Self-renewal signaling pathways and differentiation therapies of glioblastoma stem cells (Review)

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Abstract. Glioblastoma multiforme (GBM) is a primary brain tumor with a high mortality rate and a median survival time of ~14 months from the initial diagnosis. Although progress has been made in the currently available therapies, the treatment of GBM remains palliative. GBM contains subsets of GBM stem cells (GSCs) that share numerous neural stem/progenitor cell characteristics, such as expression of stem cell markers, self-renewal and multi-lineage differentiation capacity, thus contributing to the heterogeneity and complexity of these tumors. GSCs are potentially associated with tumor initiation and they are considered as the driving force behind tumor formation, as they possess tumor-propagating potential and exhibit preferential resistance to radiotherapy and chemotherapy. Targeting self-renewal signaling pathways in cancer stem cells may effectively reduce tumor recurrence and significantly improve prognosis. The aim of the present review was to summarize the current knowledge on the self-renewal signaling pathways of GSCs and discuss potential future targeting strategies for the design of differentiation therapies.

Contents

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1. Introduction

Gliomas are the most common primary malignant tumors in the brain. Glioblastoma multiforme (GBM), also known as World Health Organization grade IV glioma, is a disease with a high mortality rate worldwide, for which there is currently no effective therapy (1). The current standard treatments for GBM include maximal surgical resection, radiotherapy and temozolomide chemotherapy. However, GBM tends to recur despite therapy, with a recurrence rate as high as 90%. The median overall survival is 15-18 months according to population-based studies, and <10% of patients remain alive at 5 years post-diagnosis (2).

GBM is a heterogeneous tumor that is characterized by high resistance to therapy, which is promoted by GBM stem cells (GSCs) (1). Cancer stem cells constitute a small cell population that is highly involved in the malignant behavior of numerous types of cancer (3), and they are defined by their functionality, including maintenance of stemness, tumor formation, tumor relapse and resistance to therapy (4). GBM follows a cellular hierarchy model, with GSCs at the top and differentiated offspring cells at the bottom of the model (5,6).

There are significant similarities between neural stem/progenitor cells (NSCs) and GSCs, such as the expression of stem cell markers (CD133, SOX2, oligodendrocyte transcription factor 2 and nestin), and the ability to differentiate into oligodendrocytes, astrocytes and neurons. However, GSCs are characterized by the expression of multiple lineage markers in one differentiated cell (7,8). GSCs harbor genetic abnormalities, which contribute to tumor invasion (9), angiogenesis (10) and radio-resistance (11). The main characteristics of GSCs are their capacity for self-renewal and differentiation (3). In a xenograft assay, GSCs displayed greater tumorigenic capacity compared with non-stem tumor cells (6). GSCs play a pivotal role in the growth and therapeutic resistance of adult human GBM (7), suggesting that GSCs may lead to tumor recurrence and, eventually, death. Thus, exploring the signaling pathways regulating GSC self-renewal and the design of therapies targeting these signaling pathways are important research objectives. In recent years, numerous different signaling pathways and potential therapeutic targets for GBM have been identified, including TGF-β, Notch, Wnt, Hedgehog (HH) and STAT3, which also play important roles in normal stem cell development and differentiation. The focus of the present

review was these five key self-renewal GSC signaling pathways and the corresponding differentiation therapies, with the aim of providing novel insight and promoting advances in the clinical therapy of GBM.

2. TGF-β signaling pathway

The TGF-β family includes polypeptides that regulate GSC maintenance and tumor differentiation (12,13). In addition, TGF-β signaling is involved in carcinogenesis and tumor development (12).

The TGF-β family comprises TGF-βs, bone morphogenetic proteins (BMPs) and other associated proteins (14). The TGF-β signaling cascade is a linear pathway from type II to type I receptor kinase to SMAD activation, resulting in the transcription of target genes in the cell nucleus. On the cell surface, TGF-β ligands bind to the transmembrane receptor serine/threonine kinase (type I and II) complex, and then type II receptor kinases [BMP receptor (BMPR)II, activin receptor (ActR)II, ActRIIB, TGF-β receptor (TβR)II and anti-Müllerian hormone receptor] and trans-phosphorylate type I receptors [anaplastic lymphoma kinase (ALK); ALK5/1/2 for TβR and ALK2/3/6 for BMPR]. The consequently activated type I receptors trigger phosphorylation of SMAD and receptor-regulated SMAD (R-SMAD) (TGF-β, R-SMAD2/3/1/5; BMP, R-SMAD1/5/8), which then form a complex with common mediator SMAD4 (Co-SMAD). The activated R-SMAD/Co-SMAD complex translocates to the nucleus to regulate gene transcription. The activation of R-SMAD is inhibited by SMAD6 or SMAD7 (14,15). It was previously demonstrated that TGF-β signaling regulates cell fate (16), and that blockade of this signaling pathway can inhibit proliferation of cancer cells and the GSC subpopulation (17). Thus, targeting the TGF-β pathway may be a meaningful treatment for GBM. The current clinical trials on drugs targeting the TGF-β signaling pathway are summarized in Table I.

BMPs. BMPs comprise a subfamily of the TGF-β superfamily, and they are secreted signaling molecules that regulate embryonic development (18). BMPRs, acting as paracrine tumor suppressors, have a flexible oligomerization pattern, which allows a greater variety of responses to ligands (19). BMPs and growth differentiation factors (GDFs) form a cystine-knot cytokine family, which shares the characteristics of the TGF-β superfamily. GDFs are extracellular factors containing a potential signaling sequence for secretion and a proteolytic processing site (20,21). BMP/GDFs exist as homodimers and heterodimers, and interact with complexes of type I and type II receptor dimers, leading to the activation of one of two competing sets of R-SMAD (22).

BMPs can cause a significant reduction in stem cell numbers in GBM. BMPs induce GSC differentiation, attenuate the expression of stemness markers, reduce self-renewal and block tumor initiation (23-25). Thus, BMPs have been proposed as potential differentiation therapies targeting GSCs, which may be used to prevent GBM growth and recurrence (26).

Gremlin 1. BMPs can influence astrocyte fate and induce loss of tumorigenicity, and they are considered as a GSC differentiation targeted therapy; however, paradoxically, tumors express high levels of BMPs (24,27). Gremlin, a protein of 184 amino acids, contains a highly conserved cysteine knot domain shared by the TGF-β superfamily (28). The antagonist gremlin 1 has been demonstrated to be specifically expressed by GSCs to protect against endogenous BMPs. Gremlin 1 blocks the differentiation effects of BMPs on GSCs and promotes the maintenance of cancer cell stemness, thereby increasing tumor formation ability. Targeting gremlin 1 results in impaired cell proliferation and self-renewal. Mechanistically, gremlin 1 mediates the downregulation of the cyclin-dependent kinase inhibitor p21WAF1/CIP1, a key GSC signaling node (29). Thus, inhibition of gremlin 1 may act synergistically with BMPs in GBM treatment. One therapy option is to engineer a BMP variant that does not bind to gremlin 1 (30). Another option is combined therapy of antibodies against gremlin 1 alongside BMP-based therapy.

TGF-β. Previous studies have demonstrated that TGF-β activity is present in aggressive and highly proliferative gliomas (31). TGF-β has been shown to induce self-renewal capacity and prevent differentiation in GSCs. Furthermore, TGF-β may play a role in GSC-mediated oncogenesis via leukemia inhibitory factor induction *in vivo* (32). Thus, blocking TGF-β signaling in GBM may be of therapeutic value.

Snail family transcriptional repressor 1 (Snail). Snail increases GBM cell proliferation and invasiveness (33). However, Snail has been shown to abolish sphere formation and tumor growth in glioma (34). It has been hypothesized that the signaling pathway through which Snail impairs self-renewal, represses stemness and promotes differentiation of GSCs involves the Snail-mediated control of the activities of the TGF-β pathway at the transcriptional level. Snail interacts with SMAD and represses TGFB1 gene expression to decrease TGF-β1 signaling activity and suppress GBM tumorigenesis (35). Thus, Snail may be a key player in TGF-β-targeted therapies.

Heme oxygenase 1 (HMOX1). The expression of HMOX1, one of the cell surface transmembrane proteins, is increased in GBM, and a high expression level is associated with increased stemness and poor prognosis in GBM (36). HMOX1 regulates differentiation through the TGF-β signaling pathway (37). Specifically, TGF-β regulates HMOX1 expression on the cell surface, and endogenous activators (such as EGFR) and inhibitors (such as PTEN) of TGF-β signaling may also interfere with the expression of HMOX1. These findings indicate that targeting HMOX1 may be a novel therapeutic approach to GBM.

Four-and-a-half LIM domains 3 (FHL3). The expression of FHL3 is downregulated in glioma (38). FHL3 is the negative target gene of poly(C)-binding protein 2 (PCBP2); knockdown of PCBP2 enhances the expression of FHL3, whereas overexpression of FHL3 attenuates cell proliferation and induces apoptosis (38). FHL3 exerts an anti-proliferative effect on GSCs and suppresses their stemness. FHL3 can inhibit the transcriptional activity of SOX4 by recruiting protein phosphatase $Mg2^{+}/Mn2^{+}$ dependent 1A, which downregulates SOX2 expression to suppress GSC tumor sphere formation

Table I. Clinical trials on compounds that target the TGF-β pathway.

Compound	Country	Design	(Refs.)
TGF-α-PE38 immunotoxin (biological)	USA	TP-38 toxin for the treatment of young patients with recurrent or progressive supratentorial high-grade glioma	(124,125)
AP12009	Austria	Phase IIb clinical trial with TGF-β2 antisense compound AP12009 for recurrent or refractory high-grade glioma	(126)
LY2157299	USA	A study combining LY2157299 with temozolomide-based radiochemotherapy in patients with newly diagnosed malignant glioma	(127-129)
GC1008 TGF-β (neutralizing antibody)	The Netherlands	Safety and imaging study of GC1008 in glioma	(133)
AP12009	USA	Efficacy and safety of AP12009 in patients with recurrent or refractory anaplastic astrocytoma or secondary glioblastoma	(126)

and self-renewal, and promotes differentiation (39,40). Thus, FHL3 plays a key role in suppressing stemness by regulating the SMAD2/3/SOX4/SOX2 pathway in glioma.

3. Notch signaling pathway

Notch proteins (Notch 1-4) are transmembrane receptors that mediate cell-cell signaling. Notch signaling can amplify and consolidate molecular differences, eventually dictating cell growth, proliferation, survival and differentiation. Notch activity affects cell differentiation, proliferation and apoptotic programs (41). Both the receptors and ligands of the Notch family are cell surface type I transmembrane proteins. Notch ligands include delta (Dl) and Serrate. Upon ligand binding, Notch receptors undergo three proteolytic cleavages. The first cleavage, S1, generates fragments and forms a heterodimeric receptor, which is inserted in the cell membrane (42). S2 occurs after the heterodimers bind to the ligand (Dl-like-1, -3 and -4, and Jagged-1 and -2). S3 is mediated by the γ-secretase complex, leading to the release of Notch intracellular domain (NICD) into the nucleus (43).

The Notch signaling pathway, including NICD, hairy/enhancer-of-split (Hes)1 and Hes related family basic helix-loop-helix (bHLH) transcription factor with YRPW motif 1 (Hey1), regulates cell stemness and differentiation. Activation of the Notch receptor rapidly inhibits the death of NSCs (44). Inhibitors and activators targeting Notch receptors and ligands that exert antitumor effects have been developed. Notch stimulation results in poorly infiltrative but highly vascularized grafts, in contrast to the highly infiltrative and poorly vascularized characteristics of GBM stem cells. This indicates that the Notch pathway is crucial for regulating GSC fate (45).

During the early stages of embryogenesis, Notch signaling serves as a critical quality control pathway to prevent premature neurogenesis and maintain pools of progenitor cells in the developing central nervous system. In the perinatal stages, Notch signaling increases progenitor cell proliferation and drives astrocyte differentiation, thereby serving a critical function in human brain development. The Notch pathway is involved in maintaining adult neural stem cells bivalently by promoting self-renewal and repressing differentiation (46,47). The activity of the Notch signaling pathway plays an instrumental role in regulating self-renewal and determining cell fate of normal NSCs (48). The Notch pathway is active in NSCs during neurogenesis, gliogenesis and tumorigenesis (49,50). It has been demonstrated that the Notch target genes Hes1 and Hes5 are strongly associated with the regulation of neurogenesis and gliomagenesis in the brain (51).

The self-renewal capacity of GSCs relies on the activity of the Notch signaling pathway. The expression level of the Notch receptor gene and its downstream activation cascade of events are associated with the phenotypic plasticity and intratumor heterogeneity of GBM cells (49). A previous study that used computational modeling methods demonstrated that the stem cell renewal induced by the Notch pathway and the antagonistic effects exerted on the p53 pathway are highly involved in maintaining the regenerative properties of the NSCs (49,52). In agreement with this, previous *in vitro* and *in vivo* studies on glioma cell lines have indicated that CD133-positive GSCs are particularly sensitive to γ-secretase inhibitors or Notch1/2 knockdown compared with CD133-negative glioma cells (53). Blocking Notch signaling or recombination signal-binding protein for immunoglobulin κJ region (RBP-κJ), which is a major transcriptional effector of this pathway, reduced clonogenicity potential in tumor sphere assays and engraftment capacity in glioma xenograft models (48). Notch activity may contribute to intratumor heterogeneity by promoting stem cell behavior in poorly differentiated subpopulations of glioma cells. Notch signaling potentially regulates multiple steps of gliomagenesis, including tumor initiation, progression and recurrence.

However, the actual sequence of regulating events and the exact mechanisms through which Notch activity controls stemness and tumorigenicity remain to be elucidated. Since Notch can promote and maintain the stem cell characteristics of brain tumors, it may represent a promising target for developing more effective therapies against glioma. A phase I clinical trial investigating the use of γ-secretase/Notch inhibition in combination with temozolomide and radiotherapy in newly diagnosed GBM or anaplastic astrocytoma demonstrated that

Table II. Clinical trials on drugs that directly target the Notch signaling pathway.

Drug	Country	Design	(Refs.)
RO4929097	USA	RO4929097, temozolomide and radiation therapy for the treatment of patients with newly diagnosed malignant glioma	(131)
RO4929097	Canada	RO4929097 for the treatment of patients with recurrent invasive gliomas	(130)
RO4929097	US	RO4929097 and bevacizumab for the treatment of patients with progressive or recurrent malignant glioma	(54,130)
RO4929097	USA	Gamma-secretase/Notch signaling pathway inhibitor RO4929097 for the treatment of patients with recurrent or progressive glioblastoma	(130)
RO4929097	USA	Gamma-secretase inhibitor RO4929097 for the treatment of young patients with relapsed or refractory solid tumors, central nervous system tumors, lymphoma, or T-cell leukemia	(132)

the addition of Notch inhibition to standard treatment was associated with certain benefits (Table II) (54), although it also clearly demonstrated that Notch inhibition, alone or combined with radiation and chemotherapy, may be insufficient for fully controlling tumor progression. However, those findings indicated that Notch may serve as a targeted biological tool that counteracts tumor stem cell-like behavior by preventing self-renewal and, possibly, angiogenesis (55).

NICD (active NOTCH). NICD regulates transcription in the cell nucleus, and is directly involved in transcriptional control by associating with the DNA-binding protein CBF1, Suppressor of Hairless, Lag-1 (also known as RBP-κJ) (48). It was previously reported that Notch1 is overexpressed in GSCs (41,45). Enhancing the protein expression and nuclear transport of NICD may upregulate Notch signaling. The canonical importin α/β pathway, which targets proteins to the nuclear pore complex and facilitates their translocation across the nuclear envelope (56), can regulate the transport of NICD into the nucleus, thus being directly involved in the Notch signaling pathway (57).

Tripartite motif-containing protein (TRIM)3. TRIM3 gene and protein expression levels are markedly reduced in GBM (58). TRIM3 expression was demonstrated to attenuate stem cell marker expression, reduce neurosphere formation and lead to an increased percentage of cells that divide asymmetrically in GBM (58). These effects of TRIM3 are mediated by downregulation of Notch signaling. In human GBM, TRIM3 suppresses Notch1 signaling, attenuates cell stemness and suppresses tumor growth. The molecular mechanism underlying the suppression of the nuclear transport of NICD involves the direct binding of TRIM3 to the importin complex α and β to reduce the nuclear import of NICD (59).

C-X-C motif chemokine ligand (CXCL)12/C-X-C chemokine receptor (CXCR)4. CXCR4 is a cell surface chemokine receptor that is closely associated with glioma growth. It is overexpressed in GSCs and plays a critical role in regulating carcinogenesis (60). CXCL12, which is a CXCR4-stimulating factor, was highly expressed in glioma cells. Blockade of the CXCL12/CXCR4 signaling axis induces apoptosis and inhibits cell cycle progression, thus promoting the survival of GBM cells (61). In GBM, Notch1 and CXCR4 are enriched in GSCs, and are co-expressed with stemness markers (41,45,60). Blocking the Notch1 signaling pathway may suppress the proliferation of GSCs, and this effect may be reversed by upregulation of CXCL12. In addition, Notch1 could directly enhance the transcription of CXCR4 (62). Decreasing Notch1 expression levels may downregulate CXCR4 expression, leading to the inhibition of the PI3K/AKT/mTOR signaling pathway, and attenuation of the ability of GSC self-renewal and GBM growth (62). Therefore, investigating the crosstalk between Notch1 and the CXCL12/CXCR4 axis may uncover more effective therapies for Notch1-targeted treatment of GBM.

Hes. The Hes family comprises bHLH-type transcriptional repressors that negatively regulate the expression of downstream target genes (such as tissue-specific transcription factors). In the nucleus, NICD associates with the nuclear proteins of the RBP-κJ family and activates the transcription of primary target genes of the Notch signaling pathway, such as Hes1-7 (63). Members of the Hes family are the best characterized transcriptional targets of Notch signaling, and negatively regulate downstream target gene expression. Thus, Hes directly affects cell differentiation (63).

Cytoplasmic polyadenylation element-binding protein 1 (CPEB1). CPEB1 is a highly conserved RNA-binding protein that specifically binds to CPE, which is indirectly involved in translational repression and activation. Previous studies demonstrated that CPEB1 could reduce sphere formation ability, downregulate the expression of stemness markers and

control cell differentiation in GSCs, and it was positively associated with the overall survival of patients with glioma (64,65). The detailed molecular mechanism of action of CPEB1 is by specifically suppressing the translation of Hes1, inducing the differentiation of GSCs at the post-transcriptional level. Thus, CPEB1 is as a critical factor involved in the Notch signaling pathway and may provide novel approaches to GSC differentiation therapy.

Hey. Hey [also known as Hes-related repressor protein (Herp), and Hey/Hesr/Hrt/CHF/gridlock], is a member of the bHLH protein family and is associated with Hes. Hey expression is directly upregulated by Notch ligand binding and has intrinsic transcriptional repression activity (63). Hes and Hey form a stable heterodimer that has DNA-binding and transcription-suppressive activities, thus regulating Notch signaling and target gene expression (66,67).

4. Wnt signaling pathway

The canonical Wnt/β-catenin pathway is a highly evolutionarily conserved signaling pathway that regulates pluripotency in stem cells (68). Frizzled proteins are receptors involved in Wnt signaling (69) that interact as co-receptors with Arrow, a low-density lipoprotein receptor-related protein that forms part of a receptor complex with Frizzled protein (70). When the Wnt ligand is activated by binding to the co-receptors, the AKT/glycogen synthase kinase (GSK)3β/adenomatous polyposis coli (APC) complex separates. The AKT/GSK3β/APC complex promotes the degradation of β-catenin, an intracellular signaling molecule. APC antagonizes the Wnt signaling pathway directly at the β-catenin effector level in several different ways: It acts as an adaptor between β-catenin and C-terminal binding protein, removes β-catenin, abrogates transcriptional transactivation, and inhibits the binding of the lymphoid enhancer-binding factor (LEF)/T-cell factor (TCF) proteins to β-catenin (71,72). Therefore, through the degradation of the AKT/GSK3β/APC complex, intracytoplasmic β-catenin becomes stable, and non-phosphorylated β-catenin accumulates in the cytoplasm and translocates to the nucleus to facilitate the transcription of target genes by interacting with the TCF and LEF transcription factors (71). The XTC-3 transcription factor mediates β-catenin-induced axis formation in Xenopus embryos. Functional interaction of β-catenin with the transcription factor LEF-1 leads to the nuclear localization of β-catenin (73-75). Wnt plays a key role in maintaining stemness in GBM cells (76,77). Thus, abnormal activation of the Wnt pathway may promote GSC self-renewal.

AKT/GSK3β. AKT is a significant driver in GBM (78). The AKT/GSK3β complex regulates the transport of β-catenin into the nucleus (71), and the separation of the complex is controlled by Wnt signaling ligands. The AKT2/GSK3β pathway generally promotes GBM cell proliferation and survival, and contributes to GSC maintenance.

Astrocyte elevated gene-1 (AEG1). The architectural transcription factor LEF-1 interacts with β-catenin (thus forming a localized complex in the nucleus), and regulates transcriptional activation and tumor growth. The complex forms a ternary complex with DNA that displays an altered DNA bend (74,79,80). AEG-1 is an oncogene that is upregulated in GBM, which plays a key role in cancer cell metastasis and regulates tumorigenesis (81). In GBM cells, the internal domain of AEG-1 directly interacts with the pleckstrin homology domain of AKT2, thus contributing to tumor cell survival and proliferation (82). It has been reported that the expression level of AEG-1 is strongly associated with the presence of stemness markers in GBM. AEG-1 promotes the translocation of β-catenin into the nucleus by forming a complex with LEF1 and β-catenin, and then activating Wnt signaling in GSCs via the AEG-1/AKT/GSK3β signaling axis (83). Thus, AEG-1 acts as a critical regulator of Wnt/β-catenin signaling to control GSC stemness and differentiation.

CD163/casein kinase (CK)2. CD163 has been reported to act as a receptor that scavenges hemoglobin by regulating the endocytosis of haptoglobin-hemoglobin complexes (84). CD163 is considered to be a marker of the tumor-associated macrophage (TAM) M2 phenotype (85). TAMs are reported to secrete pleiotrophin to stimulate GSCs and promote GBM growth through its receptor protein tyrosine phosphatase receptor type Z1 (86). A high expression level of CD163 in glioma has been demonstrated to be correlated with poor prognosis (85).

CD163 cannot directly phosphorylate AKT, since it lacks a kinase group. CK2, whose constitutive phosphorylation is required for AKT activation, can interact with CD163 and plays an essential role in CD163 signaling (87,88). CD163 is necessary for maintaining GSC stemness, and downregulation of CD163 decreases stemness marker expression in GBM by interacting directly with CK2 and then inhibiting the CK2/AKT/GSK3β/β-catenin pathway. A previous study found that anti-CD163 antibodies induce cytotoxicity against glioma cells, indicating that CD163 may serve as a therapeutic target for glioma cells, specifically GSCs (89).

Inhibitor of growth (ING)5. The ING family of epigenetic regulators (ING1-5) can target histone acetyltransferase and histone deacetylase complexes to alter histone acetylation and gene expression. The ectopic expression of ING5 increases stemness, promotes self-renewal and prevents differentiation of GSCs by enhancing PI3K/AKT activity. This suggests that ING5 may represent a valuable target for therapeutic strategies in GBM (90).

β-catenin. β-catenin, a cytoplasmic protein, has two functions: Linking cadherin-mediated cell-adhesion molecules with the cytoskeleton and participating in the Wnt signaling pathway (74). A previous study has shown that the content of β-catenin affects the Wnt signaling pathway (74). When the AKT/GSK3β/APC complex is degraded, β-catenin becomes stable and translocates into the nucleus to facilitate the transcription of target genes (91).

Cyclophilin A (CypA). CypA belongs to the peptidyl-prolyl isomerase family. CypA is a specific cytosolic protein and can form a complex with cyclosporin A to induce immunosuppression (92). It was previously demonstrated that CypA

Table III. Clinical trials on drugs that target the STAT3 transcription factor.

Drug	Country	Design	(Refs.)
STAT3 inhibitor WP1066	USA	STAT3 inhibitor WP1066 for the treatment of patients with recurrent malignant glioma or progressive metastatic melanoma in the brain	(122,123)
STAT3 inhibitor WP1066	USA	Investigational treatment with the novel JAK2/STAT3 inhibitor WP1066 of pediatric patients with any progressive or recurrent malignant brain tumor that is refractory to standard treatment and is without known cure	(122,123)

is associated with GBM growth (93). CypA has been found to promote GSC stemness, self-renewal and proliferation (94). Mechanistically, CypA binds to β-catenin and increases the interaction between β-catenin and TCF4 to regulate gene transcription (94). Thus, CypA is a potential target for glioma therapy.

5. HH signaling pathway

Classical HH signaling is required to maintain stem cell niches in the adult brain (95). HH has three gene homologs: Sonic HH (SHH), Desert HH and Indian HH. Upon inhibiting SHH signaling, the number of neural progenitors is reduced. Activation of the HH protein requires Rasp-dependent acylation (96). HH ligands initiate signaling pathways by binding to the transmembrane receptor protein patched homolog (PTCH). The HH-PTCH complex is internalized, and the inhibition of the receptor Smoothened (Smo) is abolished, thus allowing Smo activation, which induces the activation of the glioma-associated oncogene homolog (Gli) family. As a result, Gli translocates to the nucleus to regulate the transcription of target genes (97).

Gli. The Gli family consists of zinc-finger transcription factors, including Gli1, Gli2 and Gli3. Gli3 and SHH repress each other, while Gli2 and Gli1 are the SHH signaling targets. However, only Gli1 can mediate SHH-induced cell differentiation. Gli3 mostly acts as a repressor, whereas Gli2 has both activator and repressor functions (98).

Histone deacetylase (HDAC)6. HDACs are epigenetic modifiers that can affect the acetylation status (99). HDAC6 is upregulated in GSCs and plays a key role in maintaining GSC traits and reducing irradiation-induced DNA damage in GBM (100). Inhibiting HDAC6 downregulates Gli1, PTCH receptor expression and SHH signaling in GSCs. The detailed mechanism involves the inhibition of HDAC6, which inactivates the SHH/Gli1 signaling pathway, decreases GSC proliferation and induces cell differentiation (101). Furthermore, HDAC6 inhibition degrades checkpoint kinase 1 via downregulation of X-linked inhibitor of apoptosis, a transcriptional target of Gli, thus causing GSCs to differentiate, inducing cell death, decreasing DNA damage repair capacity and enhancing radiosensitivity (101,102). These findings may provide promising novel drug targets to overcome GSC stemness.

6. STAT3 signaling pathway

The STAT3 signaling pathway is involved in multiple biological processes, including cell proliferation, differentiation and self-renewal of GSCs. Cytokines and growth factors bind to their receptor, which, once dimerized, activates Janus kinase (JAK). JAK induces STATs phosphorylation, and activated STATs translocate into the nucleus to regulate target gene expression. Previous studies found that phosphorylated STAT3 interacts with the switch/sucrose non-fermentable complex in the nucleus (91,103). TRIM8, the expression of which is highly correlated with stem cell markers, is reported to activate STAT3 signaling to maintain the stemness and self-renewal of GSCs. TRIM8 activates STAT3 by suppressing the expression of the protein inhibitor of activated STAT3, and STAT3 activation can upregulate TRIM8, demonstrating that bidirectional TRIM8/STAT3 signaling is involved in the regulation of the stemness of GSCs (104).

Tetraspanin CD9, a regulator of cell adhesion, stabilizes the IL-6 receptor glycoprotein 130 (gp130) by preventing its ubiquitin-dependent lysosomal degradation, thus promoting bone marrow tyrosine kinase gene on chromosome X/STAT3 signaling in GSCs. Disrupting CD9 or gp130 can inhibit the self-renewal of GSCs and promote their differentiation (105). Currently, there are various ongoing clinical trials in USA investigating the targeting of STAT3 with the small molecule inhibitor WP1066 (Table III).

7. Inhibitor of differentiation 1 (ID1) and its association with other pathways in GSCs

ID1 is highly expressed in GSCs and is involved in the TGF-β, Wnt and SHH signaling pathways. ID proteins are transcriptional regulators that are implicated in cell fate determination and differentiation of stem-like cells (106). Ubiquitination-specific proteases and cyclooxygenase-2-derived prostaglandin E2 have been reported to positively regulate the stability of ID1, and to promote GSC maintenance and treatment resistance (107,108). ID1 induces cell proliferation and promotes self-renewal through increasing cyclin E, the target molecule of cullin 3. Cullin 3 interacts with Gli2 and dishevelled segment polarity protein 2, and induces their degradation through ubiquitination. Loss of cullin 3 is the common signaling node in the Wnt and SHH signaling pathways through ID1 (109).

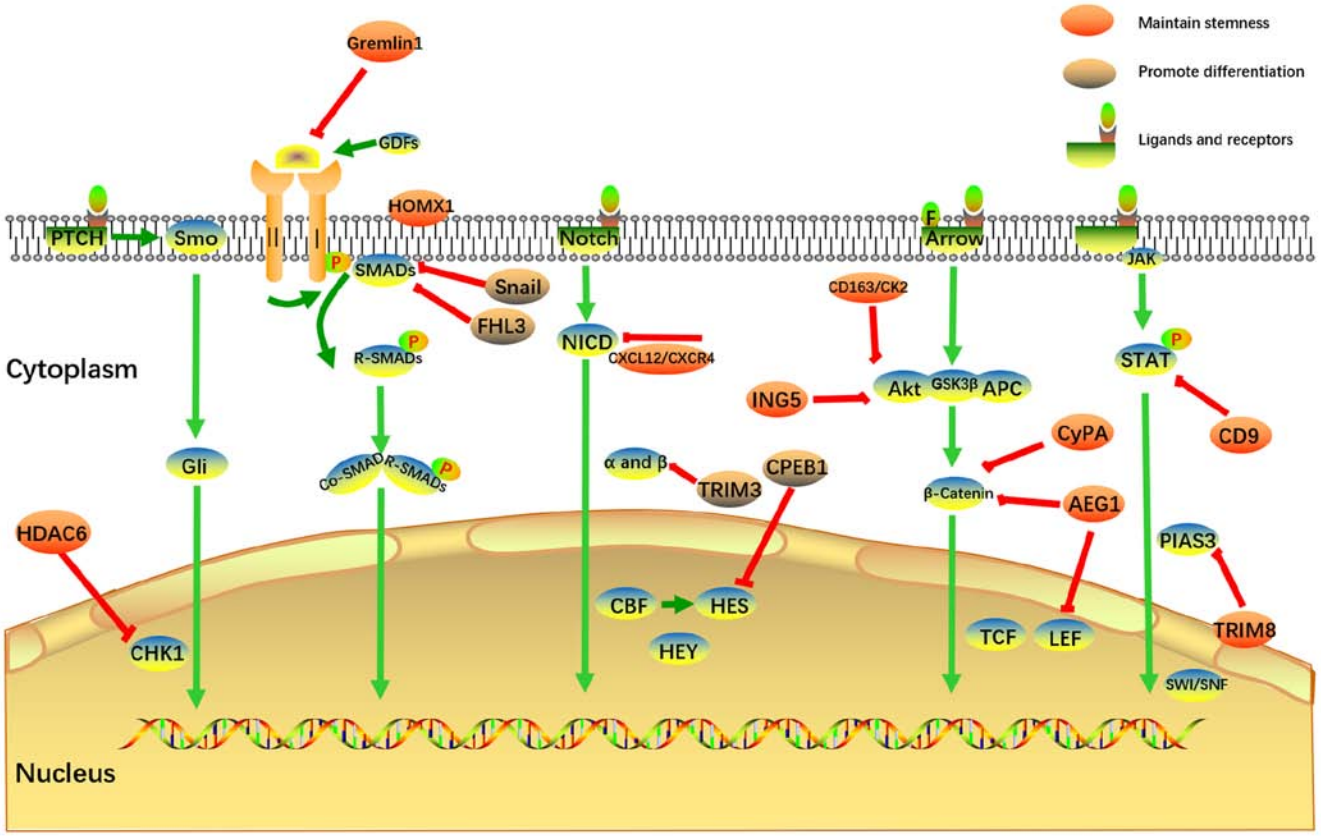

Figure 1. Schematic diagram of dysregulated signaling pathways in glioblastoma stem cells. Proteins highlighted in red, including gremlin 1, HOMX1, CXCL12/CXCR4, AEG1, CD163/CK2, ING5, CypA, HDAC6, CD9 and TRIM8, play a role in stemness maintenance. Proteins highlighted in blue, including Snail, FHL3, TRIM3 and CPEB1, play a role in stem cell differentiation. HOMX1, heme oxygenase 1; CXCL12, C-X-C motif chemokine ligand 12; CXCR4, C-X-C chemokine receptor 4; AEG1, astrocyte elevated gene-1; ING5, inhibitor of growth 5; CypA, cyclophilin A; HDAC6, histone deacetylase 6; TRIM8, tripartite motif containing 8; FHL3, Four-and-a-half LIM domains 3; TRIM3, tripartite motif containing 3; CPEB1, cytoplasmic polyadenylation element-binding protein 1; Hes, hairy/enhancer-of-split; Hey, Hes related family bHLH transcription factor with YRPW motif; TCF, T-cell factor; LEF, lymphoid enhancer-binding factor.

ID1 was previously found to inhibit BMP-mediated GSC differentiation through BMPRII and to maintain GSC traits (110). BMPs bind to a cognate high-affinity type II receptor (BMPRII) to phosphorylate the type I receptor (BMPRI). Activated BMPRI initiates downstream signaling by phosphorylating R-SMAD. ID1 could decrease BMPRII expression and the phosphorylation of its downstream signaling molecules SMAD1, SMAD5 and SMAD8 in cells (15,16). These results indicate that targeting ID1-driven intrinsic stemness signaling may be an effective therapeutic strategy for GBM.

8. Conclusion

GBM is a primary brain tumor with a high mortality rate, for which there is currently no effective therapy. Previous studies have found that GSCs promote the heterogeneity and treatment resistance of GBM. The main characteristics of GSCs are their capacity for self-renewal and differentiation. Therefore, elucidating the mechanism through which GSCs regulate the self-renewal response is meaningful in order to design therapeutic approaches targeting the self-renewal signaling pathways. The focus of the present review was five key self-renewal GSC signaling pathways, including TGF-β, Notch, Wnt, HH and STAT3, and the corresponding therapeutic targets, and the aim was to provide novel insight to enable advances in clinical therapy. Among these signaling pathways, gremlin 1, HMOX1, CXCL12/CXCR4, AEG1, CD163/CK2, ING5, CypA, HDAC6, CD9 and TRIM8 can increase stemness, promote self-renewal and prevent differentiation of GSCs. Therefore, their corresponding inhibitors may represent a novel type of therapeutic approach to glioma. On the other hand, Snail, FHL3, TRIM3 and CPEB1 may promote differentiation of GSCs. Thus, their corresponding agonists should be further investigated in this context. The aforementioned self-renewal pathways and corresponding differentiation-targeting treatments of GSCs are summarized in Fig. 1. Considering its complexity, the crosstalk between these pathways is not shown in Fig. 1. The current clinical trials targeting the TGF-β, Notch and STAT3 pathways are summarized in Tables I-III.

Based on the aforementioned findings, there are numerous potential treatments that are currently being explored. There are also emerging signaling pathways under investigation that may uncover potential treatment targets for GBM. In GSCs, lysine demethylase (KDM)1A (111), the transcription factors forkhead box G1 and transducin-like enhancer of split 1 (40,112), hypoxia-inducible factors (113), proliferating cell nuclear antigen (PCNA)-associated factor (PAF) (114), MEK partner-1 (MP1) (115), erythropoietin-producing hepatocellular receptors (116), progranulin (PGRN) (117) and DNA polymerase delta subunit 2 (POLD2) (118) are all

overexpressed, and maintain GSC self-renewal capacity and stemness. In addition to the aforementioned classical pathways, the molecular mechanisms through which these factors maintain stemness require deeper and more comprehensive investigation. For example, PAF promotes the maintenance of self-renewal ability and stemness by interacting with PCNA, and regulates PCNA-associated DNA translesion synthesis (114), while MP1 contributes to GSC stemness by driving ERK activity (115).

Other factors play a unique role in the damage and repair of DNA, such as POLD2 and PGRN (117,118). Previous studies have demonstrated that PGRN promotes DNA repair through activator protein 1 transcription factor, cFos and JunB (117). In terms of their relevance to treatment, the knockdown of these molecules can reduce GSC stemness and induce their differentiation. Based on the identification of these factors that maintain stemness, corresponding inhibitors may be developed to target GSCs. For example, a series of inhibitors have already been developed and evaluated. Two novel KDM1A-specific inhibitors (NCL-1 and NCD-38) were found to significantly reduce GSCs-driven tumor progression by inducing the activation of the unfolded protein response pathway (111). GLPG1790, a small-molecule ephrin receptor inhibitor, completely blocks ephrin type-A receptor 2 signaling and exerts antitumor effects (116). Similarly, GSC gap junctions also have pro-tumorigenic effects depending on connexin expression (119). However, to the best of our knowledge, the detailed mechanisms remain elusive and further research is needed in this field.

In addition to the aforementioned factors that maintain stemness, other factors promote differentiation, and regulating their activity may be of value in the context of differentiation therapy. For example, MAPK phosphatase 1 (MKP1), a dual-specificity phosphatase, acts as a negative inhibitor of JNK, ERK1/2 and p38 MAPK. High levels of MKP1 expression impair self-renewal and induce differentiation in GSCs (120). The let-7 miRNA family has also been shown to induce GSC differentiation. The mechanism is as follows: Its recognition elements may be bound by insulin-like growth factor 2 mRNA-binding protein 2, which prevents let-7 target gene silencing and impairs the maintenance of GSC stemness (121).

In summary, inhibitors of the factors found to maintain stemness may be developed in the future to provide possible differentiation therapies. For the factors that can promote differentiation, increasing their expression levels is an important method for targeting GSCs. It is expected that more clinically feasible differentiation treatments will be developed in the future in order to improve GBM treatment efficacy and prognosis.

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Authors' contributions

JJ, FG and ML performed the literature search. JJ, FG, CCC and ML wrote the manuscript. All the authors have read and approved the final manuscript.

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Competing interests

The authors declare that they have no competing interests.

References

1. Prager BC, Bhargava S, Mahadev V, Hubert CG and Rich JN: Glioblastoma Stem Cells: Driving Resilience through Chaos. Trends Cancer 6: 223-235, 2020.
2. Weller M, Cloughesy T, Perry JR and Wick W: Standards of care for treatment of recurrent glioblastoma - are we there yet? Neuro Oncol 15: 4-27, 2013.
3. Jordan CT: Cancer stem cells: Controversial or just misunderstood? Cell Stem Cell 4: 203-205, 2009.
4. Kondo T, Setoguchi T and Taga T: Persistence of a small subpopulation of cancer stem-like cells in the C6 glioma cell line. Proc Natl Acad Sci USA 101: 781-786, 2004.
5. Choi SA, Lee JY, Phi JH, Wang KC, Park CK, Park SH and Kim SK: Identification of brain tumour initiating cells using the stem cell marker aldehyde dehydrogenase. Eur J Cancer 50: 137-149, 2014.
6. Singh SK, Hawkins C, Clarke ID, Squire JA, Bayani J, Hide T, Henkelman RM, Cusimano MD and Dirks PB: Identification of human brain tumour initiating cells. Nature 432: 396-401, 2004.
7. Galli R, Binda E, Orfanelli U, Cipelletti B, Gritti A, De Vitis S, Fiocco R, Foroni C, Dimeco F and Vescovi A: Isolation and characterization of tumorigenic, stem-like neural precursors from human glioblastoma. Cancer Res 64: 7011-7021, 2004.
8. Li Z, Wang H, Eyler CE, Hjelmeland AB and Rich JN: Turning cancer stem cells inside out: An exploration of glioma stem cell signaling pathways. J Biol Chem 284: 16705-16709, 2009.
9. Cheng L, Wu Q, Guryanova OA, Huang Z, Huang Q, Rich JN and Bao S: Elevated invasive potential of glioblastoma stem cells. Biochem Biophys Res Commun 406: 643-648, 2011.
10. Folkins C, Shaked Y, Man S, Tang T, Lee CR, Zhu Z, Hoffman RM and Kerbel RS: Glioma tumor stem-like cells promote tumor angiogenesis and vasculogenesis via vascular endothelial growth factor and stromal-derived factor 1. Cancer Res 69: 7243-7251, 2009.
11. Bao S, Wu Q, McLendon RE, Hao Y, Shi Q, Hjelmeland AB, Dewhirst MW, Bigner DD and Rich JN: Glioma stem cells promote radioresistance by preferential activation of the DNA damage response. Nature 444: 756-760, 2006.
12. Liu Z, Bandyopadhyay A, Nichols RW, Wang L, Hinck AP, Wang S and Sun LZ: Blockade of Autocrine TGF-β signaling inhibits stem cell phenotype, survival, and metastasis of murine breast cancer cells. J Stem Cell Res Ther 2: 1-8, 2012.
13. Xi Q, Wang Z, Zaromytidou AI, Zhang XH, Chow-Tsang LF, Liu JX, Kim H, Barlas A, Manova-Todorova K, Kaartinen V, *et al*: A poised chromatin platform for TGF-β access to master regulators. Cell 147: 1511-1524, 2011.
14. Derynck R and Zhang YE: Smad-dependent and Smad-independent pathways in TGF-beta family signalling. Nature 425: 577-584, 2003.

15. Massagué J: How cells read TGF-beta signals. Nat Rev Mol Cell Biol 1: 169-178, 2000.
16. Oshimori N and Fuchs E: The harmonies played by TGF-β in stem cell biology. Cell Stem Cell 11: 751-764, 2012.
17. Liang Y, Zhu F, Zhang H, Chen D, Zhang X, Gao Q and Li Y: Conditional ablation of TGF-β signaling inhibits tumor progression and invasion in an induced mouse bladder cancer model. Sci Rep 6: 29479, 2016.
18. Furuta Y, Piston DW and Hogan BL: Bone morphogenetic proteins (BMPs) as regulators of dorsal forebrain development. Development 124: 2203-2212, 1997.
19. Gilboa L, Nohe A, Geissendörfer T, Sebald W, Henis YI and Knaus P: Bone morphogenetic protein receptor complexes on the surface of live cells: A new oligomerization mode for serine/threonine kinase receptors. Mol Biol Cell 11: 1023-1035, 2000.
20. Lee SJ: Identification of a novel member (GDF-1) of the transforming growth factor-beta superfamily. Mol Endocrinol 4: 1034-1040, 1990.
21. McPherron AC and Lee SJ: GDF-3 and GDF-9: Two new members of the transforming growth factor-beta superfamily containing a novel pattern of cysteines. J Biol Chem 268: 3444-3449, 1993.
22. Rider CC and Mulloy B: Bone morphogenetic protein and growth differentiation factor cytokine families and their protein antagonists. Biochem J 429: 1-12, 2010.
23. Chirasani SR, Sternjak A, Wend P, Momma S, Campos B, Herrmann IM, Graf D, Mitsiadis T, Herold-Mende C, Besser D, *et al*: Bone morphogenetic protein-7 release from endogenous neural precursor cells suppresses the tumourigenicity of stem-like glioblastoma cells. Brain 133: 1961-1972, 2010.
24. Piccirillo SG, Reynolds BA, Zanetti N, Lamorte G, Binda E, Broggi G, Brem H, Olivi A, Dimeco F and Vescovi AL: Bone morphogenetic proteins inhibit the tumorigenic potential of human brain tumour-initiating cells. Nature 444: 761-765, 2006.
25. Raja E, Komuro A, Tanabe R, Sakai S, Ino Y, Saito N, Todo T, Morikawa M, Aburatani H, Koinuma D, *et al*: Bone morphogenetic protein signaling mediated by ALK-2 and DLX2 regulates apoptosis in glioma-initiating cells. Oncogene 36: 4963-4974, 2017.
26. Tso JL, Yang S, Menjivar JC, Yamada K, Zhang Y, Hong I, Bui Y, Stream A, McBride WH, Liau LM, *et al*: Bone morphogenetic protein 7 sensitizes O6-methylguanine methyltransferase expressing-glioblastoma stem cells to clinically relevant dose of temozolomide. Mol Cancer 14: 189, 2015.
27. Lee J, Son MJ, Woolard K, Donin NM, Li A, Cheng CH, Kotliarova S, Kotliarov Y, Walling J, Ahn S, *et al*: Epigenetic-mediated dysfunction of the bone morphogenetic protein pathway inhibits differentiation of glioblastoma-initiating cells. Cancer Cell 13: 69-80, 2008.
28. Namkoong H, Shin SM, Kim HK, Ha SA, Cho GW, Hur SY, Kim TE and Kim JW: The bone morphogenetic protein antagonist gremlin 1 is overexpressed in human cancers and interacts with YWHAH protein. BMC Cancer 6: 74, 2006.
29. Yan K, Wu Q, Yan DH, Lee CH, Rahim N, Tritschler I, DeVecchio J, Kalady MF, Hjelmeland AB and Rich JN: Glioma cancer stem cells secrete Gremlin1 to promote their maintenance within the tumor hierarchy. Genes Dev 28: 1085-1100, 2014.
30. Tate CM, Pallini R, Ricci-Vitiani L, Dowless M, Shiyanova T, D'Alessandris GQ, Morgante L, Giannetti S, Larocca LM, di Martino S, *et al*: A BMP7 variant inhibits the tumorigenic potential of glioblastoma stem-like cells. Cell Death Differ 19: 1644-1654, 2012.
31. Bruna A, Darken RS, Rojo F, Ocaña A, Peñuelas S, Arias A, Paris R, Tortosa A, Mora J, Baselga J, *et al*: High TGFbeta-Smad activity confers poor prognosis in glioma patients and promotes cell proliferation depending on the methylation of the PDGF-B gene. Cancer Cell 11: 147-160, 2007.
32. Peñuelas S, Anido J, Prieto-Sánchez RM, Folch G, Barba I, Cuartas I, García-Dorado D, Poca MA, Sahuquillo J, Baselga J, *et al*: TGF-beta increases glioma-initiating cell self-renewal through the induction of LIF in human glioblastoma. Cancer Cell 15: 315-327, 2009.
33. Yang HW, Menon LG, Black PM, Carroll RS and Johnson MD: SNAI2/Slug promotes growth and invasion in human gliomas. BMC Cancer 10: 301, 2010.
34. Savary K, Caglayan D, Caja L, Tzavlaki K, Bin Nayeem S, Bergström T, Jiang Y, Uhrbom L, Forsberg-Nilsson K, Westermark B, *et al*: Snail depletes the tumorigenic potential of glioblastoma. Oncogene 32: 5409-5420, 2013.
35. Caja L, Tzavlaki K, Dadras MS, Tan EJ, Hatem G, Maturi NP, Morén A, Wik L, Watanabe Y, Savary K, *et al*: Snail regulates BMP and TGFβ pathways to control the differentiation status of glioma-initiating cells. Oncogene 37: 2515-2531, 2018.
36. Teh JL and Chen S: Glutamatergic signaling in cellular transformation. Pigment Cell Melanoma Res 25: 331-342, 2012.
37. Ghosh D, Ulasov IV, Chen L, Harkins LE, Wallenborg K, Hothi P, Rostad S, Hood L and Cobbs CS: TGFβ-responsive HMOX1 expression is associated with stemness and invasion in glioblastoma multiforme. Stem Cells 34: 2276-2289, 2016.
38. Han W, Xin Z, Zhao Z, Bao W, Lin X, Yin B, Zhao J, Yuan J, Qiang B and Peng X: RNA-binding protein PCBP2 modulates glioma growth by regulating FHL3. J Clin Invest 123: 2103-2118, 2013.
39. Han W, Hu P, Wu F, Wang S, Hu Y, Li S, Jiang T, Qiang B and Peng X: FHL3 links cell growth and self-renewal by modulating SOX4 in glioma. Cell Death Differ 26: 796-811, 2019.
40. Bulstrode H, Johnstone E, Marques-Torrejon MA, Ferguson KM, Bressan RB, Blin C, Grant V, Gogolok S, Gangoso E, Gagrica S, *et al*: Elevated FOXG1 and SOX2 in glioblastoma enforces neural stem cell identity through transcriptional control of cell cycle and epigenetic regulators. Genes Dev 31: 757-773, 2017.
41. Artavanis-Tsakonas S, Rand MD and Lake RJ: Notch signaling: Cell fate control and signal integration in development. Science 284: 770-776, 1999.
42. Blaumueller CM, Qi H, Zagouras P and Artavanis-Tsakonas S: Intracellular cleavage of Notch leads to a heterodimeric receptor on the plasma membrane. Cell 90: 281-291, 1997.
43. Stockhausen MT, Kristoffersen K and Poulsen HS: The functional role of Notch signaling in human gliomas. Neuro Oncol 12: 199-211, 2010.
44. Androutsellis-Theotokis A, Leker RR, Soldner F, Hoeppner DJ, Ravin R, Poser SW, Rueger MA, Bae SK, Kittappa R and McKay RD: Notch signalling regulates stem cell numbers in vitro and in vivo. Nature 442: 823-826, 2006.
45. Guichet PO, Guelfi S, Teigell M, Hoppe L, Bakalara N, Bauchet L, Duffau H, Lamszus K, Rothhut B and Hugnot JP: Notch1 stimulation induces a vascularization switch with pericyte-like cell differentiation of glioblastoma stem cells. Stem Cells 33: 21-34, 2015.
46. Kanamori M, Kawaguchi T, Nigro JM, Feuerstein BG, Berger MS, Miele L and Pieper RO: Contribution of Notch signaling activation to human glioblastoma multiforme. J Neurosurg 106: 417-427, 2007.
47. Chowdhury S and Sarkar RR: Exploring Notch pathway to elucidate phenotypic plasticity and intra-tumor heterogeneity in fliomas. Sci Rep 9: 9488, 2019.
48. Basak O, Giachino C, Fiorini E, Macdonald HR and Taylor V: Neurogenic subventricular zone stem/progenitor cells are Notch1-dependent in their active but not quiescent state. J Neurosci 32: 5654-5666, 2012.
49. Patel AP, Tirosh I, Trombetta JJ, Shalek AK, Gillespie SM, Wakimoto H, Cahill DP, Nahed BV, Curry WT, Martuza RL, *et al*: Single-cell RNA-seq highlights intratumoral heterogeneity in primary glioblastoma. Science 344: 1396-1401, 2014.
50. Ge W, Martinowich K, Wu X, He F, Miyamoto A, Fan G, Weinmaster G and Sun YE: Notch signaling promotes astrogliogenesis via direct CSL-mediated glial gene activation. J Neurosci Res 69: 848-860, 2002.
51. Bansod S, Kageyama R and Ohtsuka T: Hes5 regulates the transition timing of neurogenesis and gliogenesis in mammalian neocortical development. Development 144: 3156-3167, 2017.
52. Armesilla-Diaz A, Bragado P, Del Valle I, Cuevas E, Lazaro I, Martin C, Cigudosa JC and Silva A: p53 regulates the self-renewal and differentiation of neural precursors. Neuroscience 158: 1378-1389, 2009.
53. Fan X, Khaki L, Zhu TS, Soules ME, Talsma CE, Gul N, Koh C, Zhang J, Li YM, Maciaczyk J, *et al*: NOTCH pathway blockade depletes CD133-positive glioblastoma cells and inhibits growth of tumor neurospheres and xenografts. Stem Cells 28: 5-16, 2010.
54. Xu R, Shimizu F, Hovinga K, Beal K, Karimi S, Droms L, Peck KK, Gutin P, Iorgulescu JB, Kaley T, *et al*: Molecular and clinical effects of Notch inhibition in glioma patients: A phase 0/I trial. Clin Cancer Res 22: 4786-4796, 2016.
55. Tanaka S, Nakada M, Yamada D, Nakano I, Todo T, Ino Y, Hoshii T, Tadokoro Y, Ohta K, Ali MA, *et al*: Strong therapeutic potential of γ-secretase inhibitor MRK003 for CD44-high and CD133-low glioblastoma initiating cells. J Neurooncol 121: 239-250, 2015.
56. Goldfarb DS, Corbett AH, Mason DA, Harreman MT and Adam SA: Importin alpha: A multipurpose nuclear-transport receptor. Trends Cell Biol 14: 505-514, 2004.
57. Huenniger K, Krämer A, Soom M, Chang I, Köhler M, Depping R, Kehlenbach RH and Kaether C: Notch1 signaling is mediated by importins alpha 3, 4, and 7. Cell Mol Life Sci 67: 3187-3196, 2010.

58. Chen G, Kong J, Tucker-Burden C, Anand M, Rong Y, Rahman F, Moreno CS, Van Meir EG, Hadjipanayis CG and Brat DJ: Human Brat ortholog TRIM3 is a tumor suppressor that regulates asymmetric cell division in glioblastoma. Cancer Res 74: 4536-4548, 2014.
59. Mukherjee S, Tucker-Burden C, Zhang C, Moberg K, Read R, Hadjipanayis C and Brat DJ: *Drosophila* brat and human ortholog TRIM3 maintain stem cell equilibrium and suppress brain tumorigenesis by attenuating Notch nuclear transport. Cancer Res 76: 2443-2452, 2016.
60. Gagliardi F, Narayanan A, Reni M, Franzin A, Mazza E, Boari N, Bailo M, Zordan P and Mortini P: The role of CXCR4 in highly malignant human gliomas biology: Current knowledge and future directions. Glia 62: 1015-1023, 2014.
61. Calinescu AA, Yadav VN, Carballo E, Kadiyala P, Tran D, Zamler DB, Doherty R, Srikanth M, Lowenstein PR and Castro MG: Survival and proliferation of neural progenitor-derived glioblastomas under hypoxic stress is controlled by a CXCL12/CXCR4 autocrine-positive feedback mechanism. Clin Cancer Res 23: 1250-1262, 2017.
62. Yi L, Zhou X, Li T, Liu P, Hai L, Tong L, Ma H, Tao Z, Xie Y, Zhang C, *et al*: Notch1 signaling pathway promotes invasion, self-renewal and growth of glioma initiating cells via modulating chemokine system CXCL12/CXCR4. J Exp Clin Cancer Res 38: 339, 2019.
63. Iso T, Kedes L and Hamamori Y: HES and HERP families: Multiple effectors of the Notch signaling pathway. J Cell Physiol 194: 237-255, 2003.
64. Tay J and Richter JD: Germ cell differentiation and synaptonemal complex formation are disrupted in CPEB knockout mice. Dev Cell 1: 201-213, 2001.
65. Yin J, Park G, Lee JE, Park JY, Kim TH, Kim YJ, Lee SH, Yoo H, Kim JH and Park JB: CPEB1 modulates differentiation of glioma stem cells via downregulation of HES1 and SIRT1 expression. Oncotarget 5: 6756-6769, 2014.
66. Iso T, Sartorelli V, Poizat C, Iezzi S, Wu HY, Chung G, Kedes L and Hamamori Y: HERP, a novel heterodimer partner of HES/E(spl) in Notch signaling. Mol Cell Biol 21: 6080-6089, 2001.
67. Iso T, Sartorelli V, Chung G, Shichinohe T, Kedes L and Hamamori Y: HERP, a new primary target of Notch regulated by ligand binding. Mol Cell Biol 21: 6071-6079, 2001.
68. Sokol SY: Maintaining embryonic stem cell pluripotency with Wnt signaling. Development 138: 4341-4350, 2011.
69. Bhanot P, Brink M, Samos CH, Hsieh JC, Wang Y, Macke JP, Andrew D, Nathans J and Nusse R: A new member of the frizzled family from *Drosophila* functions as a Wingless receptor. Nature 382: 225-230, 1996.
70. Wehrli M, Dougan ST, Caldwell K, O'Keefe L, Schwartz S, Vaizel-Ohayon D, Schejter E, Tomlinson A and DiNardo S: arrow encodes an LDL-receptor-related protein essential for Wingless signalling. Nature 407: 527-530, 2000.
71. Korinek V, Barker N, Morin PJ, van Wichen D, de Weger R, Kinzler KW, Vogelstein B and Clevers H: Constitutive transcriptional activation by a beta-catenin-Tcf complex in APC$^{-/-}$ colon carcinoma. Science 275: 1784-1787, 1997.
72. Hamada F and Bienz M: The APC tumor suppressor binds to C-terminal binding protein to divert nuclear beta-catenin from TCF. Dev Cell 7: 677-685, 2004.
73. Molenaar M, van de Wetering M, Oosterwegel M, Peterson-Maduro J, Godsave S, Korinek V, Roose J, Destrée O and Clevers H: XTcf-3 transcription factor mediates beta-catenin-induced axis formation in Xenopus embryos. Cell 86: 391-399, 1996.
74. Behrens J, von Kries JP, Kühl M, Bruhn L, Wedlich D, Grosschedl R and Birchmeier W: Functional interaction of beta-catenin with the transcription factor LEF-1. Nature 382: 638-642, 1996.
75. Huber O, Korn R, McLaughlin J, Ohsugi M, Herrmann BG and Kemler R: Nuclear localization of beta-catenin by interaction with transcription factor LEF-1. Mech Dev 59: 3-10, 1996.
76. Takahashi-Yanaga F and Kahn M: Targeting Wnt signaling: Can we safely eradicate cancer stem cells? Clin Cancer Res 16: 3153-3162, 2010.
77. Rajakulendran N, Rowland KJ, Selvadurai HJ, Ahmadi M, Park NI, Naumenko S, Dolma S, Ward RJ, So M, Lee L, *et al*: Wnt and Notch signaling govern self-renewal and differentiation in a subset of human glioblastoma stem cells. Genes Dev 33: 498-510, 2019.
78. Sonoda Y, Ozawa T, Aldape KD, Deen DF, Berger MS and Pieper RO: Akt pathway activation converts anaplastic astrocytoma to glioblastoma multiforme in a human astrocyte model of glioma. Cancer Res 61: 6674-6678, 2001.
79. Morgan RG, Ridsdale J, Payne M, Heesom KJ, Wilson MC, Davidson A, Greenhough A, Davies S, Williams AC, Blair A, *et al*: LEF-1 drives aberrant β-catenin nuclear localization in myeloid leukemia cells. Haematologica 104: 1365-1377, 2019.
80. Chen J, Liu G, Wu Y, Ma J, Wu H, Xie Z, Chen S, Yang Y, Wang S, Shen P, *et al*: CircMYO10 promotes osteosarcoma progression by regulating miR-370-3p/RUVBL1 axis to enhance the transcriptional activity of β-catenin/LEF1 complex via effects on chromatin remodeling. Mol Cancer 18: 150, 2019.
81. Brown DM and Ruoslahti E: Metadherin, a cell surface protein in breast tumors that mediates lung metastasis. Cancer Cell 5: 365-374, 2004.
82. Hu B, Emdad L, Bacolod MD, Kegelman TP, Shen XN, Alzubi MA, Das SK, Sarkar D and Fisher PB: Astrocyte elevated gene-1 interacts with Akt isoform 2 to control glioma growth, survival, and pathogenesis. Cancer Res 74: 7321-7332, 2014.
83. Hu B, Emdad L, Kegelman TP, Shen XN, Das SK, Sarkar D and Fisher PB: Astrocyte elevated Gene-1 regulates β-catenin signaling to maintain glioma stem-like stemness and self-renewal. Mol Cancer Res 15: 225-233, 2017.
84. Kristiansen M, Graversen JH, Jacobsen C, Sonne O, Hoffman HJ, Law SK and Moestrup SK: Identification of the haemoglobin scavenger receptor. Nature 409: 198-201, 2001.
85. Ostuni R, Kratochvill F, Murray PJ and Natoli G: Macrophages and cancer: From mechanisms to therapeutic implications. Trends Immunol 36: 229-239, 2015.
86. Shi Y, Ping YF, Zhou W, He ZC, Chen C, Bian BS, Zhang L, Chen L, Lan X, Zhang XC, *et al*: Tumour-associated macrophages secrete pleiotrophin to promote PTPRZ1 signalling in glioblastoma stem cells for tumour growth. Nat Commun 8: 15080, 2017.
87. Ritter M, Buechler C, Kapinsky M and Schmitz G: Interaction of CD163 with the regulatory subunit of casein kinase II (CKII) and dependence of CD163 signaling on CKII and protein kinase C. Eur J Immunol 31: 999-1009, 2001.
88. Di Maira G, Salvi M, Arrigoni G, Marin O, Sarno S, Brustolon F, Pinna LA and Ruzzene M: Protein kinase CK2 phosphorylates and upregulates Akt/PKB. Cell Death Differ 12: 668-677, 2005.
89. Chen T, Chen J, Zhu Y, Li Y, Wang Y, Chen H, Wang J, Li X, Liu Y, Li B, *et al*: CD163, a novel therapeutic target, regulates the proliferation and stemness of glioma cells via casein kinase 2. Oncogene 38: 1183-1199, 2019.
90. Wang F, Wang AY, Chesnelong C, Yang Y, Nabbi A, Thalappilly S, Alekseev V and Riabowol K: ING5 activity in self-renewal of glioblastoma stem cells via calcium and follicle stimulating hormone pathways. Oncogene 37: 286-301, 2018.
91. Zhu Q, Shen Y, Chen X, He J, Liu J and Zu X: Self-renewal signalling pathway inhibitors: Perspectives on therapeutic approaches for cancer stem cells. OncoTargets Ther 13: 525-540, 2020.
92. Handschumacher RE, Harding MW, Rice J, Drugge RJ and Speicher DW: Cyclophilin: A specific cytosolic binding protein for cyclosporin A. Science 226: 544-547, 1984.
93. Sun S, Wang Q, Giang A, Cheng C, Soo C, Wang C, Liu L and Chiu R: Knockdown of CypA inhibits interleukin-8 (IL-8) and IL-8-mediated proliferation and tumor growth of glioblastoma cells through down-regulated NF-κB. J Neurooncol 101: 1-14, 2011.
94. Wang G, Shen J, Sun J, Jiang Z, Fan J, Wang H, Yu S, Long Y, Liu Y, Bao H, *et al*: Cyclophilin A maintains glioma-initiating cell stemness by regulating Wnt/β-catenin signaling. Clin Cancer Res 23: 6640-6649, 2017.
95. Machold R, Hayashi S, Rutlin M, Muzumdar MD, Nery S, Corbin JG, Gritli-Linde A, Dellovade T, Porter JA, Rubin LL, *et al*: Sonic hedgehog is required for progenitor cell maintenance in telencephalic stem cell niches. Neuron 39: 937-950, 2003.
96. Micchelli CA, The I, Selva E, Mogila V and Perrimon N: Rasp, a putative transmembrane acyltransferase, is required for Hedgehog signaling. Development 129: 843-851, 2002.
97. Takebe N, Harris PJ, Warren RQ and Ivy SP: Targeting cancer stem cells by inhibiting Wnt, Notch, and Hedgehog pathways. Nat Rev Clin Oncol 8: 97-106, 2011.
98. Ruiz i Altaba A and Altaba A: Combinatorial Gli gene function in floor plate and neuronal inductions by Sonic hedgehog. Development 125: 2203-2212, 1998.
99. Baylin SB and Jones PA: Epigenetic determinants of cancer. Cold Spring Harb Perspect Biol 8: a019505, 2016.

100. Marampon F, Megiorni F, Camero S, Crescioli C, McDowell HP, Sferra R, Vetuschi A, Pompili S, Ventura L, De Felice F, *et al*: HDAC4 and HDAC6 sustain DNA double strand break repair and stem-like phenotype by promoting radioresistance in glioblastoma cells. Cancer Lett 397: 1-11, 2017.
101. Yang W, Liu Y, Gao R, Yu H and Sun T: HDAC6 inhibition induces glioma stem cells differentiation and enhances cellular radiation sensitivity through the SHH/Gli1 signaling pathway. Cancer Lett 415: 164-176, 2018.
102. Auzmendi-Iriarte J, Saenz-Antoñanzas A, Mikelez-Alonso I, Carrasco-Garcia E, Tellaetxe-Abete M, Lawrie CH, Sampron N, Cortajarena AL and Matheu A: Characterization of a new small-molecule inhibitor of HDAC6 in glioblastoma. Cell Death Dis 11: 417, 2020.
103. Dolatabadi S, Jonasson E, Lindén M, Fereydouni B, Bäcksten K, Nilsson M, Martner A, Forootan A, Fagman H, Landberg G, *et al*: JAK-STAT signalling controls cancer stem cell properties including chemotherapy resistance in myxoid liposarcoma. Int J Cancer 145: 435-449, 2019.
104. Zhang C, Mukherjee S, Tucker-Burden C, Ross JL, Chau MJ, Kong J and Brat DJ: TRIM8 regulates stemness in glioblastoma through PIAS3-STAT3. Mol Oncol 11: 280-294, 2017.
105. Shi Y, Zhou W, Cheng L, Chen C, Huang Z, Fang X, Wu Q, He Z, Xu S, Lathia JD, *et al*: Tetraspanin CD9 stabilizes gp130 by preventing its ubiquitin-dependent lysosomal degradation to promote STAT3 activation in glioma stem cells. Cell Death Differ 24: 167-180, 2017.
106. Lasorella A, Benezra R and Iavarone A: The ID proteins: Master regulators of cancer stem cells and tumour aggressiveness. Nat Rev Cancer 14: 77-91, 2014.
107. Lee JK, Chang N, Yoon Y, Yang H, Cho H, Kim E, Shin Y, Kang W, Oh YT, Mun GI, *et al*: USP1 targeting impedes GBM growth by inhibiting stem cell maintenance and radioresistance. Neuro Oncol 18: 37-47, 2016.
108. Cook PJ, Thomas R, Kingsley PJ, Shimizu F, Montrose DC, Marnett LJ, Tabar VS, Dannenberg AJ and Benezra R: Cox-2-derived PGE2 induces Id1-dependent radiation resistance and self-renewal in experimental glioblastoma. Neuro Oncol 18: 1379-1389, 2016.
109. Jin X, Jeon HM, Jin X, Kim EJ, Yin J, Jeon HY, Sohn YW, Oh SY, Kim JK, Kim SH, *et al*: The ID1-CULLIN3 axis regulates intracellular SHH and WNT signaling in glioblastoma stem cells. Cell Rep 16: 1629-1641, 2016.
110. Jin X, Jin X, Kim LJY, Dixit D, Jeon HY, Kim EJ, Kim JK, Lee SY, Yin J, Rich JN, *et al*: Inhibition of ID1-BMPR2 Intrinsic Signaling Sensitizes Glioma Stem Cells to Differentiation Therapy. Clin Cancer Res 24: 383-394, 2018.
111. Sareddy GR, Viswanadhapalli S, Surapaneni P, Suzuki T, Brenner A and Vadlamudi RK: Novel KDM1A inhibitors induce differentiation and apoptosis of glioma stem cells via unfolded protein response pathway. Oncogene 36: 2423-2434, 2017.
112. Dali R, Verginelli F, Pramatarova A, Sladek R and Stifani S: Characterization of a FOXG1:TLE1 transcriptional network in glioblastoma-initiating cells. Mol Oncol 12: 775-787, 2018.
113. Semenza GL: Dynamic regulation of stem cell specification and maintenance by hypoxia-inducible factors. Mol Aspects Med 47-48: 15-23, 2016.
114. Ong DST, Hu B, Ho YW, Sauvé CG, Bristow CA, Wang Q, Multani AS, Chen P, Nezi L, Jiang S, *et al*: PAF promotes stemness and radioresistance of glioma stem cells. Proc Natl Acad Sci USA 114: E9086-E9095, 2017.
115. Kwon SJ, Kwon OS, Kim KT, Go YH, Yu SI, Lee BH, Miyoshi H, Oh E, Cho SJ and Cha HJ: Role of MEK partner-1 in cancer stemness through MEK/ERK pathway in cancerous neural stem cells, expressing EGFRviii. Mol Cancer 16: 140, 2017.
116. Gravina GL, Mancini A, Colapietro A, Delle Monache S, Sferra R, Vitale F, Cristiano L, Martellucci S, Marampon F, Mattei V, *et al*: The small molecule Ephrin receptor inhibitor, glpg1790, reduces renewal capabilities of cancer stem cells, showing anti-tumour efficacy on preclinical glioblastoma models. Cancers (Basel) 11: 359, 2019.
117. Bandey I, Chiou SH, Huang AP, Tsai JC and Tu PH: Progranulin promotes Temozolomide resistance of glioblastoma by orchestrating DNA repair and tumor stemness. Oncogene 34: 1853-1864, 2015.
118. Xu Q, Hu C, Zhu Y, Wang K, Lal B, Li L, Tang J, Wei S, Huang G, Xia S, *et al*: ShRNA-based POLD2 expression knockdown sensitizes glioblastoma to DNA-Damaging therapeutics. Cancer Lett 482: 126-135, 2020.
119. Hitomi M, Deleyrolle LP, Mulkearns-Hubert EE, Jarrar A, Li M, Sinyuk M, Otvos B, Brunet S, Flavahan WA, Hubert CG, *et al*: Differential connexin function enhances self-renewal in glioblastoma. Cell Rep 11: 1031-1042, 2015.
120. Arrizabalaga O, Moreno-Cugnon L, Auzmendi-Iriarte J, Aldaz P, Ibanez de Caceres I, Garros-Regulez L, Moncho-Amor V, Torres-Bayona S, Pernía O, Pintado-Berninches L, *et al*: High expression of MKP1/DUSP1 counteracts glioma stem cell activity and mediates HDAC inhibitor response. Oncogenesis 6: 401, 2017.
121. Degrauwe N, Schlumpf TB, Janiszewska M, Martin P, Cauderay A, Provero P, Riggi N, Suvà ML, Paro R and Stamenkovic I: The RNA binding protein IMP2 preserves glioblastoma stem cells by preventing let-7 target gene silencing. Cell Rep 15: 1634-1647, 2016.
122. Iwamaru A, Szymanski S, Iwado E, Aoki H, Yokoyama T, Fokt I, Hess K, Conrad C, Madden T, Sawaya R, *et al*: A novel inhibitor of the STAT3 pathway induces apoptosis in malignant glioma cells both in vitro and in vivo. Oncogene 26: 2435-2444, 2007.
123. Ott M, Kassab C, Marisetty A, Hashimoto Y, Wei J, Zamler D, Leu JS, Tomaszowski KH, Sabbagh A, Fang D, *et al*: Radiation with STAT3 blockade triggers dendritic cell-T cell interactions in the glioma microenvironment and therapeutic efficacy. Clin Cancer Res 26: 4983-4994, 2020.
124. Lim D, Kim KS, Kim H, Ko KC, Song JJ, Choi JH, Shin M, Min JJ, Jeong JH and Choy HE: Anti-tumor activity of an immunotoxin (TGFα-PE38) delivered by attenuated Salmonella typhimurium. Oncotarget 8: 37550-37560, 2017.
125. Sampson JH, Akabani G, Archer GE, Berger MS, Coleman RE, Friedman AH, Friedman HS, Greer K, Herndon JE II, Kunwar S, *et al*: Intracerebral infusion of an EGFR-targeted toxin in recurrent malignant brain tumors. Neuro Oncol 10: 320-329, 2008.
126. Hau P, Jachimczak P, Schlingensiepen R, Schulmeyer F, Jauch T, Steinbrecher A, Brawanski A, Proescholdt M, Schlaier J, Buchroithner J, *et al*: Inhibition of TGF-beta2 with AP 12009 in recurrent malignant gliomas: from preclinical to phase I/II studies. Oligonucleotides 17: 201-12, 2007.
127. Rodon J, Carducci MA, Sepulveda-Sánchez JM, Azaro A, Calvo E, Seoane J, Braña I, Sicart E, Gueorguieva I, Cleverly AL, *et al*: First-in-human dose study of the novel transforming growth factor-β receptor I kinase inhibitor LY2157299 monohydrate in patients with advanced cancer and glioma. Clin Cancer Res 21: 553-560, 2015.
128. Zhang M, Lahn M and Huber PE: Translating the combination of TGFβ blockade and radiotherapy into clinical development in glioblastoma. OncoImmunology 1: 943-945, 2012.
129. Zhang M, Kleber S, Röhrich M, Timke C, Han N, Tuettenberg J, Martin-Villalba A, Debus J, Peschke P, Wirkner U, *et al*: Blockade of TGF-β signaling by the TGFβR-I kinase inhibitor LY2109761 enhances radiation response and prolongs survival in glioblastoma. Cancer Res 71: 7155-7167, 2011.
130. Pan E, Supko JG, Kaley TJ, Butowski NA, Cloughesy T, Jung J, Desideri S, Grossman S, Ye X and Park DM: Phase I study of RO4929097 with bevacizumab in patients with recurrent malignant glioma. J Neurooncol 130: 571-579, 2016.
131. Yahyanejad S, King H, Iglesias VS, Granton PV, Barbeau LM, van Hoof SJ, Groot AJ, Habets R, Prickaerts J, Chalmers AJ, *et al*: NOTCH blockade combined with radiation therapy and temozolomide prolongs survival of orthotopic glioblastoma. Oncotarget 7: 41251-41264, 2016.
132. Tolcher AW, Messersmith WA, Mikulski SM, Papadopoulos KP, Kwak EL, Gibbon DG, Patnaik A, Falchook GS, Dasari A, Shapiro GI, *et al*: Phase I study of RO4929097, a gamma secretase inhibitor of Notch signaling, in patients with refractory metastatic or locally advanced solid tumors. J Clin Oncol 30: 2348-2353, 2012.
133. den Hollander MW, Bensch F, Glaudemans AW, Oude Munnink TH, Enting RH, den Dunnen WF, Heesters MA, Kruyt FA, Lub-de Hooge MN, Cees de Groot J, *et al*: TGF-β Antibody Uptake in Recurrent High-Grade Glioma Imaged with 89Zr-Fresolimumab PET. J Nucl Med 56: 1310-1314, 2015.