has been hypothesized that this heterogeneity in GBM tumor accounts for the plasticity of glioblastoma stem cells [36]. Increasing accumulation of SOX2-expressing GSCs induces drug resistance. Specifically, resistance is achieved through induction of EGFR phosphorylation, leading to activation of the EGFR/PI3K/AKT/ERK cascade, which promotes stemness development through expression of SOX2 in GSCs [112]. GSCs are resistant to radiotherapy, due in part to efficient DNA repair. methyltransferase-like 3 (METTL3) that was found to be important for the radiation resistance demonstrated by the GSCs. The METTL3-SOX2 axis enhances the radioresistance phenotype by supporting SOX2 as a key effector of METTL3 signaling [79]. Hyperbaric oxygen (HBO) is the most promising way to alleviate the hypoxic environment in GBM. HIF1α, HIF2α and SOX2 have been shown to be highly expressed under hypoxic conditions and contribute to the increase in GBM and chemoresistance. However, it was found that the expression of HIF1α, HIF2α and SOX2 was reduced after HBO and that it promoted GBM cell proliferation during cell cycle progression, albeit with a decrease in stem properties, and thus to HIF1α/HIF2α – induced chemosensitization through inhibition of SOX2 (Wang, Gong, et al, 2021) [113]. In summary, SOX2 actively confers resistance of several cancer cells to chemotherapeutic agents. SOX2 is therefore an interesting therapeutic target to overcome drug resistance.

## Therapeutic Targeting of SOX2 In GBM

In most human cancers including GBM, SOX2 functions as an oncoprotein that activates multiple proliferative and anti-apoptotic signaling cascades, promoting tumorigenesis, metastasis, and drug resistance. Targeting SOX2 is therefore an effective cancer treatment strategy. However, since SOX2 is an undruggable transcription factor, progress in discovering selective SOX2 inhibitors has been very limited, although several approaches to combat SOX2 have been attempted. Gene therapy, immunotherapy, stem cell therapy and CRISPR/Cas9 technology aim to deliver a gene that produces cancer cell toxicity or induces cancer cell apoptosis, enhances host immune response and corrects the mutated genome, thus reducing/curing GBM.

## Preclinical Studies Targeting SOX2 In GBM

The current standard therapy for GBM is surgical resection combined with radiation and chemotherapy. Temozolomide (TMZ), an oral alkylating agent, is the most commonly used chemotherapeutic drug in the treatment of GBM [114] which prolongs patient survival by 12–15 months [4]. The function of SOX2 in TMZ chemoresistance has been decoded during recent years. Therefore, cells with increased SOX2 expression are more resistant to TMZ, while its suppression makes GBM cells more sensitive to this factor [112]. Elevated SOX2 levels have been used as a marker for the proneural subtype, which has been shown to be the most resistant subtype to current therapeutic chemo- and

radio- therapy [115]. The intervention of SOX2 in drug resistance has been validated by different mechanisms. These facts, as well as its important role in GSC regulation, suggest that SOX2 may be a key factor responsible for resistance to current chemotherapy and postulate that targeting its activity may provide an interesting new therapeutic approach to cure GBM patients. However, SOX2 is being used directly or indirectly by several strategies to combat GSCs. The GBM microenvironment is highly heterogeneous and consists of many cell types including astrocytes and endothelial cells as well as tumor cells, which are responsible for increased resistance to standard chemotherapeutic agents such as TMZ and radiotherapy [116]. Therefore, it is of great importance to study how drug treatment affects the expression of GBM cell lineage markers in multicellular tumor (MCTS) models. Furthermore, to our knowledge, the effect of chemotherapeutic agents on the expression of GBM stem cell markers has not previously been investigated in the MCTS model. To fill this gap, it was observed that treatment with Lonafarnib (LNF), TMZ or their combination significantly reduced the spheroid size of MCTS and significantly increased the expression stem markers as NESTIN, SOX2, CD133, NANOG and Oct4 [117]. Also, the natural compounds berbamine and arcyriaflavin A (ArcA), are known to have antiproliferative and anticancer effects by reducing TMZ-induced SOX2 expression levels [118]. This is consistent with NS398, a COX-2 inhibitor, alone or in combination with TMZ [119].

Several experimental, epidemiological and clinical studies have shown significant effects of aspirin in the treatment of cancer. The authors reported for the first time that aspirin potently inhibits the proliferation and invasiveness of GSCs and reduces their stemness characteristics in vitro. Although a more detailed study is needed. These effects are related to the reduction of cyclins D1 and Rb1 Protein phosphorylation and involve the significant down-regulation of SOX2, Stat3 and Survivin at the protein and RNA levels. The results of this study suggest that aspirin could be useful as adjuvant therapy in the management of patients with GBM (Pozzoli et al, 2019) [120]. This is consistent with the use of Graphene Oxide (GO) that effectively inhibits the proliferation and induces the differentiation of GSCs by decreasing stemness-related gene expression as SOX2 (Wang et al, 2020) [121]. Ikushima et al suggest that TGF-β inhibitors could be used in combination with conventional drug therapies and radiotherapy to reduce the aggressiveness of GBM (Ikushima et al, 2009) [16]. Metformin (Met) is an oral biguanide used in the clinical management of type 2 diabetes. In addition to its antidiabetic effects, Met inhibits tumor growth in a variety of cancer types, including GBM. TGF-β1 induced an EMT-like process and increased the migratory and invasive capacity of GBM cells. Met inhibited this EMT-like process and the TGF-β1-induced stem-like properties of cancer (Song et al, 2018) [122]. Moreover, Resveratrol, a natural plant compound, apparently inhibited the EMT-induced self-renewal capacity of GSCs and inhibited EMT-induced cancer stem cell markers such as SOX2, suggesting that resveratrol is able to suppress the stem cell-like properties generated by EMT in GBM cells via TGF-β1/Smads signaling (Ji et al, 2015) [123]. TGF-β, in turn, activates various downstream signals, including Smad, MAPK, and PI3K/Akt signals, thus affecting these collateral pathways (Song et al, 2019) [124]. Combined inhibition of PI3K/Akt/mTOR and SHH pathways was superior to inhibition of the individual pathway in inhibiting GBM growth by targeting glioblastoma-initiating cells (GICs), resulting in tumor growth inhibition and a regulation of the expression of pluripotency promoting factors and stem cell markers such as SOX2 (Nanta et al, 2019) [125].

Treatment with arsenic trioxide (As2O3) which is a drug belongs to the general group of medicines called antineoplastics in GSCs induced dose-dependent apoptosis and significantly reduced SOX2 expression at the transcriptional level. Furthermore, overexpression of SOX2 can protect GSCs from As2O3-induced apoptosis, indicating the involvement of SOX2 downregulation in As2O3-induced apoptosis (Sun & Zhang, 2011) [126]. Izorhapontigenin (ISO), a compound with antioxidant properties, was recently identified as the active anticancer compound in Chinese herbs. ISO treatment resulted in the induction of expression of miR-145, a regulator of SOX2, which in turn inhibits translation of the SOX2 protein, resulting in a drastic down-regulation of SOX2 protein expression, lower cyclin D1 levels and growth inhibition. Identification of the miR-145/SOX2/cyclin D1 axis is crucial for ISO-mediated growth inhibition of PDGS (Xu et al, 2016) [127]. Polydatin (PD), a monocrystalline compound originally extracted from Polygonum cuspidatum, is characterized by exceptional and significant antitumor activities. PD has been shown to inhibit cell proliferation, migration, invasion and stemness and promote apoptosis in GBM cells with extremely low cytotoxicity to normal human cells. Furthermore, PD's cytotoxicity was demonstrated by reducing multiple components of the EGFR-AKT/ERK1/2/STAT3-SOX2/Snail signaling pathway (major EMT regulatory factors) in GBM cells (Chen et al, 2020) [128]. In addition, PBI-05204 is a specially formulated herbal medicinal product consisting of Nerium Oleander extract modified with supercritical CO2, which has the ability to cross the blood-brain barrier and penetrate into brain tissue. The amount and size of newly formed neurospheres as well as the expression of SOX2, CXCR4 and CD44 have been shown to be significantly decreased in the presence of PBI-05204, thus reducing self-renewal and GSC stemness by negatively regulating the expression of PI3k/ Akt/ mTOR pathway (Colapietro et al, 2020) [129]. These results were consistent with another studies that shows the cytotoxic effects and anticancer therapeutic efficacy of Cannabidiol, Cannabigerol (Lah et al, 2022) [130], hydrazinobenzoylcurcumin (HBC) (Shin

et al, 2019) [131] and phytochemical 4-acetylantroquinonol B (4-AAQB) (Liu et al, 2018) [132], a derivative of mono-acetylated antroquinonol, which is a bioactive extract of *Antrodia camphorate,* on GSCs by significantly decreasing the expression of SOX2 and thus significantly inhibited their self-renewal ability. Moreover, Numerous studies have shown that ginsenoside have anti-inflammatory, anti-diabetic and anti-cancer effects. ginsenoside metabolite compound K (CK) showed that it not only significantly inhibits growth but also metastatic ability of GBM cells, arrests cell cycle progression and apoptosis by blocking PI3K/Akt/mTOR-mediated signaling pathways induced in human GBM cells. In addition, CK suppressed the self-renewal ability and invasiveness of stem cells (GSCs) and led to down-regulation of GSC markers such as SOX2. Thus, these results suggest that CK has the potential to clear GSCs by regulating cell surface glycoproteins and stem-regulating transcription factors in GSCs (Lee et al, 2017) [133].

Salinomycin, an antibiotic commonly used in agricultural feed, has been shown to target CSCs in several cancer types and GBM. A study by Magrath et al. showed that salinomycin reduces the expression of SOX2 at both the transcriptional and translational levels. In addition to inducing apoptosis in the caspase 3-dependent and independent method by which salinomycin induces cell death in GBM. The current results suggest that salinomycin is a potent chemotherapeutic drug candidate capable of treating GBM by targeting both mass tumor cells and CSCs (Magrath et al, 2020) [134].

Actinomycin D (ACTD) has widespread cytotoxic effects in several GBM models. Taylor et al. found in their preclinical study that ACTD is not only highly potent in recurrent mouse GBM models, but is also well tolerated by differentiated noncancerous mouse cells both in vitro and in vivo. In vitro, low concentration actinomycin D induces cell death, inhibits self-renewal, invasion, and migration in murine NSCs and GSC recurrence lines TS9 and TD2, and significantly reduces tumor volume in GBM model, subcutaneous recurrence, and tumor growth in orthotopic GBM models. ACTD downregulates SOX2 in preclinical GBM models, suggesting that ACTD may specifically target SOX2-expressing stem cells. Also, SOX2 expression was significantly downregulated in ACTD-treated mice. Taken together, these data suggest that ACTD has a specific effect on SOX2 down-regulation or preferential killing of SOX2-expressing cells in vivo (Taylor et al, 2020) [135]. Xihuang Pill, a well-known traditional Chinese medicine in “Chinese Pharmacopoeia” (National Drug Approval No. Z11020073), destabilized the CD133/EGFR/Akt/mTOR cascade, resulting in reduced stemness enrichment of GBM by down-regulation of SOX2 (Xu et al, 2023) [136]. Ivacaftor, which is widely used to treat cystic fibrosis, acts as a potent inhibitor of GSC maintenance by specifically and potently suppressing GSCs and by directly downregulating the expression of stem cell marker genes, including CD133, CD44, and SOX2. For the first time, ivacaftor has been shown to inhibit GSC cell proliferation, self-renewal and tumor xenograft growth, and induces cell apoptosis (Liu et al, 2021) [137]. Combinations of compounds that target non-redundant GBM signaling pathways or with cytotoxic agents can act synergistically and induce GBM cellular death. There is hope that such combinations will enable promising therapies that significantly improve patient outcomes.

## Conclusion And Future Perspectives

SOX2 has the capability to act as an indispensable factor in tumorigenesis which increases its prognostic and therapeutic values in terms of patient survival. Its role as a biomarker, prognostic marker, metastatic marker, and therapeutic target in various cancers, including GBM, is well studied. The dynamic expression of SOX2 is tightly controlled by a complex regulatory network at multiple levels including transcription, post-transcription, and post-translation. SOX2 also interacts with multiple signaling pathways to assure convenient control of important biological processes, including progression of cell cycle, apoptosis, autophagy, and EMT. In promoting or inhibiting tumor growth, SOX2, as a direct or indirect factor, regulates different signaling pathways that manifest different phenotypes. Irregular expression of SOX2 impairs ESC self-renewal and leads to defective proliferation. As a critical tumor marker, SOX2 could be a potential breakthrough in cancer treatment in the future. Preclinical studies using both cell cultures and genetically engineered mouse models propose that SOX2 is an oncogenic protein. Based on the existing studies, we identified the relevant functions of SOX2 in GBM and set a foundation for future related studies and research. Unfortunately, there are currently no known small molecules that bind to SOX2. Promising approaches are transcriptional regulation and/or post-transcriptional repression of SOX2 by miRNA regulation. There are also encouraging results with SOX2 immunotherapy or the combination of tyrosine kinase and signaling pathways inhibitors. These results provide preclinical evidence that inhibition of SOX2 activity is an effective strategy in the treatment of GBM. To determine its clinical effect, more robust and comprehensive preclinical results and clinical studies with the proposed combination therapy in patients with GBM whose biopsies show increased SOX2 expression are needed.

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