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Chapter 19

Chemotherapeutic Agent for Glioma

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http://dx.doi.org/10.5772/54353

1. Introduction

Glioma is the most common primary tumors of the central nervous system, accounting approximately for 30% of entire CNS tumors, and classified into four clinical grades as I to IV. The most aggressive and lethal tumors is glioblastoma multiforme (GBM) with median survival of only 14.6 months, mainly because of limited effects of conventional post-surgical chemotherapeutic agents and irradiation [1]. In this chapter, we summarize chemotherapeutic agents for glioma focusing on their mechanism of anti-tumor action and the acquisition of resistance to the agents.

2. Temozolomide

2.1. Mechanism of action

Temozolomide (TMZ) is an alkylating agent which is applied to the treatment of malignant glioma including GBM. TMZ induces DNA methylation of guanine at O^6 position (O6-MG; 6% of adducts formed), as well as 7-methylguanine (N7-MG; 70% of adducts formed), and 3-methyladenine (N3-MA; 9% of adducts formed) [2]. O6-MG incorrectly pairs with thymine and triggers the mismatch repair (MMR) system leading to double strand break of the genome that result in the arrest of cell cycle and induction of apoptosis. N7-MG and N3-MA are removed by the methylpurine glycosylase followed by AP endonuclease which are the first two enzymes in the base excision repair (BER) pathway. Efficient BER system functions and repairs DNA lesions in normal and tumor cells. 573 patients with newly diagnosed as GBM were randomly assigned to be treated by radiotherapy alone or by radiotherapy plus continuous daily medication of temozolomide [3]. At a median follow-up of 28 months, the median survival was 14.6 months with radiotherapy plus temozolomide and 12.1 months

with radiotherapy alone. The unadjusted hazard ratio for death in the radiotherapy-plus-temozolomide group was 0.63 (95 percent confidence interval, 0.52 to 0.75; P<0.001 by the log-rank test). The two-year survival rate was 26.5 percent with radiotherapy plus temozolomide and 10.4 percent with radiotherapy alone.

2.2. MGMT – a key molecule for TMZ resistance

MGMT specifically removes the methyl/alkyl group from the O^6-position of guanine and restore the guanine to its normal form escaping from DNA strand breaks (Fig. 1). Thus, the expression of MGMT in tumors has a protective effect against alkylating agents-dependent cell death correlating between MGMT activity and TMZ resistance. MGMT expressing tumor cells exhibit 4- to 10-folds increase of resistance to TMZ, BCNU, and their related compounds [4]. MGMT-mediated repair is unique compared with other DNA repair pathways because : (a) it acts alone without relying on any other proteins or cofactors; (b) it transfers the alkyl group to an internal cysteine residue in the protein, acting as both a transferase and an acceptor of the alkyl-group; (c) it inactivates itself after receiving the alkyl-group from guanine, and thus, it is a suicidal protein; (d) it repairs in a stoichiometric fashion. As one molecule of MGMT removes one alkyl molecule, an excess of DNA adducts at the O^6-position could completely deplete MGMT. MGMT is ubiquitously expressed in normal human tissues [5] but is overexpressed in all types of human tumors, including colon cancer, glioma, lung cancer, breast cancer, leukemia, lymphomas, and myeloma. These properties make MGMT as an important drug resistance factor and an ideal target for suppression of drug resistance [2].

2.3. Regulation of MGMT

2.3.1. Promoter methylation

It is well known that MGMT expression levels vary widely in tumor cells [6; 7]. Hypermethylation of CpG islands within the promoter region is associated with epigenetic inactivation of the MGMT. In the EORTC trial with 206 GBM patients, MGMT promoter methylation was observed in 45% cases [8]. In cases with methylated MGMT promoter which means negative of MGMT expression, TMZ was effective as median survival was 21.7 months treated with TMZ and RT compared with 15.3 months with only RT (P = 0.007). A study of German Glioma Network (GGN) also showed that MGMT promoter methylation was associated with prolonged progression-free survival (PFS) and OS in patients receiving TMZ [9]. Several other studies have also shown predictive and prognostic significance of MGMT promoter methylation in GBM [10].

2.3.2. Transcriptional regulation

In the MGMT promoter region, there are several specific sequences for the binding of transcription factors including SP1, GRE, AP-1, and NF-κB, thus MGMT can be induced by glucocorticoids, cyclic AMP, protein kinase C activators, and NF-κB [11; 12; 13; 14]. p53 is also reported to suppress MGMT expression by directly binding to the MGMT or by suppressing the transcription factor of SP1 [15; 16]. In addition, MGMT expression can be induced by ra-

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diation or other forms of DNA damages [17]. However, physiological roles and regulation of MGMT induction is not elucidated.

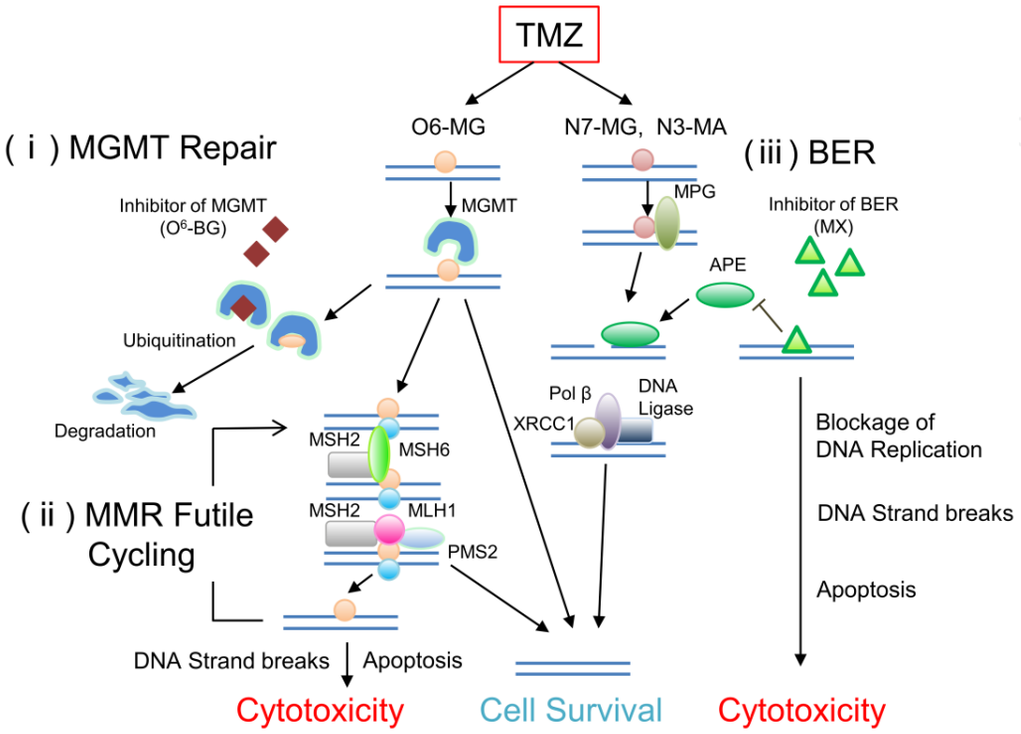

Figure 1. MGMT and other DNA repair mechanisms deal with DNA damage produced by the methylating therapeutic drug, temozolomide (TMZ), in human cells. TMZ and related drugs cause potentially cytotoxic DNA lesions such as O6-methylguanine (O6-MG, orange circle) and N7-methylguanine (N7-MG, brown circle). (i), MGMT (O6-MG DNA methyltransferase) removes the O6-alkylguanine DNA adduct through covalent transfer of the alkyl group to the conserved active-site cysteine and restores the guanine to normal. After receiving a methyl-group from O6-MG, MGMT is inactivated, and subject to ubiquitin-mediated degradation. A similar suicidal enzyme reaction occurs when MGMT transfers and accepts an alkyl-group from O6-benzylguanine (O6-BG), a therapeutic strategies. (ii), if an O6-MG DNA adduct escapes MGMT repair, it would form a base pair with thymine (blue circle) during DNA replication. The mismatched base pair of the persistent O6-MG with thymine is recognized by the mismatch repair pathway, resulting in futile cycles of repair leading to cell death. (iii), N7-MG DNA adducts (> 70% of total DNA adducts formed by TMZ) are efficiently repaired by the base excision repair (BER) pathway, and normally they contribute little to the cytotoxicity of TMZ. Methoxyamine binds to AP sites produced by methylpurine glycosylase (MPG), the first step in BER processing. Methoxyamine-bound AP sites are refractory to AP endonuclease (APE, green circle) cleavage, resulting in the blockage of the BER pathway. This leads to strand breaks, disrupted replication, and increased cytotoxicity of TMZ. Figure 1 is adapted from L. Liu et al. Clin Cancer Res. 2006;12(2):328-331.

2.3.3. Post-transcriptional regulation

MGMT protein was reported to be degraded *via* the ubiquitin proteolytic pathway [18]. According to the recent study, the correlation between MGMT promoter methylation and MGMT protein expression was poor ($p = 0.27$) [19]. *In silico* analysis predicted potential binding sites for several miRNAs within the 3'UTR of MGMT, suggesting a mechanism for post-transcriptional regulation of MGMT.

2.4. Candidate drugs for combination with TMZ

Strategies to potentiate the effecacy of TMZ by suppressing MGMT or BER pathway have been examined. Pseudosubstrates of MGMT such as O^6-benzylguanine were expected to suppress drug resistance by depleting MGMT [20; 21; 22]. However, clinical trials did not show significant restoration of TMZ sensitivity in patients with TMZ-resistant GBM [23]. IFN-β down-regulates the expression of MGMT and sensitizes resistant glioma to TMZ and phase II study has been started [15; 24].

We discovered post-transcriptional regulation of MGMT by signal transducer and activator of transcription-3 (STAT3) and demonstrated that STAT3 inhibitor or STAT3 knockdown potentiated TMZ efficacy in TMZ-resistant GBM cell lines [25] (Fig 2). Furthermore, immunohistochemical analysis of 44 malignant glioma specimens demonstrated significant positive correlation between expression levels of MGMT and phosphorylated STAT3 (pSTAT3) ($p<0.001$, $r=0.58$) (Fig 2). Therefore, STAT3 inhibitor might be one of the candidate reagents for combination therapy with TMZ for TMZ-resistant GBM patients.

2.5. Other molecules involving TMZ resistance

In spite of the correlation between promoter methylation of MGMT and temozolomide sensitivity, survival time of the patients who have methylated promoters of MGMT is still short and this suggests the involvement of other mechanism in TMZ resistance. Especially, key molecules of MMR, BER, and Fanconi anemia repair pathway such as MSH6 [26; 27], N-methyl purine DNA glycosylase (MPG) [28], DNA polymerase β (Polβ) [28], alkylpurine-DNA-N-glycosylase (APNG) [29] and FANCD1/BRCA2 [30] have been reported to affect to TMZ resistance. The unfolded protein response regulator GRP78/BiP was shown to act as a novel target for increased chemosensitivity in malignant gliomas [31]. Inhibition of Y-box binding protein-1 (YB-1) slows the increased growth of GBM and sensitizes to temozolomide independent of MGMT [32]. High levels of HOXA9/HOXA10 gene expression were associated with a shorter survival in pediatric high-grade glioma patient samples. [33]. Phosphatase and tensin homologue (PTEN) deficiency in GBM confers resistance to radiation and temozolomide that is reversed by the protease inhibitor nelfinavir [34].

Agent	Mechanism of Action	References
O^6-benzylguanine	pseudo-substrate of MGMT	Quinn JA, *et al.* J Clin Oncol. 27: 1262-1267, 2009.
Interferon-β	MGMT inhibition	Natsume A, *et al.* Cancer Res. 65: 7573-7579, 2005.
STAT3 inhibitor	MGMT inhibition	Kohsaka S, *et al.* Mol Cancer Ther. In press.
Levetiracetam	MGMT inhibition	Bobustuc GC, *et al.* NeuroOncol. 12:917-927, 2010.
PARP inhibitor(ABT888)	BER pathway inhibition	Palma JP , *et al.* Clin Cancer Res. 15:7277-7290, 2009.
Methoxyamine	BER pathway inhibition	Yan L, *et al.* Clin Cancer Res. 13:1532-1539, 2007.

Table 1. Candidate drugs for combiunation with TMZ. STAT3 indicates signal transducer and activator of transcription-3; PARP, poly(ADP-ribose) polymerase; BER, base excision repair.

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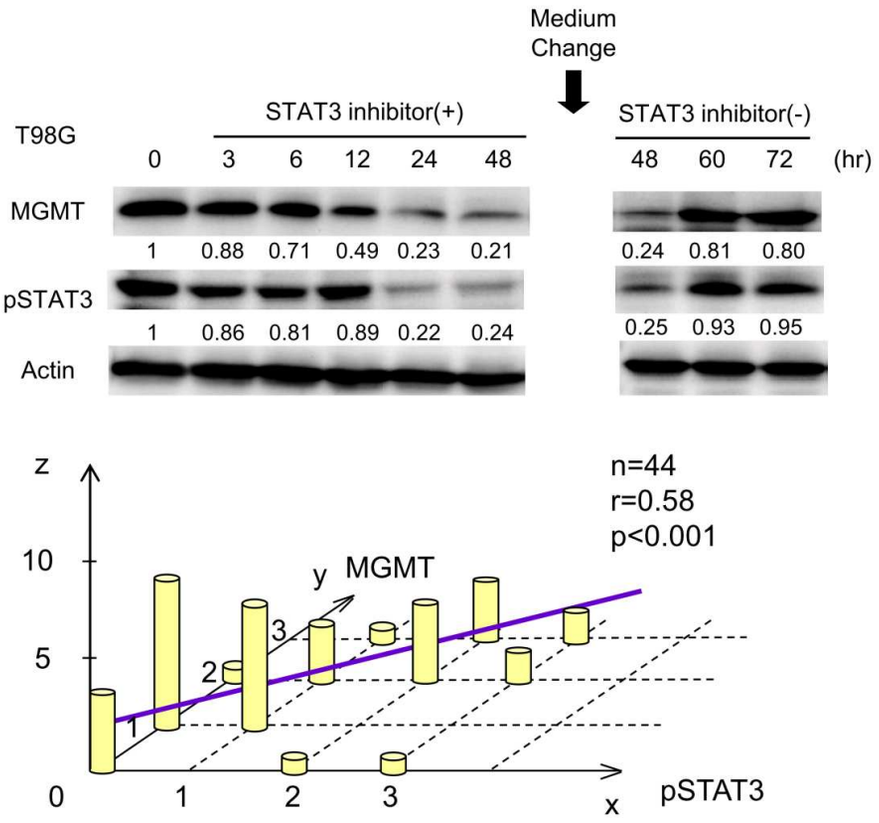

Figure 2. Correlation between expression levels of MGMT and phosphorylated STAT3. (A) Immunoblot analysis of MGMT in T98G treated with 200 mM of STAT3 inhibitor VI. Duration of the treatment is indicated at the top as 0 to 48hr. Medium change indicated removal of STAT3 inhibitor. The level of pSTAT3 was also evaluated (middle panel). Actin is shown as a loading control (bottom panel). (B) Correlation between pSTAT3 and MGMT in 44 cases of malignant glioma specimens. x and y axes indicate score of positivity of pSTAT3 and MGMT, respectively. z axis indicates the number of cases. n=44, correlation coefficient r=0.58, p<0.001. (A) Immunoblot analysis of MGMT in T98G treated with 200 mM of STAT3 inhibitor VI. Duration of the treatment is indicated at the top as 0 to 48hr. Medium change indicated removal of STAT3 inhibitor. The level of pSTAT3 was also evaluated (middle panel). Actin is shown as a loading control (bottom panel). (B) Correlation between pSTAT3 and MGMT in 44 cases of malignant glioma specimens. x and y axes indicate score of positivity of pSTAT3 and MGMT, respectively. z axis indicates the number of cases. n=44, correlation coefficient r=0.58, p<0.001.

3. Targeted molecular agents

3.1. Therapeutic targets in GBM

Identification of biological mechanisms contributing to GBM oncogenesis contributes to provide appropriate targeted therapies to improve patient outcomes. In a large-scale multidimensional analysis performed by the Cancer Genome Atlas involving, the most frequent gene amplifications were: epidermal growth factor receptor (EGFR) and platelet-derived growth factor receptor α (PDGFRα), 2 transmembrane receptors with tyrosine kinase activity; cyclin-dependent kinase 4 (CDK4), and murine double minute (MDM)2 and MDM4 which are suppressors for p53 [35]. The most frequent homozygous gene deletions were

CDKN2A, CDKN2B, and CDKN2C, which encode tumor suppressor proteins that suppress CDK4 and CDK6, phosphatase and tensin homolog (PTEN), a tumor suppressor that inhibits phosphatidylinositol-3 kinase (PI3K) signaling such as retinoblastoma (RB1), a cell-cycle inhibitor as PARK2, a regulator of dopaminergic cell death, and neurofibromin 1 (NF1), a negative regulator of the RAS signal transduction pathway. The most frequently mutated genes were p53, PTEN, NF1, EGFR, human epidermal growth factor receptor 2 (HER2), RB1, and PIK3R1 and PIK3CA-2 components/regulators of the PI3K signaling pathway. This study shows that several genes encoding proteins which are involved in signaling pathways of receptor tyrosine kinases/PI3K, and p53 and the cyclin/RB1, are considerably altered in GBM (Fig. 3). Another study has identified characteristic mutations in the active site of isocitrate dehydrogenase 1 (IDH1) in 12% of patients with GBM. IDH1 mutations occurred in a high proportion of young patients and in the majority of secondary GBM cases and were associated with increased OS (3.8 years), compared with wild-type IDH1 (1.1 years) [36]. This may be due to increased tumor sensitivity to chemotherapy, although a large controlled series in the German Glioma Network did not find any association between prolonged survival of patients with tumors with IDH1 mutations and administration of a specific therapy [9]. Mutation of the IDH1 active site prevents conversion of isocitrate to α-ketoglutarate but allows the mutated enzyme to catalyze the nicotinamide dinucleotide phosphate-dependent reduction of α-ketoglutarate to R(-)-2-hydroxyglutarate (2HG) [37]. Accumulated 2HG appears to act as an oncometabolite that contributes to glioma formation and malignant progression. This observation is supported by data from patients with inherited 2-hydroxyglutaric aciduria, in whom deficient 2HG dehydrogenase causes an accumulation of brain 2HG. These patients have an increased risk of developing brain tumors, possibly because of increased production of reactive oxygen species [38].

3.1.1. EGFR

EGFR is one of the most attractive therapeutic targets in GBM. Approximately 50% of GBM overexpress EGFR and 25% express a constitutively active mutated form of EGFR known as EGFRvIII, which has a large deletion in the extracellular domain and renders the receptor ligand independent for signaling [39]. Overexpression of EGFR is more common in primary tumors than in secondary GBM [40]. The deletion also renders a unique codon, which is not found in the wild-type receptor, thereby creating a tumor-specific epitope that can be exploited for therapeutic targeting. Increased EGFR signaling drives tumor cell proliferation, invasiveness, motility, angiogenesis, and inhibition of apoptosis.

3.1.1.1. Gefitinib, Erlotinib, Lapatinib and Cetuximab

Small-molecules of EGFR inhibitor such as gefitinib and erlotinib are well tolerated in patients with malignant gliomas, phase II trials have so far shown limited clinical benefit of erlotinib in patients with either recurrent or newly diagnosed GBM, either in combination regimens [41; 42; 43; 44] or as monotherapy [45]. Neither the EGFR/HER-2 inhibitor lapatinib [46], nor the monoclonal antibody against EGFR, cetuximab [47], have proven to be effective. Attempts to identify biomarkers to predict response to EGFR inhibitors have yielded

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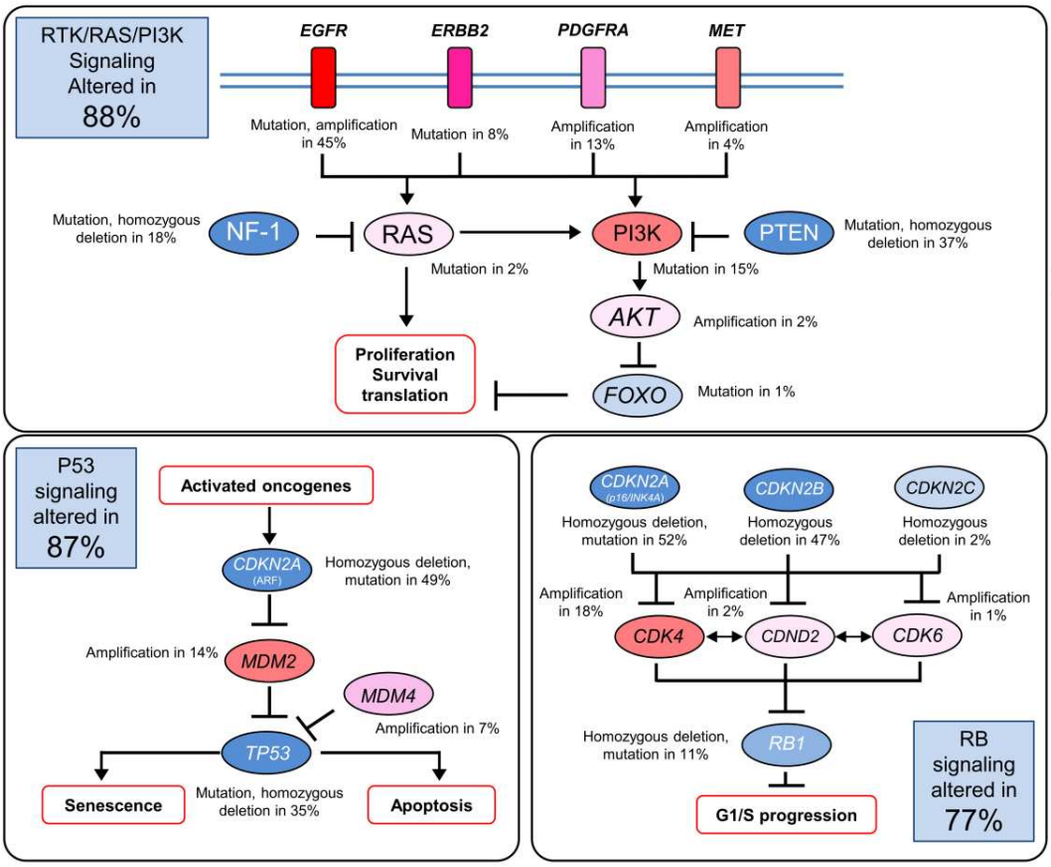

Figure 3. Genetic Alterations in Glioblastoma Occur Frequently in 3 Cellular Signaling Pathways. DNA alterations and copy number changes in the following signaling pathways are indicated in (a) receptor tyrosine kinase (RTK), RAS, and phosphoinositol–3–kinase (PI3K); (b) p53 tumor suppressor; and (c)retinoblastoma (Rb) tumor suppressor. Activating genetic alterations are shown in red. Genetic alterations that lead to a loss of function are indicated in blue. In each pathway, the altered components, the type of alteration, and the percentage of tumors carrying each alteration are shown. Blue boxes contain the total percentages of glioblastomas with alterations in at least 1 known component gene of the designated pathway. Figure 3 is adapted from The Cancer Genome Atlas Research Network. Comprehensive genomic characterization defines human glioblastoma genes and core pathways. Nature 2008;455(7216):1061–1068.

conflicting results. There is no convincing evidence of a correlation between the drug efficacy and the expression levels of EGFR in tumor tissue. In a phase I study, patients with gliomas expressing high levels of EGFR and low levels of activated AKT had better responses to erlotinib than did those with low EGFR expression and high levels of activated AKT [48]. Another study have shown significant correlation of therapeutic response of erlotinib and the presence of EGFR deletion mutant variant III [49]. However, not all studies confirmed these initial observations to predict the sensitivity to EGFR inhibitors [45].

3.1.2. PDGFR

PDGFR is a receptor tyrosine kinase with α and β isoforms. Overexpression of PDGFRα has been demonstrated in astrocytoma and GBM, indicating a potential role in tumor develop-

ment [50]. Several PDGFR-targeting agents have been developed that may have therapeutic potential against tumors with elevated PDGFR expression.

3.1.2.1. Sorafenib, Imatinib and Tandutinib

Sorafenib is an orally available antiangiogenic agent that inhibits tumor cell growth and proliferation by blocking the action of intracellular and receptor kinases, including PDGFR, RAF kinase, VEGFR2, and c-KIT [51]. In human GBM cell lines, sorafenib inhibited proliferation synergistically in combination with bortezomib, a proteosome inhibitor [52], and rottlerin, an experimental inhibitor of protein kinase C [53]. A phase II trial found that first-line TMZ and radiotherapy followed by TMZ plus sorafenib was tolerated by patients with GBM, although preliminary efficacy data for this regimen (median PFS duration, 6 months; 12-month PFS rate, 16%) were similar to data for standard therapy.

Imatinib mesylate, a small-molecule inhibitor for PDGFR, ABL, and c-KIT, was reported to have significant antitumor activity both *in vitro* and *in vivo* as orthotopic glioma models (Kilic et al 2000). Especially, preclinical trials suggested that Imatinib have shows growth inhibition in a subpopulation of CXCL12-expressing GBM cells [54] and radiosensitizes them [55]. However, in phase II trials involving recurrent GBM, imatinib alone or combined with hydroxyurea had limited antitumor activity [56; 57; 58; 59; 60].

Tandutinib is an orally active inhibitor of PDGFR, FLT3, and c-KIT tyrosine kinase activity. Although no preclinical data was available for tandutinib in GBM, 2 early-phase trials are assessing tandutinib in recurrent/progressive GBM as monotherapy or combined with bevacizumab. As correlation between increased gene expression levels of PDGFR and preclinical data for therapeutic efficacy was reported, PDGFR may be a promising target for treating GBM. However, the available clinical data suggest otherwise. Trial data of combination regimens involving PDGFR inhibitors are awaited [61].

3.1.3. VEGFR

There are multiple reasons for adapting anti-angiogenic drugs to the treatment of malignant gliomas. Malignant glioma exhibits higher vascularization which is one of the pathological hallmarks of GBM. One of the difficulties of developing effective treatments for gliomas has been poor drug penetration through the blood-brain barrier. The dense network of angiogenic vessels in GBM typically display structural, functional, and biochemical abnormalities, including large endothelial cell fenestrae, deficient basement membrane, decreased pericytes and smooth muscle cells, haphazard interconnections with saccular blind-ended extensions, complex tortuosity, and dysregulated transport pathways [62; 63; 64; 65; 66; 67]. Therefore, by targeting the tumor vasculature, it is possible to bypass this dependence on drugs to pass the blood-brain barrier to reach their targets. Further, there is also both experimental [68] and clinical [69; 70] evidence that anti-angiogenic drugs can decrease vasogenic edema and patients' requirement for corticosteroids which contributes to morbidity in this population.

The VEGF family of growth factors and their respective receptors are the best characterized proangiogenic proteins in glioma. The VEGF family includes 6 secreted glycoproteins

(VEGF-A, VEGF-B, VEGF-C, VEGF-D, VEGF-E, and placenta growth factor [PlGF]). VEGF-A, the best characterized member, typically localizes adjacent to pseudopalisading necrosis in GBM [71], and the levels of VEGF-A is increased in higher grade of glioma [72; 73], and is associated with poor prognosis [74]. The VEGF receptor (VEGFR) family includes VEGFR-1 (Flt-1), VEGFR-2 (KDR), VEGFR-3, neuropilin-1 (NRP-1), and NRP-2, which exhibit different binding affinities of the VEGF homologs. VEGFR-1 and VEGFR-2 regulate angiogenesis, whereas VEGFR-3 regulates lymphangiogenesis. Vascular endothelial growth factor production and secretion by tumor cells is stimulated mainly by hypoxia, and malignant gliomas are rapidly growing and innately hypoxic tumors. More specifically, VEGF-A binds to VEGFR-2 expressed in blood vessels, which promotes endothelial cell migration and proliferation results in new blood vessel formation in a manner of paracrine signaling loop.

3.1.3.1. Bevacizumab

Bevacizumab, a recombinant humanized monoclonal antibody composed of human immunoglobulin G1 (IgG1; 93%) and murine VEGF-binding complementarity-determining regions (7%), binds all isoforms of VEGF with high affinity and specificity [75]. Despite initial reluctance to evaluate bevacizumab in patients with brain tumors owing to concerns of intracranial hemorrhage, a series of 29 patients with recurrent malignant glioma treated with bevacizumab and irinotecan showed no significant hemorrhage with remarkable tumor regression as radiographic response rate of 66% compared with ordinal chemotherapeutic reagents as rates of 9% [76; 77]. These results led rigorous prospective clinical trials of bevacizumab in recurrent malignant gliomas. The combination of bevacizumab and irinotecan was studied in single-arm phase 2 trials for recurrent malignant glioma (n = 33) and GBM (n = 35), showing response rates as 61% and 57%, and progression-free survival (PFS) at 6 months as 55% and 46% [78; 79], respectively. These results were compared with previous rates of PFS at 6 months as 9% to 15% for recurrent GBM and 17% to 31% for recurrent malignant gliomas [80]. A large phase 2 trial randomized 167 patients of recurrent GBM to analyze efficacy of combination of either bevacizumab or bevacizumab with irinotecan. This noncomparative randomized study showed radiographic response rates as 28% and 38%, and a PFS at 6 months of 43% and 50%, respectively [69]. In addition, patients treated with bevacizumab often exhibit less vasogenic edema and decreased corticosteroid dependence secondary to neutralization of VEGF, a known vascular permeability factor. Another phase 2 trial involved bevacizumab monotherapy in 48 heavily pretreated patients with recurrent GBM [70]. The radiographic response rate was 35%, and the PFS6 rate was 29%. Ongoing phase 3 studies are evaluating the combination of bevacizumab with temozolomide and radiotherapy. The results will be of great interest because of the uncertainty regarding the impact of bevacizumab on overall survival. Combinations of bevacizumab and other chemotherapeutics or targeted molecular drugs are also currently in clinical trials.

3.1.3.2. Aflibercept

VEGF Trap (aflibercept) sequesters all isoforms of VEGF-A and PDGF as a soluble, recombinant, decoy receptor, composed of the second Ig domain of VEGFR-1 and the third Ig do-

main of VEGFR-2 bound to the hinge region of the Fc portion of human IgG1 [81]. Single arm phase II study of aflibercept in recurrent malignant glioma was proceeded [82]. 42 patients with GBM and 16 patients with malignant glioma who had received concurrent radiation and temozolomide therapies, and adjuvant temozolomide were enrolled at first relapse. The 6-month progression-free survival rate was 7.7% for GBM cohort and 25% for patients with malignant glioma. Overall radiographic response rate was 24% (18% for GBM and 44% for malignant glioma). The median PFS was 24 weeks for patients with malignant glioma (95% CI, 5 to 31 weeks) and 12 weeks for patients with GBM (95% CI, 8 to 16 weeks). A total of 14 patients (25%) were removed from the study for toxicity, on average less than 2 months from treatment initiation. This study suggested Aflibercept monotherapy had moderate toxicity and minimal evidence of single-agent activity in unselected patients with recurrent malignant glioma.

3.1.3.3. Cediranib

Several inhibitors for VEGFR tyrosine kinase have shown significant antiangiogenic and antitumor activity in preclinical GBM models [83; 84; 85; 86; 87; 88], which may also enhance cytotoxic therapy [89; 90; 91]. In addition, several these agents are undergoing evaluation in phase I/II clinical trials, but only cediranib has advanced to phase III investigation. In an initial phase II study of single-agent cediranib (45 mg/d), 27% of patients with recurrent malignant glioma exhibited a radiographic response and a 6-month PFS was 26%. In addition, cediranib induced rapid normalization of tumor vasculature, including decreased diameter of microvessels and diminished permeability, which reversed after cediranib interruption. Adverse events including hypertension and fatigue were observed, and nearly half of the patients required a dose reduction or interruption of therapy because of its toxicity [92].

3.1.3.4. Mechanisms of resistance to antiangiogenic therapy

Although antiangiogenic therapies prolong PFS of GBM patients, further progression of disease is inevitable. Progression of tumors under antiangiogenic therapy cannot often be treated successfully thereafter, and most patients die of the disease within a few months. In the cediranib study, serum levels of the proangiogenic factors bFGF, stromal-derived factor 1 (SDF1), and soluble VEGFR2 increased at the time of failure [93]. The alternative proangiogenic pathways depends on these angiogenic factors may drive angiogenesis in the setting of VEGFR inhibition. Furthermore, for many gliomas, particularly malignant gliomas, there is often little evidence for vascular proliferation. As the individual infiltrating tumor cell tends to grow along normal cerebral vasculature, and thus there is no need for tumor-associated angiogenesis. Indeed, there is at least a theoretical concern that inhibition of angiogenesis in malignant glioma may prevent the formation bulky tumor but has little effect on sparsely infiltrative GBM cells results in little impact on OS of patients. Early clinical and radiographic observations of patients treated with bevacizumab suggest that this may be the case [94; 95]. Another concern is recent laboratory evidence that suggests that inhibition of VEGF may actually increase invasiveness of tumor cells [96]. The infiltrative tumor cells are often responsible for relapse leading to the death of patients.

Combination of antiangiogenic and anti-invasion therapy may delay disease progression. Studies of co-administration of cediranib (pan-VEGFR inhibitor) with cilengitide (integrin inhibitor) and bevacizumab (neutralizing VEGF antibody) with dasatinib (PDGFRβ inhibitor) are ongoing. Another potential mechanism of resistance to antiangiogenic therapies involves increased PDGF signaling. PDGF stabilizes neovasculature by recruiting pericytes and facilitating pericyte-endothelial cell interactions [97]. Preclinical data suggest that dual VEGFR/PDGFR inhibition potentiates antiangiogenic efficacy and reduces resistance to therapy [98], and this approach is currently being evaluated in clinical trials.

3.1.4. c-MET

Aberrant signaling by hepatocyte growth factor (HGF) and its receptor MET has been observed in various tumors including GBM, and potential involvement in tumorigenesis and metastasis has been reported [99]. Recently c-MET overexpression was detected in 18 (29%) of 62 GBM with shorter median survival durations than those of little or no expression of c-MET (median durations of survival, 11.7 vs 14.3 months) [100].

3.1.4.1. AMG102 and PF02341066

Inhibitors of HGF or c-MET have shown preclinical activity against GBM cell lines [99]. The anti-HGF antibody AMG102 enhanced TMZ-induced inhibition of growth of GBM cell line *in vitro* and *in vivo* as xenografts [101]. However, phase II trial suggests AMG 102 monotherapy did not significantly suppress tumor growth of recurrent GBM [102]. PF02341066, an orally available ATP-competitive inhibitor of c-MET inhibited growth and c-MET phosphorylation of GBM in preclinical studies [103]. This molecule is currently under clinical investigation in patients with advanced cancers.

3.1.5. PI3K and related pathways

PI3K plays a role in intracellular signaling pathways regulating in cell survival, growth, and proliferation. Activated PI3K is recruited to the cell membrane where it mediates signaling after activation of receptor tyrosine kinases. Downstream targets include AKT for cell proliferation and survival; glycogen synthase kinase-3 (GSK-3) for regulation of c-MYC; and mammalian target of rapamycin (mTOR) for regulation of protein synthesis and negative regulator of PI3K. In malignant glioma, PI3K/Akt/mTOR signaling is frequently activated because of the stimulation of receptor tyrosine kinases as EGFR, PDGFR, and mesenchymal-epithelial transition factor (MET), mutation of oncogenic PI3K subunits, and/or loss of PTEN tumor suppressor activity. Therefore inhibiting the PI3K pathway may have therapeutic potential.

3.1.5.1. NVP-BEZ235 and Enzastaurin

NVP-BEZ235, an orally available kinase inhibitor for PDK1, mTOR, and PI3K, induced G1 arrest of a GBM cell line *in vitro* and enhanced TMZ efficacy *in vivo* [104]. NVP-BEZ235 treatment is currently in phase I trials involving patients with solid tumors.

Enzastaurin, a PKC/PI3K/AKT inhibitor, suppressed proliferation and induced apoptosis *via* a caspase-dependent mechanism in GBM cells in vitro [105]. *In vivo* models showed that enzastaurin combined with radiotherapy synergistically reduced tumor volume, radiation-induced satellite tumor formation, upregulation of VEGF expression, neovascularization, and GSK-3β phosphorylation [106]. In phase II study of enzastaurin in patients with recurrent heavily pretreated GBM showed that objective radiographic responses occurred in 25% of patients [107]. The subsequent phase III trial comparing lomustine and enzastaurin at first or second recurrence was the first phase III trial to evaluate a targeted therapy for recurrent GBM. Enzastaurin was well tolerated and had a better hematologic toxicity profile but did not have superior efficacy compared with lomustine in patients with recurrent GBM [108].

3.1.6. SRC and SRC-Family kinases

SRC and SRC-Family Kinases (SFKs) are frequently activated in GBM [109] frequently due to their overexpression [110]. SRC and SFKs are promiscuous regulators of multiple signaling pathways for cell proliferation, adhesion, migration, and invasion, which are important processes in tumor invasion and metastasis.

3.1.6.1. Dasatinib

Dasatinib is a potent inhibitor of SRC and SFKs and has been approved for the treatment of certain types of leukemia on the basis of inactivation of BCR-ABL [111]. Dasatinib also inhibits c-KIT and PDGFR [112]. In GBM cells, dasatinib inhibited migration and induced autophagy, resulted in cell death which was enhanced by combination with TMZ [111; 113]. Dasatinib inhibited invasion, promoted tumor regression, induced apoptosis in EGFRvIII-expressing GBM, and enhanced the activity of anti-EGFR antibodies [111]. Trials of dasatinib are ongoing in GBM and several solid tumors. A phase I/II trial involving patients with newly diagnosed GBM is assessing dasatinib combined with radiotherapy and concomitant TMZ, followed by adjuvant dasatinib plus TMZ. Trials of dasatinib for treatment of recurrent GBM include a phase II trial of dasatinib monotherapy, a phase I trial of dasatinib in combination with erlotinib, and a randomized phase I/II trial of dasatinib in combination with CCNU that has started its phase I component with patients who have recurrent GBM.

3.1.7. Integrin

Integrin plays key roles regulating cell adhesion, migration, and invasion. In addition to a role for matrix-cell contact, integrin also activate intracellular signals including SRC-dependent pathway. In various tumors, integrin has an established role in metastasis and angiogenesis [114]. Therefore, targeting integrin function may have potential for treating GBM.

http://dx.doi.org/10.5772/54353

PRIMARY TARGET	AGENT	OTHER TARGETS	MECHANISM OF ACTION
EGFR	Gefitinib (ZD1839)		TKI
	Erlotinib (OSI-774)		TKI
	Lapatinib (GW-572016)	HER-2	TKI
	PF-00299804	HER-2, HER-4	TKI (irreversible)
	BIBW2992	HER-2, HER-4	TKI (irreversible)
	Cetuximab		Monoclonal antibody
	Nimotuzumab		Monoclonal antibody
EGFRvIII	CDX110		Vaccine
PDGFR-α	IMC3G3		Monoclonal antibody
PDGFR-β	Imatinib	BCR/Abl, c-Kit	TKI
	Dasatinib	Src, BCR/Abl, c-Kit, ephrin A2	TKI
	Tandutinib (MLN518)	Flt3, c-Kit	TKI
VEGF-A	Aflibercept (VEGF Trap)	VEGF-B, PlGF	Soluble decoy receptor
	Bevacizumab		Monoclonal antibody
VEGFR-2	Cediranib (AZD2171)	All VEGFR subtypes, PDGFR-β ,c-Kit	Adnectin
	CT-322	All VEGFR subtypes	TKI
	Pazopanib	All VEGFR subtypes, PDGFR-α And β, c-Kit	TKI
	Sorafenib	VEGFR-3, B-Raf, PDGFR-β , c-Kit, Ras, p38α	TKI
	Sunitinib	PDGFR-β, Flt3, c-Kit	TKI
	Vandetanib (ZD6474)	EGFR	TKI
c-Met	XL-184	VEGFR	TKI
HGF/SF	17-AAG		Monoclonal antibody
PI3K	XL765	mTOR	STKI
PKC	Enzastaurin (LY31761)		STKI
mTOR	Sirolimus (rapamycin)		mTOR inhibitor
	Everolimus (RAD001)		mTOR inhibitor
	Temsirolimus (CCI-779)		mTOR inhibitor
	Ridaforolimus (AP23573)		mTOR inhibitor
SRC	Dasatinib		TKI
Integrins	Cilengitide (EMD121974)		Synthetic RGD peptide
HDAC	Vorinostat (SAHA)		HDAC inhibitor
	Valproic acid		HDAC inhibitor
	LBH589		HDAC inhibitor

Table 2. Targeted molecular agents currently in clinical development for high-grade glioma TKI indicates tyrosine kinase inhibitor; SAHA, suberoylanilide hydroxamic acid; RGD, arginine-glycine-aspartate; STKI. serine-threnoine kinase inhibitor; PKC, protein kinase C.

3.1.7.1. Cilengitide

Cilengitide is a specific αV integrin inhibitor in clinical development. In a phase I/IIa trial, cilengitide combined with the current standard of therapy in patients with newly diagnosed GBM was well tolerated, with 6-month PFS as 69%. Methylation of promoter of O6-methylguanine-DNA methyltransferase (MGMT) predicts a higher likelihood of achieving 6-month PFS, as shown by increases in the durations of PFS and OS to 13.4 months and 23.2 months, respectively, compared with 3.4 and 13.1 months for patients without MGMT promoter methylation [115]. On the basis of these findings, a similar regimen is being compared with radiotherapy/TMZ alone in the phase III CENTRIC trial in patients with newly diagnosed GBM with hypermethylated MGMT promoter. In a phase IIa study of recurrent GBM, cilengitide monotherapy was well tolerated but was largely inactive (6-month PFS rate, 15%); long-term disease stabilization was seen in a small subset of patients: 10% were progression free for 12 months, and 5% were progression free for 24 months [116].

3.1.8. Histone deacetylase inhibitor

Histone deacetylases (HDACs) are involved in multiple processes to lead malignant phenotype of glioma including maintanance of stemness, angiogenesis, and resistance to DNA damage.

3.1.8.1. Vorinostat

Vorinostat is an orally available inhibitor of class I and II HDAC approved for advance cutaneous T cell lymphoma. In a phase II study of recurrent GBM, vorinostat monotherapy was well tolerated and had modest clinical activity (6-month PFS rate, 15.2%; median OS duration, 5.7 months) [117]. Vorinostat is currently being evaluated for use in newly diagnosed and recurrent GBM as a combination therapy.

4. Conclusion

Although TMZ prolonged the survival of GBM patients, GBM are still immortal disease with extremely poor prognosis because of acquisition of TMZ resistance. Therefore, other therapeutic agents which suppress MGMT expression or attenuate TMZ resistance are highly desired. As the efficacy of single agent of targeted molecular therapy seems to be limited, combination therapy should be evaluated since multi-pathway is involved in the chemoresistance in GBM. An 'tailor-made' selection of chemotherapeutic agents for each GBM patients based on molecular analysis is essential to obtain maximum efficacy of chemotherapeutic agents.

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