Journal of Cell Science & Therapy

Wu et al., J Cell Sci Ther 2015, 6:3
DOI: 10.4172/2157-7013.1000207

Review Article Open Access

Targeting Glioma Stem Cells for Therapy: Perspectives and Challenges

Shaofang Wu, Norihiko Saito, W.K. Alfred Yung and Dimpy Koul*

Brain Tumor Center, Departments of Neuro-Oncology, The University of Texas M. D. Anderson Cancer Center, Houston, Texas, USA

***Corresponding author:** Dimpy Koul, Department of Neuro-Oncology, Unit 1001, The University of Texas M. D. Anderson Cancer Center, 1515 Holcombe Blvd., Houston, TX 77030, USA, Tel: 713-834-16202; Fax: 713-794-4999; E-mail: dkoul@mdanderson.org

Rec date: Nov 4, 2014; **Acc date:** June 15, 2015; **Pub date:** June 18, 2015

Abstract

Glioblastoma multiforme (GBM, WHO grade IV) is the most aggressive and lethal subtype of primary brain tumor with a median overall survival of 15 months from the time of diagnosis. Recent studies indicate that some neoplastic cells within human high-grade glioma have the capacity for self-renewal and multi-lineage differentiation, properties associated with normal neural stem cells. These stem-like tumor cells known as GBM stem cells (GSCs) are responsible for tumor progression and recurrence. Therefore, GSCs are attractive targets for novel glioma therapies. Mounting studied have evidenced that some molecular signaling pathways (including EGFR, PI3K, PDGFR, TGF and Notch.), which are critically important for GSCs self-renew and proliferation, are activated by genetically mutation or amplification in GSCs. Targeting these molecules might be promising novel treatment strategies to eliminate GSCs, however, crosstalk and compensation between different signaling pathways as well as intratumoral heterogeneity make it more complicate and a big challenge.

Keywords: Glioma stem cells; Neoplastic cells; Intratumoral heterogeneity; Multi-lineage

Introduction

GBM is poorly differentiated and resistant to traditional chemotherapeutic and radiotherapeutic approaches. The current standard treatment for GBM includes surgical resection of the primary tumor followed by radiotherapy and chemotherapy with temozolomide (TMZ) [1,2]. However, despite this aggressive treatment regimen, the prognosis for this disease remains poor, with a median overall survival of less than 15 months [3-5]. Recent evidence indicates that GBMs include a population of glioma stem cells (GSCs) that are responsible for the initiation, propagation, and recurrence of gliomas, and thus represent a major target of cancer therapy [6-9].

Although GSCs share properties with NSCs, subtle differences reside at both genetic and epigenetic levels, which lead to immortal growth, a block in differentiation and invasiveness.

Verhaak et al. clustered 1,740 genes expression data from 202 samples and divided GBM into four subtypes-proneural, neural, classical and mesenchymal--with 210 gene signatures for each subtype [10]. PDGFRA abnormalities, isocitrate dehydrogenase 1 (IDH1) and TP53 mutations are enriched in Proneural subtype. Mesenchymal subtype is associated with deletions of NF1. Amplification EGFR was observed in 97% of the Classical subtype. These signatures identified in different subtypes could be regarded either as biomarkers for prognosis or as potential therapeutic targets. This clustering is close to the previously described molecular subclasses of high-grade glioma [11]. Whether these GBM subgroups origin from the same initiating cell like previously proposed neural stem cell [12] or multiple stem cell-like populations remains to be established.

Recently, studies have identified several critical signaling pathways, including TGF-β, Notch, PI3K, activated in GSCs and are therefore promising therapeutic targets for GBM (Figure 1). Novel molecular strategies have been designed both in preclinical and clinical settings to target and interfere with those pathways with the hope to achieve longer patient survival. This review will highlight some of the signaling pathways activated in GSCs and important to the survival and proliferation of GSCs with emphasis on possible strategies for targeting these pathways for GBM therapy.

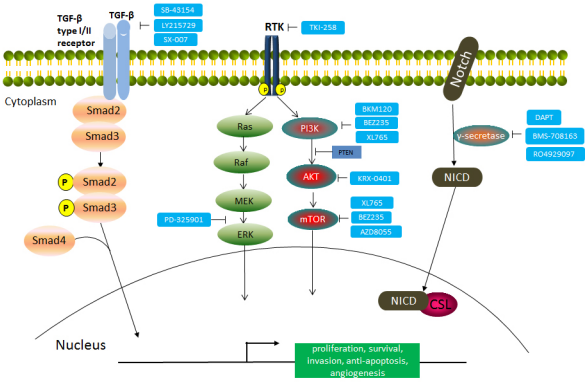

Figure 1: Schematic representation of the PI3K, MEK, Notch and TGFβ pathway with their inhibitors. TGF-β binds to and activates heterotetrameric TGF-β receptors, and leads to activation of Smad2 and Smad3. The phosphorylated Smad2/Smad3 complex interacts with Smad4 to form a heteromeric complex that translocates to the nucleus and regulates the transcription of a diverse array of genes. Both MEK-ERK signaling and PI3K signaling are activated by RTK. Notch is cleaved by γ-secretase and releases Notch intracellular domain (NICD). The NICD translocates to the nucleus, interacts with the CSL, and then converts CSL from a transcriptional repressor to an activator.

Notch Signaling

Notch signaling is a conserved pathway that plays a critical role in multiple cellular processes involved in cancer and development, including cell proliferation, survival, angiogenesis, migration, differentiation, and cell fate decisions [13,14].

Four Notch receptors (Notch1-4) and 5 Notch ligands (Jagged1 and Jagged2 [JAG1 and JAG2] and Delta-like 1, 3, and 4 [DLL-1, 3, and 4]) have been identified in mammals. Notch receptors are transmembrane receptors that contain both intracellular and extracellular regions. The extracellular regions of Notch receptors contain epidermal growth factor (EGF)-like repeats that are essential for ligand binding and juxtamembrane repeat motifs known as Lin-12/Notch repeats [15]. Notch signaling requires several proteolytic cleavages before activation. The first cleavage (at site S1) is regulated by a furin-like convertase [16,17]. Once the Notch receptor is activated by its ligand, cleavage occurs at the second proteolytic cleavage site (S2) of the Notch extracellular region by ADAM metallopeptidase domain 17 (ADAM17), also known as tumor necrosis factor-α-converting enzyme, an enzyme that belongs to the ADAM protein family of disintegrins and metalloproteases. Notch ligand binding can also induce a third proteolytic cleavage at the S3 site of the transmembrane region by γ-secretase; this cleavage releases the Notch intracellular domain (NICD) [18]. The NICD translocates to the nucleus, interacts with the DNA-binding protein Cbf1/Suppressor of hairless/Lag-1 (CSL), promotes dissociation of corepressor complexes from CSL, and then converts CSL from a transcriptional repressor to an activator [19,20].

Previous studies indicate that Notch signaling contributes to GBM progression. Kanamori et al. found that components of Notch signaling pathways were overexpressed in 51% of 35 primary GBMs, and genetic or pharmacological inhibition of Notch signaling selectively inhibited growth in cells exhibiting Notch pathway deregulation [21]. Consistently, Zhang et al. also reported that activation of Notch signaling promotes cell proliferation and colony formation in the human GBM cell line SHG-44 [22]. In addition, Notch cross talk with other signaling pathways promote GBM progression. For example, Notch1 promotes the invasive and migratory properties of GBM cells by stimulating β-catenin and NF-κB signaling [23]. Notch1 also mediates GBM cell proliferation and survival through the Akt-mammalian target of rapamycin (mTOR) signaling axis [24]. Blocking Notch pathway by γ-secretase inhibitors was reported to deplete CD133+ GBM cells, reduce neurosphere growth, and inhibit xenograft tumor growth through decreased Akt and STAT3 phosphorylation [25]. Combination of the Notch inhibitor MRK003 and the Akt inhibitor MK-2206 effectively inhibited GBM invasiveness [26].

In GSCs, the Notch signaling pathway plays an important role in cell proliferation. SiRNA targeting Notch-1 attenuated GSC proliferation *in vitro* and tumor growth *in vivo* [27]. Notch signaling pathway showed critical role in GSC maintenance and presented GBM subtype specificity. Saito et al. used several γ-secretase inhibitors (DAPT, BMS-708163, and RO4929097) to investigate the effects of Notch pathway inhibition in 16 GSC cell lines [15]. Interestingly, only proneural GSCs were sensitive to γ-secretase inhibitors. Further study found that 17 genes representing active Notch signaling components including NOTCH1, NOTCH3, hairy and enhancer of split 1 (HES1), mastermind-like 1 (MAML1), DLL-3, and JAG2 were highly expressed in proneural GSCs. Analysis of the Cancer Genome Atlas expression data identified a large percentage (43.9%) of GBM tumors with proneural expression signatures showing high Notch pathway activation; these findings suggest that γ-secretase inhibitors might be used for treating that particular GBM subtype [15]. Moreover, γ-secretase inhibitors impaired GSC maintenance and induced GSC differentiation [15]. These data suggest that pharmacological inhibition of the Notch signaling pathway may substantially improve GBM patient outcomes, particularly in patients with the proneural GBM subtype.

In addition, Notch signaling pathway was related with resistance to traditional GBM therapy. For example, Notch signaling promotes GSC radioresistance as γ-secretase inhibitors rendered GSCs more sensitive to radiation, and knockdown of Notch1 or Notch2 also resensitized GSCs to radiation and inhibited xenograft tumor formation [28]. Inhibition of the Notch signaling pathway also increased the sensitivity of CD133+ GSCs to TMZ [29]. These two studies suggested a cross talk between Notch signaling and DNA damage signaling, although the underlying mechanisms remain to be further elucidated.

PI3K/Akt Signaling

Phosphatidylinositol 3-kinase (PI3K) pathway plays extensive role in multiple cellular functions such as cell proliferation, differentiation, motility, survival, and metabolism, and is one of the most commonly activated signaling pathway in human cancers [30,31]. This pathway therefore presents both an opportunity and a challenge for cancer therapy.

PI3K is activated by receptor tyrosine kinases (RTKs), such as EGFR. PI3K binds to phosphotyrosine residues on activated RTKs, and induces a conformational change of p85, then releases the inhibition from the catalytic subunit p110. Activated PI3K phosphorylates phosphatidylinositol 4,5-bisphosphate (PIP2) to phosphatidylinositol 3,4,5-trisphosphate (PIP3), which further recruits Akt to cell membrane and Akt is then phosphorylated by PDK1 (3-phosphoinositide-dependent kinase) and the mammalian target of rapamycin complex 2 (mTORC2). After activation, AKT is recruited to the cellular membrane and, in turn, phosphorylates, activates, or inhibits numerous target proteins involved in cell growth, proliferation, motility, and survival [30,32].

EGFR is amplified in approximately 45% of GBM cases and in 97% of the classical GBM [10,33]. PIK3CA (the p110a subunit of PI3K) or PIK3R1 (the p85 regulatory subunit of PI3K) is amplified or mutated in approximately 15% of GBM cases [34-36]. PTEN, the negative regulator of PI3K pathway, is inactivated by mutations, chromosomal deletions, or epigenetic gene silencing in about 40% of GBM cases [37]. Combined together, the aberrant activation of PI3K pathway by alterations of EGFR, PTEN, PIK3CA or PI3KR1 genes have been detected in over 63% of primary and 31% of secondary GBM [36-38] highlighting the critical role of PI3K pathway in GBM.

Recently, **Brennan** group reported that independent focal amplification of two or more RTKs (most commonly PDGFRA and EGFR) was identified in 34 of 463 cases in The Cancer Genome Atlas (TCGA) GBM dataset [39]. More interestingly, dual-color fluorescence in situ hybridization (FISH) performed on eight samples with EGFR and PDGFRA amplification revealed distinct tumor cell subpopulations amplified for only one RTK [39]. This important discovery may partially explain the failure of clinical trials of inhibitors targeting EGFR and PDGFR in GBM. Simultaneous targeting both EGFR and PDGFR would be necessary for inhibiting PI3 kinase pathway in GBM with intratumoral heterogeneity.

Because of the aberrant hyperactivation of the PI3K pathway in GBM and its pivotal role for tumor progression, inhibition of this pathway components is an attractive target for GBM therapeutics. Many of the PI3K inhibitors currently in pre- or clinical development inhibit all of the catalytic subunit isoforms of class IA PI3Ks (p110a, p110b, and p110g). For example, BKM120 (Novartis), a pan-class I PI3K inhibitor, causes dose-dependent growth inhibition in GBM cell lines (including U87, U251, LN229, LN18, and D54) regardless of their phosphatase and tensin homolog (PTEN) and/or epidermal growth factor receptor (EGFR) status. Furthermore, when given orally to mice with intracerebral tumor xenografts, BKM120 prolonged their survival and was well tolerated [40]. Because BKM120 can cross the blood-brain barrier, it is an attractive option for the treatment of GBM. Similarly, NVP-BEZ235, a dual PI3K/mTOR inhibitor, has antitumor activity in human GBM [41]. Several other PI3K inhibitors are being developed and evaluated [42,43].

Multiple studies have demonstrated that the PI3K/Akt pathway not only promotes GBM progression but also contributes chemoresistance. For example, activation of PI3K signaling supports GBM migration and survival [44] and also enhances chemoresistance to TMZ [45]. Chen et al. found that blockade of the PI3K/Akt pathway by LY294002 enhances the cytotoxicity of TMZ in GBM cells [46]. The PI3K/mTOR dual inhibitor NVP-BEZ235 increased the radio-sensitivity of GSCs *in vitro* by inducing apoptosis and by increasing levels of BID, Bax, and active caspase-3, and decreased level of Bcl-2 [47].

Besides, PI3K/Akt pathway is also activated by and cross talks to other signaling pathways. For example, CD133 phosphorylation mediates CD133's interaction with the p85 subunit of PI3K, activates the PI3K/Akt pathway, and promotes the tumorigenicity of GSCs [48]. ADAM17 induces CD133+ GSC invasion and migration through EGFR/PI3K/Akt pathway activation [49]. The cross talk between PI3K/mTOR pathway and the MEK/ERK pathway was reported to be important for the maintenance of GSCs [50]. When the PI3K pathway is inhibited by BKM120 or NVP-BEZ235, ERK signaling is activated in GSCs, this indicates that ERK signaling may be a mechanism of resistance to PI3K inhibition [51]. Consequently, a combination of NVP-BEZ235 and PD-325901 (an ERK inhibitor) is more effective at inhibiting GSC proliferation than either treatment alone [51]. Taken together, these data highlight the importance of the cross talk of PI3K signaling pathway with other signaling pathways in GBM, and combinational targeting the PI3K pathway with related pathways may be an effective therapeutic strategy in GBM.

TGF-β Signaling

Transforming growth factor beta (TGF-β) belongs to a family of cytokines that includes the bone morphogenic proteins, activins, and nodals, all of which regulate embryonic development and tissue homeostasis [52]. TGF-β binds to and activates heterotetrameric transmembrane serine/threonine kinase receptors composed of the type I and II TGF-β receptors (TβRI and TβRII) [53]. Activated TβRI phosphorylates Smad2 and Smad3, which then interact with Smad4 to form a heteromeric complex that translocates to the nucleus. The Smad complex interacts directly with Smad-binding elements and associates with other transcription factors, coactivators, or corepressors, thus regulating the transcription of a diverse array of genes [54].

Disruption of the TGF-β pathway has been implicated in GBM progression via promotion of cell proliferation, angiogenesis, tumor invasion, metastasis, and immune suppression [55]. Zhang et al. found that the TGF-β expression level was positively correlated with microvascular density and angiogenesis in GBM [56]. Consistently, another study also showed TGF-β promoted angiogenesis via the c-Jun N-terminal kinase pathway and macrophage infiltration in a zebrafish GBM xenograft model [57]. Lu et al. showed that TGF-β can promote invasion and migration of GBM cells through activation of ADAM17 [58]. In addition, another study from the same group indicated that TGF-β-induced microRNA 10a/10b expression promotes GBM cell migration through the suppression of PTEN [59] suggesting a cross talk between TGF-β between PI3K/pTEN/Akt pathway.

A number of studies have shown that TGF-β plays a crucial role in the regulation of GSCs. Ye et al. showed that TGF-β1 can increase the invasiveness of human GBM-derived CD133+ GSCs [60]. Peñuelas et al. found that TGF-β promotes self-renewal of GSCs through Smad-dependent up regulation of leukemia inhibitory factor (LIF) and activation of the Janus kinase (JAK)-STAT pathway [61]. Beside the Smad-dependent pathway, TGF-β also cross talk with other pathway and activated non-Smad signaling to regulate GSCs. Wang et al. demonstrated that TGF-β and interleukin-1β cooperated to induce up regulation of a subset of stemness factor proteins (Bmi-1, Notch2, nestin, and LIF) and induced a GSC phenotype *in vivo* [62]. In addition, autocrine TGF-β signaling was reported to play an important role in maintaining the tumorigenicity of GSCs through the TGF-β/Sox4/Sox2 pathway [63]. These findings suggest that inhibition of TGF-β signaling may inhibit the capacity of GSCs to initiate brain tumors. Indeed, TGF-β receptor inhibitors decreased the CD44(high)/Id1(high) GSC population through the repression of DNA-binding proteins and the transcriptional regulators Id1 and Id3 [64], and treatment with LY364947, a selective ATP-competitive inhibitor of TGF-βRI, reversed GSC radiation resistance and improve therapeutic response in patients [65].

Conclusions and Perspectives

GBM is a very complex tumor composed of a heterogeneous mix of cells including GSCs. GBM recurrence is attributed to GSC resistance to conventional radiation therapy and chemotherapy. Therefore, current treatments targeting the bulk of the tumor are insufficient. However, therapies that directly target GSC also failed in clinical trials. There are several possibilities: 1) crosstalk and compensation between different signaling pathways: complimentary or collateral pathways are activated upon targeting one specific signaling and used by GSCs as predominant mechanism of escape. A study by Sunayama et al. showed that suppression of either the MEK/ERK or PI3K/mTOR pathway in glioblastoma stem-like cells induced activation of the other collateral pathway [50].

The study also indicated mutually inhibitory crosstalk between these two pathways, giving a clue that and combinational disruption of these pathways would be a rational and effective strategy for GBM therapy [50]. 2) GBM is characterized by a high degree of intratumoral heterogeneity. Amplification of multiple RTKs, either co-amplification or independent focal amplification of two or more RTKs (most commonly PDGFRα and EGFR), was identified within individual GBMs and simultaneous inhibition of both EGFR and PDGFR was necessary for disruption of PI3 kinase pathway activity in the mixed population [39]. 3) In gliomas, it is hypothesized that some differentiated tumor cells have the ability to revert to stem cell-like

cells [66,67]. In this case, combination of radiation/chemotherapy against the bulk tumor together with targeting signaling pathways aberrantly activated and required for the function of GSC population would be more reasonable and effective treatment.

Compound	Manufacture	Target	Comments	Trial number
XL147	Exelixis	pan-PI3K	completed phase I	NCT01240460
PX866	Oncothyreon	pan-PI3K	ongoing phase II for patients with glioblastoma multiforme at time of first relapse or progression	NCT01259869
BKM120	Novartis	pan-PI3K	ongoing phase II trial for patients with recurrent Glioblastoma and activated PI3K pathway	NCT01339052
XL765	Exelixis	PI3K/mTOR	completed phase I	NCT01240460
KRX-0401	Keryx	AKT	ongoing phase II trial for recurrent/progressive malignant Gliomas	NCT00590954
AZD8055	AstraZeneca	mTORC1/2	completed phase I trial for adults with recurrent gliomas	NCT01316809
RO4929097	Roche	NOTCH	ongoing phase II and pharmacodynamic trial for patients with recurrent/progressive Glioblastoma	NCT01122901
LY2157299	Eli Lilly	TGFβ	ongoing phase 1b/2a study combining LY2157299 with standard Temozolomide-based Radiochemotherapy in patients with newly diagnosed malignant glioma	NCT01220271
TKI-258	Novartis	RTK	ongoing phaseI/II study for patients with recurrent or progressive Glioblastoma	NCT01753713 NCT01972750
Erlotinib	Genentech/ Roche	EGFR	pilot study of EGFR inhibition using high dose administration of Erlotinib weekly for recurrent malignant Gliomas with EGFR variant III mutation	NCT01257594
BSI-201	Sanofi-Aventis	PARP-1	ongoing phase I/II trial of BSI-201 plus temozolomide in patients with newly diagnosed malignant Glioma	NCT00687765
AZD2281	AstraZeneca	PARP	ongoing phase I trial of olaparib in combination with extended low-dose oral Temozolomide in patients with relapsed Glioblastoma	NCT01390571
LDE225	Novartis	Smoothened	ongoing phase Ib of oral LDE225 in combination with BKM 120 in patients with advanced solid tumors including recurrent Glioblastoma Multiforme	NCT01576666

Table 1: Clinical trials of targeted therapies in GBM.

Additional attention should also be paid to interaction between tumor stem cells and the microenvironment (endothelial cells, extracellular matrix, cytokines, nitric oxide, oxygen levels) which promote self-renewal of GSCs and might influence the response of tumor stem cells to targeting therapies [68,69]. Therefore, an optimal therapeutic strategy should be directed against GSCs functions, in combination with therapies possibly affecting microenvironmental factors that sustain GSCs.

Nevertheless, recent studies has made tremendous progress in understanding the signaling pathways involved in GSCs, and various approaches towards targeted therapy have been tested (Table 1). As discussed above, results in preclinical models targeting PI3K, Notch, TGF-β signaling alone or in combination with traditional therapy (radiotherapy or TMZ) were proved to be effective to eliminate GSCs and inhibit GBM progression. Further *in vitro* and *in vivo* experiments as well as clinical trials are warranted to assess the success of these approaches. To identify which patient populations will benefit from these inhibitors and to optimize the combination of inhibitors of two or more pathways, as well as combination of targeting specific pathway with conventional radiation therapy, chemotherapy, and anti-angiogenic therapies based on genetic and molecular characteristics of the tumors would be challenges but also promising to improve GBM therapy.

References

1. Behin A, Hoang-Xuan K, Carpentier AF, Delattre JY (2003) Primary brain tumours in adults. Lancet 361: 323-331.
2. Stupp R, Mason WP, van den Bent MJ, Weller M, Fisher B, et al. (2005) Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. N Engl J Med 352: 987-996.
3. Johnson DR, O'Neill BP (2012) Glioblastoma survival in the United States before and during the temozolomide era. J Neurooncol 107: 359-364.
4. Davis FG, Freels S, Grutsch J, Barlas S, Brem S (1998) Survival rates in patients with primary malignant brain tumors stratified by patient age and tumor histological type: an analysis based on Surveillance, Epidemiology, and End Results (SEER) data, 1973-1991. J Neurosurg. 88: 1-10.
5. Carro MS, Lim WK, Alvarez MJ, Bollo RJ, Zhao X, et al. (2010) The transcriptional network for mesenchymal transformation of brain tumours. Nature 463: 318-325.
6. Rich JN (2007) Cancer stem cells in radiation resistance. Cancer Res 67: 8980-8984.

7. Sanai N, Alvarez-Buylla A, Berger MS (2005) Neural stem cells and the origin of gliomas. N Engl J Med 353: 811-822.
8. Singh SK, Clarke ID, Hide T, Dirks PB (2004) Cancer stem cells in nervous system tumors. Oncogene 23: 7267-7273.
9. Stiles CD, Rowitch DH (2008) Glioma stem cells: a midterm exam. Neuron 58: 832-846.
10. Verhaak RG, Hoadley KA, Purdom E, Wang V, Qi Y, et al. (2010) Integrated genomic analysis identifies clinically relevant subtypes of glioblastoma characterized by abnormalities in PDGFRA, IDH, EGFR, and NF1. Cancer Cell 17: 98-110.
11. Phillips HS, Kharbanda S, Chen R, Forrest WF, Soriano RH, et al. (2006) Molecular subclasses of high-grade glioma predict prognosis, delineate a pattern of disease progression, and resemble stages in neurogenesis. Cancer Cell 9: 157-73.
12. Galli R, Binda E, Orfanelli U, Cipelletti B, Gritti A, et al. (2004) Isolation and characterization of tumorigenic, stem-like neural precursors from human glioblastoma. Cancer Res 64: 7011-7021.
13. Kopan R, Ilagan MX (2009) The canonical Notch signaling pathway: unfolding the activation mechanism. Cell 137: 216-233.
14. Kageyama R, Ohtsuka T (1999) The Notch-Hes pathway in mammalian neural development. Cell Res 9: 179-188.
15. Saito N (2014) A High Notch Pathway Activation Predicts Response to gamma Secretase Inhibitors in Proneural Subtype of Glioma Tumor-Initiating Cells. Stem Cells 32: 301-312.
16. Logeat F, Bessia C, Brou C, LeBail O, Jarriault S, et al. (1998) The Notch1 receptor is cleaved constitutively by a furin-like convertase. Proc Natl Acad Sci U S A 95: 8108-8112.
17. Stockhausen MT, Kristoffersen K, Poulsen HS (2010) The functional role of Notch signaling in human gliomas. Neuro Oncol 12: 199-211.
18. Brou C, Logeat F, Gupta N, Bessia C, LeBail O, et al. (2000) A novel proteolytic cleavage involved in Notch signaling: the role of the disintegrin-metalloprotease TACE. Mol Cell 5: 207-216.
19. Kao HY, Ordentlich P, Koyano-Nakagawa N, Tang Z, Downes M, et al. (1998) A histone deacetylase corepressor complex regulates the Notch signal transduction pathway. Genes Dev 12: 2269-2277.
20. Guruharsha KG, Kankel MW, Artavanis-Tsakonas S (2012) The Notch signalling system: recent insights into the complexity of a conserved pathway. Nat Rev Genet 13: 654-666.
21. Kanamori M, Kawaguchi T, Nigro JM, Feuerstein BG, Berger MS, et al. (2007) Contribution of Notch signaling activation to human glioblastoma multiforme. J Neurosurg 106: 417-427.
22. Zhang XP, Zheng G, Zou L, Liu HL, Hou LH, et al. (2008) Notch activation promotes cell proliferation and the formation of neural stem cell-like colonies in human glioma cells. Mol Cell Biochem 307: 101-108.
23. Zhang X, Chen T, Zhang J, Mao Q, Li S, et al. (2012) Notch1 promotes glioma cell migration and invasion by stimulating Î²-catenin and NF-Î°B signaling via AKT activation. Cancer Sci 103: 181-190.
24. Zhao N, Guo Y, Zhang M, Lin L, Zheng Z (2010) Akt-mTOR signaling is involved in Notch-1-mediated glioma cell survival and proliferation. Oncol Rep 23: 1443-1447.
25. Fan X, Khaki L, Zhu TS, Soules ME, Talsma CE, et al. (2010) NOTCH pathway blockade depletes CD133-positive glioblastoma cells and inhibits growth of tumor neurospheres and xenografts. Stem Cells 28: 5-16.
26. Jin R, Nakada M, Teng L, Furuta T, Sabit H, et al. (2013) Combination therapy using Notch and Akt inhibitors is effective for suppressing invasion but not proliferation in glioma cells. Neurosci Lett 534: 316-321.
27. Wang J, Wang C, Meng Q, Li S, Sun X, et al. (2012) siRNA targeting Notch-1 decreases glioma stem cell proliferation and tumor growth. Mol Biol Rep 39: 2497-2503.
28. Wang J, Wakeman TP, Lathia JD, Hjelmeland AB, Wang XF, et al. (2010) Notch promotes radioresistance of glioma stem cells. Stem Cells 28: 17-28.
29. Ulasov IV, Nandi S, Dey M, Sonabend AM, Lesniak MS (2011) Inhibition of Sonic hedgehog and Notch pathways enhances sensitivity of CD133(+) glioma stem cells to temozolomide therapy. Mol Med 17: 103-112.
30. Engelman JA (2009) Targeting PI3K signalling in cancer: opportunities, challenges and limitations. Nat Rev Cancer 9: 550-562.
31. Lei Z, Tan IB, Das K, Deng N, Zouridis H, et al. (2013) Identification of molecular subtypes of gastric cancer with different responses to PI3-kinase inhibitors and 5-fluorouracil. Gastroenterology. 145: 554-65.
32. Vivanco I, Sawyers CL (2002) The phosphatidylinositol 3-Kinase AKT pathway in human cancer. Nat Rev Cancer 2: 489-501.
33. Mellinghoff IK, Wang MY, Vivanco I, Haas-Kogan DA, Zhu S, et al. (2005) Molecular determinants of the response of glioblastomas to EGFR kinase inhibitors. N Engl J Med 353: 2012-2024.
34. Gallia GL, Rand V, Siu IM, Eberhart CG, James CD, et al. (2006) PIK3CA gene mutations in pediatric and adult glioblastoma multiforme. Mol Cancer Res 4: 709-714.
35. Broderick DK, Di C, Parrett TJ, Samuels YR, Cummins JM, et al. (2004) Mutations of PIK3CA in anaplastic oligodendrogliomas, high-grade astrocytomas, and medulloblastomas. Cancer Res 64: 5048-5050.
36. Cancer Genome Atlas Research Network (2008) Comprehensive genomic characterization defines human glioblastoma genes and core pathways. Nature 455: 1061-1068.
37. Masica DL, Karchin R (2011) Correlation of somatic mutation and expression identifies genes important in human glioblastoma progression and survival. Cancer Res 71: 4550-4561.
38. Koul D (2008) PTEN signaling pathways in glioblastoma. Cancer Biol Ther 7: 1321-1325.
39. Szerlip NJ, Pedraza A, Chakravarty D, Azim M, McGuire J, et al. (2012) Intratumoral heterogeneity of receptor tyrosine kinases EGFR and PDGFRA amplification in glioblastoma defines subpopulations with distinct growth factor response. Proceedings of the National Academy of Sciences, 109: 3041-3046.
40. Koul D, Fu J, Shen R, LaFortune TA, Wang S, et al. (2012). Antitumor activity of NVP-BKM120—a selective pan class I PI3 kinase inhibitor showed differential forms of cell death based on p53 status of glioma cells. Clinical Cancer Research, 18: 184-195.
41. Liu TJ, Koul D, LaFortune T, Tiao N, Shen RJ, et al. (2009) NVP-BEZ23, a novel dual phosphatidylinositol 3-kinase/mammalian target of rapamycin inhibitor, elicits multifaceted antitumor activities in human gliomas. Mol Cancer Ther 8: 2204-2210.
42. Wu P, Su Y, Liu X, Zhang L, Ye Y, et al. (2011) Synthesis and biological evaluation of novel 2-arylamino-3-(arylsulfonyl)quinoxalines as PI3KÎ± inhibitors. Eur J Med Chem 46: 5540-5548.
43. Wu P, Su Y, Guan X, Liu X, Zhang J, et al. (2012) Identification of novel piperazinylquinoxaline derivatives as potent phosphoinositide 3-kinase (PI3K) inhibitors. PLoS One 7: e43171.
44. Lötsch D, Steiner E, Holzmann K, Spiegl-Kreinecker S, Pirker C, et al. (2013) Major vault protein supports glioblastoma survival and migration by upregulating the EGFR/PI3K signalling axis. Oncotarget 4: 1904-1918.
45. Zhang LH, Yin AA, Cheng JX, Huang HY, Li XM, et al. (2015) TRIM24 promotes glioma progression and enhances chemoresistance through activation of the PI3K/Akt signaling pathway. Oncogene 34: 600-610.
46. Chen L, Han L, Shi Z, Zhang K, Liu Y, et al. (2012) LY294002 enhances cytotoxicity of temozolomide in glioma by down-regulation of the PI3K/Akt pathway. Mol Med Rep 5: 575-579.
47. Wang WJ, Long LM, Yang N, Zhang QQ, Ji WJ, et al. (2013) NVP-BEZ23, a novel dual PI3K/mTOR inhibitor, enhances the radiosensitivity of human glioma stem cells in vitro. Acta Pharmacol Sin 34: 681-690.
48. Wei Y, Jiang Y, Zou F, Liu Y, Wang S, et al. (2013) Activation of PI3K/Akt pathway by CD133-p85 interaction promotes tumorigenic capacity of glioma stem cells. Proc Natl Acad Sci U S A 110: 6829-6834.
49. Chen X, Chen L, Chen J, Hu W, Gao H, et al. (2013) ADAM17 promotes U87 glioblastoma stem cell migration and invasion. Brain Res 1538: 151-158.

50. Sunayama J, Matsuda K, Sato A, Tachibana K, Suzuki K, et al. (2010) Crosstalk between the PI3K/mTOR and MEK/ERK pathways involved in the maintenance of self-renewal and tumorigenicity of glioblastoma stem-like cells. Stem Cells 28: 1930-1939.
51. Wu S (2013) Identification of molecular mechanisms of resistance to PI3K pathway inhibition to improve therapeutic targeting of glioblastoma. Neuro Oncology 15: 30.
52. Seoane J (2006) Escaping from the TGFbeta anti-proliferative control. Carcinogenesis 27: 2148-2156.
53. Xu J, Lamouille S, Derynck R (2009) TGF-beta-induced epithelial to mesenchymal transition. Cell Res 19: 156-172.
54. Zavadil J, Böttinger EP (2005) TGF-beta and epithelial-to-mesenchymal transitions. Oncogene 24: 5764-5774.
55. Thiery JP (2002) Epithelial-mesenchymal transitions in tumour progression. Nat Rev Cancer 2: 442-454.
56. Zhang J, Yang W, Zhao D, Han Y, Liu B, et al. (2014) Correlation between TSP-, TGF-Î² and PPAR-Î³ expression levels and glioma microvascular density. Oncol Lett 7: 95-100.
57. Yang XJ, Chen GL, Yu SC, Xu C, Xin YH, et al. (2013) TGF-beta1 enhances tumor-induced angiogenesis via JNK pathway and macrophage infiltration in an improved zebrafish embryo/xenograft glioma model. Int Immunopharmacol 15: 191-198.
58. Lu Y, Jiang F, Zheng X, Katakowski M, Buller B, et al. (2011) TGF-Î²1 promotes motility and invasiveness of glioma cells through activation of ADAM17. Oncol Rep 25: 1329-1335.
59. Liu S, Sun J, Lan Q (2013) TGF-I²-induced miR10a/b expression promotes human glioma cell migration by targeting PTEN. Mol Med Rep 8: 1741-1746.
60. Ye XZ, Xu SL, Xin YH, Yu SC, Ping YF, et al. (2012) Tumor-associated microglia/macrophages enhance the invasion of glioma stem-like cells via TGF-Î²1 signaling pathway. J Immunol 189: 444-453.
61. Peñuelas S, Anido J, Prieto-Sánchez RM, Folch G, Barba I, et al. (2009) TGF-beta increases glioma-initiating cell self-renewal through the induction of LIF in human glioblastoma. Cancer Cell 15: 315-327.
62. Wang L, Liu Z, Balivada S, Shrestha T, Bossmann S, et al. (2012) Interleukin-1Î² and transforming growth factor-Î² cooperate to induce neurosphere formation and increase tumorigenicity of adherent LN-229 glioma cells. Stem Cell Res Ther 3: 5.
63. Ikushima H, Todo T, Ino Y, Takahashi M, Miyazawa K, et al. (2009) Autocrine TGF-beta signaling maintains tumorigenicity of glioma-initiating cells through Sry-related HMG-box factors. Cell Stem Cell 5: 504-514.
64. Anido J, Sáez-Borderías A, Gonzàlez-Juncà A, Rodón L, Folch G, et al. (2010) TGF-Î² Receptor Inhibitors Target the CD44(high)/Id1(high) Glioma-Initiating Cell Population in Human Glioblastoma. Cancer Cell 18: 655-668.
65. Hardee ME, Marciscano AE, Medina-Ramirez CM, Zagzag D, Narayana A, et al. (2012) Resistance of glioblastoma-initiating cells to radiation mediated by the tumor microenvironment can be abolished by inhibiting transforming growth factor-beta. Cancer Res 72: 4119-4129.
66. Chen R, Nishimura MC, Bumbaca SM, Kharbanda S, Forrest WF, et al. (2010) A hierarchy of self-renewing tumor-initiating cell types in glioblastoma. Cancer Cell 17: 362-375.
67. Gupta PB, Chaffer CL, Weinberg RA (2009) Cancer stem cells: mirage or reality? Nat Med 15: 1010-1012.
68. Gürsel DB, Berry N, Boockvar JA (2012) The contribution of Notch signaling to glioblastoma via activation of cancer stem cell self-renewal: the role of the endothelial network. Neurosurgery 70: N19-21.
69. Zhu TS, Costello MA, Talsma CE, Flack CG, Crowley JG, et al. (2011) Endothelial cells create a stem cell niche in glioblastoma by providing NOTCH ligands that nurture self-renewal of cancer stem-like cells. Cancer Res 71: 6061-6072.