

Anticancer Mechanism of Curcumin on Human Glioblastoma

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Abstract: Glioblastoma (GBM) is the most malignant brain tumor and accounts for most adult brain tumors. Current available treatment options for GBM are multimodal, which include surgical resection, radiation, and chemotherapy. Despite the significant advances in diagnostic and therapeutic approaches, GBM remains largely resistant to treatment, with a poor median survival rate between 12 and 18 months. With increasing drug resistance, the introduction of phytochemicals into current GBM treatment has become a potential strategy to combat GBM. Phytochemicals possess multifarious bioactivities with multitarget sites and comparatively marginal toxicity. Among them, curcumin is the most studied compound described as a potential anticancer agent due to its multi-targeted signaling/molecular pathways properties. Curcumin possesses the ability to modulate the core pathways involved in GBM cell proliferation, apoptosis, cell cycle arrest, autophagy, paraptosis, oxidative stress, and tumor cell motility. This review discusses curcumin's anticancer mechanism through modulation of Rb, p53, MAPK, P13K/Akt, JAK/STAT, Shh, and NF-κB pathways, which are commonly involved and dysregulated in preclinical and clinical GBM models. In addition, limitation issues such as bioavailability, pharmacokinetics perspectives strategies, and clinical trials were discussed.

Keywords: curcumin; glioblastoma; anticancer; molecular signaling mechanism

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1. Introduction

Brain tumors can be classified into grade I and II (benign, low-grade), grade III (malignant, high-grade) such as anaplastic astrocytoma, and grade IV (highly aggressive and malignant) such as glioblastoma (GBM) [1]. GBM is the most common and aggressive form of malignant primary adult brain tumor [2]. In the United States alone, the annual age-adjusted incidence of GBM is 3.22 per 100,000 persons based on registry data from 2012 to 2016 [2]. Based on the 2016 WHO classification of the central nervous system tumors, GBM is classified as a grade IV diffuse glioma. GBM is further classified into isocitrate dehydrogenase-wildtype (IDH-wildtype), IDH-mutant, and not otherwise specified (NOS) [3]. IDH-wildtype or primary (de novo) GBM accounts for 90% of the total proportion of GBM cases [3]. The IDH-mutant or secondary GBM, which may arise from a lower grade diffuse glioma, only accounts for about 10% of the total GBM cases [3]. Primary GBM is more common in elderly patients (median age of 62 years), while secondary GBM preferentially arises in younger patients (median age of 44 years) [3].

The standard care for newly diagnosed GBM patients is surgical resection, followed by radiotherapy (60Gy in 30 fractions) with concurrent oral administration of temozolomide (TMZ), followed by six cycles of adjuvant [4]. Additionally, monoclonal antibody bevacizumab and other alkylating agents such as carmustine, lomustine, nimustine, and fotemustine are used in GBM treatment [4]. Unfortunately, these treatments

often prove ineffective, given the poor prognosis outcomes of GBM with a five-year survival rate under 10% and a median survival rate of around 12 to 18 months [2,5]. The high infiltration degree of GBM often causes surgical resection incapable of fully resecting the GBM tumor, leaving the residual presence of microscopic foci [6,7]. Moreover, the GBM tumors often develop chemo- and radio-resistance with the formation of glioma stem cells, leading to GBM recurrence [8]. In TMZ-resistant GBM tumors, numerous molecular pathways such as nuclear factor kappa light chain enhancer of activated B cells (NF- κ B), p53, and JAK-STAT are found to be commonly dysregulated [8]. In addition, several clinical complications such as pancytopenia, pyrexia, wound healing complications, multi-organ failure, or even death are observed following the chemo-radiation and immunotherapy treatment [8–10].

Thus, in recent years, scientists have been focusing on phytochemicals as potential therapeutic agents in cancer management to minimize drug toxicity and side effects. Flavonoids represent the most common and widely distributed phytochemicals in fruits and vegetables. Various flavonoids such as tannins, quinones, stilbenes, and curcuminoids possess antioxidant, anti-inflammatory, antiviral, antimutagenic, and, most importantly, anticancer properties [11,12]. Among them, curcuminoids (especially curcumin) have been gaining immense attention because of its anticarcinogenic, antitumor, antioxidant, and anti-inflammatory actions [13–15]. Curcuminoids are a family of active compounds found in the turmeric rhizome (*Curcuma longa*), an Indian spice commonly used in cooking. Natural curcuminoids are composed of curcumin, bisdemethoxycurcumin, and demethoxycurcumin in a proportion of 77:3:17 [16]. Among them, curcumin is the most abundant compound and has been widely studied as a potential therapeutic agent in chronic diseases, such as neurodegenerative, cardiovascular, pulmonary, metabolic, and autoimmune diseases [17]. For instance, curcumin was able to restore oxidative stress and DNA methyltransferase (DNMT) functions against diabetic retinopathy [15]. Curcumin also acts as a wound healing promoting agent by facilitating collagen synthesis and fibroblast migration [18]. Several pre-clinical and clinical studies also reported its anticancer effects in colorectal cancer [19,20], pancreatic cancer [21], lung cancer [22], and GBM [23]. Curcumin can modulate multiple cellular signaling pathways and molecular targets involved in GBM tumor growth, migration, invasion, cell death, and proliferation [24–27]. Retinoblastoma (Rb), p53, MAP kinase (MAPK), P13K/Akt, JAK/STAT, sonic hedgehog (Shh), and NF- κ B pathways are the most common targeted dysregulated pathways found in GBM and modulated by curcumin [28–34]. Moreover, curcumin is highly lipophilic and able to cross the blood–brain barrier (BBB) [35,36].

To date, numerous review studies have suggested curcumin as a potential drug for GBM. However, a greater focus on curcumin's anticancer potential in molecular signaling pathways that are commonly dysregulated in GBM is needed to provide a more comprehensive understanding of its therapeutic effects. This review includes the initial until recent pre-clinical and clinical studies of curcumin's mechanisms of action in modulating several molecular pathways such as Rb, p53, MAPK, P13K/Akt, JAK/STAT, Shh, and NF- κ B pathways. This review paper also discusses curcumin's related issues such as low bio-availability, pharmacokinetics, and the perspective strategies to overcome these issues.

2. Dysregulated Signaling Pathways Associated with GBM Pathogenesis

Almost all GBMs are found to have dysregulated Rb, p53, JAK/STAT, MAPK, P13K/Akt, Shh, and NF- κ B pathways. Thus, the following section discusses the mechanisms of action modulated by curcumin via these molecular signaling pathways involved in GBM cell proliferation, apoptosis, cell cycle arrest, autophagy, paraptosis, oxidative stress, and cell motility. The reported observation of in vitro and in vivo studies of curcumin against GBM are summarized in Table 1.

Table 1. Signaling pathways and mechanism of actions targeted by curcumin in vitro and in vivo against glioblastoma (GBM).

Molecular Pathway	GBM Model (In Vitro or In Vivo)	Pathway Mechanism Targeted by Curcumin OR Mechanism of Actions Induced by Curcumin	References
Rb	DBTRG	Induced G1/S phase arrest by upregulating CDKN2A/p16 and downregulating the expression of RB protein	[28]
P53	DBTRG	Induced G2/M phase arrest by upregulating p21 and downregulating cdc2, followed by increasing of p53 protein	[28]
	U251	Induced G2/M phase arrest by upregulating ING4 expression	[37]
	C6	Induced G0/G1 phase arrest and apoptosis by upregulating p53 and p21Waf/Cip1 protein levels	[38]
	U87MG	Enhanced anticancer effect of ETP and TMZ through downregulation of the p53 protein expression	[39]
	A172	Induced paraptosis by regulating the ER-related miRNAs that interact with the p53-BCL2 pathway	[40]
	KSN60	Decreased p21 expression and increased cyclin B1	[41]
	U251MG (KO)	Induced apoptosis through increased BAX:BCL2 ratio via mitochondria-mediated pathway	[42]
JAK/STAT	Tu-2449	Suppressed cell proliferation by inducing G2/M cell cycle arrest and inhibited cell invasion through downregulation of STAT3 target genes <i>c-Myc</i> , <i>MMP-9</i> , <i>Snail</i> and <i>Twist</i> , <i>Ki67</i>	[29]
	Tu-9648		
	Tu-251		
	C6B3F1 mice	Reduced growth and midline crossing of intracranially implanted tumors and proliferation of tumor, increased tumor-free long term survival rate by 15% and 38%	[43]
	A-172		
	MZ-18	Inhibited cell proliferation, migration, invasion by decreasing expression of phosphorylated STAT3 protein and its target genes <i>c-Myc</i> and <i>Ki67</i>	
	MZ-54		[44]
	MZ-256		
	MZ-304		
MAPK	U251	Induce epigenetic modifications through suppression of STAT3 protein activity, followed by <i>RANK</i> promoter methylation along with <i>RANK</i> activation	[30]
	U87		
	Glio3	Induction of intracellular ROS production through downregulation of STAT3 activity	
	Glio9		[45,46]
	U87MG	Inhibited invasion of GBM cells through downregulation of MAP kinase pathway along with decreased PMA-induced mRNA expression of <i>MMP-1</i> , <i>-3</i> , <i>-9</i> , and <i>-4</i>	
	U373MG		
	CRT-MG		[30]
	Glio3	Decreased GSC viability through induction of P38, ERK, and JNK activity	
	Glio9		
P13K/Akt	U87MG	Inhibited cell proliferation by upregulating <i>Egr-1</i> expression through ERK and JNK pathway	[47]
	U87MG	Induced autophagy through induction of ERK1/2 pathway	[48]
	U373MG		[49]
	C6	Inhibited neuroinflammatory effect through inhibition of LPS-induced CCL2 expression via JNK pathway	
	U138MG		
	U87	Induced cell cycle arrest in G2/M phase and apoptosis by inhibiting Akt phosphorylation on Ser473	[33]
	U373		
	C6		
	C6 mice	Reduced tumor size and increased number of apoptotic cells in tumor	[31]
P13K/Akt	U251	Inhibited cell proliferation, migration, and invasion by decreasing P13K/mTOR protein expression and restoring PTEN expression	
	U87		
	U87 xenograft	Inhibited tumor growth and increased PTEN expression	[48,50]
	U87MG		
	GL261	Induced autophagy through suppression of AKT/mTOR/p70S6K pathway	
	F98		[51]
	U373MG		
	U87 xenograft	Inhibited tumor growth and induced autophagy	
	U118MG	Enhanced anti-proliferation, anti-migration, and proapoptotic activities of ACNU against GBM by suppressing P13K/Akt pathway	
P13K/Akt	U251MG		[51]
	U87MG		

Shh	U87	Inhibited GBM cell proliferation, migration, and invasion through suppressing core components and GLI1-dependent target genes in Shh/GLI1 pathway	[32]
	T98G	Inhibited GBM growth and prolonged the survival rate	
	U87 xenograft	Promoted expression of mi-R326 and enhanced inhibition of SHH/GLI1 pathway	[52]
	U251	Inhibited GBM growth and prolonged the survival rate	
NF-κB	U138MG	Induced apoptosis through inhibition of NF-κB survival pathways by downregulating the antiapoptotic proteins	[33]
	C6	Decreased brain tumors (growth/size/) without reported tissues, metabolic, oxidative, or hematology toxicity	
	C6-implanted rats		
	U118MG		
	U251MG	Blocked GBM tumor growth by inhibiting NF-κB/COX-2 pathway	[51]
	U887MG		
	T98G	Induced apoptosis through downregulation of NF-κB, IAPs, and upregulation expression of IκBα	[53]
	NP-2	Induced tumor cell death through downregulation of NF-κB activity and its regulated protein cyclin D1	[54]
	GBM 8401	Induced apoptosis through inhibition of NF-κB activity	[55]
	C6	Induced cytotoxic and antiproliferative activity of PTX through inhibition of NF-κB signaling pathway	[38]
	U87MG	Induced apoptosis through suppression of apoptosis protein inhibitor and down-regulation of anti-apoptotic NF-κB dependent genes	[42]

2.1. Retinoblastoma (RB) Pathway

The RB pathway plays a central role in cell proliferation by regulating the cell cycle [56]. This pathway mainly consists of five components, which are CDKN2A/p16Ink4a, cyclin D1, cyclin-dependent protein kinases (cdk4/6), RB-family of pocket proteins (RB, p107, p130), and E2F [57,58]. CDKN2A/p16 is a negative regulator that competes with cyclin D1 to bind to and inhibit the activity of CDK4/6. This, in turn, can induce cell cycle arrest at the G1/S phase by inhibiting phosphorylation of RB protein by the cyclin D1/cdk4/6 complex. The unphosphorylated RB protein binds to E2F protein to inhibit the activation of E2F-regulated gene expression, thereby inhibiting cell cycle progression, DNA replication, and nucleotide biosynthesis [57].

Many of the important components of this pathway are frequently altered in many cancer cells, including GBM [59]. According to The Cancer Genome Atlas (TCGA) pilot project, most of the GBM acquired mutations include homozygous deletion or mutation of *CDKN2A/p16* and *RB1*, and amplification of *CDK4*, *CDK6*, and *cyclin D*, which are associated with the RB signaling pathway [60]. *RB* promoter methylation and gene silencing are found in GBMs and are more frequently reported in secondary GBMs than primary GBMs [61]. The inhibition of the RB pathway via silencing/suppression of its component proteins increases etoposide-induced DNA double strand breaks, p53 activation, and TMZ-induced GBM apoptosis [62–65]. Additionally, the inhibition of cyclin D1 can down-regulate P-glycoprotein (pgp) expression, which may help to overcome chemoresistance in GBMs [63]. Thus, the RB signaling pathway is an important drug targeted pathway to improve GBM prognosis and patient outcomes.

As shown in Figure 1a, curcumin inhibits the RB signaling pathway by increasing the negative regulator CDKN2A/p16Ink4a activity, which then suppresses the phosphorylation of RB protein. To date, Chin-Cheng Su and colleagues demonstrated that curcumin significantly inhibited the RB pathway in DBTRG glial cells in a time- and concentration-dependent manner [28]. In this study, curcumin treatment upregulated CDKN2A/p16 and downregulated the phosphorylated RB protein. It has been shown that CDKN2A/p16 protein compete with cyclin D1 to bind to CDK4/6 protein, which then inhibits phosphorylation of RB protein. Unphosphorylated RB protein could not dissociate from its repressor E2F to permit transcription of G1 genes for proceeding from G1 to S phase.

2.2. P53 Pathway

P53 is a tumor suppressor protein that can activate cell cycle arrest or induce cell apoptosis to prevent damaged cells from further dividing and growing [66]. Following DNA damage, p53 is activated to induce transcription of p21Waf/Cip1, a cyclin-dependent kinase inhibitor [58]. This P21Waf/Cip1 protein can induce G1/S and G2/M arrest by binding to and inhibiting the activity of Cdc2, cyclin-CDK2, -CDK1, and CDK4/6 complexes. This allows the damaged cells to undergo DNA repair prior to mitosis. The p53 activity can be inhibited by MDM2, the transcription of which is induced by TP53 through a negative feedback loop [67]. However, P14ARF, which is located in part of the CDKN2A locus, can bind to and inhibit MDM2 from binding to the N-terminal transactivation domain of TP53 [58,68]. On the other hand, activated p53 protein can activate the pro-apoptotic BH3-only members of the Bcl-2 protein family [69]. These pro-apoptotic proteins bind and inhibit the pro-survival Bcl-2 proteins to initiate the pro-apoptotic multi-BH domain members of the Bcl-2 family, such as BAX and BAK, to induce cell apoptosis.

Dysregulation of p53 pathway in GBM is mostly due to *TP53* mutation, amplification of *MDM2*, or loss of expression of *CDKN2A-p14ARF* [70]. The TCGA project demonstrated that the p53 signaling pathway is altered in most GBM samples with the association of *TP53* mutation or homozygous deletion, *P14ARF* deletions, and amplification of *MDM2* and *MDM4* [60]. According to WHO, the *TP53* mutation is more commonly seen in secondary GBM and is higher in proportion to primary GBM [3]. The clinical study showed that most of the sample cells (from GBM patients with age around 56) were Bcl-2 positive, and most of the Bcl-2 positive cloned cells acquired chemoresistance [71]. Thus, various strategies have been developed to target the p53 pathway, such as inhibition of pro-survival genes or MDM2/p53 interaction, degradation of mutant p53, and restoration of wildtype p53 [72–74].

As shown in Figure 1b, curcumin upregulates the expression of p53, p21, and ING4 and increases the BAX:BCL2 ratio to induce cell cycle arrest and apoptosis. Curcumin upregulates the expression of p53 and p21 in a time- and concentration-dependent manner, which then induces G2/M arrest in DBTRG cells [28]. Curcumin induces p53 activity by upregulating p21Waf/Cip1 and ING4 protein expression [37]. The upregulation of ING4 expression can increase p53 acetylation at Lys-382 and protein stability [75,76]. The acetylation of p53 inhibits its interaction with MDM2, which eventually induces cell cycle arrest and apoptosis, as observed in U251 cells [37]. In a different study, curcumin enhances paclitaxel (PTX) activity in C6 cells by increasing TP53 and p21 gene expression. In response to the increase of TP53 and p21 gene expression, the cell population in the G0/G1 phase increases while the cell population in the S phase decreases, indicating G0/G1 phase arrest [38]. Moreover, curcumin suppresses A172 cell viability by inducing paraptosis through the regulation of genes associated with the endoplasmic reticulum (ER) stress response [40]. Interestingly, in this study, interaction network analysis (with IPA software) revealed that the altered levels of ER-related miRNAs interact with p53-BCL2 pathways. Thus, it was suggested that the p53-BCL2 pathway might be involved in curcumin anticancer mechanisms. Additionally, curcumin potentiates the cytotoxic and apoptosis-inducing effect of etoposide and TMZ through downregulation of p53 mRNAs and upregulation of BAX-Bcl-2 in T98G and U87MG cells [39,42,55].

However, a contradictory finding showed that curcumin did not induce cell cycle arrest, as it enhanced cyclin B1 and decreased p21 expression in the radioresistant KNS60 and U251MG(KO) cells [41]. These radioresistant cells usually have a high basal p53 level, but the expression of p53 decreased following curcumin treatment. These results showed that mechanism actions of curcumin in radioresistant GBM cells are different.

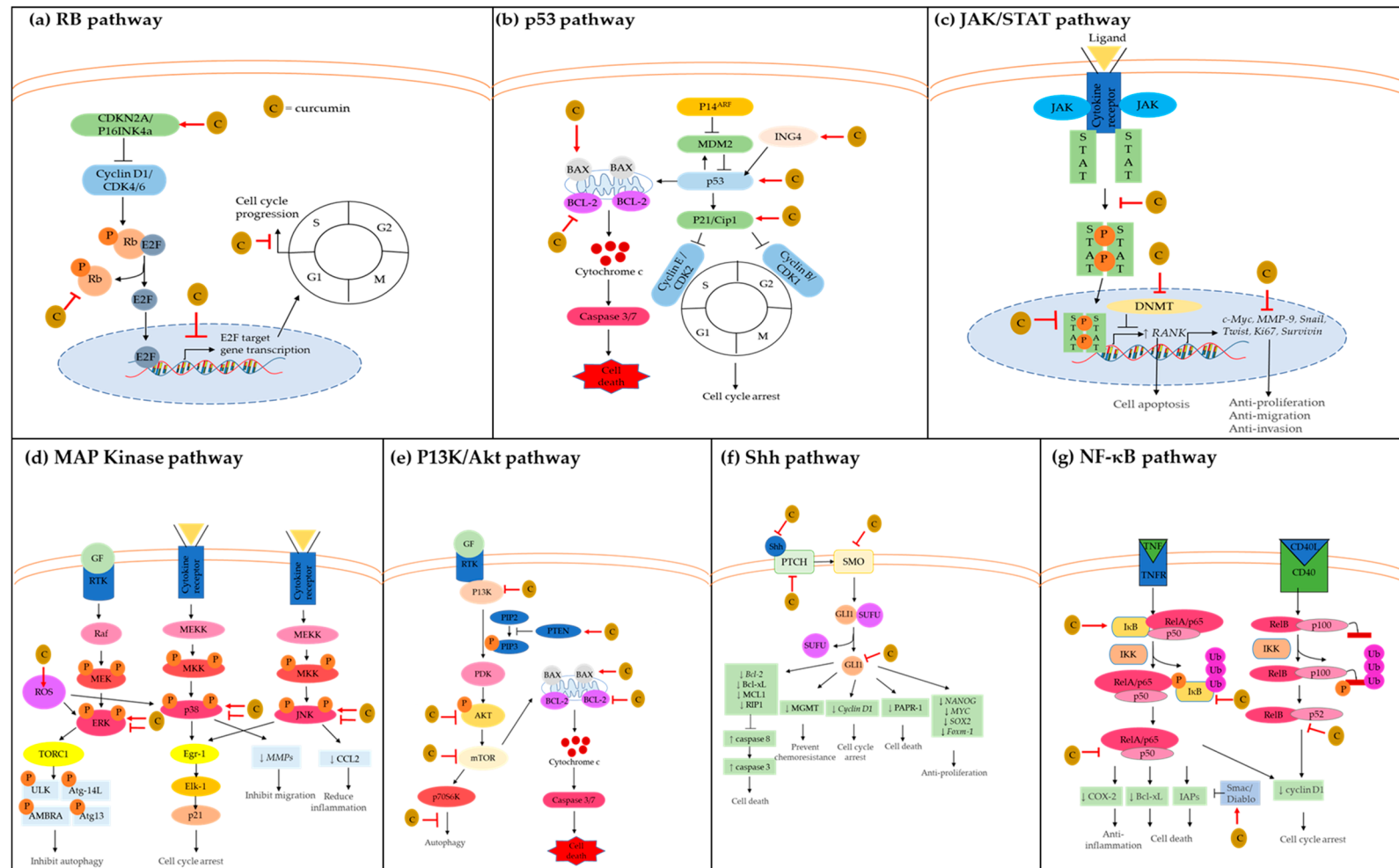


Figure 1. Mode of curcumin actions as anti-cancer agent on the key molecular targets in GBM. Curcumin possesses anti-cancer properties by inhibiting signaling pathways and their downstream molecular targets; (a) retinoblastoma (RB) pathway; (b) p53 pathway; (c) JAK1,2/STAT3 pathway; (d) MAP kinase pathway; (e) P13K/Akt pathway; (f) Shh pathway; (g) NF-κB pathway. Molecular targets and signaling pathways that are upregulated and downregulated by curcumin are noted by using → and ⊥, respectively.

2.3. JAK/STAT Pathway

Cytokines and growth factors can activate the Janus kinase/signal transducers and activators of transcription (JAK/STAT) pathway to regulate cell proliferation, differentiation, migration, and apoptosis. This pathway involves the activation of growth factor receptor kinases, phosphorylation, dimerization, and translocation of STAT proteins into the nucleus to activate the downstream target genes. JAKs (Jak1, JAK2, JAK3, Tyk2) are the cytoplasmic tyrosine kinases that relay intracellular signals originating from extracellular receptors [77–79]. Following JAKs activation and phosphorylation of the tyrosine residues on receptors, STAT is activated through its recruitment and binding to the phosphorylated tyrosine residues. The activation of STATs only lasts from a few minutes to several hours under normal physiological conditions. However, aberrant activation of STAT signaling is found in many GBM tissues compared with normal human astrocytes, white matter, and normal adjacent tissue to the tumor [29,80,81]. Studies showed that the inhibition of either JAK or STAT phosphorylation is associated with reduced levels of anti-apoptotic proteins, resulting in apoptosis in GBM cells [29,43,81–83].

As shown in Figure 1c, curcumin can inhibit STAT activation and its downstream target genes involved in cell proliferation, migration, and invasion. Curcumin inhibits JAK1,2/STAT3 tyrosine-phosphorylation, and STAT3 target genes such as *c-Myc*, *MMP-9*, *Snail*, *Twist*, and *Ki67*, which in turn decrease GBM cell migration, invasion, and proliferation [29]. In the same study, curcumin significantly decreased the tumor cell proliferation and growth of the mid-line crossing in the intracranially implanted tumor-bearing mice compared with the control diet. Moreover, curcumin treatment resulted in 15% and 38% tumor-free long-term survival in Tu-2449-bearing mice and Tu-9648-bearing mice, respectively, where all control mice died. Consistently, another study supported that curcumin is capable of inhibiting cell proliferation through the inhibition of STAT3 protein along with reduction *c-Myc* and *Ki-67* transcription in several glioma cell lines [43]. Moreover, curcumin can inhibit the DNMT analogue M.Sss1 to demethylate the RANK CpG sites and transcriptionally upregulate RANK gene expression in U251 cells [44]. This study further reported that curcumin induces RANK expression through STAT3 suppression. Activation of RANK has been known to be associated with pro-apoptotic and anti-tumorigenesis activities [84,85]. Additionally, inhibition of STAT3 can result in suppression of STAT3-DNMT1 interaction, which then demethylates tumor suppressor gene promoters [86]. Other than that, curcumin decreases the Tyr705 and increases the Ser737 phosphorylated form of STAT3 in human patient-derived GSC lines [30]. This causes the inactivation of STAT3 proteins via suppression of its nucleus translocation, which suppresses the activation of its downstream target genes, such as *survivin*, which inhibits GBM cell proliferation.

2.4. MAP Kinase Pathway

The mitogen-activated protein kinase (MAPK) pathway is a three-layer signaling cascade. This MAPK cascade is comprised of MAPK3, which activates MAPKK2 through serine/threonine phosphorylation, which then activates MAPK through tyrosine/threonine phosphorylation within a conserved Thr–Xxx–Tyr motif in the activation loop of the kinase domain [87]. There are at least 11 members of the MAPK superfamily, which are divided into three main groups: the extracellular signal-regulated protein kinases (ERK), c-Jun N-terminal kinases (JNK), and p38s [88]. Generally, ERK genes are activated by growth factors and mitogen, and the signaling cascades include RAF as MAPKKK and MEK as MAPK. The activation of ERK signaling promotes cell growth, apoptosis, differentiation, and development [89]. While p38s and JNK are activated by stress, inflammatory cytokines, and growth factors [90], their signaling cascades include MEKK as MAPKKK and MKK as MAPKK. Activation of either p38s or JNK genes may support inflammation, cell apoptosis, cell motility, growth, and chromatin remodeling. ERK, p38, and JNK signaling pathways favor both anti-apoptotic and pro-apoptotic proteins, depending

on the cell type and condition [91]. Hence, the aberrant activation or deactivation of this MAPK pathway can promote abnormal cell proliferation, contributing to tumorigenesis.

Studies showed that targeting the MEK-ERK1/2 pathway is one of the approaches to block adhesion of GBM cells onto gelatin/collagen component of ECM, therefore decreasing the proliferation and migration of GBM cells [92,93]. The high expression of p38 had a positive correlation with the glioma's malignancy grade, while suppressing p38 expression inhibited proliferation and induced apoptosis in GBM cells [94]. Ken-ichiro Matsuda and colleagues discovered that self-renewing stem-like GBM cells have elevated JNK phosphorylation levels, accompanied by increased c-JUN phosphorylation at the cognate JNK phosphorylation site [95]. Treatment with JNK inhibitor reduced the self-renewing ability of the stem-like GBM cells, suggesting JNK is needed for self-renewal in vitro and in vivo. Several studies showed that the inhibition of ERK, JNK, and p38 MAPK pathways induced GBM cell cycle arrest and inhibited cell proliferation [96–99].

Curcumin can modulate the MAPK signaling pathway to regulate cell proliferation, tumorigenesis, apoptosis, and inflammation, as shown in Figure 1d. So-Young Kim and colleagues discovered that curcumin potently inhibited glioma invasion by inhibiting all the MAPK pathways (JNK, p38, ERK), which then suppressed phorbol myristate acetate (PMA)-induced mRNA expression of *MMP-1*, *-3*, *-9*, and *-14* in U87MG and U373MG cells [45,46]. Overexpression of the matrix metalloproteinases (MMPs) facilitates migration and invasion of malignant brain tumor cells to the surrounding brain tissues. These MMPs are upregulated in human malignant gliomas [100]. Among the MMPs, MMP-9 is the most common enzyme that promotes brain tumor invasion and is frequently found in GBM [101]. MMP-1 protein level is increased with the tumor grade and correlated with increased glioma invasiveness [102]. At the same time, the activation of MMP-3 can degrade the brain's hyaluronic acid-rich matrix, which leads to the invasion and migration of tumor cells [103]. Additionally, MMP-14 is a membrane-bound protease that can remodel the ECM to stimulate proMMP-2 activation. Curcumin treatment (10 μ M) inhibits cell invasion by more than 90% in U87MG and U373MG cells [45].

In contrast, curcumin increases the phosphorylated ERK, p38, and c-Jun proteins levels, which decreases GBM stem cell (GSCs) proliferation, sphere-forming ability, and colony-forming potential [30]. Curcumin can also promote MAPK pathway activation through the induction of reactive oxygen species (ROS) [30]. The production of intracellular ROS can induce the activation of ERK and p38 MAPK pathways through oxidative modification of intracellular kinases and inactivation of the MAPK phosphatases [104,105]. Moreover, curcumin induced Egr-1 expression through the activation of p38, ERK, and JNK pathways, which mediated the transactivation of Elk-1 in U87MG human GBM cells [47]. Egr-1 binds directly to the p21 promoter to stimulate p21 transcription, inhibiting CDK activity and resulting in cell cycle arrest. Elk-1 is the direct target gene of the p38, ERK, and JNK pathways, which can form a complex with serum response factor on the serum response element of the Egr-1 promoter to activate the p53-independent transcriptional activation of p21Waf/Cip1 protein. Additionally, curcumin can induce autophagy in GBM cells through the inhibition of the ERK1/2 pathway [48]. Another study suggests that the inhibition of the ERK pathway can lead to suppression of TORC1, which plays an important role in inhibiting autophagy initiation through the phosphorylation of Atg13, ULK, AMBRA, and Atg-14L [106]. In a C6 orthotropic xenograft, curcumin suppressed the phosphorylated JNK1 and JNK2 levels, which decreased lipopolysaccharide-induced CCL2 production [49]. The overexpression of CCL2 can contribute to GBM progression by inducing encephalopathy with mild perivascular leukocyte infiltration, impaired BBB function, and increased expression of proinflammatory cytokine expression [107].

2.5. P13K/AKT Pathway

The phosphatidylinositol-3-kinase (P13K)/Akt signaling pathway is critical in regulating cell growth, cell cycle arrest, apoptosis, and mRNA translation to maintain normal

physiological conditions. There are three different classes of P13Ks, namely Class I, II, and III, which are categorized accordingly to their different structure and specific substrates [108,109]. Class I P13Ks are heterodimers consisting of a p110 catalytic subunit and p85 adaptor subunit [110,111]. Notably, Class I P13Ks is the most common type of P13K, which is incriminated in human cancer. The binding of cytokines or growth factors to the corresponding receptors results in the tyrosine residue autophosphorylation, followed by P13K binding protein recruitment. Upon allosteric activation of the p110 catalytic subunit, P13K catalyzes the phosphorylation of PtdIns(4,5) P2 (PIP2) to PtdIns(3,4,5) P3 (PIP3), which then recruits a subset of signaling proteins with pleckstrin homology (PH), such as AKT and PDK1, to initiate cell proliferation pathways. Protein-phosphatase and tensin homologue (PTEN) act to dephosphorylate PIP3 into PIP2 to prevent activation of the downstream kinases [110,111]. One of Akt's common target proteins is mTOR, which regulates cell growth and proliferation by promoting biosynthesis of multiple proteins such as cyclin D1, HIF, and VEGF [110]. The mTORC1 activates S6K and inactivates 4EBP1, promoting the production and translation of proteins to promote cell growth. While the mechanism of mTORC2 is less well clarified, it has the same responsibility in promoting cell proliferation [110].

Studies have reported that mutations of the core genes involved in P13K/AKT pathways are commonly found in human GBM tissues [60,112]. Alfeu Zanotto-Filho and colleagues reported that the P13K/Akt pathway are highly upregulated (seven- to eight-fold) in C6 and U138MG cell lines compared to the normal astrocytes cells [33]. Consistently, data from the TCGA pilot project showed that most of the GBM samples acquire homozygous deletion or mutation of *PTEN*, *P13K* mutation, and amplification of *AKT* and *FOXO* genes [60]. Another report indicated that genetic alterations such as loss of heterozygosity (LOH), mutation, and methylation have been identified in most GBM patients. LOH or *PTEN* mutation is positively associated with the poor survival of GBM patients [113]. It was reported that the delivery of Akt small-molecule inhibitor to inhibit the P13K/AKT pathway effectively suppressed the growth of both stem and non-stem GBM cell populations [114].

As seen in Figure 1e, the P13K/Akt signaling pathway and its key molecular targets are inhibited by curcumin to prevent GBM progression. Curcumin inhibits 80% of the P13K/Akt pathway's constitutive activation by suppressing the phosphorylation of Akt proteins on Ser473 [33]. Inhibition of the P13K/Akt pathway resulted in the induction of G2/M phase arrest as an early step of the apoptotic mechanism, which could probably explain how curcumin spared the non-transformed and its selectivity towards the tumor cells. In the same study, curcumin decreased GBM tumor size and increased apoptotic tumor cells in C6-implanted Wistar rats. Most importantly, curcumin did not cause any tissue toxicity in the rats' liver, kidney, lungs, or heart. Other than that, curcumin inhibited the P13K/Akt pathway by increasing PTEN expression, which decreased p-Akt and p-mTOR expression, leading to cell apoptosis [31]. In the same study, curcumin also inhibited GBM tumor growth by increasing PTEN protein expression in the U87 xenograft model.

Additionally, curcumin induces autophagy by inhibiting the AKT/mTOR/p70S6K pathway in GBM cell lines and xenograft models [48,50]. In these studies, curcumin significantly decreased the levels of P13Kp85, phosphoP13Kp85, total Akt, p-AKT, mTOR, and p-mTOR. mTOR is not only a major effector of cell growth and proliferation, but it can also inhibit autophagy events in its active form [115]. Thus, inhibiting expression of P13K and AKT, which regulate mTOR expression, is a feasible strategy to induce autophagy-cell death in GBM cells. Notably, curcumin downregulates Bcl-2 and upregulates BAX, leading to the release of cytochrome-c and caspase-3 activation. Curcumin also enhances the anti-cancer effects of nimustine hydrochloride (ACNU) against GBM by inhibiting the phosphorylation of P13K and the AKT (serine/threonine) [51,116].

2.6. Sonic Hedgehog (Shh) Pathway

The hedgehog (Hh) signaling pathway is critical for embryonic development, organogenesis, regeneration, and homeostasis for adult tissue [117]. There are three main types of Hh proteins, which are sonic hedgehog (Shh), Indian hedgehog (Ihh), and desert hedgehog (Dhh). The activation of the Shh pathway can occur either through canonical or non-canonical signaling pathways [117]. The canonical Shh activation occurs by ligand-dependent interaction when Shh binds to the patched transmembrane receptor (PTCH) [117,118]. Following this binding, PTCH is incapable of inhibiting the second transmembrane protein, smoothened (Smo). Smo signals the suppressor of fused (SUFU), which is the negative regulator of glioma-associated oncogene homologue (GLI), to release and activate GLI. The activated GLI translocates into the nucleus and modulates downstream gene expression. On the other hand, the non-canonical Shh activation occurs through either GLI-independent mechanisms or Smo-independent mechanisms [117,118]. In the GLI-independent mechanism, Smo is no longer inhibited by the PTCH, and therefore it can stimulate the release of calcium ions from the ER to control the growth of the actin cytoskeleton [118]. In contrast, the Smo-independent mechanism is involved in cyclin B activation to increase cell proliferation and survival [118].

Under normal physiological conditions, the Shh pathway is minimally active in differentiated adult tissue, as it is a highly conserved development pathway. The Shh pathway is frequently associated with GBM tumorigenesis [119–122]. Studies have reported that most of the GBM patient tissues samples exhibited an aberrant activation of Hh signaling with the presence of GLI1 in both nucleus and cytoplasm [119,123]. Among GLI family members, overexpression of GLI1 is mostly associated with poor prognosis in several cancers, including GBMs [32,124–127]. GLI1 protein can upregulate several target genes such as *PTCH1*, *CycD1*, *MYC*, *Bcl-2*, *NANOG*, and *SOX2* to promote cell proliferation, apoptosis, angiogenesis, and stem cell self-renewal [128–130]. A study showed that inhibiting GLI1 alone significantly decreases the metabolic activity of GBM cells to reduce chemoresistance [119]. This study also revealed that inhibiting the expression of GLI1 proteins can elevate the nuclear p53 level in U87MG cells. Additionally, the overexpression of Smo is significantly associated with poor prognosis in GBM patients [131]. Smo expression inhibition, which suppresses GBM proliferation, migration, invasion, and tumorigenesis, further supports this observation [132].

As shown in Figure 1f, curcumin is a potent inhibitor of the SHH/GLI signaling pathway by downregulating the Shh, Smo, PTCH, and GLI protein levels and its downstream target genes such as *cyclin D1*, *Bcl-2*, and *Foxm-1* [32,52,133]. Curcumin inhibits GBM cell proliferation, colony formation, migration, and induced apoptosis through downregulation of both mRNA and protein levels of SHH/GLI1 signaling (Shh, Smo, and GLI1) in U87 and T98G cells [32]. Curcumin also inhibits GLI nuclear translocation, which deactivates its downstream target genes including *cyclin D1*, *Bcl-1*, and *Foxm-1*. The combination treatment of curcumin and miR-326 can further reduce the tumor volume and prolong the survival period of U87-bearing mice by inhibiting GLI1 proteins compared with miR-326 or curcumin treatment alone [52]. In the same study, curcumin-treated GBM cells significantly decreased the expression of GLI1 protein, and this observation was enhanced with combination treatment with miR-326. The curcumin and miR-326 treatment also increased the expression of caspase-3 cleaved anti-poly ADP ribose polymerase 1 (PARP-1) caspase-in GBM cells. Simultaneously, the pro-survival proteins BCL-XL, MCL1, and RIP1 were decreased compared to the control and curcumin only-treated GBM cells.

2.7. NF-κB Pathway

NF-κB is a family of highly conserved transcription factors that regulate the transcription of various genes involved in cellular activities. There are four members under this NF-κB family: NF-κB1(p50/p105), NF-κB2(p52/p100), Rel-like domain-containing protein A (RelA/p65), and c-rel [134]. They form a dimeric complex (either homodimers

or heterodimers) and bind to the specific sequences of DNA called response elements (RE) for the transcription of gene involved in cell proliferation, apoptosis, and inflammatory response [133,135]. NF- κ B activation can occur through two major signaling pathways: the canonical and the non-canonical NF- κ B signaling pathways [136,137]. The canonical pathway is mediated through the nuclear translocation of p50, RelA, and c-Rel into the nucleus and binding to the targeted DNA sequences. In contrast, the non-canonical NF- κ B pathway selectively responds to stimulus and activates p100-sequestered NF- κ B members, predominantly via translocation of NF- κ B p52 and Rel B into the nucleus. NF- κ B members normally bind to the DNA sequences of anti-apoptotic, pro-survival, and immune response genes. Several studies have demonstrated that human GBM cells have aberrant NF- κ B activity to maintain their tumorigenic activity [138–143].

NF- κ B p65 subunit is overexpressed in 81% of 69 samples of GBM and is frequently noted in high-grade compared to low-grade astrocytomas [139]. The constitutive activation of NF- κ B p65 is detected in 93% of the GBM cells as compared to normal astrocytes. Studies conducted by Baisakhi et al. showed that inhibition of NF- κ B activity resulted in decreased IL-8 transcription, which then inhibited GBM cell invasion and migration [140]. A study conducted by Denise Smith et al. demonstrated that GBM cells that are transfected with short hairpin inhibitory RNAs of RelA and c-Rel for six days displayed reduced tumor growth, signifying the role of RelA and c-Rel in GBM [144]. These studies highlight the importance of inhibiting the overactivation of NF- κ B subunits as molecular targets in GBM.

Curcumin modulates the NF- κ B pathway to confer the anti-inflammation, anti-proliferation, and apoptotic activity in GBM cells, as shown in Figure 1e. Curcumin can down-regulate NF- κ B activity by decreasing the expression of anti-apoptotic protein Bcl-xL in GBM cell lines [33]. Reducing the Bcl-xL triggers mitochondrial depolarization, which precedes the losses in mitochondrial membrane integrity. This suggests that curcumin induces mitochondrial-mediated apoptosis in GBM following the inhibition of NF- κ B pathways. Other than mitochondrial depolarization, curcumin promotes cell cycle arrest in the G2/M phase prior to cell apoptosis. Most importantly, curcumin acts irrespective of the p53 or PTEN mutational status of the cells. Both PTEN and p53 mutated cells had the same experimental outcomes compared with the wild-type cells after being treated with curcumin. This shows that curcumin exerts p53-independent cell death via inhibition of NF- κ B pathways. In the same study, the inhibition of NF- κ B by curcumin increases the number of apoptotic cells in tumors, further reducing the tumor size and hemorrhagic areas in C6-implanted Wistar rats. This was in line with other studies showing that curcumin increases the I κ B inhibitor proteins and decreases the expression of NF- κ B-regulated genes that contribute to GBM chemoresistance [51,53,145]. Curcumin also enhances the anti-cancer effect of nimustine hydrochloride (ACNU) by suppressing the phosphorylation of I κ B, p65, and p50, which then decreases COX-2 expression [51]. Additionally, curcumin's antiproliferative activity might be facilitated through the downregulation of cyclin D1, since the promoter of cyclin D1 is regulated by NF- κ B [54]. In the study conducted by Tzuu-Yuan and colleagues, curcumin increased NF- κ B transcription factor inhibition in a concentration-dependent manner in GBM 8401 cells [55].

Curcumin improves the cytotoxic effect of PTX by reducing the phosphorylation of I κ B and suppressing NF- κ B p65 nuclear translocation to inhibit cell growth in C6 rat glioma cells [38]. Furthermore, curcumin upregulates the pro-apoptotic molecular Smac/Diablo to suppress NF- κ B and IAPs (cIAP-1 and cIAP-2), which induces apoptosis [42]. Studies suggest a positive feedback system between NF- κ B and IAPs, as IAPs can be upregulated by NF- κ B and vice versa [146–148]. Hence, downregulation of both NF- κ B and IAPs protein might further suppress GBM tumorigenesis.

3. Issues of Curcumin Bioavailability and Methods to Overcome Them

Despite the promising anticancer mechanisms, curcumin efficacy is hindered by its low bioavailability. Various studies have reported that very low curcumin concentration

was detected in blood, tumors, or extraintestinal tissues [149–151], which may be due to the poor absorption, rapid metabolism, chemical instability, and rapid systemic elimination characteristics of curcumin [16]. A study reported that orally-administered curcumin at a dose of 500 mg/kg only had 0.06 µg/mL maximum serum concentration, indicating only 1% oral bioavailability [152]. Due to its chemical structure, curcumin has low solubility in neutral or acidic pH. It is fully protonated, unlike in alkaline conditions where it can be hydrolyzed, especially in the intestinal (pH 6.8). Additionally, rapid metabolism and systemic elimination happen through the formation of glucuronides and sulphates by conjugation in the intestine. Studies reported that free curcumin was undetectable, but curcumin glucuronides and sulphates were highly detected in most of the subjects' serum samples who had been administered with curcumin, and this indicates rapid metabolism of curcumin [153,154].

The first step of pharmaceutical strategies is to improve curcumin solubility and its absorption to overcome this problem. The incorporation of curcumin in solid dispersion, nanoparticles, micelles, conjugates, liposomes, and phytosomal formulations have increased curcumin's solubility and absorption rate in GBM cells [155–161]. Studies showed that curcumin-loaded noisome nanoparticles (CM-NP) can more effectively suppress the viability, proliferation, and migration of GSCs by inducing cell cycle arrest and apoptosis [155]. The CM-NP also efficiently increases ROS-suppression of tumor growth and inhibits monocyte chemoattractant protein 1 (MCP1) to reduce the invasiveness of GSCs compared to curcumin alone. Additionally, rats injected with curcumin-loaded PLGA nanoparticles have significantly smaller tumor size after five days of injection, while the group injected with curcumin alone displayed no significant change [156]. Another *in vivo* study reported that the combination of antisense-oligonucleotide against miR-21 with curcumin-loaded DP micelle complex reduced the tumor volume more effectively than single therapy curcumin or miR21ASO alone [157].

Additionally, relative to natural curcumin, solid lipid curcumin particles can promote cell death and DNA fragmentation by increasing the levels of caspase-3, Bax, and p53 with downregulation of Bcl-2, c-Myc, and Akt proteins in GBM cell lines [159]. The antibody-conjugated biodegradable polymeric nanoparticles (Mab-PLGA NPs) could enhance the photodynamic efficiency of curcumin on DKMG/EGFRvIII GBM cells compared to curcumin loaded biodegradable polymeric nanoparticles alone (56% vs. 24%) [158]. Furthermore, curcumin analogue induces FBXL2-mediated AR ubiquitination, ROS, lipid peroxidation, and suppression of glutathione peroxidase 4 to inhibit growth of TMZ-sensitive and -resistant GBM *in vitro* and *in vivo* [160].

Moreover, curcumin liposomes can significantly improve the anti-tumor effects of curcumin by enhancing the uptake effects, apoptosis effects, and endocytic effects of C6 glioma cells and C6 glioma stem cells. Curcumin liposomes were also shown to inhibit tumor growth and increase the survival period of brain glioma-bearing mice [161]. Other than that, a study showed that curcumin-loaded targeted liposomes cross the BBB two-fold higher than the non-targeted liposomes loaded with curcumin [162]. Curcumin-loaded targeted liposomes can more effectively inhibit GBM tumor growth and increase the survival rate of U87 GBM tumor-bearing mice compared to free curcumin as well as the non-targeted liposome-loaded curcumin [162]. Additionally, curcumin phytosome meriva (CCP) has been shown to improve curcumin bioavailability [163], which then help to activate natural killer cells and mediate elimination of GBM and GBM stem cells [164]. Based on the results of the preclinical studies, the use of the conjugate, nanoparticles, micelles, solid lipid, analogues, liposomes, or phytosomal formulation could certainly be clinically developed to benefit GBM patients.

4. Clinical Trials

Currently, there is only one clinical study investigating the curcumin effects on 13 newly diagnosed pre-operative GBM patients [23]. In 2014, this clinical study reported the highest serum and intratumoral concentrations of curcumin detected using the micellar

curcumin formulation. It was reported that the intratumoral concentration of curcumin detected might not be sufficient to cause short-term antitumor effects. Still, it might help to control tumor growth in a long-term way. Moreover, intratumoral inorganic phosphate was significantly increased by curcumin. This might indicate increased demand for high-energy phosphates or mitochondrial dysfunction, since inorganic phosphate is used for ATP generation [23]. In addition, the side effects of taking curcumin are significantly less severe than the current chemotherapeutic drugs [23]. Thus, the oral administration of micellar curcumin is relatively safer and well-tolerated. However, this clinical trial only involved a small number of patients with a small dose of curcumin. Further clinical trials should be carried out to strengthen the statistical validity with a larger sample size. In the future, phase I/II clinical trials should be carried out to determine the safety and ideal dosage for GBM treatment, and phase III to IV to examine curcumin's efficacy and potential side effects with a larger sample population. Other than that, randomized controlled trials can be carried out to avoid bias and provide higher accuracy results.

5. Conclusions and Future Perspectives

Taken together, curcumin possesses the ability to modulate various core signaling pathways that are commonly dysregulated in GBM. However, among these signaling pathways, a greater emphasis on Rb and Shh pathways could be of focus for future pre-clinical studies, since the current data are still limited. Additionally, the contradictory findings on curcumin modulation of the p53 pathway warrant future investigation and suggest that curcumin use may be selective against radioresistant GBM tumor. Nevertheless, the combination of curcumin with standard chemotherapeutic drugs mainly results in the modulation of multiple signaling pathways that promote their anti-cancer effects. Curcumin's ability to modulate the major signaling pathways while promoting the efficacy of standard chemotherapeutic drugs warrants its use as a potential nutraceutical-based adjuvant drug for GBM treatment.

Since the clinical studies of curcumin in GBM patients are lacking, it is worthwhile for future clinical studies to incorporate curcumin as a potential neo-adjuvant in GBM. Although issues such as bioavailability, poor absorption, and rapid systemic elimination may hinder its efficacy, the pre-clinical use of nanodelivery has shown great promise while increasing the efficacy of curcumin and chemotherapeutic drugs. Considering this, a greater emphasis should also be given towards the nanoformulations of curcumin in future clinical studies, in combination with the standard chemotherapeutic drugs. Therefore, the multimodal modulation of signaling pathways via nanoformulation of targeted curcumin delivery that can synergize chemotherapeutic drugs efficacy may provide a clinical perspective in GBM therapy.

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