Investigating the therapeutic role and molecular biology of curcumin as a treatment for glioblastoma

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Abstract

Objectives: Despite the aggressive standard of care for patients with glioblastoma multiforme, survival rates typically do not exceed 2 years. Therefore, current research is focusing on discovering new therapeutics or rediscovering older medications that may increase the overall survival of patients with glioblastoma. Curcumin, a component of the Indian natural spice, turmeric, also known for its antioxidant and anti-inflammatory properties, has been found to be an effective inhibitor of proliferation and inducer of apoptosis in many cancers. The goal of this study was to investigate the expanded utility of curcumin as an antiglioma agent.

Methods: Using the PubMed MeSH database, we conducted a systematic review of the literature to include pertinent studies on the growth inhibitory effects of curcumin on glioblastoma cell lines based on Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines.

Results: A total of 19 *in vitro* and five *in vivo* studies were analyzed. All of the studies indicated that curcumin decreased glioblastoma cell viability through various pathways (i.e. decrease in prosurvival proteins such as nuclear factor κB, activator protein 1, and phosphoinositide 3 kinase, and upregulation of apoptotic pathways like p21, p53, and executor caspase 3). Curcumin treatment also increased animal survival compared with control groups.

Conclusions: Curcumin inhibits proliferation and induces apoptosis in certain subpopulations of glioblastoma tumors, and its ability to target multiple signaling pathways involved in cell death makes it an attractive therapeutic agent. As such, it should be considered as a potent anticancer treatment. Further experiments are warranted to elucidate the use of a bioavailable form of curcumin in clinical trials.

Keywords: antiproliferation, apoptosis, bioavailability, blood–brain barrier, cancer stem-like cells, curcumin, glioblastoma, toxicity

Introduction

Glioblastoma multiforme (GBM) is the most common primary brain tumor affecting approximately 9000 new people each year [Ohgaki and Kleihues, 2005]. Patients are typically treated with maximal safe surgical resection followed by adjunctive chemoradiation therapy [Grossman and Batara, 2004]. Despite advances in surgical and combination chemoradiotherapy techniques, patients with glioblastoma survive less than 2 years [Grossman and Batara, 2004; Robins *et al.* 2007]. In an attempt to address this poor prognosis, recent studies have pointed to new potential targets, including cancer stem cells for treatment of glioblastoma after surgical resection [Cheshier *et al.* 2009]. These studies indicate that glioma stem cells may be responsible for GBM regrowth, resistance to chemoradiation, and the phenotypic heterogeneity of the tumor. Therefore, there has been a recent search for chemotherapeutics that may broadly modulate and attenuate malignant properties of glioblastoma stem cells.

As a result of its wide applicability in other malignancies, such as breast, colon, bladder, cervical, and prostate cancer [Hatcher *et al.* 2008; Sharma *et al.* 2005; Aggarwal *et al.* 2003], curcumin is being investigated as a potential treatment for

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gliomas. Both *in vitro* and *in vivo* experiments demonstrate that curcumin is an effective inhibitor of GBM proliferation, invasion, and viability [Zhuang *et al.* 2012; Perry *et al.* 2010; Zanotto-Filho *et al.* 2011a, 2013; Weissenberger *et al.* 2010]. Several of these studies have demonstrated its effect on the programmed cell death pathways either by inducing apoptosis or autophagy [Karmakar *et al.* 2006, 2007; Zhuang *et al.* 2012]. Furthermore, curcumin has been demonstrated to affect a variety of signaling pathways thereby downregulating tumor resilience proteins, such as nuclear factor κB (NFκB), activator protein 1 (AP-1), and phosphoinositide 3 kinase (PI3K), and upregulating apoptotic and tumor-suppressing proteins, such as p21, p53, and caspase 3 [Choi *et al.* 2008; Dhandapani *et al.* 2007; Karmakar *et al.* 2007; Huang *et al.* 2010; Su *et al.* 2010].

Despite the large and growing body of evidence of curcumin's efficacy *in vitro* and *in vivo* on various tumors such as gliomas, the weak characterization of curcumin's effects on GBM stem cells prevents its use in clinical trials. Therefore, we conducted a systematic review to elucidate curcumin's biochemical mechanisms of action and to summarize the current evidence on its efficacy on treating gliomas in order to determine if curcumin could be a potential treatment option for GBM and other gliomas.

Materials and methods

Study selection

Using the MeSH database system of PubMed, a literature search was performed between the years 2005 and 2015 for all articles that included the keywords *curcumin*, *glioblastoma*, and *glioma* (i.e. 'curcumin' [MeSH] and 'glioma' [MeSH]; 'curcumin' [MeSH] and 'glioblastoma' [MeSH]). The articles were limited to English, and both *in vitro* and *in vivo* trials using curcumin as a treatment for malignant gliomas were included. Studies analyzing the effect of curcumin on medulloblastomas or studies where curcumin was modified using extrinsic chemicals were excluded. Studies where curcumin was encapsulated in nanoparticles to improve bioavailability were included, but are specified to differentiate from experiments using natural curcumin. Technique or methodology papers, commentaries, and editorials were omitted. Forty-one articles were identified from this initial screen. One duplicate was identified and excluded. Studies were screened and selected according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines [Moher *et al.* 2009]. A diagram illustrating how each study was included is demonstrated in Figure 1. A final total of 19 studies were included in our review. No clinical trials were found.

Data extraction

All studies which fit the inclusion criteria stated above were compiled and grouped into *in vitro* or *in vivo* or both. The *in vitro* trials (n=19) were evaluated based on tumor cell line culture, curcumin administration, and methodology. The *in vivo* trials (n=5) were evaluated based on host type, implant location, tumor size reduction, and outcome. Both groups were analyzed for proliferation indices, apoptotic induction, and effect on cell cycle. Some *in vivo* studies were found to quantify survival time.

Not all studies used the same tumor-derived cell lines or the same curcumin treatments; however, differing protocols and cell lines were accounted for in qualitative analysis. Similarly, not all *in vivo* studies use the same number of initial cells or implant in the same location. Accordingly, some comparative analysis is limited due to an inherent lack of uniformity in methodology. Quantitative data were specified when reported in the research. No statistical tests were performed.

Results of literature search

Our search on PUBMED returned 59 research studies (see Figure 1). After the duplicate was eliminated and the remaining articles were screened for the inclusion criteria stated above, 19 articles were included in our review. Of these, 19 articles were included in the *in vitro* group and five articles were included in the *in vivo* group. All *in vivo* studies also included *in vitro* experiments. Tested cell lines, curcumin treatment, methodology, and results for *in vitro* and *in vivo* studies are summarized in Tables 1 and 2, respectively.

In all studies examined, curcumin showed a reduction in cell viability. All but one study which measured cell death, showed that curcumin induces cell death by either inducing apoptosis (type I) or inducing autophagy (type II) [Senft *et al.* 2010]. It was also demonstrated that curcumin inhibited migration and invasion in all studies in which these tests were conducted [Weissenberger *et al.* 2010; Senft *et al.* 2010; Perry *et al.* 2010]. Curcumin was shown to broadly

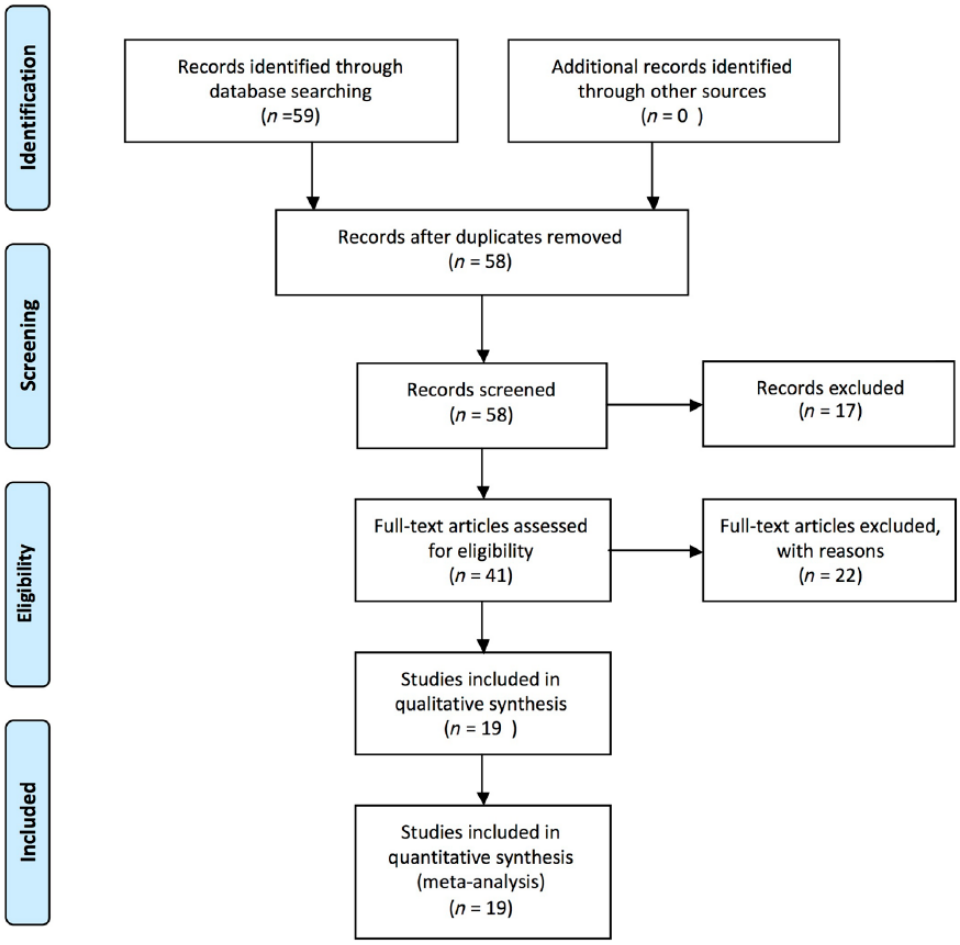

Figure 1. The PRISMA flow diagram for systematic reviews summarizes the process used to identify, screen, and include articles for this review.

affect tumor-signaling mechanisms by suppressing prosurvival proteins (NFκB, AP-1, and PI3K) and upregulating apoptotic pathways (p21, p53, and executor caspase 3) [Huang *et al.* 2010, 2012; Karmakar *et al.* 2006, 2007; Zanotto-Filho *et al.* 2011a; Su *et al.* 2010; Liu *et al.* 2007]. *In vivo* studies confirmed the results of *in vitro* studies and showed overall reduction in tumor growth [Zanotto-Filho *et al.* 2011a, 2013; Weissenberger *et al.* 2010; Zhuang *et al.* 2012; Perry *et al.* 2010] and even prevention of tumor formation in some cases [Purkayastha *et al.* 2009; Zanotto-Filho *et al.* 2011a; Weissenberger *et al.* 2010]. Additionally, all *in vivo* studies which recorded survival rate reported an increased survival in the curcumin group compared with control groups [Zanotto-Filho *et al.* 2013; Zhuang *et al.* 2012; Weissenberger *et al.* 2010; Perry *et al.* 2010].

All studies both *in vitro* and *in vivo* showed that curcumin inhibited cell proliferation or cell viability. In these experiments, curcumin reduced overall cell viability by inducing apoptosis [Thani *et al.* 2012; Huang *et al.* 2010, 2012; Ramachandran *et al.* 2012], inducing arrest in the cell cycle at G2/M [Liu *et al.* 2007; Panchal *et al.* 2008], or both [Luthra *et al.* 2009; Zanotto-Filho *et al.* 2011a, 2013; Lim *et al.* 2011]. Curcumin was also found to interact synergistically with other common chemotherapeutics in five separate studies [Castonguay *et al.* 2012; Zanotto-Filho *et al.* 2011a, 2015; Ramachandran *et al.* 2012; Dhandapani *et al.* 2007]. The summary of these findings is expressed in Table 3.

Discussion

Originally used over 4000 years ago in ancient Ayurvedic medicine, curcumin is an antioxidant isolated from the *Curcuma longa* plant, and has been recently 'rediscovered' as an anti-inflammatory therapeutic for many diseases,

Table 1. *In vitro* results of curcumin's effects on glioblastoma multiforme.

Author and year	Tissue origin	Curcumin administration		Analysis	Results	Apoptosis	Cell cycle	Notes
		IC_{50} (μM)	Duration					
Dhandapani *et al.* [2007]	T98G U87MG T67 C6	–	2, 4, 6 days	Cell viability NFκB AP-1 c-Jun	↓ ↓ ↓ ↓	Yes	N/A	–
Wu *et al.* [2013]	U251 U87	–	24 h	RANK mRNA qPCR RANK promoter methylation STAT3	↑ ↓ ↓	N/A	N/A	Curcumin reactivates RANK expression by inhibiting STAT3
Thiyagarajan *et al.* [2013]	C6	50	24 h	Cell viability	↓	N/A	N/A	–
Ramachandran *et al.* [2012]	U87 D283	37.3 (U87) 28.7 (D283)	72 h	Cytotoxicity assay Apoptosis mRNA-Bax/Bcl-2 ratio	↑ ↑ ↑	Yes	N/A	Curcumin and turmeric force
Zanotto-Filho *et al.* [2013]	C6 U251	30 (C6) 60 (U251)	24, 48, 96 h	Cell viability	↓	Yes	G2/M arrest	Curcumin loaded nanoparticles uptake is higher after 24 h Nanoparticle loaded curcumin increased cytotoxicity in U251 line
Zanotto-Filho *et al.* [2011a]	U138MG C6 U87 U373	29(U138MG) 25 (C6) 19 (U87) 21 (U373	36 h	Cell viability NFκB PI3K	↓ ↓ ↓	Yes	G2/M arrest	Curcumin synergizes with anticancer drugs and is efficacious in a variety of GBM cell lines
Weissenberger *et al.* [2010]	Tu-2449 Tu-9648 Tu-251	–	–	STAT3 target gene transcription JAK1, 2/STAT3 tyrosine phosphorylation Cell proliferation Migration and invasion assay	↓ ↓ ↓ ↓	N/A	G2/M arrest	–
Manju and Sreenivasan [2011]	C6	97	24 h	Cell viability	↓	N/A	N/A	Magnetic nanoparticles enhances cytotoxicity of curcumin
Thani *et al.* [2012]	U373	41	48 h	Cell viability MMP assay	↓ ↓	Yes	N/A	–
Zhuang *et al.* [2012]	C6	–	–	Cell viability CCL2 mRNA Phospho-JNK	– ↓ ↓	N/A	N/A	Anti-inflammatory pathway of curcumin in CNS depends on inhibition of CCL2 through JNK pathway
Huang *et al.* [2012]	8401	22.7	24 or 48 h	Cell proliferation M.M.P. DNA fragmentation assay WB-pro-caspase Caspase 3, 8, 9 NFκB transcription factor	↓ ↓ ↑ ↓ ↑ ↓	Yes	Sub-G1 arrest	Curcuminoids refers to curcumin and its related demethoxy compounds

Table 1. (Continued)

Author and year	Tissue origin	Curcumin administration		Analysis	Results	Apoptosis	Cell cycle	Notes
		IC_{50} (μM)	Duration					
Senft *et al.* [2010]	A-172 MZ-18 MZ-54 MZ-256 MZ-304	–	–	Cell proliferation C-myc Cell migration Cell invasion	↓ ↓ ↓ ↓	No	N/A	Curcumin is efficacious on both newly diagnosed and recurrent GBM
Su *et al.* [2010]	DBTRG	43.7 30.4	24 h 48 h	Aberrant P53 Aberrant RB PI3K	↓ ↓ –	Yes	G2/M Arrest	–
Perry *et al.* [2010]	U87	11.6	72 h	Endothelial cell migration Endothelial cell formation Cell proliferation	↓ ↓ ↓	N/A	N/A	Transcytosis of curcumin across BBB was noted
Fong *et al.* [2010]	C6	–	–	ATP binding cassette transport (side populations)	↓	NA	NA	Decrease in side populations suggests curcumin's role in inhibiting stem cells
Luthra *et al.* [2009]	U87	–	–	Bcl-2 binding DNA fragmentation	↑ ↑	Yes	G2/M Arrest	–
Choi *et al.* [2008]	U87MG	–	–	Egr-1 mRNA, protein p21	↑ ↑	N/A	G1 Arrest	Cell cycle G1 arrest *via* p53 independent induction of p-21, and a concomitant reduction in cyclin D
Panchal *et al.* [2008]	C6	–	–	Cell proliferation Gluthathione S-transferase Heme-oxygenase 1	↓ ↑ ↑	N/A	N/A	–
Zhuang *et al.* [2012]	SU-2 SU-3	N/A	3 days	GIC self-renewal Differentiation Autophagy Cell proliferation	↓ ↑ ↑ ↓	N/A	N/A	Curcumin induces autophagy and differentiation of GICs

AP-1, activator protein 1; ATP, adenosine triphosphate; bax, bcl-2 associated x gene; BBB, blood–brain barrier; bcl-2, B-cell lymphoma 2; p21, cyclin-dependent kinase inhibitor 1; CCL2, chemokine ligand 2; Egr-1, early growth response protein 1; GBM, glioblastoma multiforme; GIC, glioma-initiating cell; IC50, half-maximal inhibitory concentration (i.e. drug concentration resulting in fifty percent viability compared to control); JAK, janus kinase; JNK, c-Jun N-terminal kinase; MMP, matrix metalloproteinase; M.M.P., mitochondrial membrane potential; mRNA, messenger ribonucleic acid; N/A, not applicable; NFκB, nuclear factor κB; p53, protein 53; PI3K, phosphatidylinositol 3-kinases; qPCR, quantitative polymerase chain reaction; RANK, receptor activator of nuclear factor κB; RB, retinoblastoma gene; RT-PCR, real time polymerase chain reaction; STAT3, signal transducer and activator of transcription 3; WB, western blot.

Table 2. *In vivo* results of curcumin's effects on glioblastoma multiforme.

Author and year	Tissue origin	Implant location	Days of treatment	Delivery method	Decrease in tumor size (%)	Control survival	Treatment survival	Notes
Zanotto-Filho *et al.* [2013]	C6	Striatum	14 days	IP	50%	30 days	40 days	Nanoparticle-mediated curcumin administration increases rat survival and efficacy of curcumin administration at lower dosages
Zhuang *et al.* [2012]	SU-2 SU-3	Caudate	5 weeks	IP	NR	NR	NR	Curcumin-treated mice survived >120 days compared with control <90 days
Zanotto-Filho *et al.* [2011a]	C6	Striatum	10 days	IP	73%	NR	NR	Increased apoptosis in implanted cells with minimum toxicity Four mice had undetectable tumors after treatment
Weissenberger *et al.* [2010]	Tu-2449 Tu-9648	Striatum	80 days	Oral	NR	20–37 days	20–40 days	Curcumin increased tumor-free survival by 15% Decreased contralateral tumor spread and tumor growth
Perry *et al.* [2010]	U87	Flank Caudate putamen	29 days 29 days	IP IP	47.5% N/A	N/A 20.9 days	N/A 23.4 days	Curcumin prolonged survival in intracerebral *in vivo* model

IP, intraperitoneal; NR, not reported.

Table 3. Curcumin as an adjunct treatment.

Study	Primary agent	Notes
Dhandapani *et al.* [2007]	Cisplatin Etoposide Camptothecin Doxorubicin Radiation (5 Gy)	Decreased cell viability Increased DNA fragmentation Radiation: increased cell death
Castonguay *et al.* [2012]	Ruthenium letrozole	Increased autophagy
Zanotto-Filho *et al.* [2011b]	Cisplatin Doxorubicin	Synergism of apoptosis
Ramachandran *et al.* [2012]	Temozolomide etoposide	Potentiation of apoptosis
Zanotto-Filho *et al.* [2015]	Temozolomide + resveratrol	Synergism of apoptosis *via* inhibition of autophagy

including dermatologic conditions, upper respiratory tract infections, and malignancies [Hatcher *et al.* 2008; Sharma *et al.* 2005; Lee *et al.* 2013]. It has been proposed that curcumin's biochemical structure (two phenol rings connected through a long network of conjugated pi bonds) contributes to its antineoplastic properties [Lee *et al.* 2013]. Curcumin has been demonstrated to possess marked antiproliferative and proapoptotic effects on a variety of malignancies *in vitro* (leukemia and breast cancers) and *in vivo* (skin, stomach, colon, and liver) [Sharma *et al.* 2005; Hatcher *et al.* 2008;

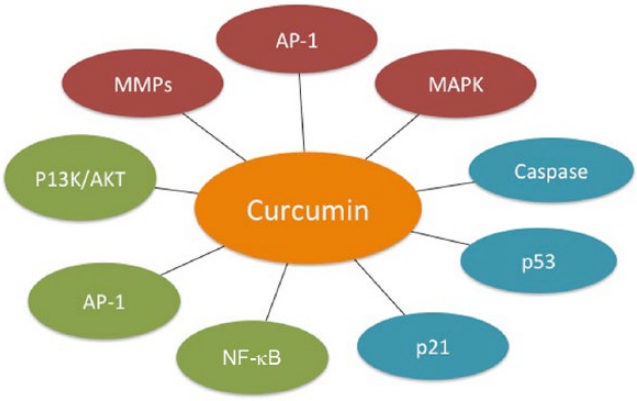

Figure 2. Curcumin modulates cellular pathways in glioblastoma multiforme. Green circles indicate prosurvival pathways, red circles indicate invasion and angiogenesis, and blue circles indicate apoptosis or cell cycle arrest. AP-1, activator protein 1; MAPK, mitogen-activated protein kinase; MMP, matrix metalloproteinase; NFκB, nuclear factor B; PI3K, phosphoinositide 3 kinase.

Aggarwal *et al.* 2003; Lee *et al.* 2013]. As a result, curcumin is now being used in clinical trials for colon and pancreatic adenocarcinoma, multiple myeloma, and Alzheimer's disease [Hatcher *et al.* 2008; Aggarwal *et al.* 2003; Cheng *et al.* 2001].

For glioblastoma, the use of curcumin has been recently examined. Based on the results of our review, it is evident that curcumin may be an effective therapeutic agent for malignant gliomas. These studies demonstrate curcumin's inhibitory effect on malignant glioma cell viability or proliferation as well cell invasion. Furthermore, the *in vivo* studies support a role for curcumin in treating malignant gliomas by reducing tumor growth and increasing survival. Curcumin's potential as a strong therapeutic agent for GBM may be its ability to broadly affect multiple targets and cellular pathways (Figure 2).

Cellular pathways affected by curcumin

Caspase family. Caspases are cysteine proteases, which are essential mediators of the apoptotic pathway and as such are useful markers to examine apoptosis [Fulda and Debatin, 2006]. Synthesized as mature, inactive proteins, they become active upon cleavage in response to apoptotic stimuli. Initiation of the mitochondrial-mediated (intrinsic) or receptor-mediated (extrinsic) apoptotic pathways induces caspase activity and cell death. Activation of caspase 3, the execution caspase, is the last protease in both the intrinsic and extrinsic pathways. It is responsible for the proteolytic cleavage of key proteins, and ultimately contributes to DNA fragmentation and cell demise [Fulda and Debatin, 2006]. Curcumin treatment increased caspase three activity and GBM cell death multiple cell lines [Huang *et al.* 2010, 2012; Su *et al.* 2010; Zanotto-Filho *et al.* 2011a; Karmakar *et al.* 2006, 2007]. Furthermore, increased levels of caspase 8 and caspase 9 upon curcumin treatment indicate that both the extrinsic and intrinsic apoptotic pathways are activated, respectively [Huang *et al.* 2010, 2012; Karmakar *et al.* 2006, 2007].

NFκB pathway. NFκB is a transcription factor which binds to DNA and induces gene expression, thereby causing inflammation, increased invasion, angiogenesis, proliferation, and resistance to apoptosis [Aggarwal 2004]. NFκB has been shown to be abnormally overactive in GBM, as much as six to seven times, compared with healthy astrocytes, and many other chemoresistant cancers [Dhandapani *et al.* 2007; Huang *et al.* 2010; Zanotto-Filho *et al.* 2011b]. It has been suggested that the overactivity of this transcription factor may play a role in chemoradiation resistance [Karmakar *et al.* 2006; Zanotto-Filho *et al.* 2011a]. Nevertheless, curcumin has been demonstrated to inhibit overactive NFκB in malignant gliomas and induce apoptosis [Zanotto-Filho *et al.* 2011a, 2011b; Karmakar *et al.* 2006, 2007; Dhandapani *et al.* 2007; Huang *et al.* 2010, 2012; Woo *et al.* 2005]. Karmakar and colleagues reported direct downregulation of NFκB following curcumin treatment; as well as indirect inhibition through increasing cytosolic levels of Smac/Diablo, which decreased levels of inhibitor-of-apoptosis proteins affecting translation of NFκB and favoring apoptosis [Karmakar *et al.* 2007].

PI3K/AKT pathway. Phosphatase and tensin homilog deletion, mutations in receptor tyrosine kinases (such as epidermal growth factor receptor amplification), and gain of function activity of PI3K contribute to overactivation of the PI3K/AKT signaling pathway in GBM. Mutations in at least one of these genes occurs in approximately two thirds of primary GBM cases and one third of secondary GBM cases, making the PI3K/AKT pathway a very attractive therapeutic target [Rao *et al.* 2010; Wen *et al.* 2012]. In fact, Zanotto-Filho and colleagues found that although AKT activity was seven to eight times greater in GBM cell lines compared with normal astrocytes, curcumin treatment markedly decreased phosphorylated AKT levels and significantly reduced GBM cell viability with no simultaneous decrease in viability of healthy astrocytes [Zanotto-Filho *et al.* 2011a]. Furthermore, Aoki and colleagues showed that the addition of recombinant full-length AKT1 attenuated curcumin-induced cell death in U87-MG and U373-MG cells [Aoki *et al.* 2007].

Matrix metalloproteinase. Matrix metalloproteinases (MMPs) have been found to be a major factor in the invasiveness and migration ability of malignant gliomas and have been positively correlated with histological grade [Mercapide *et al.* 2003; Koul *et al.* 2001]. MMPs are responsible for breaking down the extracellular matrix and diminishing the extracellular matrix barrier, potentially allowing malignant gliomas to invade and migrate into the surrounding healthy brain cells. Some MMPs have been found in much higher levels in glioblastoma tissue samples compared with healthy astrocytes [Sawaya *et al.* 1998]. Curcumin inhibits the overactive MMPs in GBM *in vitro* [Woo *et al.* 2005; Kim *et al.* 2005; Weissenberger *et al.* 2010; Thani *et al.* 2012]. Kim and colleagues showed curcumin had an inhibitory effect on the expression of several MMPs in GBM cell lines by downregulating mRNA expression of MMP-1, -3, -9, and -14, suppressing AP-1-mediated transcriptional activity and inhibiting mitogen-activated protein kinase activity; furthermore, the authors also showed that 10 μM curcumin prevented over 90% of U87MG GBM cell invasion *in vitro* [Kim *et al.* 2005].

p53 tumor suppressor. The tumor suppressor gene p53 is a cycle regulator protein associated with suspension of cell growth and induction of apoptosis [Bieging *et al.* 2014]. In many GBM samples, p53 has been found to be suppressed or absent [Rao *et al.* 2010]. In several of the studies included in our review, p53 was upregulated by curcumin *in vitro* as early as 8 h following treatment [Su *et al.* 2010; Liu *et al.* 2007]. Su and colleagues demonstrated that curcumin was able to inhibit proliferation by dose or duration-dependent upregulation of p53 expression, resulting in either intrinsic apoptosis or p53-mediated cell cycle arrest at G2/M [Su *et al.* 2010]. Furthermore, Liu and colleagues concluded that curcumin was a potent inhibitor of proliferation by increasing p53 activity and inhibiting the cell cycle at G2/M phase in a p53-dependent fashion [Liu *et al.* 2007].

Cell cycle arrest. Unrestrained cell proliferation is a hallmark of cancer and GBM is no exception. Amplifications or deletions of genes involved in cell cycle control and alterations in critical cell signaling pathways all contribute to this very aggressive tumor [Rao *et al.* 2010]. Curcumin inhibited proliferation by disrupting the cell cycle through G2/M cell cycle arrest [Liu *et al.* 2007; Luthra *et al.* 2009; Panchal *et al.* 2008; Su *et al.* 2010; Zanotto-Filho *et al.* 2011a; Lim *et al.* 2011], as well as sub-G0/G1 cycle arrest [Choi *et al.* 2008; Huang *et al.* 2012]. Curcumin-induced G2/M arrest has been reported *via* many mechanisms. Both Liu and colleagues and Su and colleagues show G2/M arrest *via* upregulation of p53 and p21 [Liu *et al.* 2007; Su *et al.* 2010]. G2/M arrest associated with inhibition of Bcl-2 has also been reported [Luthra *et al.* 2009]. Even genetic regulation has been suggested by Pachal and colleagues, who shows downregulation of cdc2a, a gene involved in the transition for G2/M [Panchal *et al.* 2008]. Choi and colleagues found that curcumin induces arrest at G1 due to an upregulation of p21 and resulting inhibition of cyclin D1, which is responsible for the transition from G1 to S phase in the cell cycle [Choi *et al.* 2008].

In vivo studies of curcumin therapy

Reduction of tumor size. Several *in vivo* studies demonstrate that curcumin can significantly reduce tumor volume in rodent models of GBM [Perry *et al.* 2010; Zanotto-Filho *et al.* 2011a, 2013; Aoki *et al.* 2007; Zhuang *et al.* 2012; Weissenberger *et al.* 2010]. Using the C6 glioma model, curcumin restricted brain tumor growth in immune-competent rats with up to 73% reduction in tumor volume compared with the control group [Zanotto-Filho *et al.* 2011a]. Curcumin also significantly inhibited proliferation within

treatment groups as median tumor volume proliferation decreased from 12.7 fold to 3.5 fold with treatment in U87-MG xenografted nude mice [Aoki *et al.* 2007]. Mechanisms of curcumin-mediated inhibition of tumor growth have been attributed to the downregulation of MMP-9 [Perry *et al.* 2010], as well as an increase in autophagy as indicated by increased LC3 staining of the curcumin treated tumor [Aoki *et al.* 2007; Zhuang *et al.* 2012]. A more recent study by Zanotto-Filho and colleagues corroborates the increase in LC3 and autophagy seen *in vivo* by Aoki and colleagues [Zanotto-Filho *et al.* 2015]. However, Zanotto-Filho and colleagues suggest this curcumin-induced autophagy may be a protective response, and blocking it may potentiate its other cytotoxic effects. Curcumin was also found to significantly reduce endothelial cell proliferation within the tumor, indicating that curcumin was able to inhibit GBM-induced angiogenesis, suggesting an additional mechanism by which curcumin treatment modulates tumor growth [Perry *et al.* 2010].

Increased model animal survival. Studies also report a survival benefit with curcumin treatment in *in vivo* rodent models [Perry *et al.* 2010; Weissenberger *et al.* 2010; Zhuang *et al.* 2012; Zanotto-Filho *et al.* 2013]. Zhuang and colleagues studied a group of mice, which were observed until moribund, or until 120 days. The study plotted survival time on Kaplan–Meier curves, which showed the curcumin-treated group (300 mg/kg/day) having a significantly longer survival time than the control. Both groups, each with a different line of glioma-initiating cell, showed similar survival times, with over 70% of the treatment group still surviving at day 120, and less than 20% of the control group surviving by day 90 after tumor implantation [Zhuang *et al.* 2012]. They hypothesized that the extended survival in the treatment group was a result of inhibition of proliferation and induction of glioma initiating cell differentiation. Curcumin-treated groups had smaller, less invasive, and more confined lesions, whereas the controls had larger more extensive lesions with more infiltrating malignant cells [Zhuang *et al.* 2012]. Zanotto-Filho and colleagues also showed an increase in survival rate with smaller doses of curcumin of 50 mg/kg/day, and as low as 1.5 mg/kg/day with nanoparticle-encapsulated curcumin [Zanotto-Filho *et al.* 2013].

Tumor prevention. Besides reducing tumor burden and increasing survival, curcumin has also been tested as a preventative therapeutic [Zanotto-Filho *et al.* 2011a; Purkayastha *et al.* 2009; Weissenberger *et al.* 2010; Perry *et al.* 2010]. Primarily, Purkayastha and colleagues demonstrated that intracerebral injections of curcumin prevented tumor formation in 80% of treatment mice after intracerebral inoculation of melanoma cells [Purkayastha *et al.* 2009]. Subsequently, Zanotto-Filho and colleagues discovered a similar marked reduction in GBM tumor development (only 60% developed tumors in the treatment group *versus* 100% in the control group) when treated with intraperitoneal curcumin after tumor implantation [Zanotto-Filho *et al.* 2011a]. Weissenberger and colleagues even reported a tumor preventive effect in two glioma cell lines with dietary curcumin 7 days before tumor inoculation: 15% and 38% of the treatment groups had tumor-free long-term survival, whereas 100% of control mice from both experimental groups died [Weissenberger *et al.* 2010]. These findings are useful in evaluating that curcumin may be effective in prevention of malignant glioma recurrence after surgical resection.

Blood–brain barrier permeability. Although penetration of the blood–brain barrier (BBB) remains an obstacle for treatment of malignant gliomas, the fact that curcumin when administered *via* intraperitoneal injection [Zanotto-Filho *et al.* 2011a; Zhuang *et al.* 2012; Perry *et al.* 2010], intravenously [Purkayastha *et al.* 2009], or included in the diet [Weissenberger *et al.* 2010] was efficacious in orthotopic glioma models suggests it can cross the BBB. Specifically, Purkayastha and colleagues demonstrated that a tail vein injection of 200 µl of a 667 µM curcumin solution (given the total volume of fluids in a 35 g mouse to be 4 ml, the authors estimate a final curcumin concentration of 35 µM) reached concentrations of 50 fmol in the forebrain in just 30 min without evidence of toxicity in healthy brain cells [Purkayastha *et al.* 2009]. Achieving therapeutic concentrations of curcumin in plasma and brain tissue remains a challenge for scientists using the native curcumin compound. Typically, *in vitro* studies require higher levels of curcumin to demonstrate efficacy; however, curcumin has a low bioavailability due to poor mucosal absorption and rapid metabolism [Anand *et al.* 2007]. In fact, it is has been recently demonstrated that high-dose oral curcumin administration of 8 g/day produced maximum concentrations of 2 µM in the blood [Cheng *et al.* 2001]. Nevertheless, Weissenberger and colleagues demonstrated that the equivalent

dose in mice (an estimated 100 μg of daily dietary curcumin) reduced malignant glioma growth and significantly increased long-term survival compared with control diet mice [Weissenberger *et al.* 2010].

Methods to increase the levels of curcumin in plasma and brain include using novel formulations or combining with the bioavailability enhancer, piperine [Shoba *et al.* 1998]. Piperine, a black pepper extract, was found to increase the bioavailability of curcumin in both animals and humans. Coadministration of piperine with curcumin increased the bioavailability of curcumin in healthy volunteers by 2000% without adverse effects [Shoba *et al.* 1998].

In a recent preclinical study in rats, the potential of nanoparticle encapsulated curcumin (nanocurcumin), liposomal curcumin, and polylactic glycolic acid co-polymer curcumin as a treatment strategy for neuropathic insults was evaluated [Chiu *et al.* 2011]. Following intravenous administration of all curcumin formulations curcumin was detected in multiple brain regions reaching as high as 0.5% of the injected material [Chiu *et al.* 2011]. Due to curcumin's hydrophobic properties and poor absorption, several groups have studied this strategy of using nanoparticle-encapsulated curcumin to enhance bioavailability [Manju and Sreenivasan, 2011; Zanotto-Filho *et al.* 2013; Chiu *et al.* 2011; Lim *et al.* 2011]. In addition, curcumin hybrids such as CNB-001 have been recently generated to extend the bioavailability and therapeutic window for curcumin [Lapchak and McKim, 2011].

Toxicity to normal tissue. Multiple trials with curcumin have shown it to be well tolerated and safe. Out of all *in vivo* studies discussed here, there were no side effects reported. A phase I clinical trial showed no treatment-related toxicity with up to 8 g of oral curcumin per day, which resulted in serum concentrations of 1.5–3.5 μM [Cheng *et al.* 2001]. However, cytotoxic effects in healthy astrocytes have not been seen at concentrations below 120 μM [Zanotto-Filho *et al.* 2011a].

Effect on cancer stem cells. Currently, one of the most promising models of treating glioblastoma is aimed at developing personalized treatment, targeting specific unique pathways in each tumor sample [Wolff *et al.* 2012; Idbaih *et al.* 2007; Huse and Holland, 2010; Huse *et al.* 2011]. Although curcumin seems to broadly affect cancer pathways, there may be a growing role for curcumin in personalized treatment. Presently, there are only a few studies that evaluate the effect of curcumin on GBM stem cells or tumor precursor cells. These studies support the ability of curcumin to induce differentiation of tumor precursor cells into healthy neural cells [Zhuang *et al.* 2012; Fong *et al.* 2010].

GBM stem cells represent a small population of cells within the tumor responsible for driving tumor growth and contributing to chemo and radioresistance [Cheshier *et al.* 2009]. Although GBM stem cells display marked heterogeneity, they are still studied as prospective therapeutic targets after surgical resection [Cheshier *et al.* 2009; Waters *et al.* 2010]. In order to assess individual patient heterogeneity, a topic for future investigation lies in experimenting and characterizing patient-derived stem cell lines from patients with glioblastoma and evaluating the efficacy of curcumin on these stem cells. Successful targeting of GBM stem cells may be necessary to prevent tumor regrowth and patient relapse (Figure 3).

Adjuvant chemoradiation. While curcumin holds the promise to one day be an option as chemotherapy for treating GBM, it has also been shown to be an excellent adjuvant to current chemoradiation treatment (Table 3) [Dhandapani *et al.* 2007; Castonguay *et al.* 2012; Ramachandran *et al.* 2012; Zanotto-Filho *et al.* 2011a, 2015]. Zanotto-Filho and colleagues treated U138 MG glioma cells with curcumin followed by one of two common chemotherapeutic agents (cisplatin and doxorubicin) for 48 h and found that cell viability was markedly reduced compared with either chemotherapy or curcumin alone [Zanotto-Filho *et al.* 2011a]. Similarly, Dhandapani and colleagues showed that curcumin in adjunct with cisplatin, doxorubicin, etopiside, or camptothecin, greatly decreased cell viability and increased DNA fragmentation in both T98G and U87MG glioma cells [Dhandapani *et al.* 2007]. A more recent study by Zanotto-Filho and colleagues investigated the synergistic effect of curcumin with the standard of care chemotherapy, temozolomide [Zanotto-Filho *et al.* 2015]. While they report additive effects rather than synergistic effects, adding resveratrol improved the efficacy of curcumin plus temozolomide by increasing apoptosis.

In addition, Dhandapani and colleagues exposed curcumin-treated T98G and U87MG cells to 5 Gy of irradiation resulting in over 50% cell death induction, 20% greater than just curcumin

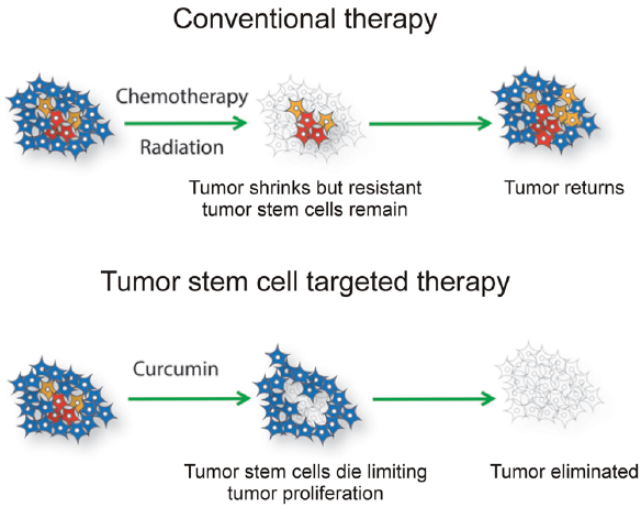

Figure 3. Curcumin targets glioblastoma multiforme stem cells. The stem cell theory of cancer predicts that successful elimination of tumor stem cells is necessary to prevent tumor regrowth and patient relapse.

treatment and over 40% greater than 5 Gy irradiation alone [Dhandapani *et al.* 2007]. Therefore, it is likely that curcumin may potentiate both standard radiation and chemotherapy regimens for high-grade gliomas.

Conclusion

Curcumin may be an effective GBM treatment as demonstrated by several preclinical studies. Curcumin has potent abilities to inhibit cell proliferation, migration, and invasion, to induce apoptosis, differentiation in glioma-initiating cells, and to cross the BBB. In combination with standard treatment, curcumin may potentiate adjuvant chemoradiation therapy for malignant gliomas. Our review indicates that curcumin remains a viable therapeutic for targeting patient-derived GBM stem cells. GBM stem cells are reported to be responsible for maintaining tumor growth, chemo- and radioresistance and regrowth of tumor following surgery, therefore elimination of this cell population is necessary for successful treatment of GBM. Future studies must characterize the effects and molecular mechanisms of action of curcumin on GBM stem cells. In addition, new approaches must be discovered to increase curcumin's bioavailability and reach therapeutic levels in the brain. However, our review shows curcumin to be safe, even at high doses, and to interact synergistically with commonly used chemotherapeutics. Based on our review, curcumin may be a potential safe treatment option for patients with GBM; however clinical trials must be performed to evaluate treatment efficacy.

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