Cancer Chemotherapy and Pharmacology
https://doi.org/10.1007/s00280-020-04109-w

REVIEW ARTICLE

Advances in histone deacetylase inhibitors in targeting glioblastoma stem cells

R. Gajendra Reddy[1] · Unis Ahmad Bhat[1] · Sumana Chakravarty[2,3] · Arvind Kumar[1,3]

Received: 19 March 2020 / Accepted: 26 June 2020
© Springer-Verlag GmbH Germany, part of Springer Nature 2020

Abstract
Glioblastoma multiforme (GBM) is a lethal grade IV glioma (WHO classification) and widely prevalent primary brain tumor in adults. GBM tumors harbor cellular heterogeneity with the presence of a small subpopulation of tumor cells, described as GBM cancer stem cells (CSCs) that pose resistance to standard anticancer regimens and eventually mediate aggressive relapse or intractable progressive GBM. Existing conventional anticancer therapies for GBM do not target GBM stem cells and are mostly palliative; therefore, exploration of new strategies to target stem cells of GBM has to be prioritized for the development of effective GBM therapy. Recent developments in the understanding of GBM pathophysiology demonstrated dysregulation of epigenetic mechanisms along with the genetic changes in GBM CSCs. Altered expression/activity of key epigenetic regulators, especially histone deacetylases (HDACs) in GBM stem cells has been associated with poor prognosis; inhibiting the activity of HDACs using histone deacetylase inhibitors (HDACi) has been promising as mono-therapeutic in targeting GBM and in sensitizing GBM stem cells to an existing anticancer regimen. Here, we review the development of pan/selective HDACi as potential anticancer agents in targeting the stem cells of glioblastoma as a mono or combination therapy.

Keywords Glioblastoma multiforme · Cancer stem cell · Epigenetic therapeutics · Histone deacetylase inhibitors

Introduction

Glioblastoma multiforme (GBM), most frequently reported primary brain malignancy categorized as GBM IDH-wild type, GBM IDH-mutant, and GBM NOS tumors (WHO classification), is a lethal and aggressive grade IV glioma (WHO classification) with global prevalence [1–4]. GBM comprises about 16% of all primary brain tumors and constitutes a major part (about 54%) of gliomas with a median survival of 15 months post diagnosis [2, 3]. GBM is mostly managed in a case-dependent manner: contemporary treatment regimen is mostly palliative medical care and includes maximal surgical resection of tumor, followed by concomitant radiation and Temozolomide (TMZ) therapy, and long-term maintenance with adjuvant Temozolomide chemotherapy [5–7]. Despite some therapeutic advancement in the anticancer treatment of non-CNS cancers, GBM tumor has remained medically intractable due to impediments posed by a limited understanding of GBM microenvironment intricacies and restricted permeability of anticancer drugs to brain and GBM tumors [8–10]. Furthermore, similar to the presence of heterogeneity in non-CNS cancers [11–13], there is mounting evidence that shows the presence of cellular heterogeneity in GBM [14–18]and several cellular studies have established the existence of a distinct subpopulation of cancer stem cells (stem cell-like cancer cells or tumor stem cells or tumor-initiating cells) within GBM tumors [19–21]. Such Glioblastoma CSCs are highly potent in tumor initiation, progression, maintenance, invasion, angiogenesis, immune response modulation, intracellular drug efflux, and possess efficient metabolic plasticity and repair machinery [21–28]. These characteristics of GBM stem cells pose greater therapeutic resistance to existing anticancer therapeutics [23, 24],

R. Gajendra Reddy and Unis Ahmad Bhat are equal contribution.

✉ Arvind Kumar
akumar@ccmb.res.in

[1] CSIR-Centre for Cellular and Molecular Biology, Habsiguda, Uppal Road, Hyderabad 500007, Telangana, India

[2] Applied Biology Division, CSIR-Indian Institute of Chemical Technology, Tarnaka, Uppal Road, Hyderabad 500007, Telangana, India

[3] Academy of Scientific and Innovative Research (AcSIR), Ghaziabad 201002, Uttar Pradesh, India

and necessitates the development of alternative therapeutic strategies to effectively treat GBM and improve quality of life post-diagnosis [19, 28]. There is growing interest to augment current treatment modalities by targeting these CSCs with HDACito develop better and effective anticancer therapeutics for GBM treatment [29, 30]. In this review, we have focused on the use of histone deacetylase inhibitors (HDACi)—an epigenetic drug in targeting GBM stem cells for the development of effective anticancer therapeutics.

CSCs and GBM

Cellular heterogeneity and hierarchy of cells in hematological malignancies and other cancers have been well established [11–17, 31, 32]. Recent application of technological advancements of single-cell sequencing experiments to cancer research has been pivotal in advancing molecular understanding of cellular heterogeneity in cancers, provided a foundational basis for the phylogeny of cancer cells and augmented clonal evolution concept in cancers viz. copy number evolution and point mutation evolution in tumors of different origins [12, 31, 33–43]. Such studies have been instrumental in identifying a rare population of cells (usually less than 1% of cells and characterized as cancer stem cells) within cancer mass and have demonstrated the presence of common founder mutation for different lineage trajectories within cancer tissues [12, 35, 37]. Many other studies have also described similar rare cell sub-populations within cancer mass as CSCs [21, 44–51]. These cells have been shown to possess self-renewal properties [52–54], tumor-initiating potentials [52, 55]and are often implicated in treatment resistance to a wide range of anticancer agents in many cancers [22, 24, 56, 57]. Identification and characterization of these CSCs in solid tumors have remained debatable due to usage of conventional marker-dependent strategies that could not account for their small number [58–61], high variability in marker gene expression [62–66], overlapping molecular profiles with other cancer cells [48, 67, 68], functional heterogeneity [69], metabolic plasticity [70–72] and potential to switch from differentiated tumor cells, and vice versa [36, 73–75]. Despite these challenges, a large number of reports [76–82] have described the identification and characterization of CSCs at the molecular level to much finer details. Similar to the presence of CSCs in non-CNS cancers, GBM also nurtures subpopulations of cells that have been characterized as GBM stem cells [21, 83–85] and have been shown to be potentially originating from neural stem/progenitor cells [86–88], non-stem cancer cells and from local brain cells [75, 89–92] (Fig. 1). GBM CSCs express a wide range of markers like CD133 [21, 93–95], SOX2 [96–98], Nestin [95, 99], Musashi1 [100, 101], Bmi-1 [102–104], SALL4 [105, 106], OCT-4 [97, 107, 108], STAT3 [109], NANOG [110, 111], c-Myc [112, 113], and some of these markers are common either to embryonic stem cells and/or to adult neural stem cells [114, 115]. Although GBM stem cells share many markers with neural stem cells and non-stem cancer cells, still GBM stem cells are distinguishable due to the expression of embryonic stem cell markers [114] and dysregulation of specific signaling pathwaysvizWnt, Hedgehog-Gli, RTK-Akt, STAT3,TGF-β Notch signaling and bone morphogenetic protein (BMPs) pathway [28, 75, 116–119]. In addition to the expression of

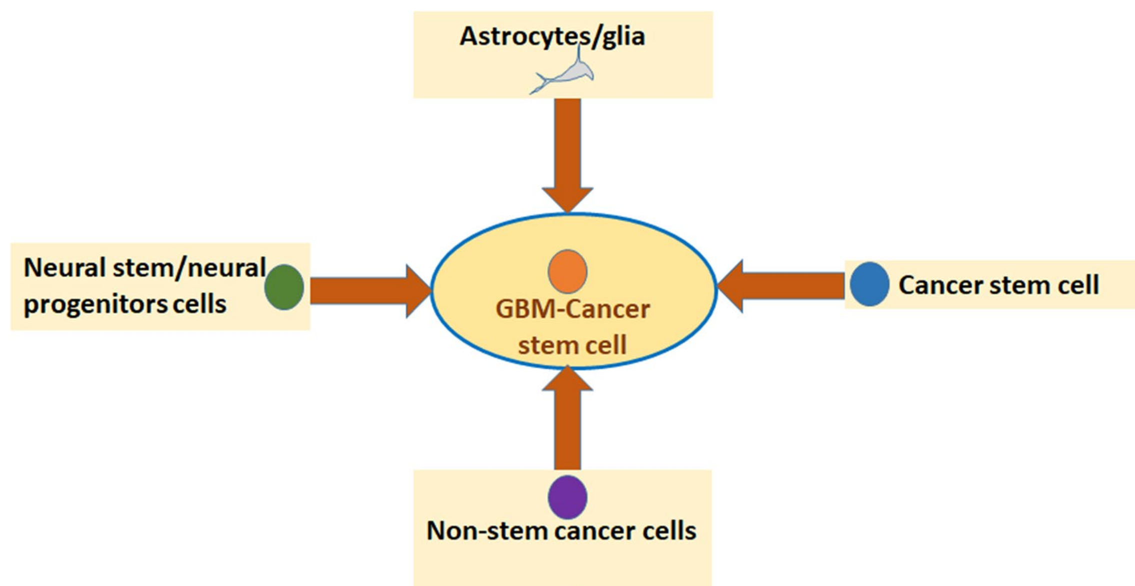

Fig. 1 Origin of Cancer stem cells in GBM tumors. Cancer stem cells formed from aberrant genetic or epigenetic dysregulation of neural stem/progenitors, non stem cancer cells, glia and from cancer stem cells

differential markers than non-stem cancer cells, GBM stem cell-associated genetic and epigenetic [120, 121]signatures provide for high tumorigenicity, capability to resist niche-induced differentiation [117]and for greater therapeutic resistance to conventional anticancer agents [22, 24, 122]. Studies suggest that anticancer treatment results in transient quiescence in GBM stem cells followed by enrichment and/or enhanced proliferation of GBM stem cells, which in turn result in much aggressive, resistant and recurrent glioma [123]. Recent efforts have established the role of epigenetic and chromatin remodeling mechanisms in regulating GBM stem cell transitioning between slow-cycling persistent and fast cycling forms in response to kinase inhibitors, and this transitioning has been shown in mediating resistance to anticancer agents [122]. Next-generation sequencing technologies have been instrumental in identifying distinct epigenome states associated with molecular profiles of GBM stem cells in different transitional states [124]. Along with other regulatory mechanisms, several reports demonstrated differential regulation of histone and non-histone acetylation—a key regulatory modification in GBM tumors [125].

Histone deacetylases in GBM cancer stem cell

HDACs are the epigenetic enzymes that dynamically regulate the acetylation status of histones and non-histone proteins by removing acetyl moieties from specific lysine residues. HDACs mediate deacetylation of histone proteins which in turn alters chromatin conformation dynamics towards transcriptionally non-permissive chromatin resulting in downregulation of gene expression [126]. Furthermore, HDACs regulate various cellular activities by deacetylation of non-histone proteins like STATs, NF-kB, P53, FOXO, HSP90, tubulin, etc.[127, 128]. HDACs are generally categorized into four different classes: class I (HDAC 1, 2, 3 and 8), class II (class IIa – 4, 5, 7, 9 and class IIb- 6, 10), class III (sirt1-7) and class IV (HDAC 11). Among these, class I, class II and class IV are Zinc dependent, while class III is NAD^+ dependent. HDACs have been implicated in many GBM tumors [129–131], hematological [132–136] and in several other solid malignancies [129, 137, 138]. Aberrant HDAC expression/activities [139–142] regulate tumor progression, invasion, poor prognosis and survival outcome [143–145], and changes in HDACs expression level have been reported in several gliomas [141, 143–147]. Furthermore, resistance to anticancer therapy has been associated with the expression of HDAC4, HDAC6 and HDAC8 [130, 131, 143], and Knockdown of HDAC1 and 2 induces anticancer effects in glioma tumors [146]. In addition, differential expression/activity of HDACs has been reported in GBM CSCs compared to non-stem cancer cell or neural stem/progenitor cells and dysregulated HDAC expression has been associated with altered signaling mechanisms like sonic hedgehog (SHH) pathway (essential for stemness, viability and radio-resistance) and in some cases correlates with glioma progression [130, 143]. Furthermore, increased levels of SIRT1, SIRT2 [148, 149], class III (NAD-dependent) HDACs, has been reported in CSC of GBM compared to NSC and normal brain cells. CD133 positive GBM cells express increased levels of SIRT1, SIRT2, SIRT4 and decreased levels of SIRT6 in comparison to CD133 negative populations [148]. In addition, SIRT1 knockdown enhances radio-sensitivity of GBM stem cells and reduces tumor volume with the positive therapeutic outcome on CD133 positive GBM tumors [148]. The interplay of SIRT3 and TRAP1 in GBM CSCs mediates enhanced metabolic plasticity, essential for reducing ROS production, maintaining mitochondrial functions and metabolic adaptions to GBM tumor microenvironment [150]. Thus, the role of HDACs in CSC functions of GBM is intriguing as a potential therapeutic candidate in targeting the CSC and development of better anticancer therapeutics for GBM.

HDAC inhibitors in GBM cancer stem cells

In a pursuit to expand the repertoire of existing anticancer treatment, there have been efforts to sensitize GBM stem cells and restore aberrant gene expression by complementing existing treatment modalities with epigenetic therapeutics [151, 152]. Among various epigenetic targets, targeting Histone deacetylase (HDAC) activity by the use of various small molecule chemical HDACi in combination with existing anticancer treatments has been emerging as a promising anticancer strategy in preclinical studies [153]. HDACi exhibits anticancer function by increasing the acetylation levels of histone and non-histone proteins; eventually enhancing DNA alkylating agent-induced chromatin damage on de-condensed chromatin and also by mitigating cancer-associated gene silencing by regulating the transcriptional activation of various genes involved in apoptosis, cell cycle arrest and proliferation [154, 155]. Research on these lines has resulted in the development of many HDAC inhibitors with different class specificities, and interestingly some of them cross the blood–brain barrier (BBB) at physiologically tolerable concentrations. Several studies have demonstrated the development of pan/selective HDAC inhibition as adjuvant therapy in sensitizing GBM stem cells to existing treatments. Potent anticancer effects of HDACi is being explored at various stages of preclinical and clinical trials as a combination or monotherapy in GBM tumors.

HDACi as a mono-therapeutic in GBM stem cells

HDAC inhibitors have been potential therapeutic candidates for treating GBM tumors and recent molecular research efforts further support the emerging anticancer effects of

various HDACi on GBM. HDACi act as chemo/radio-sensitizers and target GBM CSCs to potentiate anticancer properties of conventional anticancer therapies. As a mono therapeutic anti-GBM agent, HDACi has been shown to reduce cancer stem cell burden in GBM tumors (Fig. 2) by modulating molecular pathways regulating stemness, proliferation, differentiation, cell cycle arrest, apoptosis, autophagy, vasculogenic mimicry of CSCs, etc. [143, 156–158] (Fig. 3).

Vorinostat, also known as suberoylanilidehydroxamic acid (SAHA), is a class I and class II pan HDACi. It has been in clinic for treating cutaneous T-cell lymphoma (CTCL) and is being actively investigated for treating various solid malignancies including GBM. As a mono therapeutic agent, Vorinostat targets both CSCs and non-stem cancer cells of GBM. Orzan et al. (2011) have shown Vorinostat induced downregulation of EZH2 (Enhancer of zeste homolog 2) expression—a PRC (polycomb repressor complex 2) associated main catalytic enzyme – regulates stemness, differentiation and apoptosis in cancer stem cell of GBM. Overall, Vorinostat treatment reduces stemness (low CD133

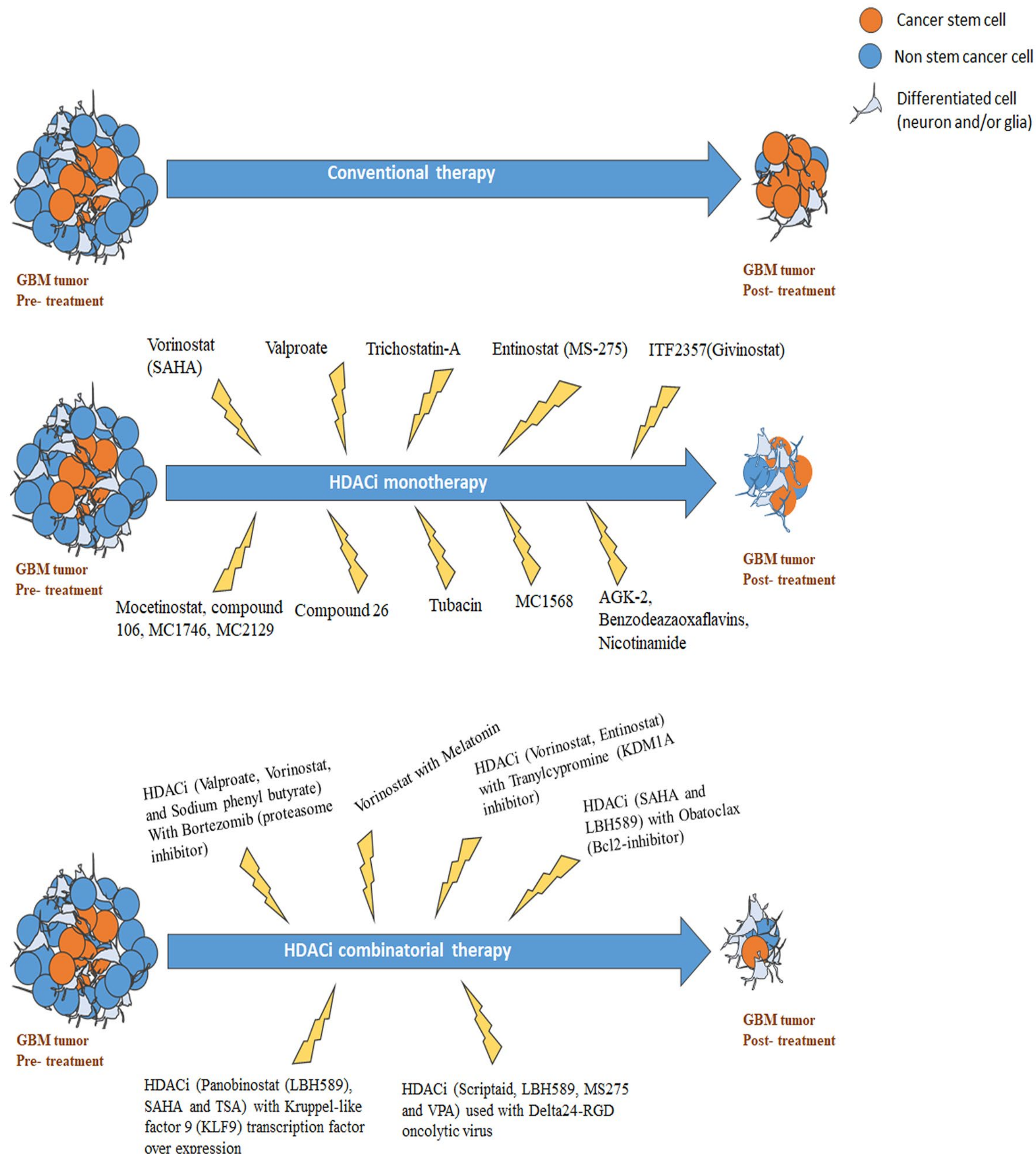

Fig. 2 Therapeutic treatment of GBM with **a** Conventional anticancer therapy (Radiation and Temozolomide), **b** HDACi monotherapy and **c** HDACi combinatorial treatment. The CSCs in GBM were reduced by HDACi monotherapy and combinatorial therapy

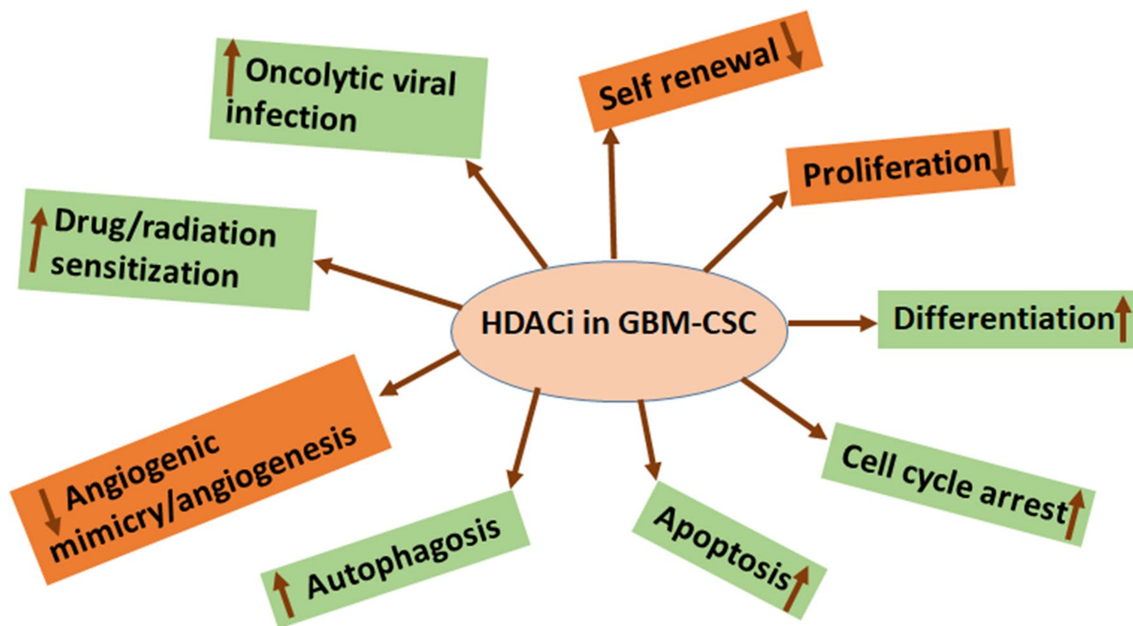

Fig. 3 Therapeutic role of HDACi in cancer stem cells of GBM tumors

markers), promotes differentiation and apoptosis in GBM CSCs [159]. In addition to this, Hsu et al. (2016) reported Vorinostat induced decrease in cell viability, cell growth, sphere and colony formation potential in CSCs of human glioblastoma line U87MG. Vorinostat treatment promotes apoptosis by inducing expression of cleaved PARP and caspase 3. Treatments with milder concentrations of Vorinostat results in cell cycle arrest with more G1 cells compared to S and G2/M cells [156] and has been found to decrease tube formation (vasculogenic mimicry) in GBM patient-derived stem cells [157]. Similar other reports also demonstrated Vorinostat-mediated increase in global histone H4 acetylation levels and significant induction of p21WAF1 and γ-H2AX proteins -markers of cell growth arrest and DNA damage response (DDR pathway), respectively.

Valproate, a short-chain fatty acid, inhibits class I and class IIa HDACs. It has been in clinical usage as an anticonvulsant and a mood stabilizer. Valproate also exhibits its anticancer activity and capacity to target the CSCs of GBM. Riva et al. (2016) have shown temporal and cell line-dependent effects of Valproate on GBM CSCs. Valproate alone is a potent cytotoxic agent and induces differentiation in proliferating stem cells. Short-term and long-term treatments with Valproate also affect genome methylation levels although such changes are not consistent among various cancer stem cell lines. Valproate treatment to patient GBM derived CSCs in in vitro has been shown not only to decrease stemness marker expression like CD133, Nanog, Oct3/4, ZFP 342, Sox2 and telomerase reverse transcriptase but also to promote neuronal (increased expression of TUJ1) and glial (increased GFAP expression) differentiation markers, besides reducing the overall proliferation [158]. Also, Valproate fails to reduce cancer cell viability and sensitization to TMZ treatment in a subtype of GBM stem cell lines [160].

Treatment with Trichostatin A (TSA), another class of HDACi, to patient-derived GBM CSCs in vitro results in repression of stem cell markers viz. CD133, Nanog, Oct3/4, ZFP 342, Sox2 and overall promotion of neuronal (increased expression of TUJ1) and glial (increased GFAP expression) differentiation markers, besides reduction in proliferation [158]. In other systems like U87 derived stem cells, TSA alone reduces neurosphere formation, proliferation and promotes differentiation, besides affecting the cell viability [161]. TSA affects the activation of DNER/Deltex signaling pathway, which in turn reduces stemness (reduced CD133 expression) and proliferation in neurosphere assay and colony formation assay, increases apoptosis and induces differentiation markers like TUJ1 and GFAP expression in GBM CSCs [162]. TSA significantly decreases tube formation (vasculogenic mimicry) in GBM patient-derived stem cells [157].

Like TSA, Entinostat (MS-275) negatively regulates stemness (reduced CD133 expression) and proliferation (reduced neurosphere forming properties) and promotes differentiation via DNER/Deltex signaling pathway in GBM CSCs [162]. MS-275 significantly decreases tube formation (vasculogenic mimicry) in GBM patient-derived stem cells [157]. Recent efforts to screen and develop novel HDACi led to identification of compound 26, an analog of Entinostat,

as a blood–brain barrier permeable HDAC inhibitor capable of decreasing cell viability, inducing apoptosis, inhibiting sphere formation and promoting cell cycle arrest, besides increasing H3 acetylation in patient-derived GBM stem cells. Compound 26 HDACi has been found to extend the survival time when used in combination with TMZ [152], in orthotopic intracranial tumor models.

Similarly, ITF2357(Givinostat)—a pan HDACi- BBB penetrant, has been shown to attenuate tumor growth [163] and cell viability by promoting the apoptosis and autophagy in GBM CSCs [164]. On similar lines, ITF2357 (Givinostat) reduces neurosphere formation in patient-derived GBM stem cells and promotes differentiation [163].

Tubacin, a selective HDAC6i, is shown to have therapeutic importance in CNS diseases including brain tumors. Tubacin acts as anticancer agent by inhibiting HDAC6, which is often increased in GBM CSCs and has been implicated in mediating radio-resistance. Besides inhibiting HDAC6 activity in GBM CSCs, tubacin treatment also increases acetylation levels of tubulin. Furthermore, it reduces the stemness by affecting the SHH pathway and promotes differentiation, apoptosis and radiation sensitivity [143]. In addition to nonspecific pan HDACi, use of specific HDAC inhibitors like Tubacin has also been shown to reduce GBM stem cell viability by promoting apoptosis and enhancing response to radiotherapy [143].

GBM tumors also develop resistance to anti-angiogenic therapeutics by developing angiogenic mimicry. HDACi MC1568 significantly decrease tube formation (vasculogenic mimicry) in GBM patient-derived stem cells [157]. In addition, treatment with mocetinostat, compound 106, MC1746, MC2129, results in histone H4 hyper-acetylation and significant induction of p21WAF1 and γ-H2AX proteins, markers of growth arrest and DDR pathway, respectively, with a slight increase of the cleaved PARP-1 levels, as evident by western blot analysis.

As mentioned in the previous section, apart from Zinc-dependent class 1 and class2 HDAC enzymes, Sirtuins—NAD dependent class III HDACs—have been implicated in the pathophysiology of GBM and GBM CSCs [149, 150]. Sirtuin inhibitors like Ex-527(SIRT1 selective), AGK-2 (SIRT2 selective) and Benzodeazaoxaflavins (SIRT1/SIRT2 inhibitor) have been evaluated for the anticancer activity in GBM CSCs. AGK-2 and Benzodeazaoxaflavins analogues show potent anti-proliferative activity in patient-derived CSCs of GBM [165]. Interestingly, Nicotinamide-induced inhibition of Sirtuins increases Trap1 acetylation which negatively affects metabolic plasticity and maintenance of CSCs [150].

Other malignant brain tumors (medulloblastoma) driven by CSCs have also been shown to be sensitive to HDAC inhibition and a pan HDAC inhibitor sodium butyrate in combination with Etoposide-induced significant reduction in colony formation assay, reduced neurosphere formation ability of medulloblastoma cells, reduced cell viability and promoted neuronal (Gria2) and glial (GLAST) lineage differentiation [166]. HDACi from hydroxamic acids (Vorinostat and Panobinostat) to benzamideEntinostat and short-chain fatty acid VPA have been shown to effectively sensitize ependymoma stem cells, which usually pose resistance to chemotherapy, to standard anticancer drugs like Vincristine (VCR), Cisplatin (CDDP) and Temozolomide (TMZ) [167]. Also, in ependymoma tumors the resistance is shown by CSCs to established anticancer drugs like temozolomide, vincristine and cisplatin, but the cells respond to HDACi SAHA treatment, showing attenuation in neurosphere formation ability of CSCs and promoting neuronal differentiation [167].

HDACi as a combinatorial therapeutics in GBM stem cells

Though HDACi as mono-therapeutic agents exhibit promising anticancer activity, efforts are underway to boost their anticancer activities in targeting the CSCs with combinatorial usage of existing anti-cancer drugs. Several studies have demonstrated the synergistic effect of combinatorial usage of HDACi with anticancer drugs in targeting CSCs of GBM (Fig. 2). Asklund et al. (2012) have demonstrated the application of HDACi Valproate, Vorinostat and Sodium phenylbutyrate along with the FDA approved drug Bortezomib (a proteasome inhibitor) in GBM stem cell lines TB101 and R11. These HDACi in combination with Bortezomib at clinically relevant drug concentrations have shown a synergistic effect in reducing cell viability of GBM CSCs. In addition, HDACi Vorinostat along with Bortezomib reduced colony formation and increased apoptosis in GBM stem cell lines [168]. Similar studies from Sung et al. (2019) showed a synergistic effect of co-treatment with Vorinostat and Melatonin on human Glioma CSC cell lines GSC267 and GSC23. Vorinostat and Melatonin treatment reduced the expression of Transcription factor EB (TFEB) and increased the expression of cleaved PARP and phosphorylated γH2AX. Such co-treatments also reduced the number and size of tumor spheres formed by CSCs of GBM. Along similar lines, combined treatment severely compromised the tumorigenic potential of CSCs in orthotopic xenograft tumors induced by CSCs and resulted in prolonged survival [169]. Singh et al. (2015) reported epigenetic therapeutics based combinatorial treatment like Pan HDACi—Vorinostat and class I HDACi—Entinostat in combination with LSD1/KDM1A (lysine specific demethylase1) inhibitor Tranylcypromine, which resulted in reduced cell viability in patient-derived CSC of GBM, reducing TP53 and TP73 expression in mice GBM xenograft tumor model, reducing the tumor size and

promoting the survival of animals [153]. Valproate with Salinomycin has shown anticancer activity in GBM-CSC recently [170].

HDACi (SAHA and LBH589) have shown a synergistic effect with Obatoclax—(a BCL2 inhibitor in clinical trials for treating solid and circulatory tumors) in targeting GBM cells. Interestingly, subtypes of GBM with BCL2 family member's overexpression have been observed to develop resistance to radio-sensitizing effect of these HDACi but have responded positively to similar treatment supplemented/ reinforced with Obatoclax. The combinatorial application of SAHA and LBH589 with BCL2 inhibitor Obatoclax affected networks of genes linked to cell death and survival, cell morphology, cell cycle and resulted in increased caspase activity, LC3BI/II conversion and showed a synergistic effect to induce apoptosis and autophagy in GBM CSCs [171].

HDACi mediated synergistic effect is not restricted to only anti-cancer chemical agents but has also been reported with biological agents. Tung et al. (2018) reported synergistic effects of HDACi LBH589, SAHA and TSA and overexpression of transcription factor Kruppel-like factor 9 (KLF9) in inducing cell death by apoptosis and necrosis in human GBM stem cell lines [172]. Similar synergistic actions of HDACi have been observed with the oncolytic viral sensitizers like Delta 24-RGD. HDACi like Scriptaid, LBH589, MS275 and VPA used with Delta 24-RGD virus synergistically enhanced oncolytic ability of Delta 24-RGD oncolytic virus therapy in GBM CSCs [173].

Clinical trials of HDACi for glioblastoma tumors

Many HDACi have been approved by the FDA and are in clinic for treating circulatory malignancies which are mostly driven by CSCs. The FDA approved HDACi for treating various circulatory cancers include Vorinostat and FK228 (Romidepsin) for treating cutaneous T-cell lymphoma (CTCL), Belinostat (PXD101) for peripheral T-cell lymphoma (PTCL) and Panobinostat (Farydak) for the treatment of multiple myeloma [174]. Interestingly, various promising HDACi are being evaluated in pre/clinical trials for treating GBM (Table 1) and some other cancer stem cell-driven solid malignancies. Few of the HDACi in clinical trials for treating GBM and other brain tumors exhibit potent anticancer activity in combination with radio and/or chemotherapy. However, in these studies, HDACi is being evaluated as a generalized anticancer agent and not for specifically targeting GBM stem cells. Future studies are warranted to evaluate anti-cancer stem cell properties of HDACi in clinical trials and such studies might provide better indices for evaluation of HDACi in targeting GBM stem cells.

Table 1 Clinical trial data of HDACi in treating Glioblatoma tumors (source: ClinicalTrials.gov)

Treatment/Intervention	Clinical trial phase
Vorinostat+Pembrolizumab+Temozolomide+Radiotherapy	Phase 1
Valproic Acid+Temozolomide+Radiotherapy	Phase 3
Vorinostat+Bortezomib	Phase 2
Vorinostat	Phase 2
Belinostat+Temozolomide+Radiotherapy	Phase 2
Vorinostat+Temozolomide+Radiotherapy	Phase 1, 2
Vorinostat+Bortezomib+Irinotecan	Phase 1
Vorinostat+Bevacizumab	Phase 1, 2
Vorinostat+Isotretinoin+Temozolomide	Phase 1, 2
Valproic acid+Temozolomide+Radiotherapy	Phase 2
Vorinostst+Bevacizumab+Temozolomide	Phase 1, 2
Vorinostat+Temozolomide	Phase 1
Vorinostat+Radiotherapy	Phase 1, 2
Vorinostat+Bevacizumab	Phase 2

HDACi anticancer drug efficacy and CSCs of GBM

Despite the therapeutic advancement in anticancer treatment, success of anticancer drugs in treating GBM is generally low due to many limitations: ability to cross the blood–brain barrier at toxicologically permissible concentrations, effective blood to CSF diffusion, blood to tumor barrier permeability and maintenance of pharmacologically effective drug concentration within tumor core, physiologically relevant diffusion rates in brain and tumor parenchyma, duration of effective plasma concentration, drug metabolism, elimination from the brain and to overcome resistance exhibited by CSCs of GBM [175, 176]. In general, CSCs pose a major obstacle in treating solid malignancies and often mediate refractory resistance to radio cum chemotherapies due to specially fortified survival mechanisms, ranging from physical avoidance of drugs to better drug-induced damage repair mechanisms and enhanced metabolic plasticity. Presence of CSCs in hypoxic tumor core microenvironment often provides physical protection from drug exposure [177]. Such hypoxia-associated niche microenvironment not only protects CSCs from the generation of excessive reactive oxygen

species on radiation therapy due to limited availability of oxygen but also generates signaling cascades from stromal connective tissues to activate other resistance mechanisms in CSCs upon exposure to an anticancer agent [177]. In case of physical exposure to cytotoxic drugs, CSCs deploy drug efflux mechanisms to reduce molecular exposure to the drugs in order to escape from drug-induced toxicity. CSCs of GBM contain efflux transport systems like multidrug resistance (MDR) associated protein family including ATP binding cassette (ABC) transporters. Two of these transporters, P-glycoprotein (P-gp) and MDR protein (MRP) are major components that limit drug penetration into the CNS and to GBM stem cells [177, 178]. In addition to this, CSCs limit drug-induced damage by expressing enzymes for drug inactivation or by repressing enzymes needed for conversion of prodrugs to an active form. GBM stem cells are metabolically more plastic and can reversibly transition from fast-dividing phenotype to slow dividing phenotypes to avoid favorable conditions for the drug action [179]. Many cancer-targeting drugs are DNA alkylating agents and induce DNA damage in cancer cells. CSCs overexpress checkpoint and DNA repair proteins viz. ATM, Chk1/2, p53, BRCAs and XRCC5 and are better adapted to repair damages caused by the drugs and as such upregulate pro-survival and anti-apoptotic molecular programs.

HDACi has been effective in restricting some of these above-mentioned resistance mechanisms in cancerous cells, e.g. use of HDACi Vorinostat, Trichostatin A, Entinostat not only sensitizes cancer cells to DNA damage agent but also limits the development of resistance to DNA alkylating agent by reducing expression of DNA repair-related proteins like RAD51, RAD52, BRCA1/2, CtIP, Ku70, Ku86, DNA-PKCs, XRCC4 and DNA ligase 4 [180]. HDACi like Vorinostat has been shown to induce the accumulation of DNA double-strand breaks in normal and transformed cells. Also, Vorinostat preferentially targets cancer cells via upregulation of thioredoxin binding protein (a negative regulator of ROS scavenging protein thioredoxin)(TRX) and promotes the accumulation of ROS for effective damage to cancer cells [180]. In relation to drug efflux mechanisms, certain HDACi viz. Valproic acid, Apicidin, Romidepsin and Sodium butyrate increase expression of P-glycoprotein (P-gp) and MDR protein (MRP) in several cancer cells and might diminish expected response in combination therapy [180]. Although multidrug resistance-related transporters are upregulated by certain HDACi, only Romidepsin has been characterized as a substrate of P-gp [181]. HDACi Vorinostat and Oxamflatin circumvent drug efflux mechanisms mediated by P-gp or MRP successfully and target cancer cells in P-gp expressing independent manner [180]. Despite being tolerant to drug efflux mechanisms, CSCs develop resistance to HDACi also by activating various signaling pathways in response to HDACi exposure: resistance to Vorinostat, sodium butyrate, Valproic acid and Entinostat in BCL2 expressing cancer cells, activation of NF-kB by Vorinostat, Trichostatin A, Entinostat and Panobinostat reduce their efficacy in certain cancer cells, expression of retinoic signaling-associated proteins like RARα or PRAME (Preferentially expressed antigen in melanoma) cause resistance to Entinostat, Vorinostat and butyrate in certain cancers [180]. In addition, resistance to HDACi has been observed in some specific genetic backgrounds like R132H mutation in isocitrate dehydrogenase 1($IDH1^{R132H}$) GBM cells and resistance developed due to the elevated levels of Octyl-2HG which in turn mediates NANOG expression [182]. Similarly, BCL2 overexpressed GBM stem cells have shown resistance to the radiosensitizing effect of HDAC inhibitors (SAHA and LBH589) [171]. In spite of these drug resistance-related challenges, HDACi has been promising against cancer resistance and few reports demonstrated potential of HADCi in overcoming drug resistance like Trichostatin in eliminating resistant cells during transient resistance states to Temozolomide in GBM treatment [179]. However, detailed mechanisms of HDACi resistance specifically in GBM CSCs is not/poorly known and needs to be explored for the development of effective treatment strategies.

Conclusion and future directions

The encouraging preclinical and clinical research data intriguing the researchers for further exploration of HDACi as promising futuristic addition to the contemporary anticancer regimens, in sensitizing and targeting cancer stem cells for GBM treatment. Although HDACis as combinatorial therapeutics sensitize GBM CSCs to classical anticancer agents, it fails to circumvent drug-induced cytotoxicity in non-cancerous tissues and also in the development of drug resistance to HDACi, as has been reported in few studies on GBM tumors [171, 182]. Furthermore, the use of HDACi does not elicit a similar progressive response in all patient studies owing to inter-tumoral enormous heterogeneity. These limitations mostly obscure potential of HDACi in targeting GBM stem cells and necessitate further investigations into the understanding of individual HDACs in the stem cell biology of GBM. Also, more precise prognostic molecular markers of GBM stem cells have to be identified and used to evaluate individual HDACi treatment response. In addition to pan HDAC inhibition, the role of individual HDACs and associated molecular pathways in GBM CSCs need further explorations [183]. Such studies could be instrumental in prioritizing individual HDACi with specific class selectivity over non-selective HDACis and would provide for minimized off-target toxicity.

In order to expand the repertoire of brain available HDACi, low HDACi BBB permeability issue should be

improvised by better designing molecular architecture for efficient BBB permeability, CNS enrichment and brain retention. The futuristic design of next-generation HDACi should be focused on improving selectivity and efficacy in targeting CSCs of GBM; designing of HDACi equipped with surface recognition cap groups to uniquely expressed surface receptors in CSCs should be explored to increase therapeutic efficacy, bioavailability and decrease in side effects. Designing hybrid HDACi by conjugating with other pharmacophoric moieties may provide for multi functionalities to modulate multifactorial oncogenic targets. HDACi in combination with other anti-cancer drugs for targeting GBM stem cells targeting the drug efflux ability, DNA repair machinery, angiogenic mimicry or angiogenesis and others may provide better treatment outcomes. In addition, the HDACi dose optimization and time of treatment have to be considered for increasing the therapeutic efficacy of HDACi in GBM treatment. These aforementioned provisions need to be potentiated and improvised for GBM drug delivery to tumor core with the use of tissue or cell-selective drug accumulation approaches like nanoparticle [184, 185] or liposome formulations [186, 187] of HDACi. Moreover, the heterogeneity of CSCs and the presence of actively proliferating and quiescent CSCs in GBM are also observed. Thus, it is important to design HDACis targeted towards the proliferative as well as quiescent cells alike. Designing novel HDACi which can overcome the prevailing limitations to effectively target CSCs of GBM might not seem to be a distant dream.

Acknowledgements This work was supported by the Council of Scientific and Industrial Research (CSIR) in-house grants to CSIR-CCMB (to AK) and CSIR-IICT (to SC). R Gajendra Reddy acknowledges the DST-SERB for the National Post-Doctoral Fellowship. Unis Ahmad Bhat acknowledges Department of Biotechnology, Government of India National health Initiative in Glia Research Project (BT/PR27426/MED/122/140/2018) for a senior research fellowship.

Author contributions All the authors were involved in conception, drafting and critically reviewed the manuscript. All the authors have read and approved the final manuscript.

Compliance with ethical standard

Conflict of interest None.

References

1. Louis DN, Perry A, Reifenberger G, von Deimling A, Figarella-Branger D, Cavenee WK, Ohgaki H, Wiestler OD, Kleihues P, Ellison DW (2016) The 2016 world health organization classification of tumors of the central nervous system: a summary. Acta Neuropathol 131(6):803–820. https://doi.org/10.1007/s00401-016-1545-1
2. Ostrom QT, Gittleman H, Farah P, Ondracek A, Chen Y, Wolinsky Y, Stroup NE, Kruchko C, Barnholtz-Sloan JS (2013) CBTRUS statistical report: Primary brain and central nervous system tumors diagnosed in the United States in 2006–2010. Neuro Oncol. https://doi.org/10.1093/neuonc/not151
3. Tamimi AF, Juweid M (2017) Epidemiology and outcome of glioblastoma. In: Glioblastoma. Codon Publications. https://doi.org/10.15586/codon.glioblastoma.2017.ch8
4. Holland EC (2000) Glioblastoma multiforme: the terminator. Proc Natl Acad Sci U S A 97(12):6242–6244. https://doi.org/10.1073/pnas.97.12.6242
5. Fernandes C, Costa A, Osório L, Lago RC, Linhares P, Carvalho B, Caeiro C (2017) Current standards of care in glioblastoma therapy. In: Glioblastoma. Codon Publications. https://doi.org/10.15586/codon.glioblastoma.2017.ch11
6. Hou LC, Veeravagu A, Hsu AR, Tse VC (2006) Recurrent glioblastoma multiforme: a review of natural history and management options. Neurosurg Focus 20(4):E5. https://doi.org/10.3171/foc.2006.20.4.2
7. Stupp R, Mason WP, van den Bent MJ, Weller M, Fisher B, Taphoorn MJ, Belanger K, Brandes AA, Marosi C, Bogdahn U, Curschmann J, Janzer RC, Ludwin SK, Gorlia T, Allgeier A, Lacombe D, Cairncross JG, Eisenhauer E, Mirimanoff RO, European Organisation for R, Treatment of Cancer Brain T, Radiotherapy G, National Cancer Institute of Canada Clinical Trials G (2005) Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. N Engl J Med 352(10):987–996. https://doi.org/10.1056/NEJMoa043330
8. Woodworth GF, Dunn GP, Nance EA, Hanes J, Brem H (2014) Emerging insights into barriers to effective brain tumor therapeutics. Front Oncol 4:126. https://doi.org/10.3389/fonc.2014.00126
9. De Vleeschouwer S, Bergers G (2017) Glioblastoma: to target the tumor cell or the microenvironment? In: Glioblastoma. Codon Publications. https://doi.org/10.15586/codon.glioblastoma.2017.ch16
10. Audia A, Conroy S, Glass R, Bhat KP (2017) The impact of the tumor microenvironment on the properties of glioma stem-like cells. Front Oncol 7:143
11. McGranahan N, Swanton C (2017) Clonal heterogeneity and tumor evolution: past, present, and the future. Cell 168(4):613–628. https://doi.org/10.1016/j.cell.2017.01.018
12. Navin N, Kendall J, Troge J, Andrews P, Rodgers L, McIndoo J, Cook K, Stepansky A, Levy D, Esposito D, Muthuswamy L, Krasnitz A, McCombie WR, Hicks J, Wigler M (2011) Tumour evolution inferred by single-cell sequencing. Nature 472(7341):90–94. https://doi.org/10.1038/nature09807
13. Prasetyanti PR, Medema JP (2017) Intra-tumor heterogeneity from a cancer stem cell perspective. Mol Cancer 16(1):41. https://doi.org/10.1186/s12943-017-0600-4
14. Alves TR, Lima FR, Kahn SA, Lobo D, Dubois LG, Soletti R, Borges H, Neto VM (2011) Glioblastoma cells: a heterogeneous and fatal tumor interacting with the parenchyma. Life Sci 89(15–16):532–539. https://doi.org/10.1016/j.lfs.2011.04.022
15. Bernstock JD, Mooney JH, Ilyas A, Chagoya G, Estevez-Ordonez D, Ibrahim A, Nakano I (2019) Molecular and cellular intratumoral heterogeneity in primary glioblastoma: clinical and translational implications. J Neurosurg. https://doi.org/10.3171/2019.5.JNS19364
16. Brown DV, Stylli SS, Kaye AH, Mantamadiotis T (2019) Multilayered heterogeneity of glioblastoma stem cells: biological and clinical significance. Adv Exp Med Biol 1139:1–21. https://doi.org/10.1007/978-3-030-14366-4_1
17. Sottoriva A, Spiteri I, Piccirillo SG, Touloumis A, Collins VP, Marioni JC, Curtis C, Watts C, Tavare S (2013) Intratumor heterogeneity in human glioblastoma reflects cancer evolutionary

dynamics. Proc Natl Acad Sci U S A 110(10):4009–4014. https://doi.org/10.1073/pnas.1219747110
18. Lee JK, Wang J, Sa JK, Ladewig E, Lee HO, Lee IH, Kang HJ, Rosenbloom DS, Camara PG, Liu Z, van Nieuwenhuizen P, Jung SW, Choi SW, Kim J, Chen A, Kim KT, Shin S, Seo YJ, Oh JM, Shin YJ, Park CK, Kong DS, Seol HJ, Blumberg A, Lee JI, Iavarone A, Park WY, Rabadan R, Nam DH (2017) Spatiotemporal genomic architecture informs precision oncology in glioblastoma. Nat Genet 49(4):594–599. https://doi.org/10.1038/ng.3806
19. Lathia JD, Mack SC, Mulkearns-Hubert EE, Valentim CL, Rich JN (2015) Cancer stem cells in glioblastoma. Genes Dev 29(12):1203–1217. https://doi.org/10.1101/gad.261982.115
20. Qiang L, Yang Y, Ma Y-J, Chen F-H, Zhang L-B, Liu W, Qi Q, Lu N, Tao L, Wang X-T (2009) Isolation and characterization of cancer stem like cells in human glioblastoma cell lines. Cancer Lett 279(1):13–21
21. Singh SK, Clarke ID, Terasaki M, Bonn VE, Hawkins C, Squire J, Dirks PB (2003) Identification of a cancer stem cell in human brain tumors. Cancer Res 63(18):5821–5828
22. Chen J, Li Y, Yu TS, McKay RM, Burns DK, Kernie SG, Parada LF (2012) A restricted cell population propagates glioblastoma growth after chemotherapy. Nature 488(7412):522–526. https://doi.org/10.1038/nature11287
23. Eramo A, Ricci-Vitiani L, Zeuner A, Pallini R, Lotti F, Sette G, Pilozzi E, Larocca LM, Peschle C, De Maria R (2006) Chemotherapy resistance of glioblastoma stem cells. Cell Death Differ 13(7):1238–1241. https://doi.org/10.1038/sj.cdd.4401872
24. Bao S, Wu Q, McLendon RE, Hao Y, Shi Q, Hjelmeland AB, Dewhirst MW, Bigner DD, Rich JN (2006) Glioma stem cells promote radioresistance by preferential activation of the DNA damage response. Nature 444(7120):756–760. https://doi.org/10.1038/nature05236
25. Bao S, Wu Q, Sathornsumetee S, Hao Y, Li Z, Hjelmeland AB, Shi Q, McLendon RE, Bigner DD, Rich JN (2006) Stem cell–like glioma cells promote tumor angiogenesis through vascular endothelial growth factor. Can Res 66(16):7843–7848
26. Nakai E, Park K, Yawata T, Chihara T, Kumazawa A, Nakabayashi H, Shimizu K (2009) Enhanced MDR1 expression and chemoresistance of cancer stem cells derived from glioblastoma. Cancer Invest 27(9):901–908
27. Vlashi E, Lagadec C, Vergnes L, Matsutani T, Masui K, Poulou M, Popescu R, Della Donna L, Evers P, Dekmezian C (2011) Metabolic state of glioma stem cells and nontumorigenic cells. Proc Natl Acad Sci 108(38):16062–16067
28. Cheng L, Bao S, Rich JN (2010) Potential therapeutic implications of cancer stem cells in glioblastoma. Biochem Pharmacol 80(5):654–665
29. Safa AR, Saadatzadeh MR, Cohen-Gadol AA, Pollok KE, Bijangi-Vishehsaraei K (2016) Emerging targets for glioblastoma stem cell therapy. J Biomed Res 30(1):19
30. Hosein AN, Lim YC, Day B, Stringer B, Rose S, Head R, Cosgrove L, Sminia P, Fay M, Martin JH (2015) The effect of valproic acid in combination with irradiation and temozolomide on primary human glioblastoma cells. J Neurooncol 122(2):263–271
31. Francis JM, Zhang CZ, Maire CL, Jung J, Manzo VE, Adalsteinsson VA, Homer H, Haidar S, Blumenstiel B, Pedamallu CS, Ligon AH, Love JC, Meyerson M, Ligon KL (2014) EGFR variant heterogeneity in glioblastoma resolved through single-nucleus sequencing. Cancer Discov 4(8):956–971. https://doi.org/10.1158/2159-8290.CD-13-0879
32. Perus LJM, Walsh LA (2019) Microenvironmental Heterogeneity in Brain Malignancies. Front Immunol 10:2294. https://doi.org/10.3389/fimmu.2019.02294
33. Baslan T, Kendall J, Ward B, Cox H, Leotta A, Rodgers L, Riggs M, D'Italia S, Sun G, Yong M, Miskimen K, Gilmore H, Saborowski M, Dimitrova N, Krasnitz A, Harris L, Wigler M, Hicks J (2015) Optimizing sparse sequencing of single cells for highly multiplex copy number profiling. Genome Res 25(5):714–724. https://doi.org/10.1101/gr.188060.114
34. Gawad C, Koh W, Quake SR (2014) Dissecting the clonal origins of childhood acute lymphoblastic leukemia by single-cell genomics. Proc Natl Acad Sci U S A 111(50):17947–17952. https://doi.org/10.1073/pnas.1420822111
35. Hou Y, Song L, Zhu P, Zhang B, Tao Y, Xu X, Li F, Wu K, Liang J, Shao D, Wu H, Ye X, Ye C, Wu R, Jian M, Chen Y, Xie W, Zhang R, Chen L, Liu X, Yao X, Zheng H, Yu C, Li Q, Gong Z, Mao M, Yang X, Yang L, Li J, Wang W, Lu Z, Gu N, Laurie G, Bolund L, Kristiansen K, Wang J, Yang H, Li Y, Zhang X, Wang J (2012) Single-cell exome sequencing and monoclonal evolution of a JAK2-negative myeloproliferative neoplasm. Cell 148(5):873–885. https://doi.org/10.1016/j.cell.2012.02.028
36. Klevebring D, Rosin G, Ma R, Lindberg J, Czene K, Kere J, Fredriksson I, Bergh J, Hartman J (2014) Sequencing of breast cancer stem cell populations indicates a dynamic conversion between differentiation states in vivo. Breast Cancer Res 16(4):R72. https://doi.org/10.1186/bcr3687
37. Li Y, Xu X, Song L, Hou Y, Li Z, Tsang S, Li F, Im KM, Wu K, Wu H, Ye X, Li G, Wang L, Zhang B, Liang J, Xie W, Wu R, Jiang H, Liu X, Yu C, Zheng H, Jian M, Nie L, Wan L, Shi M, Sun X, Tang A, Guo G, Gui Y, Cai Z, Li J, Wang W, Lu Z, Zhang X, Bolund L, Kristiansen K, Wang J, Yang H, Dean M, Wang J (2012) Single-cell sequencing analysis characterizes common and cell-lineage-specific mutations in a muscle-invasive bladder cancer. Gigascience 1(1):12. https://doi.org/10.1186/2047-217X-1-12
38. Patel AP, Tirosh I, Trombetta JJ, Shalek AK, Gillespie SM, Wakimoto H, Cahill DP, Nahed BV, Curry WT, Martuza RL, Louis DN, Rozenblatt-Rosen O, Suva ML, Regev A, Bernstein BE (2014) Single-cell RNA-seq highlights intratumoral heterogeneity in primary glioblastoma. Science 344(6190):1396–1401. https://doi.org/10.1126/science.1254257
39. Potter NE, Ermini L, Papaemmanuil E, Cazzaniga G, Vijayaraghavan G, Titley I, Ford A, Campbell P, Kearney L, Greaves M (2013) Single-cell mutational profiling and clonal phylogeny in cancer. Genome Res 23(12):2115–2125. https://doi.org/10.1101/gr.159913.113
40. Suzuki A, Matsushima K, Makinoshima H, Sugano S, Kohno T, Tsuchihara K, Suzuki Y (2015) Single-cell analysis of lung adenocarcinoma cell lines reveals diverse expression patterns of individual cells invoked by a molecular target drug treatment. Genome Biol 16:66. https://doi.org/10.1186/s13059-015-0636-y
41. Wang Y, Waters J, Leung ML, Unruh A, Roh W, Shi X, Chen K, Scheet P, Vattathil S, Liang H, Multani A, Zhang H, Zhao R, Michor F, Meric-Bernstam F, Navin NE (2014) Clonal evolution in breast cancer revealed by single nucleus genome sequencing. Nature 512(7513):155–160. https://doi.org/10.1038/nature13600
42. Xu X, Hou Y, Yin X, Bao L, Tang A, Song L, Li F, Tsang S, Wu K, Wu H, He W, Zeng L, Xing M, Wu R, Jiang H, Liu X, Cao D, Guo G, Hu X, Gui Y, Li Z, Xie W, Sun X, Shi M, Cai Z, Wang B, Zhong M, Li J, Lu Z, Gu N, Zhang X, Goodman L, Bolund L, Wang J, Yang H, Kristiansen K, Dean M, Li Y, Wang J (2012) Single-cell exome sequencing reveals single-nucleotide mutation characteristics of a kidney tumor. Cell 148(5):886–895. https://doi.org/10.1016/j.cell.2012.02.025
43. Yu C, Yu J, Yao X, Wu WK, Lu Y, Tang S, Li X, Bao L, Li X, Hou Y, Wu R, Jian M, Chen R, Zhang F, Xu L, Fan F, He J, Liang Q, Wang H, Hu X, He M, Zhang X, Zheng H, Li Q, Wu H, Chen Y, Yang X, Zhu S, Xu X, Yang H, Wang J, Zhang X, Sung JJ, Li Y, Wang J (2014) Discovery of biclonal origin and a novel

oncogene SLC12A5 in colon cancer by single-cell sequencing. Cell Res 24(6):701–712. https://doi.org/10.1038/cr.2014.43
44. Al-Hajj M, Wicha MS, Benito-Hernandez A, Morrison SJ, Clarke MF (2003) Prospective identification of tumorigenic breast cancer cells. Proc Natl Acad Sci U S A 100(7):3983–3988. https://doi.org/10.1073/pnas.0530291100
45. Shipitsin M, Polyak K (2008) The cancer stem cell hypothesis: in search of definitions, markers, and relevance. Lab Invest 88(5):459–463. https://doi.org/10.1038/labinvest.2008.14
46. Shackleton M, Quintana E, Fearon ER, Morrison SJ (2009) Heterogeneity in cancer: cancer stem cells versus clonal evolution. Cell 138(5):822–829. https://doi.org/10.1016/j.cell.2009.08.017
47. Engelmann K, Shen H, Finn OJ (2008) MCF7 side population cells with characteristics of cancer stem/progenitor cells express the tumor antigen MUC1. Cancer Res 68(7):2419–2426. https://doi.org/10.1158/0008-5472.CAN-07-2249
48. Ginestier C, Hur MH, Charafe-Jauffret E, Monville F, Dutcher J, Brown M, Jacquemier J, Viens P, Kleer CG, Liu S, Schott A, Hayes D, Birnbaum D, Wicha MS, Dontu G (2007) ALDH1 is a marker of normal and malignant human mammary stem cells and a predictor of poor clinical outcome. Cell Stem Cell 1(5):555–567. https://doi.org/10.1016/j.stem.2007.08.014
49. Hirschmann-Jax C, Foster AE, Wulf GG, Nuchtern JG, Jax TW, Gobel U, Goodell MA, Brenner MK (2004) A distinct "side population" of cells with high drug efflux capacity in human tumor cells. Proc Natl Acad Sci U S A 101(39):14228–14233. https://doi.org/10.1073/pnas.0400067101
50. Dalerba P, Dylla SJ, Park IK, Liu R, Wang X, Cho RW, Hoey T, Gurney A, Huang EH, Simeone DM, Shelton AA, Parmiani G, Castelli C, Clarke MF (2007) Phenotypic characterization of human colorectal cancer stem cells. Proc Natl Acad Sci U S A 104(24):10158–10163. https://doi.org/10.1073/pnas.0703478104
51. Giustacchini A, Thongjuea S, Barkas N, Woll PS, Povinelli BJ, Booth CAG, Sopp P, Norfo R, Rodriguez-Meira A, Ashley N, Jamieson L, Vyas P, Anderson K, Segerstolpe A, Qian H, Olsson-Stromberg U, Mustjoki S, Sandberg R, Jacobsen SEW, Mead AJ (2017) Single-cell transcriptomics uncovers distinct molecular signatures of stem cells in chronic myeloid leukemia. Nat Med 23(6):692–702. https://doi.org/10.1038/nm.4336
52. O'Brien CA, Kreso A, Jamieson CH (2010) Cancer stem cells and self-renewal. Clin Cancer Res 16(12):3113–3120. https://doi.org/10.1158/1078-0432.CCR-09-2824
53. Al-Hajj M, Clarke MF (2004) Self-renewal and solid tumor stem cells. Oncogene 23(43):7274–7282. https://doi.org/10.1038/sj.onc.1207947
54. Reya T, Morrison SJ, Clarke MF, Weissman IL (2001) Stem cells, cancer, and cancer stem cells. Nature 414(6859):105–111. https://doi.org/10.1038/35102167
55. Dieter SM, Ball CR, Hoffmann CM, Nowrouzi A, Herbst F, Zavidij O, Abel U, Arens A, Weichert W, Brand K, Koch M, Weitz J, Schmidt M, von Kalle C, Glimm H (2011) Distinct types of tumor-initiating cells form human colon cancer tumors and metastases. Cell Stem Cell 9(4):357–365. https://doi.org/10.1016/j.stem.2011.08.010
56. Bai X, Ni J, Beretov J, Graham P, Li Y (2018) Cancer stem cell in breast cancer therapeutic resistance. Cancer Treat Rev 69:152–163. https://doi.org/10.1016/j.ctrv.2018.07.004
57. Corbin AS, Agarwal A, Loriaux M, Cortes J, Deininger MW, Druker BJ (2011) Human chronic myeloid leukemia stem cells are insensitive to imatinib despite inhibition of BCR-ABL activity. J Clin Invest 121(1):396–409. https://doi.org/10.1172/JCI35721
58. Ishizawa K, Rasheed ZA, Karisch R, Wang Q, Kowalski J, Susky E, Pereira K, Karamboulas C, Moghal N, Rajeshkumar NV, Hidalgo M, Tsao M, Ailles L, Waddell TK, Maitra A, Neel BG, Matsui W (2010) Tumor-initiating cells are rare in many human tumors. Cell Stem Cell 7(3):279–282. https://doi.org/10.1016/j.stem.2010.08.009
59. Patrawala L, Calhoun T, Schneider-Broussard R, Zhou J, Claypool K, Tang DG (2005) Side population is enriched in tumorigenic, stem-like cancer cells, whereas ABCG2+ and ABCG2- cancer cells are similarly tumorigenic. Cancer Res 65(14):6207–6219. https://doi.org/10.1158/0008-5472.CAN-05-0592
60. Schatton T, Murphy GF, Frank NY, Yamaura K, Waaga-Gasser AM, Gasser M, Zhan Q, Jordan S, Duncan LM, Weishaupt C (2008) Identification of cells initiating human melanomas. Nature 451(7176):345
61. Roesch A, Fukunaga-Kalabis M, Schmidt EC, Zabierowski SE, Brafford PA, Vultur A, Basu D, Gimotty P, Vogt T, Herlyn M (2010) A temporarily distinct subpopulation of slow-cycling melanoma cells is required for continuous tumor growth. Cell 141(4):583–594
62. Eun K, Ham SW, Kim H (2017) Cancer stem cell heterogeneity: origin and new perspectives on CSC targeting. BMB Rep 50(3):117–125. https://doi.org/10.5483/bmbrep.2017.50.3.222
63. Botchkina IL, Rowehl RA, Rivadeneira DE, Karpeh MS, Crawford H, Dufour JU, Botchkina WLAJYYGI (2009) Phenotypic subpopulations of metastatic colon cancer stem cells: genomic analysis. Cancer Genom Proteom 6(1):19–29
64. Pece S, Tosoni D, Confalonieri S, Mazzarol G, Vecchi M, Ronzoni S, Bernard L, Viale G, Pelicci PG, Di Fiore PP (2010) Biological and molecular heterogeneity of breast cancers correlates with their cancer stem cell content. Cell 140(1):62–73. https://doi.org/10.1016/j.cell.2009.12.007
65. Proia TA, Keller PJ, Gupta PB, Klebba I, Jones AD, Sedic M, Gilmore H, Tung N, Naber SP, Schnitt S, Lander ES, Kuperwasser C (2011) Genetic predisposition directs breast cancer phenotype by dictating progenitor cell fate. Cell Stem Cell 8(2):149–163. https://doi.org/10.1016/j.stem.2010.12.007
66. Beier D, Hau P, Proescholdt M, Lohmeier A, Wischhusen J, Oefner PJ, Aigner L, Brawanski A, Bogdahn U, Beier CP (2007) CD133(+) and CD133(-) glioblastoma-derived cancer stem cells show differential growth characteristics and molecular profiles. Cancer Res 67(9):4010–4015. https://doi.org/10.1158/0008-5472.CAN-06-4180
67. Zhou BB, Zhang H, Damelin M, Geles KG, Grindley JC, Dirks PB (2009) Tumour-initiating cells: challenges and opportunities for anticancer drug discovery. Nat Rev Drug Discov 8(10):806–823. https://doi.org/10.1038/nrd2137
68. Floor S, Van Staveren W, Larsimont D, Dumont JE, Maenhaut C (2011) Cancer cells in epithelial-to-mesenchymal transition and tumor-propagating–cancer stem cells: distinct, overlapping or same populations. Oncogene 30(46):4609–4621
69. Hope KJ, Jin L, Dick JE (2004) Acute myeloid leukemia originates from a hierarchy of leukemic stem cell classes that differ in self-renewal capacity. Nat Immunol 5(7):738–743. https://doi.org/10.1038/ni1080
70. Luo M, Wicha MS (2015) Metabolic plasticity of cancer stem cells. Oncotarget 6(34):35141
71. Snyder V, Reed-Newman TC, Arnold L, Thomas SM, Anant S (2018) Cancer stem cell metabolism and potential therapeutic targets. Front Oncol 8:203
72. Ye F, Zhang Y, Liu Y et al (2013) Protective properties of radio-chemoresistant glioblastoma stem cell clones are associated with metabolic adaptation to reduced glucose dependence. PLoS One 8(11):e80397. https://doi.org/10.1371/journal.pone.0080397
73. Brooks MD, Burness ML, Wicha MS (2015) Therapeutic implications of cellular heterogeneity and plasticity in breast cancer. Cell Stem Cell 17(3):260–271. https://doi.org/10.1016/j.stem.2015.08.014

74. Friedmann-Morvinski D, Verma IM (2014) Dedifferentiation and reprogramming: origins of cancer stem cells. EMBO Rep 15(3):244–253
75. Safa AR, Saadatzadeh MR, Cohen-Gadol AA, Pollok KE, Bijangi-Vishehsaraei K (2015) Glioblastoma stem cells (GSCs) epigenetic plasticity and interconversion between differentiated non-GSCs and GSCs. Genes Dis 2(2):152–163. https://doi.org/10.1016/j.gendis.2015.02.001
76. Abbaszadegan MR, Bagheri V, Razavi MS, Momtazi AA, Sahebkar A, Gholamin M (2017) Isolation, identification, and characterization of cancer stem cells: A review. J Cell Physiol 232(8):2008–2018. https://doi.org/10.1002/jcp.25759
77. Ho DW, Tsui YM, Sze KM, Chan LK, Cheung TT, Lee E, Sham PC, Tsui SK, Lee TK, Ng IO (2019) Single-cell transcriptomics reveals the landscape of intra-tumoral heterogeneity and stemness-related subpopulations in liver cancer. Cancer Lett 459:176–185. https://doi.org/10.1016/j.canlet.2019.06.002
78. Humphries HN, Wickremesekera SK, Marsh RW, Brasch HD, Mehrotra S, Tan ST, Itinteang T (2017) Characterization of cancer stem cells in colon adenocarcinoma metastasis to the liver. Front Surg 4:76. https://doi.org/10.3389/fsurg.2017.00076
79. Jonasson E, Ghannoum S, Persson E, Karlsson J, Kroneis T, Larsson E, Landberg G, Stahlberg A (2019) Identification of breast cancer stem cell related genes using functional cellular assays combined with single-cell RNA sequencing in MDA-MB-231 cells. Front Genet 10:500. https://doi.org/10.3389/fgene.2019.00500
80. Parte SC, Batra SK, Kakar SS (2018) Characterization of stem cell and cancer stem cell populations in ovary and ovarian tumors. J Ovarian Res 11(1):69. https://doi.org/10.1186/s13048-018-0439-3
81. Pan S, Zhan Y, Chen X, Wu B, Liu B (2019) Identification of biomarkers for controlling cancer stem cell characteristics in bladder cancer by network analysis of transcriptome data stemness indices. Front Oncol 9:613. https://doi.org/10.3389/fonc.2019.00613
82. Rodriguez-Meira A, Buck G, Clark SA, Povinelli BJ, Alcolea V, Louka E, McGowan S, Hamblin A, Sousos N, Barkas N, Giustacchini A, Psaila B, Jacobsen SEW, Thongjuea S, Mead AJ (2019) Unravelling intratumoral heterogeneity through high-sensitivity single-cell mutational analysis and parallel RNA sequencing. Mol Cell. https://doi.org/10.1016/j.molcel.2019.01.009
83. Chen R, Nishimura MC, Bumbaca SM, Kharbanda S, Forrest WF, Kasman IM, Greve JM, Soriano RH, Gilmour LL, Rivers CS, Modrusan Z, Nacu S, Guerrero S, Edgar KA, Wallin JJ, Lamszus K, Westphal M, Heim S, James CD, VandenBerg SR, Costello JF, Moorefield S, Cowdrey CJ, Prados M, Phillips HS (2010) A hierarchy of self-renewing tumor-initiating cell types in glioblastoma. Cancer Cell 17(4):362–375. https://doi.org/10.1016/j.ccr.2009.12.049
84. Piccirillo SG, Combi R, Cajola L, Patrizi A, Redaelli S, Bentivegna A, Baronchelli S, Maira G, Pollo B, Mangiola A, DiMeco F, Dalpra L, Vescovi AL (2009) Distinct pools of cancer stem-like cells coexist within human glioblastomas and display different tumorigenicity and independent genomic evolution. Oncogene 28(15):1807–1811. https://doi.org/10.1038/onc.2009.27
85. Lottaz C, Beier D, Meyer K, Kumar P, Hermann A, Schwarz J, Junker M, Oefner PJ, Bogdahn U, Wischhusen J, Spang R, Storch A, Beier CP (2010) Transcriptional profiles of CD133+ and CD133- glioblastoma-derived cancer stem cell lines suggest different cells of origin. Cancer Res 70(5):2030–2040. https://doi.org/10.1158/0008-5472.CAN-09-1707
86. Lee JH, Lee JE, Kahng JY, Kim SH, Park JS, Yoon SJ, Um JY, Kim WK, Lee JK, Park J, Kim EH, Lee JH, Lee JH, Chung WS, Ju YS, Park SH, Chang JH, Kang SG, Lee JH (2018) Human glioblastoma arises from subventricular zone cells with low-level driver mutations. Nature 560(7717):243–247. https://doi.org/10.1038/s41586-018-0389-3
87. Facchino S, Abdouh M, Bernier G (2011) Brain cancer stem cells: current status on glioblastoma multiforme. Cancers 3(2):1777–1797
88. Jackson EL, Garcia-Verdugo JM, Gil-Perotin S, Roy M, Quinones-Hinojosa A, VandenBerg S, Alvarez-Buylla A (2006) PDGFRα-positive B cells are neural stem cells in the adult SVZ that form glioma-like growths in response to increased PDGF signaling. Neuron 51(2):187–199
89. Auffinger B, Tobias A, Han Y, Lee G, Guo D, Dey M, Lesniak M, Ahmed A (2014) Conversion of differentiated cancer cells into cancer stem-like cells in a glioblastoma model after primary chemotherapy. Cell Death Differ 21(7):1119
90. Liu YC, Lee IC, Chen PY (2018) Biomimetic brain tumor niche regulates glioblastoma cells towards a cancer stem cell phenotype. J Neurooncol 137(3):511–522. https://doi.org/10.1007/s11060-018-2763-2
91. Suva ML, Rheinbay E, Gillespie SM, Patel AP, Wakimoto H, Rabkin SD, Riggi N, Chi AS, Cahill DP, Nahed BV, Curry WT, Martuza RL, Rivera MN, Rossetti N, Kasif S, Beik S, Kadri S, Tirosh I, Wortman I, Shalek AK, Rozenblatt-Rosen O, Regev A, Louis DN, Bernstein BE (2014) Reconstructing and reprogramming the tumor-propagating potential of glioblastoma stem-like cells. Cell 157(3):580–594. https://doi.org/10.1016/j.cell.2014.02.030
92. Nduom EK-E, Hadjipanayis CG, Van Meir EG (2012) Glioblastoma cancer stem-like cells–implications for pathogenesis and treatment. Cancer J (Sudbury, Mass) 18(1):100
93. Yan X, Ma L, Yi D, Yoon JG, Diercks A, Foltz G, Price ND, Hood LE, Tian Q (2011) A CD133-related gene expression signature identifies an aggressive glioblastoma subtype with excessive mutations. Proc Natl Acad Sci U S A 108(4):1591–1596. https://doi.org/10.1073/pnas.1018696108
94. Brescia P, Ortensi B, Fornasari L, Levi D, Broggi G, Pelicci G (2013) CD133 is essential for glioblastoma stem cell maintenance. Stem cells 31(5):857–869
95. Zhang M, Song T, Yang L, Chen R, Wu L, Yang Z, Fang J (2008) Nestin and CD133: valuable stem cell-specific markers for determining clinical outcome of glioma patients. J Experim Clin Cancer Res 27(1):85
96. Gangemi RMR, Griffero F, Marubbi D, Perera M, Capra MC, Malatesta P, Ravetti GL, Zona GL, Daga A, Corte G (2009) SOX2 silencing in glioblastoma tumor-initiating cells causes stop of proliferation and loss of tumorigenicity. Stem Cells 27(1):40–48
97. Guo Y, Liu S, Wang P, Zhao S, Wang F, Bing L, Zhang Y, Ling EA, Gao J, Hao A (2011) Expression profile of embryonic stem cell-associated genes Oct4, Sox2 and Nanog in human gliomas. Histopathology 59(4):763–775. https://doi.org/10.1111/j.1365-2559.2011.03993.x
98. Lee Y, Kim KH, Kim DG et al (2015) FoxM1 promotes stemness and radio-resistance of glioblastoma by regulating the master stem cell regulator Sox2. PLoS One 10(10):e0137703. https://doi.org/10.1371/journal.pone.0137703
99. Jin X, Jin X, Jung J-E, Beck S, Kim H (2013) Cell surface Nestin is a biomarker for glioma stem cells. Biochem Biophys Res Commun 433(4):496–501
100. Iacopino Fortunata et al (2014) Isolation of cancer stem cells from three human glioblastoma cell lines: characterization of two selected clones. PloS one 9(8):e105166. https://doi.org/10.1371/journal.pone.0105166
101. Lagadec C, Vlashi E, Frohnen P, Alhiyari Y, Chan M, Pajonk F (2014) The RNA-binding protein Musashi-1 regulates proteasome subunit expression in breast cancer-and glioma-initiating cells. Stem Cells 32(1):135–144

102. Venugopal C, Li N, Wang X, Manoranjan B, Hawkins C, Gunnarsson T, Hollenberg R, Klurfan P, Murty N, Kwiecien J (2012) Bmi1 marks intermediate precursors during differentiation of human brain tumor initiating cells. Stem Cell Res 8(2):141–153
103. Sibin M, Lavanya C, Bhat DI, Rao N, Geethashree N, Vibhuti W, Chetan G (2015) CD133 and BMI1 expressions and its prognostic role in primary glioblastoma. J Genet 94(4):689–696
104. Vora P, Seyfrid M, Venugopal C, Qazi MA, Salim S, Isserlin R, Subapanditha M, O'Farrell E, Mahendram S, Singh M (2019) Bmi1 regulates human glioblastoma stem cells through activation of differential gene networks in CD133+ brain tumor initiating cells. J Neurooncol 143(3):417–428
105. Zhang L, Yan Y, Jiang Y, Cui Y, Zou Y, Qian J, Luo C, Lu Y, Wu X (2015) The expression of SALL4 in patients with gliomas: high level of SALL4 expression is correlated with poor outcome. J Neurooncol 121(2):261–268
106. Bradshaw A, Wickremsekera A, Tan ST, Peng L, Davis PF, Itinteang T (2016) Cancer stem cell hierarchy in glioblastoma multiforme. Front Surgery 3:21
107. Du Z, Jia D, Liu S, Wang F, Li G, Zhang Y, Cao X, Ling EA, Hao A (2009) Oct4 is expressed in human gliomas and promotes colony formation in glioma cells. Glia 57(7):724–733. https://doi.org/10.1002/glia.20800
108. Ikushima H, Todo T, Ino Y, Takahashi M, Saito N, Miyazawa K, Miyazono K (2011) Glioma-initiating cells retain their tumorigenicity through integration of the Sox axis and Oct4 protein. J Biol Chem 286(48):41434–41441
109. Sherry MM, Reeves A, Wu JK, Cochran BH (2009) STAT3 is required for proliferation and maintenance of multipotency in glioblastoma stem cells. Stem Cells 27(10):2383–2392. https://doi.org/10.1002/stem.185
110. Niu CS, Yang Y, Cheng CD (2013) MiR-134 regulates the proliferation and invasion of glioblastoma cells by reducing Nanog expression. Int J Oncol 42(5):1533–1540. https://doi.org/10.3892/ijo.2013.1844
111. Zbinden M, Duquet A, Lorente-Trigos A, Ngwabyt SN, Borges I, Ruiz i Altaba A (2010) NANOG regulates glioma stem cells and is essential in vivo acting in a cross-functional network with GLI1 and p53. EMBO J 29(15):2659–2674. https://doi.org/10.1038/emboj.2010.137
112. Herms JW, von Loewenich FD, Behnke J, Markakis E, Kretzschmar HA (1999) c-myc oncogene family expression in glioblastoma and survival. Surg Neurol 51(5):536–542. https://doi.org/10.1016/s0090-3019(98)00028-7
113. Zheng H, Ying H, Yan H, Kimmelman AC, Hiller DJ, Chen AJ, Perry SR, Tonon G, Chu GC, Ding Z, Stommel JM, Dunn KL, Wiedemeyer R, You MJ, Brennan C, Wang YA, Ligon KL, Wong WH, Chin L, dePinho RA (2008) Pten and p53 converge on c-Myc to control differentiation, self-renewal, and transformation of normal and neoplastic stem cells in glioblastoma. Cold Spring Harb Symp Quant Biol 73:427–437. https://doi.org/10.1101/sqb.2008.73.047
114. Field M, Alvarez A, Bushnev S, Sugaya K (2010) Embryonic stem cell markers distinguishing cancer stem cells from normal human neuronal stem cell populations in malignant glioma patients. Clin Neurosurg 57:151–159
115. Cheng J-X, Liu B-L, Zhang X (2009) How powerful is CD133 as a cancer stem cell marker in brain tumors? Cancer Treat Rev 35(5):403–408
116. Piccirillo S, Vescovi A (2007) Bone morphogenetic proteins regulate tumorigenicity in human glioblastoma stem cells. In: Cancer Stem Cells. Springer, pp 59–81. https://doi.org/10.1007/2789_2007_044
117. Lee J, Son MJ, Woolard K, Donin NM, Li A, Cheng CH, Kotliarova S, Kotliarov Y, Walling J, Ahn S, Kim M, Totonchy M, Cusack T, Ene C, Ma H, Su Q, Zenklusen JC, Zhang W, Maric D, Fine HA (2008) Epigenetic-mediated dysfunction of the bone morphogenetic protein pathway inhibits differentiation of glioblastoma-initiating cells. Cancer Cell 13(1):69–80. https://doi.org/10.1016/j.ccr.2007.12.005
118. Sandberg CJ, Altschuler G, Jeong J, Stromme KK, Stangeland B, Murrell W, Grasmo-Wendler UH, Myklebost O, Helseth E, Vik-Mo EO, Hide W, Langmoen IA (2013) Comparison of glioma stem cells to neural stem cells from the adult human brain identifies dysregulated Wnt- signaling and a fingerprint associated with clinical outcome. Exp Cell Res 319(14):2230–2243. https://doi.org/10.1016/j.yexcr.2013.06.004
119. Huang Z, Cheng L, Guryanova OA, Wu Q, Bao S (2010) Cancer stem cells in glioblastoma—molecular signaling and therapeutic targeting. Protein Cell 1(7):638–655
120. Pang B, Xu J, Hu J, Guo F, Wan L, Cheng M, Pang L (2019) Single-cell RNA-seq reveals the invasive trajectory and molecular cascades underlying glioblastoma progression. Mol Oncol 13(12):2588–2603. https://doi.org/10.1002/1878-0261.12569
121. Lai A, Kharbanda S, Pope WB, Tran A, Solis OE, Peale F, Forrest WF, Pujara K, Carrillo JA, Pandita A (2011) Evidence for sequenced molecular evolution of IDH1 mutant glioblastoma from a distinct cell of origin. J Clin Oncol 29(34):4482
122. Liau BB, Sievers C, Donohue LK, Gillespie SM, Flavahan WA, Miller TE, Venteicher AS, Hebert CH, Carey CD, Rodig SJ, Shareef SJ, Najm FJ, van Galen P, Wakimoto H, Cahill DP, Rich JN, Aster JC, Suva ML, Patel AP, Bernstein BE (2017) Adaptive chromatin remodeling drives glioblastoma stem cell plasticity and drug tolerance. Cell Stem Cell. https://doi.org/10.1016/j.stem.2016.11.003
123. Tamura K, Aoyagi M, Ando N, Ogishima T, Wakimoto H, Yamamoto M, Ohno K (2013) Expansion of CD133-positive glioma cells in recurrent de novo glioblastomas after radiotherapy and chemotherapy. J Neurosurg 119(5):1145–1155
124. Zhou D, Alver BM, Li S, Hlady RA, Thompson JJ, Schroeder MA, Lee JH, Qiu J, Schwartz PH, Sarkaria JN, Robertson KD (2018) Distinctive epigenomes characterize glioma stem cells and their response to differentiation cues. Genome Biol 19(1):43. https://doi.org/10.1186/s13059-018-1420-6
125. Murata H, Yoshimoto K, Hatae R, Akagi Y, Mizoguchi M, Hata N, Kuga D, Nakamizo A, Amano T, Sayama T, Iihara K (2015) Detection of proneural/mesenchymal marker expression in glioblastoma: temporospatial dynamics and association with chromatin-modifying gene expression. J Neurooncol 125(1):33–41. https://doi.org/10.1007/s11060-015-1886-y
126. Ayer DE (1999) Histone deacetylases: transcriptional repression with SINers and NuRDs. Trends Cell Biol 9(5):193–198
127. Spange S, Wagner T, Heinzel T, Krämer OH (2009) Acetylation of non-histone proteins modulates cellular signalling at multiple levels. Int J Biochem Cell Biol 41(1):185–198
128. Singh BN, Zhang G, Hwa YL, Li J, Dowdy SC, Jiang S-W (2010) Nonhistone protein acetylation as cancer therapy targets. Expert Rev Anticancer Ther 10(6):935–954
129. Li Y, Seto E (2016) HDACs and HDAC Inhibitors in Cancer Development and Therapy. Cold Spring Harb Perspect Med. https://doi.org/10.1101/cshperspect.a026831
130. Marampon F, Megiorni F, Camero S, Crescioli C, McDowell HP, Sferra R, Vetuschi A, Pompili S, Ventura L, De Felice F (2017) HDAC4 and HDAC6 sustain DNA double strand break repair and stem-like phenotype by promoting radioresistance in glioblastoma cells. Cancer Lett 397:1–11
131. Santos-Barriopedro I, Li Y, Bahl S, Seto E (2019) HDAC8 affects MGMT levels in glioblastoma cell lines via interaction with the proteasome receptor ADRM1. Genes Cancer 10(5–6):119–133. https://doi.org/10.18632/genesandcancer.197
132. Pillonel V, Reichert N, Cao C, Heideman MR, Yamaguchi T, Matthias G, Tzankov A, Matthias P (2016) Histone deacetylase

1 plays a predominant pro-oncogenic role in Eμ-myc driven B cell lymphoma. Sci Rep 6:37772
133. Pinazza M, Ghisi M, Minuzzo S, Agnusdei V, Fossati G, Ciminale V, Pezzè L, Ciribilli Y, Pilotto G, Venturoli C (2018) Histone deacetylase 6 controls Notch3 trafficking and degradation in T-cell acute lymphoblastic leukemia cells. Oncogene 37(28):3839–3851
134. Wells CE, Bhaskara S, Stengel KR et al (2013) Inhibition of histone deacetylase 3 causes replication stress in cutaneous T cell lymphoma. PLoS One 8(7):e68915. https://doi.org/10.1371/journal.pone.0068915
135. Tharkar-Promod S, Johnson DP, Bennett SE, Dennis EM, Banowsky BG, Jones SS, Shearstone JR, Quayle SN, Min C, Jarpe M (2018) HDAC1, 2 inhibition and doxorubicin impair Mre11-dependent DNA repair and DISC to override BCR-ABL1-driven DSB repair in Philadelphia chromosome-positive B-cell precursor acute lymphoblastic leukemia. Leukemia 32(1):49–60
136. Johnson DP, Spitz GS, Tharkar S, Quayle SN, Shearstone JR, Jones S, McDowell ME, Wellman H, Tyler JK, Cairns BR (2015) HDAC1, 2 inhibition impairs EZH2-and BBAP-mediated DNA repair to overcome chemoresistance in EZH2 gain-of-function mutant diffuse large B-cell lymphoma. Oncotarget 6(7):4863
137. Ouaissi M, Silvy F, Loncle C, Ferraz da Silva D, Martins Abreu C, Martinez E, Berthezene P, Cadra S, Le Treut YP, Hardwigsen J, Sastre B, Sielezneff I, Benkoel L, Delgrande J, Ouaissi A, Iovanna J, Lombardo D, Mas E (2014) Further characterization of HDAC and SIRT gene expression patterns in pancreatic cancer and their relation to disease outcome. PLoS ONE 9(9):e108520. https://doi.org/10.1371/journal.pone.0108520
138. Park SY, Jun JA, Jeong KJ, Heo HJ, Sohn JS, Lee HY, Park CG, Kang J (2011) Histone deacetylases 1, 6 and 8 are critical for invasion in breast cancer. Oncol Rep 25(6):1677–1681. https://doi.org/10.3892/or.2011.1236
139. Campos B, Bermejo JL, Han L, Felsberg J, Ahmadi R, Grabe N, Reifenberger G, Unterberg A, Herold-Mende C (2011) Expression of nuclear receptor corepressors and class I histone deacetylases in astrocytic gliomas. Cancer Sci 102(2):387–392
140. Dali-Youcef N, Froelich S, Moussallieh F-M, Chibbaro S, Noël G, Namer IJ, Heikkinen S, Auwerx J (2015) Gene expression mapping of histone deacetylases and co-factors, and correlation with survival time and 1 h-hrmas metabolomic profile in human gliomas. Sci Rep 5:9087
141. Liu Q, Zheng JM, Chen JK, Yan XL, Chen HM, Nong WX, Huang HQ (2014) Histone deacetylase 5 promotes the proliferation of glioma cells by upregulation of Notch 1. Molecular Med Rep 10(4):2045–2050
142. Zhu J, Wan H, Xue C, Jiang T, Qian C, Zhang Y (2013) Histone deacetylase 3 implicated in the pathogenesis of children glioma by promoting glioma cell proliferation and migration. Brain Res 1520:15–22
143. Yang W, Liu Y, Gao R, Yu H, Sun T (2018) HDAC6 inhibition induces glioma stem cells differentiation and enhances cellular radiation sensitivity through the SHH/Gli1 signaling pathway. Cancer Lett 415:164–176
144. Li S, Liu X, Chen X, Zhang L, Wang X (2015) Histone deacetylase 6 promotes growth of glioblastoma through inhibition of SMAD2 signaling. Tumour Biol 36(12):9661–9665. https://doi.org/10.1007/s13277-015-3747-x
145. Lin TW, Chen MT, Lin LT, Huang PI, Lo WL, Yang YP, Lu KH, Chen YW, Chiou SH, Wu CW (2017) TDP-43/HDAC6 axis promoted tumor progression and regulated nutrient deprivation-induced autophagy in glioblastoma. Oncotarget 8(34):56612–56625. https://doi.org/10.18632/oncotarget.17979
146. Wang XQ, Bai HM, Li ST, Sun H, Min LZ, Tao BB, Zhong J, Li B (2017) Knockdown of HDAC1 expression suppresses invasion and induces apoptosis in glioma cells. Oncotarget 8(29):48027–48040. https://doi.org/10.18632/oncotarget.18227
147. Yang R, Wu Y, Wang M, Sun Z, Zou J, Zhang Y, Cui H (2015) HDAC9 promotes glioblastoma growth via TAZ-mediated EGFR pathway activation. Oncotarget 6(10):7644–7656. https://doi.org/10.18632/oncotarget.3223
148. Chang CJ, Hsu CC, Yung MC, Chen KY, Tzao C, Wu WF, Chou HY, Lee YY, Lu KH, Chiou SH, Ma HI (2009) Enhanced radiosensitivity and radiation-induced apoptosis in glioma CD133-positive cells by knockdown of SirT1 expression. Biochem Biophys Res Commun 380(2):236–242. https://doi.org/10.1016/j.bbrc.2009.01.040
149. Sayd S, Thirant C, El-Habr EA, Lipecka J, Dubois LG, Bogeas A, Tahiri-Jouti N, Chneiweiss H, Junier M-P (2014) Sirtuin-2 activity is required for glioma stem cell proliferation arrest but not necrosis induced by resveratrol. Stem Cell Rev Rep 10(1):103–113
150. Park H-K, Hong J-H, Oh YT, Kim SS, Yin J, Lee A-J, Chae YC, Kim JH, Park S-H, Park C-K (2019) Interplay between TRAP1 and sirtuin-3 modulates mitochondrial respiration and oxidative stress to maintain stemness of glioma stem cells. Can Res 79(7):1369–1382
151. Yamashita AS, da Costa RM, Borodovsky A, Festuccia WT, Chan T, Riggins GJ (2019) Demethylation and epigenetic modification with 5-azacytidine reduces IDH1 mutant glioma growth in combination with temozolomide. Neuro-oncology 21(2):189–200
152. Grinshtein N, Rioseco CC, Marcellus R, Uehling D, Aman A, Lun X, Muto O, Podmore L, Lever J, Shen Y (2016) Small molecule epigenetic screen identifies novel EZH2 and HDAC inhibitors that target glioblastoma brain tumor-initiating cells. Oncotarget 7(37):59360
153. Singh MM, Johnson B, Venkatarayan A, Flores ER, Zhang J, Su X, Barton M, Lang F, Chandra J (2015) Preclinical activity of combined HDAC and KDM1A inhibition in glioblastoma. Neuro-oncology 17(11):1463–1473
154. Bose P, Dai Y, Grant S (2014) Histone deacetylase inhibitor (HDACI) mechanisms of action: emerging insights. Pharmacol Ther 143(3):323–336
155. Wanczyk M, Roszczenko K, Marcinkiewicz K, Bojarczuk K, Kowara M, Winiarska M (2011) HDACi–going through the mechanisms. Front Biosci 16:340–359
156. Hsu C-C, Chang W-C, Hsu T-I, Liu J-J, Yeh S-H, Wang J-Y, Liou J-P, Ko C-Y, Chang K-Y, Chuang J-Y (2016) Suberoylanilide hydroxamic acid represses glioma stem-like cells. J Biomed Sci 23(1):81
157. Pastorino O, Gentile MT, Mancini A, Del Gaudio N, Di Costanzo A, Bajetto A, Franco P, Altucci L, Florio T, Stoppelli MP (2019) Histone deacetylase inhibitors impair vasculogenic mimicry from glioblastoma cells. Cancers 11(6):747
158. Alvarez AA, Field M, Bushnev S, Longo MS, Sugaya K (2015) The effects of histone deacetylase inhibitors on glioblastoma-derived stem cells. J Mol Neurosci 55(1):7–20
159. Orzan F, Pellegatta S, Poliani P, Pisati F, Caldera V, Menghi F, Kapetis D, Marras C, Schiffer D, Finocchiaro G (2011) Enhancer of Zeste 2 (EZH2) is up-regulated in malignant gliomas and in glioma stem-like cells. Neuropathol Appl Neurobiol 37(4):381–394
160. Riva G, Butta V, Cilibrasi C, Baronchelli S, Redaelli S, Dalprà L, Lavitrano M, Bentivegna A (2016) Epigenetic targeting of glioma stem cells: Short-term and long-term treatments with valproic acid modulate DNA methylation and differentiation behavior, but not temozolomide sensitivity. Oncol Rep 35(5):2811–2824
161. de Almeida SF, Caesar L, Jaeger M, Nör C, Abujamra AL, Schwartsmann G, de Farias CB, Brunetto AL, da Costa Lopez PL, Roesler R (2014) Inhibitory activities of trichostatin A in

U87 glioblastoma cells and tumorsphere-derived cells. J Mol Neurosci 54(1):27–40
162. Sun P, Xia S, Lal B, Eberhart CG, Quinones-Hinojosa A, Maciaczyk J, Matsui W, DiMeco F, Piccirillo SM, Vescovi AL (2009) DNER, an epigenetically modulated gene, regulates glioblastoma-derived neurosphere cell differentiation and tumor propagation. Stem Cells 27(7):1473–1486
163. Marampon F, Leoni F, Mancini A, Pietrantoni I, Codenotti S, Letizia F, Megiorni F, Porro G, Galbiati E, Pozzi P (2019) Histone deacetylase inhibitor ITF2357 (givinostat) reverts transformed phenotype and counteracts stemness in in vitro and in vivo models of human glioblastoma. J Cancer Res Clin Oncol 145(2):393–409
164. Angeletti F, Fossati G, Pattarozzi A, Würth R, Solari A, Daga A, Masiello I, Barbieri F, Florio T, Comincini S (2016) Inhibition of the autophagy pathway synergistically potentiates the cytotoxic activity of givinostat (ITF2357) on human glioblastoma cancer stem cells. Front Mol Neurosci 9:107
165. Rotili D, Tarantino D, Carafa V, Paolini C, Jörg S, Jung M, Botta G, Di Maro S, Novellino E, Steinkühler C (2012) Benzodeazaoxaflavins as sirtuin inhibitors with antiproliferative properties in cancer stem cells. J Med Chem 55(18):8193–8197
166. Nör C, Sassi FA, de Farias CB, Schwartsmann G, Abujamra AL, Lenz G, Brunetto AL, Roesler R (2013) The histone deacetylase inhibitor sodium butyrate promotes cell death and differentiation and reduces neurosphere formation in human medulloblastoma cells. Mol Neurobiol 48(3):533–543
167. Milde T, Kleber S, Korshunov A, Witt H, Hielscher T, Koch P, Kopp H-G, Jugold M, Deubzer HE, Oehme I (2011) A novel human high-risk ependymoma stem cell model reveals the differentiation-inducing potential of the histone deacetylase inhibitor Vorinostat. Acta Neuropathol 122(5):637
168. Asklund T, Kvarnbrink S, Holmlund C, Wibom C, Bergenheim T, Henriksson R, Hedman H (2012) Synergistic killing of glioblastoma stem-like cells by bortezomib and HDAC inhibitors. Anticancer Res 32(7):2407–2413
169. Sung GJ, Kim SH, Kwak S, Park SH, Song JH, Jung JH, Kim H, Choi KC (2019) Inhibition of TFEB oligomerization by co-treatment of melatonin with vorinostat promotes the therapeutic sensitivity in glioblastoma and glioma stem cells. J Pineal Res 66(3):e12556
170. Booth L, Roberts JL, Conley A, Cruickshanks N, Ridder T, Grant S, Poklepovic A, Dent P (2014) HDAC inhibitors enhance the lethality of low dose salinomycin in parental and stem-like GBM cells. Cancer Biol Ther 15(3):305–316
171. Pont LMB, Spoor JK, Venkatesan S, Swagemakers S, Kloezeman JJ, Dirven CM, van der Spek PJ, Lamfers ML, Leenstra S (2014) The Bcl-2 inhibitor Obatoclax overcomes resistance to histone deacetylase inhibitors SAHA and LBH589 as radiosensitizers in patient-derived glioblastoma stem-like cells. Genes Cancer 5(11–12):445
172. Tung B, Ma D, Wang S, Oyinlade O, Laterra J, Ying M, Lv S-Q, Wei S, Xia S (2018) Krüppel-like factor 9 and histone deacetylase inhibitors synergistically induce cell death in glioblastoma stem-like cells. BMC Cancer 18(1):1025
173. Pont LMB, Kleijn A, Kloezeman JJ, van den Bossche W, Kaufmann JK, de Vrij J, Leenstra S, Dirven CM, Lamfers ML (2015) The HDAC inhibitors scriptaid and LBH589 combined with the oncolytic virus Delta24-RGD exert enhanced anti-tumor efficacy in patient-derived glioblastoma cells. PLoS ONE 10(5):e0127058
174. Suraweera A, O'Byrne KJ, Richard DJ (2018) Combination therapy with histone deacetylase inhibitors (HDACi) for the treatment of cancer: achieving the full therapeutic potential of HDACi. Front Oncol 8:92. https://doi.org/10.3389/fonc.2018.00092
175. Siegal T (2013) Which drug or drug delivery system can change clinical practice for brain tumor therapy? Neuro-oncology 15(6):656–669
176. Siegal T (2014) Which Drug or Drug Delivery Method Can Change Clinical Practice for Brain Tumor Therapy? In: Drug Delivery to the Brain. Springer, pp 657–682. https://doi.org/10.1093/neuonc/not016
177. Zhao J (2016) Cancer stem cells and chemoresistance: The smartest survives the raid. Pharmacol Ther 160:145–158
178. Di C, Zhao Y (2015) Multiple drug resistance due to resistance to stem cells and stem cell treatment progress in cancer. Experim Therapeutic Med 9(2):289–293
179. Rabé M, Dumont S, Álvarez-Arenas A, Janati H, Belmonte-Beitia J, Calvo GF, Thibault-Carpentier C, Séry Q, Chauvin C, Joalland N (2020) Identification of a transient state during the acquisition of temozolomide resistance in glioblastoma. Cell Death Dis 11(1):1–14
180. Lee J-H, Choy ML, Marks PA (2012) Mechanisms of resistance to histone deacetylase inhibitors. In: Advances in cancer research, vol 116. Elsevier, pp 39–86. https://doi.org/10.1016/b978-0-12-394387-3.00002-1
181. Xiao JJ, Foraker AB, Swaan PW, Liu S, Huang Y, Dai Z, Chen J, Sadée W, Byrd J, Marcucci G (2005) Efflux of depsipeptide FK228 (FR901228, NSC-630176) is mediated by P-glycoprotein and multidrug resistance-associated protein 1. J Pharmacol Exp Ther 313(1):268–276
182. Kim G-H, Choi SY, Oh T-I, Kan S-Y, Kang H, Lee S, Oh T, Ko HM, Lim J-H (2019) IDH1R132H Causes Resistance to HDAC Inhibitors by Increasing NANOG in Glioblastoma Cells. Int J Mol Sci 20(11):2679
183. Reddy RG, Surineni G, Bhattacharya D, Marvadi SK, Sagar A, Kalle AM, Kumar A, Kantevari S, Chakravarty S (2019) Crafting carbazole-based vorinostat and tubastatin-a-like histone deacetylase (HDAC) inhibitors with potent in vitro and in vivo neuroactive functions. ACS Omega 4(17):17279–17294
184. Elechalawar CK, Bhattacharya D, Ahmed MT, Gora H, Sridharan K, Chaturbedy P, Sinha SH, Jaggarapu MMCS, Narayan KP, Chakravarty S (2019) Dual targeting of folate receptor-expressing glioma tumor-associated macrophages and epithelial cells in the brain using a carbon nanosphere–cationic folate nanoconjugate. Nanoscale Adv 1(9):3555–3567
185. Kumari S, Bhattacharya D, Rangaraj N, Chakarvarty S, Kondapi AK, Rao NM (2018) Aurora kinase B siRNA-loaded lactoferrin nanoparticles potentiate the efficacy of temozolomide in treating glioblastoma. Nanomedicine 13(20):2579–2596
186. Bhunia S, Vangala V, Bhattacharya D, Ravuri HG, Kuncha M, Chakravarty S, Sistla R, Chaudhuri A (2017) Large amino acid transporter 1 selective liposomes of L-DOPA functionalized amphiphile for combating glioblastoma. Mol Pharm 14(11):3834–3847
187. Saha S, Venu Y, Bhattacharya D, Kompella SD, Madhusudana K, Chakravarty S, Ramakrishna S, Chaudhuri A (2017) Combating established mouse glioblastoma through nicotinylated-liposomes-mediated targeted chemotherapy in combination with dendritic-cell-based genetic immunization. Adv Biosyst 1(1–2):1600009

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.