BRD4: An emerging prospective therapeutic target in glioma

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INTRODUCTION

Gliomas are the most common primary intracranial tumors, accounting for more than 30% of central nervous system neoplasias.2 Gliomas have been traditionally classified by the World Health Organization (WHO) into grades I–IV, which describe them in terms of histopathological lineage as either astrocytoma, oligodendroglioma, or oligoastrocytoma.1 Grades I and II are considered low-grade gliomas, while grades III and IV are considered high-grade gliomas. Most patients have high-grade gliomas, and higher grades generally have a less favorable prognosis.2 The traditional standard management of gliomas involves observation, surgery, chemotherapy, and/or radiotherapy.2

In 2016, a new classification of the WHO recommended molecular diagnosis of the metabolic gene isocitrate dehydrogenase (IDH) mutational status in gliomas. IDH-mutated and IDH wild-type tumors are driven by different oncogenic pathways and respond differently to current therapeutic regimens.1 IDH mutant gliomas are often represented by low-grade cases and have better prognosis. Moreover, IDH mutant tumors are more likely to undergo total resection with low incidence of long-term neurological deficits. Postoperative adjuvant radiotherapy combined with vincristine and/or temozolomide (TMZ)-based chemotherapy is recommended.1 Additionally, the WHO 2016 classification also added a new subtype under grade IV gliomas, that is, H3K27M mutant tumors, which mainly occur in children and younger patients and are characterized by poor prognosis.3

The efficacy of traditional therapeutic approaches is limited, especially for glioblastoma (GBM) cases.4 New treatments have been developed, such as tumor-treating fields (by low-intensity, alternating, electric fields delivered by transducer arrays applied to the scalp for antimitotic therapy),5 immune checkpoint inhibitors (anti-CTLA-4, anti-PD-1, anti-PD-L1),5 angiogenesis inhibitors (bevacizumab),6 IDH inhibitors (enasidenib and ivosidenib),6 oncolytic viruses,7 and tumor vaccines.8

It is unclear whether other genetic changes, apart from IDH mutations, can condition glioma progression; however, epigenetic regulatory mechanisms are gaining a growing interest. Epigenetic modifications are heritable and reversible alterations to DNA, which do not involve changes in the nucleotide sequence. Epigenetics play an important role in maintaining homeostasis in normal cells via changes in CpG island methylation patterns and histone modifications that regulate gene expression. Epigenetic proteins have recently emerged as novel anti-cancer targets. Among these, the bromodomain and extraterminal (BET) family of proteins has gained increasing attention due to its critical role in recruiting chromatin-regulating enzymes and “reading” chromatin to control gene expression in cancer development.9 In particular, rearrangement or mutations of tumor-associated genes regulated by bromodomain-containing protein 4 (BRD4) have been identified in glioma studies.

This review focuses on the role of BRD4 dysfunction in promoting glioma development. In addition, the application of small-molecule inhibitors targeting specific BET proteins, as a novel therapeutic strategy for glioma patients, is discussed.

BET BROMODOMAIN PROTEINS

The BET family consists of four proteins: BRD2, BRD3, BRD4, and BRDT (specifically expressed in testis).5 The BET proteins are characterized by two tandem, conserved, bromodomain motifs (BD1 and BD2), one extraterminal (ET) domain, and a C-terminal motif
The bromodomain structure consists of four reverse parallel α-helices and two circular structures, one αa loop and a BC loop, connecting the helices. These structures form a hydrophobic cavity that recognizes acetylated lysine residues. Each BET bromodomain binds to two acetyl-lysine histone markers. In addition, BET proteins interact with the transcription elongation complex and with other transcription factors. In summary, BET proteins are key “readers” of epigenetic information in both normal and transformed cells.

BRD4: THE MOST STUDIED BET PROTEIN
BRD4 is a double bromodomain (BD1 and BD2)-containing protein. In addition, BRD4 comprises an additional terminal clustering domain (ET), a CTM, and several conserved motifs. Multiple studies have indicated a clear role for BRD4 in the regulation of transcription by interaction with acetylated histones (e.g., H3K9ac, H3K14ac, H3K27ac, H4K5ac, H4K8ac, H4K12ac, and H4K16ac) and non-histonic proteins (e.g., RelA subunit of NF-kB, TWIST, GATA1, Cyclin T1, γ-DAX, ERG). Moreover, and possibly most importantly for cancer, BRD4 is involved in the recruitment of the transcriptional machinery to gene targets (such as c-Myc, Tp53, c-Jun, Stat3, NPM1, FoxO3, Ap2, BCL2, BCL6, PAX5, CD44 and CDK6). BRD4 is widely expressed in human tissues, cancer cells, cancer-associated fibroblasts, and chronic inflammatory fibroblasts (e.g., liver, lung, cardiovascular, and renal). In fact, BRD4 is considered the dominant transcriptional regulator within the BET protein family, and it plays a significant role in the onset and progression of malignant human diseases, especially by controlling tumor cell proliferation and apoptosis, making it the most studied BET protein in cancer therapy.

BRD4 INHIBITORS
BRD4 is not only crucial for the regulation of gene transcription, but it is also a keeper of genome stability. Thus, BET inhibitors have a wide spectrum of prospects for the treatment of cancer. Although BRD4 inhibitors have shown exciting antitumor effects, the selectivity of these drugs needs improvement. In fact, since current BRD4 inhibitors all bind to the bromodomain structure (also found in other BET family proteins), none is selective for BRD4 only.

Small-molecule inhibitors of the first generation bind to BET bromodomains as competitors of acetyl-lysine, thus displacing BET protein complexes from chromatin. According to drug selectivity, BRD4 small-molecule inhibitors are subdivided into pan-bromodomain inhibitors (which do not discriminate between the two bromodomains), BD1-selective inhibitors, and BD2-selective inhibitors (Figure 2). The pan-bromodomain inhibitors belonging to the triazoloazepine or isoxazole derivative classes are the most studied. JQ1 was the first BRD4 bivalent triazoloazepine inhibitor developed in 2010. JQ1 has strong inhibitory effects on both BD1 and BD2, with a 50% inhibitory concentration (IC50) of 77 nM and a diffusion constant (Kd) of 50 nM. Two other well-known BRD4 pan-bromodomain inhibitors are OTX015 and TEN-010. OTX015 has shown efficacy not only in primary hematological and solid cancers but also in refractory or relapsed hematological cancers. While the latter inhibitors are triazoloazepines, I-BET151 is the most promising isoxazole-based BRD4 inhibitor (IC50 of 794 nM). I-BET151 showed beneficial effects in the treatment of GBM and leukemia. In addition, CPI-0610, PLX51107, and INCBO543294 belong to isoxazole-based BRD4 inhibitors.

As the inhibition of individual BET bromodomains will lead to different transcriptional and functional outcomes, chemists have continued to optimize BRD4 inhibitors with the aim to improve...
their selectivity. BD1 selective inhibitors, such as GSK778, MS-436, Olimone, and BI-2536, as well as the BD2 selective inhibitors RVX-208, RVX-297, GSK046, and ABBV-744 have been produced.\(^{27,42}\)

The second-generation BRD4 inhibitors are mainly synthesized by proteolysis targeting chimera (PROTAC) technology. This approach implicates the use of a molecule that binds both the protein to be degraded and the E3 ubiquitin ligase. Such bridging between the target protein and E3 ligase mediated by PROTAC promotes its ubiquitination and proteasomal degradation.\(^{35}\) For example, BET degraders, called dBETs, bind to both the E3 ubiquitin ligase cereblon (similar to the drug thalidomide) and BET proteins.\(^{43}\) As a second-generation BET degrader, dBET6 is a highly cell-permeable inhibitor of BET bromodomains. Compared to JQ1\(^{44}\) and other BET bromodomain inhibitors (BBIs),\(^{44}\) dBET6 is substantially more active.

A variety of BRD4 inhibitors have entered clinical trials. We summarize all clinical trials with BRD4 inhibitors, including one for glioma patients, in Table 1. Actually, the half-life of JQ1 is relatively short, which has hindered its further application in vivo.\(^{18}\) dBET6 is a newly developed drug, which has not been used in clinical trials yet. In addition to these, a series of new drugs, including I-BET762 (GSK525762),\(^{45}\) I-BET151 (GSK2820151),\(^{46,47}\) and OTX015/MK-8628,\(^{46}\) have improved risk-benefit profiles in multiple cancers. However, most of the BRD4 inhibitors listed in Table 1 have been tested in GBM cell lines and mouse tumor models. Only one clinical trial was conducted (ClinicalTrials.gov: NCT02296476) by the application of OTX015 in recurrent GBM, after standard front-line therapy failure. Participants received 80, 120, or 160 mg of oral OTX015 administered once daily every day in a 28-day cycle for up to six cycles. Unfortunately, this study was terminated due to lack of clinical efficacy and not due to safety reasons.

The side effects and drug resistance profiles of BRD4 inhibitors still deserve attention. In clinical studies, BRD4 inhibitors have shown adverse reactions, including thrombocytopenia, fatigue, headache, and hyperbilirubinemia. Additionally, low expression of BRD4 may be associated with a decreased number of hematopoietic cells, skin hyperplasia with abnormal hair follicles, and intestinal crypt rupture with loss of secretory cells. Moreover, BRD4 inhibitors target BRD2/3 simultaneously, and low expression of BRD2/3 resulting from BRD4 inhibition therapy may relate to neuronal defects and obesity.\(^{50}\) Mechanisms of BRD4 inhibitor resistance were identified in a preclinical model, which may be related to activation of oncogenic pathways, such as WNT/β-catenin/MYC (acute myeloid leukemia [AML]), Hedgehog/GLI2/MYC (pancreatic cancer), mitogen-activated protein kinase (MAPK) (colorectal cancer), or RAS/BCL2 (lymphoma).\(^{50}\)

### BET PROTEINS IN GLIOMA

The dysregulation of BET proteins, in particular BRD4, has been implicated in the development of cancers, including gliomas.\(^{36}\) Numerous BRD4 inhibitors have been studied in recent years, and some are currently in clinical trials. Recent clinical data demonstrated that BRD4 is a valuable target for treating glioma.\(^{47}\) Interestingly, BRD4 inhibitors effectively penetrate the blood-brain barrier (BBB)\(^{46,48,49}\) and target glioma tumor tissues, while having little effects on normal brain tissue.\(^{46}\)

### BRD4 EXPRESSION IN GLIOMA

The expression of BRD4 in glioma tissues and cell lines is significantly higher than in normal tissues and cells.\(^{36}\) In 2014, Pastori et al.\(^{40}\) first discovered that BRD4 mRNA is significantly overexpressed in glioma. NanoString technology was used to determine the expression of 40 bromodomain proteins in 27 gliomas compared to 9 control samples isolated from epilepsy patients. BRD4 expression was 1.8-fold higher in glioma samples relative to controls, and the p value was less than 0.05. Wang et al.\(^{50}\) also found that BRD4 expression is increased in glioma compared to adjacent tissues. Analyses of online datasets indicated that BRD4 expression was negatively correlated with the overall survival of glioma patients. Many subsequent studies have explored the role of BRD4 in glioma.\(^{50–52}\) Genome-wide transcriptional analysis of BRD4-regulated genes and pathways included multiple cellular processes, such as cell cycle and apoptosis in human glioma cells.\(^{52}\)

### BRD4 INHIBITOR THERAPY FOR GLIOMA

Knockdown of BRD4 by lentivirus or small interfering RNA (siRNA) has been proven to affect the occurrence and development of glioma.\(^{50–52}\) The use of BRD4 inhibitor monotherapy for glioma shows satisfactory results in both glioma cell lines or glioma stem cells (GSCs) (Table 2).
Many studies have investigated the effects of JQ1 in glioma. In fact, the therapeutic use of JQ1 for treating glioma was first reported in 2013. Cheng et al.53 discovered the antitumor effects of JQ1 by treating ex vivo cultures derived from xenografts of primary GBM cells. JQ1 induced G1 cell-cycle arrest and apoptosis by affecting the expression of c-Myc, p21 (CIP1/WAF1), hTERT, Bcl-2, and Bcl-xL. JQ1 is effective on glioma cell lines with different mutational status. Liu et al.54 demonstrated that JQ1 suppressed aggressive growth of GBM cells carrying oncogenic epidermal growth factor receptor (EGFR) mutations. Additionally, since the H3K27M histone mutation is commonly found in diffuse intrinsic pontine glioma (DIPG), BRD4 inhibitor is considered a potential therapeutic target for this disease.55 JQ1 induces growth arrest through a typical decrease of c-Myc transcription in H3K27M DIPG cell lines.

In vitro, a xenograft mouse model of DIPG treated with JQ1 for 10 days also exhibited significantly reduced tumor size and prolonged animal survival.

Previous studies reported an association of BRD4 with the expression of inducible nitric oxide (NO) synthase (iNOS) in macrophages and microglia. In GBM, Fahey et al.56 found that JQ1 could greatly improve the clinical outcome of photodynamic therapy (PDT) by inhibiting iNOS expression. In fact, PDT is an effective approach to treat GBM. However, PDT oxidative challenge results in prolonged upregulation of iNOS in GBM U87 cells, and endogenous NO generated by iNOS promotes proliferation, invasion, and resistance to apoptosis in GBM cells. JQ1 can inhibit iNOS expression, endogenous NO production, and malignant biological behavior of GBM cells better than iNOS inhibitors. In mechanistic terms, NF-kB-regulated iNOS expression in GBM cells, and BRD4 can act as a NF-kB co-activator, which explains JQ1-induced enhancement of PDT cytotoxicity. In fact, JQ1 at minimally toxic concentrations significantly suppressed the upregulation of iNOS, survivin (a potent inhibitor of apoptosis), and Bcl-xL (a NF-kB-regulated anti-apoptotic protein) in response to PDT.56

Additional BRD4 inhibitors appear to be useful in the treatment of glioma.

Pastori et al. suggested that inhibition of BET protein is an effective way to reduce GBM cell proliferation. The disruption of BRD4 expression in GBM cells impaired cell cycle progression at the G1/S transition point. Similarly, I-BET151 inhibited glioma cell proliferation in vitro and in vivo. Importantly, I-BET151 is as potent an inhibitor of GBM cell proliferation in vitro as TMZ, which is the main treatment currently administered to GBM patients. Therefore,
### Table 2. Summary of the progress of BRD4 inhibitor monotherapy in glioma

| No. | Author | Research objects | Mutation of cell line | Treatment regimen | Mechanisms | Related pathways | Genes/targets | Conclusion |
|-----|--------|------------------|-----------------------|-------------------|------------|-----------------|--------------|------------|
| 1   | Cheng et al. | T4105, T4302, and T4597 primary GBM xenograft cell lines | N/A | JQ1 | G1 cell cycle arrest (+), apoptosis (+) | N/A | c-Myc, p21 (CIP1/WAF1), hTERT, Bcl-2, and Bcl-xL | (1) JQ1 induced G1 cell cycle arrest and apoptosis (2) JQ1 treatment resulted in changes in expression of c-Myc, p21 (CIP1/WAF1), hTERT, Bcl-2, and Bcl-xL |
| 2   | Liu et al. | U87, U87 EGFRvIII, LN229, U373, GBM6 cell lines | EGFRvIII mutations | JQ1 | apoptosis (+) | SOX9 and FOXG1 | c-Myc, CDKN1A (p21), TUBB3 (Tuj1), and MAP2 | (1) JQ1 induces growth arrest through the canonical reduction in c-Myc transcription (2) mice treated with JQ1 for 10 days exhibited significantly reduced tumor size and prolonged animal survival |
| 3   | Piunti et al. | SF8628, SF7761 and SU-DIPG-IV, pcGBM2, SF9402, and SP9427 primary pediatric human cell lines and mice | H3K27M mutations | JQ1 | proliferation (−) | N/A | c-Myc, CDK1, Bcl-2, and Bcl-xL | JQ1 showed better inhibition of iNOS expression, endogenous NO production, and malignant biological behavior of GBM tumor cells than did iNOS inhibitors |
| 4   | Fahey et al. | U87 and U251 GBM cell lines | N/A | JQ1 | proliferation (−), invasion (−) | N/A | p21cip1, HEXIM-1 | (1) JQ1 inhibited the proliferation and self-renewal of GSCs (2) JQ1 has notable anti-tumor effects against GBM, which may be mediated via the VEGF/PI3K/AKT signaling pathway (3) JQ1 significantly inhibited the growth of GSC tumors in vivo |
| 5   | Wen et al. | CSC2078 and CSC1589 primary murine GSC lines and mice | N/A | JQ1 | proliferation (−), self-renewal (−) | VEGF/PI3K/AKT pathway | MMP, AKT downstream target genes | (1) JQ1 inhibited the proliferation of GBM cells by reducing cell cycle progression at the G1/S phase (2) JQ1 is as potent at inhibiting GBM cell proliferation as TMZ (3) JQ1 can significantly reduce expression levels of IncRNA HOTAIR |
| 6   | Pastori et al. | U87MG, A172, SW1783, and UM20 (from patient) GBM cell lines and mice | N/A | I-BET151 | G1/S cell cycle arrest (+) | p21cip1, HEXIM-1 | c-MYC, CDK1, Bcl-2, and Bcl-xL | (1) I-BET151 inhibits the proliferation of GBM cells by reducing cell cycle progression at the G1/S phase (2) I-BET151 is as potent at inhibiting GBM cell proliferation as TMZ (3) I-BET151 can directly reduce expression levels of IncRNA HOTAIR |
| 7   | Pastori et al. | LN18, U87MG, A172, and T98G GBM cell lines and mice | N/A | I-BET151 | proliferation (−) | N/A | HOTAIR, MEG3, NEAT1, DGR5 | (1) I-BET151 is able to reduce proliferation and self-renewal of GSCs (2) BRD4 is mainly located at the promoter region of Notch1 and may be involved in the process of tumor metabolism (3) I-BET151 eliminated GSC tumorigenicity in the mouse intracranial models |
| 8   | Tao et al. | U87, U251 GBM cell line and GSCs (CD133*) and mice | N/A | I-BET-151 | proliferation (−), self-renewal (−) | Notch1, NICD, Hes1 pathway | Notch1, NICD, Hes1 | (1) OTX015 inhibits the proliferation of GBM cells (2) OTX015 significantly increases survival in GBM mice (3) OTX015 can effectively penetrate the blood-brain barrier |
| 9   | Berenguer-Daizé et al. | U87MG, T98G, UI18 cell lines and mice | N/A | OTX015 | cell cycle arrest (+) | Ras/Akt/mTOR pathway | c-MYC, CDK1, BRD2, BRD3, SESN3, HEXIM-1, HIST2H2BE, HIST1H2BK, MTHFDIIL, HIST2H4A, and HIST1H2BJ | (1) OTX015 inhibits the proliferation of GBM cells (2) OTX015 significantly increases survival in GBM mice (3) OTX015 can effectively penetrate the blood-brain barrier |

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BRD4 inhibitor therapy for GSCs

Multiple studies have demonstrated that BRD4 regulates stem cell differentiation. In particular, Wen et al.51 focused on the antitumor effects of JQ1 on GSCs. JQ1 and siRNAs targeting BRD4 (siBRD4) could inhibit GSC proliferation and self-renewal in vitro. In addition, JQ1 significantly inhibited the growth of GSC tumor xenografts in mice. Mechanistic studies suggest that JQ1 inhibited the development of GSC by interfering with the VEGF/phosphatidylinositol 3-kinase (PI3K)/Akt signaling pathway. In fact, JQ1 inhibited VEGF receptor-2 phosphorylation in GSCs, thereby reducing PI3K and AKT activity. In addition, JQ1 inhibits the expression of MMP, thereby reducing extracellular matrix degradation and angiogenesis in GBM. Additionally, the treatment with JQ1 induced apoptosis by activating AKT downstream target genes.

A signaling pathway involving BRD4 and Notch1 was also found to reduce GSC proliferation and self-renewal.32 In vitro, I-BET151 reduces the proliferation and self-renewal of GSCs. Mechanistic studies showed that BRD4 is largely associated with the promoter region of Notch1, which may be involved in tumor metabolism. Finally, I-BET151 obstructed the tumorigenic potential of GSCs in intracranial injection mouse models.

In conclusion, BRD4 inhibitors inhibit the development of glioma or GSCs by interfering with cell cycle progression, proliferation, and/or apoptosis in vivo and in vitro. This has been associated with interference with NF-κB, Akt and Notch signaling pathways, transcriptional regulation of oncogenes (such as c-Myc, Bcl-2, and Bcl-xL), lncRNA expression, and inhibition self-renewal of GSCs (Figure 3). Since I-BET151 is as potent at inhibiting GMB cell proliferation as TMZ,10 BRD4 inhibitors might represent an alternative substitute for TMZ in GMB patients.

Table 2. Continued

| No. | Author | Research objects | Mutation of cell line | Treatment regimen | Mechanisms | Related pathways | Genes/targets | Conclusion |
|-----|--------|------------------|----------------------|------------------|------------|-----------------|---------------|------------|
| 10  | Xu et al.14 | A172, T98G, U87, U138, U251, and U343 GMB cell lines and mice | N/A | dbET6 | G2/M cell cycle arrest (+) | N/A | CEBPB, RUNX1, FOXL2, and STAT3 | (1) dBET6 interfered with GBM transcriptional regulation, which was controlled by BET and E2F1 |
|     |        |                  |                      |                  |            |                 |               | (2) dBET6 inhibits GBM cell proliferation better than JQ1, I-BET151, and OTX015 |

NO, nitric oxide; iNOS, inducible NO synthase; GSC, glioma stem cell; DIPG, diffuse intrinsic pontine glioma; N/A, not applicable.

In 2016, Berenguer-Daizé et al.46 found that the novel BRD4 inhibitor, OTX015 (MK-8628), showed better anti-proliferative effects than its analog JQ1. Results in three additional glioma cell lines supported the anti-proliferative effects of OTX015. Furthermore, single oral administration of OTX015 significantly increased survival in mice bearing in situ or ectopic U87MG xenografts. Surprisingly, OTX015 effectively penetrated the BBB, and the concentration of OTX015 in glioma tumor tissue was 7- to 15-fold higher than in surrounding normal tissue.

In 2018, Xu et al.14 explored the effects of dBET6, a new generation BRD4 inhibitor that chemically degrades BET proteins. G2/M cell cycle transition, cancer cell self-renewal, and the tumorigenic potential of GCSs were significantly inhibited by dBET6. In addition, dBET6 drastically reduced BET protein genomic occupancy, RNA Pol II activity, and permissive chromatin marks. Mechanistic research showed that dBET6 interfered with glioma transcriptional regulation, which is controlled by BET and E2F1. Moreover, dBET6 inhibits glioma cell proliferation better than traditional BET inhibitors (e.g., JQ1, I-BET151, OTX015) and overcomes both intrinsic and acquired resistance to traditional BBIs. More than 70% of the glioma cell lines showed intrinsic refractoriness to at least one BBI. Moreover, glioma cells can develop adaptive tolerance and cross-resistance to BBIs after prolonged treatment. BBI resistance of glioma cells may relate to Sp15 amplicon or TERT expression being significantly upregulated. Since glioma cells with either intrinsic or acquired BBI resistance still relied on the presence of BET proteins, cell lines with acquired resistance to BBIs may still be sensitive to the activity of the BET protein degraders. Notably, RNA sequencing (RNA-seq) uncovered that dBET6 and JQ1 elicited different transcriptomic responses in glioma cells. dBET6-responsive events in acquired resistant cell lines to BBIs were largely unaffected, indicating that dBET6 can overcome acquired insensitivity to BBIs by degrading the BET protein in glioma cells.14

BRD4 protein inhibitors might be considered as an alternative therapy for TMZ-resistant GBM patients.60 BRD4 can also promote tumorigenesis and development of GBM cells in vitro and in vivo via interfering noncoding RNA. Researchers analyzed the differential expression of long noncoding RNAs (lncRNAs) in GBM using Heliocos single-molecule sequencing. A subset of glioma-specific, BET-regulated lncRNAs, including HOX transcript antisense RNA (HOTAIR), was identified. I-BET151 significantly reduced HOTAIR levels and restored the expression of several other lncRNAs downregulated in GBM. In contrast, the overexpression of HOTAIR in GBM cells treated with I-BET151 abrogated the anti-proliferative effects of the BET inhibitor. In addition, chromatin immuno precipitation analysis demonstrated that BRD4 directly bound to the HOTAIR promoter region, suggesting that BET proteins can directly regulate lncRNA expression.41

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COMBINATION THERAPY INCLUDING BRD4 INHIBITOR FOR GLIOMA

Cancers are often developing resistance to monotherapies. Notably, recent studies have shown that combinatorial therapeutic regimens including BRD4 inhibitors can show striking effects (Table 3).

Combination therapy including BRD4 inhibitor for glioma cells

JQ1-based combination therapies are the most studied. For glioma cell lines, the association of JQ1 with EPZ6438 (EZH2 inhibitor)/panobinostat (histone deacetylase inhibitor)/THZ1 (CDK7 inhibitor)/ICG-001 (CREB-binding protein inhibitor) achieved higher inhibition of tumor growth compared to each inhibitor alone. The mechanism may be related to the activation of caspase-3/7 induced by panobinostat plus JQ1. Notably, the treatment with ICG-001 or JQ1 alone may lead to the activation of a subset of detrimental DIPG super enhancers (SEs), whereas the combinatorial treatment prevented the unintentional activation of these SEs. Indeed, this approach rescued the effect of single treatments on enhancer-driven oncogenes in H3K27M-mutated DIPG; however, it showed little effect in H3 wild-type DIPG cells.

Similar to the previous study, the combination of OTX015 with panobinostat inhibited proliferation and promoted apoptosis in GBM cell lines as well. Studies have indicated that BRD4 inhibitors also improve the glioma response to currently used chemotherapy drugs. For instance, in U87 glioma cells, Berenguer-Daizé et al. showed synergistic anti-tumor effects of OTX015 in association with SN38 (an active metabolite of irinotecan), with TMZ, or with everolimus. In vivo, OTX015 combined with TMZ improved mouse survival better than either single agent in the treatment of glioma. I-BET151 also could augment the effect of TMZ on GBM cells by inducing apoptosis. An increased amount of reactive oxygen species and superoxide anions resulted in caspase-3 activation, possibly associated with decreased superoxide dismutase (SOD) activity and increasing PUMA expression in GBM cells. These findings underscore the relevance of approaches tackling GBM by existing chemotherapy drugs in combination with BET inhibitors.

Gene amplification and/or mutation of the EGFR is found in nearly 60% of gliomas. EGFR variant III (EGFRvIII) signaling promotes interleukin (IL)-6 secretion in GBM cells. The crosstalk between the wild-type receptor and its constitutively active mutant form, EGFRvIII, limits sensitivity to EGFR tyrosine kinase inhibitors (TKIs) through an interclonal communication mechanism mediated by IL-6. IL-6 activates NF-κB via paracrine and/or autocrine pathways, leading to BRD4-dependent expression of the prosurvival protein survivin (BIRC5) and attenuation of sensitivity to EGFR TKIs. Both in vivo and in vitro experiments confirmed...
| No. | Author | Research objects | Mutation of cell line | Treatment regimen | Mechanisms | Related pathways | Genes/targets | Conclusions |
|-----|--------|------------------|---------------------|------------------|------------|----------------|--------------|-------------|
| 1   | Zhang et al. | DIPG cell lines isolated from the dorsal forebrain of mouse embryos and mice | H3K27M mutations | JQ1 + EPZ6438 (EZH2 inhibitor) | proliferation (−) | N/A | p16Ink4a, Igf2bp2, and HOXA10 | (1) in vitro and in vivo, the combination of JQ1 and EPZ6438 exhibited better inhibition of the tumor growth compared to use the inhibitor alone (2) the inhibition was performed by blocking proliferation and promoting cell apoptosis |
| 2   | Nagaraja et al. | SU-DIPG-IV: H3.1-K27M; SU-DIPG-VIII: JHH-DIPG1, SF7761; H3.3-K27M patient-derived DIPG cell lines and mice | H3K27M mutations | JQ1 + panobinostat (HDAC inhibitor) | proliferation (−) | N/A | NTRK3, LINGO1, ASCL1, SYT4, SYT17, MYT1, MYRF, and SALL3 | (1) JQ1 together with panobinostat synergistically inhibited cell proliferation and induced apoptosis the combination of JQ1 and THZ1 had a synergistic inhibitory effect on cell viability |
| 3   | Nagaraja et al. | SU-DIPG-IV: H3.1-K27M; SU-DIPG-VIII: JHH-DIPG1, SF7761; H3.3-K27M patient-derived DIPG cell lines and mice | H3K27M mutations | JQ1 + THZ1 (CDK7 inhibitor) | proliferation (−) | N/A | ETS1, ELF4, MGA, SOX10, and HES5 | combined treatment of JQ1 and ICG-001 induced stronger cytotoxic effects than did either drug alone in H3K27M-mutated DIPG cell lines |
| 4   | Wiese et al. | VUMC-DIPG-10 DIPG cell line and SF188 pedHGG cell lines | H3K27M mutations | JQ1 + ICG-001 (CREB-binding protein inhibitor) | proliferation (−) | N/A | MYC, MYC-associated factor X, JUND | TNF pathway, PI3K/mTOR pathway, insulin receptor pathway, biosynthesis of antibiotics and FoxO signaling pathway caspase-3, caspase-7, CCND1, MKI67, TOP2A, FOXO3, p21, and BNIP3 markedly inhibited cell proliferation and induced apoptosis |
| 5   | Meng et al. | U87 and U251 GBM cell lines | N/A | JQ1 + panobinostat | proliferation (−) | N/A | | (Continued on next page) |
| No. | Author          | Research objects                                      | Mutation of cell line | Treatment regimen  | Mechanisms       | Related pathways | Genes/targets    | Conclusions                                                                                                                                 |
|-----|-----------------|------------------------------------------------------|-----------------------|------------------|------------------|------------------|-----------------|----------------------------------------------------------------------------------------------------------------------------------------------|
| 6   | Lam et al.      | GMB tumor-bearing mice                                | N/A                   | JQ1 + TMZ (loaded by Tf-NPs) | DNA damage (+)  | N/A              | N/A             | (1) TMZ plus JQ1 is additive in gliomas                                                                                                                                                               |
|     |                 |                                                      |                       |                  |                  |                  |                 | (2) treatment of tumor-bearing mice with Tf-NPs loaded with TMZ and the JQ1 leads to increased DNA damage and apoptosis                                                                             |
|     |                 |                                                      |                       |                  | apoptosis (+)    |                  | N/A             | (3) Tf-NPs loaded with TMZ and JQ1 show a decrease of 1.5- to 2-fold in tumor burden and an increase in survival compared to equivalent free-drug dosing                                                  |
| 7   | Wang et al.     | CSC2078, CSC1534, and CSC1589 murine GSCs and mice    | N/A                   | JQ1 + RGFP966 (HDAC inhibitor) | proliferation (−) | GLI1/IL-6/STAT3 pathway | c-Myc, cyclin D1, Bcl-2, Bcl-xL, p21, Bim, and Bax | (1) JQ1/RGFP966 combination can suppress GSC growth by blocking the GLI1/IL-6/STAT3 signaling axis in vitro                                                                 |
|     |                 |                                                      |                       |                  |                  |                  |                 | (2) In vivo, the JQ1/RGFP966 combination caused stronger tumor growth suppression than did either drug alone                                                                                           |
| 8   | Meng et al.     | U87 and U251 GBM cell lines                           | N/A                   | OTX015 + panobinostat | proliferation (−) | N/A              | CCND1, MKI67, TOP2A, FOXO3, p21, and BNIP3 | markedly inhibited cell proliferation and induced apoptosis                                                                                                                                            |

(Continued on next page)
| No. | Author | Research objects | Mutation of cell line | Treatment regimen | Mechanisms | Related pathways | Genes/targets | Conclusions |
|-----|--------|------------------|----------------------|------------------|------------|-----------------|---------------|-------------|
| 9   | Berenguer-Daizé et al. | U87MG, T98G, and U118 cell lines and mice | N/A | OTX015 + SN38/TMZ/everolimus (cell lines) | G2/M cell cycle arrest (+) | transcriptional regulatory network involving CEBPB, RUNX1, FOSL2, and STAT3 | genes with E2F binding, RNA Pol II, H3K27ac, H3K4me3, H3K27me3, and H3K9me3 | (1) showed synergistic antitumor effect (2) improved survival of mice by suppressing tumorigenic ability over either single agent |
| 10  | Ishida et al. | NCH644, NCH690, and NCH421K stem-like GBM cells | N/A | OTX015 + imipridones (AKT/ERK inhibitor) | proliferation (−) | mTORC1 pathway | c-Myc, Bcl-2, Bcl-xL, Mcl-1, mTOR, 4EBP1, S6K, S6 | Induced apoptosis in stem-like GBM cells |
| 11  | Zanca et al. | U87, mAstr-Ink4a/Arf⁻/⁻, U178, and U373 GBM cell lines and mice | EGFRvIII mutations | JQ1/ I-BET-151/ I-BET-762 + EGFR TKIs | proliferation (−) | NF-κB pathway | NF-κB and survivin | IL-6 mediates resistance to EGFR TKIs in GBM and BRD4 inhibitors restores sensitivity of GBM cells to EGFR TKIs (1) I-BET151 could augment the effect of TMZ on GBM cells (2) I-BET151 increased the TMZ-induced apoptosis in GBM cells by enhancing the activities of caspase-3 |
| 12  | Yao et al. | U251 and U87 GBM cell lines and mice | N/A | I-BET151 + TMZ | apoptosis (+) | N/A | PUMA | I-BET151 increased the amount of reactive oxygen species, superoxide anions with a decrease of activity of SOD, and the anti-oxidative properties of GBM cells; I-BET151 also induced increased PUMA expression | pedHGG, pediatric high-grade glioma, HDAC: histone deacetylase; Tf-NP, transferrin-functionalized nanoparticle. |
that the treatment with BRD4 inhibitors (JQ1, I-BET-151, and I-BET-762) increases tumor cell sensitivity to EGFR TKIs. Thus, BRD4 inhibitors can be combined with EGFR TKIs for the treatment of GBM.

Delivery systems (e.g., nanoparticles) help to improve drug passing across the BBB. Transferrin-functionalized nanoparticles (Tf-NPs) were found capable of crossing the BBB in mice and delivering drugs to GBM tumor tissue. Tf-NPs loaded with TMZ and JQ1 reduced GBM tumor burden and survival rate in mice by 1.5- to 2-fold compared with the same dose of free drugs. Additionally, GBM mice treated with Tf-NPs loaded with drugs show reduced systemic drug toxicity. Thus, nanoscale platforms for delivering combination therapies (e.g., JQ1 or OTX015 + TMZ) have an emerging role in the treatment of gliomas.

Combination therapy including BRD4 inhibitor for GSCs

In recent years, the combination of BRD4 inhibitors and other innovative drugs has been also investigated in GSCs. Wang et al. found that a combination of JQ1 and RGFP966 (HDAC3 inhibitor) could suppress GSC growth by blocking the GLI1/IL-6/STAT3 signaling axis. In fact, HDAC3 inhibition upregulated the acetylation of H3K27, which allowed the recruitment of BRD4 to the GLI1 gene promoter to induce its expression. JQ1, instead, inhibited the transcription of GLI1 gene, thereby blocking the GLI1/IL-6/STAT3 pathway. In vivo, a combination of JQ1 and RGFP966 showed a stronger inhibitory effect on GSC growth than either single drug.

Imipridones are a novel class of AKT/extracellular signal-regulated kinase (ERK) inhibitors that display limited therapeutic efficacy against GBMs. The expression of c-Myc can predict the response to imipramine. Since BRD4 is known to regulate the expression of c-Myc to control cancer cell proliferation, the effect of a BRD4 inhibitor combined with imipramine in the treatment of GBM was further explored. Ishida et al. discovered that the inhibition of c-Myc by OTX015 sensitizes stem-like GBM cells to imipridone-induced apoptosis in vitro and in vivo.

In general, the combination of BRD4 inhibitors can improve the glioma responsiveness not only to current chemotherapy drugs but also to innovative drugs, by impairing cell proliferation and/or promoting apoptosis, through signaling pathways such as STAT3, NF-κB, and mTOR (Figure 4). BRD4 inhibitors showed adverse effects such as thrombocytopenia, fatigue, gastrointestinal symptoms, and hyperbilirubinemia in the clinical trial. However, there have been few clinical studies using a BRD4 inhibitor for the treatment of glioma or other brain disease; hence, the side effects of BRD4 inhibitors in humans are still largely unknown. Clinical trials with BRD4 inhibitors in combination therapies for glioma are warranted. Moreover, the
application of new drug carriers should be assessed in order to help these molecules to pass across the BBB.

CONCLUSIONS AND FUTURE PERSPECTIVES

Much evidence surveyed in this study shows that BRD4 is a transcriptional and epigenetic regulator that plays a pivotal role in glioma development. BRD4 inhibitors (or the knockdown of BRD4 expression) significantly inhibit the malignant behavior of glioma cells or GSCs, resulting in effective therapeutic targeting of these tumors. Thus, BRD4 inhibitors can significantly delay or even reverse the malignant progression of gliomas. When combined with other innovative drugs, BRD4 inhibitors can induce cell cycle arrest, inhibit oncogenic pathways, activate tumor suppressor genes, and interfere with the transcriptional regulatory activity of BET and E2F1. In particular, the therapy with BRD4 inhibitors, alone or in combination, has an inhibitory effect on glioma cells or GSCs, resulting in effective therapeutic targeting of these tumors.

A number of important issues still need to be addressed. First, the molecular mechanisms of action of BRD4 inhibitors need to be further studied. Second, clinical trials applying BRD4 inhibitors in the treatment of glioma need to be carried out to assess their efficacy and side effects in humans. Third, some inhibitors, such as JQ1, can inhibit BRD4, BRD3, and BRD2 at the same time; notably, whether BRD2 or BRD3 plays any role in glioma also needs to be clarified. Finally, the application of delivery systems should be assessed in order to further enhance the transit of BRD4 inhibitors across the BBB. In fact, since BRD4 inhibitors and TMZ achieve similar therapeutic effects on glioma cells in vitro, it is exciting to speculate that the treatment with BRD4 inhibitors in association with delivery systems could represent an amenable alternative for the therapy of TMZ-resistant glioma patients.

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AUTHOR CONTRIBUTIONS

H. You conceived the hypothesis and revised the manuscript. H. Yang, L. Wei, Y. Xun, and A.P. Yang did the literature search. H. Yang and L. Wei wrote the manuscript. H. Yang and H. You prepared the tables and figures.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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