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Review

Advances in epigenetic glioblastoma therapy

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ABSTRACT

Glioblastoma multiforme (GBM) is the most lethal primary brain tumor in adults despite contemporary gold-standard first-line treatment strategies. This type of tumor recurs in virtually all patients and no commonly accepted standard treatment exists for the recurrent disease. Therefore, advances in all scientific and clinical aspects of GBM are urgently needed. Epigenetic mechanisms are one of the major factors contributing to the pathogenesis of cancers, including glioblastoma. Epigenetic modulators that regulate gene expression by altering the epigenome and non-histone proteins are being exploited as therapeutic drug targets. Over the last decade, numerous preclinical and clinical studies on histone deacetylase (HDAC) inhibitors have shown promising results in various cancers. This article provides an overview of the anticancer mechanisms of HDAC inhibitors and the role of HDAC isoforms in GBM. We also summarize current knowledge on HDAC inhibitors on the basis of preclinical studies and emerging clinical data.

INTRODUCTION

Glioblastoma multiforme (GBM) is an aggressive, highly invasive, vascularized brain tumor [1]. Despite multimodal treatment, prognosis is unfortunately very poor; less than 5% of patients surviving at 5 years following initial diagnosis. Standard regimen includes maximum safe surgical resection followed by chemoradiation therapy [2]. Genetics, epigenetics, bacterial infection, and many other factors influence GBM oncogenesis, but the molecular mechanism underlying gliomagenesis is poorly understood [3, 4]. Conventional chemotherapy has limited efficacy in GBM due to poor blood-brain barrier (BBB) penetration, intratumor heterogeneity, intrinsic GBM resistance, and nonspecific toxicity [1, 5]. Based on successful preclinical studies, many clinical trials have tested the efficacy of novel therapies, but improved survival for patients with GBM has been limitedly achieved over the past few decades [6]. Therefore, further work is urgently required to discover novel therapeutic targets and develop more effective combination strategies for GBM treatment.

Histone deacetylase (HDAC) inhibitors have evoked great interest for the treatment of numerous malignancies because they are able to change transcriptomic profiles to promote tumor cell death. Hallmark features of GBM, including enhanced proliferation, invasion and migration, angiogenesis, and resistance to apoptosis, are targeted by HDAC inhibitors. The HDAC inhibitors vorinostat, panobinostat, valproic acid (VPA), and entinostat are well-studied epigenetic agents that effectively radiosensitize various tumors, including GBM [7]. HDAC inhibitor is among the successful examples of epigenetic therapy. Several HDAC inhibitors are US FDA approved, including the hydroxamic acid-based compounds vorinostat, panobinostat, belinostat, and the depsipeptide romidepsin for hematological malignancies [8]. Vorinostat [9-12] and VPA [13] are currently being tested in clinical trials on GBM as either monotherapies or combination therapies. The other FDA-approved epigenetic drugs, azacytidine and decitabine, DNA methyltransferase inhibitors [14], have not been clinically tested to evaluate its anticancer effect on GBM. Although drugs targeting histone methyltransferases and demethylases have considerable potential, their specific effects and the stability of such effect must be elucidated in greater detail to develop as antitumor agents. [15]. To the best of our knowledge, no drugs that target histone methylation or epigenetic readers are FDA approved or under clinical trials. To date, of the epigenetic agents, only HDAC inhibitors have been investigated in clinical trials as antitumor agents against GBM. Thus, this review focuses on recent studies that

highlight the role of HDAC isoforms and discuss the preclinical and clinical data on HDAC inhibitors as therapeutic agents for GBM.

GLIOBLASTOMA MULTIFORME

Glioblastoma is the most common malignant primary tumor of the central nervous system in adults. GBM represents approximately half of all gliomas and 15% of primary brain tumors [1]. WHO grade IV GBM, which is the highest grade glioma, is divided into two major classes: "primary" and "secondary." A vast majority of GBMs arise *de novo* as primary GBMs in elderly patients. Secondary GBMs, those that arise from a pre-existing glioma of WHO grade II or III, are less frequent [16, 17]. Most secondary GBMs develop in younger patients (< 45 years). The disease incidence continues to increase with age and the median age at diagnosis is 64 years. Survival rates are poor; only approximately 34% of patients survived for 1 year, 12% for 2 years, and less than 5% for 5 years from the time of diagnosis. Older age and incomplete surgical resection are associated with poor survival [18, 19]. GBM remains one of the deadliest of malignancies, with limited treatment options and a high rate of recurrence [2, 20, 21].

While histologically identified ischemic necrosis and elevated microvascular proliferation, GBM is more accurately characterized and distinguished by its genomic and epigenomic profiles [19]. The Cancer Genome Atlas (TCGA) created a gene expression-based molecular classification system in which GBM is categorized into mesenchymal, classical, neural, and proneural subtypes [22]. These subtypes were compared with the corresponding normal neural cell types to determine the possible cellular origin for each of these tumors; correlations between subtype and clinical response were determined. TCGA research network reported that three signaling pathways are frequently modified in GBM: receptor tyrosine kinase (RTK)/Ras/phosphoinositide 3-kinase (PI3K), p53, and retinoblastoma (Rb) signaling. In adults, components of the RTK/Ras/PI3K pathway are mutated in 88% of GBMs, those of the p53 pathway in 87%, and those of the Rb pathway in 78%. Mutations such as amplification of the epidermal growth factor receptor (EGFR) can be found in 45% of GBMs, gain of PI3K function in 15%, and loss of phosphatase and tensin homolog (PTEN) in 36% [23, 24]. These discoveries have led to a better understanding of the molecular signature of GBM and have revealed numerous consistent changes in genes and pathways [4, 16, 22, 25, 26]. However, there is still an unmet need to translate these findings into clinical practice, identify predictive biomarkers, and improve outcomes for patients with GBM.

CURRENT STANDARD TREATMENT

The current first-line standard regimen for GBM is an aggressive combination therapy, including maximum

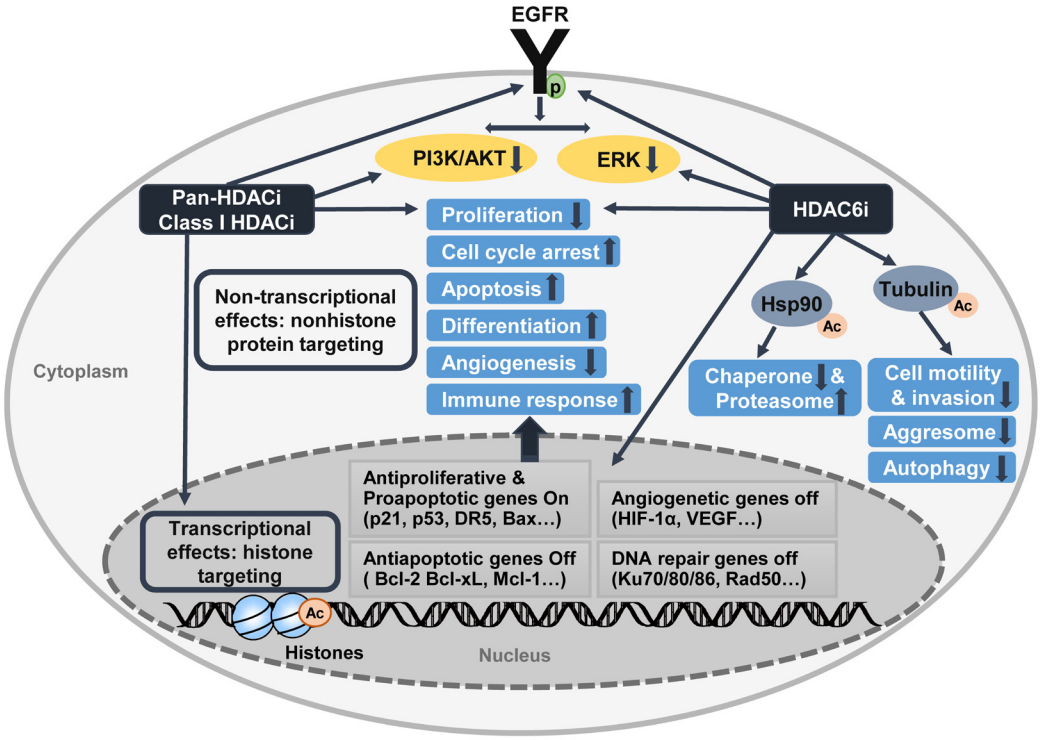

Figure 1: Antitumoral activity of HDAC inhibitors

safe surgical resection and adjuvant radiotherapy with concurrent and adjuvant temozolomide chemotherapy [2, 16]. Surgical resection is often compromised by the diffusely infiltrative nature of gliomas, which recur even after gross total resection. In addition, these tumors often invade critical neurological structures, precluding complete surgical resection [19, 27]. Radiation therapy following surgery increases the median survival times ranged from 14 to 36 weeks [28]. The benefits of treatment with radiation were initially established using whole brain radiotherapy, but improved technology (e.g., field radiation therapy) has markedly reduced the associated side effects [19]. A total radiation dose of 60 Gy delivered to the tumor provides the maximum survival benefit [29].

The addition of the alkylating agent temozolomide to postoperative radiation or concurrent temozolomide and radiotherapy is the only chemotherapeutic regimen that significantly improves the overall survival (OS) of patients with GBM. The methylation status of MGMT (O^6-methylguanine-DNA methyltransferase), a DNA-repair gene, is used as a GBM predictor because it is the major relevant biomarker for the response to temozolomide treatment [24]. Silencing of MGMT expression by promoter methylation impairs the ability of tumor cells to repair the DNA damage induced by temozolomide, further decreasing tumor cell survival [30]. Patients whose tumors have the unmethylated MGMT gene promoter also experience a modest but less significant benefit from the addition of temozolomide. Thus, combined treatment with temozolomide and radiation remains the standard regimen for all patients with GBM [19, 31]. However, the improved 2-year survival with temozolomide treatment is only in 27% [24], which is still unsatisfactory.

Currently, bevacizumab (Avastin) is the only approved therapeutic agent for the treatment of patients with recurrent GBM. Bevacizumab is a humanized therapeutic antibody that specifically binds to vascular endothelial growth factor (VEGF)-A, disrupting VEGF-VEGF receptor interaction and preventing angiogenesis [32, 33]. Because GBM tumors are particularly vascular and overexpress numerous angiogenic factors, antiangiogenic therapy is efficient. A phase II trial of combined treatment with bevacizumab and irinotecan (a topoisomerase 1 inhibitor) in recurrent GBM showed increased OS from 4.1 to 9.2 months [11]. The 6-month and 12-month survival rates were 77% and 31%, respectively [33-35]. However, patients who had received previous bevacizumab therapy had shorted PFS and OS. Bevacizumab was subsequently investigated in phase III trials for newly diagnosed GBM, but there was no effect on overall patient survival. In addition, phase III trials are currently being tested to evaluate the efficacy of bevacizumab with temozolomide and radiotherapy for newly diagnosed GBM (NCT00884741 and NCT00943826) [19, 36].

HDAC EXPRESSION IN GBM

The human genome contains 18 known HDACs, which are grouped into four classes on basis of phylogenetic analysis [37, 38]: class I (HDAC1, 2, 3 and 8), IIa (HDAC4, 5, 7, 9) and IIb (HDAC6, 10), III (SIRT1-7) and VI (HDAC11). The HDAC family is separated into Zn^{2+}-dependent (classes I, II and IV) and Zn^{2+}-independent (class III), nicotinamide-adenine dinucleotide-dependent enzymes. Class I, II and IV HDACs are also referred to as classical HDACs. Most HDAC inhibitors available as anticancer agents target class I and II HDACs. Class I HDACs are primarily nuclear proteins and have histones as principle target substrates. Class I HDACs are ubiquitously expressed in all tissues whereas class II HDACs are tissue-specifically expressed [39]. Class II HDACs shuttle between the nucleus and cytoplasm and have histone and non-histone proteins as primary targets. HDAC11 (class IV) is phylogenetically most closely related to HDAC3 and 8 but also has some features of class II HDACs [40]. Class III HDAC is also called sirtuins (SIRT) and comprises seven SIRT isoforms (SIRT1-7), which differ in their substrate specificities, functions and subcellular localization [41].

HDACs are overexpressed and mutated in various solid and hematologic malignancies and play key roles in tumorigenesis [38, 39]. The expression of individual HDACs is largely inversely correlated with disease-free and overall survival rates. The aberrant expression of HDACs correlates with a poor prognosis [42]. However, the expression and functions of HDACs in GBM are not well characterized. Recent studies have begun to focus on the expression patterns of HDACs in GBM. GBM cells and primary GBM tissues exhibit slightly and variably increased HDAC1, 3 and 6 expression levels compared to non-neoplastic brain tissues at both the mRNA and protein levels [43]. This result was further confirmed in silico using the REMBRANDT glioma dataset available through a GlioVis online application. In particular, HDAC1 and 3 expression levels correlate with WHO tumor grades, with the highest expression levels occurring in GBM samples. Furthermore, Kaplan-Meier survival curve analyses show that HDAC3 expression is associated with a poor survival of in GBM patients. Another study demonstrates that HDAC9 is overexpressed in prognostically poor GBM patients using TCGA and French's datasets [44]. This result was further confirmed in primary GBM tissues and cell lines. Class III HDAC SIRT2 positively correlates with GBM malignant progression and inversely correlated with the survival time of patients with GBM [45]. In contrast, SIRT1 and 6 are downregulated in GBM tissues and cell lines [46-48]. The role of SIRT in GBM is currently under debate due to conflicting findings suggesting that SIRT acts as a tumor suppressor or as an oncogene [49, 50]. SIRT inhibitors have not been clinically tested to evaluate

Table 1: Antitumor activity of HDAC inhibitors

Biological effects	Key effects of HDAC inhibitors
• *Direct effects on tumor cells*	
Cell death	• Induction of apoptosis through the intrinsic and extrinsic apoptosis pathways • Enhanced ROS production and decreased production of free radical scavengers • Immunogenic cell death
DNA damage and repair	• Accumulation of DNA damage through transcriptional downregulation or impaired function of DNA repair proteins
Cell cycle arrest	• Induction of cell cycle arrest
Senescence	• Induction of senescence
Autophagy	• Induction of autophagy
Differentiation	• Induction of differentiation
Tumor immunogenicity	• Enhanced immunogenicity • Enhanced antigenicity of tumor cells
• *Indirect effects on tumor cells*	
Immunomodulation	• Inhibition of dendritic cell differentiation and function • Cytotoxicity to macrophages, neutrophils, and eosinophils • Induction of apoptosis in proliferating B cells • Increased tumor killing by NK cells and cytotoxic T cells • Increased differentiation and function of $CD8^+$ T cells • Inhibition immunosuppressive functions of regulatory T cells • Suppression of inflammatory cytokine production • Increased expression of PD-L1
Inhibition of angiogenesis	• Suppressed expression of pro-angiogenic genes
Inhibition of metastasis	• Suppressed expression of pro-metastatic genes • Increased expression of anti-metastatic genes
Glucose metabolism	• Inhibition of glucose utilization

Abbreviations: NK, natural killer; ROS, reactive oxygen species; PD-L1, programmed death-ligand 1.

their anticancer effect on GBM. Thus, this review focuses on Zn^{2+}-dependent HDACs and their inhibitors.

HDAC INHIBITORS AS CANCER THERAPY FOR GBM

HDAC inhibitors are classified as epigenetic agents that target the aberrant epigenetic characteristics of tumor cells. Epigenetic alterations modulate cellular phenotype through changes in gene expression without modifying the DNA sequence [19]. HDAC inhibitors are known as effective therapeutic anticancer agents *via* multiple mechanisms, including the induction of cell-cycle arrest, differentiation, senescence, intrinsic and extrinsic apoptosis, mitotic cell death, autophagic cell death, generation of reactive oxygen species, inhibition of angiogenesis and metastasis, and improvement in tumor immunity [8, 51] (Table 1 and Figure 1). Because these diverse effects on cancer cells overlap, HDAC inhibitors are very attractive as single agents and in combination with other therapies (Table 2). HDAC inhibitors are a promising class of therapeutic agents that are under investigation for treating different types of tumors, including GBM.

PRECLINICAL STUDIES OF HDACS AND HDAC INHIBITORS IN GBM

Pan-Histone deacetylase inhibitors as radiosensitizers

Several preclinical studies have revealed that HDAC inhibitors act as potent radiosensitizers in various cancers, including GBM [52-55], breast cancer [56], colorectal cancer [57], head and neck cancer [58], non-small-cell lung cancer [59], melanoma [60], and prostate cancer [53]. The exact molecular mechanism underlying HDAC inhibitor-induced radiosensitization remains elusive. However, evidence suggests that it partially involves the inhibition of the DNA damage repair response [19]. HDAC inhibitors

Table 2: Preclinical studies on HDAC inhibitors as therapeutic agents for GBM

HDAC inhibitor	Chemotherapeutic or biological agents	Radiation therapy (RT)	Function	Ref
Valproic acid	-	RT	Protection of normal hippocampal neurons Radiosensitizer up to 12 h after post-irradiation	[67]
Vorinostat (SAHA)	-	RT	Induction of chromatin decondensation Increased DNA DSBs Induction of apoptosis	[65,66]
	Bcl2 inhibitor (obatoclax)	RT	Synergistic apoptotic GBM cell death Overcome resistance to SAHA as a radiosensitizer	[66]
	KDM1A inhibitor (tranylcypromine)	-	Synergistic apoptotic GBM cell death	[68]
	PARP inhibitor (olaparib)	-	Decline of DDR marker expressions Impaired cell cycle progression Synergistic apoptotic GBM cell death	[6]
Panobinostat (LBH589)	-	RT	Induction of chromatin decondensation Increased DNA DSBs Induction of apoptosis	[64, 66]
	Bcl2 inhibitor (obatoclax)	RT	Synergistic apoptotic GBM cell death Overcome resistance to LBH589 as a radiosensitizer	[66]
Entinostat (MS-275)	-	RT	Minimal radiosensitizer after post-irradiation	[53]
HDAC6 inhibitors Ricolinostat (ACY-1215), Tutastatin A, CAY10603	Temozolomide	-	Inactivation of the EGFR pathway Inhibition of cell proliferation Induction of apoptosis Impaired spheroid formation Overcome resistance to TMZ	[80]

block DNA double-strand break (DSB) repair following radiation, as evidenced by the continuous expression of phosphorylated H2AX (γH2AX) Several HDAC inhibitors delay the dispersal of γH2AX foci in irradiated cells [52, 53, 55, 57, 59, 60]. Despite the undefined mechanism for this defective DNA DSB repair process, HDAC inhibitors may affect at least two components of this repair process. They induce the downregulation of DNA repair proteins, including Ku70, Ku80, Ku86, Rad50, and Rad51 [59, 62]. Also, the binding of HDACs to DNA damage response proteins may play a key role in HDAC inhibitor-induced radiosensitization [19, 63].

Furthermore, HDAC inhibitors may influence the response of tumor cells to radiation by changing the chromatin structure. Pan-HDAC inhibitor LBH589 (panobinostat) treatment induced chromatin relaxation and this chromatin decondensation correlated with increased levels of DNA DSBs and radiosensitivity [19, 64]. Thus, HDAC inhibitor-induced chromatin decompaction may increase DNA DSBs induced by radiation, ultimately increasing tumor cell death. Another pan-HDAC inhibitor, suberoylanilide hydroxamic acid (SAHA; vorinostat), has shown a similar effect in GBM [65]. DNA damage response markers and antiapoptotic proteins predict the radiosensitization efficacy of vorinostat and panobinostat in patient-derived GBM cells [66]. Responses to SAHA and LBH589 correlate with pChk2 and Bcl-xL levels. In patient-derived GBM stem-like cells, the Bcl-2 inhibitor obatoclax is reported to abrogate resistance to SAHA and LBH589 as radiosensitizers [66]. Other class I HDAC inhibitors (MS-275 and VPA) were also tested for administration after irradiation, with differing results. MS-275 displayed only minimal radiosensitization after irradiation [19, 53]. In contrast, VPA effectively radiosensitized cells when administered up to 24 h after radiation treatment, although the augmented degree was not strong compared with that of cells treated both before and after irradiation [19, 67]. Therefore, these data indicate that sufficiently high HDAC inhibitor levels should be maintained in tumor cells both before and after irradiation because the cells seek to repair the DNA damage induced by radiation [19, 29]. Overall, HDAC inhibitors seem to prevent DNA DSB repair, resulting in increased tumor cell death.

Pan-HDAC inhibitors as combination drugs

In addition to radiosensitizers, HDAC inhibitors have been used as chemosensitizers in GBM [68, 69]. SAHA affects gene expression patterns and proliferation of glioma cells. After SAHA treatment in GBM cell lines, the expression level of many proapoptotic, antiproliferative genes (*DR5, TNFα, p21,* and *p27*) increased and that of

Table 3: Current clinical trials on HDAC inhibitors in GBM

HDAC inhibitor	Chemotherapeutic or biological agents	Radiation therapy (RT)	Type of malignancy	Phase	Trial identifier
Valproic acid	Temozolomide	RT	GBM that has not been previously treated with chemotherapy or radiation	2	NCT00302159
Vorinostat	-	-	Progressive or recurrent glioblastoma	2	NCT00238303
	Temozolomide	-	Malignant glioma: glioblastoma	1	NCT00268385
	Temozolomide	RT	Newly diagnosed glioblastoma	1,2	NCT00731731
	Temozolomide + isotretinoin	-	Recurrent glioblastoma	1,2	NCT00555399
	Bortezomib	-	Progressive, recurrent glioblastoma	2	NCT00641706
	Bevacizumab	-	Recurrent glioblastoma	2	NCT01738646
	Temozolomide + bevacizumab	-	Recurrent glioblastoma	1,2	NCT00939991
	Bevacizumab + irinotecan	-	Recurrent glioblastoma	1	NCT00762255
Belinostat	Temozolomide	RT	Newly diagnosed glioblastoma	2	NCT02137759
Romidepsin	-	-	Recurrent high grade gliomas: glioblastoma	1,2	NCT00085540

many antiapoptotic, progrowth genes (*CDK2, CDK4*, and the genes encoding cyclins D1 and D2) decreased [70]. HDAC1/2 and histone H3K4 demethylase (LSD1/KDM1) are components of common nuclear corepressor complexes and the acetylation status of adjacent histone residues affects the activity of LSD1 [71]. These results provide the rationale for using dual inhibitors of LSD1 and HDAC for cancer treatment. The inhibition of LSD1 renders GBM cells sensitive to SAHA [68]. Combined treatment with tranylcypromine and SAHA synergistically induces apoptotic cell death in GBM cells. These data suggest that LSD1 and HDACs cooperatively modulate the key pathways of GBM cell death and verify the combined administration of LSD1 and HDAC inhibitors as a therapeutic strategy for GBM.

Several studies report constitutively active DNA damage response in malignant gliomas caused by continuous oxidative and replicative stress [6, 72-74]. In addition to constitutive activation of DNA damage response, genomic instability causes therapeutic resistance and high recurrence rates. Inhibition of poly (ADP-ribose) polymerase (PARP) efficiently eradicates GBM cells, either alone or in combination with chemoradiation [74, 75]. PARP inhibition increases the radiosensitivity of radioresistant GBM cells. Currently, a phase I trial of olaparib (AZD2281; a potent inhibitor of PARP1/2) in conjunction with temozolomide is being investigated in patients with relapsed GBM (ClinicalTrials.gov ID: NCT01390571) [6]. The expression of all DNA damage response markers (BRCA1, Rad51, and PARP1) was observed to further decrease when combined with SAHA and olaparib. This combination treatment synergistically reduced GBM cell survival, induced apoptosis, and inhibited cell-cycle progression [6]. These data also provide a preclinical rationale for combined treatment with SAHA and olaparib, which are already under investigation individually in clinical trials.

Isoform-selective HDAC inhibitors

Most of the GBM studies to date have focused on testing the antitumor effects of pan-HDAC inhibitors such as vorinostat and VPA rather than evaluating the role of HDAC in GBM. Despite some encouraging results from preclinical studies, early clinical trials showed only modest therapeutic benefits. Therefore, the value of pan-HDAC inhibitors in clinical practice is needed for further verification in larger prospective trials to address the function of each HDAC isoform in GBM. Few recent studies investigated the role of HDAC isoforms in GBM. New molecules that target individual HDACs are under preclinical development (such as PCI-34051, which targets HDAC8) or clinical trials (such as ACY-1215, which targets HDAC6).

HDAC6

HDAC6 belongs to class IIb HDAC family. This enzyme deacetylates various substrates, including cortactin, Hsp90, and α-tubulin in the cytoplasm and nucleus [37]. HDAC6 controls both epigenetic and non-epigenetic mechanisms by shuttling between these two cellular compartments. An increasing number of

studies suggest that HDAC6 is also a pivotal regulator of cancer-related signaling pathways, including the EGFR, mitogen-activated protein kinase (MAPK), protein kinase B, and p53 signaling pathways. These findings indicate HDAC6 as a potential therapeutic target for cancer therapy [76]. Aberrant expression patterns of HDAC6 are found in various cancers, including breast cancer [77], oral squamous cell carcinoma [78], ovarian cancer [79], GBM [80], and mouse tumor models. Recently, Wang et al. reported that HDAC6 increases proliferation and imparts temozolomide resistance in GBM [80]. HDAC6 is overexpressed in GBM tissues and cell lines. HDAC6 overexpression facilitates the proliferation and spheroid formation of GBM cells and renders GBM cells resistant to temozolomide. Conversely, knockdown or inactivation of HDAC6 prevents cell proliferation, induces apoptosis, hinders spheroid formation, and renders GBM cells more sensitive to temozolomide. Moreover, temozolomide resistance is associated with activation of EGFR and increased expression of HDAC6. The HDAC6 inhibitors (ACY-1215, tubastatin A, and CAY10603) abrogate temozolomide resistance by decreasing and inactivating EGFR protein. These data imply that the inhibition of HDAC6 is a novel approach for treating GBM and overcoming resistance to temozolomide. ACY-1215 (ricolinostat), a leading HDAC6-selecitve inhibitor, is currently being tested in advanced clinical trials for hematological malignancies (myeloma and lymphoid malignancies) [81, 82]. Thus, these studies and the fact that ACY-1215 is already under clinical trials imply that HDAC6 inhibitors are worthy of consideration for further clinical tiral in GBM patients.

HDAC9

HDAC9 is a member of the class IIa HDAC family and controls regulatory T cell function, cardiac growth, and muscle differentiation [83-85]. It has been reported that HDAC9 expression is significantly upregulated in cervical cancer [86], medulloblastoma [87], acute lymphoblastic leukemia [88], and GBM [44]. HDAC9 is overexpressed in GBM patients who have a poor prognosis. HDAC9 promotes GBM proliferation and tumor formation *via* activation of the transcription coactivator with PDZ-binding motif (TAZ)-mediated EGFR pathway [44]. HDAC9 directly interacts with TAZ, an oncogene and an essential downstream effector of the Hippo pathway. Depletion of HDAC9 reduces the expression of TAZ. A significant effort is underway to find new molecules targeting class IIa HDACs, including HDAC9. However, to date, no HDAC9-specific inhibitors are available. Nevertheless, these results provide new evidence of a promising target for GBM treatment.

HDAC2

HDAC2 is a member of the class I HDAC family. High expression of HDAC2 has been reported in GBM cells [89]. Depletion of HDAC2 by siRNA suppresses proliferation, migration, and invasion of GBM cells and renders the cells sensitive to temozolomide. HDAC2 depletion significantly downregulates the mRNA and protein expression of MRP1 with no effect on ABCB1 and ABCG2. Schisandrin B, a specific inhibitor of MRP1, further enhances the temozolomide sensitivity in HDAC2 knockdown GBM cells. This finding suggests that HDAC2 is a viable target for GBM therapy and improves the efficiency of temozolomide therapy. However, to date, no HDAC2-specific inhibitors are available.

CLINICAL TRIALS OF HDAC INHIBITORS IN GBM

Vorinostat, depsipeptide, panobinostat, and belinostat are the FDA-approved HDAC inhibitors for cancer therapy; these drugs are used specifically for the treatment of refractory cutaneous T-cell lymphoma (CTCL), peripheral T-cell lymphoma (PTCL), and multiple myeloma [8]. Numerous clinical trials are evaluating the safety and efficacy of other HDAC inhibitors, used singly or in combination, for the treatment of various malignancies [19] (https://clinicaltrials.gov). In general, the side effects of HDAC inhibitors include dehydration, diarrhea, fatigue, nausea, thrombocytopenia, lymphopenia, neutropenia, and prolonged QT [19, 90]. Despite favorable toxicity profiles and reversible adverse effects, HDAC inhibitors seem to be not sufficient as monotherapies in solid tumors compared with current standard cancer therapies, partly because of their poor pharmacokinetic properties [11, 91]. However, the potential of HDAC inhibitors as cancer therapeutic agents is apparent from clinical trials combining HDAC inhibitors with chemotherapies or targeted therapies. Table 3 summarizes the ongoing clinical trials of HDAC inhibitors in GBM.

Vorinostat

Vorinostat is a small-molecule inhibitor of human class I and II HDACs that was the first FDA-approved HDAC inhibitor for the treatment of refractory CTCL. It has been reported that vorinostat can penetrate BBB and possesses antitumor effects in glioma models [70, 92]. Vorinostat is the most advanced HDAC inhibitor to enter clinical trials in GBM and is well tolerated as a monotherapy as well as combination therapy in recurrent GBM. A phase II trial tested the efficacy of vorinostat in patients with recurrent GBM [11, 33]. A total complete response (CR) or partial response (PR) occurred in only 3% of patients. Median progression-free survival (PFS) was 1.9 months and 6-month PFS was 17%. This trial showed modest monotherapy activity of vorinostat with a median OS of 5.7 months in recurrent GBM. There are multiple ongoing phase II trials of vorinostat in conjunction with targeted agents, temozolomide, and

radiotherapy. A phase I trial of vorinostat in conjunction with temozolomide was well tolerated in patients with high-grade glioma, although thrombocytopenia and a related grade V hemorrhage were dose-limiting toxicities (ClinicalTrials.gov ID: NCT00268385). A phase I/II trial of vorinostat with radiotherapy and concomitant temozolomide demonstrated reasonable tolerability in newly diagnosed GBM, although the phase II efficacy information is not yet published (NCT00731731). A phase I/II trial of vorinostat, temozolomide, and isotretinoin in recurrent GBM is underway (NCT00555399). Another phase II trial investigated the effects of combination treatment of vorinostat with bortezomib (a proteasome inhibitor) in recurrent GBM [10, 33]. However, the trial was stopped because patients did not obtain 6-month PFS on interim analysis. The reduction of antitumor activity of bortezomib in GBM is likely due to lack of penetration of the BBB [93]. A phase I trial of vorinostat in combination with bevacizumab and irinotecan (a topoisomerase I inhibitor) in recurrent GBM found the same maximum tolerated dose for vorinostat with less thrombocytopenia. In addition, this study showed improved PFS and OS compared to that of vorinostat alone (NCT00762255) [9]. A phase II trial of vorinostat and bevacizumab for recurrent GBM is ongoing (NCT01738646).

Panobinostat

Panobinostat (LBH589) is a potent, small-molecule inhibitor of class I, II, and IV HDACs that was FDA-approved for the treatment of multiple myeloma [94]. Panobinostat shows antitumor and antiangiogenic effects in glioma. A phase II trial of panobinostat in combination with bevacizumab in recurrent GBM was well tolerated. However, the trial was terminated because combination regimen did not significantly improve PFS at 6 month compared to historical controls of bevacizumab monotherapy (NCT00859222) [95].

Valproic acid

VPA is a class I HDAC inhibitor as well as an antiepileptic drug [19], has a low toxicity profile [96] and effectively crosses BBB [97]. VPA showed impressive preclinical efficacy as a radiosensitizer in glioma cells at a dose comparable to that achievable clinically [98-101]. In contrast, it had a radioprotective effect on normal brain tissue and hippocampal neurons [102, 103]. Several retrospective studies analyzed the effects of VPA on the survival of GBM patients [104-106]. Although these results have suggested favorable effects of VPA, whether VPA improves the OS of GBM patients is debatable [107]. However, a phase II trial of VPA, temozolomide, and concurrent radiotherapy for GBM patients was investigated and promising results were recently reported [13]. The median OS is reported to be 29.6 months in patients with newly diagnosed GBM. The most common grade III/IV toxicities of the combination regimen are metabolic and laboratory toxicities (8%), neurological toxicity (11%), and blood and bone marrow toxicity (32%) (NCT00302159) [107], which seem to be well tolerated. Based on the considerable preclinical and retrospective data, VPA is considered to be one of the most promising agents for GBM treatment, but prospective data are still limited [107]. Further investigations are needed to assess its efficacy and clarify the optimal treatment.

Romidepsin

Romidepsin (FK228) is a class I HDAC inhibitor [108] and was the second FDA-approved HDAC inhibitor for the treatment of refractory CTCL and PTCL [109]. It induces apoptosis and inhibits proliferation and metastasis of GBM cells [110]. Romidepsin was studied in a phase I/II trial on patients with recurrent high-grade gliomas, but at the standard dose and schedule, it was ineffective for patients with recurrent GBM (NCT00085540) [111].

CONCLUSIONS

Despite advances in therapeutics and diagnostics, the prognosis of GBM is still poor, and clinically relevant biomarkers have not been established. Due to the heterogeneity of GBM tumors, new strategies have shown clinical limits in terms of efficacy and side effects. We need to understand the complexity of GBM to offer insight into the prognosis and management of this incurable disease. GBM tumorigenesis and chemoresistance are mediated by multiple factors, suggesting that multitargeted strategies are more efficient. Therefore, classification of GMB patients based on genetic, epigenetic, and transcriptional profiling data might be beneficial for selecting drugs for their treatment and predicting patient outcomes. Indeed, progress in the molecular classification of GBM contributes to develop more effective targeted therapeutic agents and combination strategies and to predict patient outcome. However, this progress is still unsatisfactory. We should pursue new discoveries that come from basic science and translate these scientific findings into effective clinical practice.

Abbreviations

BBB; blood-brain barrier, CR; complete response, CTCL; cutaneous T-cell lymphoma, DSB; DNA double-strand break, EGFR; epidermal growth factor receptor, GBM; glioblastoma multiforme, HDAC; histone deacetylase, LSD1/KDM1A; lysine-specific histone demethylase 1A, MAPK; mitogen-activated protein kinase, MGMT; O^6-methylguanine-DNA methyltransferase, OS;

overall survival, PARP; poly(ADP-ribose) polymerase, Rb; retinoblastoma, PFS; progression-free survival, PKB/Akt; protein kinase B, PR; partial response, PTCL; peripheral T-cell lymphoma, RTK; receptor tyrosine kinase, SAHA; suberoylanilide hydroxamic acid, Sirtuin; SIRT, TCGA; The Cancer Genome Atlas, VPA; valproic acid, VEGF; vascular endothelial growth factor.

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CONFLICTS OF INTEREST

The authors declare no competing financial interest.

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