

Mitochondrial Dysfunction in Gliomas: Pharmacotherapeutic Potential of Natural Compounds

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Abstract: Gliomas are the most common primary brain tumors either benign or malignant originating from the glial tissue. Glioblastoma multiforme (GBM) is the most prevalent and aggressive form among all gliomas, associated with decimal prognosis due to its high invasive nature. GBM is also characterized by high recurrence rate and apoptosis resistance features which make the therapeutic targeting very challenging. Mitochondria are key cellular organelles that are acting as focal points in diverse array of cellular functions such as cellular energy metabolism, regulation of ion homeostasis, redox signaling and cell death. Eventual findings of mitochondrial dysfunction include preference of glycolysis over oxidative phosphorylation, enhanced reactive oxygen species generation and abnormal mitochondria mediated apoptotic machinery are frequently observed in various malignancies including gliomas. In particular, gliomas harbor mitochondrial structure abnormalities, genomic mutations in mtDNA, altered energy metabolism (Warburg effect) along with mutations in isocitrate dehydrogenase (IDH) enzyme. Numerous natural compounds have shown efficacy in the treatment of gliomas by targeting mitochondrial aberrant signaling cascades. Some of the natural compounds directly target the components of mitochondria whereas others act indirectly through modulating metabolic abnormalities that are consequence of the mitochondrial dysfunction. The present review offers a molecular insight into mitochondrial pathology in gliomas and therapeutic mechanisms of some of the promising natural compounds that target mitochondrial dysfunction. This review also sheds light on the challenges and possible ways to overcome the hurdles associated with these natural compounds to enter into the clinical market.

Keywords: Apoptosis, cytotoxicity, glioma, mitochondrial dysfunction, mitochondrial membrane potential, natural compounds, oxidative stress, warburg effect.

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1. INTRODUCTION

Glioma is a broad term that encompasses all the tumors either benign or malignant that arise from glial cells. In 2012, worldwide 256,213 individuals were diagnosed with primary malignant brain tumors with higher incidence rates in developed countries than developing countries [1]. In US, an estimated 68,470 new cases of primary malignant and non-malignant brain and CNS tumors and 13,770 deaths are expected to occur in 2015. Glioma is common primary brain tumor accounting for 28% of all brain tumors and 80 % of all malignant tumors [2]. Although its relative incidence is low; poor prognosis, negative impact on quality of life and cognitive function of patients makes its management a challenging task.

Gliomas have different cellular origins, and classified as oligodendroglioma, astrocytoma, ependymoma, glioblastoma, mixed glioma and few other histological types. WHO classified gliomas from grade I-IV with increasing rate of dedifferentiation and malignant nature. Glioblastoma, also known as “grade IV astrocytoma” or glioblastoma multiforme

(GBM) which is highly prevalent (54.7 %) of all gliomas and the most aggressive form with poor prognosis [1]. GBM has decimal survival rates with only 4.7 % of patients live longer than 5 years [3]. Exposure to high doses of ionizing radiation and presence of few rare genetic syndromes caused by inherited gene mutations are only two accepted risk factors for pathogenesis of gliomas. Numerous reports suggest negative correlation of allergies and atopic diseases to the incidence of gliomas indicating protective effect of allergies [4]. Surgery, radiotherapy, temozolimide based chemotherapy are mainstay treatment options in current clinical management of glioma. However, most of the gliomas are surgically unresectable and diagnosed at later stage and their high level of resistance to conventional chemotherapy, radiotherapy marks the gliomas as one of the numerous cancers that are difficult to treat. Despite of recent developments in these therapies, currently GBM has median survival of 14.6 months from diagnosis. The existence of blood brain barrier, complex network of multiple altered signaling pathways, presence of glioma stem cells are the main causative factors for the poor outcome with current therapies [5]. Hence more efficacious therapies for the treatment of malignant gliomas are urgently warranted. The current review is an attempt to summarize the overall pathophysiology of gliomas with respect to mitochondrial dysfunction. This review also discusses the importance of natural products as potential cytotoxic agents targeting mitochondrial derangements in glioma cells.

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1.1. Molecular Pathology of Gliomas

Recent advances in techniques of genomic and proteomic analyses have contributed to our identification and understanding of molecular pathology of malignant gliomas. Based on currently available knowledge in molecular alterations in gliomas, various classification systems have been established which provide a basis for more targeted therapies [6-8]. The cancer genomic atlas (TCGA) has grouped glioblastomas relying on proteomic data into 3 classes: one showing epidermal growth factor receptor (EGFR) mutations or amplifications, second, having ligand driven platelet derived growth factor activation, third with loss of RAS regulator NF1 [6]. Another classification provided by Verhaak *et al.*, categorized glioblastoma into four subtypes: classical, mesenchymal, proneural and neural through genomic profiling [7]. They are defined as follows: Classical- EGFR amplification and amplification of chromosome 7 with loss of chromosome 10; Mesenchymal-increase in frequency of Nf1 mutations/ deletion/ low levels of expression along with loss of Phosphatase and tensin homolog (PTEN); Proneural- focal amplifications in platelet derived growth factor alpha (PDGFRA) and isocitrate dehydrogenase I (IDH1) point mutations, TP53 mutations; Neural- Most similar to normal brain tissue expression patterns.

Other researchers used various criteria such as IDH1 mutations, immunohistochemistry to classify the glioblastomas. These classification systems based on molecular markers discern the more appropriate management of the glioblastomas using targeted therapies [8, 9].

In recent years, numerous molecular alterations were identified and added to the core of molecular landscape of gliomas. The most common deregulations include mutations in P53, IDH1, alpha thalassemia/mental retardation syndrome X-linked (ATRX), telomerase reverse transcriptase (TERT), H3 histone, family 3(H3F3) genes, change in methylation patterns of O-6-methylguanine-DNA methyltransferase (MGMT), P16 promoters, Expression of platelet derived growth factor receptor alpha (PDGFRA), oligodendrocyte transcription factor 2 (OLIG2) [10]. Three main core pathways that are frequently altered in >75% of glioblastomas were identified by The Cancer Genome Atlas (TCGA) as i) receptor tyrosine kinase/RAS/phosphatidylinositol 3 kinase (RTK/RAS/PI3K) signaling ii) p53 iii) retinoblastoma (RB) signaling networks [11].

Understanding molecular abnormalities in malignant gliomas has paved a way for the targeted therapies. Aberrations in epidermal growth factor receptor (EGFR), platelet derived growth factor receptor (PDGFR), vascular endothelial growth factor receptor (VEGFR) such as amplifications, mutations are frequently occurring phenomena in glioblastomas resulting in enhanced angiogenesis, migration and survival [12].

2. MITOCHONDRIAL PATHOPHYSIOLOGY IN GLIOMAS

Mitochondria are the key cellular organelles that play essential role in various functions such as regulation of cellular metabolism, redox signaling, ionic homeostasis and cell death. In addition to its well established role as

“powerhouse of cell”, recently it has been accepted as “motor of cell death” due to its crucial role in programmed cell death or apoptosis [13]. These are unique double membrane enclosed organelles having their own genetic material and undergo their own biogenesis process with no synchrony with the cell cycle. Due to their fundamental roles in key processes such as the production of energy currency ATP for cellular maintenance, generation of reactive oxygen species (ROS) and execution of cell death pathways, mitochondria has been implicated in numerous pathological processes such as cancer, neurodegenerative diseases, obesity, diabetes and aging.

Mitochondria are considered as semi-autonomous organelles in the eukaryotic cellular system having circular DNA encoding genes for proteins of critical importance [14]. Being a seat for primary cellular ATP and ROS production, changes in the mitochondrial functioning can have dire consequences on cellular fate and thus study of the role and regulation of mitochondria in glioma cancers is of primary concern [15]. Mitochondrial function is found to be altered/ impaired to variable extent in various types of glioblastomas such as astrocytoma and ependymoblastoma. Structural and functional impairment in the glial mitochondria is cumulative effect of altered haemodynamic, mitogenic, apoptotic and bioenergetic signaling, which are the common core features of cancer cells [16]. Structural changes to the mitochondria like partial/total cristolysis, swelling of mitochondria, altered dynamics like fission and fusion cycles of the mitochondria results in a heterogeneous group of mitochondria in various grades of glioma [17]. These structural derangements clearly indicate the compromised oxidative phosphorylation capacity and energy coupling in glioma cell lines.

Malignant glioma cells derived from *in vitro* cultures and humans primarily depend on cytosolic ATP produced from aerobic glycolysis instead of mitochondria derived ATP, a switch in the energetic preference popularly termed as “Warburg hypothesis” [18]. Their dependence on less energy deriving pathways unlike their healthy counterparts exposes the degree of functional impairment in the mitochondrial function and its inability to utilize ketones and fatty acids for the production of ATP apart from glucose [19]. This property of exclusive dependency on glucose for energy production was harvested for therapeutic targeting of paediatric GBM through ketogenic diet in several patients, where many cases of symptomatic relief and improved tumor management were observed [20]. Some animal models of gliomas have shown pro apoptotic, anti proliferative, anti inflammatory and anti angiogenic profile in the presence of glucose restriction, which strongly warrants the presence of deranged, decoupled mitochondria and inefficiency in ketone metabolism for energy production in the glioblastoma cells [21]. High energy demand along with the lack of proper redox buffering system against mitochondrial ROS generation further makes glioma cells susceptible for apoptotic damage (Fig. 1). Glioma cells attempt for an alternative energy deriving pathways in the absence of glucose, especially from fatty acids, which results in enhanced ROS output. These ROS in the presence of impaired antioxidant defenses, such as reduced GSH levels in gliomas can execute apoptosis and thus prevent tumor progression [22]. This therapeutic benefit

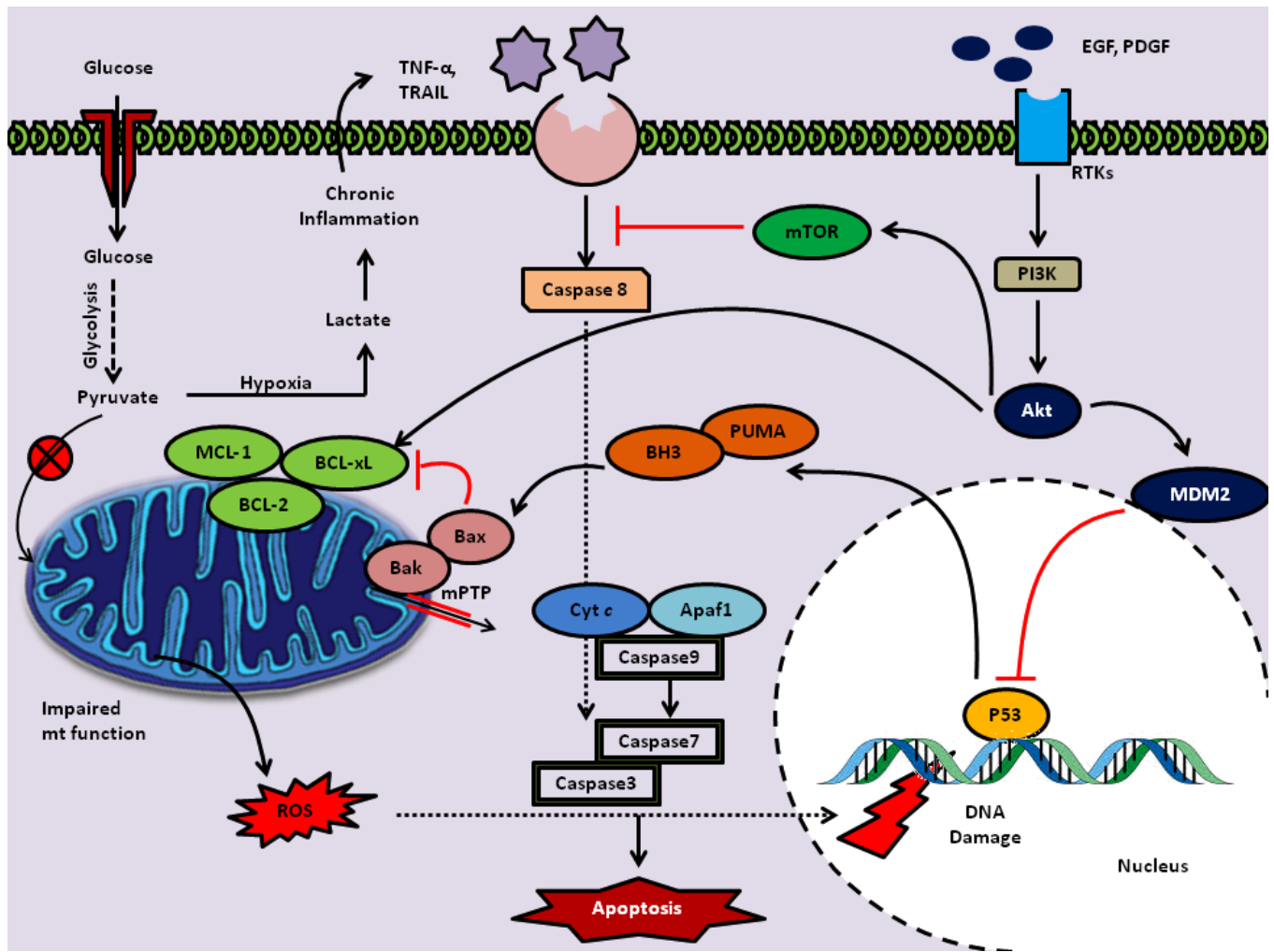


Fig. (1). Mitochondrial dysfunction in gliomas- Evasion of Apoptosis: Impaired mitochondrial function due to the genomic mutations in mtDNA leads to impaired function of electron transport chain thus enhancing generation of ROS. This elevated cellular oxidative stress leads to the damage of nuclear genome resulting in activation of p53. This process in turn promotes P53 mediated generation of apoptotic response through release of p53 up regulated modulator of apoptosis (PUMA) and Bcl-2 homology domain 3 proteins (BH3) which acts on Bcl2 family apoptotic proteins Bax, Bak and mitochondria mediated apoptotic events. However, gliomas with enhanced growth factor signaling through RTKs results in PI3K/ Akt pathway activation that causes MDM2 mediated degradation of P53. Glioma cells harboring P53 mutations are also resistant to the ROS mediated apoptotic pathway. As glioma cells preferentially generate energy through the glycolysis over krebs cycle, they generate large amounts of pyruvate, and it becomes lactate under hypoxic conditions generating chronic inflammatory state that leads to upregulation of TNF-alpha, TRAIL. These ligands trigger apoptosis through activating caspase 8 by death receptor pathway. Enhanced PI3K/Akt activated signaling blocks this extrinsic pathway through mTOR mediated inhibition.

of ROS mediated apoptosis in the presence of glucose restriction further warrants the potential of targeting of energy metabolism for glioma therapy.

Impaired mitochondrial metabolic capacity in glioma cells was evident through identification of mutations in genes coding for Isocitrate dehydrogenase (IDH) [23]. This enzyme is responsible for reductive carboxylation of α -keto glutarate to isocitrate, which subsequently get isomerized to citrate, the principal component of Krebs cycle. It has been identified that most of the glioblastomas show a mutation in the gene encoding the cytosolic form of IDH1. IDH1 mutations occurring at nucleotides that code for arginine, R132 are frequent in most of the diffuse and secondary gliomas [24]. Mutations in IDH2, the mt homolog of IDH1

also found to occur at R172 and known to be associated with loss of enzymatic activity [25]. Both the type of mutations leads to accumulation of 2-hydroxy glutarate, an oncometabolite which is used as a biomarker [26]. Mutations in the NADPH linked mitochondrial isoforms of IDH1 and IDH2 leads to impaired energy production in the mitochondria and thus provides an evidence for mitochondrial dysfunction in gliomas and other tumor types [16].

Apoptosis is a type of primary cellular death mechanism characterized by nuclear condensation, formation of apoptotic bodies followed by phagocytosis. Several anti cancer agents work predominantly by executing apoptosis and thus halt malignant progression of tumors [27]. Impairment in the apoptotic machinery either through

genetic mutations or altered cellular signaling is the ploy mechanism employed by cancer cells responsible for chemo resistance [28]. Glioma cells have been found to have impaired apoptotic signaling primarily due to mutations in the genes of p53 and B-cell lymphoma like 2 (BCL-2) homologue of proteins. P53 is considered as “the guardian of the genome”, known for its active role in DNA repair, apoptosis and cell cycle regulation. P53 through promotion of p53 up regulated modulator of apoptosis (PUMA), phorbol-12-myristate-13-acetate-induced protein 1 (NOXA) and Bcl-2 homology domain 3 proteins (BH3) and apoptotic peptidase activating factor-1 (Apaf-1), helps in executing apoptosis [29]. Inactivating mutations in the p53 gene were found to be common in 30-50% of human gliomas [30]. Along with p53 mutations, mutations in the genes coding for Bcl-2 homologue family of proteins are also common feature of malignant gliomas. Bcl-2 family proteins have variable effect on mitochondrial membrane modulation *via* stabilization or permeabilization and thus classified into anti apoptotic and pro apoptotic proteins respectively. Immunohistochemical studies have shown elevated expression of anti-apoptotic Bcl-2 protein in human glioma tumor samples [31]. Indeed there is a paradoxical elevation of the pro-apoptotic Bcl-2-associated X protein (Bax), which suggests the abnormal interaction among the Bcl-2 family member proteins corresponding to occurrence of mitochondrial membrane dysfunction in malignant gliomas. Mutations in the other anti apoptotic members such as Bcl-2, Bcl-xl and Mcl-1 were also responsible for resistance to radiotherapy and chemotherapy in GBM [32]. In addition to the aforementioned mutations in the intrinsic apoptotic machinery, mutations in the upstream cellular pathways regulating apoptosis were also found to be observed in gliomas. Human gliomas were identified to be characterized by enhanced receptor tyrosine kinase (RTKs) expression on the cellular surfaces [33]. Enhanced growth factor signaling through RTKs leads to phosphoinositide 3-kinase (PI3K) recruitment and corresponding activation of protein kinase Akt, which exerts multitude of cellular effects. Akt through its ability to phosphorylate various proteins that potentiate the action of anti-apoptotic BCL-2 family members, by negative regulation of p53 by murine double minute 2 (MDM2) inhibits intrinsic pathway of apoptosis [34]. It also indirectly inhibits extrinsic pathway of apoptosis through mTOR activation (Fig. 1).

Cardiolipin is an important phospholipid constituent present in inner mitochondrial membrane. It is a dimeric phospholipid with four different acyl chains combined to give variants of cardiolipin molecular species [35]. It is highly concentrated at the contact sites of outer and inner mitochondrial membranes and also at electron transport chain (ETC). Its association with supra molecular complexes of respirasome components is highly essential for mitochondrial function [36]. Several experimental and clinical evidences clearly have shown improper cardiolipin biosynthesis and corresponding mitochondrial functional ETC impairment is associated with gliomas. Molecular lipidomic technologies have clearly indicated asymmetric distribution of different molecular species of cardiolipin corresponds to suboptimal functioning of respiratory complexes [37]. Cardiolipin is also found to affect the opening of mitochondrial permeability transition pore (MPTP), a multimeric channel responsible for

release of apoptosis executioners such as cytochrome *c*, SMAC and Omi. Cardiolipin rich sites of mitochondrial membrane help to recruit pro apoptotic BCL-2 family of proteins and interaction of Bax with MPTP [34]. Thus the degree of faulty cardiolipin synthesis and its function indirectly affects the mitochondrial metabolic capacity and intrinsic apoptosis execution which were found to be impaired/ malfunctioned in certain grades of glioma cells.

It has been also identified that mitochondrial membrane permeability is regulated by cytosolic structural proteins especially those from tubulin family. Class III β -tubulin isotype (β III-tubulin) is found to be associated with voltage dependent anion channel (VDAC) on outer mitochondrial membrane and its regulation [38]. Tubulin is found to block VDAC by phosphorylation and reduces metabolite movement across the membrane and thus halts mitochondrial function in tumor cells [39]. Indeed β III-tubulin is identified to be an important pro survival factor for cancer cells which acts in concert with other cellular pro survival factors and is hence responsible for cancer chemo resistance [40]. The pro survival effect mediated by β III-tubulin is mediated through adoption of cells to oxidative stress and energy deprivation. This way of VDAC regulation by tubulin offers another mechanism of mitochondrial dysfunction in gliomas [16]. Along with mitochondrial tubulins, alterations in other members of the tubulin family are also found to be associated with mitochondrial association with endoplasmic reticulum, cancer cell migration and metastasis [41, 42].

Autophagy is a vital cell death pathway carried out by lysosomes for recycling the damaged cellular components. Various cancers have been shown to have impaired or aberrant autophagic signaling which results in tumor progression and pathogenesis [43]. Recent studies on *in vitro* cultures of glioma cells have found that certain cytotoxic agents such as selenite and inhibitor of growth (ING4) can execute autophagy through mitochondrial ROS generation and mitochondrial membrane hyperpolarization and corresponding activation of mitophagy [44, 45]. The absence of endogenous activation of mitophagy in response to enormous generation of cancer cells suggests an impaired homeostatic regulation of autophagic/mitophagic pathways in glioblastoma cells.

3. PHYTOCHEMICALS TARGETING MITOCHONDRIAL DYSFUNCTION IN GLIOMAS

The main goal in the drug discovery lies in the discovery and development of efficacious, safe and affordable chemotherapeutic agents. Bioactive natural compounds are of great importance in the drug discovery as they are in agreement with these objectives. Recent studies in the last decades have revealed that anticancer activity of numerous natural compounds in gliomas by specifically targeting mitochondrial dysfunction. Some of these compounds act on various components of mitochondria directly, affecting its oxidative phosphorylation and apoptotic signaling and others act indirectly by modulation of metabolic abnormalities occurred as a result of mitochondrial dysfunction (Fig. 2). Table 1 represents the list of natural compounds, their model systems, cytotoxic range and mechanisms identified responsible for their observations.

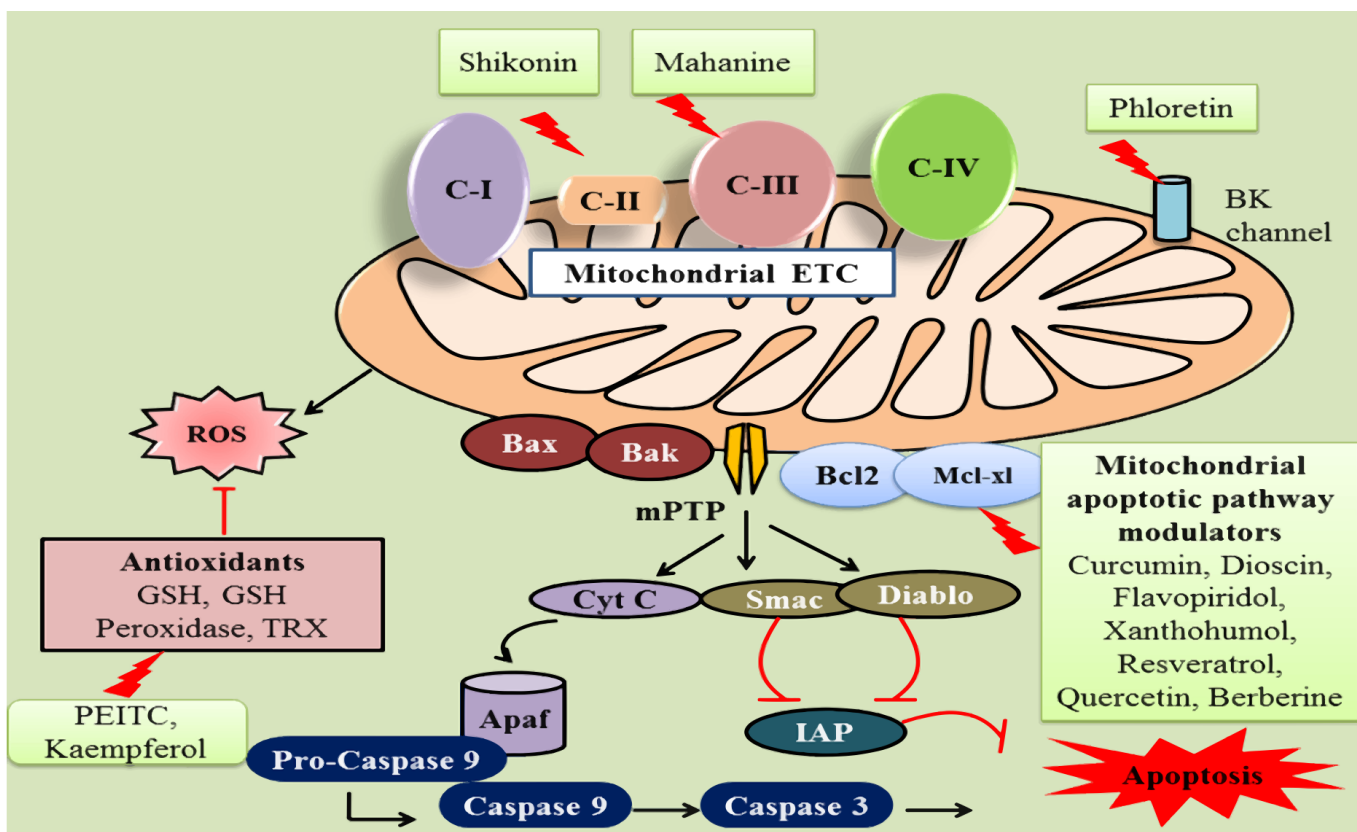


Fig. (2). Natural compounds targeting mitochondrial dysfunction in gliomas: Phytochemicals are known to exert anti cancer effect in several gliomas through affecting multitude functions of mitochondria at various levels. Shikonin, Mahanine were identified to inhibit ETC complexes II, III respectively and thus create cellular oxidative stress. Cellular antioxidants such as GSH, thioredoxin, GSH peroxidase are necessary to regulate the ROS levels and create normal redox balance. PEITC, Kaempferol disrupts these antioxidant systems in cancer cells. BK channels (Functional big potassium channels) exist in membranes of mitochondria, endoplasmic reticulum and maintain normal osmotic balance in cell. Phloretin causes opening of these BK channels which leads to osmotic damage to mitochondria and thus induces swelling and vacuolization which consequently triggers paraptosis like death. Bcl2 family apoptotic proteins, mitochondrial permeability transition pore are important players in mitochondria mediated apoptosis. Natural compounds such as Curcumin, Dioscin, Resveratrol and Berberine triggers generation of ROS and loss of mitochondrial membrane potential to release cytochrome c, Smac, Diablo in to cytosol. The released Cytocrome c in cytosol forms complex with Apaf-1 and promotes the activation of caspase 9 which in turn activates final executioner caspase 3 to induce apoptotic cell death.

3.1. Curcumin

Curcumin, a diferuloylmethane, a naturally occurring polyphenolic yellow pigment obtained from the plant turmeric is widely used in medicine and culinary traditions in India. Accumulating evidence suggests its antioxidant, anti-inflammatory, anti-proliferative properties [46, 47]. Curcumin induced mitochondria mediated apoptotic pathway by the release of cytochrome c, elevated Bax-Bcl2 ratio, cleavage of Bid to tBid and activated caspase 8, 9 and 3 in U87MG cells [48]. Curcumin was also found to sensitize the resistant U87MG cells to the TNF-related apoptosis-inducing ligand (TRAIL) mediated apoptosis at sub lethal concentrations by triggering both extrinsic and intrinsic apoptotic pathways through release of cytochrome c from mitochondria and activation of caspases 3,8,9 [49]. In another study in human glioblastoma T98G cells, curcumin enhanced Bax-Bcl2 ratio, induced release of cytochrome c,

apoptosis inducible Factor (AIF), second mitochondria-derived activator of caspases (Smac) from the mitochondria, activated caspase 9 and indicating role of mitochondria mediated apoptosis [50]. Curcumin increased mitochondrial membrane permeability, resulting in reduced respiration and ATP synthesis in isolated rat liver mitochondria. The observed outcome was found to be due to opening of mitochondrial membrane permeability transition pore (MPTP) by oxidation of membrane thiol groups and low Ca^{++} levels in mitochondria [51].

3.2. Mahanine

Mahanine is a carbazole alkaloid, derived from asian vegetable plants such as *Micromelum minutum*, *Murraya koenigii* etc., Recent studies suggest many pharmacological properties of mahanine such as anti mutagenicity, antibacterial activity, and anticancer properties in various tumor models

Table 1. Natural compounds targeting mitochondrial dysfunction by in various experimental models of gliomas.

S. No.	Name of Natural Compound	Experimental Model System	Observed Cytotoxic Range	Probable Mechanisms of Action	Observations	Refs.
1.	Curcumin	U87MG glioblastoma cell line	25-50µM	Enhanced Bax-Bcl2 ratio, caused cytochrome c, Smac, Diablo release, Decreased cIAP2, caspase 9 activation	Decrease in cell viability, induction of apoptosis	[48]
		U87 MG cells along with TRAIL	Sublethal conc.	Release of cytochrome c, caspase 9, 3,8 activation	Sensitizes to cytotoxic action of TRAIL, induction of apoptosis	[49]
		T98G glioblastoma multiforme	25- 50µM	Caused cytochrome c, smac, Diablo, AIF release, Enhanced Bax-Bcl2 ratio, decreased cIAP1,cIAP2, caspase 9 activation	Decrease in cell viability, induction of apoptosis	[50]
2.	Mahanine	U87MG, LN229 <i>In vivo</i> U87 MG xenografts	5-20 µM	Mitochondrial electron transport chain complex III inhibition, decreased oxygen consumption, elevated ROS, decreased MMP, activation and up regulation of Chk1/chk2, down regulation of CDK4/CDK6, cyclin D1/D3, CDC25A	Decrease in proliferation, G0/G1 arrest, decreased invasion, induction of differentiation <i>in vivo</i> Reduced tumor burden in models	[54]
3.	PEITC (Phenethyl isothiocyanate)	GBM 8041 cells	4-8 µM	ROS generation, rise in [Ca ²⁺] _i , promotion of Fas, FasL, FADD, TRAIL, caspases 8,9,3, increase in apoptotic proteins (Bax, Bak, Bid) inhibition of anti apoptotic proteins(Bcl2, Bcl-xl), release of cytochrome c, AIF, Endo G, decrease in mitochondrial membrane potential,	Inhibition of growth, induction of apoptosis	[61]
		U87MG cells	2.5 µM	Enhanced ROS generation, up regulation of DR5, enhanced activities of caspase 3, 8, 9, increase in mitochondrial superoxide levels	Enhancement of cytotoxicity of TRAIL at sub toxic concentrations of PEITC	[62]
		Ln229 cells	10, 20 µM	Induction of ROS, inhibition of SOD and glutathione and enhancement of caspase 3 activity	Inhibition of proliferation, induction of apoptosis, cell cycle arrest	[63]
4.	Aloe emodin	U87	58.6 µg/ml	Collapse of mitochondrial membrane potential, S phase cell cycle arrest	Decrease in cell proliferation, induction of apoptosis	[68]
5.	Dioscin	Rat glioma c6 cells <i>in vitro</i> and <i>in vivo</i>	1.25-5 µg/ml <i>in vitro</i> and 30mg/kg oral <i>in vivo</i>	ROS generation, Ca ⁺⁺ release, mitochondrial structural changes, mitochondrial permeability changes, release of cytochrome c, PDCD5, increase of Bax, Bak, Bid Decrease of Bcl-2, Bcl-xl, increased activity of caspase 9, 3	Inhibition of cell proliferation, Induction of apoptosis, <i>in vivo</i> - Increase of survival time of rodents, decrease of tumor volume	[72]
6.	α-bisabolol	U87	2.5-10 µM	Dissipation of mitochondrial membrane potential, release of cytochrome c, PARP cleavage	Inhibition of cell viability, induction of apoptosis	[73]
7.	Dantron	C6	10-100 µM	Induction of ROS, disruption of mitochondrial membrane potential, release of cytochrome c, AIF, Endo G, increase in caspase 3, 9 activities	Decrease in cell viability, induction of apoptosis	[74]
8.	Flavopiridol	Murine glioma GL261 <i>in vitro</i> and <i>in vivo</i>	100-400nM <i>in vitro</i> and <i>in vivo</i> 5mg/ kg	Mitochondrial damage, release of cytochrome c, nuclear translocation of apoptosis inducing factor	Inhibition of cell growth and inhibition of migration <i>In vivo</i> decrease of tumor volume	[76, 77]
9.	Xanthohumol	T98G cells	1-50 µM	ROS generation, depolarization of mitochondria, mitochondrial permeability transition, cytochrome c release, decrease in Bcl-2, activation of caspase-9,	Decrease of cell viability, induction of apoptosis	[79]
10.	Shikonin	U87MG cells	2-8 µM	Induction of ROS, disruption of mitochondrial membrane potential, mitochondrial superoxide generation, inhibition of complex II of mitochondrial ETC, GSH depletion, catalase down regulation, SOD1 up regulation, modulation of Bcl-2 family proteins	Induction of apoptosis	[81, 82]

Table 1. contd....

S. No.	Name of Natural Compound	Experimental Model System	Observed Cytotoxic Range	Probable Mechanisms of Action	Observations	Refs.
11.	Resveratrol	U251 cells	10-100 µM	Release of cytochrome <i>c</i> from mitochondria, activation of caspase 9, up regulation and translocation of Bax to mitochondria	Cytotoxic action, induction of apoptosis, inhibition of proliferation	[84]
12.	Quercetin	U373MG	25-100 µM	Decrease in mitochondrial membrane potential, upregulation and translocation of P53 to mitochondria, release of cytochrome <i>c</i> , increase in activities of caspase 9, 3.	Inhibition of cell proliferation, induction of apoptosis and cytoprotective autophagy	[86]
13.	Hydroxygenkwanin	c6 glioma	25 µM	Loss of mitochondrial membrane potential, mitochondrial damage including swelling, over expression of Bak, Bid, reduced expression of Bcl-xl	Inhibition of cell proliferation, apoptosis	[89]
14.	Alantolactone	U87, U373, LN229 Kumming mice	40 µM 100mg/kg/ day	ROS generation, dissipation of mitochondrial membrane potential, cardioplin oxidation, GSH depletion, release of cytochrome <i>c</i> , upregulation of p53, Bax, down regulation of Bcl2, increase in activities of caspase 9, 3	Induction of cell death, apoptosis induction No hepatotoxicity, nephrotoxicity	[91]
15.	Kaempferol	T98G, U373MG, LN229	50 µM	ROS generation, loss of mitochondrial membrane potential, down regulation of Bcl2	Inhibition of cell viability, induction of apoptosis, potentiation of doxorubicin's cytotoxic effects	[93]
16.	Honokiol	DBTRG-05MG	20-80 µM	Increased ROS accumulation, decrease of mitochondrial membrane potential, release of cytochrome <i>c</i> , increase in activities of caspase 9, 3 and elevation of intracellular calcium	Induction of apoptosis	[94, 95]
17.	Parthenolide	U87MG, U373 Intracerebral glioblastoma xenograft model U138MG, U87, U373 and C6	0.1-50 µM 10mg/kg/day i.p daily for 3 weeks 5-50 µM	Increased expression of Bax, Bak, down regulation of Bcl2, Increased activities of caspase 9, 3 Reduction in VEGF, MMP-9 Loss of mitochondrial membrane potential, release of cytochrome <i>c</i> , decrease in Bcl-xl	Reduction of proliferation, suppression of invasion and angiogenesis Reduction of tumor growth, angiogenesis Decrease in cell viability, induction of apoptosis	[97] [97] [98]
18.	Phloretin	U251, T9 glioma	50, 100 µM	Mitochondrial swelling, loss of ATP, induction of paraptosis (cellular swelling along with vacuolization), BK channel activation, over expression of heat shock proteins(HSP 60, 70,90)	Reduction in cell viability	[101, 102]
19	Gossypol	U87, U373, MZ-54 U87MG-luc2 xenograft along with TMZ	10-30 µM 30mg/kg/day	Induction of autophagic cell death, decrease in mitochondrial membrane potential, release of cytochrome <i>c</i> Decrease in TUNEL positive, Ki67positive cells, decrease in micro vessel density	Cytotoxic action, autophagic cell death Reduction of tumor burden, increase in apoptosis	[103] [104]
20	Berberine	T98G	50-200 µM 50, 100 mg/kg/day	Enhanced oxidative stress, Ca ²⁺ levels, loss of mitochondrial membrane potential, enhanced Bax-Bcl2 ratio, increased caspase 9,3 activities Senescence induction, decrease in Ki67 and EGFR	Decreased cell viability, Induction of apoptosis Inhibition of tumor growth	[107] [108]

(AIF- Apoptosis inducing factor; Bax- BCL2-Associated X Protein; Bak- Bcl-2 antagonist/killer-1; Bid- BH3 interacting domain death agonist; BK channel- Big Potassium channel; CDC25A- cell division cycle 25 homolog A; CDK- Cyclin-dependent kinase; cIAP- Cellular inhibitor of apoptosis 2; Diablo- Direct IAP binding protein with low pI; DR5- Death Receptor 5; Endo G- Endonuclease G; ETC-Electron transport chain; Fas- Apoptosis stimulating fragment; FasL –Fas ligand; FADD- Fas-Associated protein with Death Domain; MMP9- Matrix metalloproteinase 9; PARP- Poly ADP ribose polymerase; PDCD5- Programmed cell death protein 5; ROS-reactive oxygen species; Smac- Second Mitochondria-Derived Activator of Caspases; SOD- Superoxide dismutase; VEGF- Vascular endothelial growth factor; TRAIL- TNF-related apoptosis-inducing ligand; TUNEL- Terminal deoxynucleotidyl transferase dUTP nick end labeling).

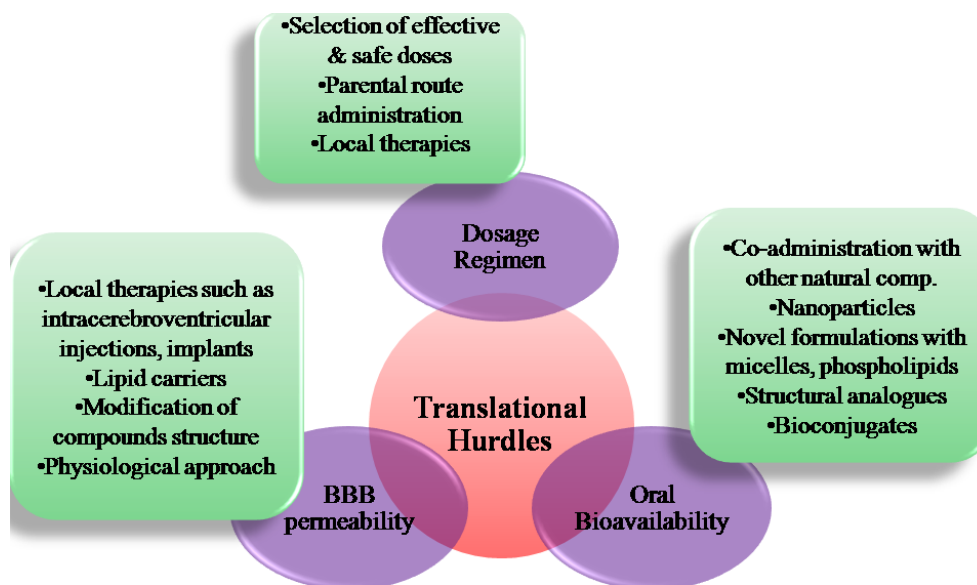


Fig. (3). Translational challenges and possible solutions for the natural compounds to enter clinic for the management of gliomas (BBB- Blood Brain Barrier).

[52, 53]. Mahanine has shown potent antiproliferative effects in both *in vitro* and *in vivo* models of GBM without having effect on normal astrocytes. In U87MG, LN229 cell lines, it demonstrated specific inhibition of mitochondrial ETC complex III thus inducing generation of ROS and its associated DNA damage response. This mediates up regulation and activation of Chk1/Chk2 leading to its G0/G1 arrest. N-acetyl cysteine (NAC) mediated scavenging of ROS and knockdown of Checkpoint kinases 1/2 (chk1/chk2) decreased propensity of mahanine's inducing ability of G0/G1 arrest. These events clearly establish the essential role of mahanine's induced ROS generation and its downstream activation of chk1/chk2 in its anti proliferative activity. Furthermore, mahanine also reduced invasion and induced differentiation of glioblastoma cells [54].

3.3. Phenethylisothiocyanate (PEITC)

PEITC is an important member of group of naturally occurring isothiocyanates, a group of chemicals containing isothiocyanate group (N=C=S) that are abundant in many cruciferous vegetable plants such as cabbage, broccoli, watercress *etc.* Numerous reports suggest chemopreventive and anticancer potential of PEITC in a variety of human cancers with less toxicity towards normal cells [55-59]. The selective cytotoxicity of PEITC at least in part was attributed to its ROS modulating effects. PEITC initially depletes glutathione (GSH), glutathione-S-S-glutathione (GSSG) and later form adducts with glutathione and exports it from the cell thus completely disrupting total glutathione antioxidant system. Furthermore it also inhibits glutathione peroxidase system [60]. Lee *et al.*, Chou *et al.*, Su *et al.*, reported that PEITC exhibits potential antitumor efficacy against a variety of human glioma cell lines through apoptosis induction. *In vitro* studies with PEITC in human GBM (GBM8401) cells suggest the possible involvement of both death receptor and mitochondria mediated death

pathway in the apoptosis induction. PEITC treatment enhanced ROS generation, disrupted mitochondrial membrane potential leading to the release of various apoptotic modulatory proteins from mitochondria such as cytochrome *c*, Endo G and apoptosis inducing factor (AIF). It also enhanced pro- apoptotic proteins (Bax, Bid and Bak) and decreased anti- apoptotic protein levels (Bcl-2, Bcl-x1) and increased caspase-9, 8, 3 activities. Furthermore, subtoxic concentrations of PEITC also sensitized glioma cells to cytotoxicity of TRAIL through increased total ROS generation, increased mitochondrial superoxide levels [61, 62]. In another study, PEITC reduced components of cellular antioxidant system such as GSH expression and activity of SOD in LN 229 cells [63]. In a study, chen *et al.*, reported that PEITC suppresses mitochondrial respiration through disruption of its electron transport chain at complex I by an early degradation of NADH dehydrogenase Fe-S-protein-3 and rapidly depletes GSH levels in human leukemia cells [64].

3.4. Aloe Emodin

Aloe emodin is one of the biologically active compounds isolated from leaves of *A.vera*. It has shown anticancer activity in multiple types of cancers [65, 66]. It suppressed the proliferation of U87 cells in time and dose dependent manner and altered mitochondrial membrane potential leading to mitochondria mediated apoptosis [67]. Genomic analysis carried out in Aloe emodin treated U87 cells revealed the upregulation of the important genes involved in mitochondrial apoptotic pathway and mitochondrial dynamics. Such genes of potential relevance include SHANK-associated RH domain interactor (SHARPIN) and mitochondrial fission 1 protein (FIS1) [68].

3.5. Dioscin

Dioscin, a natural steroidal saponin is isolated from medicinal plants of *Dioscorea* species. Research in past

decades has suggested its wider range of pharmacological properties such as hepatoprotective, anticancer, anti-inflammatory activities [69-71]. Dioscin has shown promising anticancer activity in c6 rat glioma in both *in vitro* and *in vivo* models by inducing oxidative stress. Treatment with dioscin induced ROS generation, Ca^{2+} stress which leads to mitochondrial dysfunction. ROS accumulation further resulted in mitochondrial injury including alterations in mitochondrial structure such as decomposition of mitochondrial double membrane, swelling of mitochondria and mitochondrial permeability transition and change in mitochondrial membrane potential. Dioscin induced apoptosis through mitochondrial dependent pathway by release of cytochrome c, Programmed cell death 5 (PDCD-5), down regulated expression of anti-apoptotic factors such as Bcl-2, Bcl-xl, up regulated expression of pro-apoptotic factors such as Bax, Bak, Bid and increased activities of caspase-3. *In vivo*, Dioscin treatment significantly decreased tumor volume and extended survival time of rats in rat allograft model of glioma [72].

3.6. α -bisabolol

α -bisabolol, a sesquiterpene alcohol present in essential oil possesses potent antitumor properties in human glioma U87 cells. α -bisabolol induced time and dose dependent cytotoxic effect through intrinsic apoptotic pathway as evident by dissipation of mitochondrial membrane potential, release of cytochrome c and PARP cleavage in U87 cells [73].

3.7. Dantron

Dantron (formerly known as danthron) is a natural anthraquinone derivative obtained from Chinese medicine Rhubarb. It was withdrawn from the market as laxative by US FDA in 2009 due to its carcinogenic activity. Dantron induced cell death in c6 rat glioma cells through mitochondrial dependent pathway by inducing ROS generation, collapse of mitochondrial transmembrane potential and release of cytochrome c, AIF, Endo G and increase in caspase 3, 9 levels [74].

3.8. Flavopiridol

Flavopiridol, a potent cyclin dependent kinase inhibitor is a semisynthetic flavone, originally isolated from *Dysoxylum binectariferum*, a plant used in Indian traditional medicine. Flavopiridol has shown efficacy in many solid and hematological malignancies with multiple mechanisms of action such as cell cycle arrest and apoptosis and regulation of genetic expression [75]. Ew Newcomb *et al.*, demonstrated that flavopiridol inhibits growth of murine glioma GL261 cells both *in vitro* and *in vivo* by altering expression of CDK4, cyclin D and p21 and induces apoptosis by mitochondrial mediated apoptotic pathway. Flavopiridol caused mitochondrial damage such as swelling of mitochondria with displaced and distorted cristae and induced release of cytochrome c and nuclear translocation of AIF [76, 77].

3.9. Xanthohumol

Xanthohumol is a major prenylated flavanoid constituent of flowers of Hop plant (*Humulus lupulus*) which is used as

raw material in beer to preserve and to give its distinct flavor. Recent experimental findings have identified its numerous biological effects including chemopreventive and chemotherapeutic effects in wide variety of cancers [78]. In T98G human glioblastoma cell line, xanthohumol decreased cell viability by inducing apoptosis through mitochondrial dependent pathway by oxidative stress. Xanthohumol depolarized mitochondria and triggered mitochondrial permeability transition as evident by release of cytochrome c and decrease of mitochondria associated anti-apoptotic protein Bcl-2 thus leading to caspase-9 mediated apoptosis. It was found that xanthohumol induced ROS generation subsequently resulted in its effects on mitochondrial mediated apoptotic events [79].

3.10. Shikonin

Shikonin is a natural naphthoquinone pigment originated from *Lithospermum erythrorhizon*, a plant which is frequently used in traditional Chinese medicine for the treatment of various inflammatory conditions. Recent pharmacological reports suggest that shikonin exhibits strong cytotoxic action in numerous types of malignancies. Selective accumulation of Shikonin in mitochondria and its disruption of mitochondrial membrane potential were one of the major mechanisms of shikonin induced cell death in U937 leukemia cell line [80]. Study report by CH Chen *et al.*, indicated that a similar mitochondrial dependent mechanism has been involved in antitumor activity of shikonin in glioma cell lines. Shikonin treatment induced ROS generation which eventually leads to disruption of mitochondrial membrane potential. Shikonin triggered disruption of complex II of mitochondrial electron transport chain that has led to generation of mitochondrial originated superoxide radicals. ROS generation also resulted in depletion of GSH, down regulation of catalase along with up regulation of SOD1, modulation of Bcl-2 family apoptosis related proteins [81, 82].

3.11. Resveratrol

Resveratrol, chemically trans-3,4,5-trihydroxystilbene is a dietary phytoalexin present in abundant amounts in grapes, peanuts, red wine etc. Accumulating evidence suggest that resveratrol exhibits multiple pharmacological effects such as antioxidant, anti-inflammatory, anti-aging, chemopreventive and chemotherapeutic effects [83]. Resveratrol induced cell death through triggering apoptosis in u251 glioma cell lines by mitochondrial dependent pathway along with other mechanisms such as cell cycle arrest. Exposure to resveratrol has triggered release of cytochrome c, activated caspase 9 along with up regulation and translocation of pro-apoptotic family member Bax to mitochondria [84].

3.12. Quercetin

Quercetin is a dietary flavanoid that is present in large amounts in various fruits, vegetables, nuts and seeds. Pharmacological studies revealed the potential of quercetin as anticancer agent in many tumor models by multiple mechanisms [85]. Quercetin has exhibited potent antitumor action in the U373 glioma cells by inhibition of proliferation and induction of apoptosis after 48, 72 hr of treatment. The apoptosis has been mediated through mitochondria as

evident by increase in activities of caspase 9 and caspase 3 and increase in cleavage of poly ADP ribose polymerase (PARP) protein. Quercetin treatment induced loss of mitochondrial membrane potential along with up regulation and translocation of p53 to mitochondria which further results in the release of cytochrome *c* from mitochondria. Experimental findings suggested that quercetin at initial stages promoted cytoprotective autophagy as combination with autophagy inhibitor chloroquine further enhanced its apoptosis effects through both intrinsic and extrinsic pathways [86]. However, in a recent study by LL Zamin *et al.*, reported that quercetin treatment at 50mg/kg/day has promoted tumor growth in one rat glioma model. The contrasting effects between *in vitro* and *in vivo* models could be due to several reasons such as low concentration of quercetin in brain after treatment (0.53 μM in brain tissue), modulating effects on immune system, low stability and formation of conjugates of quercetin *in vivo* [87].

3.13. Hydroxygenkwanin

Hydroxygenkwanin is one of the bioactive flavanoids isolated from the flower buds of *Daphne genkwa*, a plant that is frequently used in traditional Chinese medicine for variety of inflammatory ailments [88]. Hydroxygenkwonin alone, or its combination with apigenin, another flavanoid has displayed antiglioma effects *in vitro* by mitochondria modulating effects. In combination treatment and alone, they exerted anti-proliferative, apoptotic effects on rat c6 glioma cell lines by loss of mitochondrial membrane potential, mitochondrial damage such as swelling of mitochondria and disappearance of cristae. At molecular level, over expression of Bid, Bak along with down regulation of Bcl-xl proteins were observed in this treatment approach [89].

3.14. Alantolactone

Alantolactone is a sesquiterpene lactone compound, isolated from many medicinal plant species such as *Inula helinium*, *Inula japonica*, *Aucklandia lappa*, *Radix Inulae* etc. Alantolactone has shown wide spectrum of pharmacological effects such as antimicrobial, antifungal, anti inflammatory and anticancer effects [90]. In a recent study, M Khan *et al.*, reported that alantolactone has exhibited growth inhibitory effects *in vitro* in U87, U373, LN229 glioma cell lines by induction of oxidative stress and mitochondrial dysfunction. In U87 cell line, alantolactone has triggered apoptotic cell death by generation of ROS that in turn leading to depletion of GSH, dissipation of mitochondrial membrane potential, oxidation of cardiolipin all of which culminates in cell death. At molecular level, alantolactone treatment caused up regulation of p53, Bax and down regulation of Bcl2 along with release of cytochrome *c*. Furthermore, alantolactone has increased activities of caspase 9, 3 indicating mitochondria mediated apoptosis. *In vivo* studies proved that alantolactone crosses blood brain barrier and didn't exert any hepatotoxic, nephrotoxic effects indicating its promise as effective chemotherapeutic agent in treatment of gliomas [91].

3.15. Kaempferol

Kaempferol, a phytoestrogen flavanoid present in fruits and vegetables has been reported to possess wide variety of

biological effects including antioxidant, anti-inflammatory, chemopreventive and chemotherapeutic effects [92]. Kaempferol has been found to induce apoptosis in U87MG, LN229, T98G glioma cells. The anticancer effects of kaempferol in glioma might be due to elevation of intracellular oxidative stress, suppression of oxidant scavengers super oxide dismutase (SOD-1), thioredoxin (TRX-I) and loss of mitochondrial membrane potential, decreased expression of Bcl2. In addition to that, Kaempferol inhibited migration of glioma cells through ROS modulation effect. Furthermore, Kaempferol augmented doxorubicin cytotoxic effect through enhancement of ROS levels and decreasing cellular efflux of doxorubicin [93].

3.16. Honokiol

Honokiol, a bioactive constituent isolated from genus *Mangolia* has been reported to exhibit multiple pharmacological actions including anti-angiogenesis, anti-inflammatory, anti-proliferative effects. In a study in DBTRG-05MG glioblastoma cells, honokiol has found to induce apoptosis through mitochondria mediated apoptotic pathway. Honokiol treatment induced ROS generation, enhanced intracellular Ca^{++} release, reduced mitochondrial membrane potential, released cytochrome *c* into cytosol and increased activities of caspase 9,3 [94]. In another study, J J Jeong *et al.*, has shown that honokiol also modulated Bcl2 apoptotic family proteins in the induction of apoptosis. Furthermore, honokiol is proved to have the ability to cross the blood brain barrier (BBB), and blood- cerebrospinal fluid (BCSFB) suggesting its strong potential in the therapy of malignant brain tumors [95].

3.17. Parthenolide

Parthenolide, a sesquiterpene lactone is the principal bioactive constituent from plant *Tanacetum parthenium* (Feverfew) [96]. It inhibits nuclear factor light chain kappa beta (NF- κ B) by inhibiting inhibitory kappa beta (I κ B) kinase as well as by direct binding to p65 in the NF- κ B complex. Parthenolide has displayed strong anti-invasion, anti-angiogenesis and anti-proliferative effects in glioblastoma cells *in vitro*. The involvement of mitochondria mediated signaling in apoptosis induction effect by parthenolide has been evident by the decrease in mitochondrial membrane potential, release of cytochrome *c* along with activation of caspases 9 and 3. The over expression of Bax, Bak, including reducing expression of Bcl2 also indicate the role of mitochondria in the cytotoxic effects of parthenolide. Furthermore, *in vivo* intracerebral glioblastoma xenograft mouse model, parthenolide effectively reduced tumor growth, angiogenesis processes indicating its possible use in glioblastoma therapy [97, 98].

3.18. Phloretin

Phloretin, a dihydrochalcone flavanoid present abundantly in apples has known to possess many interesting biological properties such as anti-atherosclerotic, antioxidant, antimicrobial activities [99, 100]. Phloretin is a well known BK channel (functional big potassium channels) activator and has been reported to cause cytotoxic effect in U251, T9 glioma cells. Phloretin induced cellular swelling and vacuolization

at initial hours of treatment along with mitochondrial and endoplasmic reticulum swelling due to osmotic imbalance created by opening of BK channels. This damage on mitochondria leads to loss of ATP, over expression of heat shock proteins Hsp 60, 70, 90 which progressed to cytotoxic effect in glioma cells and release of danger signals such as High mobility group box-1 (HMGB-1) protein which increases tumor immunity [101, 102].

3.19. Gossypol

Gossypol, a natural polyphenolic compound obtained from cotton seeds is reported as BH3 mimetic with Pan Bcl-2 inhibitory action. Bcl2 family proteins suppress both apoptosis and autophagy. Pharmacological investigations have revealed that gossypol induces autophagic cell death along with inducing mitochondrial dysfunction in U87, U373, MZ-54 glioblastoma cell lines. Gossypol treatment caused decrease in mitochondrial membrane potential along with release of cytochrome c [103]. In addition to that, gossypol augmented the cytotoxic action of Temozolimide *in vivo* glioma DBTRG-05MG models and also showed anti angiogenesis and anti invasive properties alone and in combination *in vitro* [104]. Gossypol was tested in clinical trials in patients of glial tumors and proved to be well tolerated at the dose of 10mg bid PO. This treatment displayed low, measurable response in already pretreated patients with recurrent tumors that are associated with poor prognosis [105]. Moreover, gossypol was also tested in other 2 early phase clinical trials I) as single agent in phase 2 trial in patients of progressive / recurrent GBM (NCT00540722), II) in combination with Temozolimide (TMZ) with/ without radiation therapy in Phase 1 trial in patients of newly diagnosed GBM (NCT010390403). Although these clinical trials were completed, so far results were not accessible [104].

3.20. Berberine

Berberine is a natural isoquinoline alkaloid isolated from various medicinal herbs *Berberis vulgaris*, *Berberis aquifolium*, *Berberis aristata*, *Hydrastis canadensis* and *Tinospora cordifolia*. It displayed wide variety of pharmacological effects including antioxidant, antimicrobial, anthelmintic and antitumor effects [106]. Berberine has shown remarkable anti glioma effects in various *in vitro* and *in vivo* models by multiple mechanisms such as mitochondria mediated apoptosis, G1 cell cycle arrest and induction of senescence and endoplasmic reticulum stress. In T98G cells, berberine has reduced cell viability through enhanced ROS generation and increase in intracellular Ca^{2+} levels. Significant activation of mitochondria dependent apoptotic pathway has also been observed in berberine treated T98G cells as evident by loss of mitochondrial membrane potential, enhanced Bax/Bcl-2 ratio and increase in activities of caspase 9,3. Furthermore, berberine treatment also showed anti-invasive and anti-metastasis properties and potently inhibited growth of tumor xenografts [107-109]. In a recent study, mitochondriotropic derivatives of berberine, synthesized by addition of moderate length alkyl chains have shown efficacy in inhibition of glioma cell proliferation at low micromolar range along with potent suppression of invasion

and metastasis. These derivatives have enhanced lipophilicity and localized into mitochondria and triggered ROS generation [110].

Growing literature suggests the prooxidant property of the phytoconstituents i.e., the ability to oxidize the cellular system depend upon numerous factors such as concentration, presence of metal ions and pH [111]. Usually at higher concentrations and in the presence of metal ions, phytochemicals exhibit this property generating enhanced amounts of ROS in *in vitro* biological systems. For instance, curcumin at 25-100 μ M significantly increased cellular ROS levels in human hepatoma Hep3B cells in time dependent manner [112]. This prooxidant property is recently attributed as one of major mechanisms for the antitumor properties of plant polyphenols [113]. Moreover, the selective cytotoxicity of many phytoconstituents might be mediated through prooxidant action of these compounds as cancer cells are more vulnerable to ROS mediated cytotoxicity than their normal counterparts [114].

4. NATURAL COMPOUNDS FOR THE TREATMENT OF GLIOMAS: TRANSLATIONAL HURDLES

Hitherto, most of these natural compounds except gossypol are under preclinical evaluation for their efficacy for anti-glioma activity, safety and their effects on metabolism. Translation of these possible chemotherapeutic agents from bench to clinic for the treatment of gliomas offers many challenges such as bioavailability, BBB permeability, selection of optimal dosage regimen etc. According to the new stringent regulations of US patent and trademark office (USPTO) for biological patents, complete dosage regimen for treatment of a disease should be established for a natural compound which is both expensive and time consuming to make them patentable. Hence battery of preclinical tests on various aspects such as pharmacokinetics, pharmacodynamics, toxicity effects both *in vitro* and *in vivo* including genotoxicity, mutagenicity and safety pharmacology are needed to be performed to get approved by food and drug administration (FDA) and to enter into clinical markets [115]. Growing body of evidence suggest that many of these aforementioned natural products are well tolerated without significant organ toxicities in both animal and human studies at pharmacological doses [116, 114]. However, dosage schedule and route of administration are also crucial to ascertain bioavailability, efficacy and patient compliance in clinical trials without significant toxicities and thus need to be carefully determined.

Poor oral bioavailability is a major obstacle for a number of natural products for their clinical utility [117]. Solubility, GI membrane permeability, metabolism and chemical stability are contributed as important factors for their low bioavailability. For instance, Results from both human and animal studies demonstrate the lower plasma concentrations of curcumin after its oral intake. Poor aqueous solubility, rapid metabolism and rapid systemic elimination are prime factors contributing for less bioavailability of curcumin [118]. Many researchers have made several attempts to enhance the bioavailability of the natural compounds by various approaches such as co-administration with other

natural compounds like piperine, nanoparticle administration, novel formulations with use of micelles, phospholipid complexes and development of structural analogues and bioconjugates [119]. Parenteral route of administration eliminates the need for the proper aqueous solubility and permeability through gastrointestinal membrane hence can be useful in the cases where natural compounds have poor oral absorption. However, in parental routes of administration such as i.p and i.v routes, half-lives of compounds in the plasma are affected by the rate of metabolism and excretion and their tissue distribution patterns. In many cases, these polyphenolic natural compounds undergo phase II metabolism and these conjugates have reasonable half lives in plasma and can hydrolyse to parent compounds thus ensuring clinical efficacy [117]. The clinical application of these aforementioned approaches and alternative route of administrations might enhance the bioavailable fraction of the administered compound and improve their biological efficacy in the clinical studies.

Another important factor for successful efficacious therapy in gliomas is the drug's ability to cross BBB - physiological barrier formed by tight junctions between capillary endothelial cells. Only small molecules (usually 400- 500 Daltons) with appropriate lipophilic nature and charge diffuse through BBB while large compounds with >1 Kilodaltons are unable to permeate through BBB [120]. Several delivery approaches were investigated to enhance the delivery of the compounds through BBB. Among them, invasive approach includes various methods such as physically breaching the BBB and injecting or implanting the drug into the brain by intra cerebroventricular injection, convention enhanced delivery and use of intracerebral implants. Moreover, modifications of compound's structure to enhance its lipophilicity, usage of lipid carriers were also successfully employed to bypass BBB. An alternative approach; physiological approach takes advantage of the receptors expressed in BBB to facilitate receptor mediated transcytosis. Tagging with endogenous ligands or antibodies targeting to those receptors such as transferrin receptor, insulin receptor, LDL receptor, nicotinic acetylcholine receptors were evaluated to augment the brain delivery of therapeutic compounds [121, 122].

Local delivery of the drugs into gliomas by methods such as either direct bolus injection or continuous infusion and controlled release methods by using carriers allow direct delivery of drug into tumors achieving maximum efficacy [123]. This method offers advantages such as lower systemic toxicity due to minimal dose requirement and allows entry of the hydrophilic large molecular drugs to tumors that are having lower BBB diffusion properties. Few chemotherapeutic drugs and proteins like TNF- α were injected into gliomas directly in clinical trials. However none of the aforementioned phytoconstituents were directly administered into gliomas [124]. The disadvantages of this administration include lower patient compliance, possible chances of intracranial hemorrhage, central nervous system toxicity and infection. These limitations can be possibly avoided by selecting optimal dosage schedule and utility of the best surgical practices and patient care and the use of advanced methods including drug impregnated microchips, local gene therapy

[125]. Thus the testing of novel compounds in *in vitro* and *in vivo* models to check their ability to cross BBB and implementation of strategies such as local delivery with novel methods to enhance their delivery into brain is necessary to achieve the desirable therapeutic outcome in the treatment of brain tumors.

The aforesaid clinical challenges if addressed properly by conducting large scale controlled clinical trials with these natural compounds paves a way for them to clinic. All of these phytoconstituents exhibited potent anti-glioma action in preclinical studies through multitude of biological effects such as decrease in cell viability, apoptosis induction, inhibition of invasion and migration. Moreover, they also enhance the anticancer activity in the gliomas when given in combination with other standard drugs such as temozolamide and TRAIL. Interestingly functional expression of P-glycoprotein pump (P-gp), an efflux pump that extrudes the drugs has been observed in brain endothelial cells in BBB [126]. Further, enhancement of the CNS effects of drugs such as loperamide, methadone (P-gp substrates) was observed when they are co-administered with the P-gp inhibitors like quinidine, verapamil [127, 128]. As numerous phytoconstituents such as quercetin, kaempferol were reported to inhibit P-gp, possible consideration of combination strategies of chemotherapeutic agents that are P-gp substrates along with these phytochemicals might be beneficial in increasing the permeability of the anticancer therapeutics thus may exhibit synergistic anticancer effects [129]. In the view of these attributes, these phytoconstituents can be directly used as drug or adjuvant therapy in combination with other standard chemotherapeutic agents to achieve desirable therapeutic outcome.

5. FUTURE DIRECTIONS

Recent studies have focused on the discovery and synthesis of compounds that are capable of selectively accessing mitochondria - mitochondriotropics- with aim of either inducing or preventing cellular death [130]. Mitocans-one class of such mitochondriotropics- are the anticancer agents that selectively target the mitochondria, destabilize them and thus exhibit cytotoxicity [131]. Many natural products target mitochondria at multiple nodes such as direct interaction with key molecules in mitochondria or indirect alteration of metabolic consequences arise as a result of mitochondrial dysfunction. Further selective targeting of such natural compounds to enhance their efficacy as antitumor agents has been achieved by tagging them with membrane -permeant cations such as triphenyl phosphonium (TPP) which selectively localizes into regions of negative potential. Recently, such mitochondria targeted analogues of natural compounds like resveratrol, quercetin, vitamin E were prepared and reported to have enhanced selective cytotoxic action owing to their enhanced ROS generation and specific targeting of mitochondrial components in variety of malignancies [132-134]. Future investigations of such mitocans are highly warranted in both *in vitro* and *in vivo* models of gliomas. Clinical success of anticancer agents also depends upon an important factor "therapeutic index" i.e., the selectivity between targeting cancer and normal cells. Hence identification and exploitation of differences

between cancer cells and normal cells such as elevated ROS, abnormal antioxidant system balance, deregulated mitochondria mediated apoptotic signaling in cancer cells when compared to normal cells are necessary for the safe and efficacious therapy in targeting mitochondrial dysfunction.

6. CONCLUDING REMARKS

Mitochondria are attractive pharmacological targets owing to their notable roles in cellular metabolism, regulation of redox signaling, ion homeostasis and induction of apoptotic cell death mechanism. Gliomas frequently harbor mitochondrial alterations such as shift from oxidative phosphorylation to glycolysis (Warburg effect), enhanced oxidative stress, abnormalities in mitochondrial membrane potential and apoptotic machinery, mitochondrial genome mutations along with somatic mutations in tricarboxylic acid cycle gene IDH. Plant derived phytochemicals target mitochondria by either directly binding to mitochondrial components or indirectly affecting metabolic alterations as a result of mitochondrial dysfunction. Targeting and disruption of mitochondrial electron transport chain by selective inhibition of various electron transport chain complexes, enhancement of ROS levels, disruption of cellular antioxidant system, targeting of Bcl2 anti-apoptotic family proteins and ion channels in the membrane of mitochondria are some identified mechanisms of action of mitochondria targeted natural compounds. These natural compounds have shown promise in treatment of gliomas in *in vitro* and *in vivo* models by their mitochondria specific effects and thus warrant further preclinical and clinical development in the treatment of gliomas. Critical observation of pharmacokinetics, bioavailability and tissue distribution patterns of each compound and implementation of innovative methods to enhance their bioavailability are highly warranted for their clinical success.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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