Chapter

Drug Repositioning for the Treatment of Glioma: Current State and Future Perspective

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Abstract

Gliomas are the most common primary brain tumors. Among them, glioblastoma (GBM) possesses the most malignant phenotype. Despite the current standard therapy using an alkylating anticancer agent, temozolomide, most patients with GBM die within 2 years. Novel chemotherapeutic agents are urgently needed to improve the prognosis of GBM. One of the solutions, drug repositioning, which broadens the indications of existing drugs, has gained attention. Herein, we categorize candidate agents, which are newly identified as therapeutic drugs for malignant glioma into 10 classifications based on these original identifications. Some drugs are in clinical trials with hope. Additionally, the obstacles, which should be overcome in order to accomplish drug repositioning as an application for GBM and the future perspectives, have been discussed.

Keywords: glioma, glioblastoma, drug repositioning, chemotherapy, temozolomide, existing drugs, pre-drugs

1. Introduction

Many diseases require the development of new drugs for effective treatment. The relevance of drug repurposing in medical science has progressively grown recently. The increasing interest in drug repurposing is realized based on the increase of related academic publications.

Annually, approximately 23 per 100,000 people suffer from tumors of the central nervous system (CNS). Gliomas, which account for 25% of all CNS tumors, are the most common primary brain tumors, and most are malignant [1]. Glioblastoma (GBM) is a malignant glioma with the worst prognosis, as it accounts for 60% of all gliomas and is classified as grade IV by the World Health Organization (WHO) [1, 2]. Despite aggressive therapies, the median overall survival (OS) of patients who suffer from GBM is only 15–18 months [1, 3].

The current treatments for GBM are maximum surgical resection and adjuvant chemoradiotherapy. The first-line agent for chemotherapy is temozolomide (TMZ), an imidazotetrazinone derivative [4]. TMZ acts as a major groove-directed deoxyribonucleic acid (DNA)-alkylating agent, and its molecular weight is only 194 Da [4]. A phase III clinical trial revealed that concomitant and adjuvant TMZ with radiotherapy is effective for the treatment of patients with primary GBM [5].

Approximately half of the cases of GBM have a methylation of the O^6-methylguanine-DNA methyltransferase (MGMT) promoter, and these cases are associated with a favorable outcome after concomitant and adjuvant TMZ with radiotherapy [6]. MGMT potentially removes methyl adducts at the O^6 position of guanine and indicates resistance to alkylating agents; however, the methylation of the MGMT promoter interferes with MGMT activity and induces glioma cell death [6].

Clinical trials have revealed the therapeutic benefit of bevacizumab (BEV), a recombinant, humanized, monoclonal antibody against vascular endothelial growth factor (VEGF), in patients with cancer [7]. Large-scale clinical studies have been performed to investigate the therapeutic effects of BEV in patients with newly diagnosed GBM [8, 9]. However, the clinical benefits of BEV in patients with glioma are still unknown.

Research into drug repositioning for GBM is now expanding, although no effective drug has yet been reported with strong and solid evidence. Herein, we focus on candidate agents with therapeutic effects in malignant glioma and that have undergone clinical trials to evaluate their efficacy in patients. We also describe the issues of drug repositioning in malignant glioma.

2. Current candidate agents for glioma

Here, we categorize candidate agents into 10 classifications (**Table 1**).

2.1 Antidiabetic drugs

2.1.1 Metformin

The intracellular metabolic pathway of cancer cells differs from that of normal cells, as represented by the Warburg effect, and is considered as a cancer therapeutic target. Metformin is a biguanide antidiabetic drug that exerts a hypoglycemic effect via the suppression of gluconeogenesis in the liver and promotion of glucose uptake in the muscle and adipose tissues. The antitumor effects of metformin are widely known and reported in various cancers, such as breast cancer [10].

Basic research with metformin in glioma cells and glioma stem-like cells (GSCs) has shown that metformin targets multiple pathways (**Figure 1**). Metformin activates AMP-activated protein kinase (AMPK) via the inhibition of oxidative phosphorylation in mitochondrial complex I, which increases the AMP/ATP ratio, thereby inhibiting the mammalian target of rapamycin (mTOR) and promoting apoptosis [11, 12]. The metformin-mediated activation of AMPK, followed by the activation of forkhead box O3 (FOXO3), induces GSC differentiation and reduces tumorigenicity [13]. The Cancer Genome Atlas has reported missense mutations in isocitrate dehydrogenase (IDH) genes 1 and 2. D-2-Hydroxyglutarate (D-2HG), a cancer metabolite produced by the mutant IDH protein, contributes to the development and progression of cancer. The conversion of glutamine to α-ketoglutarate (αKG) is catalyzed by glutamate dehydrogenase (GDH), and the inhibition of GDH by metformin reduces the production of D-2HG in glioma with the IDH 1/2 mutation [14]. Chloride intracellular channel 1 (CLIC1) is involved in the progression of various cancers, including GBM [15–17]. CLIC1 is involved in the regulation of the G1/S transition, and metformin causes G1 cell cycle arrest in GSCs by the selective inhibition of CLIC1 [18].

An epidemiological study using the Clinical Practice Research Datalink reported that the use of metformin is not associated with a reduced risk of glioma [19]. In a

	Candidate agent	Original indication disease	Mechanism of original disease	Mechanism of anti-glioma effect	CT	Refs.
2.1 Antidiabetic drugs	2.1.1 Metformin	Diabetes mellitus	Suppress gluconeogenesis in the liver	Activate AMPK Inhibit glutamate dehydrogenase	NY	[10–20]
2.2 Antihypertensive drugs	2.2.1 Angiotensin II receptor blocker	Hypertension	Block angiotensin II receptor	Inhibit vascular endothelial growth factor	III	[21–25]
	2.2.2 β-blocker	Hypertension	Block β receptor	Decrease cAMP levels	NY	[26, 27]
	2.2.3 Calcium channel blocker	Hypertension	Block calcium channel	Inhibit hippo pathway	NY	[28, 29]
2.3 Antiepileptic drugs	2.3.1 Valproic acid	Epilepsy	Block sodium channel	Inhibit GSK3β	NY	[31–39]
	2.3.2 Levetiracetam	Epilepsy	Block calcium channel	Inhibit MGMT expression	II	[40, 41]
2.4 Pesticides	2.4.1 Chloroquine	Malaria (*Plasmodium* spp.)	Inhibit heme polymerization	Inhibit TGF-β and NF-κB	II	[42–46]
	2.4.2 Pentamidine	Pneumocystis pneumonia	Inhibition of glucose metabolism, protein synthesis, amino acid transport and ribonucleic acid synthesis	Unknown	NY	[47–49]
2.5 Antipsychotic drugs	2.5.1 Fluvoxamine	Depression	Selective serotonin reuptake inhibitor	Suppress the activity of actin polymerization regulators	NY	[50–57]
	2.5.2 Fluspirilene	Schizophrenia	Dephenylbutylpiperidine	Inhibit activation of STAT3	NY	[58–62]
2.6 Antineoplastic drugs	2.6.1 Eribulin	Breast cancer	Inhibit of microtubule activity	Inhibitor of telomerase reverse transcriptase- RNA-dependent	II	[63–76]
2.7 Anti-inflammatory drugs	2.7.1 Acetylsalicylic acid drugs	Fever, inflammation disease	Inhibit cyclooxygenase	Activate connexin 43, suppress Wnt/β-catenin/T-cell factor signaling and SHH/GLI1 pathway	NY	[77–81]
	2.7.2 Sulfasalazine	Rheumatoid arthritis	Block activation of NF-κB	Block activation of NF-κB	I/II	[82–86]
2.8 Multiple drug combination therapy	2.8.1 CLOVA cocktail	–	–	Inhibit GSK3β	I/II	[87–90]
	2.8.2 CUSP9* treatment	–	–	Suppress multiple molecule pathway	NY	[91, 92]
	2.8.3 FTT cocktail	–	–	Inhibit ROCK2/moesin/β-catenin pathway, suppress TGF-β	NY	[93–96]

	Candidate agent	Original indication disease	Mechanism of original disease	Mechanism of anti-glioma effect	CT	Refs.
2.9 Other drugs	2.9.1 Disulfiram	Alcoholism	Inhibitor of ALDH	Inhibiting polo-like kinese-1	II	[97–101]
	2.9.2 Statins	Dyslipidemia	Inhibited 3-hydroxy-3-methylglutaryl-coenzyme A reductase	Activate transcription factor-2 and c-jun, suppress ERK	NY	[102–106]
2.10 Pre drugs	2.10.1 Kenpaullone	-	-	Inhibit GSK3β	NY	[107–111]
	2.10.2 2-Fluoropalmitic acid	-	-	Dephosphorylate ERK, suppress MMP-2	NY	[112–115]

ALDH, aldehyde dehydrogenase; AMPK, AMP-activated protein kinase; CT, clinical trial; ERK, extracellular signal-regulated kinase; GLI1, glioma-associated oncogene homolog 1; GSK3β, glycogen synthase kinase 3β; MGMT, O^6-methylguanine-DNA methyltransferase; MMP-2, matrix metalloproteinase-2; NF-κB, nuclear factor- kappaB; NY, not yet; ROCK2, Rho-associated protein kinase 2; SHH, sonic hedgehog; STAT3, signal transducer and activator of transcription 3; TGF-β, tumor growth factor-β.

Table 1.
The list of candidate agents.

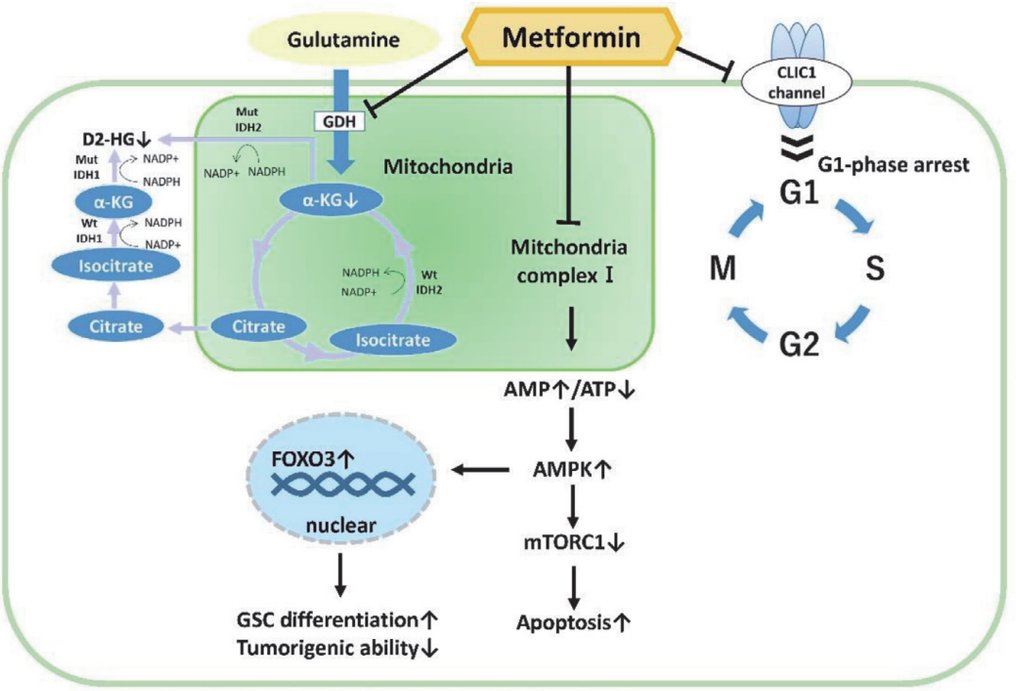

Figure 1.
Antitumor mechanisms of metformin in glioma. The inhibition of oxidative phosphorylation in mitochondrial complex I induces the inhibition of mammalian target of rapamycin complex 1 (mTORC1) and activation of FOXO3 via activating AMPK. The inhibition of GDH reduces the D-2HG production via αKG reduction in IDH 1/2 mutation glioma. Selective inhibition of CLIC1 causes G1 cell cycle arrest in GSCs.

pooled analysis that included 1731 patients from large-scale randomized controlled trials, the use of metformin was not significantly associated with OS or progression-free survival (PFS) in patients with newly diagnosed GBM [20]. Although the results of existing retrospective and epidemiological studies are somewhat discouraging, randomized clinical trials are underway, and we expect to see encouraging results in the future.

2.2 Antihypertensive drugs

2.2.1 Angiotensin II (AT2) receptor blocker

AT2 plays a major role in the renin-angiotensin-aldosterone system and regulates vascular homeostasis, mainly via the activation of angiotensin I receptor (AT1R) and AT2 receptor. Recent studies have revealed that AT2 has roles in cell proliferation, differentiation, apoptosis, and migration. Furthermore, AT2 induces angiogenesis via the stimulation of growth factors such as VEGF, which suggests that AT2 is a target for cancer therapy [21, 22]. Rivera et al. first reported the presence of AT1R in glioma cells and demonstrated that the selective blockade of AT1R with losartan in C6 glioma rats exerts antitumor effects via the inhibition of tumor growth and angiogenesis [22]. The group also showed that treatment with losartan inhibits tumor growth via the inhibition of VEGF and promotes apoptosis in vitro and in vivo [23]. A retrospective analysis of 81 patients with newly diagnosed GBM showed that the administration of an AT2R blocker or angiotensin-converting enzyme (ACE) inhibitor with the current treatment is associated with reduced brain edema and steroid requirements and improved clinical outcomes [24]. Nevertheless, the ASTER trial (NCT01805453), a randomized, placebo-controlled trial, which included losartan to the current treatment for patients with

GBM, did not show any difference in steroid requirements or a significant increase in the median OS [25].

2.2.2 β-Blocker

Tewarie et al. summarized previous preclinical and clinical studies about the effects of β-blockers on gliomas and noted reduced cell proliferation via a decrease in cAMP levels, time-dependent cell cycle arrest, and reduced cell migration [26]. However, in a retrospective cohort study of 218 patients with recurrent GBM, Johansen et al. observed no correlation between the usage of β-blockers and OS and PFS [27].

2.2.3 Calcium channel blocker

The altered expression and activity of specific Ca^{2+} channels and pumps have been reported in malignant gliomas [28]. Amlodipine, a commonly used antihypertensive drug, was shown to inhibit tumor growth by the inhibition of YAP/TAZ signaling via the hippo pathway, which is involved in tumor malignancy by the activation of store-operated Ca^{2+} entry. This allows intracellular Ca^{2+} influx [29]. Most research on calcium signaling in GBM is recent and further study is warranted.

2.3 Antiepileptic drugs

A common symptom of GBM is epilepsy, which occurs in half of all cases; thus, patients are often treated with antiepileptic drugs, such as valproic acid (VPA) and levetiracetam (LEV) (**Figure 2**). Enzymatic modifications of histone proteins that regulate gene expression have been investigated as therapeutic drug targets. Histones are modified by histone acetyltransferase (HAT) and histone deacetylase (HDAC). A HDAC inhibitor (HDACi) enhances the acetylation by HAT and causes a hyperacetylated state, which exerts multiple antitumor effects such as cell differentiation, apoptosis, cell cycle arrest, sensitivity to chemotherapy, and inhibition of migration and angiogenesis [30].

2.3.1 Valproic acid

Recently, VPA has been shown to be an effective HDACi and has been proposed as a drug for cancer treatment [31]. VPA inhibits the proliferation of glioma cells and enhances radiosensitivity by increasing hyperacetylation in vitro and in vivo [32]. Another antitumor effect of VPA is the induction of apoptosis by the inhibition of GSK3β via the activation of Akt/ERK [33]. According to several studies, the inhibition of GSK3β suppresses survival and proliferation and induces apoptosis in human GBM cells [34]. However, some meta-analyses have revealed that the clinical benefit of VPA combination treatment in patients with GBM was contraindicated [35–39], and further studies are warranted.

2.3.2 Levetiracetam

LEV has been shown to increase HDAC1 transcription, recruit the mSin3A/HDAC1 corepressor complex on the MGMT promoter, and inhibit MGMT expression through the direct binding of p53 to the MGMT promoter [40]. Thus, LEV inhibits glioma cell proliferation and significantly potentiates the cytotoxic effects of TMZ in glioma cells and GSCs [40, 41]. A phase II clinical trial (NCT02815410) is ongoing and the results are expected in the future.

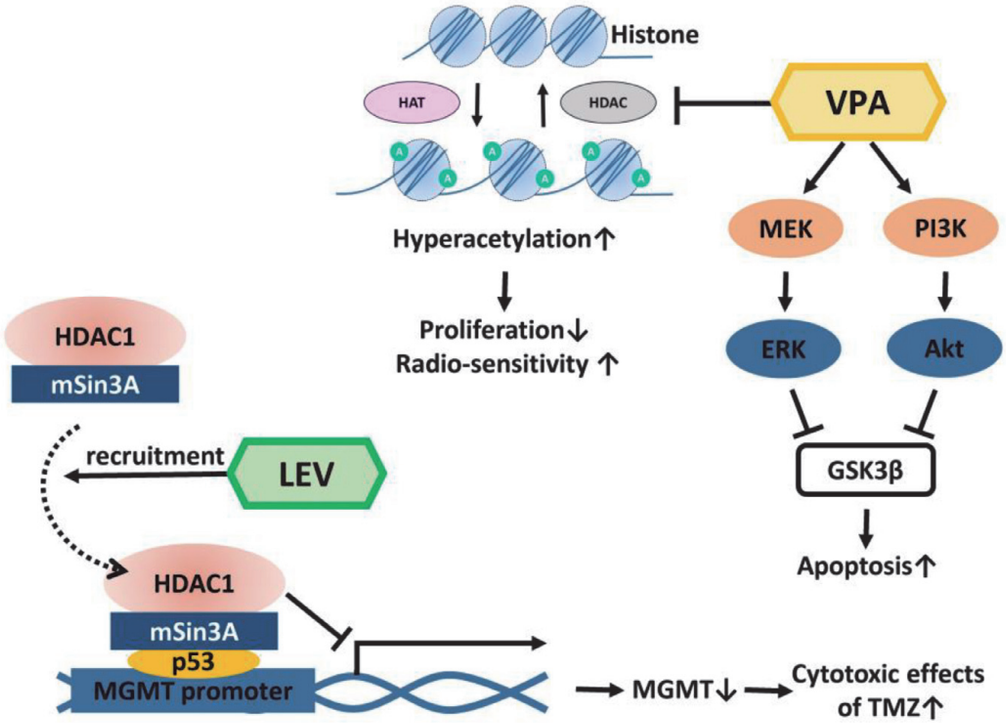

Figure 2.
Antitumor mechanisms of VPA and LEV in glioma. ***VPA:*** *hyperacetylation of histones via the inhibition of HDAC suppresses cell proliferation and increases radiosensitivity. The activation of Akt/extracellular signal-regulated kinase (ERK) inhibits glycogen synthase kinase-3β (GSK3β) and induces apoptosis.* ***LEV:*** *recruitment of the mSin3A/HDAC1 corepressor complex and direct binding to the MGMT promoter via p53. Abbreviations: HAT, histone acetyltransferase; MEK, mitogen-activated protein kinase/ERK kinase; PI3K, phosphoinositide 3-kinase; TMZ, temozolomide.*

2.4 Pesticides

2.4.1 Chloroquine (CHQ)

CHQ is a therapeutic drug for the treatment of malaria [42]. This agent has antitumor effects for some cancer cells, including glioma cells [43]. However, the mechanism of the antitumor effect of CHQ in glioma is not well known. Some studies have suggested that CHQ leads to cancer cell death by controlling autophagy [44], and recent research has revealed more effects of CHQ treatment. CHQ adjusts the metabolism of amino acids and inhibits glycogenesis [42]. CHQ administration also induces the alteration of mitochondrial membrane potential in glioma cells and causes apoptosis [45]. Some studies have investigated the molecular signaling associated with CHQ treatment. The molecular signaling changes in glioma cells caused by CHQ include the inhibition of the signaling pathway of transforming growth factor-β (TGF-β) and nuclear factor-kappaB (NF-κB), which play a role in tumorigenesis [42, 45]. CHQ treatment also suppresses glioma cell invasion by the inhibition of matrix metalloproteinase-2 (MMP-2) and improved radiosensitivity by the accumulation of glioma cells in the G2/M phase [45]. Based on the results of these in vitro studies, clinical trials that investigated the therapeutic effects of CHQ in patients with glioma have been conducted [43]. In a randomized trial (double-blind, placebo-controlled) of patients with primary GBM, there were no statistically significant differences between the CHQ treatment group and the placebo group; however, the death rate in the CHQ group was half as large as that in the placebo group [46]. Further clinical trials are in progress (NCT03243461, NCT02432417, and NCT02378532).

2.4.2 Pentamidine

Pentamidine is effective in the treatment of pneumonia caused by *Pneumocystis jirovecii*. This drug exerts its therapeutic effects via the inhibition of glucose metabolism, protein synthesis, amino acid transport, and ribonucleic acid (RNA) synthesis [47]. Previous studies have shown the therapeutic effects of pentamidine in various cancers [48]. One in vitro study revealed that pentamidine suppressed cancer activity via the inhibition of phosphatase of regenerating liver (PRL) [48] and the inhibition of PRL phosphatase suppressed the activation of Akt and ERK [49]. Based on these studies, we investigated the effect of pentamidine in glioma cells and GSCs. Pentamidine suppressed the proliferation of glioma cells and GSCs and reduced the stemness of GSCs. Additionally, there are clinical benefits to repurposing pentamidine as the therapeutic drug for malignant glioma, because the current chemoradiotherapy sometimes induces lymphopenia as a side effect and patients might suffer from pneumonia caused by *P. jirovecii*. Further research to investigate the molecular mechanism of pentamidine is in underway. In the future, clinical trials are warranted to determine the benefit of pentamidine for patients with malignant glioma.

2.5 Antipsychotic drugs

2.5.1 Fluvoxamine

Fluvoxamine has been used as an antidepressant since 1986 and is widely applied in the treatment of anxiety disorders owing to its selective serotonin reuptake inhibitor activity, which helps maintain sufficient serotonin levels in the brain to function [50, 51]. Recently, a new screening method for the quantitative determination of actin polymerization showed that fluvoxamine inhibits the formation of F-actin, which induces lamellipodial protrusions, focal adhesions, and stress fibers at the edge of GBM and is essential for the migration and invasion of GBM cells into normal brain tissues [52–54]. The molecular signal changes in fluvoxamine-treated glioma cells are achieved by the suppression of the activity of actin polymerization regulators, focal adhesion kinases, and mTOR complex 2 [55, 56]. The daily administration of fluvoxamine to an intracranial xenograft mouse model significantly prolongs survival and blocks the infiltration of tumor cells into normal brain tissues in vivo [57]. Therefore, fluvoxamine disrupts focal adhesion and actin depolymerization, blocks the migration and invasion ability of GBM cells, and prolongs patient survival. Fluvoxamine is a potentially effective anti-invasive drug for the treatment of glioma.

2.5.2 Fluspirilene

Fluspirilene, a member of the diphenylbutylpiperidine class of drugs, is an effective, traditional, long-acting antipsychotic [58, 59]. Fluspirilene displays an effective Ca^{2+} channel blocking activity [60] and inhibits synaptic transmission; thus, fluspirilene can mitigate a seizure [58]. However, recent studies have shown a new effect of fluspirilene against some incurable cancers, such as hepatocellular carcinoma [61] and GBM [62]. Fluspirilene has been identified as a potential anti-GSC drug. An in vitro investigation has shown that fluspirilene not only attenuates the cell viability, stemness, sphere-forming ability, and proliferation of GSCs but also suppresses the invasion of GBM cells via the inhibition of signal transducer and activator of transcription 3 (STAT3) activity and its nuclear reduction in GBM cells [62]. In vivo, fluspirilene significantly decreases tumor volume and prolongs

survival in an intracranial xenograft mouse model [62]. These results suggest that fluspirilene is a potential novel anti-glioma candidate.

2.6 Antineoplastic drugs

2.6.1 Eribulin

Eribulin, a non-taxane inhibitor of microtubule dynamics [63, 64], was approved by the US Food and Drug Administration (FDA) in 2010 for the treatment of stage 4 breast cancer [65]. Eribulin prevents the growth of tumor cells via the inhibition of microtubule activity during cell mitosis and induces M-phase arrest, which result in cell apoptosis (**Figure 3**) [66, 67]. Eribulin also reduces the aberrance of the vascular microenvironment of a tumor [68]. Based on these effects on various cancers, recent studies have demonstrated that eribulin sensitizes a tumor to radiation via eribulin-induced M-phase arrest and causes more DNA damage than radiation alone. This induces an increase in cleaved caspase-3 and cleaved poly-ADP ribose polymerase levels and results in mitotic catastrophe (**Figure 3**) [69, 70]. An in vivo study of the concomitant administration of radiation with eribulin showed that this combination prolongs the survival of the intracranial xenograft GBM mouse model [71]. Eribulin also suppresses vascular remodeling and normalizes the radiation-induced aberrant vascular microenvironment in the xenograft mouse model [71]. A growing evidence indicates that a telomerase reverse transcriptase (TERT) promoter mutation, a common mutation in GBM [72], maintains telomerase activity to evade telomere shortening; thus, tumor cells overcome replicative senescence and proliferate infinitely [73] telomerase-independent RNA-dependent RNA polymerase (RdRP) activity [74, 75]. Eribulin has been identified as a specific inhibitor of TERT-RdRP through drug screening [76]. Thus, TERT-targeting therapies would be a novel direction to treat glioma (**Figure 3**). Both in vitro and in vivo experiments using eribulin to treat gliomas have shown that eribulin exerts an anticancer activity and suppresses glioma proliferation through its function as a TERT-RdRP inhibitor, in addition to its microtubule inhibitor activity. Now, eribulin is in a phase II doctor-led clinical trial in recurrent GBM (UMIN ID: 000030359).

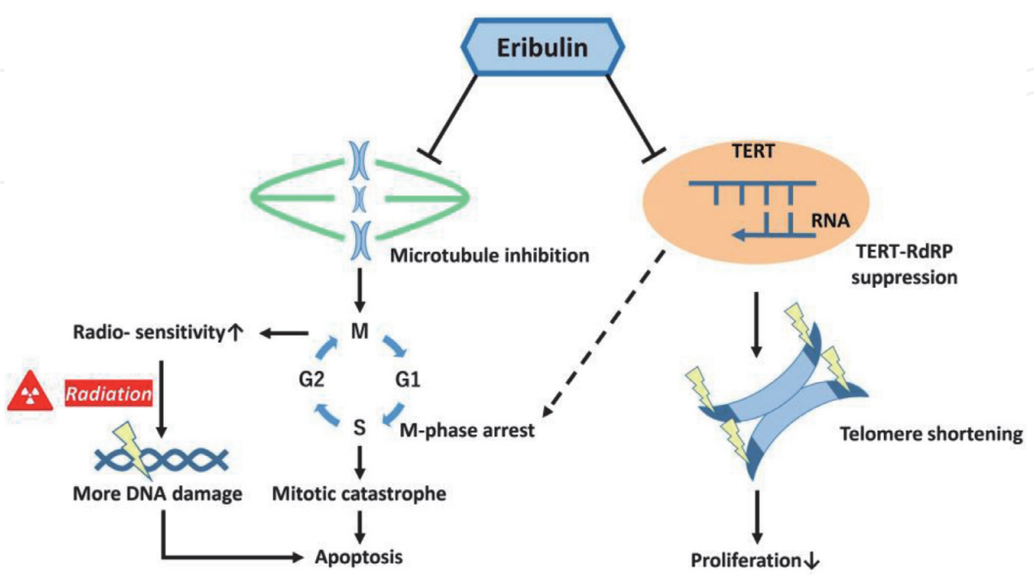

Figure 3.
Antitumor mechanisms of eribulin. The effect of eribulin against glioblastoma multiforme. Eribulin suppresses microtubule activity and induces M-phase arrest, which makes cells more radiosensitive and ends up with apoptosis. Eribulin also suppresses proliferation by inhibiting the TERT-RdRP activity.

2.7 Anti-inflammatory drugs

2.7.1 Acetylsalicylic acid (ASA)

ASA, a nonsteroidal anti-inflammatory drug, is used worldwide. Previous studies have shown the molecular signaling changes by aspirin (**Figure 4**). ASA exerts an anticancer via the inhibition of prostaglandin, including prostaglandin E2 (PGE2), synthesis through the acetylation, and inhibition of cyclooxygenase [77, 78]. ASA treatment suppresses the invasion of glioma cells via the activation of the expression of connexin 43 (Cx43), which is a major gap junction protein in astrocytes. Cx43 is normally suppressed by PGE2. Thus, ASA-treated glioma cells would overexpress Cx43 and the invasion would be inhibited [79]. Other studies have revealed that ASA suppresses the Wnt/β-catenin/T-cell factor (TCF) signaling pathway, which plays a key role in glioma progression [79]. Wnt/β-catenin/TCF pathway suppression would suppress glioma via the regulation of downstream genes, *c-myc* and *cyclin D1*. ASA inhibits the sonic hedgehog (SHH)/glioma-associated oncogene homolog 1 (GLI1) pathway and adjusts the epithelial-to-mesenchymal transition [80]. The SHH/GLI1 pathway is also associated with recovery from the damage by TMZ [80]. Based on these studies, a retrospective cohort study was performed to investigate the therapeutic effect of ASA in patients with malignant glioma. The results revealed that the use of ASA is associated with a higher OS and PFS in patients with WHO grade III glioma; however, there was no difference in OS and PFS in patients with WHO grade IV glioma [81]. In the future, prospective multicenter randomized studies are warranted to determine the effect of ASA in malignant glioma.

2.7.2 Sulfasalazine (SAS)

SAS, which is approved for the treatment of rheumatoid arthritis and inflammatory bowel diseases, may be a therapeutic drug for malignant glioma [82]. SAS exerts anti-inflammatory effects by blocking the activation of NF-κB and the X_c^- antiporter system, which usually causes the uptake of cystine, release of glutamate, and increase in the levels of reactive oxygen species (ROS) [83]. NF-κB is activated in GBM tissues and promotes cell proliferation and survival. SAS blocks

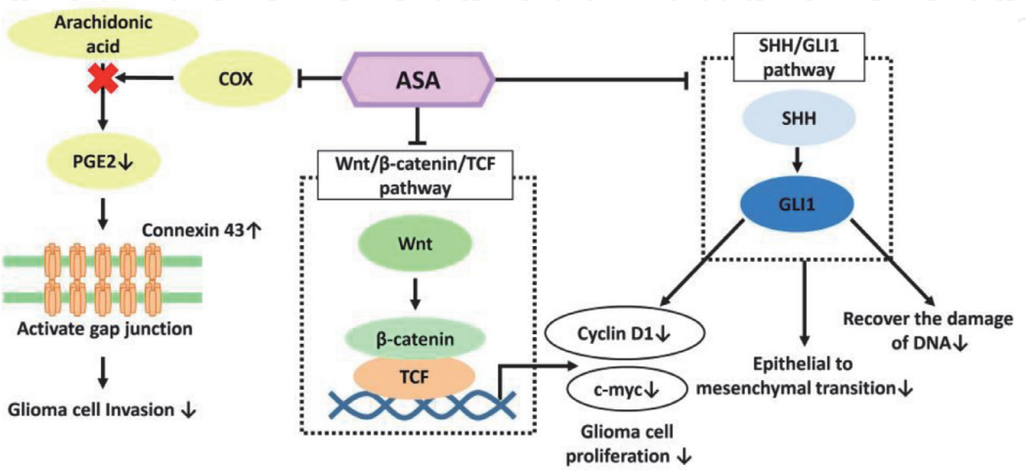

Figure 4.
Antitumor mechanisms of acetylsalicylic acid. ASA indicates multimodal effects for glioma cells. ASA suppresses the invasion of glioma cells by activating the expression of connexin 43. ASA also suppresses c-myc *and* cyclin D1 *through Wnt/β-catenin/TCF pathway and interfered the recovery of DNA damages and adjusted the epithelial-to-mesenchymal transition through SHH/GLI1 pathway. Abbreviation: PGE2, prostaglandin E2.*

the cell cycle and induces apoptosis in vitro and inhibits the growth of brain tumors in mouse xenograft models [83, 84]. However, a phase I/II study of the current therapy with SAS for patients with recurrent malignant glioma showed no clinical benefit of SAS [85]. Recently, a phase I/II study of the current therapy with SAS in patients who were newly diagnosed GBM was performed [86], which showed that there is no increase in OS and PFS in the current therapy with SAS group compared to the current therapy group. Results suggest that this new regimen would improve seizure control; however, the therapeutic effect of SAS would be limited.

2.8 Multiple-drug combination therapy

A combination therapy with different drugs targeting on multiple molecules that contribute to malignancy is rational and enhances antitumor effects, reduces side effects, and avoids resistance. This section provides an overview of the treatment of recurrent GBM with multiple existing drugs (**Figure 5**).

2.8.1 CLOVA cocktail

The CLOVA cocktail, composed of cimetidine, lithium, olanzapine, and valproate, targets dysregulated GSK3β in GBM [87–89]. The therapeutic effects of GSK3β inhibition are the suppression of tumor cell survival and proliferation, synergy with TMZ and irradiation, attenuation of invasion, and induction of GSC differentiation via various pathways [90]. Olanzapine stimulates AMPK catabolic action, followed by the induction of p53-dependent autophagy. VPA, as an HDACi, enhances the effect of radiation. A phase I/II clinical study to investigate the efficacy and safety of the CLOVA cocktail in patients with TMZ-resistant recurrent GBM revealed that this regimen is well tolerated and results in a higher OS than the control group treated with TMZ alone [87].

2.8.2 CUSP9 treatment*

The rational of the coordinated undermining of the survival paths active in GBM by nine repurposed drugs [aprepitant, artesunate, auranofin, captopril, celecoxib, disulfiram (DSF), itraconazole, ritonavir, and sertraline], termed CUSP9*, was developed to prevent therapeutic resistance in tumor cells. CUSP9* targets the diverse complementary redundant pathways to render tumor cells susceptible to the cytotoxic effects of TMZ [91] by the simultaneous administration of nine drugs with low-dose daily TMZ. Each drug exerts different inhibitory effects on the 17 molecules and pathways shown in **Figure 5**. Auranofin and DSF increase the level of intracellular reactive oxygen species [96]. Recently, the experimental CUSP9* strategy with TMZ was shown to suppress the stemness of GSCs and tumorigenesis via the blockade of the Wnt/β-catenin pathway [92].

2.8.3 FTT cocktail

A unique therapeutic approach to reprogram and reverse cancer cells to normal somatic cells has attracted attention. The combination of fasudil, tranilast, and TMZ was identified to reprogram GBM cells into neuronal like cells [93]. GBM cells treated with the FTT cocktail show normal neuronal morphology, gene expression, and electrophysiological properties and lower malignancy than untreated cells. This might be caused by the synergistic effect of the three drugs [93]. In addition, the FTT cocktail suppresses tumor growth and prolongs survival in a GBM xenograft

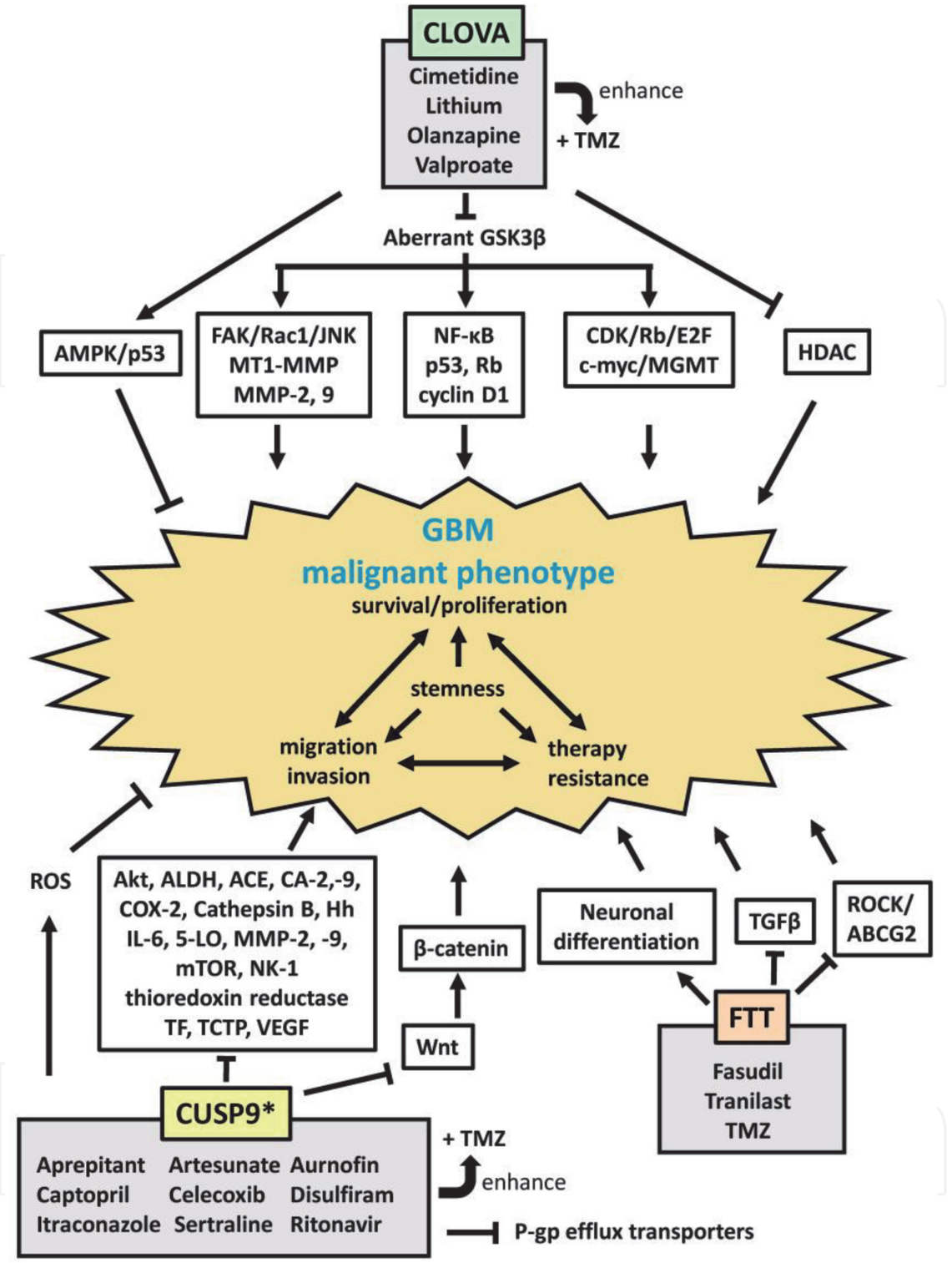

Figure 5.
Multiple molecular-targeted therapies by multiple-drug treatment with temozolomide. Multiple existing drug combination, CLOVA cocktail, CUSP9 treatment, and FTT cocktail, targets multiple signaling pathways which attribute GBM malignant phenotype. Abbreviations: 5-LO, 5-lipoxygenase; ABCG2, ATP-binding cassette super-family G member 2; ACE, angiotensin-converting enzyme; ALDH, aldehyde dehydrogenase; AMPK, adenosine monophosphate; CA, carbonic anhydrase; CDK, cyclin-dependent kinase; COX, cyclooxygenase; FAK, focal adhesion kinase; GSK3β, glycogen synthase kinase-3β; HDAC, histone deacetylase; HH, hedgehog; JNK, c-Jun N-terminal kinase; MGMT, O^6-methylguanine-DNA methyltransferase; MMP, matrix metalloproteinase; MT, membrane type; mTOR, mammalian target of rapamycin; NK-1, neurokinin-1; NF-κB, nuclear factor-kappaB; P-gp, P-glycoprotein; ROCK, rho-associated protein kinase; ROS, reactive oxygen species; TCTP, translationally controlled tumor protein; TF, tissue factor; TGF-β, transforming growth factor-β; TMZ, temozolomide; VEGF, vascular endothelial growth factor.*

model more than TMZ alone. Fasudil inhibits the ROCK2/moesin/β-catenin pathway in TMZ-resistant glioma cell lines and downregulates the ATP-binding cassette super-family G member 2 transporter to increase sensitivity to TMZ [94]. The inhibition of ROCK with mTOR inhibition exerts neuronal reprogramming more effectively in vitro and in vivo than the inhibition of ROCK alone [95], which suggests the possibility of more drug combinations. Tranilast alone inhibits glioma progression via TGF-β restriction [96]. Although the mechanism underlying the tumor-suppressive function of the FTT cocktail is not fully elucidated, this cocktail might improve the current therapy for malignant glioma.

2.9 Other drugs

2.9.1 Disulfiram

DSF, the FDA-approved drug for the treatment of alcohol abuse, may be a therapeutic drug for GBM. DSF is an irreversible inhibitor of aldehyde dehydrogenase [97], which is a functional marker of cancer stem cells [98]. An in vitro study revealed that DSF is an inhibitor of MGMT and enhances the efficacy of alkylator-induced tumor death [99]. Another study revealed that DSF suppresses the growth and self-renewal of GSCs via the inhibition of polo-like kinase-1, which controls cell progression and cytokinesis [97]. The activity of DSF is potentiated by copper and induces GSC death [100]. However, an open-label, single-arm phase II study of TMZ plus DSF for patients with recurrent TMZ-resistant GBM showed that the objective response rate is 0% and DSF combination therapy would have only limited therapeutic effects for patients with GBM [101].

2.9.2 Statins

Statins, a therapeutic drug for dyslipidemia, inhibit 3-hydroxy-3-methylglutaryl-coenzyme A reductase. Some statins have a therapeutic effect on glioma cells [102, 103]. In vitro, simvastatin induces the apoptosis of C6 glioma cells by phosphorylation of activating transcription factor-2 and c-Jun [102]. Lovastatin suppresses the proliferation and migration of glioma cell lines via the suppression of the activation of ERK [103]. A retrospective cohort study suggested that the long-term prediagnostic statin intake increases the OS in patients with GBM [104]. Another retrospective cohort study suggested that statin intake is associated with fewer seizures in patients with GBM [105]. However, other cohort studies have not indicated a survival relationship between malignant glioma and statin intake. Finally, a meta-analysis of these retrospective cohort studies revealed that statins do not increase the PFS and OS in patients with GBM [106]. In the future, prospective multicenter randomized studies are warranted to determine the effect of statins in malignant glioma.

2.10 Pre-drugs

2.10.1 Kenpaullone

Kenpaullone, a potent and nonselective inhibitor of GSK3 [107], is a serine/threonine kinase that regulates numerous signaling pathways involved in cell cycle control, proliferation, differentiation, and apoptosis [108, 109]. Kenpaullone treatment inhibits glioma cell proliferation, suppresses anti-apoptotic mechanisms in the mitochondria, inhibits pro-survival factors, and

attenuates the stemness and viability of GSCs via the downregulated activity of GSK3β [88, 110, 111]. The combination of low-dose kenpaullone with TMZ enhances cytotoxicity against glioma via the induction of c-Myc-mediated apoptosis [110]. These results suggest that kenpaullone is a potential compound for the treatment of glioma.

2.10.2 2-Fluoropalmitic acid (2-FPA)

Recently, 2-FPA, a new fatty acid inhibitor compound, has been identified as a potential anti-glioma agent through a drug screening system for drugs that target cancer stem cells using existing drug libraries [62]. As an active chemical compound, the safety of 2-FPA for normal brain cells has not yet been revealed. There are no reports that have mentioned the effect of 2-FPA in other cancers. An in vitro investigation using GSCs and GBM cells [112] has shown that 2-FPA suppresses the viability and sphere-forming ability of GSCs; inhibits the proliferation of GBM cells via the dephosphorylation of ERK, which is essential for the proliferation and invasion of glioma [113]; and blocks the invasion of GBM cells via the suppression of the activity of MMP-2, which plays an important role in cell invasion [114]. In addition to its mono activity against glioma, the combination of 2-FPA with TMZ synergistically enhances the efficacy of TMZ against glioma in vitro via the increase in MGMT promoter methylation and downregulation of MGMT, the main and predominant reasons for TMZ resistance [115], which suggest that combination therapy may be one strategy to improve TMZ efficacy and overcome resistance. Overall, 2-FPA is a potential therapeutic agent against GBM. To extend these results, physiological studies are required.

3. Issues of drug repositioning for glioma

Despite these studies, some problems remain in drug repositioning for the treatment of glioma because of the uniqueness of this brain tumor.

The biggest problem is the penetration of the blood-brain barrier (BBB), which restricts the passage of molecules, including candidate agents. The BBB is a multilayered barrier between the blood and brain tissues to regulate the environment of the brain. The BBB has a good permeability for nutrients that are required for nerve cells [116]. Additionally, the BBB adjusts the ionic composition and the concentration of neurotransmitters, such as neuroexcitatory amino acids, to maintain the optimal environment for synapses. If ions and neurotransmitters spread into the CNS in an uncontrolled manner, the synapse is insufficiently stimulated and brain tissue is damaged [116]. The BBB also prevents the penetration of macromolecules more than 400–500 Da to exclude neurotoxic molecules [117]. Some plasma proteins induce the apoptosis of nerve cells [116]. This multilayered barrier blocks these proteins and would block the penetration of candidate agents. To overcome this problem, techniques are being explored. Some studies have investigated a new drug delivery system that uses an ultrasound-sensitizing nanoparticle complex, as preliminary studies have revealed that an ultrasound with microbubbles could open the BBB locally [118]. Other studies have evaluated the usefulness of convention-enhanced delivery therapy, which is a local infusion therapeutic technique to directly introduce a drug to brain neoplasms [119, 120].

A malignant glioma has features that are different from those in other malignant tumors. First, a malignant glioma has heterogeneity. Some malignant cancers, such as acute leukemia, are homogeneous; thus, the appropriate candidate agent would induce remission because “the weak point” of all tumor cells is the same.

However, a malignant glioma is a complicated aggregation, once called "glioblastoma multiforme" [121]. If one candidate agent exerts therapeutic effects for some glioma cells, other resistant glioma cells would multiply. To overcome this problem, several previous studies have performed multiple-drug combination therapy. This therapy would focus on multiple therapeutic targets at once with minimal side effects [85]; however, currently, there are no combination treatments that can replace the current treatments. Second, despite its clinical aggressiveness, 60–70% of the tumor cells in malignant glioma are in the nonproliferating phase [122]. This indicates that not only heterogeneous cells but also the cell cycle must be considered because resting cells indicate resistance to chemoradiotherapy [122]. Based on this, some studies have focused on candidate agents that can change the phase of the cell cycle [18, 45].

4. Perspective

The strategy to discover the most effective drug is the key to accomplish a successful drug repositioning. One of the main methods is an in vitro or in vivo drug screening system in which target cells are treated by various existing drugs and the alteration to the malignant phenotype, such as by cytotoxicity, is analyzed. Drugs that exert cytotoxicity in GBM cells, especially GSCs, at low concentrations would be good candidates. Since the previous reports mention that GSCs were the cause of recurrence of GBM [100], GSCs can be good target. Lower drug concentration can minimize side effects. However, to achieve this strategy, appropriate experimental resources, including candidate agents, drug screening systems, and established cell lines are required. Epidemiological discovery is another option, such as the measurement of the incidence of a certain disease in the population to which specific drugs are administered. Serendipity is an important factor in this strategy. For instance, a prospective cohort study revealed a lower cancer incidence in people with schizophrenia [123]. This led us to the idea that antipsychotic drugs possess therapeutic effects against cancers including glioma [57, 62]. However, the most efficient method might be mutual molecular and structure analyses between target cells and drugs using artificial intelligence (AI). Different biochemical and mathematical techniques have been designed and optimized to accurately infer links between target cells and drugs. Drug-target interaction prediction is an important part of most rational drug repositioning pipelines. The major target molecules for malignant glioma are Akt, ERK, and STAT3, which sustain malignant phenotype [62, 103, 113].

The supply of research resources is also important. Pharmaceutical companies hold the materials for drug repositioning such as drug libraries and useful knowledge for bringing new drugs to market. Thus, a collaboration between researchers who establish efficient screening systems and pharmaceutical companies that own various drugs, including those that failed in clinical trials, can lead to a successful drug repositioning.

Although drug repositioning may be useful in the future, there are hurdles to the transition of this research into clinical practice owing to financial problems. Drug repositioning involves reinvestment in inexpensive drugs with expired patents; therefore, the benefits to pharmaceutical companies are small, which results in a reluctance to cooperate to broaden the indications of their drugs. This is especially true for rare diseases, such as glioma. Currently, the only way for researchers to raise public and private funds is by themselves, and they must conduct physician-led clinical trials without the support of pharmaceutical companies. An effective system in which the government supports drug repositioning is required to

overcome the issue of budget constraints. From an economic perspective, it would be beneficial to patients and countries to treat patients with inexpensive drugs with expired patents.

After the appearance of TMZ, drug development for GBM has stagnated. A huge advance in the treatment of patients with GBM can be expected if effective drugs are identified via drug repurposing.

5. Conclusion

Drug repositioning is a useful research strategy to identify the therapeutic agents for glioma. Here, we discuss the current drug repositioning and its perspective for glioma treatment. Despite many efforts to date, no agents are widely used in the current clinical practice. For breaking down the current situation, appropriate screening system, suitable animal model, well-designed clinical trials, and tight collaboration with pharmaceutical companies are warranted. From now on, the drastic progress in this field would be occurred by new methods including AI.

Conflict of interest

All authors declare no conflict of interests for this article.

Abbreviations

2-FPA	2-fluoropalmitic acid
AI	artificial intelligence
AMPK	AMP-activated protein kinase
ASA	acetylsalicylic acid
AT1R	angiotensin I receptors
AT2	angiotensin II
BBB	blood-brain barrier
BEV	bevacizumab
CHQ	chloroquine
CLIC1	chloride intracellular channel 1
CNS	central nervous system
Cx43	connexin 43
D-2HG	D-2-hydroxyglutarate
DSF	disulfiram
ERK	extracellular signal-regulated kinase
FDA	Food and Drug Administration
GBM	glioblastoma
GDH	glutamate dehydrogenase
GLI1	glioma-associated oncogene homolog 1
GSC	glioma stem-like cell
GSK3	glycogen synthase kinase 3
HAT	histone acetyltransferase
HDAC	histone deacetylase
HDACi	histone deacetylase inhibitor
IDH	isocitrate dehydrogenase
LEV	levetiracetam
MGMT	O^6-methylguanine-DNA methyltransferase

MMP-2	matrix metalloproteinase-2
mTOR	mammalian target of rapamycin
NF-κB	nuclear factor-kappaB
OS	overall survival
PFS	progression-free survival
PGE2	prostaglandin E2
PRL	phosphatase of regenerating liver
RdRP	RNA-dependent RNA polymerase
ROCK	Rho-associated protein kinase
SAS	sulfasalazine
SHH	sonic hedgehog
STAT3	signal transducer and activator of transcription 3
TCF	T-cell factor
TERT	telomerase reverse transcriptase
TGF-β	transforming growth factor-β
TMZ	temozolomide
VEGF	vascular endothelial growth factor
VPA	valproic acid
WHO	World Health Organization

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