Molecular Cancer

REVIEW

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Glioblastoma multiforme: insights into pathogenesis, key signaling pathways, and therapeutic strategies

Ashkan Pouyan[1], Masoud Ghorbanlo[2], Masoud Eslami[3], Majid Jahanshahi[4], Ehsan Ziaei[5], Ali Salami[6], Khatere Mokhtari[7], Koorosh Shahpasand[8,9], Najma Farahani[8], Tohid Emami Meybodi[10,11*], Maliheh Entezari[8], Afshin Taheriazam[8,12*], Kiavash Hushmandi[8,13*] and Mehrdad Hashemi[8*]

Abstract

Glioblastoma multiforme (GBM) is the most prevalent and aggressive primary brain tumor in adults, characterized by a poor prognosis and significant resistance to existing treatments. Despite progress in therapeutic strategies, the median overall survival remains approximately 15 months. A hallmark of GBM is its intricate molecular profile, driven by disruptions in multiple signaling pathways, including PI3K/AKT/mTOR, Wnt, NF-κB, and TGF-β, critical to tumor growth, invasion, and treatment resistance. This review examines the epidemiology, molecular mechanisms, and therapeutic prospects of targeting these pathways in GBM, highlighting recent insights into pathway interactions and discovering new therapeutic targets to improve patient outcomes.

Keywords Glioblastoma multiforme, Signaling pathways, Therapeutic resistance, Molecular mechanisms, Targeted therapy

*Correspondence:
Tohid Emami Meybodi
tohid.emami@sbmu.ac.ir
Afshin Taheriazam
a.taheriazam@iautmu.ac.ir
Kiavash Hushmandi
houshmandi.kia7@ut.ac.ir
Mehrdad Hashemi
mhashemi@iautmu.ac.ir
[1] Department of Neurosurgery, Faculty of Medicine, Zahedan University of Medical Sciences, Zahedan, Iran
[2] Department of Anesthesiology, School of Medicine, Iran University of Medical Sciences, Tehran, Iran
[3] Department of Neurosurgery, Kerman University of Medical Sciences, Kerman, Iran
[4] Department of Neurosurgery, School of Medicine, Iran University of Medical Sciences, Tehran, Iran
[5] Department of Neurosurgery, Faculty of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran
[6] Department of Neurosurgery, Faculty of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran
[7] Department of Cell and Molecular Biology and Microbiology, Faculty of Biological Science and Technology, University of Isfahan, Isfahan, Iran
[8] Farhikhtegan Medical Convergence Sciences Research Center, Farhikhtegan Hospital Tehran Medical Sciences, Islamic Azad University, Tehran, Iran
[9] Department of Laboratory Medicine and Pathology, Institute for Translational Neuroscience, University of Minnesota Medical School, Minneapolis, MN, USA
[10] Neuroscience Research Center, Iran University of Medical Sciences, Tehran, Iran
[11] Functional Neurosurgery Research Center, Shohada Tajrish Hospital, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[12] Department of Orthopedics, Faculty of Medicine, Tehran Medical Sciences, Islamic Azad University, Tehran, Iran
[13] Department of Epidemiology, University of Tehran, Tehran, Iran

Introduction to GBM

Glioblastoma multiforme (GBM) represents the most prevalent form of aggressive brain cancer in adults. It is characterized by rapid growth and invasiveness, often leading to poor prognosis despite aggressive treatment [1–3]. Over the past three decades, the survival rates for individuals diagnosed with GBM have experienced only limited improvement [4].

Astrocytomas with IDH mutations (mut), categorized as grade 2, 3, or 4, are now considered distinct entities [5]. Histopathologically, GBM is marked by diffuse neoplastic infiltration into nervous tissue, with a necrotic core and cells exhibiting astroglial-like characteristics, such as angular nuclei and euchromatin [6]. The blood–brain barrier (BBB) structure, coupled with the high heterogeneity of GBM, provides a survival advantage to tumor cells [7, 8].

Despite multimodal treatment approaches, GBM generally takes an aggressive course. Recurrence happens in 75–90% of cases, typically within 2–3 cm of the original lesion's margins, and multiple lesions are seen in about 5% of cases after treatment [2]. Historically, the management of GBM has focused on achieving the most extensive possible surgical resection, followed by adjuvant radiation therapy (RT), or primary RT for tumors deemed inoperable. Over the last two decades, the introduction of temozolomide (TMZ) as a chemotherapeutic agent, along with the application of a non-invasive tumor-treating field device, has shown clinical efficacy, contributing to significant improvements in patient outcomes. These advancements, however, remain insufficient to overcome the aggressive nature of GBM, and further innovations in treatment strategies are necessary to enhance survival and quality of life for patients [9–11]. Other treatment options that have demonstrated therapeutic activity include bevacizumab, lomustine, carmustine, and the PCV regimen, which combines procarbazine, lomustine, and vincristine [12–15].

As per the 2020 NCCN Guidelines, the recommended standard treatment for GBM in patients aged 70 years or younger with a favorable performance status involves a comprehensive approach. This includes brain RT combined with TMZ during both the concurrent and adjuvant phases. The use of alternating electric field therapy (tumor-treating fields) is also recommended as part of the regimen. Importantly, these guidelines apply regardless of the methylation status of the MGMT promoter, emphasizing the broad applicability of this treatment strategy for the specified patient group [10, 11, 13, 16]. MGMT is a key DNA repair enzyme that plays a vital role in preserving genomic integrity by repairing DNA damage caused by alkylating agents. Specifically, it removes alkyl groups from the O-6 position of guanine bases, a critical site of damage caused by various chemotherapeutic agents, including TMZ. This repair function serves as a major resistance mechanism, as high MGMT expression or unmethylation of the MGMT promoter enables tumor cells to repair the DNA damage induced by alkylating agents, thereby reducing the effectiveness of chemotherapy. In contrast, low MGMT expression, often resulting from MGMT promoter methylation, is linked to better chemotherapy response, as the DNA damage remains unrepaired, leading to tumor cell death [13, 17]. MGMT promoter methylation is a crucial biomarker in high-grade gliomas, as it is linked to improved survival outcomes in patients treated with alkylating chemotherapeutic agents like TMZ or lomustine. This epigenetic modification results in reduced MGMT expression, impairing the tumor's ability to repair DNA damage caused by these agents. Consequently, the tumors become more vulnerable to the cytotoxic effects of alkylating chemotherapy, leading to better treatment responses and improved survival. Notably, MGMT promoter methylation remains a strong predictor of chemotherapy efficacy, even in older adult populations, who might otherwise experience poorer treatment outcomes due to age-related decline in performance status or comorbidities [18–22]. Tumors with H3K27 mutations are significantly less likely to exhibit MGMT promoter methylation and are associated with a poorer prognosis [23, 24]. In contrast, tumors with MGMT promoter methylation generally respond better to alkylating chemotherapies like TMZ, which is linked to a more favorable prognosis. Although the H3F3A G34 mutation, often considered a distinct genetic alteration in GBMs, is present in some tumors, these tumors do not exhibit a worse prognosis compared to other IDH-wildtype GBMs. This suggests that the beneficial effect of MGMT promoter methylation in terms of chemotherapy response may offset the negative prognostic implications typically associated with the H3F3A G34 mutation [13, 24, 25].

MGMT promoter methylation in GBM serves as both a prognostic and predictive biomarker, helping to evaluate the likelihood of treatment response to alkylating chemotherapy. This is especially important in GBM treatment regimens that include RT and alkylating agents such as TMZ and lomustine. In the small Phase II UKT-03 trial, which involved 31 patients with newly diagnosed GBM, the combination of postoperative RT, TMZ, and lomustine resulted in a promising median overall survival of 34.3 months. This outcome highlights the potential benefits of this treatment approach, particularly in patients with MGMT promoter methylation, who are more likely to respond positively to alkylating agents. The term "OS" refers to overall survival, a common metric in clinical research and oncology used to measure the duration

from diagnosis or the start of treatment until death from any cause. This median OS significantly surpassed the 23.4-month median OS reported for MGMT promoter-methylated GBM patients treated with RT and TMZ in the EORTC-NCIC trial [26]. Building on the survival benefits observed with combination alkylating agents in MGMT promoter-methylated GBM, the Phase III CeTeG/NOA-09 trial further explored the effectiveness of combining lomustine with TMZ and RT in patients with newly diagnosed MGMT promoter-methylated GBM. This study randomized patients aged 18–70 with a Karnofsky Performance Status (KPS) of 70 or higher to receive one of two treatment regimens: RT combined with lomustine and TMZ, or RT with TMZ alone. The trial aimed to determine whether adding lomustine to the standard TMZ and RT regimen could offer a survival benefit compared to the current standard of care [13, 27, 28].

GBMs primarily affect the cerebral hemispheres in adults, while in children, they are less common and typically occur in the brainstem. These tumors are categorized into primary and secondary types, with approximately 90% of cases being primary, which develop de novo, especially in older patients. In contrast, secondary GBMs arise from lower-grade astrocytomas and are more commonly observed in younger individuals. The genetic characteristics of primary and secondary GBMs differ significantly, as IDH1 mutations are frequently found in secondary GBMs but are absent in primary tumors [29]. Common genetic alterations in GBM include loss of heterozygosity (LOH) on the 10q chromosome arm, which is present in 60–90% of cases [30, 31]. Other genetic alterations and deletions affecting the p53 gene can be found in approximately 85.3–87% of GBM cases [32]. Alterations in the p53 gene are more commonly seen in secondary GBMs than in primary GBMs. Secondary GBMs, which typically develop from lower-grade astrocytomas, often contain mutations in the TP53 gene, a tumor suppressor that regulates the cell cycle and apoptosis in response to DNA damage. These p53 mutations enable the tumor to bypass normal cell cycle checkpoints, promoting uncontrolled cell growth. In contrast, primary GBMs, which arise de novo and are typically IDH-wildtype, usually exhibit alterations in other genes, such as EGFR amplification or PTEN loss, rather than mutations in TP53 [29]. Mutations in EGFR and PDGFR play a critical role in the development of GBM. EGFR, a receptor tyrosine kinase (TK) that regulates vital cellular functions such as proliferation, differentiation, and survival, is mutated in approximately 40–57% of GBM cases. These mutations often result in constitutive activation of the receptor, leading to enhanced signaling that promotes tumor growth and resistance to apoptosis. Similarly, PDGFR, a cell surface TK receptor involved in cell growth, migration, and angiogenesis, is altered in about 60% of GBM cases. Changes in PDGFR contribute to abnormal signaling pathways that drive tumor progression, including angiogenesis and increased cell proliferation. These mutations in EGFR and PDGFR are commonly targeted in therapies aimed at inhibiting the aberrant signaling in GBM. However, resistance to targeted therapies remains a challenge due to the tumors' complexity and heterogeneity [32–35]. Additional mutations occur in the MDM2 gene in 10–15% of GBM cases, as well as in the PTEN gene, which is mutated in 20–34% of cases [36–38]. Genomic studies carried out by the Cancer Genome Atlas Research Network have uncovered further alterations in key signaling pathways that are closely associated with the development and progression of GBM. These findings provide valuable insights into the molecular mechanisms underlying GBM and highlight potential therapeutic targets that could lead to more effective treatment strategies for this challenging malignancy [32, 33]. It is important to recognize that mutations in GBM may not solely affect a single cellular pathway; rather, they can arise from alterations across multiple pathways, as previously discussed [39]. Figure 1 depicts three key mechanisms of GBM invasion and emphasizes the roles of neurons (blue) and astrocytes (yellow) in each stage. (A) Vessel Co-option: In the initial phase, GBM cells infiltrate the brain and co-opt pre-existing blood vessels to support tumor growth without initiating angiogenesis. During this phase, astrocytes (yellow) closely interact with GBM cells, offering structural and metabolic support via their connections to blood vessels. Neurons (blue) are largely unaffected, but their proximity to the co-opted vessels puts them at risk of disrupted signaling and nutrient deprivation due to the tumor's metabolic demands; (B) Vessel Invasion: As the tumor advances, GBM cells invade the blood vessel walls, compromising vascular integrity. Astrocytes play a dual role in this phase by aiding GBM invasion by releasing pro-invasive factors and attempting to preserve vascular stability. Neurons, however, start experiencing functional impairments as the invasion disrupts their blood supply, causing localized hypoxia and excitotoxic damage; (C) BBB Breakdown: In the advanced stage, GBM induces a breakdown of the BBB, increasing vascular permeability and allowing harmful substances and immune cells to infiltrate the brain. Astrocytes, which typically maintain BBB integrity, become overwhelmed and lose their protective function. This disruption accelerates GBM progression by facilitating nutrient and oxygen supply to the tumor and helping it evade immune responses. Neurons, now exposed to a compromised environment, suffer further damage, including oxidative stress and impaired

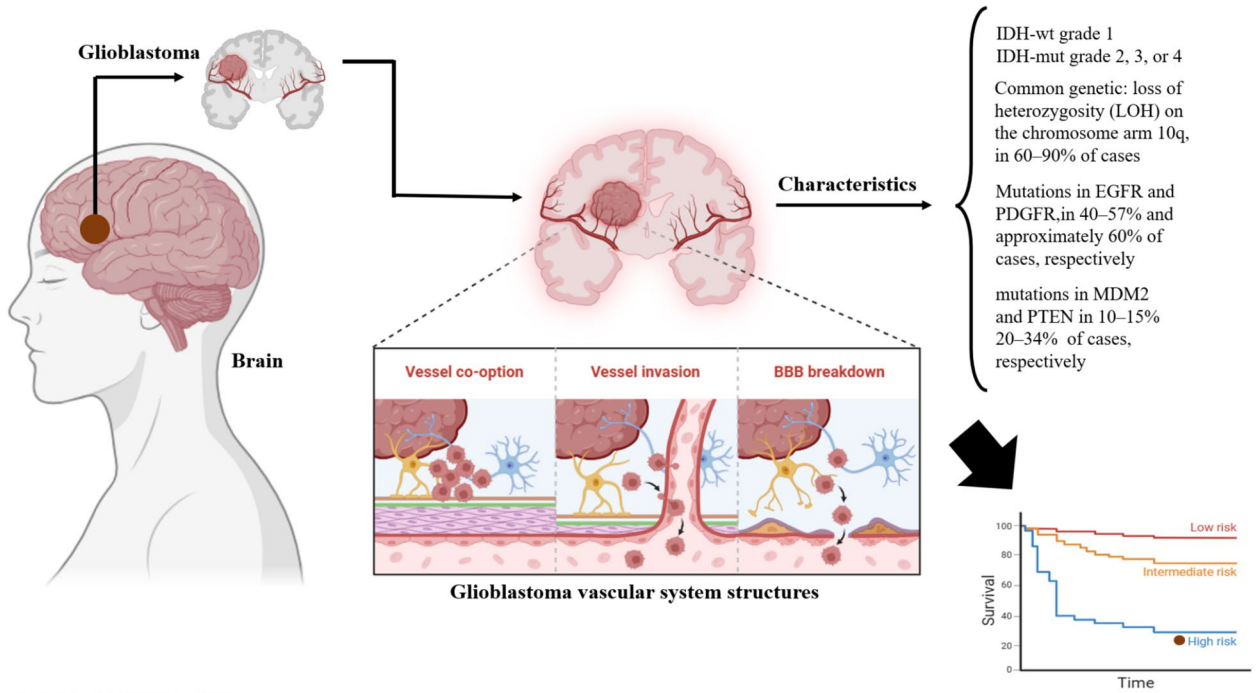

Fig. 1 An overview of GBM progression. Key mechanisms include vessel co-option, where tumor cells utilize pre-existing vessels without inducing angiogenesis, and vessel invasion, which compromises vascular integrity and leads to neuronal hypoxia. In advanced stages, the breakdown of the BBB increases vascular permeability, facilitating tumor growth. Genetic alterations are crucial in GBM, with EGFR and PDGFR mutations promoting proliferation and angiogenesis, while IDH1 status affects prognosis. Common genetic changes, such as LOH on chromosome 10q, and mutations in PTEN, MDM2, and TERT, disrupt tumor suppression and enhance malignancy. Understanding these mechanisms is essential for the development of targeted therapies

synaptic function, contributing to the neurological deficits frequently seen in GBM patients [40]. Understanding these molecular and vascular mechanisms is essential for developing innovative therapeutic strategies that target both the tumor's microenvironment and its genetic drivers. In a survival curve with three distinct lines representing high-risk, medium-risk, and low-risk categories, GBM would most likely fall into the high-risk group. Due to its aggressive nature, poor prognosis, and rapid progression, GBM generally exhibits significantly lower survival rates compared to other central nervous system tumors. This categorization is influenced by its genetic and molecular profile (Fig. 1).

Epidemiology

Epidemiological data from the Central Brain Tumor Registry of the United States reveal a complex pattern in brain tumor incidence. While the overall incidence of brain tumors is significant, malignant tumors make up a smaller proportion but are associated with a higher mortality rate. Gliomas, especially GBMs, are the most common malignant brain tumors, while meningiomas are the most frequently diagnosed non-malignant tumors. This distinction underscores the importance of ongoing research and the development of enhanced management strategies to address the public health challenges posed by brain tumors [5, 41–51]. GBM accounts for about 15% of all brain tumors and primarily affects adults between the ages of 45 and 70 [52]. Results from pivotal clinical trials demonstrated that the median survival for patients treated with a combination of radiotherapy and TMZ was 14.6 months, in contrast to 12.1 months for those undergoing radiotherapy alone. These findings underscore the potential benefit of adding TMZ to standard radiotherapy in improving survival outcomes for patients with GBM [10].

The 5-year overall survival rate for patients receiving radiotherapy combined with TMZ was 9.8%, compared to just 1.9% for those undergoing radiotherapy alone [26]. Currently, the only established risk factor for GBM is exposure to high doses of ionizing radiation [53]. It is important to note that individuals with asthma and other allergic conditions have a reduced risk of developing GBM. Furthermore, certain genotypes associated with an increased risk of asthma are also linked to a lower risk of GBM [4]. Several studies have indicated an inverse relationship between the use of NSAIDs and the incidence of GBM [54–56]. A study conducted in 2020, along with

other research, found that valganciclovir significantly improved outcomes in patients with newly diagnosed GBM [57–59]. While these findings are promising, they need validation through larger, randomized studies in the future (Fig. 2). The latest WHO classification underscores the increasing importance of GBM genotyping. The broader use of molecular profiling, combined with advances in machine learning techniques, improves the accuracy of prognosis prediction and the assessment of responses to targeted therapies [60]. The discovery of novel mutations in GBM opens new possibilities for developing targeted therapies and improves the ability to link specific mutations to distinct clinical outcomes. This enables more accurate diagnosis and better prognostication of disease severity. In 2017, the cIMPACT-NOW (Consortium to Inform Molecular and Practical Approaches to CNS Tumor Taxonomy) initiative was launched to assess and recommend updates to the WHO classification of brain tumors. The consortium's goal is to refine the molecular classification of brain tumors, ensuring that the latest genetic and molecular insights are incorporated into diagnostic criteria, thereby facilitating more personalized treatment strategies and improved patient management [46].

The 2021 WHO criteria and nomenclature place a stronger emphasis on the critical role of molecular genetics in diagnosing GBM. Tumors with IDH mutations, which were previously classified as diffuse astrocytoma, anaplastic astrocytoma, or GBM, are now categorized as IDH-mutant astrocytomas, with grades II, III, or IV. This reclassification represents a shift in the diagnostic approach, where the grading of IDH-mutant diffuse astrocytic tumors is based not only on histological features but also on molecular markers. A key marker is the homozygous deletion of CDKN2A/B, which automatically assigns the tumor a CNS WHO grade IV, regardless of the presence of microvascular proliferation or necrosis. This update improves the accuracy of glioma grading and enables more precise prognostic predictions and personalized treatment strategies [5].

The current classification of GBM, IDH-wildtype, now includes key molecular markers such as TERT promoter mutations, EGFR gene amplification, and the combined chromosomal alteration of +7/−10 (gain of chromosome 7 and loss of chromosome 10) as diagnostic criteria. These molecular features are vital for differentiating GBM, IDH-wildtype, from other types of gliomas. Specifically, GBM, IDH-wildtype is diagnosed in adult patients with IDH-wildtype diffuse astrocytic gliomas when one or more of the following characteristics are present: microvascular proliferation, necrosis, TERT promoter mutation, EGFR gene amplification, or the chromosomal alteration +7/−10. This updated molecular framework enhances the accuracy of GBM diagnosis by reflecting the tumor's genetic alterations, which is crucial for selecting appropriate, individualized treatment strategies. This approach highlights the shift towards precision medicine in GBM, enabling clinicians to tailor therapies based on the tumor's molecular profile [5].

Signaling pathways in GBM

In GBM, changes and/or increased activity in crucial signaling pathways such as Wnt, TGF-β, VEGF, EGFR, CDKN2A, NF-κB, and the PI3K/AKT/mTOR pathway are believed to play a role in the disease's pathogenesis and contribute to the tumor's aggressive behavior (Figs. 3 and 4, Table 1).

Genetic and epigenetic alterations in GBM

It is crucial to recognize that human malignant gliomas, including glioblastomas, typically do not rely on a single oncogene or tumor suppressor gene for their initiation and progression. This inherent complexity may account for the limited success of therapies targeting only one specific molecular alteration in clinical trials, as the multifaceted nature of these tumors requires more comprehensive therapeutic strategies [7, 9, 76].

Epigenetic modifications play a pivotal role in the development of GBM, with approximately 50% of newly diagnosed cases showing methylation of the MGMT promoter. This methylation event is significant because it influences the tumor's response to treatments, particularly chemotherapy, by silencing the MGMT gene, which is involved in DNA repair. The MGMT gene encodes a DNA repair enzyme that counteracts the effects of alkylating agents, like TMZ, by removing alkyl groups from guanine residues in DNA [77].

GBM may show co-deletion of chromosomes 1p and 19q, which is associated with increased chemosensitivity and a better prognosis in oligodendroglioma. This highlights its potential impact on tumor behavior and treatment response [78]. As a result, it has been proposed that GBMs with an oligodendroglioma component and 1p/19q co-deletion may also have prognostic significance. However, this hypothesis has not yet been fully confirmed through extensive studies [79]. In one study, the frequency of 1p/19q co-deletion in GBM was found to be 3%. However, neither this co-deletion nor isolated mutations showed any correlation with improved survival, suggesting that they do not have prognostic value in this context [80]. The TP53 gene, known for encoding the tumor suppressor protein p53, is one of the most commonly mutated genes across various cancers. In GBM, TP53 mutations are the second most frequent, occurring in approximately 28.3% of cases, following PTEN, which is mutated in around 30.7% of GBM patients. These

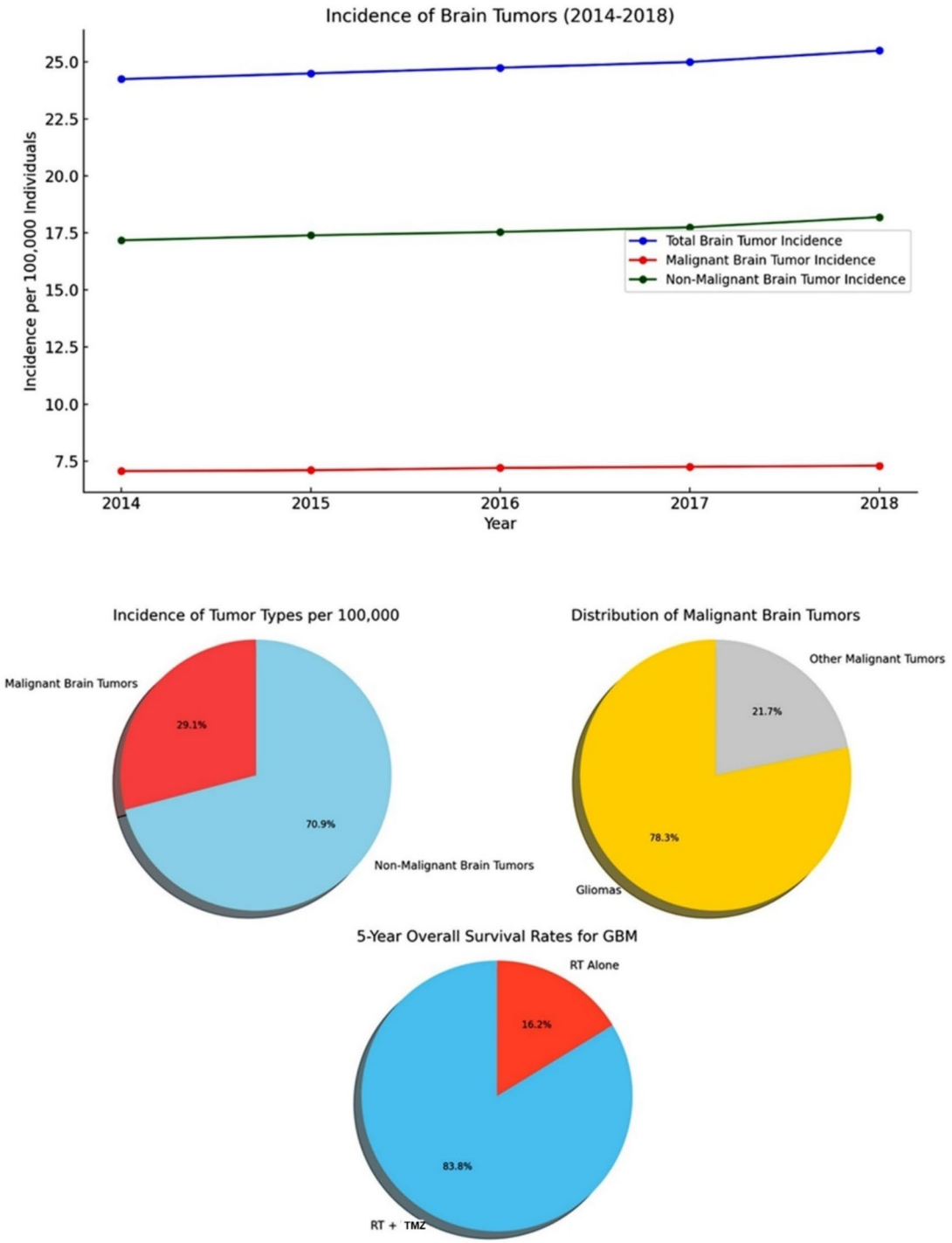

Fig. 2 Epidemiology of GBM. This figure offers an overview of brain and CNS tumor distribution from 2014 to 2018, categorized by tumor behavior (benign or malignant) and histological type. The upper section presents the overall distribution of tumor behaviors, while the lower section focuses on malignant tumors, particularly gliomas. Additionally, the figure includes a pie chart comparing the five-year survival rates of GBM patients treated with RT alone versus those treated with a combination of radiation therapy (RT) and temozolomide (TMZ), highlighting the significant improvement in survival with the combination therapy

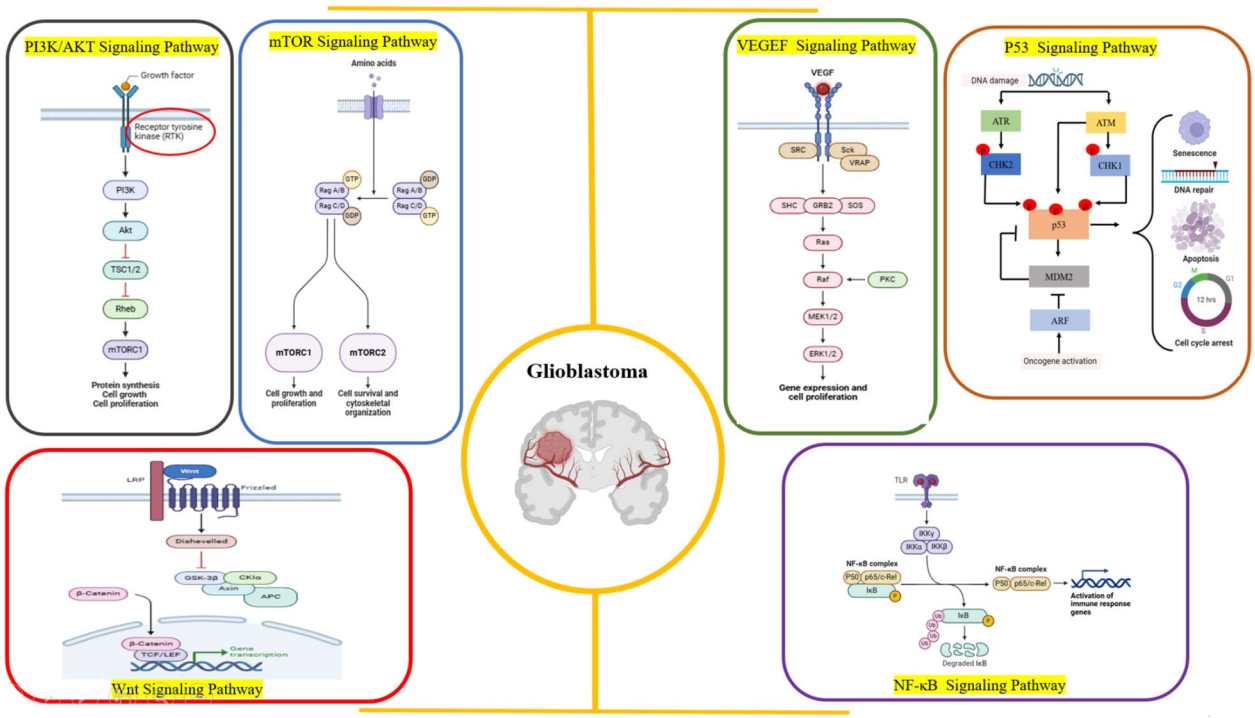

Fig. 3 Signaling pathways in GBM. Selected signaling pathways, such as VEGF, Wnt, NF-κB, mTOR, PI3K/AKT, and P53, are highlighted as examples of pathways studied in GBM

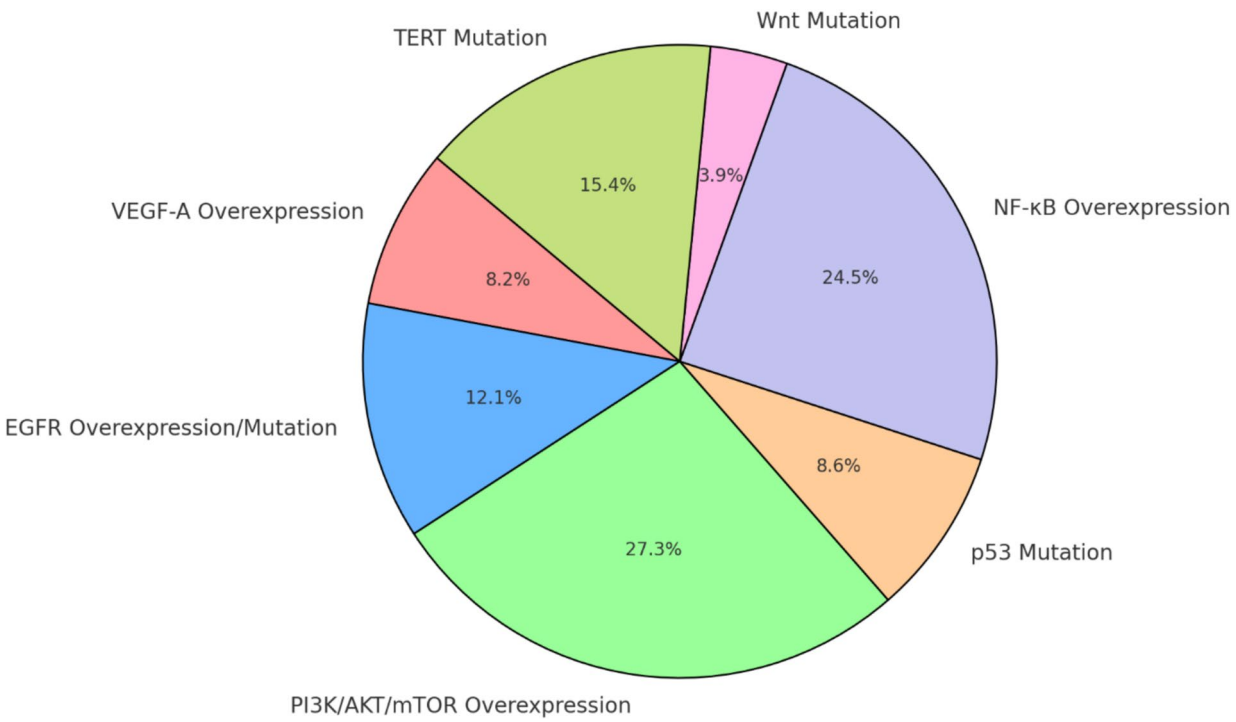

Fig. 4 Pie chart illustrates the prevalence of various molecular mechanisms in GBM

Table 1 Current and Potential Treatment Strategies

Molecular mechanism	Current and possible treatment strategies	(Refs.)
VEGF-A	Bevacizumab is a humanized monoclonal IgG1 antibody targeting VEGF-A	[61, 62]
EGFR	Therapeutic strategies targeting EGFR or its constitutively active mutant form, VEGFR, include a variety of approaches such as TK inhibitors, monoclonal antibodies, vaccines, and RNA-based therapies	[63–65]
PI3K/AKT/mTOR	BKM120 and PX-866 are inhibitors targeting PI3K, while perifosine specifically inhibits Akt. Rapamycin and its derivatives, including everolimus, temsirolimus, and ridaforolimus, act as mTORC1 inhibitors	[66]
p53	Nutlin analogs, such as MI77301, RG7388, RG7112, CGM097, AMG232, andMK8242 function as inhibitors of the MDM2/p53 interaction. Additionally, PRIMA-1 reactivates p53 functionality by altering the folding of mutant p53 proteins to restore the wild-type conformation	[67, 68]
NF-κB	NF-κB inhibitors include parthenolide, CBL0137, and BAY 11–7082, along with amentoflavone, which also exhibits inhibitory effects on the NF-κB pathway	[69–71]
Wnt	Although many molecularly targeted drugs have progressed to early-phase clinical trials, none have yet reached the market for widespread use	[72, 73]
TERT	A study showed that in IDH-wildtype patients, the presence of a pTERT mutation could serve as a predictor for those likely to benefit from adjuvant chemotherapy or radiotherapy, leading to improved survival	[74, 75]

mutations in both genes contribute significantly to the aggressive nature and poor prognosis of GBM [67]. Consistent with its essential role in preventing oncogenesis, mutations that impair the function of wild-type p53 are frequently observed in human malignant tumors [81]. Data from the TCGA project showed that about 85% of GBM cases involve disruption of the p53 signaling pathway, which includes key components such as CDKN2A, MDM2, and TP53 [33].

GBM tumors contain a variety of immunoregulatory cell populations, including ontogenetically distinct macrophages, such as Sall1 + tumor-associated microglia and Sall1-negative monocyte-derived macrophages. These tumors also exhibit immunosuppressive T regulatory cells expressing CCR8 and dysfunctional T-cell subsets, which are characterized by increased levels of inhibitory molecules like CTLA-4 and PD-1 [82]. Computational analyses have classified GBM tumors into three distinct subgroups based on immune response features, as outlined in Table 2.

Therapeutic targets of pathways

Genomic studies of GBM have unveiled several critical signaling pathways and genetic mutations that drive the tumor's progression. These include pathways responsible for regulating cell cycle checkpoints and apoptosis, as well as signaling pathways like TGF-β, NF-κB, and Notch. Additionally, key pathways involved in growth factor signaling and RAS activation, such as the PI3K/AKT/mTOR pathway, EGFR, PTEN/AKT signaling, and the CDKN2A pathway, play a pivotal role in the pathogenesis of GBM. These alterations collectively contribute to the aggressive nature and therapeutic resistance of GBM [84, 85].

The presence of multiple dysregulated signaling pathways in GBM underscores the idea that tumors rely on the disruption of various molecular targets, which collectively influence tumor biology. This complexity suggests that GBM progression is driven by the alteration of numerous interconnected pathways, making it challenging to target a single molecular alteration for effective treatment. EGFR is critical in regulating various cellular functions, such as cell division, migration, adhesion, differentiation, and apoptosis. Upon ligand binding and activation, EGFR triggers several downstream signaling pathways, particularly the PI3K-AKT-mTOR pathway. These pathways are key cancer cell proliferation and survival drivers, promoting tumor growth. Additionally, the

Table 2 Immune response-related subgroups in GBM

Subgroup	Characteristics	Cell Types and Features	Ref.s
Negative	Low immune cell presence	Enrichment of TCGA-proneural cells	[8, 83]
		Amplification of CDK4-MARCH9	
Humoral	High presence of B-cells and CD4 + T-cells	Enrichment in TCGA-mesenchymal cells	
Cellular-like	Elevated activity related to "negative regulation of T-cell activation"	Cluster of gamma delta T-cells	
		Enrichment of TCGA-classical cells	
		Samples with high macrophage content	

activation of these pathways increases resistance to various therapeutic approaches, including chemotherapy and targeted therapies, making treatment outcomes more challenging in cancers with EGFR dysregulation [63].

EGFR amplification and/or protein-level overexpression are frequently observed in 35–45% of GBM cases [86]. The amplification of the active mutant EGFRvIII, which is defined by an in-frame deletion of exons 2–7, is a key characteristic of GBM and is found in roughly 50% of cases. This alteration plays a significant role in driving the aggressive nature of the tumor by activating downstream signaling pathways involved in cell proliferation, survival, and invasion [64]. Despite the pivotal roles of EGFR and its variant EGFRvIII in GBM pathogenesis, clinical trials using EGFR TK inhibitors and antibodies have shown limited therapeutic success [87].

The overexpression of EGFR and EGFRvIII has been associated with increased proliferation and migration of GBM cells, contributing to the tumor's aggressive nature [88]. Additionally, EGFRvIII expression has been demonstrated to promote and expedite angiogenesis in preclinical in vivo GBM models [89]. Several potential therapies targeting EGFR or EGFRvIII are currently under development or undergoing clinical trials [87].

Due to the limitations of EGFR-targeted therapies, preclinical studies investigating EGFR biology in GBM have predominantly utilized models where EGFR and/or EGFRvIII are ectopically overexpressed in non-amplified GBM cell lines. These models allow for the subsequent targeted inhibition of these overexpressed proteins to evaluate the therapeutic potential and efficacy of EGFR inhibitors in overcoming resistance and improving treatment outcomes in GBM [90]. Another significant challenge is the heterogeneous distribution of EGFR within tumors, which leads to variable expression levels and differential treatment sensitivity, ultimately contributing to therapeutic failure [91] (Table 3).

Regorafenib acts on multiple receptor TKs, including KIT, RET, RAF-1, BRAF, and the mutant BRAF V600E. Additionally, it inhibits PDGFR and FGFR, both critical components in the tumor microenvironment (TME) and drivers of cancer progression. This broad range of targets highlights regorafenib's potential as a versatile therapeutic option for treating cancers influenced by these pathways [97–99]. Beyond its effects on tumor angiogenesis, oncogenesis, and the TME, regorafenib also modulates immune responses by targeting macrophages. It inhibits CSF-1R, a key regulator of macrophage differentiation, survival, and function. By blocking CSF-1R, regorafenib disrupts the development and maintenance of tumor-associated macrophages (TAMs), reducing their presence within the TME. This alteration in the tumor's immune landscape may enhance antitumor immune responses. With this dual mechanism—suppressing tumor growth and modulating immune cell activity—regorafenib shows promise for improving therapeutic outcomes, particularly in tumors dependent on macrophage-mediated immune suppression [100, 101]. Xenograft models of various cancer types, such as lung, melanoma, pancreatic, and ovarian cancers, have demonstrated regorafenib's efficacy in inhibiting tumor growth, suggesting its broad applicability across malignancies. In a GBM xenograft model, regorafenib also exhibited potent anti-angiogenic effects by targeting VEGFRs involved in angiogenesis. This disruption of tumor vasculature limits nutrient and oxygen supply, contributing to tumor growth inhibition. These findings underscore regorafenib's potential as a therapeutic agent with both direct anti-tumor activity and anti-angiogenic effects across a diverse range of cancers [97, 102] (Fig. 5).

Relapse of GBM continues to pose a significant challenge in neuro-oncology. The REGOMA trial highlighted regorafenib's potential, demonstrating a significant improvement in overall survival compared to lomustine, with median survival times of 7.4 months and 5.6 months, respectively [14, 102].

The trial enrolled 119 patients with relapsed GBM to evaluate regorafenib's efficacy. The findings revealed a longer overall survival of 7.4 months in the regorafenib group versus 5.6 months in the control group receiving lomustine. Moreover, the regorafenib arm exhibited a statistically significant improvement in 6-month progression-free survival compared to the control. However, concerns have been raised about certain aspects of trial design. Notably, the outcomes in the control arm were suboptimal compared to other studies on lomustine in relapsed GBM. Additionally, the exclusive inclusion of IDH-mutated patients in the regorafenib arm raises

Table 3 Overview of EGFR overexpression and targeted therapy strategies in GBM

Therapeutic Approaches	Mechanism of Action	Clinical Efficacy	Limitations	Ref.s
Erlotinib	EGFR TKI	Limited efficacy in EGFR overexpression cases	Ineffective as a standalone treatment; marginal benefits post-radiation	[92]
Gefitinib	EGFR TKI	Anti-tumor activity independent of EGFR expression	Modest clinical effects in Phase II trials	[93–95]
Depatux-M	Antibody–drug	Similar efficacy to control group; HR 1.04	Failed to achieve primary endpoint in trials	[96]

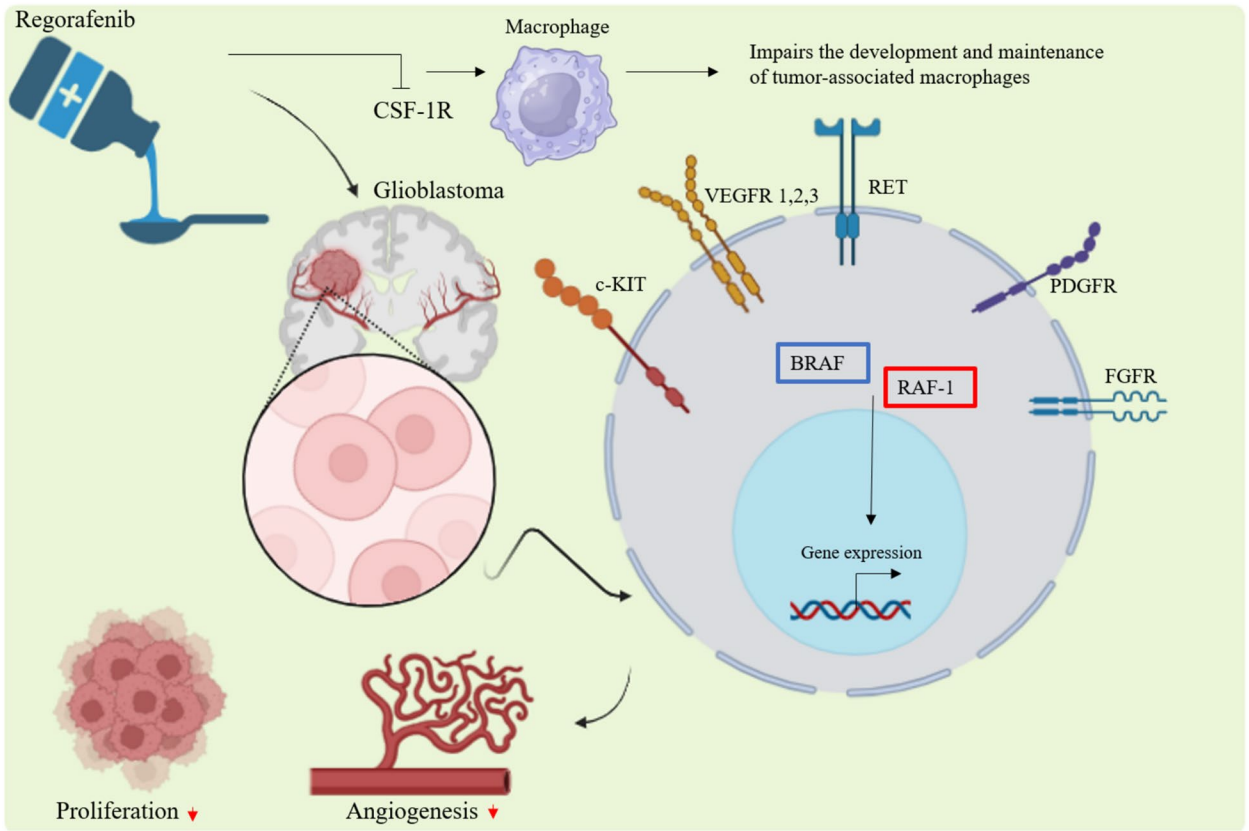

Fig. 5 Regorafenib targets key pathways involved in tumor progression, including stromal kinases (FGFR, PDGFR) that regulate the TME, angiogenic kinases (VEGFRs, Tie-2) critical for blood vessel formation and stabilization, and oncogenic kinases (RET, KIT) that drive tumor cell proliferation and survival. A notable mechanism of action is its inhibition of CSF-1R on macrophages, which disrupts the development and maintenance of tumor-TAMs. By targeting TAMs, regorafenib diminishes its role in promoting tumor growth and immune evasion, shifting the TME toward an anti-angiogenic and anti-proliferative state. Additionally, regorafenib's inhibition of VEGFRs and Tie-2 enhances its anti-angiogenic effects by reducing the formation of blood vessels necessary for tumor growth and metastasis

concerns about the generalizability of the results, given the potential differences in treatment response between IDH-mutated and IDH-wildtype patients. The lack of centralized pathology and molecular review further adds to uncertainties regarding patient selection and tumor characterization, potentially impacting the reliability of the results [103, 104].

A recent Phase II study comparing patient-reported outcomes between regorafenib and lomustine showed that regorafenib did not adversely affect health-related quality of life. This finding suggests that regorafenib may be a viable treatment option for patients without compromising their overall well-being during therapy. This indicates that, while regorafenib offers survival benefits for patients with relapsed GBM, it does so without compromising their overall well-being or daily functioning compared to lomustine treatment. These results are particularly significant for clinicians seeking treatment options that effectively balance therapeutic efficacy with preserving the patient's quality of life. They further underscore the clinical relevance of regorafenib as a viable treatment alternative for GBM, demonstrating its ability to provide therapeutic benefits with minimal negative effects on patients' quality of life [105]. Despite its incorporation into clinical practice for treating relapsed GBM, the molecular mechanisms that dictate patient sensitivity to regorafenib remain poorly understood. A preliminary retrospective analysis from the REGOMA trial team, which involved transcriptional profiling of tumor specimens, identified a mini-signature that was associated with improved overall survival in patients treated with regorafenib. This finding offers some insight into the potential biomarkers that could predict better treatment outcomes, though further research is needed to validate these results and uncover the underlying mechanisms [106]. This signature consists of five biomarkers: increased mRNA levels of HIF1A and CDKN1A, along with decreased expression of miR-93-5p, miR-3607-3p, and miR-301a-3p. It was derived from RNA analyses of tumor samples obtained

from 72 patients, with 36 patients receiving regorafenib and 36 treated with lomustine. These biomarkers may offer valuable insights into identifying patients who are more likely to respond favorably to regorafenib, though further validation is required to confirm their predictive accuracy [107]. Additionally, recent findings by Jiang and colleagues revealed that regorafenib induces autophagic arrest in GBM cells, providing valuable insights into its anti-tumor mechanism. This highlights a potential pathway through which regorafenib exerts its therapeutic effects, further supporting its role in GBM treatment [108].

Despite the promising results from the REGOMA trial, the clinical benefit of regorafenib in treating relapsed GBM remains uncertain. While some patients show a favorable response to regorafenib, its efficacy is inconsistent across the diverse and heterogeneous GBM population. A more comprehensive understanding of the molecular factors driving regorafenib responsiveness is essential to optimize its use in this complex disease [14, 104, 109].

A 2024 study [102] reported several active clinical trials investigating regorafenib in GBM[105, 110–114]. One ongoing Phase II trial, titled Regorafenib in Bevacizumab-Refractory Recurrent GBM, is active but not recruiting participants. Another trial, Regorafenib in Patients with Relapsed GBM (IOV-GB-1–2020 REGOMA-OSS), is actively enrolling patients, though its phase has not been specified. Additionally, a Phase II trial is evaluating the combination of regorafenib and nivolumab for safety and efficacy; this study is also active but not recruiting. Lastly, the GBM AGILE trial, a Phase II/III adaptive and innovative study, is actively recruiting participants to explore advanced therapeutic approaches for GBM. These ongoing trials underscore the persistent efforts to investigate regorafenib in various therapeutic contexts, reflecting a broader commitment to improving outcomes for this aggressive and challenging cancer.

Future research into RTKs in GBM therapy has identified a unique fusion event in specific glioma subtypes. IDH wild-type (IDHwt) gliomas, defined by the absence of IDH mutations, represent a heterogeneous group requiring further subclassification. Notably, approximately 3–5% of adult IDHwt diffuse gliomas harbor an oncogenic chromosomal translocation involving the fusion of the FGFR3 and TACC3 genes. This alteration produces the FGFR3-TACC3 fusion protein, which drives tumor progression by activating key pathways related to cell proliferation, survival, and angiogenesis [115–117]. FGFR3, a member of the fibroblast growth factor receptor (FGFR) family, plays a critical role in regulating various physiological cellular processes, including development, survival, migration, and angiogenesis. Through interactions with specific fibroblast growth factors (FGFs), FGFR3 activates downstream signaling pathways essential for maintaining tissue homeostasis, such as RAS/MAPK, PI3K/Akt, and STAT3. These pathways influence cell differentiation, proliferation, and tissue remodeling. Dysregulation of FGFR3, through mutations or fusions, can contribute to oncogenesis by promoting unchecked cell growth and resistance to apoptosis [118]. Under normal conditions, FGF binding to FGFR triggers receptor dimerization and phosphorylation of intracellular TK domains, initiating several signaling cascades vital for processes like embryogenesis and wound healing. However, aberrations in FGFR signaling, including mutations and fusions, disrupt this balance, contributing to tumorigenesis and cancer progression [118]. Altered FGFR signaling, commonly through missense mutations, has been implicated in several cancers, including gliomas [119, 120]. TACC3, on the other hand, is essential for maintaining mitotic spindle stability during cell division. Its disruption can lead to chromosomal instability, a hallmark of cancer [121, 122]. The close proximity of FGFR3 and TACC3 on chromosome 4p16 predisposes them to fusion events, as observed in gliomas, further linking their dysregulation to tumorigenesis [123].

The FGFR3-TACC3 (F3T3) fusions were first identified by Singh et al. in 2012 through transcriptome analysis of cultured glioma cells derived from nine GBM patients [115]. By utilizing split reads and split inserts, the researchers were able to identify intrachromosomal rearrangements that resulted in in-frame fusion transcripts. These fusion transcripts combined the N-terminal region of FGFR3 with the C-terminal region of TACC3, generating the FGFR3-TACC3 fusion. This fusion has been implicated in driving tumorigenesis and is considered a potential therapeutic target in cancers where it occur [115]. The predicted fusion protein consists of the intracellular tyrosine kinase (TK) domain of FGFR3, which is fused in-frame with the coiled-coil (C–C) domain of TACC3 located upstream. This structural rearrangement is thought to contribute to abnormal signaling and cell growth, potentially driving tumorigenesis by enhancing the activity of the FGFR3 pathway through the altered fusion protein [124]. Tumor cells with this gene fusion showed elevated expression levels of the chimeric protein [115, 125].

The identification of F3T3 fusions as a key oncogenic driver in certain GBM cases highlights a unique therapeutic vulnerability. Targeting FGFR signaling pathways with inhibitors such as fexagratinib (AZD4547) and erdafitinib (JNJ-42756493) demonstrated efficacy in inhibiting the growth of glioma stem cells expressing F3T3 at low nanomolar concentrations. Moreover, these inhibitors significantly extend survival in mice harboring

F3T3 glioma xenografts. These findings suggest that FGFR-targeted therapies could provide a promising strategy for treating GBM patients with F3T3 fusions, offering potential improvements in patient outcomes [115, 126].

GBMs are characterized by significant subclonal heterogeneity, with neighboring cells frequently activating distinct RTKs [127, 128]. F3T3 GBMs are particularly noteworthy because of the consistent and widespread expression of the F3T3 fusion protein within tumors, coupled with its mutual exclusivity with other RTK alterations [116, 124, 129–131]. Another challenge in GBM treatment is the temporal instability of molecular targets, which can be lost during tumor recurrence [132]. However, studies of paired primary and recurrent tumors have shown that the F3T3 fusion is consistently maintained upon recurrence, reinforcing its potential as a durable therapeutic target [126, 130, 133, 134].

The FGFR3-TACC3 (F3T3) fusion is recognized as a critical actionable target in GBM treatment and is prominently featured in the current EANO guidelines. Its retention upon tumor recurrence and its potential for targeted inhibition underscore its promise as a therapeutic avenue for this aggressive malignancy [135]. On the ESMO Scale for Clinical Actionability of Molecular Targets (ESCAT), F3T3 is categorized as a Tier IIB target, reflecting the availability of drugs with demonstrated antitumor activity, though the extent of their benefit remains uncertain [125, 135, 136].

The first evidence of FGFR inhibitor (FGFRi) efficacy in F3T3 gliomas emerged during a Phase I clinical trial, in which two patients with recurrent F3T3 GBM were treated with erdafitinib. This trial demonstrated promising results, highlighting the potential of FGFR inhibition as a therapeutic strategy for targeting F3T3 GBM, particularly in patients with alterations in the FGFR3-TACC3 fusion [137]. Both patients demonstrated clinical improvement during the trial. One patient achieved disease stabilization, evidenced by a reduction in relative cerebral blood volume (rCBV), while the other experienced a partial response. Notably, disease control was maintained for 115 and 135 days, respectively, further supporting the potential therapeutic benefit of erdafitinib in treating recurrent F3T3 GBM [126].

A promising area of research in F3T3 gliomas involves targeting oxidative phosphorylation (OXPHOS) metabolism. This approach stems from findings in medullary thyroid cancer (MTC), where tumors dependent on OXPHOS for energy production demonstrate synthetic lethality when treated with mitochondrial inhibitors [83]. Similar effects have been observed in F3T3 glioma cells, where mitochondrial inhibitors significantly reduce cell viability [138]. Building on these preclinical insights, the OPTIMUM trial (NCT04945148), a phase II clinical study, is evaluating the efficacy of metformin, a widely used and well-tolerated mitochondrial inhibitor, in combination with standard first-line chemoradiation for newly diagnosed OXPHOS-dependent GBMs [139].

There is limited evidence supporting the efficacy of MEK inhibitors (MEKi) in mitigating FGFR-driven activation of the MAPK pathway in gliomas with FGFR alterations. However, trametinib, a MEK inhibitor, has been tested in two pediatric glioma patients with FGFR rearrangements. Although extensive studies are lacking, these initial cases indicate that MEKi may offer a potential therapeutic strategy for targeting FGFR-related pathway alterations in gliomas. Further investigation is necessary to evaluate the broader applicability and effectiveness of this approach, particularly in pediatric populations [125, 140, 141].

Innovative strategies for treating F3T3 gliomas include RNA interference (RNAi) techniques [142]. Additionally, emerging nanotechnologies, such as nanocarriers for targeted drug delivery, show promise in improving the delivery of FGFR inhibitors to the brain while minimizing systemic toxicity [143, 144].

In summary, gliomas with F3T3 fusions represent a distinct subgroup of IDH wild-type gliomas, characterized by unique molecular features, including specific oncogenic pathways, altered metabolism, and distinct clinical, histological, and radiological profiles. These fusions are targetable with FGFR inhibitors (FGFRi), which have shown limited but encouraging efficacy in phase I and II clinical trials for recurrent disease. Given the scarcity of effective treatments for recurrent diffuse gliomas, systematic screening for F3T3 gene fusions in newly diagnosed IDH wild-type diffuse gliomas is highly recommended. This recommendation aligns with the European Association of Neuro-Oncology (EANO) guidelines, which classify F3T3 fusion screening as a high-priority diagnostic tool (ESCAT level 2). Identifying these fusions can provide crucial prognostic insights and guide therapeutic strategies in this challenging patient population. Current research efforts focus on enhancing the efficacy of targeted TK inhibitors (TKIs) and exploring alternative approaches, such as metabolic reprogramming [125].

Targeting signaling pathways in GBM

NF-κB pathway

The persistent and aberrant activation of the NF-κB signaling pathway is a hallmark of GBM. This chronic hyperactivation promotes oncogenesis by driving tumor growth and invasion, inhibiting apoptosis, and increasing resistance to therapy [145, 146]. The p65-p50 heterodimer is the most common NF-κB dimer, regulating gene transcription by binding to specific DNA sequences.

Subtle variations in how these dimers interact with target sequences enable precise control of cellular activity by NF-κB [147]. Under normal conditions, NF-κB dimers remain inactive in the cytoplasm due to their association with inhibitory proteins, primarily IκBα, which block NF-κB's nuclear localization sequence and prevent its translocation to the nucleus. Upon activation, NF-κB dimers enter the nucleus and bind to κB sites in the regulatory regions of target genes, modulating cellular processes [148]. In neurons, NF-κB plays a critical role in maintaining neuronal health, supporting synaptic development, and facilitating plasticity-related functions [149]. In cancers, including gliomas, the NF-κB pathway is frequently activated and regulates inflammation, cell survival, proliferation, and immune responses. In gliomas, dysregulation of NF-κB signaling can arise from various mechanisms, such as mutations in upstream signaling components (e.g., receptors and kinases) or alterations in inhibitory proteins like IκB, which normally suppress NF-κB activation. Chronic inflammation and interactions with the TME further enhance NF-κB signaling, contributing to glioma cell survival and therapy resistance. This dysregulation is associated with increased tumor aggressiveness and poorer clinical outcomes in glioma patients [70, 150, 151] (Table 4 and Fig. 6).

MYC transcription factors, part of the Myc Proto-Oncogene family, play a critical role in regulating cell growth, proliferation, differentiation, and apoptosis. These factors are essential for brain development and are implicated in most human cancers [157, 158]. In GBM, MYC is highly expressed, with its overexpression correlating with higher tumor grades [159, 160]. While NF-κB and MYC share overlapping functions, studies indicate that NF-κB signaling can modulate MYC activity [161–163].

Key transcription factors such as KLF4, OCT4, SOX2, and MYC—often referred to as the "four factors"—are potential regulators of stemness in cancer stem cells (CSCs) [164]. In GBM stem cells (GSCs), MYC, N-MYC, and OCT4 are expressed, while KLF4 is not. OCT4, a marker of high-grade GBM, enhances glioma cell proliferation and colony formation [165].

At the cellular level, TNFα acts as a pro-tumor cytokine, promoting GSC viability even when co-treated with TMZ, a standard chemotherapeutic drug [166]. Interestingly, while TNFα activates NF-κB signaling, its inhibition with PDTC does not affect the mRNA expression levels of MYC, MAX, N-MYC, or L-MYC [166]. These findings underscore the potential of targeting the NF-κB signaling pathway and/or MYC in GSCs as a promising therapeutic strategy. By inhibiting these key pathways, it may be possible to suppress the proliferative and invasive characteristics of GBM, offering a new avenue for treatment and potentially improving patient outcomes. Both NF-κB and MYC are key drivers of tumorigenesis, contributing to the maintenance of GSCs, which are often responsible for tumor recurrence and treatment resistance. Inhibiting these pathways could reduce tumor growth, prevent invasion, and enhance the efficacy of GBM therapies [166].

Inhibition of canonical NF-κB activity in patient-derived GSC cultures significantly reduces tumor-sphere

Table 4 Key mechanisms underlying the disruption of NF-κB signaling in GBM

Mechanism	Description	Impact on GBM	Ref.s
Constitutive NF-κB Activation	Persistent hyperactivation of NF-κB signaling	Promotes tumor growth, invasion, suppresses apoptosis, and contributes to therapy resistance	[145, 146]
NF-κB p65-p50 Heterodimer	Binds to specific gene sequences to regulate gene transcription	Regulates gene transcription for cell activity; implicated in oncogenesis	[147]
Inhibitory Factors IκBα, IκBβ, IκBε	Bind NF-κB dimers to prevent nuclear translocation	Loss of inhibition leads to NF-κB activation and gene expression promoting oncogenic processes	[148]
p65 Subunit Overexpression	High expression in GBM cases	Overexpressed in 81% of GBM cases, contributing to oncogenicity	[69]
EGFR and PDGFR Interaction	Interplay with NF-κB signaling pathways	Drives GBM growth and invasion	[152]
Loss of Tumor Suppressors (e.g., Neurofibromin 1)	Loss of regulatory mechanisms on PI3K and NF-κB	Disrupted NF-κB activation due to increased PI3K activity	[153]
Krüppel-like Factor 6 Disruption	Normally acts as a negative regulator of NF-κB	Loss contributes to NF-κB pathway activation in GBM	[154]
PDGF Overexpression	Activates NF-κB via PI3K pathway	Promotes glioma cell proliferation and high NF-κB levels	[146, 155]
Additional Mechanisms			
	Involves factors like PIN1, MLK4, and NFKBIA gene deletion	Facilitates NF-κB pathway disruption in GBM	[156]

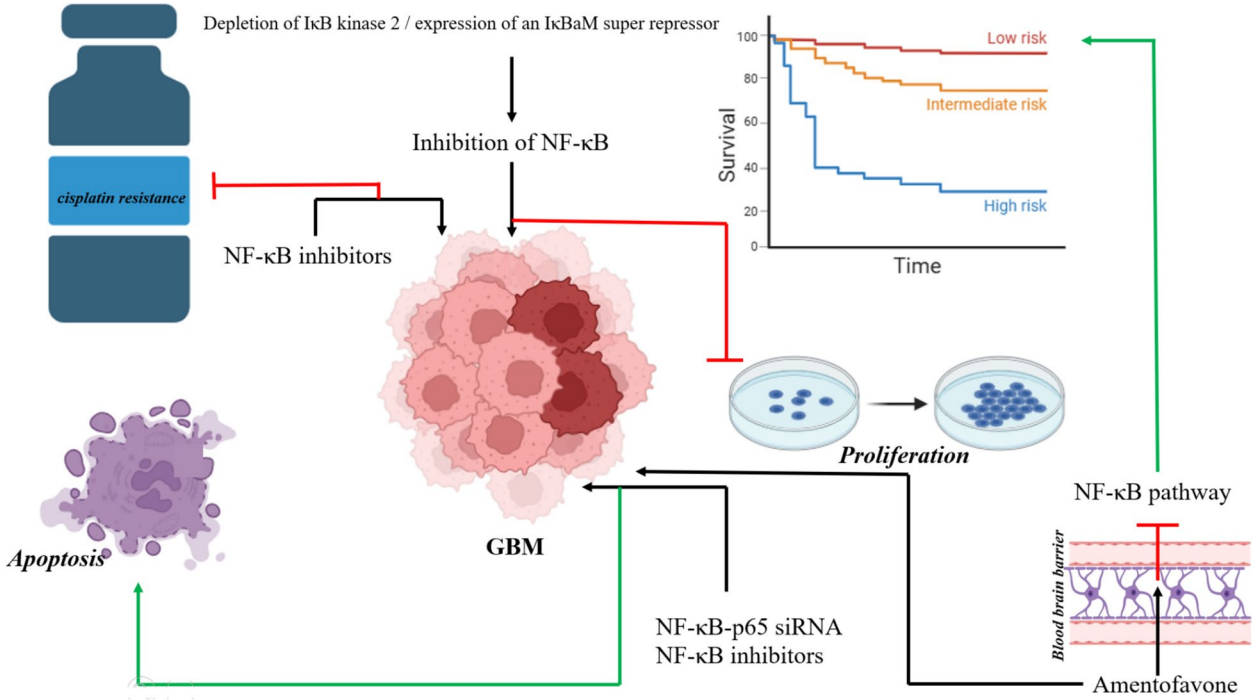

Fig. 6 The NF-κB pathway is a pivotal target in GBM therapy. Inhibition of IκB kinase 2 or the expression of an IκBαM super-repressor reduces tumor proliferation. Amentofavone suppresses NF-κB signaling, mitigating associated risks. In the Kaplan–Meier plot, the vertical axis represents survival, while the horizontal axis indicates time. The plot includes lines for low risk, intermediate risk, and high risk groups. Individuals who are placed in the low-risk category are those in whom the inhibition of the NF-kB pathway results in a reduction in risk, placing them in the low-risk group. This suggests that the suppression of the NF-kB signaling pathway plays a protective role, reducing the risk and thus categorizing these individuals as low-risk. NF-κB inhibitors and siRNA promote apoptosis and overcome cisplatin resistance, presenting a promising therapeutic approach for GBM

formation frequency [167]. Furthermore, co-stimulation with TNFα and treatment with PDTC do not provide cytoprotective effects to GSCs, reinforcing the potential of targeting MYC or NF-κB as a therapeutic strategy [166].

Immunotherapy for GBM using NK cells is gaining significant attention for therapeutic applications, as NK cells exhibit strong cytotoxic activity while causing fewer toxic side effects compared to traditional therapies [168]. A study reported a reduction in tumor volume in nine patients with malignant glioma following treatment with autologous NK cells, with no evidence of significant neurological toxicity [169]. GBM cells with stem-like traits appear particularly vulnerable to NK cell-mediated cytotoxicity due to their low MHC class I expression and the presence of ligands that activate NK receptors [170]. Notably, NK cells showed a stronger cytotoxic effect against CD133 + /Nestin + -GSCs cultured as spheres, compared to adherently cultured GSCs lacking CD133 and Nestin expression [171]. Furthermore, NK cells have been demonstrated as a promising alternative cell-based therapeutic strategy, capable of specifically targeting and eliminating GSCs [166] (Fig. 7).

Wnt pathway

The Wnt signaling pathway is an ancient and fundamental genetic program that provides crucial regulatory instructions for cell growth, differentiation, and tissue patterning. Its roles are vital during early development and are conserved across species, shaping cellular functions and structures in complex multicellular organisms [172–176]. Interaction between Wnt and Frizzled proteins activates distinct signaling pathways, categorized into a β-catenin-dependent canonical pathway and two β-catenin-independent noncanonical pathways. The canonical pathway involves the activation of β-catenin, while the noncanonical pathways—planar cell polarity and Wnt/Ca2 + —function independently of β-catenin, eliciting alternative cellular responses such as changes in cell polarity and intracellular calcium levels [173–175]. The canonical pathway is particularly known for its role in maintaining and expanding stem and progenitor cell populations and directing lineage specification in both embryonic and adult tissues [177]. Additionally, the noncanonical Wnt pathway regulates convergent proliferation during gastrulation and the migration of neural and epithelial cells [178–180].

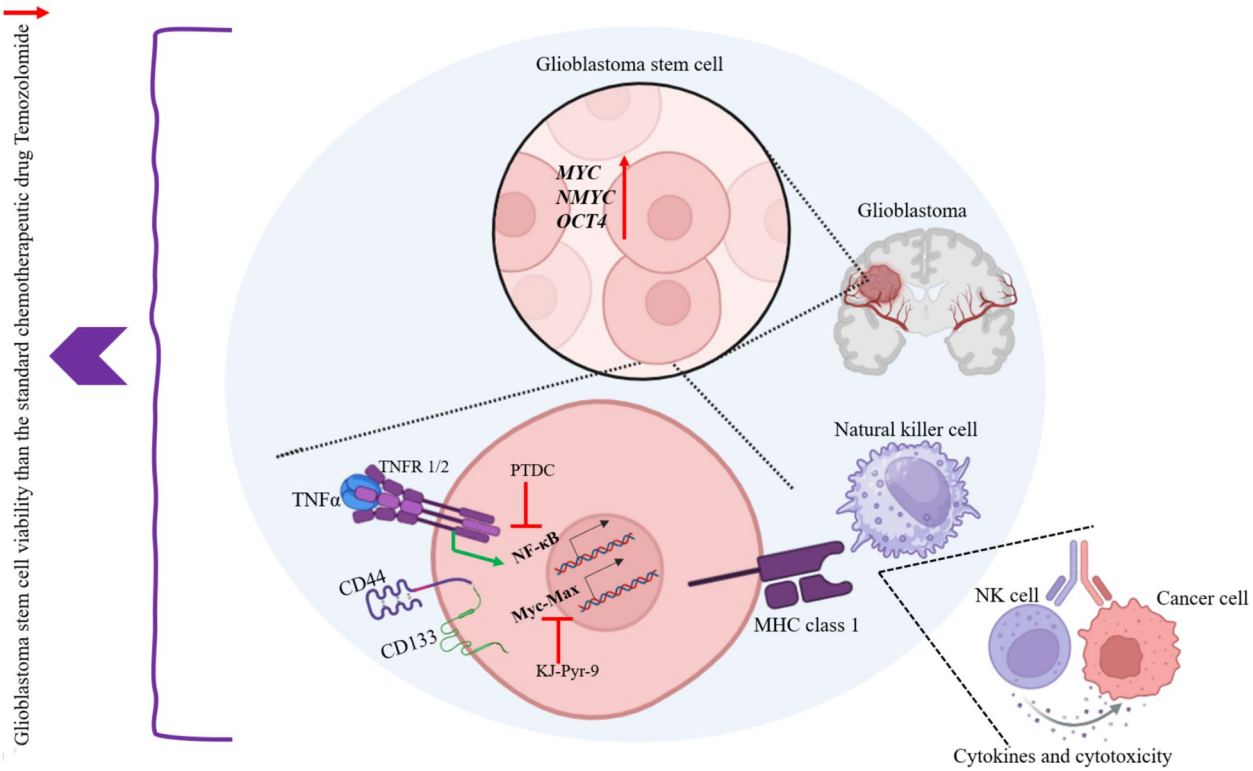

Fig. 7 Targeting GSCs holds great promise for improving the efficacy of chemotherapy and immunotherapy. Pharmacological inhibition of NF-κB using PTDC (Pyrrolidinedithiocarbamate) or suppression of MYC with KJ-Pyr-9 (a small molecule inhibitor) significantly reduces GSC viability, outperforming standard chemotherapeutic agents like TMZ, even in the absence of TNFα's cytoprotective effects. Furthermore, natural killer (NK) cells offer an effective cell-based therapeutic approach by efficiently targeting and eradicating GSCs, presenting a powerful strategy to combat GBM

Extensive research into gliomagenesis has emphasized the pivotal role of the canonical Wnt/β-catenin signaling pathway in promoting the proliferation and self-renewal of GSCs. This pathway is crucial in enhancing the tumorigenic potential of GSCs, particularly in the context of high-grade gliomas, where its activation contributes significantly to tumor growth and resistance to conventional therapies [181–183].

Although the altered expression or upregulation of Wnt5a has been linked to enhanced tumor cell invasion and metastasis in a range of solid cancers, its precise role in modulating the invasive behavior and progression of high-grade gliomas is still not fully elucidated. Further investigation is needed to clarify how Wnt5a contributes to glioma aggressiveness and whether it could serve as a potential therapeutic target for managing tumor invasion in these malignancies. Wnt5a is known to regulate critical cellular processes, such as migration, adhesion, and extracellular matrix remodeling, but its precise contribution to glioma invasion and progression is still under investigation. Elucidating this relationship could uncover new therapeutic targets by inhibiting Wnt5a-mediated signaling pathways to reduce glioma malignancy and improve patient outcomes [176, 184–186].

These observations align with prior research showing that noncanonical Wnt5a promotes glioma cell migration by modulating the expression of MMPs, which are essential for extracellular matrix (ECM) degradation [186]. Additionally, overexpression of Wnt5a in classical GSCs induced a highly migratory phenotype and an expression profile consistent with the mesenchymal GBM "invasive signature." These findings underscore the functional and molecular transition between subtypes driven by changes in Wnt5a expression within GSCs, highlighting Wnt5a as a key regulator of the invasive potential of GSCs [187].

The study revealed that human GSCs, which exhibit constitutively active AKT and a dominant-negative p53, utilize a Pax6/Dlx5 transcriptional program to control Wnt5a-mediated differentiation into endothelial-like cells. This finding highlights a potential mechanism through which GSCs influence tumor vascularization and contribute to the complex biology of gliomas, suggesting a possible target for therapeutic strategies aimed at disrupting this differentiation process. Pax6

and Dlx5 are critical transcription factors involved in developmental processes, particularly in the formation of the nervous system, limbs, and various other tissues. These findings underscore the roles of Pax6 and Dlx5 in driving the malignant transformation and vascularization of gliomas, presenting potential therapeutic targets to disrupt aberrant differentiation and invasive behavior in GSCs [188]. This aligns with prior research highlighting Wnt5a's pivotal role in regulating the differentiation of embryonic stem cells into endothelial cells during vascular development. Wnt5a also facilitates endothelial cell proliferation, survival, and migration, processes integral to angiogenesis. These findings suggest that Wnt5a supports both endothelial differentiation and the dynamic activities necessary for blood vessel formation, further emphasizing its potential as a therapeutic target for controlling GBM growth and metastasis [189–191]. Given that Dlx5 regulates Wnt5a expression during CNS development and is also expressed in GSCs, it indicates that GSCs exploit the Pax6/Dlx5-Wnt5a transcriptional axis, a developmentally controlled pathway. Activation of this pathway enables GSCs to differentiate into endothelial-like cells, contributing to the extensive infiltration of GBM cells beyond the primary tumor site. This mechanism highlights a potential link between developmental signaling pathways and the aggressive invasive behavior of GBM, providing insights into the molecular drivers of tumor spread and revealing possible therapeutic targets to inhibit tumor migration [192, 193]. Moreover, GSCs have been shown to generate vascular pericytes, which actively remodel perivascular niches. This remodeling is essential for shaping the TME, thereby promoting GBM progression and invasiveness [194] (Table 5 and Fig. 8).

Mutations in the promoter of telomerase reverse transcriptase (TERT) in GBM

TERT encodes the catalytic component of the telomerase complex, which is critical for telomerase activity. Mutations in the TERT gene promoter are frequently associated with cancer due to their impact on the catalytic subunit's function. These mutations typically involve nucleotide substitutions at two main "hot spots": position −124 and position −146 relative to the transcription start site [201].

The TERT promoter mutation (pTERTmut) was first identified in melanoma, and subsequent studies have shown its high prevalence in IDH-wildtype GBMs. Additionally, pTERTmut is observed in IDH-mutant oligodendrogliomas and oligodendrogliomas with 1p/19q co-deletion. This mutation is significant for its role in telomerase activation, which enables cancer cells to maintain telomere length, contributing to their immortality. The presence of pTERTmut serves as a key molecular marker for glioma diagnosis and prognosis, aiding in distinguishing between glioma subtypes and informing therapeutic strategies. These findings underscore the importance of pTERTmut as a marker for glioma classification [202].

GBMs are broadly categorized into molecular subtypes based on mutations in the TERTp and IDH1/2 genes, with distinct mechanisms for telomere maintenance. TERTp mutations activate telomerase by creating new

Table 5 Overview of key research on Wnt signaling pathways in GBM

Main Findings	Study Focus	Author(s)	Year	Ref.s
APC mutations found in 13% of GBM cases	APC mutation in GBM	Tang, Chao, et al	2015	[72]
Wnt pathway linked to GBM growth and chemo-resistance	Wnt pathway's role in chemotherapy resistance	Yun, Eun-Jin, et al. and Tompa, Marton, et al	2020 and 2018	[195, 196]
Links Wnt/β-catenin activity to brain development stages	Neural progenitor proliferation and differentiation	Gao, Juanmei, et al	2021	[180]
HIF-1α sustains β-catenin transcription in hypoxia	Influence of hypoxia on Wnt signaling	Mori, Hiroyuki, et al	2016	[197]
Wnt-induced differentiation affects Notch signaling	Wnt-Notch signaling interaction	Rampazzo, E., et al	2013	[198]
Antibodies like vantictumab and ipafricept tested in trials	Inhibition of Wnt signaling in clinical trials	Diamond, Jennifer R., et al	2020	[199]
Wnt-Cxcr4 required for OPC migration and CNS dispersal	Wnt-Cxcr4 in OPC migration	Tsai, et al	2016	[200]
Wnt5a regulates invasive phenotype in GSCs	Role of Wnt5a in GSC invasion	Binda, Elena, et al. and Hu, Baoli, et al	2017 and 2016	[187, 188]
Shows Wnt5a's role in enhancing GBM cell migration	Wnt5a in GBM cell lines			

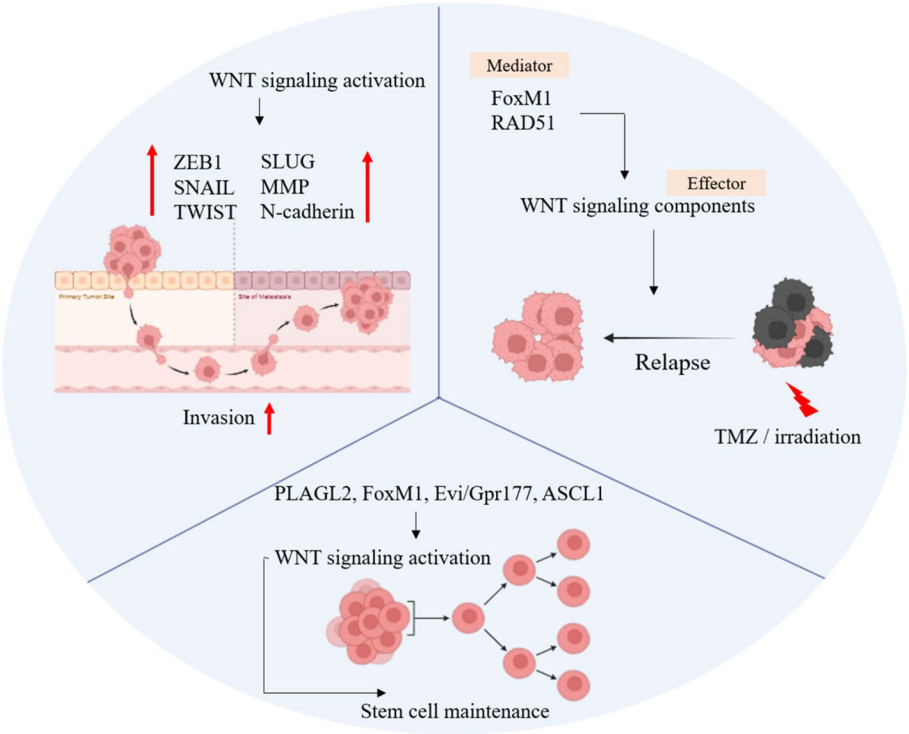

Fig. 8 WNT signaling is a key driver of GBM progression and resistance, influencing tumor initiation, advancement, and therapeutic response. Its role can be summarized in three main areas: (1) maintaining GBM stem cells (GSCs), (2) promoting tumor cell migration and invasion, and (3) contributing to multi-drug resistance. WNT signaling facilitates GSC self-renewal and survival in adverse microenvironments through regulators such as PLAGL2, FoxM1, and ASCL1. It enhances GBM aggressiveness by upregulating EMT-related genes like ZEB1, SNAIL, and MMPs. Furthermore, WNT signaling aids therapy resistance by allowing residual tumor cells to evade treatment, leading to recurrence. Targeting WNT signaling presents a promising avenue for overcoming GBM resistance and improving patient outcomes

transcription factor binding sites, leading to increased TERT expression. Conversely, mutations in the ATRX gene facilitate the alternative lengthening of telomeres (ALT). GBMs with TERTp-wildtype and IDH-wildtype lack specific genetic markers or defined telomere maintenance mechanisms [203] (Table 6 and Fig. 9).

PI3K/AKT/mTOR pathway

The PI3K/AKT/mTOR intracellular signaling pathway plays a crucial role in regulating various cellular processes, including growth, proliferation, and metabolism. This pathway is essential for maintaining cellular homeostasis and enabling responses to environmental signals, such as nutrients, growth factors, and stress. Dysregulation of PI3K/AKT/mTOR signaling is commonly observed in many cancers, contributing to tumorigenesis by promoting uncontrolled cell growth, survival, and resistance to apoptosis [66]. PI3K is categorized into three classes (I, II, and III) based on their substrate specificity and the type of products they generate. Class I PI3Ks, the most studied, are composed of regulatory and catalytic subunits that form heterodimers. These enzymes are activated by cell-surface receptors, including GPCRs and RTKs, which possess intrinsic tyrosine kinase (TK) activity. Upon ligand binding, RTKs undergo autophosphorylation at specific tyrosine residues in their cytoplasmic domains. The SH2 domains within the regulatory subunits of PI3K recognize and bind to these phosphorylated tyrosine residues, facilitating the recruitment of PI3K to the plasma membrane. This positioning enables the enzymes to interact with their substrates, leading to their activation and initiation of downstream signaling events [207]. PI3K inhibitors are categorized into three main types: pan-PI3K inhibitors, which target all isoforms of PI3K; isoform-selective inhibitors, which are designed to specifically target individual PI3K isoforms; and dual PI3K/mTOR inhibitors, which simultaneously inhibit both the PI3K and mTOR pathways. Pan-PI3K inhibitors broadly block the activity of all PI3K isoforms, while isoform-selective inhibitors focus on specific variants, offering a more targeted approach. Dual inhibitors aim to disrupt both the PI3K and mTOR pathways, which are closely linked and often dysregulated in various cancers, providing a more comprehensive blockade of the pathway's signaling network [208]. The PI3K/AKT/mTOR pathway is initiated by the activation of transmembrane

Table 6 Overview of studies on TERT in GBM and other cancers

Study No	Description of Study	Gene/Protein Targeted	Methodology	Key Findings	Ref.s
1	Further studies on hTERTC27-mediated effects on malignant tumors in vivo were conducted using human GBM xenografts in thymus-free mice. In this model, intra-tumor injection of rAAV-hTERTC27 was performed to assess its impact	hTERTC27	In vivo gene therapy	rAAV-hTERTC27 slowed GBM tumor growth, induced necrosis, apoptosis, neutrophil infiltration, and reduced microvessel density	[204]
2	Increased hTERT gene expression in high-grade glioma patients, linked to tumor aggressiveness	hTERT	Expression analysis	Patients with low hTERT levels had a progression-free survival of 24 months, whereas those with high levels had a PFS of 11 months. Targeting hTERT with siRNA reduced cell viability	[205]
3	Utilized CRISPR-associated protein 9 with sgRNA for gene editing as a pre-clinical therapeutic approach	TERT promoter mutations	Gene editing (CRISPR)	Intra-tumoral injection of adeno-associated viruses expressing sgRNA-controlled CjCas9-fused adenine base editor effectively suppressed glioma growth in tumors with TERT promoter mutations	[206]

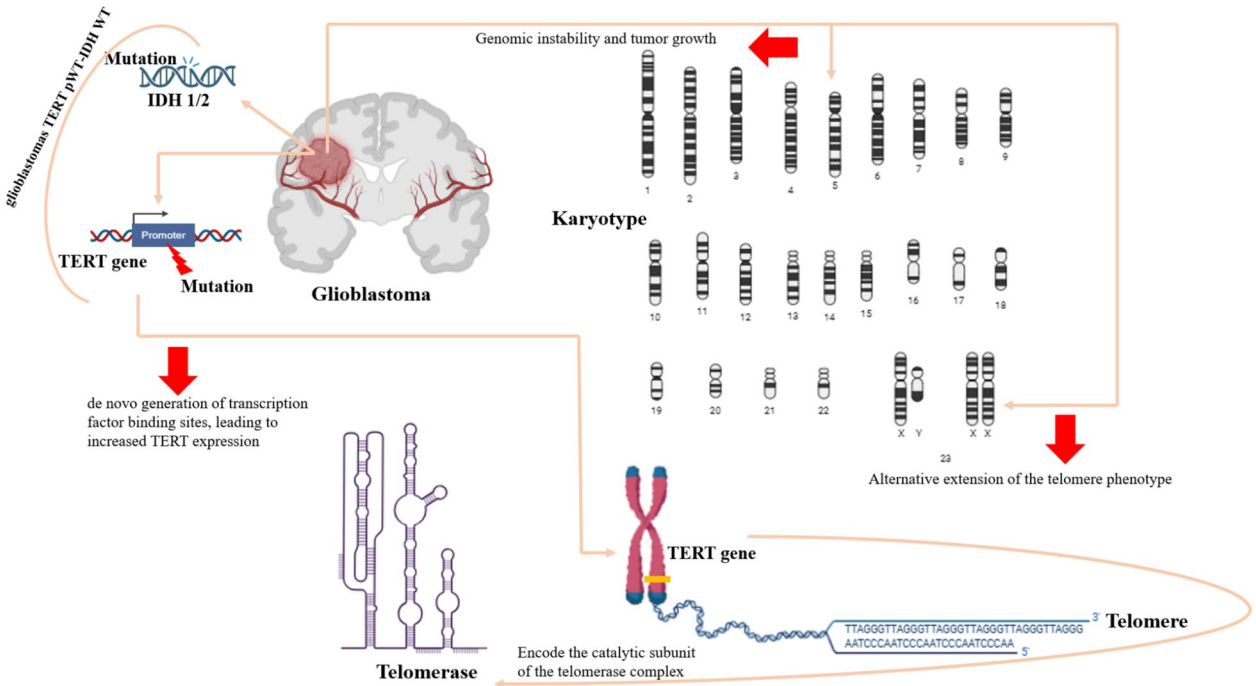

Fig. 9 Telomerase reverse transcriptase in GBM. Mutations in IDH1/2 and the TERT gene contribute to the development of TERTp-WT-IDH WT GBM, playing a critical role in telomere maintenance during DNA replication—a key process in GBM progression. While there is substantial evidence supporting the involvement of TERTp mutations in GBMs, their prognostic value remains a subject of debate. Understanding the molecular mechanisms underlying TERTp-mutated GBMs could pave the way for targeted TERT-based therapies. TERT, located on chromosome 5, encodes the catalytic subunit of the telomerase complex, significantly contributing to genomic instability and tumor progression. Additionally, on the X chromosome, it drives the alternative lengthening of telomeres phenotype. These mutations create new transcription factor binding sites, enhancing TERT expression and promoting GBM progression

receptor TK, growth factor receptors, integrins, and GPCRs. When these receptors are activated, the PI3K enzyme is recruited to the plasma membrane, where it catalyzes the conversion of phosphatidylinositol 4,5-bisphosphate (PIP2) to phosphatidylinositol 3,4,5-trisphosphate (PIP3). This lipid second messenger (PIP3) then activates downstream signaling molecules, such as AKT, which regulates various cellular processes like growth, metabolism, and survival. The cascade of events triggered by this pathway is critical for normal cellular function, and its dysregulation is implicated in various cancers and other diseases. This step is pivotal for activating downstream signaling that promotes cell survival, growth, metabolism, and migration [209]. In GBM, mutations in the *PIK3CA* gene, which encodes p110α, the catalytic subunit of class IA PI3K, occur in 4% to 27% of cases [208]. Knockdown of *PIK3CA* significantly impairs cell survival, migration, and invasion in GBM by reducing the activation of AKT and FAK, a non-receptor TK involved in cell adhesion, motility, and survival through its interactions with integrins and growth factor receptors [210]. Isoform-selective inhibitors of p110α, such as A66 and PIK-75, have demonstrated substantial inhibition of GBM cell growth, survival, and migration in vitro [211]. In the absence of PTEN, the p110β isoform becomes crucial for driving GBM cell proliferation, survival, and migration [212]. AKT-FOXO proteins may act as tumor suppressors unless they are degraded by E3 ubiquitin ligases, underscoring the importance of this pathway in tumor biology [213].

Multikinase inhibitors, designed to target multiple kinase pathways simultaneously, have been extensively studied as potential therapies for recurrent GBM. These inhibitors aim to disrupt critical tumor-associated processes, including invasion and metastasis, cancer cell proliferation and survival, and neo-angiogenesis (the formation of new blood vessels) [214, 215]. Regorafenib, a notable example, inhibits various angiogenic kinases such as VEGFR 1–3 and PDGFR-β, along with oncogenic kinases including c-KIT, RET, FGFR, and Raf. Beyond its direct effects on GBM cells, regorafenib primarily exerts therapeutic benefits through its anti-angiogenic properties and its capacity to remodel the TME via multiple mechanisms of action [102].

Regorafenib (BAY 73–4506) is an oral multikinase inhibitor targeting angiogenic, stromal, and oncogenic

RTKs. Structurally similar to sorafenib, regorafenib demonstrates enhanced pharmacological activity, contributing to its increased therapeutic potency [102, 216–218].

The PI3K/AKT/mTOR signaling pathway is crucial in controlling essential cellular functions such as growth, survival, and metabolism. When activated, AKT phosphorylates members of the FOXO subfamily of transcription factors, leading to the suppression of pro-apoptotic proteins. This inhibition of FOXO transcription factors prevents the expression of genes that promote cell death, thereby contributing to enhanced cell survival and proliferation. Dysregulation of this pathway, often through mutations or overactivation of upstream components, can lead to uncontrolled cell growth, making it a significant target in cancer therapies [219]. Akt also phosphorylates and promotes the degradation of the inhibitor of κB, activating NF-κB and increasing the transcription of pro-survival genes. Additionally, Akt regulates MDM2, which suppresses p53 activity, a crucial mediator of cell cycle arrest [220, 221].

mTOR is a key kinase in the PI3K-related family, involved in regulating cellular processes such as growth, metabolism, and survival. It functions as a central component of two distinct protein complexes: mTORC1 (mTOR Complex 1) and mTORC2 (mTOR Complex 2). mTORC1 primarily regulates protein synthesis, cell growth, and autophagy in response to nutrients, growth factors, and stress signals. On the other hand, mTORC2 is involved in controlling cell survival, metabolism, and cytoskeletal organization, and it plays a role in regulating AKT activation. The differential roles of these complexes in cellular regulation highlight their importance in maintaining homeostasis and their potential as therapeutic targets in various diseases, including cancer [222, 223]. A variety of extracellular signals, including growth factors, nutrients, and amino acids, mediate interactions between mTOR and various protein molecules. The PI3K/AKT/mTOR signaling pathway governs multiple growth signals through direct phosphorylation of downstream substrates [224]. In normal cells, receptors like RTKs, including EGFR, the insulin receptor, and G-protein-coupled receptors (GPCRs), are activated by extracellular growth factors. Upon activation, these receptors undergo conformational changes, leading to the recruitment of class I PI3Ks to the plasma membrane. At the membrane, PI3Ks catalyze the phosphorylation of phosphatidylinositol 4,5-bisphosphate [PtdIns(4,5)P2], converting it into phosphatidylinositol 3,4,5-trisphosphate [PtdIns(3,4,5)P3]. This conversion is a critical step in the activation of downstream signaling pathways that regulate cellular processes such as growth, metabolism, and survival. The accumulation of PtdIns(3,4,5)P3 at the membrane serves as a docking site for signaling proteins. PTEN, a tumor suppressor, counteracts this process by dephosphorylating PtdIns(3,4,5)P3, reverting it to PtdIns(4,5)P2 [225]. The mTOR signaling pathway is hyperactivated in nearly 90% of GBMs. However, the mTOR inhibitor rapamycin has shown limited efficacy in clinical trials, primarily due to persistent activation of mTORC2 signaling [226, 227]. Studies have found that activated mTORC2 is scarcely detectable in normal brain tissue but is significantly upregulated in GBM cell lines. Furthermore, 86% of GBM tumor samples exhibit overexpression of Rictor, a key component of the rapamycin-insensitive mTORC2 complex, with 70% displaying elevated mTORC2 activity, consistent with in vitro findings [228].

Akt regulates mTOR activation through both direct and indirect mechanisms, with mTOR functioning in two distinct complexes: mTORC1 and mTORC2. mTORC1 comprises mTOR, Raptor, mLST8, and PRAS40, and it activates S6K1, which in turn phosphorylates S6, driving increased cell proliferation and growth. Additionally, mTORC1 inhibits 4E-BP1, facilitating the assembly of the eIF4F complex and enhancing protein translation [229]. In contrast, mTORC2, composed of mTOR, Rictor, Sin1, and mLST8, is less well-characterized compared to mTORC1 [229]. However, it has been shown to activate PKC, thereby enhancing its kinase activity [230]. mTORC2 also plays roles in cell survival and the regulation of cytoskeletal dynamics [231]. Furthermore, mTOR regulates HIF1α, which induces the secretion of VEGF, thereby promoting angiogenesis [231]. Deregulation of the mTOR pathway is closely associated with radioresistance in GBM tumors, highlighting its significance in therapeutic resistance [232]. A detailed overview of mTORC1 inhibitors is provided in Table 7 (Fig. 10).

TGF-β signaling

TGF-β is a multifunctional cytokine that influences various cell types and plays a crucial role in regulating key cellular processes, including proliferation, immune response, apoptosis, and adhesion [239, 240]. TGF-β binds to TGF-βRII, initiating the formation of a heterodimeric complex with TGF-βRI, which becomes phosphorylated [241]. This activation phosphorylates SMAD proteins via TGF-βRI, triggering their activation. Activated SMAD proteins, which mediate TGF-β signal transduction, form complexes that regulate the transcription of specific target genes. These complexes play a crucial role in regulating a variety of cellular functions, including proliferation, differentiation, and apoptosis. Beyond the canonical SMAD pathway, TGF-β signaling also activates non-SMAD pathways, engaging other signaling molecules that drive diverse cellular responses. These responses are pivotal in processes like tumorigenesis, fibrosis, and immune

Table 7 Summary of mTORC Inhibitor Studies and Key Findings in GBM

Inhibitor	Study Type	Key Findings	Ref.s
Everolimus	Phase II	No notable difference in PFS was observed when compared to the control group; however, an increase in toxicities was noted	[233, 234]
Ridaforolimus	Phase I (peri-surgical)	Study suspended due to slow patient accrual and drug administration challenges post-surgery	[235]
Temsirolimus	Phase II	Median OS of 14.8 months (vs. 16.0 in control); Median PFS of 5.4 months (vs. 6.0 in control)	[236]
Second/Third-Generation Inhibitors (INK128, Torin 1 and AZD8055)	Preclinical/Exploratory	TORKi (INK128, Torin 1, AZD8055) and bivalent inhibitors target mTOR resistance mutations but face challenges in crossing BBB	[237, 238]

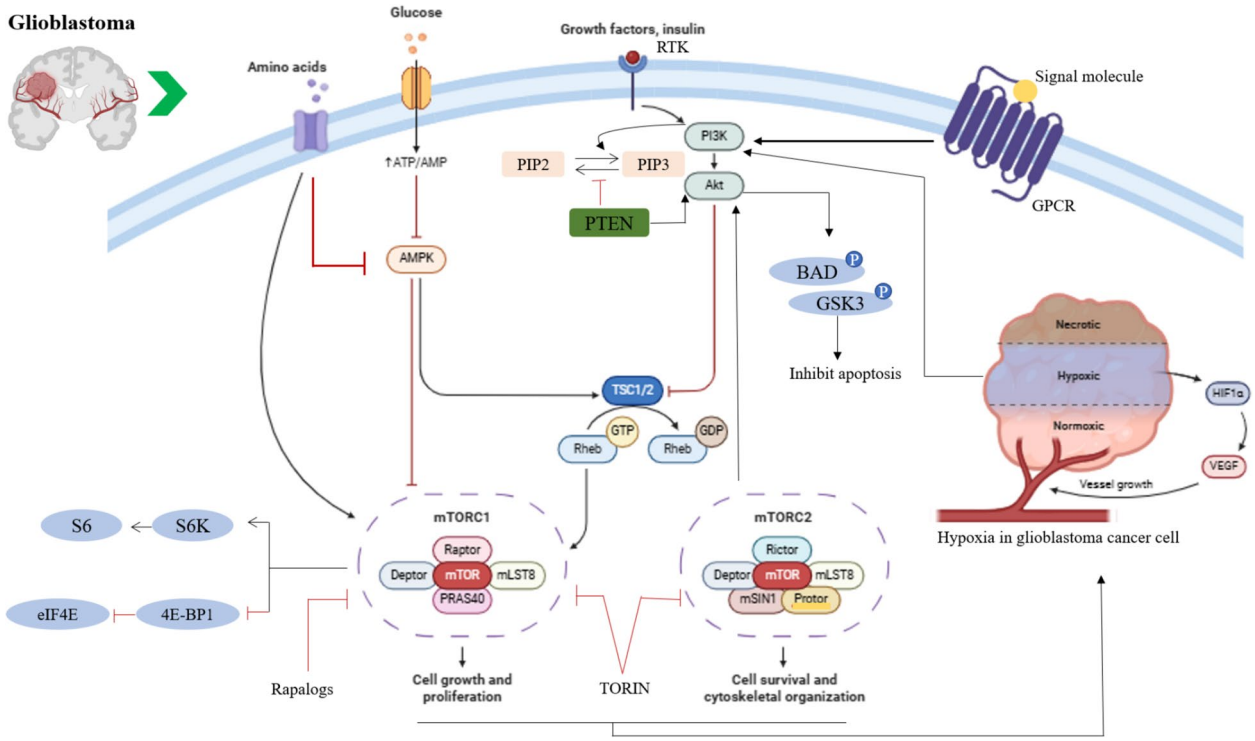

Fig. 10 The PI3K/AKT/mTOR pathway in GBM progression. This pathway plays a critical role in GBM progression. Elevated glucose levels increase the ATP/AMP ratio, inhibiting AMPK, which normally suppresses mTORC1. This inhibition activates mTORC1, driving protein synthesis, cell growth, and proliferation. RTKs activate PI3K, converting PIP2 to PIP3, which activates AKT, a central regulator of cell survival and growth. The loss of PTEN, a tumor suppressor, results in sustained AKT activation, leading to uncontrolled signaling. AKT promotes GBM cell survival by inhibiting apoptosis through the phosphorylation of targets such as BAD and GSK3. Under hypoxic conditions, HIF1 and VEGF interact with this pathway to enhance angiogenesis and metabolic adaptation, contributing to tumor aggressiveness. Inhibitors like TORIN and Rapalogs offer potential therapeutic strategies by targeting mTOR-dependent signaling in GBM

regulation. By modulating both SMAD-dependent and non-SMAD signaling routes, TGF-β signaling can influence the tumor microenvironment, promoting cancer progression, resistance to apoptosis, and the alteration of tissue architecture [241, 242]. For example, TGF-β stimulates the Ras/Raf/MEK/ERK pathway by promoting GTP loading of Ras [243]. Under normal physiological conditions, TGF-β acts as a tumor suppressor by limiting cell proliferation. However, mutations in the TGF-β signaling pathway can disrupt this growth-inhibitory function, leading to loss of cellular sensitivity and uncontrolled proliferation [241]. Dysregulated TGF-β signaling contributes to pathological processes such as inflammation, invasion, metastasis, angiogenesis, and immune evasion. In GBM, aberrant TGF-β signaling is a key factor in tumor development and progression [241]. GBM cells have been found to secrete TGF-β2, which plays a critical role in suppressing the

anti-tumor immune response, thereby facilitating tumor progression and enabling immune evasion [244].

The upregulation of TGF-β1 and TGF-β2 significantly impacts the TME by promoting immune evasion, tumor cell invasion, and treatment resistance. Enhanced TGF-β signaling activates various downstream pathways that drive glioma cell proliferation, migration, and metastasis, contributing to the aggressive behavior of GBM [245]. Furthermore, higher expression levels of TGF-β2 are associated with poorer clinical outcomes in GBM patients [246]. Mechanistically, TGF-β facilitates the mesenchymal phenotype in GBM cells through the activation of SMAD2 and ZEB1, a transcription factor critical for EMT. This activation increases the migratory and invasive capacities of glioma stem cells, further exacerbating the tumor's aggressive nature [247, 248] (Fig. 11 and Table 8).

Notch signaling

The mRNA and protein expression levels of Notch1, Notch4, Dll4, Jagged1, Hes1, Hey1, Dll1, Hey2, and CBF1 are elevated in brain tumor cells compared to normal brain tissue. This upregulation is associated with increased levels of VEGF and pAKT and decreased PTEN expression [272–276]. Notch1 expression is notably higher in patients with survival durations exceeding one year compared to those surviving less than a year. However, its overexpression correlates with poorer overall survival, suggesting a complex role for Notch1 in gliomagenesis [276–278]. Notch1 and Notch4 expression levels positively correlate with GFAP and vimentin, respectively, while Notch4 expression increases with tumor grade and is predominantly observed in primary tumors [279]. In GBM tissues, Notch2 expression correlates positively with stemness markers such as nestin and SOX2, astrocyte differentiation markers like vimentin and GFAP, and anti-apoptotic proteins including BCL6 and BCL-W. Conversely, Notch2 expression is negatively correlated with oligodendrocyte differentiation markers (e.g., Olig2, CNP, and PLP1) and pro-apoptotic proteins such as BAX and BCLAF1 [280]. Overexpression of Hey1, linked to survival and tumor grade, may result from disrupted Notch and E2F signaling pathways. Elevated Hey1 expression in neural stem cells (NSC) has been shown to promote neurosphere formation and GBM cell proliferation. In contrast, decreased expression

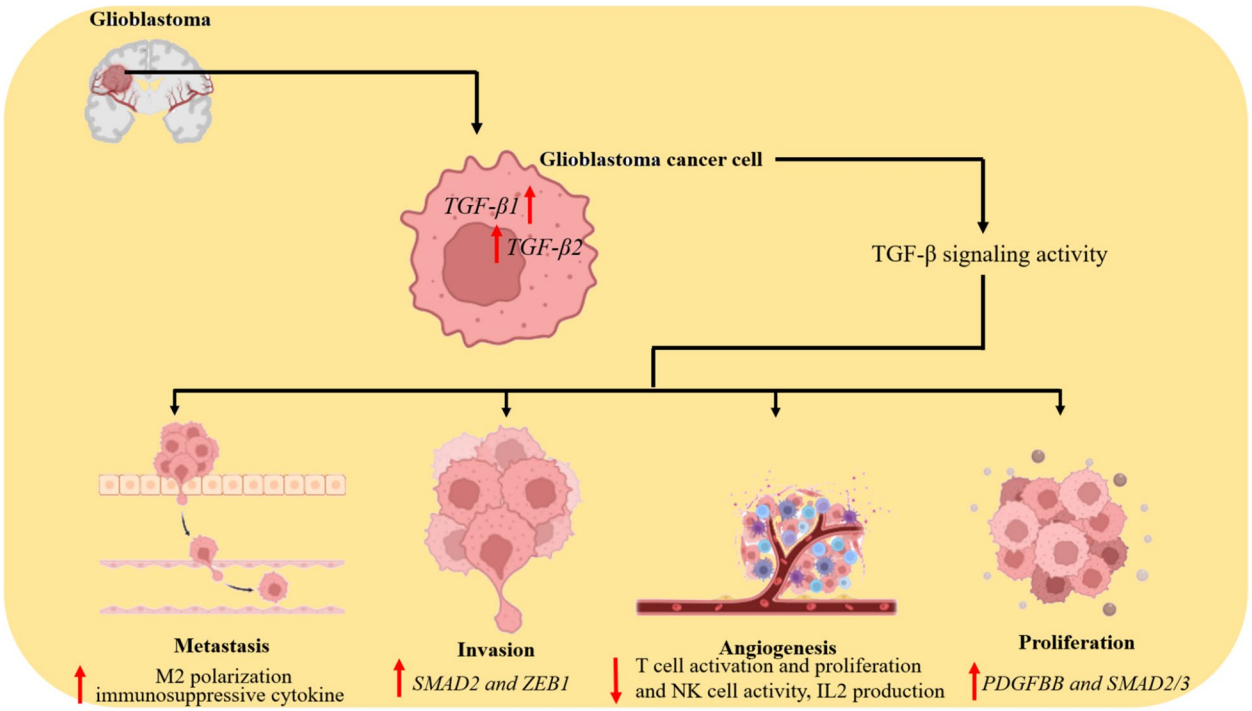

Fig. 11 TGF-β signaling in GBM progression. In GBM cells, upregulation of TGF-β1 and TGF-β2 activates the TGF-β signaling pathway, triggering a cascade of cellular events that drive tumor progression. This activation increases the expression of PDGF-BB and SMAD2/3, which enhance cell proliferation and tumor growth. Elevated SMAD2 and ZEB1 levels further promote tumor cell invasion, contributing to the aggressive and metastatic nature of GBM. TGF-β signaling also plays a crucial role in immune modulation by suppressing T cell activation and proliferation, reducing NK cell activity, and downregulating IL-2 production, thereby weakening the immune response and enabling tumor immune evasion. Additionally, TGF-β signaling drives M2 polarization of macrophages, leading to the secretion of immunosuppressive cytokines, which further enhance immune suppression within the TME

Table 8 Summary of TGF-β Family Effects in GBM

Aspect	TGF-β Effects in GBM	BMP Effects in GBM	Ref.s
Oncogenic Pathways	Acts as an oncogenic factor; high expression levels correlate with poor prognosis	BMPs act as tumor suppressors; induce differentiation and reduce tumorigenicity in GSCs	[246, 249–252]
SMAD Signaling Modulation	Enhanced by USP15 amplification; SMAD7 stabilization promotes TGF-β signaling	Promotes astrocytic differentiation via SMAD1/5 signaling	[253–257]
Cell Proliferation	Induces PDGFBB expression via SMAD2/3; promotes glioma cell growth and stemness through SOX4 and SOX2 expression	BMP4 impairs tumor-initiating capacity of GSCs and downregulates SOX2 expression in sensitive GBM cells	[249, 250, 258–260]
Invasion and Migration	Induces EMT via ZEB1, Claudin 4, and lncRNA-mediated pathways; promotes lamellipodia formation and invasion	Suppresses invasion and migration by reducing stem cell properties (e.g., CD133 expression) and EMT	[71–73, 75–80, 96]
Resistance Mechanisms	Enhances chemotherapy resistance (e.g., TMZ) by MGMT accumulation and miRNA modulation	BMP2 sensitizes resistant GBM cells to TMZ by reducing HIF1α activity and MGMT expression	[261–264]
GSC Maintenance	Maintains GSC self-renewal through NOX4, LIF, and SOX2/4 pathways; counteracts differentiation	BMP4 promotes differentiation and apoptosis via EPHA6 phosphorylation and DLX2 induction	[257, 265–268]
Microenvironment Modulation	TGF-β increases microtube networks via TSP1, supporting cell communication and invasion	BMP9 suppresses trans-differentiation of GSCs into tumor-derived endothelial cells	[269, 270]
Therapeutic Implications	TGF-β inhibitors reduce SMAD2 phosphorylation, GSC self-renewal, and EMT, offering potential therapeutic avenues	BMP signaling offers therapeutic potential by targeting GSCs for differentiation and sensitizing to chemotherapy	[254, 264, 271]

levels of Notch1, Notch2, MAML1, and p300 have been reported in some GBM studies [281, 282]. In secondary GBMs, disrupted Notch signaling, evidenced by reduced Hes1 expression, correlates with overexpression of ASCL1 [283]. Notch signaling has been linked to GBM molecular subtypes, with the mesenchymal subtype—characterized by high aggressiveness—showing significant enrichment of Notch-related genes. This enrichment is particularly evident in patients with elevated p-STAT3 levels, indicating a synergistic interaction between Notch and STAT3 pathways [284]. Additionally, Notch signaling is highly expressed in the classic GBM subtype [285]. The proneural GBM subtype, associated with IDH mutations in most tumors, typically exhibits a proneural gene expression pattern. However, only about 30% of GBMs with proneural profiles harbor IDH mutations [29]. IDH-mutant gliomas, typically lower grade, show high and uniform expression of Dll3, whereas approximately 50% of IDH-wild-type GBMs either lack Dll3 expression or exhibit it in isolated cells, primarily in non-mesenchymal areas [286, 287]. The non-canonical Notch pathway also plays a significant role in glioma development [288]. Epigenetic modifications, a hallmark of GBM, offer reversible therapeutic targets, although their role in Notch signaling remains incompletely understood. Decreased methylation of CpG islands within the *Hey1* promoter in GBM samples has been associated with *Hey1* overexpression [289]. Treatment with sodium butyrate (NaB), an HDAC inhibitor, in 4910 and 5310 xenograft cell lines reduced *Hey1* expression, increased DNMT1 levels, and induced apoptosis in GBM cells. Silencing *Hey1* significantly decreased cell invasion, migration, and proliferation [289] (Fig. 12).

Ras/MAP/ERK pathway

This signaling pathway, activated by cell surface receptors, governs essential cellular processes such as angiogenesis, proliferation, migration, and survival, which are crucial for maintaining normal tissue homeostasis and play a significant role in tumor growth and metastasis. Ras activation occurs when GDP is exchanged for GTP, triggering the activation of MAP kinases. These kinases phosphorylate downstream ERK, amplifying the signaling cascade [290]. This pathway is frequently activated in various tumors due to mutations or overexpression of cytokine receptors such as Flt-3, Kit, and Fms, in either wild-type or mutated forms [291]. Activation of the Ras/MAP/ERK pathway also stimulates HIF-1α, a critical factor in tumorigenesis, which subsequently activates VEGF to promote angiogenesis and support tumor growth [292].

In gliomas, the Ras-RAF-ERK signaling pathway is often hyperactivated, primarily due to elevated activity of upstream regulators like EGFR and PDGFR [293]. While Ras mutations are rare in gliomas, numerous studies have employed oncogenic Ras genes to explore the effects of dysregulated Ras-RAF-ERK signaling in gliomagenesis and associated phenotypic changes.

In 2000, tissue-specific viral expression vectors were engineered to activate K-Ras (G12D) and Akt expression

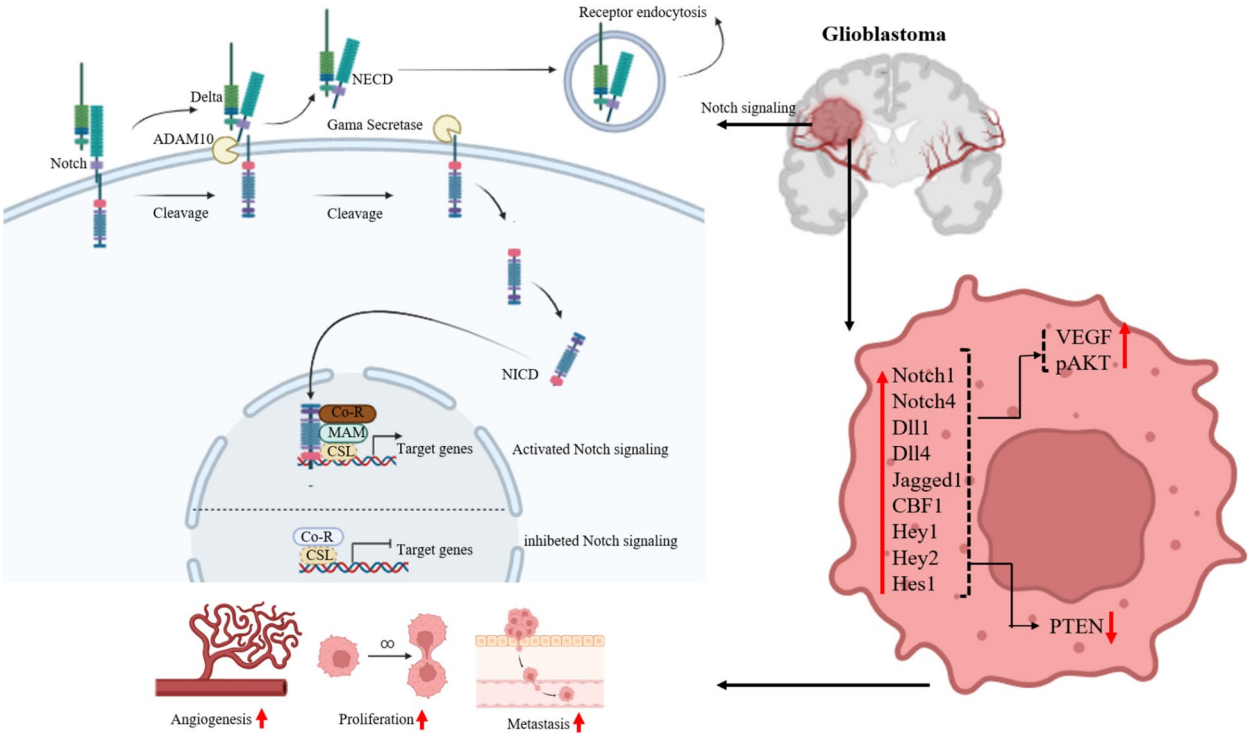

Fig. 12 Notch signaling in GBM progression. Notch signaling is activated through ligand-receptor interactions, where the Delta ligand binds to the Notch receptor. This binding triggers Notch cleavage by ADAM10 and Gamma-secretase, releasing the Notch Intracellular Domain (NICD). The NICD translocates to the nucleus, where it interacts with Co-Repressors (Co-R) and the CSL transcription factor to regulate the expression of target genes involved in proliferation, differentiation, and survival. In contrast, when NICD is absent, Notch signaling is inhibited, and Co-R and CSL complexes suppress gene expression. Dysregulation of this pathway is associated with elevated VEGF and pAKT levels and decreased PTEN expression, promoting tumor growth. These changes drive increased tumor cell proliferation, angiogenesis, and metastasis, contributing to the aggressive behavior of GBM

in astrocytes through the GFAP promoter, and in neural progenitors via the nestin promoter, within a mouse model. This method provided valuable insights into the role of these pathways in glioma pathogenesis [294]. While the expression of K-Ras G12D or Akt alone was inadequate to trigger GBM formation, their combined expression resulted in high-grade gliomas that exhibited histological features resembling human GBMs [294]. Further investigations demonstrated that co-expression of K-Ras G12D and loss of the INK4a-Arf locus was essential for transforming astrocytes and neural progenitors into GBMs, underscoring the synergistic impact of these genetic changes in promoting aggressive gliomas [295, 296]. In 2008, researchers discovered that the constitutive activation of RAF-1, when combined with either Akt activation or the loss of the INK4a-Arf locus, greatly accelerated glioma oncogenesis in mice. This study highlighted the critical role of these molecular alterations in driving the rapid development and progression of gliomas. The simultaneous activation of RAF-1 and Akt, along with the loss of tumor suppressor genes such as INK4a-Arf, creates a highly favorable environment for glioma formation, contributing to the aggressive nature of the disease. These findings emphasize the complex interplay between genetic alterations in key signaling pathways that promote glioma oncogenesis. This study highlighted the collaborative role of these genetic alterations in driving rapid glioma development [297] (Fig. 13).

p14ARF/MDM2/p53 pathway

The p53 gene encodes a protein crucial for regulating cellular stress responses by controlling the expression of target genes involved in vital processes, including cell cycle regulation, apoptosis, differentiation, senescence, DNA repair, and neovascularization [240, 298]. In response to DNA damage, p53 is activated and triggers the expression of genes such as p21Waf1/Cip1, which play critical roles in regulating cell cycle progression, particularly at the G1 phase, thereby halting the cycle to prevent the proliferation of damaged cells [299, 300]. The MDM2 gene encodes a potential transcription factor that, when overexpressed, enhances the tumorigenic potential of cells. MDM2 forms a strong complex with p53, suppressing its transcriptional activity by binding

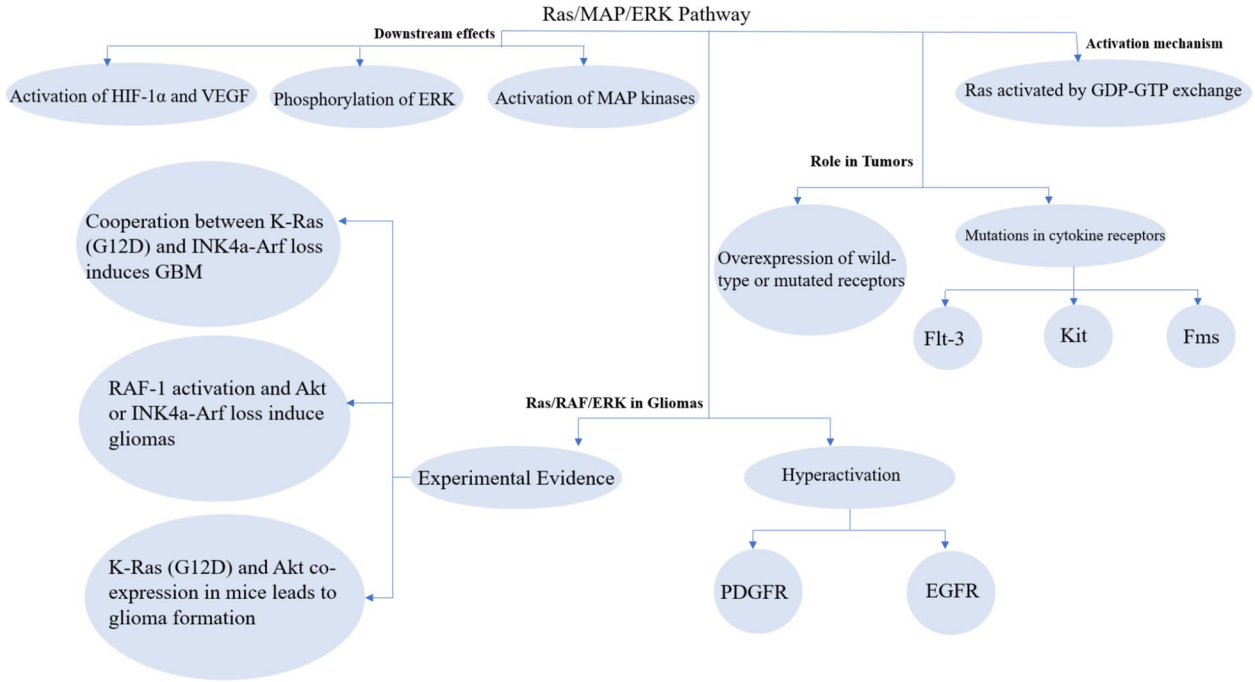

Fig. 13 Ras/MAP/ERK Pathway in GBM. This schematic illustrates the key components of the Ras/MAP/ERK pathway in GBM, including its activation mechanisms, downstream effects, contributions to tumor development, and the specific role of the Ras/RAF/ERK cascade in gliomas

to the N-terminal transactivation domain. Additionally, MDM2 facilitates p53 ubiquitination, leading to its proteasomal degradation as another regulatory mechanism [301, 302]. Conversely, wild-type p53 promotes MDM2 gene transcription, establishing a negative feedback loop that controls both p53 levels and activity [303–305]. This loop regulates MDM2 expression and ensures proper p53 function.

The p14ARF gene, located within the CDKN2A locus, encodes a protein that interacts with MDM2 to prevent p53 degradation and inhibit MDM2's transactivation functions [306–308]. In turn, p53 negatively regulates p14ARF expression [300].

The occurrence of a p53 mutation, often following IDH1/2 mutations, marks a key genetic event, except in Li-Fraumeni syndrome cases. This progression may lead to glioma cell differentiation into an astrocytic lineage, followed by chromosome 1p/19q loss, facilitating a shift toward an oligodendroglial phenotype [309, 310]. GBMs with IDH1/2 mutations often carry p53 mutations, strongly linked to tumor progression and disease-defining characteristics [311]. Around two-thirds of low-grade diffuse astrocytomas contain p53 mutations, a frequency that is similarly observed in anaplastic astrocytomas and secondary GBMs. These mutations impair the tumor-suppressive role of p53, enabling uncontrolled cell proliferation and tumor progression. In secondary GBMs, p53 mutations are pivotal for the transition from low-grade to high-grade tumors, emphasizing their role in GBM evolution and their potential as therapeutic targets [312]. While p53 mutations are less common in primary GBMs, they occur in about 25% of cases [305, 313]. In secondary GBMs, 57% of p53 mutations are concentrated in hotspot codons 248 and 273, whereas primary GBMs exhibit a broader mutation distribution across all exons, with only 17% at these hotspots. This distribution may reflect secondary genomic instability events during tumor progression. Though p53 mutations are the most frequent alterations in the p53 pathway in gliomas, other pathway genes, including p14ARF, MDM2, and MDM4, also show alterations [314]. In GBMs, loss of p14ARF expression is often associated with promoter methylation or hemizygous deletion, while mutational inactivation of p14ARF is relatively rare [315, 316]. Promoter methylation of p14ARF is more common in secondary GBMs than in primary GBMs. However, the overall frequency of p14ARF alterations does not significantly differ across GBM subtypes [315] (Fig. 14).

ATM/Chk2/p53 pathway

Recently, the ATM/Chk2/p53 pathway has garnered significant attention alongside the well-established p14ARF/MDM2/p53 pathway. Squatrito et al. demonstrated that the loss of key components in the ATM/Chk2/p53 pathway accelerates glioma progression and increases resistance to RT [317]. In response to ionizing radiation, cells

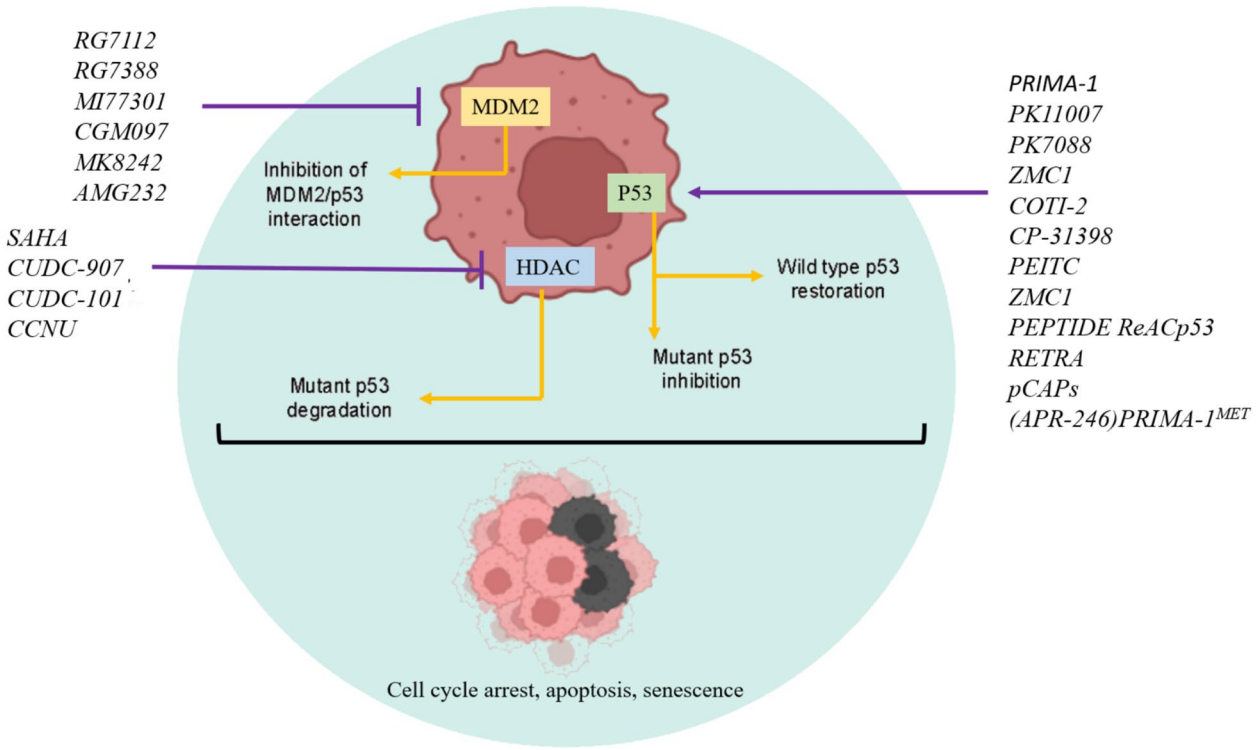

Fig. 14 The high prevalence of p53 mutations in GBM underscores their potential as key targets for precision medicine therapies. Strategies to reactivate or restore wild-type (wt) p53 function hold significant promise for treating GBM and other cancers. Therapeutic approaches focus on enhancing wt-p53 activity or counteracting gain-of-function (GOF) mutant p53. These include inhibiting the MDM2/p53 interaction to prevent wt-p53 degradation, restoring wt-p53 function in tumors with mutant p53, and targeting GOF mutant p53 for degradation. Together, these strategies highlight the potential of p53-targeted therapies to improve outcomes in GBM. (To review the function of each item mentioned in the figure, please refer to the list of abbreviations provided in the article)

activate critical sensor kinases, including ATM, ATR, and DNA-PK, which are integral to the DNA damage response. These kinases initiate repair processes that are essential for maintaining genomic stability [318, 319]. These kinases phosphorylate downstream mediators, such as checkpoint kinases Chk1 and Chk2, which subsequently activate cell-cycle checkpoints and/or apoptosis.

Chk2 independently regulates p53-dependent apoptosis and acts as a tumor suppressor, even in the absence of ATM [320–323]. While earlier studies reported a low frequency of Chk2 mutations, typically around 6% or absent altogether, the TCGA study revealed a 22% incidence of glioma patients with a single-copy loss of the chromosomal region containing Chk2. This chromosomal loss correlated with a significant reduction in Chk2 mRNA expression, suggesting that Chk2 functions as a critical tumor suppressor in a subset of glioma patients [317, 324, 325] (Fig. 15).

RB signaling pathway

The RB (Retinoblastoma Protein) pathway plays a critical role in halting cell cycle initiation and progression, functioning in tandem with the p53 pathway. The RB1 protein, a 107 kDa protein encoded by the RB1 gene located at 13q14, regulates the transition from the G1 phase to the S-phase of the cell cycle [326, 327]. Disruptions in this regulation can occur through alterations in RB1, CDK4, or CDKN2A, leading to improper G1 to S phase transitions. Such pathway inactivation is a common feature of both primary and secondary GBMs [327]. Approximately 80% of GBMs exhibit genetic alterations in this pathway, including RB1 loss (40%), homozygous CDKN2A deletions (40%), and CDK4 amplification (15%) [328], which are generally mutually exclusive [328–330]. Data from the TCGA pilot project revealed that 77% of GBMs harbor genetic alterations in the RB signaling pathway. These alterations include homozygous deletions or mutations in CDKN2A (52%), homozygous deletions in CDKN2B (47%) and CDKN2C (2%), amplification of CDK4 (18%), amplification of cyclin D2 (2%), amplification of CDK6 (1%), and mutations or homozygous deletions in RB1 (11%) [32]. However, alterations in the RB pathway alone are insufficient to drive tumor formation. For instance, EGFR amplification, which activates the

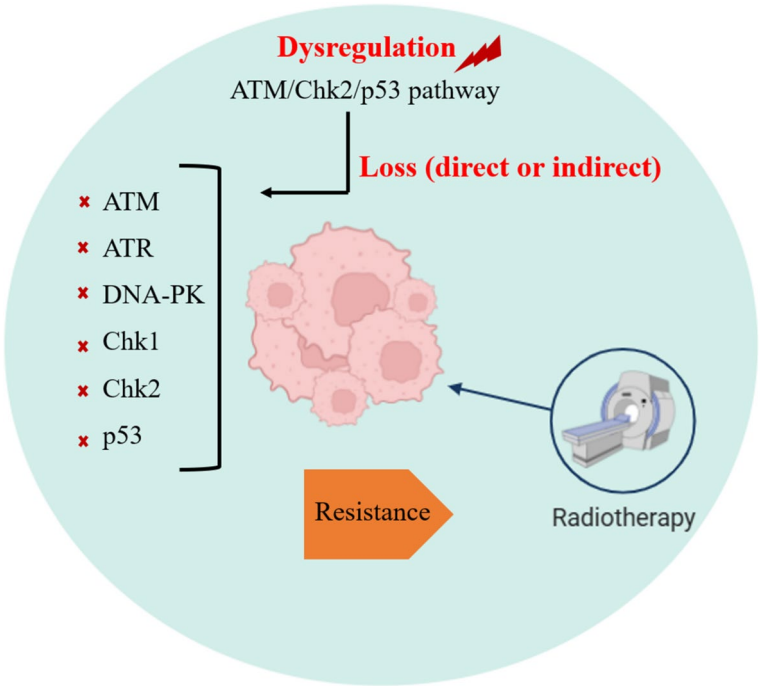

Fig. 15 ATM/Chk2/p53 pathway in GBM

PI3K pro-growth signaling cascade, frequently co-occurs with CDKN2A deletions [331, 332]. The TCGA study further indicates that CDKN2A loss is predominantly associated with the classical subtype of GBM [32].

Taken together, these findings highlight the diverse and complex roles of signaling pathways in GBM. These pathways, encompassing processes such as proliferation, apoptosis, angiogenesis, and immune evasion, interact dynamically and in a context-dependent manner, significantly impacting tumor progression, therapeutic resistance, and overall disease outcomes (Fig. 16).

Sonic hedgehog (SHH) signaling pathway

The TME and stromal components are intricately linked to tumorigenesis, metastasis, and invasion [381–383]. The TME and stroma primarily consist of endothelial cells, adipocytes, immune cells, and cancer-associated fibroblasts (CAFs) [384]. CAFs secrete soluble factors that activate cancer cells, facilitating tumor metastasis and contributing to chemotherapy resistance [385–387]. The recombinant human Sonic Hedgehog N-terminal peptide (rhSHH) activates the SHH signaling pathway, resulting in increased mRNA and protein expression of matrix metalloproteinases MMP2 and MMP9. Additionally, a positive correlation has been identified between GLI1 protein expression and MMP2 and MMP9 levels, which enhance the adhesive and invasive properties of GBM cells [387]. Gap junctions also play a pivotal role in tumor growth and progression. In an in vitro model, modulated SHH signaling was shown to influence CX43-mediated intercellular communication, emphasizing the significance of gap junctions in cancer development [388]. Modulating SMO, using an agonist (taxamine) or antagonist (cyclopamine), altered CX43 expression, impacting cellular functions. Activation of SMO promoted cell proliferation and migration, while inhibiting the CX43 channel mitigated the effects of SMO activation [388].

Primary cilia (PC) function as cellular antennae in GBM, facilitating and regulating multiple signaling pathways, particularly the SHH pathway. SHH levels are significantly elevated in GBM cells compared to normal brain tissue, with overexpression shown to promote neuroectodermal angiogenesis during mouse embryonic development [389–391]. FLT1 is markedly upregulated in GBM cells, leading to elevated SHH expression [391]. FLT1, a receptor TK with a high affinity for VEGF-A, drives tumor progression and metastasis [392]. VEGF-A, a critical factor in GBM angiogenesis, binds to FLT1 and activates a downstream signaling cascade, stimulating tumor cell proliferation, progression, and blood vessel formation [393]. Brain tumor-initiating cells secrete DHH ligands that activate the paracrine DHH/PTCH2 signaling cascade, enhancing vascular permeability, promoting angiogenesis, and contributing to GBM growth and progression [394]. Furthermore, studies have

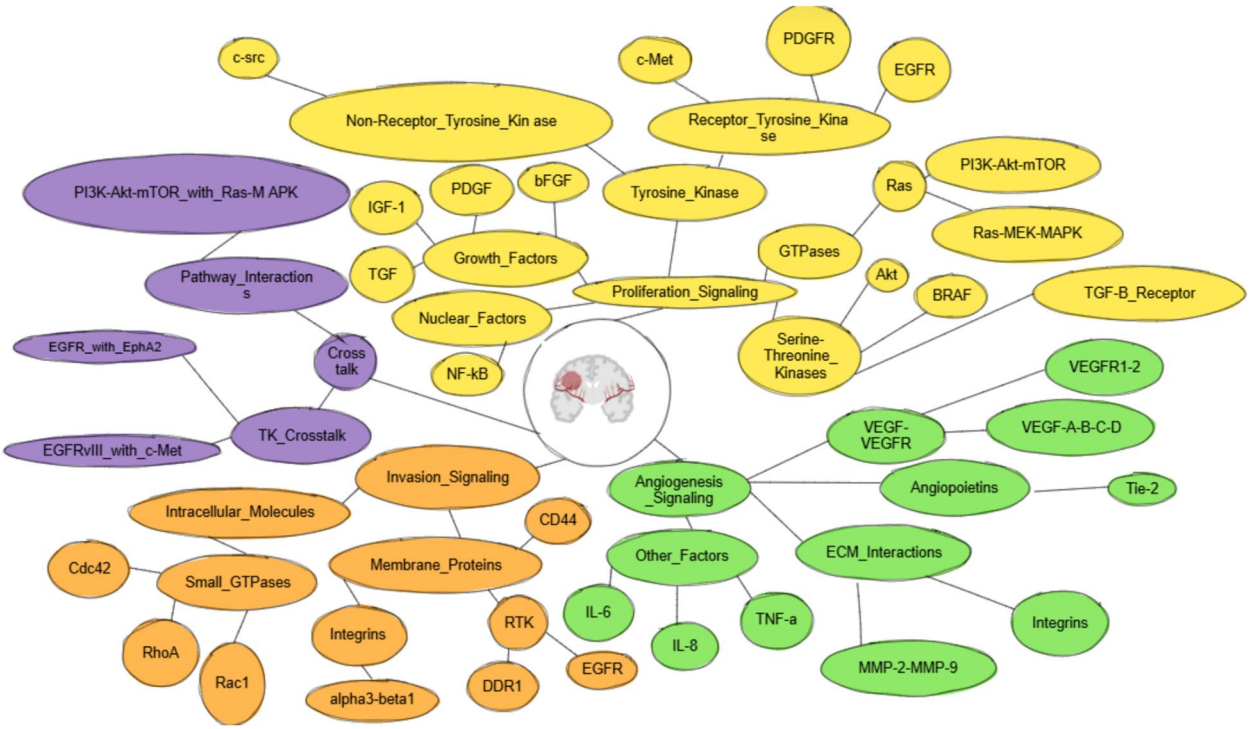

Fig. 16 Complex and dynamic interactions between signaling pathways in shaping GBM progression, therapeutic resistance, and disease outcomes (*To draw this figure, data from the reports in references [39, 146, 155, 245, 249, 294, 333–380] were used.)

reported increased CtBP2 expression and decreased zinc finger protein ZBTB18 expression in GBM tissues, with a negative correlation between these levels [395]. CtBP2 short hairpin (sh)RNA interacts with ZBTB18, causing cell cycle arrest at the G0/G1 phase and inhibiting the SHH-Gli1 signaling pathway, ultimately reducing tumor volume [395]. However, the mechanism by which CtBP2 affects SHH gene expression remains unclear. Consequently, targeting FLT1 or CtBP2 presents a promising avenue for the development of anti-metastatic therapies.

The progression of GBM is driven by a complex network of signaling pathways. Disrupting key pathways, such as the PKA-dependent PI3K/AKT and SHH/GLI1 pathways, plays a pivotal role in inhibiting GBM cell migration and invasion. This dual interference impairs critical processes associated with tumor progression and metastasis, offering a promising therapeutic strategy for GBM treatment. By inhibiting these pathways, the downstream effects that promote GBM cell migration and invasion are blocked, thereby limiting tumor progression [396, 397].

Studies have shown that the SHH signaling pathway enhances cancer cell plasticity by modulating cell adhesion to the extracellular matrix. This modulation increases cell motility and aggressiveness, driving cancer progression and leading to worse outcomes for patients [395, 396]. Statistical analysis of the TCGA dataset revealed a strong association between upregulation of the SHH pathway and significantly reduced overall survival, underscoring its role in the poor prognosis of GBM patients [391].

Hedgehog-interacting protein (HHIP) serves as an antagonist of SHH, DHH, and IHH. Immunohistochemical analysis has demonstrated that HHIP expression functions as an independent prognostic marker, with higher levels of HHIP correlating with better outcomes in GBM patients [398].

Although GLI1 was first recognized as an amplified gene in malignant human gliomas, its amplification is relatively uncommon in most cancers, including GBM [399, 400]. However, as a critical downstream target of the SHH pathway, GLI1 mRNA expression is a reliable marker of SHH pathway activity [401]. Elevated GLI1 protein levels are observed in various cancers and are frequently associated with tumor progression. This upregulation contributes to aggressive tumor behavior by driving cell proliferation, survival, and metastasis [402, 403]. Low GLI1 mRNA expression has been inversely linked to survival in GBM patients. Comparatively, GBM displays significantly lower GLI1 mRNA levels than high-SHH medulloblastoma (MB) but higher levels than low-SHH MB. Interestingly, GLI1 mRNA expression in

GBM follows a continuous distribution rather than discrete high or low categories, reflecting a nuanced role in GBM pathogenesis and the complexity of its expression [404]. GLI1 promotes its own nuclear import in GBM cells through its interaction with the transcription factor FOXM1 (Forkhead Box M1). This interaction amplifies the expression of GLI1 target genes, facilitating tumor cell growth, survival, and invasiveness. The FOXM1-mediated regulation of GLI1 underscores a critical mechanism for activating the Hedgehog signaling pathway in GBM [405].

In GBM cells, knockout of the USP48 gene inhibits cell proliferation and the expression of GLI1 target genes. USP48 prevents the proteasomal degradation of GLI1 by removing ubiquitin, stabilizing the protein. Inhibition of USP48 enhances GLI1 degradation, reducing its activity and suppressing GBM progression [403]. Furthermore, GLI1 mediates the effects of USP48 on cell proliferation and tumorigenesis. The SHH pathway also induces USP48 expression through GLI1 trans-activation, establishing a feedback loop. This loop amplifies GLI1 activity, as increased USP48 expression stabilizes GLI1, enhancing its signaling and the tumorigenic potential of GBM cells [403]. Similarly, Engrailed 1 (EN1) is highly expressed in GBM cells and tissues, where it positively regulates GLI1 levels. EN1 enhances GLI1 expression and modulates SHH signaling by regulating the length of primary cilia and the cilia transport-related protein TUB-like Protein 3. These cilia dynamics influence processes such as cell proliferation, colony formation, migration, and in vivo tumorigenesis, highlighting EN1's role in SHH signaling and its contribution to GBM aggressiveness [406]. TGLI1, a functionally enhanced form of GLI1, exhibits greater potency in promoting the expression of angiogenic heparanase. Both in vitro and in vivo studies show that TGLI1 drives angiogenesis and tumor growth in GBM more effectively than GLI1. As a novel mediator in the Hedgehog signaling pathway, TGLI1 enhances GBM angiogenesis by targeting heparanase as a transcriptional target. These findings provide insight into tumor angiogenesis and invasive growth mechanisms, positioning TGLI1 as a potential therapeutic target in GBM treatment [406].

Studies have demonstrated that activating metabolic glutamate receptor subtype 4 (mGluR4) and utilizing the compound naringin effectively inhibit GLI1 expression in cells, thereby disrupting the SHH signaling pathway. This inhibition suppresses cell proliferation and promotes apoptosis, ultimately reducing GBM cell growth. These findings suggest that modulating GLI1 expression through these mechanisms presents a potential therapeutic approach for controlling GBM progression [407, 408]. By targeting mGluR4 activation or employing compounds like naringin to inhibit GLI1, novel strategies may emerge for limiting tumor progression in GBM through SHH pathway inhibition.

Crosstalk between the mTORC1/2 and SHH signaling pathways has been identified, with evidence that both pathways influence each other's activity. This interaction implies that mTORC1/2 can modulate SHH pathway signaling and vice versa, significantly impacting cell growth, survival, and tumor progression. Targeting this crosstalk could lead to more effective therapeutic strategies, particularly in GBM, where both pathways are frequently dysregulated [409]. In GBM, increased mTORC2 activity upregulates key components of the SHH signaling pathway, including GLI1, GLI2, and PTCH1. Knocking down GLI2 via lentiviral-mediated shRNA resulted in the downregulation of genes associated with both the SHH and Wnt signaling pathways, such as leucine-rich repeat-containing G-protein coupled receptor 5. This downregulation inhibited tumor cell proliferation, reduced invasiveness, and promoted apoptosis, highlighting the potential of targeting mTORC2-mediated SHH signaling as a therapeutic approach for GBM [410]. Furthermore, the overexpression of GLI2DC, a truncated C-terminal form of GLI2, has been shown to counteract the activity of GLI transcription factors. This overexpression inhibited the proliferation of glioma-initiating cells in culture and in vivo. Additionally, targeting CDC2, a downstream factor of GLI2, effectively prevented glioma-initiating cell proliferation. This suggests a mechanism for controlling glioma cell proliferation by manipulating GLI2 activity [411]. The findings emphasize that the SHH/GLI/CDC2 signaling cascade is crucial for the proliferation and malignancy of glioma-initiating cells. Since GLI2 regulates multiple downstream oncogenic and tumor-suppressing pathways and plays a pivotal role in the neoplastic microenvironment, targeting GLI2 could simultaneously disrupt several interconnected pathways. This approach holds promise as an effective therapeutic strategy for inhibiting glioma initiation and progression by addressing the molecular networks involved in tumor growth and malignancy. The SHH, mTOR, Notch, and Wnt/β-catenin signaling pathways play pivotal roles in regulating the stemness and self-renewal capacity of glioblastoma stem cells (GSCs). However, the ability of GSCs to self-renew, differentiate abnormally, and develop resistance to radiotherapy and chemotherapy significantly contributes to GBM recurrence and its invasive nature following conventional treatments. These pathways not only maintain GSC stemness but also enhance GSC resilience against therapeutic interventions, driving the aggressive behavior and poor prognosis associated with GBM [412–416].

The mechanisms underlying GBM cell migration and invasion are highly complex, involving interrelated biological processes. Key steps include the adhesion of GBM cells to the extracellular matrix (ECM), ECM remodeling, and ECM degradation. Adhesion allows GBM cells to interact with and anchor to the ECM, providing a base for movement. ECM remodeling involves the secretion of enzymes, such as matrix metalloproteinases (MMPs), which degrade ECM components to create pathways for invasive tumor cells. ECM degradation facilitates GBM cell movement through surrounding tissues, promoting tumor spread and metastasis. These processes are intricately regulated by signaling pathways and molecular factors that enhance the invasive and migratory capacities of GBM cells [416, 417]. Similar to other malignant tumors, GBM growth, metastasis, and invasion heavily depend on tumor angiogenesis. Gliomas are often characterized by hypervascularization, which sustains tumor growth by supplying nutrients and oxygen. However, anti-angiogenesis therapies face several limitations. A significant challenge is the reactive resistance triggered by the TME, which adapts to compensate for reduced blood supply. Additionally, hypoxia, a common feature in solid tumors, activates signaling pathways that promote tumor cell invasion and metastasis, complicating the effectiveness of anti-angiogenic strategies. These adaptive responses contribute to the aggressive nature of GBM and hinder the success of conventional treatments [418, 419]. During invasion and metastasis, GBM cells undergo epithelial-mesenchymal transition (EMT), a process where they lose epithelial traits, such as cell polarity and adhesion, and acquire mesenchymal characteristics, including increased motility and invasiveness. EMT enables GBM cells to detach from the primary tumor, invade surrounding tissues, and metastasize to distant sites. This transition is a key mechanism driving GBM's aggressive behavior and is associated with therapeutic resistance and poor prognosis [420]. EMT represents a drug-resistant, low-proliferative, and transient state frequently observed in cancers, with a particularly prominent role in GBM [421–423]. Tubastatin A, an inhibitor of HDAC6, has been shown to reduce the expression of mesenchymal markers in GBM cells, thereby promoting EMT reversal. This suggests that targeting EMT-related pathways could offer promising therapeutic strategies to mitigate GBM invasiveness and improve treatment outcomes [424].

The SHH signaling pathway is closely linked to the function of the primary cilium (PC), and disrupting PC function may inhibit GBM proliferation, slow malignant progression, and improve treatment sensitivity [425]. Studies have indicated that resistance to kinase inhibitors in GBM is associated with upregulated PC activity, uncontrolled PC elongation, and aberrant activation of SHH signaling. Notably, KIF7 knockdown was found to regulate PC length and integrity, effectively re-sensitizing GBM cells to treatment [426]. Additionally, Dynarrestin has been shown to inhibit the intraflagellar transport of SMO within primary cilia, thereby suppressing SHH pathway activity. This suppression leads to reduced proliferation of neuronal precursors and tumor cells reliant on SHH signaling [427]. As a result, Dynarrestin emerges as a promising candidate for the development of anticancer therapies, particularly for targeting the SHH signaling pathway to impede tumor growth and progression (Fig. 17).

MAPK pathway

The MAPK signaling pathway components are expressed across various brain regions, generally showing overlapping expression patterns, except for MEK2, which is absent in specific areas under normal, non-pathological conditions. The cellular outcomes of MAPK pathway activation are highly context-dependent, varying based on the cellular environment, type of stimulus, and tissue condition. This complexity allows the MAPK pathway to regulate diverse cellular processes, including growth, differentiation, and survival, while contributing to pathological states when dysregulated [428]. The MAPK pathway is integral to various neurological processes, such as pain perception, memory formation, cerebellar and midbrain development, and the initiation of cortical neurogenesis. In GBM, the accumulation of precursor or NSCs, rather than post-mitotic glial cells, drives the formation of glial cells, ultimately contributing to GBM onset. Genomic analyses of GBM have revealed mutations in key signaling components that disrupt downstream kinase cascades, leading to uncontrolled cell proliferation, invasion, and metastasis. These interactions highlight the complexity of GBM biology and potential therapeutic targets [429]. The MAPK signaling pathway plays a pivotal role in various cancers, including GBM, through its hyperactivation, which drives processes such as migration, proliferation, and survival. Hyperactivation of MAPK signaling is associated with poor prognosis in multiple cancers, including colon, breast, lung, ovarian cancers, and GBM. One critical player in this pathway is EGFR, which, upon binding to its ligand, EGF, undergoes phosphorylation in its cytoplasmic domain. This phosphorylation activates adapter proteins that trigger the downstream MAPK signaling cascade. In GBM, EGFR mutations, particularly the EGFRvIII mutation, are prevalent. EGFRvIII is a missense mutation that leads to ligand-independent activation of EGFR, causing sustained MAPK pathway activation. This abnormal activation significantly contributes to tumorigenesis, with at least 20% of mutated

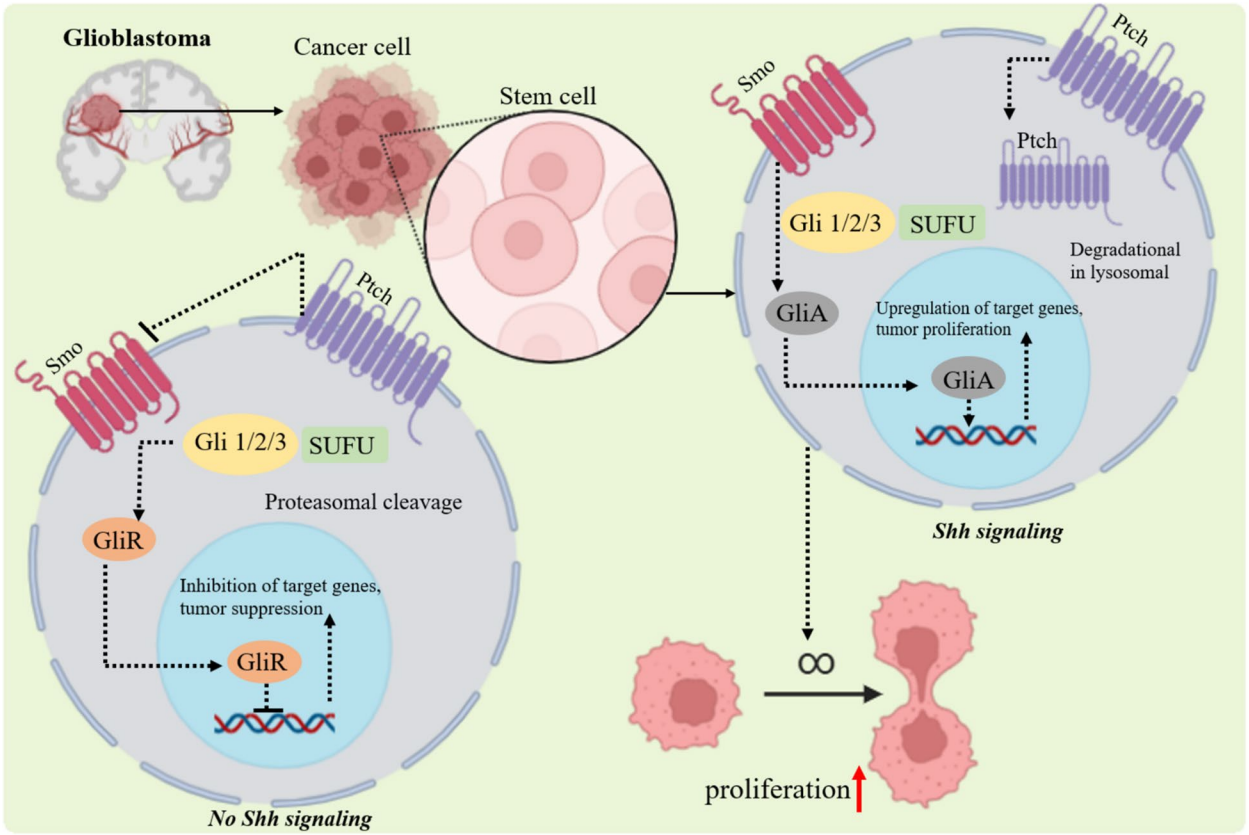

Fig. 17 SHH signaling pathway in GBM, particularly in GSCs: In the absence of the hedgehog (HH) ligand, the Ptch receptor inhibits Smo, leading to the formation of GliR, a repressor that suppresses the expression of target genes. Ptch and SUFU act as critical tumor suppressors in this context. When the HH ligand binds to Ptch, it induces the receptor's degradation, relieving the inhibition on Smo. This activates a signaling cascade through Smo, resulting in the formation of GliA, a transcription factor that drives the expression of genes promoting tumor proliferation and survival. Activation of this pathway significantly enhances tumor cell proliferation, fueling cancer growth and progression

genes in GBM linked to MAPK pathway hyperactivation. The pathway also regulates various cellular proteins by activating downstream transcription factors like CREB, which upregulates cyclin D1, a key factor in cell cycle progression. This co-activation of cell proliferation and CREB underscores the MAPK pathway's role in GBM malignancy, suggesting it as a promising therapeutic target [430]. Traditionally, the MAPK signaling pathways have been viewed as linear intracellular conduits activated by specific stimuli such as growth factors, cytokines, and environmental cues. This stepwise progression involves the sequential activation of kinases and transcription factors, regulating processes like proliferation, differentiation, and survival. However, emerging evidence suggests that the MAPK pathway operates in a more integrated and dynamic fashion, intersecting with other pathways such as PI3K/AKT, JAK/STAT, or NF-κB. This cross-talk enhances cellular plasticity, enabling nuanced regulation of processes like migration, invasion, and drug resistance—key factors in cancer progression. These findings highlight the MAPK pathway's role as an integrated signaling network, suggesting therapeutic strategies that target both MAPK signaling and its interactions with other pathways to achieve more effective tumor suppression in GBM [431]. In GBM, the MAPK pathway plays a crucial role in regulating essential tumor progression functions, including cell survival, proliferation, migration, and invasion. By controlling processes such as cell cycle progression, apoptosis resistance, and motility, the pathway significantly contributes to tumor malignancy. Targeting specific components within the MAPK pathway, such as MAPK kinases (e.g., MEK or ERK), upstream receptors (e.g., EGFR), or downstream transcription factors (e.g., CREB, c-Fos, or c-Myc), offers potential to disrupt aberrant signaling and inhibit tumor proliferation and survival. For instance, inhibiting EGFR mutations or downstream kinases like MEK and ERK can block key survival pathways, induce tumor cell death, and reduce tumor growth. These approaches emphasize the MAPK pathway's therapeutic potential in GBM and

other cancers [432]. Research also reveals local variability in MAPK pathway activation, with patterns of mutual exclusivity observed among signaling pathways. Recent studies have highlighted the role of the transcription factor CREB, supported by in situ co-expression analysis and computational studies [430, 433]. Intracellular heterogeneity in signaling pathways significantly shapes tumor behavior, influencing the development of targeted therapies and the classification of GBM subtypes. Inhibiting MAPK signaling pathways has proven crucial for effectively suppressing tumor cells in GBM [433, 434]. While current therapeutic strategies primarily focus on improving survival rates, further research is needed to explore cell-type-specific effects of MAPK activation and the relationship between these effects and the cell of origin in GBM development.

Warburg effect as a treatment for GBM

Like other cancer types, GBM cells face two primary metabolic challenges: meeting the bioenergetic and biosynthetic demands required for rapid proliferation. This metabolic reprogramming is thought to provide cancer cells with a competitive advantage, enabling accelerated growth and division [435–437]. Glucose plays a central role in this process by serving as a key source of ATP through glycolysis and mitochondrial OXPHOS. Beyond energy production, glucose also supplies crucial intermediates for biosynthetic pathways, such as ribose sugars for nucleotide synthesis, glycerol and citrate for lipid biosynthesis, nonessential amino acids, and NADPH via the oxidative pentose phosphate pathway [436]. Beyond their function as bioenergetic centers, mitochondria also produce metabolites essential for macromolecule synthesis, thereby fulfilling the biosynthetic needs of proliferating cancer cells [437].

Methylene blue (MB), synthesized in 1876, has been a staple in clinical practice for over a century, serving both diagnostic and therapeutic roles. It has been used to treat various conditions, including methemoglobinemia, malaria, ifosfamide-induced neurotoxicity, and cyanide poisoning [438, 439]. Recent studies indicate that MB enhances brain metabolism and provides neuroprotective effects in several neurodegenerative disease models, including Parkinson's disease, Alzheimer's disease, and Huntington's disease [440–444]. The role of MB as an electron carrier has been well-established, with studies demonstrating its ability to facilitate the reduction of cytochrome c in isolated mitochondria [445]. Recent studies have demonstrated that MB serves as an alternative electron carrier between mitochondrial complexes I and III, thereby increasing cellular oxygen consumption and reducing lactate production in murine hippocampal cells [440].

A previous study showed that MB is capable of accepting electrons from NADH at mitochondrial complex I and transferring them to cytochrome c, thereby offering an alternative pathway for mitochondrial electron transfer. This mechanism has been shown to reduce the extracellular acidification rate (ECAR), highlighting its role in modulating cellular metabolic activity [440]. It is hypothesized that MB counteracts the Warburg effect by shifting mitochondrial function in GBM cells from a biosynthetic role to a primarily bioenergetic one. This metabolic shift reduces the availability of intermediates required for biosynthesis and inhibits cancer cell proliferation. Supporting this hypothesis, evidence shows that MB increases ATP production, decreases NADPH levels, and induces cell cycle arrest in the S phase. Interestingly, MB's effect on lactate production is less pronounced compared to its impact on ECAR, suggesting that additional mechanisms may contribute to its effects on GBM cells. Notably, extracellular acidity itself has been implicated in promoting cancer progression, further underscoring the therapeutic potential of targeting metabolic pathways [446, 447].

Metabolic features of GBM

Glycolysis and GBM

In GBM, glycolysis is crucial for tumor growth, invasion, angiogenesis, and the development of resistance to chemotherapy and RT. Additionally, glycolytic activity significantly impacts the TME, modulating inflammatory and immune responses [448–451]. Research indicates that restoring OXPHOS as the primary energy production pathway can promote the differentiation of GBM cells into astrocytes [452, 453]. Under stressful conditions, the brain relies on alternative energy sources, such as lactic acid and ketone bodies, to maintain normal function. Recent gene expression analyses of glycolysis and mitochondrial metabolism in brain tumor samples from patients with lower-grade gliomas and GBM revealed elevated levels of glycolytic enzyme expression in GBM tissues [454]. Key enzymes involved in glycolysis and the tricarboxylic acid (TCA) cycle are essential regulators of GBM metabolism. Notably, rate-limiting glycolytic enzymes like hexokinase 2 (HK2) and pyruvate kinase M2 (PKM2) are significantly overexpressed in GBM patients, with their upregulation strongly linked to GBM progression. Elevated HK2 levels, in particular, have been shown to promote tumor growth and enhance cancer cell resistance to apoptosis [455]. A study by Zhimin Lu and colleagues demonstrated that HK2 plays a pivotal role in activating the NF-κB signaling pathway, which drives the expression of PD-L1, enabling tumor immune evasion. This mechanism involves the phosphorylation and subsequent degradation of IκBα, a key inhibitor of NF-κB

[453, 456]. Glycolysis is also influenced by other metabolic pathways. Recent studies have highlighted the role of fructolysis, a mechanism specific to the brain, in modulating the Warburg effect. Fructolysis has been found to suppress mitochondrial respiration and aerobic glycolysis while enhancing OXPHOS. This mechanism is thought to play a significant role in metastasis, particularly under hypoxic conditions [453].

Tricarboxylic acid cycle and GBM

The TCA cycle is a central metabolic pathway crucial for energy production, as it oxidizes carbohydrates, fats, and proteins to generate ATP. Additionally, it provides essential precursors for the biosynthesis of molecules critical for cell growth and division. In cancer cells, metabolic alterations, including those affecting the TCA cycle, help meet the elevated energy demands of tumors, supporting their survival and proliferation. The pyruvate dehydrogenase (PDH) complex, located in the mitochondrial matrix, plays a key role in oxidative metabolism by converting pyruvate into acetyl-CoA. PDH activity is tightly regulated by pyruvate dehydrogenase kinase (PDK), which phosphorylates and inhibits PDH, reducing pyruvate oxidation in mitochondria and increasing lactate production in the cytoplasm.

A study by Prabhu et al. demonstrated that Ras-mediated expression of PDH phosphatase (PDP) enhances PDH activity, but PDP expression is significantly reduced in GBM patients. Restoring PDP1 expression was shown to slow GBM tumor growth, highlighting its therapeutic potential. Acetyl-CoA, a key metabolite in the TCA cycle, undergoes oxidation to produce CO2 while driving energy generation. This process generates NADH and FADH2, which are oxidized in the electron transport chain (ETC), with released protons and electrons used to generate ATP via OXPHOS [453, 457, 458]. Targeting OXPHOS has emerged as a promising therapeutic strategy against tumor cells. Studies have shown that OXPHOS inhibitors, such as AG311 and Gboxin, effectively suppress tumor growth in GBM by disrupting the OXPHOS pathway. This disruption impairs ATP production, hindering the energy metabolism critical for tumor cell survival. The inhibition of OXPHOS by these compounds has shown significant potential in reducing GBM tumor growth.

In addition to energy production, α-ketoglutarate (α-KG) plays a multifaceted role in GBM metabolism. As a key intermediate in the TCA cycle, α-KG serves as a substrate for CO2 production and provides a carbon backbone for amino acid biosynthesis, including aspartate and glutamate. GBM cells adapt metabolically by importing extracellular aspartate and glutamate, converting them into α-KG to maintain TCA cycle activity. This adaptation enables GBM cells to sustain their metabolic demands even when conventional metabolic pathways are disrupted [459]. In GBM, cells predominantly rely on glycolysis for ATP production rather than the TCA cycle, a phenomenon known as the Warburg effect. This metabolic shift allows GBM cells to generate energy through glycolysis even in normal oxygen conditions. Furthermore, GBM cells repurpose TCA cycle intermediates to meet biosynthetic needs, directing carbon flux toward the production of essential biomolecules, including amino acids, lipids, and nucleotides. These adaptations support the rapid proliferation, growth, and invasive potential of GBM [453].

Pentose phosphate pathway and GBM

The pentose phosphate pathway (PPP), an essential offshoot of glycolysis, plays a pivotal role in lipid biosynthesis, as well as in the production of NADPH and nucleotides. In cancers such as GBM, upregulation of PPP-related proteins supports critical cellular functions, including providing nucleotides for DNA replication and repair and generating NADPH to enhance antioxidant defenses. This enables cancer cells to withstand oxidative stress and sustain rapid proliferation [453, 460]. Elevated expression of enzymes involved in de novo pyrimidine biosynthesis, alongside upregulation of their associated genes, has been strongly correlated with poor clinical outcomes in GBM patients. This dysregulation facilitates tumor growth by accelerating nucleotide production, essential for the survival and proliferation of rapidly dividing cancer cells. Moreover, altered pyrimidine metabolism may interact with other oncogenic pathways, further driving the aggressive behavior of GBM. Understanding these metabolic changes could lead to new therapeutic strategies targeting this vulnerability [453, 461]. PPP intermediates, including glucose-6-phosphate (G6P), 6-phosphogluconolactone (6PGL), 6-phosphogluconate (6PG), ribulose-5-phosphate (Ru5P), and ribose-5-phosphate (R5P), are central to nucleotide biosynthesis, redox homeostasis, and metabolic regulation. Disruptions in the levels of these intermediates and their associated enzymes are strongly linked to GBM progression. Notably, GBM patients exhibit increased activity of enzymes such as 6-phosphogluconolactonase (PGLS) and 6-phosphogluconate dehydrogenase (PGD), which are key to the oxidative phase of the PPP. Conversely, the activity of glucose-6-phosphate dehydrogenase (G6PD), which catalyzes the pathway's initial step, is significantly reduced in GBM. These findings suggest a reprogramming of the PPP in GBM to support the heightened biosynthetic and survival demands of tumor cells, highlighting the pathway's potential as a therapeutic target in GBM.

The upregulation of PGLS and PGD enhances the production of R5P and NADPH, which are critical for nucleotide biosynthesis and maintaining cellular energy production, thereby promoting tumor proliferation. Recent studies have also identified STAT3 as a key activator of phosphoinositide 3-kinase-activating Akt (PIKE-A). The interaction between STAT3 and PIKE-A recruits Fyn kinase, which phosphorylates STAT3, leading to increased G6PD expression. This activation drives tumor growth while suppressing cellular senescence, further fueling GBM malignancy [462]. Collectively, these findings highlight widespread upregulation of glycolysis and PPP-related genes, which supports elevated ATP and nucleotide production. This metabolic reprogramming is essential for sustaining the uncontrolled proliferation and growth characteristic of GBM cells [453].

Glutamine metabolism and GBM

Glutamine is a critical nutrient for cancer cells, providing both energy and carbon skeletons essential for their rapid proliferation. Specialized transporters on the cell membrane ensure the efficient import of glutamine, maintaining a steady supply to meet the metabolic demands of tumor growth [463]. The conversion of glutamate to glutamine is tightly regulated through the glutamine synthetase (GS) pathway, particularly during glutamine scarcity. This de novo synthesis of glutamine is vital for sustaining cell growth and supporting nucleotide biosynthesis in both GBM cell lines and astrocytes, highlighting its key role in tumor metabolism and proliferation [453, 464].

GBM exhibits profound alterations in glutamine metabolism, which are closely linked to its aggressive nature. Elevated glutamine levels are often redirected from the TCA cycle into alternative metabolic pathways that fuel tumor progression. Glutamine-derived glutamate is converted into α-ketoglutarate (α-KG), a crucial TCA cycle intermediate that supports energy production and biosynthesis. This metabolic reprogramming enables GBM cells to meet the high energy and biosynthetic demands required for rapid proliferation. The simultaneous elevation of glutamine and glucose levels in GBM underscores their indispensable roles in tumor growth and survival [465]. Research has shown that GBM cells exhibit increased glutamine uptake and utilization, even in glucose-rich conditions. Glutamine deprivation significantly reduces GBM cell viability in vitro, emphasizing its pivotal role in sustaining survival and proliferation. These findings illustrate the metabolic adaptability of GBM cells and the essential role of glutamine as a key nutrient for their aggressive growth [453, 461]. Recent studies have highlighted the potential of targeting glutamine metabolism as a therapeutic strategy for GBM. For example, the glutamine antagonist prodrug JHU-083 has been shown to effectively inhibit GBM cell proliferation while inducing profound metabolic disruptions. This treatment also suppressed mTOR signaling and downregulated Cyclin D1, a critical regulator of the cell cycle, demonstrating the therapeutic promise of targeting glutamine metabolism to disrupt tumor growth and progression [466]. Around 50% of GBM patients harbor genetic alterations in EGFR, which play a critical role in tumor progression and therapy resistance. These alterations, often involving amplifications or mutations, drive aggressive tumor behavior by promoting unchecked cell proliferation, survival, and invasion. EGFR mutations are also associated with poor prognosis, making them a significant therapeutic target in GBM [467]. A study by Yang et al. revealed that activated EGFR enhances glutamine metabolism via a pathway dependent on glutamate dehydrogenase 1 (GDH1). This pathway facilitates the conversion of glutamine to glutamate, which is then used in processes critical for GBM progression. Silencing GDH1 significantly reduced GBM cell proliferation and tumorigenesis, highlighting the pivotal role of glutamine metabolism in driving tumor growth. These findings suggest that targeting GDH1 could be a promising therapeutic approach for GBM [453, 468]. Targeting various aspects of glutamine metabolism offers potential strategies for developing effective GBM treatments. Approaches include inhibiting glutamine uptake or modulating the activity of key enzymes like GDH and glutaminase (GLS), which are central to glutamine metabolism. Additionally, targeting glutamate transporters to limit the availability of critical metabolic intermediates could disrupt tumor growth. Counteracting the effects of lactate, a byproduct of altered GBM metabolism, may also help restore the TME and inhibit tumor progression. Together, these strategies hold promise for advancing therapeutic options and improving the efficacy of existing GBM treatments [453].

Lactate metabolism and acidosis and GBM

Excessive lactate production is a hallmark of the altered metabolic state in cancer cells, primarily driven by upregulated glycolysis, even under aerobic conditions—a phenomenon known as the Warburg effect. This metabolic reprogramming results in significant lactate accumulation within the TME. Lactate concentrations in tumor tissues can be nearly 20 times higher than those in normal tissues. This elevated lactate not only reflects metabolic dysregulation but also contributes to an acidic microenvironment that promotes tumor progression by facilitating immune evasion, angiogenesis, and metastasis. Additionally, lactate serves as an alternative energy source for cancer cells, further sustaining their rapid proliferation and survival [469]. The accumulation and

secretion of acidic metabolites like lactate are mediated by monocarboxylate transporters (MCTs), which play a key role in transporting lactate and other metabolites across cell membranes. In the TME, overexpression of MCTs leads to increased lactate secretion, causing significant acidification. This acidic environment has several pro-tumorigenic effects, including enhanced tumor progression, immune evasion, and cancer cell invasiveness. The acidic conditions also impair immune cell function, such as T cells, allowing tumors to escape immune surveillance. Furthermore, low pH in the TME promotes angiogenesis, enabling tumors to form new blood vessels that support growth and metastasis. Targeting MCTs and TME acidification offers a promising therapeutic strategy to disrupt these tumor-promoting processes [470].

Lactic acidosis, frequently observed in malignant tumors like GBM, triggers a cascade of biochemical changes that alter cellular metabolism and signaling pathways. Many tumors exhibit upregulated glycolysis alongside impaired OXPHOS. This metabolic shift supports tumor cell proliferation and enables their survival under harsh microenvironmental conditions, relying heavily on the Warburg effect and resulting in excessive lactate production [453, 471]. In GBM, lactic acidosis plays a critical role in fostering drug resistance and immune evasion, further contributing to its aggressive behavior and poor prognosis [472, 473].

Fatty acid metabolism and GBM

Lipids are essential for maintaining brain structure and function, primarily by preserving cell membrane integrity and facilitating the biosynthesis of specific proteins in CNS [474–476]. Changes in fatty acid (FA) metabolism, such as enhanced FA biosynthesis, increased lipid droplet accumulation for energy storage, and upregulated FA catabolism, are well-recognized factors in tumorigenesis, cancer progression, and resistance to therapy. These metabolic adaptations supply the energy and biosynthetic precursors needed for the aggressive proliferation, survival, and invasiveness of tumor cells, enabling them to thrive in the hostile TME. Dysregulated FA metabolism thus emerges as a key driver of cancer persistence and adaptability [477]. Abnormal accumulation of lipid droplets has been observed in both GBM cell lines and patient samples, with this buildup of fatty acid metabolites correlating with reduced survival rates in GBM patients. This suggests that dysregulated lipid metabolism plays a significant role in driving tumor aggressiveness and contributing to poor clinical outcomes. Consequently, lipid metabolism may serve as both a prognostic indicator and a therapeutic target in GBM [478]. Studies have identified palmitic acid and oleic acid as the predominant fatty acids in GBM, underscoring their involvement in the metabolic processes that sustain tumor growth and progression [453, 479]. Alterations in FA metabolism are also involved in driving inflammation within GBM. Arachidonic acid, a polyunsaturated fatty acid (PUFA), serves as a precursor for bioactive molecules such as prostaglandins and leukotrienes, which play essential roles in mediating inflammatory responses. Research by Nicolaou et al. demonstrated a strong association between poor survival outcomes and elevated expression of microsomal PGE synthase 1 and prostaglandin reductase 1 mRNA, both of which are key enzymes in prostaglandin biosynthesis. These findings suggest that the upregulation of these enzymes contributes to the inflammatory TME in GBM, thereby promoting tumor progression [480]. Elevated FA levels can also facilitate cancer cell proliferation, particularly in metastatic cells that infiltrate the brain parenchyma via the BBB. One study showed that PUFAs released by inflammation-activated astrocytes serve as vital resources for metastatic cancer cells to construct their cell membranes. Conversely, another study highlighted the potential therapeutic effects of omega-3 fatty acids, which were found to induce GBM cell death and enhance radiotherapy efficacy in both in vitro and in vivo models. These contrasting findings underscore the multifaceted roles of fatty acids in GBM progression and their influence on the tumor's response to therapy [481, 482].

Cholesterol, another crucial lipid molecule, plays a vital role in various biological processes, acting as a structural component of cell membranes and a precursor for numerous metabolites. Elevated cholesterol levels have been shown to promote tumorigenesis and metastasis in cancer cells, emphasizing its importance in the progression of malignancies [480, 482]. In GBM, cholesterol metabolism is intricately linked to tumor cell survival, further highlighting its significance as a potential therapeutic target [453, 483].

Hypoxia and low oxygen levels are hallmark features of GBM, contributing significantly to tumor cell invasion, treatment resistance, and the suppression of antitumor immune responses. Under hypoxic conditions, GBM cells increase fatty acid uptake through FABP3 and FABP7. These fatty acids are subsequently stored in lipid droplets, serving as essential energy reservoirs that enable GBM cells to endure hypoxia and cycles of reoxygenation [453, 484]. Fatty acid β-oxidation (FAO) has been identified as a crucial metabolic pathway in GBM. Comprehensive analyses of metabolomic and gene expression data from GBM patient samples have highlighted FAO as a central component of the GBM metabolic network, underscoring its importance as a key metabolic node in the disease. The upregulation of FAO allows GBM cells to adapt to the dynamic TME, promoting their survival and tumor growth [485, 486]. A deeper understanding of the

metabolic reprogramming in GBM could uncover critical therapeutic targets, emphasizing the importance of integrating metabolic pathways into the design of innovative treatment strategies [453] (Table 9).

Monoamines and GBM

Emerging evidence highlights the critical role of the TME in driving the aggressive progression of GBM. Within this environment, neurotransmitters—key signaling molecules involved in synaptic communication in the brain—represent an underexplored factor. These molecules orchestrate intricate molecular pathways and regulate cellular functions across various CNS cell types, including neural stem and progenitor cells, neurons, and glial cells. Recent studies reveal that neurotransmitters influence crucial processes such as cell proliferation, quiescence, and differentiation. This regulatory capacity is particularly significant given the role of neural progenitors and glial cells, which are thought to contribute to GBM origins. Furthermore, the widespread presence of neurotransmitters throughout the CNS suggests they may have far-reaching effects on tumor biology. These insights underscore the importance of investigating neurotransmitter roles in GBM initiation and progression, as understanding their influence on TME dynamics could unveil novel therapeutic approaches and enhance strategies to combat this malignancy [494].

The roles of dopamine and serotonin signaling warrant special attention, as these neurotransmitters exert their effects through interactions with a broad range of receptors. Dopamine, synthesized in the cytoplasm of synaptic terminals, regulates various physiological processes such as mood, motivation, and motor control [495]. Similarly, serotonin signaling is mediated by a diverse array of receptor subtypes, which influence signaling pathways and functional outcomes. This diversity contributes to the complex, context-dependent effects of serotonin in normal and pathological conditions, including its role in cancer progression and modulation of the TME [496, 497]. Adding to the complexity, monoamine signaling involves extensive receptor interactions. Dopamine receptors, for instance, can form heterodimers with other dopamine receptors as well as with GPCRs such as endocannabinoid and somatostatin receptors. These interactions modulate signaling outcomes and establish intricate regulatory networks that affect cellular responses, including those linked to cancer progression and the TME [498–501]. Similarly, serotonin receptors can interact with other serotonin receptors and various GPCRs, further complicating serotonin signaling [502]. Notably, serotonin and dopamine receptors can also dimerize with each other, introducing another layer of complexity to monoamine signaling [503]. Together, dopamine and serotonin signaling form a highly complex network of receptors and cascades. Deciphering these interconnected mechanisms remains a significant challenge in neuroscience and neuro-oncology. Understanding how these signaling pathways impact cellular behavior, particularly in tumor progression and therapy response, holds substantial potential for advancing therapeutic strategies for brain tumors like GBM. The intricate receptor interactions and diverse cellular outcomes necessitate further exploration to effectively target and modulate these pathways for improved treatment options [494].

Dopamine plays a vital role in regulating the behavior of progenitor cells in the CNS, influencing both their proliferation and differentiation. Monoamines are integral components of the GBM microenvironment, yet

Table 9 Therapeutic approaches targeting metabolic vulnerabilities in GBM

Treatment Strategy	Mechanism/Target	Ref,s
Glucose Uptake Inhibition (2-DG)	2-DG inhibits glucose phosphorylation by hexokinase, blocking glucose metabolism in tumor cells	[487]
Dimethylaminomicheliolide (DMAMCL)	Alters glycolysis and decreases GBM cell proliferation by activating PKM2	[488]
CPI-613 (Devimistat)	Targets enzymes involved in energy metabolism (pyruvate dehydrogenase, alpha-ketoglutarate dehydrogenase), reducing TCA cycle metabolites	[489]
Metformin	Inhibits OXPHOS, disrupting mitochondrial energy production and inducing cell death in GBM	[490]
Gboxin	Inhibits mitochondrial complex I, disrupting OXPHOS, reducing ATP production, and increasing cellular stress in GBM	[458]
Gamitrinib (Geldanamycin)	Mitochondrial matrix inhibitor, suppresses oxygen consumption and ATP production in GBM	[491–493]
mTOR Pathway Targeting	Inhibits mTOR pathway, impacting glutamine metabolism, glucose uptake, lactate production, and cell proliferation in GBM	[453]
Lipid Metabolism Targeting (MI-1, RO-48–8071)	Inhibits lanosterol synthase, selectively killing H3-K27M-mutant diffuse intrinsic pontine glioma and GBM cells, increasing LXR ligands	[453]

their precise roles and mechanisms remain poorly understood. Insights from developmental neurobiology suggest that monoamines significantly affect the behavior of NSCs and progenitor cells. As progenitors of neurons, astrocytes, and oligodendrocytes—each responsive to neurotransmitters—NSCs are modulated by these signaling molecules. GBM cells, which originate from these progenitors, appear to leverage these signaling pathways to enhance their survival and proliferation in the CNS. Understanding how monoamines influence GBM cell behavior and tumor progression could unveil novel therapeutic targets [494].

The responsiveness of GBM to monoamines highlights its remarkable ability to adapt to the CNS microenvironment. The effects of monoamines on GBM are complex and produce diverse experimental outcomes, driven by two primary factors [494]. Furthermore, it is widely accepted that neurotransmitter signaling and expression in the brain exhibit significant dynamism. Consequently, it is unlikely that monoamines simply activate pathways in a straightforward binary on/off fashion. Instead, we propose that monoamine transmitters exert a concentration-dependent effect on GBM cells. This concentration-dependent influence has been observed for both dopamine and serotonin in numerous studies of the normal brain, and we hypothesize that similar mechanisms could also be operative in tumor [494]. Second, neurotransmitter signaling in the brain is highly dynamic, suggesting that monoamines do not merely activate pathways in a binary on/off manner. Instead, monoamines likely exert concentration-dependent effects on GBM cells. This concentration dependence, well-documented for dopamine and serotonin in studies of the normal brain, may also apply to tumor cells [504–506]. For example, oligodendrocyte precursor cells (OPCs) exhibit dose-dependent responses to dopamine agonists, with higher doses potentially inducing cytotoxicity [507]. Moreover, it is plausible that neurotransmitter concentrations vary across tumor compartments, as dopamine and serotonin neurons innervate specific brain regions in a region-dependent manner [508, 509]. Thus, the proximity of the tumor to monoamine-synthesizing nuclei could significantly influence its progression. Another critical factor is the frequency of seizures in GBM patients, which has been shown to elevate dopamine and serotonin levels [510, 511]. Increased seizure activity may lead to elevated monoamine concentrations within the TME, potentially affecting tumor growth and therapeutic response. The involvement of multiple receptor subtypes, coupled with dynamic fluctuations in neurotransmitter levels within the tumor, adds significant complexity to understanding the mechanisms by which monoamines contribute to GBM progression. This multifaceted interplay underscores the need for further investigation to identify targeted strategies for modulating monoamine signaling in GBM [494].

Despite the complex interplay between monoamines and GBM, there is growing optimism about repurposing drugs targeting these signaling pathways for brain cancer treatment. An increasing body of evidence suggests that drugs targeting dopamine and serotonin receptors hold promise as therapeutic options for GBM. Several studies have demonstrated that antipsychotic medications, which act as antagonists to these receptors, can effectively inhibit GBM cell proliferation. Notably, Dolma et al. reported that antagonizing D4 receptors suppresses GBM growth and, when combined with TMZ, significantly improves median survival in xenograft mouse models. Mechanistic studies revealed that this drug inhibits normal autophagy, shedding light on its potential mode of action. Additionally, other antipsychotic drugs, including clozapine, thioridazine, olanzapine, haloperidol, aripiprazole, and trifluoperazine, have also demonstrated efficacy in inhibiting GBM growth, further supporting the potential of this drug class in GBM therapy [494, 512–520].

The promising outcomes observed in preclinical models suggest that antipsychotics may provide a viable therapeutic strategy for GBM. However, it is essential to consider the broader implications of targeting monoamine signaling, as these pathways are critical for the normal functioning of neurons and astrocytes. These cells, which are vital for maintaining brain homeostasis, rely on precise monoamine signaling for key processes such as neurotransmission, synaptic plasticity, and glial cell function. Therefore, understanding how antipsychotics affect both tumor cells and the normal cellular components of the brain is crucial. This comprehensive approach will help minimize potential side effects and enhance the safety and effectiveness of such treatments in the context of GBM [521–524]. Therefore, any pharmacological intervention targeting monoamines in GBM is likely to lead to side effects, including mood disturbances and movement disorders. In the short term, these side effects may be manageable for patients, especially if they result in significant life extension. However, in the long term, we remain hopeful that ongoing research will clarify the complex and tumor-specific monoamine signaling mechanisms. A better understanding of these mechanisms could facilitate the development of targeted therapies that minimize side effects while optimizing therapeutic efficacy. These mechanisms may involve unique dimerization patterns and receptor configurations specific to the tumor, enabling more precise and effective treatment strategies [494].

The wide range of receptors targeted by antipsychotics, along with their complex roles in normal brain physiology, adds complexity to their direct repurposing for GBM treatment. However, this complexity also presents a unique therapeutic opportunity. The diverse binding profiles and receptor interactions of these drugs offer a broad selection of FDA-approved options, giving clinicians flexibility in customizing treatment strategies. When combined with advances in genomic and proteomic tumor profiling, these drugs show great promise for personalized medicine. For example, GBM tumors with elevated expression of dopamine receptors could be specifically targeted with antipsychotics that match these receptors' affinities. This approach not only improves treatment precision but also enhances efficacy and may reduce off-target effects. By exploring the potential applications of antipsychotics, especially considering their receptor diversity, clinicians and researchers can broaden the range of adjuvant therapies for GBM. This innovative strategy could lead to more effective, targeted treatments, ultimately improving patient outcomes for this challenging malignancy [494].

Repurposing CNS drugs, particularly those with established safety profiles and the ability to cross the BBB, presents a promising strategy for developing new therapies for GBM. A key study explored the anti-GBM effects of selected antipsychotic and antidepressant medications, assessing their impact both in vitro and in vivo. The results revealed that these drugs share a common mechanism of action against GBM, primarily through the disruption of lysosomal function. This disruption destabilizes lysosomal membranes, leading to cell death. Notably, GBM cells with functional PTEN expression exhibited increased sensitivity to these drugs, indicating a genetic context-dependent effect. Furthermore, the study emphasized a synergistic therapeutic approach by combining lysosomal function inhibitors with drugs targeting the EGFR-PI3K-Akt pathway. This combination led to a significant collapse of cellular energy metabolism and antioxidant defense systems, greatly enhancing the anti-tumor effects. These findings not only highlight the potential of CNS drugs in treating GBM but also open the door for further investigation into combination therapies that target lysosomal vulnerabilities and metabolic stress to combat this aggressive cancer [525].

There is substantial evidence suggesting that antidepressants may reduce cancer incidence and improve patients' quality of life. Notably, fluoxetine has been shown to directly bind to AMPA receptors (AMPARs), leading to transmembrane calcium influx. This increases intracellular calcium concentration ([Ca2+]i), causing mitochondrial calcium overload and triggering apoptosis. Given the overexpression of AMPARs in glioma tissues, fluoxetine appears to selectively target glioma cells. In an in vivo study, fluoxetine effectively inhibited GBM growth in Nu/Nu mice, demonstrating antitumor efficacy comparable to TMZ [526].

Conclusion

GBM remains a significant clinical challenge due to its heterogeneity, aggressive progression, and resistance to existing treatments. Recent studies have provided insights into the complex molecular mechanisms and signaling pathways—such as PI3K/AKT/mTOR, Wnt, NF-κB, and TGF-β—that drive tumor growth and therapeutic resistance. While these findings have revealed potential therapeutic targets, the intricate interactions between these pathways underscore the need for more targeted and personalized treatment approaches. Future research should focus on better understanding the crosstalk between these pathways and its role in resistance mechanisms. This knowledge could guide the development of combination therapies targeting multiple pathways and the identification of new biomarkers for early detection and treatment monitoring. Furthermore, advancements in genomic technologies, including CRISPR and next-generation sequencing, hold promise for uncovering novel therapeutic targets. By integrating these strategies, we may be able to develop more effective, long-lasting treatments, ultimately improving survival rates and quality of life for GBM patients.

Abbreviations

GBM	Glioblastoma Multiforme
IDH	Isocitrate Dehydrogenase
RT	Radiotherapy
NCCN Guidelines	National Comprehensive Cancer Network Guidelines
MGMT	O6-Methylguanine-DNA Methyltransferase
DNA	Deoxyribonucleic Acid
H3K27	Histone H3 Lysine 27 to Methionine Mutation
H3F3A G34	Histone H3.3 G34 Mutation
OS	Overall Survival
EORTC-NCIC trial	European Organisation for Research and Treatment of Cancer–National Cancer Institute of Canada Tria
KPS	Karnofsky Performance Status
USP48	Ubiquitin Specific Peptidase 48
LOH	Loss of Heterozygosity
EGFR	Epidermal Growth Factor Receptor
PTEN	Phosphatase and Tensin Homolog
PDGFR	Platelet-Derived Growth Factor Receptor
MDM2	Mouse Double Minute 2
PI3K	Phosphoinositide 3-Kinase
GOF	Gain-of-function
TERT	Telomerase Reverse Transcriptase
NSAIDs	Non-Steroidal Anti-Inflammatory Drugs
cIMPACT-NOW	Consortium to Inform Molecular and Practical Approaches to CNS Tumor Taxonomy
WHO	World Health Organization
CDKN2A/B	Cyclin-Dependent Kinase Inhibitor 2A/B
CNS	Central Nervous System
TGF-β	Transforming Growth Factor Beta
mTOR	Mammalian Target of Rapamycin
IgG1	Immunoglobulin G1
VEGF-A	Vascular Endothelial Growth Factor A

p53	Tumor Protein 53
TCGA	The Cancer Genome Atlas
TP53	Tumor Protein P53 Gene
Sall1	Spalt-like 1-positive
CCR8	C–C Chemokine Receptor Type 8
CTLA-4	Cytotoxic T-Lymphocyte-Associated Protein 4
PD-1	Programmed Death-1
MARCH9	Membrane-Associated RING-CH 9
EGFRvIII	Epidermal Growth Factor Receptor Variant III
EGFR TKI	Epidermal Growth Factor Receptor Tyrosine Kinase Inhibitor
HR	Hazard Ratio
RTKs	Receptor Tyrosine Kinase
KIT	Proto-Oncogene Receptor Tyrosine Kinase
RAF-1	Rapidly Accelerated Fibrosarcoma 1
BRAF	V-Raf Murine Sarcoma Viral Oncogene Homolog B1
CSF-1R	Colony Stimulating Factor 1 Receptor
TAMs	Tumor-Associated Macrophages
Tie-2	Tyrosine Kinase with Immunoglobulin-like and EGF-like Domains 2
RET	Rearranged During Transfection
FGFR3	Fibroblast Growth Factor Receptor 3
TACC3	Transforming Acidic Coiled-Coil Containing Protein 3
FGFs	Fibroblast Growth Factors
TK	Tyrosine Kinase
MAPK	Mitogen-Activated Protein Kinase
STAT3	Signal Transducer and Activator of Transcription 3
FGFR3-TACC3	Fibroblast Growth Factor Receptor 3-Transforming Acidic Coiled-Coil Containing Protein 3 Fusion
FGFRi	Fibroblast Growth Factor Receptor Inhibitors
MTC	Medullary Thyroid Cancer
MEKi	MEK Inhibitors
RNAi	RNA Interference
TKIs	Tyrosine Kinase Inhibitors
IκBα	Inhibitor of Nuclear Factor Kappa B Alpha
κB	Kappa B
PIN1	Peptidyl-Prolyl Isomerase 1
MLK4	Mixed Lineage Kinase 4
NFKBIA	NF-Kappa-B Inhibitor Alpha
MYC	Myelocytomatosis Proto-Oncogene
KLF4	Kruppel-Like Factor 4
OCT4	Octamer-Binding Transcription Factor 4
iPSCs	Induced Pluripotent Stem Cells
SOX2	SRY-Box Transcription Factor 2
NMYC	Neuroblastoma-Derived Myc Oncogene
GSC	Glioblastoma Stem Cell
MAX	Myc Associated Factor X
NK Cells	Natural Killer Cells
CD133 + /Nestin +	Markers for Neural Stem or Progenitor Cells
Wnt	Wingless-Related Integration Site Signaling Pathway
MMPs	Matrix Metalloproteinases
Pax6/Dlx5	Paired Box 6/Distal-Less Homeobox 5
HIF-1α	Hypoxia-Inducible Factor 1 Alpha
CXCR4	C-X-C Chemokine Receptor Type 4
OPC	Oligodendrocyte Precursor Cell
APC	Adenomatous Polyposis Coli
EMT	Epithelial-Mesenchymal Transition
SNAIL	Snail Family Transcriptional Repressor
TWIST	Twist Family BHLH Transcription Factor
SLUG	Snail Family Zinc Finger 2
N-Cadherin	Neural Cadherin
RAD51	RAD51 Recombinase
PLAGL2	Pleiomorphic Adenoma Gene-Like 2
Evi/Gpr177	Wnt Pathway Protein
ASCL1	Achaete-Scute Family BHLH Transcription Factor 1
TERTp	Telomerase Reverse Transcriptase Promoter
pTERTmut	Promoter Telomerase Reverse Transcriptase Mutation
ATRX	Alpha Thalassemia/Mental Retardation Syndrome X-Linked
PFS	Progression-Free Survival
rAAV-hTERTC27	Recombinant Adeno-Associated Virus Vector Engineered to Deliver hTERTC27
CRISPR	Clustered Regularly Interspaced Short Palindromic Repeats
GPCRs	G-Protein-Coupled Receptors
SH2 Domains	Src Homology 2 Domains
PIP2	Phosphatidylinositol 4,5-Bisphosphate
PIP3	Phosphatidylinositol 3,4,5-Trisphosphate
PIK3CA	Phosphatidylinositol-4,5-Bisphosphate 3-Kinase Catalytic Subunit Alpha
FAK	Focal Adhesion Kinase
PIK-75	A PI3K Inhibitor
AKT-FOXO	AKT/Forkhead Box O Pathway
PDGFR-β	Platelet-Derived Growth Factor Receptor Beta
c-KIT	Proto-Oncogene Receptor Tyrosine Kinase
FGFR	Fibroblast Growth Factor Receptor
mLST8	Mammalian Lethal with SEC13 Protein 8
PRAS40	Proline-Rich AKT Substrate of 40 kDa
S6K1	Ribosomal Protein S6 Kinase Beta-1
4E-BP1	4E-Binding Protein 1
eIF4F	Eukaryotic Translation Initiation Factor 4F
PKC	Protein Kinase C
BAD	BCL2-Associated Agonist of Cell Death
GSK3	Glycogen Synthase Kinase 3
AMPK	AMP-Activated Protein Kinase
ATP	Adenosine Triphosphate
AMP	Adenosine Monophosphate
TORKi	MTOR Kinase Inhibitors
BBB	Blood–Brain Barrier
SMAD	Sma- and Mad-Related Proteins
GTP	Guanosine Triphosphate
SMAD2	SMAD Family Member 2
ZEB1	Zinc Finger E-Box Binding Homeobox 1
PDGF-BB	Platelet-Derived Growth Factor Subunit BB
USP15	Ubiquitin-Specific Protease 15
BMP	Bone Morphogenetic Protein
TMZ	Temozolomide
NOX4	NADPH Oxidase 4
LIF	Leukemia Inhibitory Factor
EPHA6	Ephrin Type-A Receptor 6
DLX2	Distal-Less Homeobox 2
TSP1	Thrombospondin-1
GSCs	Glioblastoma Stem Cells
Dll4	Delta-Like Ligand 4
Jagged1	A Notch ligand involved in cell fate decisions
Hes1	Hairy and Enhancer of Split 1
Split-1	Split Enhancer of Notch-1 Pathway
Hey1	Hairy and Enhancer of Split-Related 1
Dll1	Delta-Like Ligand 1
Hey2	Hairy and Enhancer of Split-Related 2
CBF1	C Promoter Binding Factor 1
GFAP	Glial Fibrillary Acidic Protein
BCL6	B-Cell Lymphoma 6 Protein
BCL-W	A pro-survival member of the BCL-2 protein family
PLP1	Proteolipid Protein 1
BCL2	B-Cell Lymphoma 2
BAX	BCL2-Associated X Protein
BCLAF1	BCL2-Associated Transcription Factor 1
E2F	E2 Promoter Binding Factor
NSCs	Neural Stem Cells
MAML1	Mastermind-Like Transcriptional Coactivator 1
Dll3	Delta-Like Ligand 3
HDAC	Histone Deacetylase
DNMT1	DNA Methyltransferase 1
NICD	Notch Intracellular Domain
CSL	CBF1/Su(H)/Lag-1
GDP	Guanosine Diphosphate
ERK	Extracellular Signal-Regulated Kinase
Flt-3	Fms-like Tyrosine Kinase 3
Fms	Fms-Related Tyrosine Kinase 1
RAF	Rapidly Accelerated Fibrosarcoma Kinase

INK4a	Cyclin-Dependent Kinase Inhibitor 2A
Arf	Alternative Reading Frame Protein
ATM	Ataxia Telangiectasia Mutated
ATR	Ataxia Telangiectasia and Rad3-Related Protein
Rad3	DNA Damage Repair Protein
DNA-PK	DNA-dependent protein kinase and V(D)J recombination
Chk2	Checkpoint Kinase 2
RB	Retinoblastoma Protein
CDK4	Cyclin-Dependent Kinase 4
MMP2	Matrix Metalloproteinase 2
MMP9	Matrix Metalloproteinase 9
IL6	Interleukin 6
ECM	Extracellular Matrix
IL8	Interleukin 8
TNF-α	Tumor Necrosis Factor Alpha
VEGF	Vascular Endothelial Growth Factor
VEGFR	Vascular Endothelial Growth Factor Receptor
RAS	Rat Sarcoma
AKT	Protein Kinase B
RhoA	Ras Homolog Family Member A
CDC42	Cell Division Control Protein 42 Homolog
NF-κB	Nuclear Factor Kappa B
bFGF	Basic Fibroblast Growth Factor
c-SRC	Proto-Oncogene c-Src
Rac1	Ras-Related C3 Botulinum Toxin Substrate 1
DDR1	Discoidin Domain Receptor 1
RTK	Receptor Tyrosine Kinase
c-MET	Hepatocyte Growth Factor Receptor
SHH	Sonic Hedgehog
TME	Tumor Microenvironment
rhSHH	Recombinant Human Sonic Hedgehog
GLI1	Glioma-Associated Oncogene Homolog 1
CX43	Connexin 43
SMO	Smoothened
FLT1	Fms-Related Tyrosine Kinase 1
DHH	Desert Hedgehog
PTCH2	Patched 2
CtBP2	C-Terminal Binding Protein 2
ZBTB18	Zinc Finger and BTB Domain Containing 18
HHIP	Hedgehog-Interacting Protein
IHH	Indian Hedgehog
MB	Medulloblastoma
FOXM1	Forkhead Box M1
EN1	Engrailed 1
TUBB3	Tubulin Beta-3 Chain
TGLI1	Truncated GLI1
CDC2	Cell Division Cycle 2
PC	Primary Cilium
HH	Hedgehog
Ptch	Patched
Smo	Smoothened
GliR	Gli Repressor
SUFU	Suppressor of Fused
HH	Hedgehog Ligand
SHH	Sonic Hedgehog
DHH	Desert Hedgehog
IHH	Indian Hedgehog
GliA	Gli Activator
GSCs	Glioblastoma Stem Cells
MEK2	Mitogen-Activated Protein Kinase Kinase 2
CREB	CAMP Response Element Binding Protein
JAK	Janus Kinase
NADPH	Nicotinamide Adenine Dinucleotide Phosphate
MB	Methylene Blue
ECAR	Extracellular Acidification Rate
OXPHOS	Oxidative Phosphorylation
TCA	Tricarboxylic Acid Cycle
HK2	Hexokinase 2
PKM2	Pyruvate Kinase M2
PDH	Pyruvate Dehydrogenase
PDK	Pyruvate Dehydrogenase Kinase
PDP	Pyruvate Dehydrogenase Phosphatase
PPP	Pentose Phosphate Pathway
G6P	Glucose-6-Phosphate
6PGL	6-Phosphogluconolactone
6PG	6-Phosphogluconate
Ru5P	Ribulose-5-Phosphate
R5P	Ribose-5-Phosphate
PGLS	6-Phosphogluconolactonase
PGD	Phosphogluconate Dehydrogenase
G6PD	Glucose-6-Phosphate Dehydrogenase
GS	Glutamine Synthetase
GDH1	Glutamate Dehydrogenase 1
GLS	Glutaminase
MCTs	Monocarboxylate Transporters
FAOCoA	Fatty Acid Oxidation
2-DG	2-Deoxy-Glucose
DMAMCL	Dimethylaminomicheliolide
ATP	Adenosine Triphosphate
H3-K27M	Histone H3 Lysine 27 Mutation
LXR	Liver X Receptor
NSCs	Neural Stem Cells
OPCs	Oligodendrocyte Precursor Cells
FDA	The U.S. Food and Drug Administration.
AMPARs	AMPA Receptors
EORTC-NCIC trial	European Organisation for Research and Treatment of Cancer and National Cancer Institute of Canada

MDM2 inhibitors

RG7112	Small-molecule inhibitor of MDM2 that reactivates p53 by preventing its degradation.
RG7388	A next-generation MDM2 inhibitor with improved potency and selectivity for reactivating p53.
MI77301	Potent MDM2 antagonist that disrupts the MDM2-p53 interaction, restoring p53 function.
CGM097	Oral MDM2 inhibitor that stabilizes p53 and induces apoptosis in cancer cells.
MK8242	Selective MDM2 inhibitor that promotes p53-mediated apoptosis and cell cycle arrest.
AMG232	Highly potent MDM2 antagonist with activity in p53-wildtype tumors.

HDAC inhibitors

SAHA (Vorinostat)	Histone deacetylase inhibitor that induces apoptosis and inhibits tumor growth.
CUDC-907	Dual inhibitor of HDAC and PI3K pathways, enhancing anti-tumor activity.
CUDC-101	Multi-target inhibitor of HDAC, EGFR, and HER2, designed to overcome resistance mechanisms.
CCNU (Lomustine)	Alkylating agent with potential HDAC inhibitory effects, used in glioblastoma treatment.

P53 activators

PRIMA-1	Restores mutant p53 to its wild-type conformation, reactivating its tumor suppressor function.
PK11007	Small molecule that stabilizes p53 by targeting mutant forms.
APR-246 (Eprenetapopt)	Converts mutant p53 to its active form, inducing apoptosis.
PK7088	Selective activator of p53 function, targeting specific mutations.
ZMC1	Zinc metallochaperone that reactivates mutant p53 by restoring its zinc-binding capability.
COTI-2	A small molecule that reactivates p53 and inhibits PI3K/AKT signaling.
pCAPS	Synthetic peptide that stabilizes the active form of p53.
PEITC	A naturally occurring compound that restores p53 activity and induces oxidative stress in cancer cells.

CP-31398	Stabilizes the p53 protein, restoring its transcriptional activity in tumors.
PEPTIDE ReACp53	Specifically targets and refolds mutant p53 aggregates, reactivating its function.
RETRA	Small molecule that selectively kills cells with mutant p53 by disrupting mutant p53-driven pathways

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