Glioma stem cells (GSCs) are crucial in the formation, perpetuation and recurrence of glioblastomas (GBs) due to their self-renewal and proliferation properties. Although GSCs share cellular and molecular characteristics with neural stem cells (NSCs), GSCs show unique transcriptional and epigenetic features that may explain their relevant role in GB and may constitute druggable targets for novel therapeutic approaches. In this review, we will summarize the most important findings in GSCs concerning epigenetic-dependent mechanisms.

Keywords: glioblastoma, histone, DNA, methylation, acetylation, Polycomb, H3.3, HDACi

INTRODUCTION

GB is the most common and aggressive primary brain cancer in adults. Despite the combined clinical therapy of surgical resection, radiotherapy and chemotherapy with the first-line agent temozolomide (TMZ), the prognosis is still unfavorable, with a median overall survival of 15 months and a high risk of recurrence (>90%) (1). This ability to resist chemo- and radiotherapy can be explained by the presence of a subpopulation of cells within the perivascular and hypoxic niches of the tumor known as GSCs or brain tumor-initiating cells. The subventricular zone (SVZ) is a neurogenic niche containing NSCs and progenitor cells and is suspected to be the origin of different brain tumor types due to the generation of GSCs (2–4). GSCs share functional characteristics with NSCs, including the capacity for self-renewal and long-term proliferation required to maintain and propagate the tumor, respectively (5). In addition, GSCs exhibit other properties of cancer cells, such as angiogenesis, invasion and immunosuppression, that promote disease progression and complicate treatment (6). Cells positive for stemness markers (e.g., CD133) have the ability to form tumors in vivo and oncospheres in vitro (reminiscent of neurosphere-derived NSCs) (6). In fact, understanding the hallmarks of GSCs can offer novel therapeutic strategies targeted at these cells to achieve an effective treatment for this disease.

Abbreviations: BMP, Bone morphogenic protein; BRD, Brodomain; CNTF, Ciliary neurotrophic factor; DIPG, Diffuse intrinsic pontine gliomas; EED, Embryonic ectoderm development; EMT, Epithelial–mesenchymal transition; ESC, Embryonic stem cell; EZH2, Enhancer of Zeste homolog 2; GB, Glioblastoma; GSC, Glioma stem cell; HDAC, Histone deacetylase; HDACi, HDAC inhibitor; HOTAIR, HOX transcript antisense RNA; KAT, Lysine acetyltransferase; IncRNA, Long Non-coding RNA; MELK, Maternal embryonic leucine zipper kinase; MGMT, O-6-methylguanine-DNA methyltransferase; NEK2, NIMA-related kinase 2; NSC, Neural stem cell; PRC1/2, Polycomb repressive complex 1/2; SUZ12, Suppressor of Zeste 12; SVZ, subventricular zone; TMZ, Temozolomide; TUG1, Taurine upregulated gene 1; VPA, Valproic acid.
THE RELEVANCE OF EPIGENETICS IN THE REGULATION OF GENE EXPRESSION IN GSCs AND NSCs

The nucleosome is the structural unit of chromatin and is composed of 147 bp of DNA wrapped around an octamer of histones (H2A, H2B, H3, and H4). The chromatin organization and its degree of compaction are modulated by DNA and histone covalent modifications, ATP-dependent chromatin remodeling and certain non-coding RNAs (ncRNAs). Epigenetic mechanisms contribute to the cellular hierarchy of tumoral tissue in GB (7) and are crucial to understanding tumorigenesis and response to treatment in gliomas. For example, promoter hypermethylation of the O-6-methylguanine-DNA methyltransferase (MGMT) gene can predict good outcomes in TMZ treatment (8, 9). Additionally, mutations in arginine 132 of the tricarboxylic acid cycle component IDH1 (or in arginine 172 of IDH2), which are associated with longer survival, induce the overproduction of the 2-hydroxybutyrate metabolite that inhibits the α-ketoglutarate-dependent activity of epigenetic enzymes such as JumonjiC histone demethylases and TET hydroxymethylases, affecting both histone and DNA methylation (10).

The gene expression profiles of GSCs resemble those of normal NSCs (11, 12), but differential gene expression patterns between both types of cells can identify a transcriptional signature that is correlated with patient survival (13); however, copy number variations only explain a small portion of such gene expression alterations and other mechanisms (e.g., epigenetics) should be more relevant. For instance, changes in the patterns of DNA methylation, H3K27me3 and H3K4me3 are important in neural lineage differentiation (14–16), and a comparison of the genome-wide distribution of these and other epigenetic marks revealed important differences between GSCs and normal NSCs, affecting genes involved in neural differentiation and cancer processes (17, 18). These glioma-specific patterns of epigenetic marks can be found in DNA elements that are important for gene regulation:

- Bivalent promoters are considered a feature of embryonic stem cells (ESCs) due to their high prevalence in these cells (16, 19) and are characterized by the coexistence of epigenetic marks associated with active and repressed genes (generally H3K4me3 and H3K27me3). Genes under the control of such promoters are poised, i.e., maintained in silent state but ready to be activated under appropriate external or developmental stimuli (20). Genome-wide analyses identified a high diversity of bivalent regions within GSCs, which were shown to have significantly distinct patterns compared to NSCs and ESCs (17, 21). Loss of bivalency in GSCs affected a very low number of promoters but associated with the potential activation of proto-oncogenes and genes related to transcription, and the potential repression of genes linked to cell adhesion and ion channels (17). Moreover, consistent bivalent genes across several GSCs were members of the Wnt pathway and HOX family as well as potassium channels and solute carriers that can be associated with overall survival (21).

- Enhancers often regulate cell-specific gene expression and are defined by the simultaneous occupancy of H3K27ac and H3K4me1. Although enhancer patterns are relatively conserved between GSCs and NSCs, unique GSC patterns are mainly linked to genes with functions in DNA damage response, p53 signaling and angiogenesis; prominent examples are HOX cluster genes, which acquire enhancer histone modifications in GSCs and become highly expressed despite promoter methylation (22). In contrast, NSC-specific enhancers are more associated with stem cell differentiation, apoptosis and epigenetic regulation (22).

Overall, GSCs are characterized by an impairment of differentiation due to a permanent epigenetic block that maintains the self-renewal capacity of these cells (18, 23). Nonetheless, GSCs can rapidly adapt to diverse microenvironments by modulating their transcriptomes and DNA methylomes (24), indicating that such alterations are at least partially reversible, contrary to genetic variations. Reversibility of epigenetic marks was demonstrated in reprogramming experiments of glioma cells with the appropriate combination of transcription factors they can be reversed into an early embryonic state that was accompanied by a widespread resetting of cancer-associated DNA methylation (23). Still, this resetting was not sufficient to abolish the malignant behavior of these cancer cells, indicating that we need to decipher how epigenetic-related activities work in GSCs to explaining their malignancy. In the following sections we review the experimental evidences found in GSCs about the role of epigenetics in malignancy and potential treatments.

THE ROLE OF POLYCOMB REPRESSIVE COMPLEXES IN THE MAINTENANCE OF THE GSC PHENOTYPE

The Polycomb repressive complexes, essential for normal developmental processes, have been the most studied epigenetic modulators in GSCs. The most relevant findings are summarized in Figure 1A. Polycomb repressive complex 2 (PRC2) is necessary for neurogenesis at the SVZ (25, 26) and regulates the trimethylation of H3K27 thanks to the catalytic activity of Enhancer of Zeste Homolog 2 (EZH2), which transfers a methyl group from S-adenosyl methionine, in cooperation with Suppressor of Zeste 12 (SUZ12) and Embryonic Ectoderm Development (EED). Overexpression of EZH2 has proto-oncogenic implications in several cancers, including glioma, in which elevated EZH2 expression has been associated with high-grade disease and poor overall survival (27, 28). Moreover, EZH2 activity is required for GSC maintenance by targeting MYC expression (29). Even in cells derived from diffuse intrinsic pontine gliomas (DIPG), a brain pediatric cancer that can also affect young adults, in which the actions of EZH2 are inhibited by the H3K27M mutation, residual EZH2 activity is still retained at strong PRC2 targets to drive GSC proliferation (30). Therefore, it is not surprising that selective EZH2 inhibition can constitute a promising therapeutic approach, as treated GSCs can reduce the
levels of EZH2 and H3K27me3, cell proliferation and migration, the number and diameter of oncospheres, and the growth of intracranial xenotransplanted cells in mice, reverse epithelial-mesenchymal transition (EMT), potentiate the effects of TMZ and downregulate stem cell markers while increasing the expression of differentiation markers (29, 31–33).

PRC2 activity is important for other epigenetic modifications. First, trimethylation of H3K27 is a prerequisite for histone H2A monoubiquitylation by Polycomb repressive complex 1 (PRC1) (34). Within this complex, the ring finger protein BMI1 is also a glioma stemness marker, and interference of its activity affects GSC malignancy in vitro and in xenotransplanted mice and enhances radiosensitivity (35–37). Second, EZH2 can recruit DNA methyltransferases (38), which explains the hypermethylation of PRC2 targets in primary GB (39, 40).

GSC characteristics display regional variations depending on the tumor niche. Whereas the regions defined by the disruption of the blood-brain barrier in angiogenesis foci were characterized by a high expression of proneural genes, an enrichment of EZH2/SUZ12/H3K27me3 targets and GSCs primarily positive to the proneural markers SOX2 and OLG2, the hypoxic necrotic regions contained high expression of mesenchymal genes, a strong association with H2A119ub, an enrichment of BMI1 targets and GSCs primarily positive to the mesenchymal markers CD44 and YKL40 (41). Selective inhibition of either EZH2 or BMI1 was highly effective against the survival of...
pronеural and mesenchymal GSCs, respectively. Thus, the combined strategy to abolish the activity of both PRCs can target different tumor compartments, increasing the efficacy of the therapy (41).

Research on GSCs is starting to disentangle EZH2-dependent oncogenic mechanisms. In certain GSCs, astroglial differentiation mediated by the bone morphogenetic protein (BMP) and ciliary neurotrophic factor (CNTF) signaling pathways is impaired due to the silencing of the BMP receptor subtype gene BMPRIIB by hypermethylation of its promoter, mediated by the EZH2-dependent recruitment of DNMT1 (42). Whereas incubation with BMP2 or CNTF can induce an increase in the differentiation markers GFAP or β-III tubulin in cultured NSCs and GSCs, in GSCs with impaired expression of BMPRIIB, these trophic factors enhance proliferation (42). These pleiotropic actions are reminiscent of the role of the BMP signaling pathway in embryonic NSCs to promote either NSC proliferation or neuronal differentiation, depending on the expression of the BMP receptor subunit (43). To add more complexity to the EZH2 involvement in gliomagenesis, EZH2 can methylate non-histone proteins such as oncogenic STAT3. This association leads to enhanced activation of STAT3 to positively regulate GSC self-renewal and survival (44).

How PRC2 activity is deregulated in GSCs has been intensively explored. For instance, EZH2-dependent resistance of GSCs to radiotherapy can be explained by the transcriptional upregulation of EZH2 induced by maternal embryonic leucine zipper kinase (MElk) and activation of EZH2 through phosphorylation by NIMA-related kinase 2 (NEK2) (33, 45). Moreover, it has been proposed that dysfunction of miR-128 is an early event of gliomagenesis that increases the levels of both SUZ12 and BMI1, augmenting the histone modifications they regulate: H3K27me3 and H2AK119ub. These observations suggested a coordinated regulation of PRC1 and PRC2 activities. Therefore, restoring miR-128 expression diminishes proliferation and confers radiosensitivity (46). Additionally, EZH2 activity can be regulated by the IncRNA HOX transcript antisense RNA (HOTAIR), which is associated with poor survival in diverse cancers (47). In CD133+ cells, HOTAIR recruits both EZH2 and the lysine demethylase KDM1A/LSD1 to repress the tumor suppressor gene PDCD4 (48). In addition, another IncRNA, taurine upregulated gene 1 (TUG1), also binds to EZH2 and SUZ12 to repress neuronal differentiation genes such as BDNF, NGF, and NTF3 (49).

**OTHER EPIGENETIC MODULATORS**

In addition to PRCs and H3.3, other epigenetic-related factors have been implicated in the GSC phenotype and are listed in Table 1.

**HDAC INHIBITORS AS THERAPEUTIC AGENTS IN GSCs**

Considering that altered gene expression levels have been reported for histone deacetylases (HDACs) in GB (66, 67), most therapeutic approaches have been focused on histone deacetylase inhibitors (HDACis) due to their recognized antiproliferative effects in multiple cancer models and their benefits and tolerability in the amelioration of several neurological conditions in vivo at the preclinical stage; in addition, some of these compounds have been approved as therapeutic agents in other types of cancers. Histone acetylation is regulated by the opposing enzymatic activities of lysine acetyltransferases and HDACs: whereas the former enzymes transfer the acetyl group from an acetyl-CoA molecule to the lynes of the protruding histone tails (an activity that is associated with active genes), HDACs catalyze this removal, which is associated with gene repression. Inhibition of HDACs can induce cell cycle arrest, apoptosis and cellular differentiation and can interfere with cancer angiogenesis (68). One interesting target of HDACis is the phosphatase DUSP1, an inhibitor of the JNK, ERK1/2 and p38 MAPK pathways that is associated with GSC differentiation and good prognosis (69).

Among the tested HDACis in clinical trials, vorinostat/SAH, romidepsin/FK228/FR901228 and panobinostat/LBH-589
demonstrated very limited efficacy as therapeutic agents in single therapies in both newly diagnosed and recurrent GB. However, the most promising effect of HDACi-based treatment is as sensitizers to current therapeutic approaches such as radiotherapy and TMZ therapy [see (70) for a review on this topic]. Some considerations should be kept in mind to understand the potential benefits and limitations of HDACi-based treatment in vivo. First, acetylation increase by HDACi is not exclusive of histones (71); second, antineoplastic actions of HDACi can be achieved independently of (or in addition to) HDAC inhibition (72); third, the solubility of HDACi in water is usually poor, resulting in inefficient transport through the blood-brain barrier with oral administration (73); last, chemoresistance has been reported in long-term treatments (74). In any case, the prospects of using HDACi are still promising, and research on GSCs can help in elucidating the underlying anticancer mechanisms of HDAC inhibition and in proposing novel formulations to improve drug delivery (e.g., loading these hydrophobic compounds into nanomicelles) (75). Efforts are being mainly focused on valproic acid (VPA), with proven antitumoral effects (72, 76, 77). Often administered as an anticonvulsant agent to treat epilepsy in brain tumors (78), retrospective clinical studies reported that treatment with this compound increased the overall survival of GB patients (79) although this effect was not found in other reports and still remains controversial (80, 81). VPA is capable of inducing a predifferentiation state in GSCs (74) and can be combined with other antineoplastic compounds for synergistic effects, as reported for the antimitotic paclitaxel (82). However, VPA failed to sensitize GSCs to TMZ (74), although another study reported sensitization to both TMZ and nimustine (ACNU), especially in MGMT-expressing cells (83). VPA is able to modify the DNA methylomes of GSCs (74), leading to the activation of the Wnt/β-catenin pathway which was related with growth inhibition, reduced migration and EMT impairment (84). This is in conflict with the suppression of the Wnt/β-catenin pathway by SAHA, which partially rescues the downregulation of histocompatibility complex class I and antigen-processing machinery genes, as a plausible strategy to potentiate the activation of cytotoxic T cells in vivo (85).

**CONCLUDING REMARKS**

Research on the epigenetics of GSCs has the potential to elucidate the self-renewal and perpetuation mechanisms of these cells through the identification of the epigenetic program that governs aberrant gene activation and repression in cancer. Less known epigenetic modifications should be further explored, as they can provide further insights into tumorigenesis, as in the case of 5′-formylcytosine (5fC) and 5′-carboxycytosine (5caC) (22). In addition, a systematic and detailed description of direct target genes of epigenetic activities is required to understand the complex mechanisms of epigenetic dysregulation in gliomas. As we have seen through this review, multiple epigenetic activities can be involved in glioma malignancy in a complex manner; therefore, the simultaneous modulation of various epigenetic activities may be highly effective, as demonstrated by the dual inhibition of HDACs and KDM1A/LSD1 (87, 88).

**AUTHOR CONTRIBUTIONS**

All authors contributed to the article and approved the submitted version.
FUNDING
LV is supported by the Plan Propio INiBICA (Grant L19-071N-CO07) and by the Programa Estatal de Generación de Conocimiento y Fortalecimiento del Sistema Español de I+D+i, financed by the Instituto de Salud Carlos III and Fondo Europeo de Desarrollo Regional 2014–2020 (Grants CP15/00180 and P116/00722). LV is the recipient of a Miguel Servet I contract (CP15/00180) financed by the Instituto de Salud Carlos III and Fondo Social Europeo 2014–2020, Programa Estatal de Promoción del Talento y su empleabilidad in I+D+i.

REFERENCES
1. Stupp R, Hegi ME, Mason WP, van den Bent MJ, Taphoorn MJ, Janzer RC, et al. Effects of radiotherapy with concomitant and adjuvant temozolomide versus radiotherapy alone on survival in glioblastoma in a randomised phase III study: 5-year analysis of the EORTC-NCIC trial. Lancet Oncol (2009) 10(5):459–66. doi: 10.1016/S1470-2245(09)70025-7
2. Lim DA, Cha S, Mayo MC, Chen MH, Keles E, VandenBerg S, et al. Effects of glioblastoma cell secretome on cancer stem cell expansion in a co-culture system in vivo. Nat Commun (2013) 4:188–9. doi: 10.1038/ncomms2537
3. Suva ML, Riggi N, Bernstein BE. Epigenetic reprogramming in cancer. Science (2013) 339(6127):1567–70. doi: 10.1126/science.1230184
4. Chang S, Yim S, Park H. The cancer driver genes IDH1/2, JARID1C/KDM5C, and MLL1/ENL are upregulated in glioblastoma stem cells. Neoplasia (2019) 21(5):459–69. doi: 10.1016/j.neo.2019.02.003
5. Haskins WE, Zablotsky BL, Foret MR, Ihlrie RA, Alvarez-Buylla A, Eisenman RN, et al. Molecular Characteristics in MRI-Classified Group I Glioblastoma Multiforme. Front Oncol (2013) 3:318. doi: 10.3389/fonc.2013.00318
6. Vescovi AL, Galli R, Reynolds BA. Brain tumour stem cells. Nat Rev Cancer (2006) 6(6):425–36. doi: 10.1038/nrc1889
7. Heddleston JM, Hitomi M, Venere M, Flavahan WA, Yang K, Kim Y, et al. EZH2 is essential for glioblastoma cancer stem cell maintenance. Oncotarget (2013) 4(10):76. doi: 10.1186/gm377
8. Stazi G, Taglieri L, Nicolai A, Romanelli A, Fioravanti R, Morrone S, et al. DNA methylation is a critical cell-intrinsic determinant of astrocyte differentiation in the fetal brain. Dev Cell (2001) 1(6):749–58. doi: 10.1016/S1534-5807(01)00101-0
9. Jepsen K, Solum D, Zhou T, McEvilly RJ, Kim HJ, Glass CK, et al. SMRT-mediated repression of an H3K27 demethylase in progression from neural stem cell to neuron. Nature (2007) 450(7168):415–9. doi: 10.1038/ nature06270
10. Pickrell KE, Liao Y, Ely D, Tamir H, Wilson RK, Fennell T, et al. The origin of differential nucleosome modification in frontal cortex and cerebellum. Cell (2009) 137(5):881–92. doi: 10.1016/j.cell.2009.05.025
11. Wang J, Cheng P, Pavlyukov MS, Yu H, Zhang Z, Kim SH, et al. Targeting NEK2 attenuates glioblastoma growth and radiosensitivity by destabilizing histone methyltransferase EZH2. J Clin Invest (2017) 127(8):3075–89. doi: 10.1172/JCI89902
41. Jin X, Kim LJY, Wu Q, Wallace LC, Prager BC, Sanvoranart T, et al. Targeting of the Bmi-1 oncogene/stem cell renewal factor by microRNA-128 inhibits glioma proliferation and self-renewal. Cancer Res (2008) 68(22):9125–30. doi: 10.1158/0008-5472.CAN-08-2629.

42. Facchino S, Abdouh M, Chatoo W, Bernier G. BMI1 confers radioresistance to normal and cancerous neural stem cells through recruitment of the DNA damage response machinery. J Neurosci Res (2010) 30(30):10096–111. doi: 10.1002/jnr.20364.

43. Godlewski J, Nowicki MO, Bronisz A, Williams S, Otaki S, Nuovo G, et al. Targeting of the Bmi-1 oncogene/stem cell renewal factor by microRNA-128 inhibits glioma proliferation and self-renewal. Cancer Res (2008) 68(22):9125–30. doi: 10.1158/0008-5472.CAN-08-2629.

44. Vire E, Brenner C, Deplus R, Blanchon L, Fraga M, Didelot c, et al. The Polyclub group protein EZH2 directly controls DNA methylation. Nature (2006) 439(7078):871–4. doi: 10.1038/nature04431.

45. Widschwendter M, Fiegel H, Egle D, Mueller-Holzner E, Spizzo G, Marth C, et al. Epigenetic stem cell signature in cancer. Nat Genet (2007) 39(2):157–8. doi: 10.1038/ng1941.

46. Martinez R, Martin-Subero JI, Rohde V, Fernandez AF, et al. A microarray-based DNA methylation study of glioblastoma multiforme. Epigenetics (2009) 4(4):255–64. doi: 10.4161/epi.91390.

47. Jin X, Kim LJY, Wu Q, Wallace LC, Prager BC, Sanvoranart T, et al. Targeting glioma stem cells through combined BMI1 and EZH2 inhibition. Nat Med (2017) 23(11):1352–61. doi: 10.1038/nm.4415.

48. Lee J, Son MJ, Woolard K, Donin NM, Li A, Cheng CH, et al. Epigenetic-mediated dysfunction of the bone morphogenetic protein pathway inhibits differentiation of glioblastoma-initiating cells. Cancer Cell (2008) 13(6):169–80. doi: 10.1016/j.ccc.2007.12.005.

49. Hall AK, Miller RH. Emerging roles for bone morphogenetic proteins in central nervous system glioblastoma. J Neurosci Res (2004) 76(1):1–8. doi: 10.1002/jnr.20019.

50. Kim E, Kim M, Woo DH, Shin Y, Shin J, Chang N, et al. Phosphorylation of EZH2 activates STAT3 signaling via STAT3 methylation and promotes tumorigenicity of glioblastoma stem-like cells. Cancer Cell (2013) 23(6):839–52. doi: 10.1016/j.ccc.2013.04.008.

51. Kim SH, Joshi K, Ezharalasan R, Myers TR, Siu J, Gu C, et al. EZH2 protects glioma stem cells from radiation-induced cell death in a MELK/FoxM1-dependent manner. Stem Cell Rep (2015) 4(2):226–38. doi: 10.1002/stemcr.2014.12.006.

52. Peruzzi P, Bronisz A, Nowicki MO, Wang Y, Ogawa D, Price R, et al. MicroRNA-128 coordinately targets Polyclub Repressor Complexes in glioma stem cells. Neuro Oncol (2013) 15(9):1212–24. doi: 10.1093/neuonc/nos355.

53. Wang L, He Z. Functional Roles of Long Non-Coding RNAs (lncRNAs) in Glioma Stem Cells. Med Sci Monit (2015) 21:5767–73. doi: 10.12659/MSM196400.

54. Fang K, Liu P, Dong S, Guo Y, Cui X, Zhu X, et al. Magnetofection based on superparamagnetic iron oxide nanoparticle-mediated low lncRNA HOTAIR expression decreases the proliferation and invasion of glioma stem cells. Int J Oncol (2016) 49(2):509–18. doi: 10.3892/ijo.2016.3571.

55. Katsushima K, Natsume A, Ohka F, Shinjo K, Hatanaka A, Ichimura N, et al. Targeting the Notch-regulated non-coding RNA TUG1 for glioma treatment. Nat Commun (2016) 7:13616. doi: 10.1038/ncomms13616.

56. Herrmann A, Lahtz C, Song J, Aftabizadeh M, Cherryholmes GA, Xin H, et al. Integrin alpha6 signaling induces STAT3-TET3-mediated hydroxymethylation of genes critical for maintenance of glioma stem cells. Oncogene (2020) 39(10):2156–69. doi: 10.1038/s41388-019-1134-6.

57. Lucio-Eterovic AK, Cortez MA, Valera ET, Motta FJ, Queiroz RG, Machado HR, et al. Differential expression of 12 histone deacetylase (HDAC) genes in astrocytomas and normal brain tissue: class II and IV are hyperexpressed in glioblastomas. BMC Cancer (2008) 8:243. doi: 10.1186/1471-2407-8-243.

58. Lee P, Murphy B, Miller R, Menon V, Banik NL, Giglio P, et al. Mechanisms and clinical significance of histone deacetylase inhibitors: epigenetic glioblastoma therapy. Anticancer Res (2013) 35(2):615–25.

59. Eckelschager T, Pflieger M, Stiborova M, Cabo J, Heidegger C. Histone Deacetylase Inhibitors as Old Drugs in Glioblastoma. Anticancer Res (2012) 32(9):3907–14. doi: 10.2187/ncr.2012.2907.

60. Rasmussen TA, Tolstrup M, Moller HJ, Brinkmann CR, Olesen R, Erikstrup C, et al. Activation of latent human immunodeficiency virus by the histone
deacetylase inhibitor panobinostat: a pilot study to assess effects on the central nervous system. *Open Forum Infect Dis* (2015) 2(1):ofv037. doi: 10.1093/ofid/ofv037

Riva G, Butta V, Cilibrasi C, Baronechelli S, Redaelli S, Dalpra L, et al. 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. *Onco Rep* (2016) 35(5):2811–24. doi: 10.3892/or.2016.4665

Singleton WG, Collins AM, Bienemann AS, Killick-Cole CL, Haynes HR, Asby DJ, et al. Convection enhanced delivery of panobinostat (LBH589)-loaded pluronic nano-micelles prolongs survival in the F98 rat glioma model. *Int J Nanomed* (2017) 12:1385–99. doi: 10.2147/IJN.S125300

Osuka S, Takano S, Watanabe S, Ishikawa E, Yamamoto T, Matsumura A. Valproic acid inhibits angiogenesis in vitro and glioma angiogenesis in vivo in the brain. *Neurol Med Chir (Tokyo)* (2012) 52(4):186–93. doi: 10.2176/nmc.52.186

Chen Y, Tsai YH, Tseng SH. Valproic acid affected the survival and invasiveness of human glioma cells through diverse mechanisms. *J Neurooncol* (2012) 109(1):23–33. doi: 10.1007/s11060-012-0871-y

Maschio M, Aguglia U, Avanzini G, Banfi P, Buttinelli C, Capovilla G, et al. Management of epilepsy in brain tumors. *Neurol Sci* (2019) 40(10):2217–34. doi: 10.1007/s10072-019-04025-9

Ruda R, Pellerino A, Sofetti R. Does valproic acid affect tumor growth and improve survival in glioblastomas? *CNS Oncol* (2016) 5(2):51–3. doi: 10.2217/cns-2016-0004

Lu VM, Texakalidis P, McDonald KL, Mekary RA, Smith TR. The survival effect of valproic acid in glioblastoma and its current trend: a systematic review and meta-analysis. *Clin Neurol Neurosurg* (2018) 174:149–55. doi: 10.1016/j.clineuro.2018.09.019

Happold C, Gorlia T, Chinot O, Gilbert MR, Narbour LB, Wick W, et al. Does Valproic Acid or Levetiracetam Improve Survival in Glioblastoma? A Pooled Analysis of Prospective Clinical Trials in Newly Diagnosed Glioblastoma. *J Clin Oncol* (2016) 34(7):731–9. doi: 10.1200/JCO.2015.63.6563

Riva G, Baronechelli S, Paolella L, Butta V, Biunno I, Lavitrano M, et al. In vitro anticancer drug test: A new method emerges from the model of glioma stem cells. *Toxicol Rep* (2014) 1:188–99. doi: 10.1016/j.toxrep.2014.05.005

Li Z, Xia Y, Bu X, Yang D, Yuan Y, Guo X, et al. Effects of valproic acid on the susceptibility of human glioma stem cells for TMZ and ACNU. *Oncol Lett* (2018) 15(6):9877–83. doi: 10.3892/ol.2018.8551

Singleton WG, Collins AM, Bienemann AS, Killick-Cole CL, Haynes HR, Asby DJ, et al. Convection enhanced delivery of panobinostat (LBH589)-loaded pluronic nano-micelles prolongs survival in the F98 rat glioma model. *Int J Nanomed* (2017) 12:1385–99. doi: 10.2147/IJN.S125300

Osuka S, Takano S, Watanabe S, Ishikawa E, Yamamoto T, Matsumura A. Valproic acid inhibits angiogenesis in vitro and glioma angiogenesis in vivo in the brain. *Neurol Med Chir (Tokyo)* (2012) 52(4):186–93. doi: 10.2176/nmc.52.186

Chen Y, Tsai YH, Tseng SH. Valproic acid affected the survival and invasiveness of human glioma cells through diverse mechanisms. *J Neurooncol* (2012) 109(1):23–33. doi: 10.1007/s11060-012-0871-y

Maschio M, Aguglia U, Avanzini G, Banfi P, Buttinelli C, Capovilla G, et al. Management of epilepsy in brain tumors. *Neurol Sci* (2019) 40(10):2217–34. doi: 10.1007/s10072-019-04025-9

Ruda R, Pellerino A, Sofetti R. Does valproic acid affect tumor growth and improve survival in glioblastomas? *CNS Oncol* (2016) 5(2):51–3. doi: 10.2217/cns-2016-0004

Lu VM, Texakalidis P, McDonald KL, Mekary RA, Smith TR. The survival effect of valproic acid in glioblastoma and its current trend: a systematic review and meta-analysis. *Clin Neurol Neurosurg* (2018) 174:149–55. doi: 10.1016/j.clineuro.2018.09.019

Happold C, Gorlia T, Chinot O, Gilbert MR, Narbour LB, Wick W, et al. Does Valproic Acid or Levetiracetam Improve Survival in Glioblastoma? A Pooled Analysis of Prospective Clinical Trials in Newly Diagnosed Glioblastoma. *J Clin Oncol* (2016) 34(7):731–9. doi: 10.1200/JCO.2015.63.6563

Riva G, Baronechelli S, Paolella L, Butta V, Biunno I, Lavitrano M, et al. In vitro anticancer drug test: A new method emerges from the model of glioma stem cells. *Toxicol Rep* (2014) 1:188–99. doi: 10.1016/j.toxrep.2014.05.005

Li Z, Xia Y, Bu X, Yang D, Yuan Y, Guo X, et al. Effects of valproic acid on the susceptibility of human glioma stem cells for TMZ and ACNU. *Oncol Lett* (2018) 15(6):9877–83. doi: 10.3892/ol.2018.8551

Riva G, Cilibrasi C, Bazzoni R, Cadamuro M, Negroni C, Butta V, et al. Valproic Acid Inhibits Proliferation and Reduces Invasiveness in Glioma Stem Cells Through Wnt/beta Catenin Signalling Activation. *Genes (Basel)* (2018) 9(11):522. doi: 10.3390/genes9110522

Yang W, Li Y, Gao R, Xu Z, Sun T. MHC class I dysfunction of glioma stem cells escapes from CTL-mediated immune response via activation of Wnt/beta-catenin signaling pathway. *Oncogene* (2020) 39(5):1098–111. doi: 10.1038/s41388-019-1045-6

Cattaneo M, Baronechelli S, Schiffer D, Mellai M, Caldera V, Saccani GJ, et al. Down-modulation of SEL1L, an unfolded protein response and endoplasmic reticulum-associated degradation protein, sensitizes glioma stem cells to the cytotoxic effect of valproic acid. *J Biol Chem* (2014) 289(5):2826–38. doi: 10.1074/jbc.M113.527754

Singh MM, Manton CA, Bhat KP, Tsai WW, Aldape K, Barton MC, et al. Inhibition of LSD1 sensitizes glioblastoma cells to histone deacetylase inhibitors. *Neuro Oncol* (2011) 13(8):894–903. doi: 10.1093/neuonc/nor049

Singh MM, Johnson B, Venkatarayan A, Flores ER, Zhang J, Su X, et al. Preclinical activity of combined HDAC and KDM1A inhibition in glioblastoma. *Neuro Oncol* (2015) 17(11):1463–73. doi: 10.1093/neuonc/nov041

**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2020 Valor and Hervás-Corpión. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.