Sox2: regulation of expression and contribution to brain tumors

Sheila Mansouri^{*1}, Romina Nejad^{*1}, Merve Karabork², Can Ekinci²,
Ihsan Solaroglu^{2,3}, Kenneth D Aldape¹ & Gelareh Zadeh^{*1,4}

Practice points

- Sox2 is thought to play a significant role in maintaining characteristics of cancer stem cells that are thought to give rise to different cancer types and confer resistance to chemotherapy and radiation.
- The Sox2 gene is amplified in a number of human cancers including prostate cancer, lung, esophageal squamous cell carcinoma and small-cell lung cancer.
- In addition to gene amplification, overexpression of Sox2 has been reported in a number of human brain tumors including glioblastoma and medulloblastoma.
- Sox2 expression correlates positively with the malignancy grade of brain tumors and elevated levels of Sox2, and thus higher stem cell gene expression profile have been shown to correlate with poor clinical outcome in brain tumor patients.
- Sox2 expression is generally higher in the Shh group of medulloblastoma compared with the other medulloblastoma subgroups, and as observed in multiple tumor types, the stemness factors Sox2 and Nestin are important in the development and progression of medulloblastoma.
- Sox2 is the master regulator of pluripotency and maintenance of tumor stem cell properties, and is, therefore, a highly attractive molecular target for treatment of glioblastoma and medulloblastoma tumors.
- Different approaches of targeting Sox2 have been suggested, including developing a vaccination against oncoproteins presented on the surface of cancer cells for Sox2 immunotherapy, and post-transcriptional suppression of Sox2 expression by overexpressing miRNAs – such as miR-126 and -145 – that specifically target the 3' untranslated region (3' untranslated region) of Sox2 mRNA.

Tumors of the CNS are composed of a complex mixture of neoplastic cells, in addition to vascular, inflammatory and stromal components. Similar to most other tumors, brain tumors contain a heterogeneous population of cells that are found at different stages of differentiation. The cancer stem cell hypothesis suggests that all tumors are composed of subpopulation of cells with stem-like properties, which are capable of self-renewal, display

¹McFeeters-Hamilton Center for Neuro-Oncology Research, 101 College St., Toronto, ON, M5G 1L7, Canada

²School of Medicine, Koç University, Rumelifeneri Yolu, 34450, Sariyer, Istanbul, Turkey

³School of Medicine, Loma Linda University, Loma Linda, CA 92354, USA

⁴Division of Neurosurgery, Toronto Western Hospital, Toronto, M5T 2S8, Canada

*Author for correspondence: gelareh.zadeh@uhn.ca

*Authors contributed equally

resistance to therapy and lead to tumor recurrence. One of the most important transcription factors that regulate cancer stem cell properties is SOX2. In this review, we focus on SOX2 and the complex network of signaling molecules and transcription factors that regulate its expression and function in brain tumor initiating cells. We also highlight important findings in the literature about the role of SOX2 in glioblastoma and medulloblastoma, where it has been more extensively studied.

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Background

SRY (sex determining region Y)-box 2 (SOX2) is an intronless single-exon gene that is located on chromosome 3q26.3–q27, encodes a 317 amino acid protein, and is a member of the SOX (SRY-related high mobility group box) family of transcription factors that regulate many stages of mammalian development. The Sox family of proteins shares a highly conserved DNA-binding domain – known as high mobility group box domain – that contains approximately 80 amino acids (Figure 1) [1]. SOX2 plays a key role in maintenance of self-renewal and pluripotency of undifferentiated embryonic stem cells (ESCs) and plays a critical role in the maintenance of embryonic and neuronal stem cell characteristics [2,3]. Interestingly, both high and low expression of SOX2 can lead to loss of pluripotency in ESCs and this sensitivity to changes in SOX2 expression may be due to altered level of promoter/enhancer activity of SOX2 target genes [4,5]. In addition, expression of SOX2 needs to be maintained in a dynamic equilibrium with other transcription factors – such as OCT4 and NANOG – which are also associated with regulation of pluripotency and are required for the proliferation of undifferentiated ESCs. Boyer *et al.* used chromatin immunoprecipitation combined with DNA microarrays and genome-wide location analysis in human ESCs to identify DNA sequences occupied by OCT4. Surprisingly, they discovered that SOX2, OCT4 and NANOG share many of their target genes, co-occupying at least 353 genes, some of which are developmentally important transcription factors in human ESCs such as homeodomain proteins [6]. Their results further indicate that OCT4, SOX2 and NANOG function together to form autoregulatory and feed-forward regulatory loops that lead to the transcriptional regulation in stem cells, thereby contributing to their pluripotency and self-renewal.

A very promising and exciting area of research in regenerative medicine is induced pluripotency,

and SOX2 holds great promise in this field. In 2006, a breakthrough study performed by Takahashi and Yamanaka involving mouse ESCs demonstrated that differentiated somatic cells can be induced to become pluripotent and resemble ESCs that are capable of differentiating into adult cell types [7]. They discovered that concomitant expression of SOX2, OCT4, KLF4 and c-MYC transcription factors (SOKM; also known as Yamanaka factors) was sufficient for induction of pluripotency in differentiated cells, resulting in generation of what is known to be induced pluripotent stem (iPS) cells. This finding that only a set of four transcription factors are necessary and sufficient to induce pluripotency has revolutionized regenerative medicine. For example, astrocytes residing within the spinal cord can be induced to convert to double cortin (DCX)-positive neuroblasts upon introduction of SOX2 alone. These cells will in turn mature and develop into synapse-forming neurons, resulting in repair of the damaged spinal cord. Therefore, reprogramming of local astrocytes into neurons is a potential strategy for cellular regeneration after spinal cord injury [8,9].

In addition, SOX2 is thought to play a significant role in maintaining characteristics of cancer stem cells that are thought to give rise to different cancer types and confer resistance to chemotherapy and radiation [10]. Therefore, understanding the mechanism of action of SOX2 in regulation of stemness and pluripotency, in addition to its role in disease mechanism and cancer development, is a topic of intense investigation that holds promise for development of effective approaches in both regenerative medicine and cancer treatment. In this review, we aim to highlight the essential role of SOX2 in the development and progression of brain tumors by describing in detail its functions and regulation in normal brain and brain malignancies. For more comprehensive reviews on the functional role of SOX2 in ESCs and induced pluripotency please see references [11–13].

Figure 1. SOX2 protein domains and post-translational modification sites. Expression and function of SOX2 is regulated through several post-translational modifications such as ubiquitination, sumoylation, phosphorylation, methylation and acetylation. Acetylation of SOX2 at lysine residues within its DNA-binding domain leads to its export from the nucleus, thus inhibiting its transcriptional activity [62]. Phosphorylation of SOX2 at S249, S250 and S251 promotes sumoylation of SOX2, subsequently inhibiting the ability of SOX2 to bind DNA [63,64]. SOX2 is monomethylated at K119 by Set7, which inhibits SOX2 transcriptional activity and induces its ubiquitination and subsequent degradation. Phosphorylation of SOX2 at T118 by AKT1 stabilizes SOX2 by antagonizing the K119me by Set7 [66].

Regulation of SOX2 expression

SOX2 expression is regulated differently in various contexts through a complex network of transcriptional, post-transcriptional and post-translational regulators. Herein, we focus on regulation of SOX2 expression at transcriptional level through other transcription factors and signaling pathways, its post-transcriptional regulation via miRNAs, in addition to post-translation modifications.

• Transcription factors regulating SOX2 expression

Expression of SOX2 in neural progenitor cells (NPCs) is positively regulated by transcription factors that are expressed at considerably high levels during early neural development and differentiation such as AP-2, PROX1 and PAX6 [14]. In addition, cell-cycle regulators – such as E2F3a and E2F3b – have been reported to regulate SOX2 expression and control proliferation of NPCs [15,16]. Cyclin-dependent kinase

inhibitor P21 has also been found to directly bind to SOX2 enhancer and repress SOX2 expression in NPCs [16]. Taken together, the various enhancer/regulatory regions of SOX2 promoter cooperate to stringently regulate its expression from early embryonic stages to the NPC state and eventually full differentiation [17,18].

The promoter of SOX2 was also found to be hypomethylated, which leads to its aberrant expression in brain tumors such as glioblastoma (GBM), reflecting the more primitive cellular state that is also found in neural stem cells (NSCs) [19,20]. Expression of SOX2 is also regulated by other members of the SOX family transcription factors such as SOX4, which was shown to regulate SOX2 transcription via the TGF- β signaling pathway and form a cooperative complex with Oct4 at the promoter of SOX2 [21]. Subsequently, Boer *et al.* demonstrated that elevating the levels of SOX2 in cancer stem cells and ESCs inhibits the expression of SOX2-OCT-3/4 target genes due to decreased activities

of their own promoters, suggesting the existence of a negative feedback loop that controls stem cell pluripotency and self-renewal [5,22]. Collectively, these studies indicate that the levels of key pluripotency transcription factors – such as SOX2, OCT4 and NANOG – are carefully regulated in ESCs; however, the mechanisms through which the levels of these transcription factors, in particular SOX2, are regulated are poorly understood.

• Signaling pathways regulating SOX2 expression

SOX2 expression is activated through four main signaling pathways, including Shh, Wnt, FGFR and TGF- β (Figure 2) [23]. Shh pathway functions to induce cellular proliferation in the developing CNS and is also implicated in driving tumorigenesis in the brain [24]. This pathway is initiated with the binding of Shh ligand to Ptch receptor, causing repression of Ptch, and thus, activation of a G-protein-coupled receptor known as SMO. Active SMO will in turn enable the direct activation of GLI1/2, which then translocate into the nucleus and activate a number of genes that promote cellular proliferation, the most important one being SOX2.

The canonical Wnt/ β -catenin signaling pathway functions in coordination with the Shh pathway, as it also activates GLI1/2 transactivators. This cascade transduces signals through the FZD family of G-protein-coupled receptors and LRP5/LRP6 co-receptors to determine cell fate [24,25]. Through the activation of Dsh, GSK-3 β is inhibited, which in turn, leads to β -catenin stabilization and accumulation within the cytoplasm. Accumulated β -catenin then translocates into the nucleus, where it promotes proliferation by regulating the expression of proto-oncogenes, such as cyclin D1 and c-Myc (not shown in figure) and crosstalks with the Shh signaling cascade to regulate the expression of SOX2 [24,26]. The canonical Wnt/ β -catenin pathway is known to promote invasion, migration and metastasis in different types of cancer, and is upregulated in glioma tumors, breast, prostate and colorectal cancers [27–32]. In GBM, Wnt/ β -catenin pathway is a key downstream mediator of MET receptor tyrosine kinase (RTK) and contributes to the maintenance of glioma stem cell properties [32]. The Wnt/ β -catenin pathway is also implicated in medulloblastoma – characterized by mutations in β -catenin – differentiating it from the other

subgroups; Shh, characterized by *Ptch1* mutation and groups 3 and 4, which involve chromosome aberrations, specifically in chromosomes 8 and 17 [33–36].

The FGFR pathway regulates SOX2 expression through two main signaling cascades: Ras/MAPK and PI3K. Both pathways are activated upon binding of FGFs, a family of ligands that bind to four subtypes of FGFR [37]. Activation of the Ras-Raf-MAPK signaling cascade begins with RAS activation, followed by c-RAF, which then activates MEK1/2. MEK1/2 then phosphorylate and activate ERK1/2 (also known as MAPK), which travels into the nucleus and activates transcription of OCT4, SOX2 and NANOG. The PI3K-AKT-mTOR pathway regulates SOX2 expression via the activation of PI3K, which consequently activates PKB (also known as Akt). AKT activates mTOR via phosphorylation, thus translocating mTOR into the nucleus, where it regulates expression of genes involved in cell growth, proliferation and survival [38]. Frassan *et al.* used pharmacologic inhibitors of PI3K in primary medulloblastoma-derived cells, demonstrating that the PI3K-AKT-mTOR pathway is fundamental for the proliferation and survival of these cells. Moreover, their results suggest that PI3K inhibition leads to medulloblastoma-derived cell death by specifically activating the mitochondrial apoptotic cascade in the CD133⁺ cancer stem cell population, while sparing the more differentiated cells [39].

A known tumor suppressor protein that inhibits the PI3K-AKT-mTOR pathway is PTEN [40]. PTEN functions in various cellular processes such as cell-cycle progression, cell growth, migration and invasion, focal adhesion, angiogenesis and apoptosis [41,42]. Specifically, PTEN inhibits the PI3K-AKT-mTOR pathway by dephosphorylating and converting PIP3 to PIP2, which in turn inhibits various downstream signaling molecules, most importantly Akt [43]. Taken together, evidence from multiple studies suggests that PTEN mutation is associated with poor prognosis in high-grade gliomas, highlighting the importance of the PI3K-AKT-mTOR pathway and its regulation of SOX2 in these tumors.

Last, the TGF- β pathway is known to have both tumor suppressor and activating properties. In normal tissue, it functions as a tumor suppressor, but the pathway shifts to become oncogenic during tumorigenesis. This signaling cascade is initiated upon the binding of

Figure 2. Signaling pathways regulating expression of SOX2 and its downstream target genes. Expression of SOX2 is orchestrated by crosstalk between multiple signaling pathways such as Wnt, Shh, TGF- β and FGFR. Upon ligand binding to transmembrane receptors corresponding to each pathway, the signaling pathways are initiated and activated downstream effector proteins translocate into the nucleus, where they activate or repress transcription of their target genes by binding to their promoter regions.

TGF- β ligand to T β R1, subsequently activating SMAD2/3, which then activates SOX2 expression through crosstalk with other signaling pathways, specifically the PI3K and MAPK pathway (Figure 2) [44]. TGF- β is an important signaling pathway in GBM development and progression [45,46]. Functionally, the activation of canonical and noncanonical TGF- β signaling enhances glioma initiating cell tumorigenicity through SOX4 – a member of the SOXC family of transcription factors that are implicated in brain tumors – and consequent increased

expression of SOX2 [21,47]. Additionally, inhibition of TGF- β signaling dramatically decreases the tumorigenicity of glioma initiating cells by promoting their differentiation, and these effects are reversed by overexpression of SOX2 or SOX4 [21].

• Post-transcriptional regulation of SOX2 expression by miRNAs

Another layer of regulation of SOX2 expression occurs at the post-transcriptional level through several miRNA families in various biological

contexts. miRNAs are a class of small noncoding RNAs that are key post-transcriptional regulators of gene expression by targeting the 3' untranslated region (UTR) and/or coding sequence of specific mRNAs, thus, inhibiting translation or inducing degradation of these mRNAs [48]. Several miRNAs are thought to regulate the expression of *SOX2* mRNA in NSC/NPCs and also in cancer stem cells, maintaining the level of *SOX2* protein at a specific dose in order to precisely balance its expression in either the stem or differentiated states. For example, overexpression of miR-200c, which targets the conserved miR-200b/c/429 binding site within the 3' UTR of *SOX2*, was found to suppress the expression of *SOX2* in NSC/NPCs and result in exit from cell cycle and neuronal differentiation [49]. *SOX2* and E2F3 transcription factors in turn, are thought to activate the expression of murine miR-200c/141 gene cluster and lead to the negative feedback regulation of *SOX2* and miR-200 expression and their gradual decrease in postmitotic neurons [49,50]. Unlike the miR-200 miRNAs that are downregulated in differentiating NSC/NPCs, miR-9 and its complementary strand miR-9* are thought to be upregulated at the onset of neuronal differentiation and target a highly conserved sequence in the 3' UTR of *SOX2* [49,51]. Therefore, it is likely that miR-9 and miR-200c may cooperate to regulate the expression of *SOX2* and result in definitive differentiation of NSC/NPCs into neurons or glial cells.

Some miRNAs that target *SOX2* mRNA are considered tumor suppressors (suppress the expression of oncogenes), while some are oncogenic (suppress the expression of tumor suppressor genes), and thus, regulate the activity of *SOX2* in various transformed tissues [52]. For example, the tumor suppressive miR-9*, miR-126, miR-140, miR-145 and miR-638, were shown to downregulate the expression of *SOX2* in a variety of human transformed tissues [53–58]. Among brain malignancies, the expression of miR-9* is suppressed by ID4 in human GBM and glioma stem cells, which in turn results in increased expression of *SOX2* and promote self-renewal, tumorigenicity and chemoresistance in these cells [53].

Altogether, these studies indicate that decreased expression of the tumor suppressive miRNAs that have a conserved binding site within the 3' UTR of *SOX2* leads to increased expression of *SOX2* and the consequent tumorigenic transformation and elevated malignant potential of tumors and cancer stem cells. On the other hand, oncogenic

miRNAs, such as miR-126 and miR-429, that target the 3' UTR of *SOX2* are upregulated in some malignant tissues and cell lines, and their expression inversely correlates with expression of *SOX2* mRNA [59,60]. For example, miR-429, a member of the miR-200 miRNA family, directly targets a conserved binding site in the 3' UTR of *SOX2* and is upregulated in human colorectal carcinoma tissues and also shows an opposite expression pattern from that of *sox2* mRNA [60]. Therefore, miRNAs fine-tune the expression of *Sox2* and its function in regulation of pluripotency.

• Post-translational regulation of *SOX2*

Contrary to NSCs, expression and function of *SOX2* in the adult brain is almost undetectable [61] and it is thought to be regulated via a number of mechanisms, including post-translational modifications such as ubiquitination, sumoylation, phosphorylation, methylation and acetylation (Figure 1). *SOX2* is acetylated at lysine residues within its DNA-binding domain. This modification enhances the export of *SOX2* to the cytoplasm, thus inhibiting its transcriptional activity [62]. *SOX2* protein function can also be modulated by phosphorylation, which affects its activity as a transcriptional regulator. Three phosphorylation sites, S249, S250 and S251, have been identified in *SOX2* and phosphorylation at these residues promotes sumoylation of *SOX2*, subsequently inhibiting the ability of *SOX2* to bind DNA [63–65]. These findings suggest that a crosstalk may exist between two different types of post-translational modifications of *SOX2*. Fang *et al.* provided evidence that the exact level of *SOX2* protein in ESCs is maintained by a balance between methylation and phosphorylation [13,66]. Specifically, *SOX2* is monomethylated at K119 by Set7, which inhibits *SOX2* transcriptional activity and induces *SOX2* ubiquitination and subsequent degradation. The E3 ligase WWP2 specifically interacts with *SOX2* methylated at K119 through its HECT domain to promote ubiquitination of *SOX2*. In contrast, phosphorylation of *SOX2* at T118 by AKT1 stabilizes *SOX2* by antagonizing the K119me by Set7 [66]. These studies highlight the importance of an *SOX2* methylation–phosphorylation switch in regulating ESC proliferation and differentiation.

Functional roles of *SOX2* in the brain

• Regulation of neural stem cell properties

SOX2 is expressed during early stages of neurogenesis in the developing CNS within the

neural tube [67]; however, as the development progresses, SOX2 expression decreases and becomes limited to the sub-ventricular zone and sub-granular zone of dentate gyrus. This serves as a marker for NSCs, because all SOX2-expressing cells coexpress the stemness marker gene *NESTIN* [68]. Upon differentiation, astrocytes lose SOX2 but continue to express other genes in the SOX subfamily [69]. SOX2 is also highly expressed in NPCs during embryonic development, but is downregulated upon their exit from the cell cycle [70], following which NPCs become postmitotic and fully differentiate into neuronal or glial cells. Thus, NPCs can divide into one identical progenitor cell and one fully differentiated neural cell type in a process that is referred to as asymmetric cell division, a necessary hallmark of stem cells. Consequently, SOX2-expressing NPCs have the potential to generate neural precursors as well as additional SOX2-positive NPCs.

Experiments using chick spinal cord showed that suppression of SOX2 expression in NPCs leads to inhibition of cell proliferation and early neuronal differentiation [71]. Mechanistically, it is thought that the SOX family of transcription factors may form inhibitory complexes with proneural proteins, which in turn, reduces their ability to bind DNA or to transactivate their target genes [69]. Interestingly, ectopic expression of SOX2 alone can directly reprogram fibroblast cells into multipotent NPCs, underscoring the essential role for SOX2 in these cells [72,73]. Furthermore, adult NSCs, which express higher levels of SOX2 and c-MYC than ESCs, have the capacity to become iPS cells upon introduction of OCT4. Therefore, only two exogenous factors, one of which is OCT4, are sufficient to induce pluripotency in NSCs [74]. Gangemi *et al.* showed that SOX2 silencing in normal mouse ESCs causes a very high percentage of these cells to exit from the cell cycle and engage in the differentiation pathway, showing increased expression of neuronal marker genes despite culture conditions that are not permissive for differentiation of these cells [75]. Taken together, these findings suggest that SOX2 can function as a single factor for generating induced NSCs (iNSCs) from somatic cells and plays a key role in establishment and maintenance of neural progenitor cell properties.

In the adult brain, on the other hand, SOX2 is expressed to almost undetectable levels [61]. Additional studies further confirmed this by

demonstrating that SOX2 expression is negligible in neurons and glial cells of the adult brain [76]. In addition, low expression of SOX2 has been observed in some glial cells in the thalamus, hippocampus and brain stem, and this is thought to be due to incomplete differentiation of the cells in these regions. SOX2 expression has also been detected in astroglial cells of the adult rat brain and although it is difficult to establish the exact identity of these SOX2-positive astrocytes, some groups have suggested that Sox2-positive astrocytes are in fact 'neurogenic' as they reside within a 'neurogenic' niche [77]. Interestingly, these SOX2-positive astrocytes were found to resume proliferation after rat brains were subjected to injury and this is in line with the positive effect of SOX2 on cell proliferation [76].

• Role of Sox2 in brain tumors

In addition to its role in regulating pluripotency in NSC/NPCs, SOX2 expression and function is deregulated in a large number of benign and malignant tumors, especially in cancer stem cells with self-renewal and tumor-initiating properties [78,79]. This is not surprising, as normal and cancer stem cells share several features including rapid proliferation, the capacity to differentiate into multiple lineage-specific cells [80], in addition to a large overlap in their gene expression profiles [81]. *SOX2* gene is amplified in a number of human cancers including prostate cancer, lung, esophageal squamous cell carcinoma and small-cell lung cancer [82–85]. In squamous cell carcinoma, recurrent gene amplifications occur in the 3q26.3 chromosomal region in which the *SOX2* gene is also located and results in overexpression of SOX2. Therefore, SOX2 is considered an oncogene in this context and a key factor in development of lung squamous cell carcinoma, by regulating the expression of many genes that promote tumor progression [83]. In addition to gene amplification, overexpression of SOX2 has been reported in a number of human tumors including GBM, other glial tumors [19,86], breast cancer [87] and many more (Table 1). These findings suggest that SOX2 has an oncogenic role in tumors and its increased expression is generally associated with higher proliferation, invasiveness and survival of cancer cells, which in turn leads to poor prognosis of the corresponding tumor. In specific cancer types such as gastric cancer, SOX2 is downregulated and functions as a tumor suppressor, inhibiting

Table 1. Regulation and functional role of Sox2 in various cancer types.

Cancer type	Type of modification	Biological function	Ref.
Breast cancer	Altered expression, subcellular distribution, proteasomal degradation	Regulates cell proliferation, colony formation, invasion, metastasis	[27,87]
Esophageal squamous cell cancer	Gene amplification	Regulates tumor growth and proliferation of esophageal squamous cell carcinomas, promotes epithelial to mesenchymal transition	[82]
Gastric cancer	Decreased expression	Triggers apoptosis, inhibits cell proliferation and migration	[59,88]
Glioblastoma	Gene amplification, overexpression, promoter hypomethylation	Regulates invasion, migration, cell proliferation and colony formation	[19,21,53,75,76,114]
Lung squamous cell carcinoma	Gene amplification	Regulates tumor progression	[82,83]
Medulloblastoma	Gene amplification	Regulates stemness but not tumorigenicity	[127]
Oligodendroglioma	Gene amplification, overexpression	Regulates stemness and tumorigenicity	[139]

the proliferation and inducing apoptosis of gastric epithelial cells [88].

Cerebral tumors account for about 2% of all cancer types, with gliomas being the most frequent cerebral neoplasm, showing incidents of approximately 86% among all brain tumors. Gliomas are divided into four clinical grades (I–IV) with GBM (grade IV) being the most common and lethal primary CNS tumor, resulting in median survival of 12–15 months despite combination of cytoreductive surgery, chemotherapy and radiation [75,89–91]. In tumors of the CNS, SOX2 expression has been detected in astroglial, oligodendroglial and ependymal, but not neuronal lineages and correlates negatively with the ability of cancer cells to differentiate [86]. Increased SOX2 protein and mRNA levels have also been detected in neurospheres derived from pediatric tumors [86,92,93] and it is expressed together with NESTIN, MUSASHI-1 and CD133 stemness markers in neurospheres derived from gliomas [94] and gliomatosis cerebri [95]. Additionally, SOX2 expression correlates positively with the malignancy grade in brain tumors [26,61,86] and elevated levels of SOX2 have been shown to correlate with poor clinical outcome in brain tumor patients [96]. In general, cancer patients with higher stem cell gene expression profile, including higher SOX2 level, suffer from worse prognosis and decreased overall survival compared with patients who do not [97–99].

In the recent years, extensive investigations have been performed in order to understand the mechanism by which essential stem cell-associated transcription factors – such as SOX2, OCT4 and NANOG – exert their effects in cancer stem cells. For this purpose, significant efforts have been made to map the protein–protein

interaction landscapes of these transcription factors in ESCs [100–104]. The results from these investigations suggest that these pluripotency-associated transcription factors are part of a highly integrated protein–protein interaction network that is composed of many other transcription factors, proteins associated with chromatin remodeling and DNA repair, in addition to a number of RNA-binding proteins [105,106]. Moreover, these results suggest a role for SOX2 in post-transcriptional regulation of gene expression through its association with RNA-binding proteins [107] and also as a putative RNA splicing factor [108]. Given the highly diverse array of proteins that interact with SOX2, it is likely that such large-scale proteomic studies on SOX2 in brain tumors could help identify additional proteins that influence the growth of these tumors. Further experiments will also be necessary to clarify the mechanisms of these interactions and their functional significance in the development and progression of tumors.

• SOX2 in glioblastoma

SOX2 is expressed in all gliomas, and the proportion of SOX2-positive cells – ranging from 6–80% of cells in the tumor – correlates with the malignancy grade [75]. In GBM, SOX2 is intensely expressed in the most malignant component of the tumor and in highly proliferating cells of oligodendrogliomas. The gene encoding Sox2 is also amplified in approximately 14.4 and 11.1% of GBM and anaplastic oligodendrogliomas [76], respectively, compared with EGFR amplification in 36–40% [109] and loss of PTEN due to loss of heterozygosity in 60–80% of GBM cases [110]. In malignant glioma samples, the intense positive staining for SOX2 overlaps with Ki67/MIB.1-positive nuclei, which is

a gold standard marker for proliferating cells. In addition, tumor regions showing intense SOX2 staining also show frequent amplification of the *SOX2* gene. In cultured neurospheres, *SOX2* gene is often amplified, and therefore, the hypothesis that a genetic correlation exists between neurospheres and the most anaplastic regions in glioma is validated by the expression pattern of *SOX2* [76].

The extent of *SOX2* expression is also concordant with the degree of heterogeneity observed in the cell population found in gliomas, which appear in various stages of differentiation. Gangemi *et al.* demonstrated that suppression of *SOX2* expression in GBM tumor-initiating cells prevented their proliferation and reduced their tumorigenicity in long-term culture conditions and no short-term obvious effects on apoptosis, cell senescence or increased differentiation was detected. They also found that downregulation of *SOX2* using siRNA targeting the 3' UTR of *SOX2* mRNA in GBM cell lines results in reduced Ki67 expression in these cells. This effect was independent of defects in progression through the cell cycle and more likely due to loss of the ability of GBM cells to divide indefinitely, hence their reduced stemness. Reduced proliferation of these cells eventually leads to their premature exit from the cell cycle and eventual disappearance from the culture [75].

Annovazzi *et al.* conducted a study in order to evaluate *SOX2* expression, distribution and gene copy number status in normal nervous tissue, and in a number of neuroepithelial tumors and cell lines derived from primary GBM tumors by immunohistochemistry, western blotting and other molecular biology techniques. Consistent with other reports, they found that *SOX2* expression was absent in neuronal tumors such as neurocytomas and neuronally differentiated medulloblastomas. Interestingly, they demonstrated very intense *SOX2* nuclear staining within hypercellular regions with high vascular density in highly invasive areas of GBM tumors [76,86]. Consistent with other reports, they also observed a correlation between expression of *SOX2*, Ki 67/MIB.1 and *NESTIN*. These regions are thought to contain NSCs or to give rise to cells exhibiting many of the features of cancer stem cells [76,111]. These hypercellular regions in GBM may also correspond to regions composed of the highest number of dedifferentiated tumor cells, which arise as a consequence of accumulation of mutations or epigenetic events and are

characterized by expression of other stemness-associated factors such as *NESTIN*, *CD133* and *MUSASHI-1* [26,94,112]. In this regard, it is possible that *SOX2* creates a permissive environment for induction of pluripotency and tumor development [82,113].

SOX2 is thought to regulate the expression of several genes at transcriptional level (Table 2), some of which are other members of the *SOX* family of transcription factors such as *SOX1*, in addition to other proteins that regulate stem-cell fate [114,115]. In order to investigate the *SOX2* target genes specifically in GBM, Fang *et al.* performed an integrated genomic analysis using chromatin immunoprecipitation-seq, microarray profiling and miRNA sequencing in LN229 GBM cells and identified 4883 regions within the genome of these cells that bound to *SOX2* through the consensus sequence wWTGnWTw. Gene expression analysis indicated that 489 of the corresponding genes showed reduced expression upon *SOX2* silencing, some of which belong to the *SOX* family of transcription factors, in addition to two genes – brain expressed X-linked 1 and 2; *BEX1* and *BEX2* – with known tumor suppressor activity in GBM [107]. In a separate study, the same authors demonstrated that downregulation of *SOX2* results in reduced expression of multiple miRNAs, including miR-143, -145, -253-5p and -452 [114]. Their findings suggest that *SOX2* and miR-145 form a double-negative feedback regulatory loop in GBM cells, and previous reports have indicated that miR-145 downregulates *OCT4*, *SOX2* and *KLF4*, and thereby, suppresses pluripotency in human ESCs [116].

• *SOX2* in medulloblastoma

Medulloblastoma is the most common high-grade pediatric brain neoplasm [117,118], and current treatment modalities cause dramatic impairment of cognitive function, predisposing the patients to future treatment-associated types of cancer and immune dysfunction [119,120]. Prognosis for medulloblastoma patients is very poor (70–80%; 5-year survival), and despite the recent developments in therapeutic interventions, medulloblastoma is still considered one of the most life-threatening cancers in the world [121–123]. Therefore, there is a pressing need to identify novel proteins and signaling pathways that can serve as new targets for treatment of medulloblastoma. In medulloblastoma, similar to many other neural tumors, *SOX2*

Table 2. Proposed downstream Sox2 target genes in glioblastoma.

Target gene	Function	Ref.
<i>SOX1</i>	Key regulator of neuronal cell fate determination and differentiation	[114,115]
<i>SOX18</i>	Plays a role in the development of vasculature during embryogenesis	[114]
<i>BEX-1</i>	Involved in apoptosis; a tumor suppressor that is silenced in malignant gliomas	[114]
<i>ACIN1</i>	A nuclear protein that induces apoptotic chromatin condensation after activation by caspase-3	[114]
<i>BMPRI1B</i>	A member of the bone morphogenic protein receptor family of transmembrane serine/threonine kinases involved in endochondral bone formation and embryogenesis	[114]
<i>ETS1</i>	Transcriptional activators or repressors depending on many genes; involved in stem cell development, senescence and death as well as tumorigenesis	[114]
<i>SHH</i>	Involved in embryogenesis and a signaling pathway that regulates multiple cellular processes	[114]
<i>IGFBP3</i>	Prolongs half-life of insulin-like growth factors and alters their interaction with cell-surface receptors; methylation status of this gene is strongly associated with effectiveness of chemotherapy	[114]
<i>RUNX1</i>	Transcription factor involved in the development of normal hematopoiesis	[114]
<i>CDC20</i>	A regulatory protein involved in the cell cycle	[114]
<i>FGF13</i>	Involved in many processes, including embryonic development, cell growth, tissue repair, invasion and tumorigenesis	[114]
<i>UTF1</i>	A transcriptional coactivator involved in embryonic developmental timing	[114]

is expressed and its levels correlate directly with stemness properties of the tumor cells, and therefore, SOX2 is used as a marker for stemness [113,124,125]. Medulloblastomas are classified into several subgroups including the Shh and the WNT subgroup, depending on their RNA expression profiles [126]. SOX2 expression is generally higher in the Shh group compared with the other medulloblastoma subgroups [127]; and as observed in multiple tumor types, the stemness factors SOX2 and NESTIN are important in the development and progression of medulloblastomas [97,125].

Vanner *et al.* showed that relapse of medulloblastoma correlates closely with the percentage of SOX2-positive cells in the tumor tissue. They also found that SOX2-expressing cells are a quiescent population and progress slowly through the cell cycle. Upon treatment with antimetabolic and Shh-targeted therapy, however, the proportion of SOX2-positive cells increases significantly, suggesting that Sox2-positive population may play a role in resistance to therapy and may be responsible for tumor recurrence. Therefore, SOX2-positive medulloblastoma cells can be considered as medulloblastoma-propagating cells, which is further supported by their ability to self-renew and differentiate into medulloblastoma tumors *in vivo*. Additionally, gene expression profiling of SOX2-positive medulloblastoma cells indicated that these cells express

other genes, such as *GFAP*, *OLIG1*, *OLIG2*, *BLBP* and *PDGFRA*, which are also markers of NSCs. In Sox2-negative cells, on the other hand, a more differentiated gene expression profile was observed, with significantly higher expression of neuronal lineage-associated genes, such as *PAX6*, *ATOH1*, *DCX*, *RBFOX3* (NeuN) and *ZIC2* [97].

In an elegant study using the medulloblastoma cell line DAOY, Cox *et al.* employed a multidimensional protein identification technology (MudPIT) and identified 283 SOX2-interacting proteins, the majority of which are transcription factors. In the same experiment, analysis of SOX2 interactome in a GBM cell line resulted in identification of 144 interacting proteins; however, the number of proteins that overlapped between GBM and medulloblastoma cell lines was only two. They also demonstrated that knockdown of SOX2, consistent with observations in other tumor types, reduced the ability of DAOY cells to proliferate [128]. Two of the SOX2-interacting proteins – namely MSI2 and USP9X – have recently been shown to regulate cell proliferation in other cancer types [129–131] and they were also found to be necessary for the growth and survival of DAOY cells. Furthermore, overexpression of Sox2 impaired the ability of both CD133⁺ and CD133[−] DAOY cells to form neurospheres. Surprisingly, the authors also found

that elevated expression of SOX2 in DAOY cells caused them to undergo differentiation and show reduced expression of pluripotency-associated marker genes [128]. These findings suggest that SOX2 expression must be maintained at a specific level and its overexpression leads to defects in the ability of cancer stem cells to proliferate and differentiate.

Conclusion & future perspective

Brain tumors are among the most lethal malignancies, with GBM being the most common and aggressive form [75,89–91]. Medulloblastoma is the most common pediatric CNS tumor with a median survival of up to 5 years, and current therapies against this tumor type result in significant comorbidities [117,118]. These tumors are also highly resistant to radiotherapy and chemotherapeutic agents, which are widely used for treatment of other cancer types [97,132–134]. Thus, more effective therapeutic approaches need to be developed to improve patients' survival and quality of life.

The cancer stem-like cell hypothesis suggests that these cells are responsible for self-renewal, resistance to therapy and tumor recurrence [135]. It has been shown that after aggressive treatment of brain tumors with conventional therapies, the proportion of these stem-like tumor-propagating cells increases in the heterogenic tumor cell population [97,136]. Therefore, critical signaling pathways and factors regulating cancer stem cell properties that are only expressed in these cells should be investigated for targeted therapy. Glioma stem cells are also highly resistant to current treatment modalities, and are thought to contribute to recurrence of gliomas [132,133]. Therefore, the efficiency of conventional chemotherapy and radiation can be improved by directly targeting glioma stem cells to eliminate this small population of treatment-resistant cells. Thus, SOX2, as the master regulator of pluripotency and maintenance of tumor stem cell properties, is a highly attractive molecular target for treatment of GBM and medulloblastoma tumors [97,137]. Downregulation of SOX2 in glioma stem cells was shown to reduce sphere formation efficiency, inhibit cell proliferation and induce cell death. Furthermore, downregulation of SOX2 increased the sensitivity of these cells to imatinib and NVP-AEW541 [137]. Therefore, silencing SOX2 in GBM may affect proliferation and tumorigenicity. Taken together, these

findings underscore the importance of SOX2 and its immediate downstream effectors as attractive therapeutic targets for GBM (Table 2), some of which have been used to target skin squamous cell carcinoma cells [125] and targeting SOX2 has shown great potential for treatment of breast cancer [138]. Targeting SOX2-positive cells in Shh group medulloblastoma has also been shown to improve patient outcome [97]. In a screen to search for agents to which medulloblastoma cells are sensitive, Vaner *et al.* discovered mithramycin as one of the best chemicals, as it targets SOX2 and crosses the blood–brain barrier, and this ability is critical for targeting brain neoplasms. Therefore, it is likely that mithramycin and other similar drugs that target SOX2-positive cells may present an effective therapeutic approach for Shh group medulloblastomas and help to prevent tumor recurrence [97].

SOX2 has also been evaluated for targeting as an antigen for T-cell-based immunotherapy in gliomas [61]. An approach that has been suggested for SOX2 immunotherapy is vaccination and a recent study demonstrated that oncoproteins could be targeted with this approach as some intracellular antigens are presented on the surface of cancer cells. Favaro *et al.* concluded that targeting SOX2 in combination with a selected number of its downstream target genes could be an efficient way to oppose development of oligodendrogliomas, and this approach can be tested in other similar tumor types [139]. Another approach for targeting SOX2 is post-transcriptional suppression of its expression by overexpressing miRNAs – such as miR-126 and miR-145 – that specifically target the 3' UTR of SOX2 mRNA [53,55,140]. Although these therapeutic approaches appear very attractive and serve as logical targets for treatment of brain neoplasms, more effective techniques will be needed to be developed for delivery of any newly identified therapy.

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