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The Role of Mitochondria in Glioma Pathophysiology

Bartlomiej B. Ordys · Séverine Launay · Ruth F. Deighton · James McCulloch · Ian R. Whittle

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Abstract It has long been recognised that malignant tumours favour aerobic glycolysis to generate ATP and contain abnormalities of the intrinsic, mitochondria-dependent, apoptotic pathway, suggesting the involvement of dysfunctional mitochondria in tumour pathophysiology. However, the mechanisms underlying such processes in gliomas are poorly understood. Few recent studies have evaluated mitochondrial ultrastructure and proteomics in the pathophysiology of malignant gliomas. However, aberrant energy metabolism has been reported in gliomas and mitochondrial dysfunction links to glioma apoptotic signalling have been observed. Mitochondrial structural abnormalities and dysfunction in malignant gliomas is a neglected area of research. Definition of abnormalities in mitochondrial proteomics, membrane potential regulation, energy metabolism and intrinsic apoptotic pathway signalling in gliomas may open novel therapeutic opportunities.

Keyword Mitochondria · Glioma · Apoptosis · Pathophysiology · Mitochondrial dysfunction · Cell death

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B. B. Ordys · I. R. Whittle (✉)
Department of Clinical Neurosciences, Western General Hospital,
University of Edinburgh,
Edinburgh EH4 2XU, UK
e-mail: ian.whittle@ed.ac.uk

S. Launay · R. F. Deighton · J. McCulloch
Centre for Cognitive and Neural Systems,
University of Edinburgh,
1 George Square,
Edinburgh EH8 9JZ, UK

Introduction

The worldwide incidence of primary brain tumours is estimated as 7.2–12.5 per 100,000 persons per year, encompassing ~1–2% of all adult primary tumours and 23% of childhood cancers. Primary brain tumours are responsible for approximately 13,000 deaths per year and account for 2% of adult and 26% of childhood cancer deaths [1–6]. The most common type of tumour is glioma, found in 49% of cases, with an age-standardised incidence rate of ~5 per 100,000 persons per year [2, 4, 7]. The resulting individual, social, healthcare and economic burden arising from the diagnosis and management of this group of cancers is significantly extensive.

The glial cell type of origin allows histological classification of gliomas into subtypes, with the most common type being astrocytoma [2]. Further histopathological analysis is then used to grade the level of malignancy from I to IV using the World Health Organization grading system (Table 1) [6, 8–11].

Glioblastoma multiforme (GBM) is the most malignant and most frequently found gliomal tumour type in adults, diagnosed in up to 80% of glioma cases [2, 4, 6, 8]. It can arise via one of two possible pathways: de novo or through evolvement of an existing lower-grade astrocytoma [3, 6, 11, 12]. Outlook from diagnosis is extremely poor, with a median survival of 9–14 months depending on age, performance status and treatment given. Over the last 40 years, outlook has improved only modestly despite technological and therapeutic improvements in surgical resection, radiotherapy and chemotherapy [3, 12–14].

It has been well reported that malignant tumours, including gliomas, favour abnormal energy production via aerobic glycolysis (non-oxidative metabolism of glucose in the presence of oxygen) and show an inherent resistance to

Table 1 WHO grading of astrocytomas

Grade	Type	Features	Median survival time
I	Pilocytic astrocytoma	Good differentiation Well-circumscribed border	Curable
II	Diffuse astrocytoma	Moderately increased proliferation Cytological atypia Diffuse invasion	10 years
III	Anaplastic astrocytoma	Markedly increased proliferation Cytological and nuclear atypia Invasion Angiogenesis	3 years
IV	Glioblastoma multiforme	Poor differentiation Greatly increased proliferation Cytological and nuclear atypia Necrotic core Invasion Angiogenesis	9–14 months

apoptosis (programmed cell death) [6, 15, 16]. This suggests the underlying involvement of mitochondrial dysfunction in malignant tumour pathophysiology, which is not well understood.

Mitochondria are oval-shaped, membrane-enclosed intracellular organelles of 0.5- to 10-μm diameter which contain their own DNA. They have distinct structural compartments that are essential for performing specialised functions [17]. The most recognised of which is the generation of adenosine triphosphate (ATP), the chemical form of cellular energy, via aerobic respiration. This is the main energy-producing pathway of normal glial cells and the ensuing step following glycolysis in the presence of oxygen [17, 18]. Mitochondria are also involved in the regulation of cellular proliferation and apoptosis, two other important areas of dysfunction in glioma.

This paper aims to review and consolidate knowledge of the function and dysfunction of mitochondria in energy generation and apoptosis in gliomas. Insight into aberrant mitochondrial function in gliomas is essential to provide new directions in the development of novel research approaches that translate into improved therapies.

Energy Metabolism

Mitochondrial Decoupling

Mitochondrial aerobic respiration is the main source of cellular energy in glial cells [19]. It begins with enzymatic metabolism of acetyl-CoA in the mitochondrial matrix, via the tricarboxylic acid (TCA) cycle, which causes reduction of co-enzymes nicotinamide adenine dinucleotide (NAD^+) and flavin adenine dinucleotide (FAD). The reduced forms (NADH and $FADH_2$) then undergo oxidative phosphorylation by the mitochondrial respiratory chain. This is a series of five (I–V) transmembrane protein complexes spanning the inner mitochondrial membrane. Complexes I–IV act in a chain to transfer the electrons liberated by oxidation, which drives H^+ (protons) into the intermembrane space, creating a proton motive gradient across the inner membrane. Complex V (ATP synthase) utilises the energy obtained from the movement of protons along this gradient to generate ATP (Fig. 1) [17, 18].

Acetyl-CoA is mainly derived from glucose by cytoplasmic glycolysis to pyruvate and subsequent metabolism by pyruvate dehydrogenase. However, when blood glucose becomes low following prolonged fasting, the blood levels of the liver-produced ketone β-hydroxybutyrate (β-OHB) are increased, which becomes an alternative substrate for acetyl-CoA generation in glial cells [20]. It is transported across the blood–brain barrier by monocarboxylic transporters (MCTs) and is metabolised within the mitochondrial matrix directly, allowing rapid energy production capable of sustaining the brain's metabolic demands (Fig. 1) [21–24].

In contrast, gliomas show reliance on persistent aerobic glycolysis as their main source of ATP production, as exhibited by animal models, human glioma-derived cell cultures and in vivo human studies [25–27]. In fact, toxic inhibition of the mitochondrial respiratory chain in glioma cell lines has no effect on cell viability or ATP production in the presence of adequate glucose [28]. This failure of malignant tumour cells to progress to more efficient aerobic respiration for energy production was first described by the Nobel laureate Warburg and subsequently termed the *Warburg hypothesis* [29].

Glioma-derived cell lines demonstrate the presence of ketone energy metabolism pathways, but despite this, animal glioma models cannot sustain tumour growth in

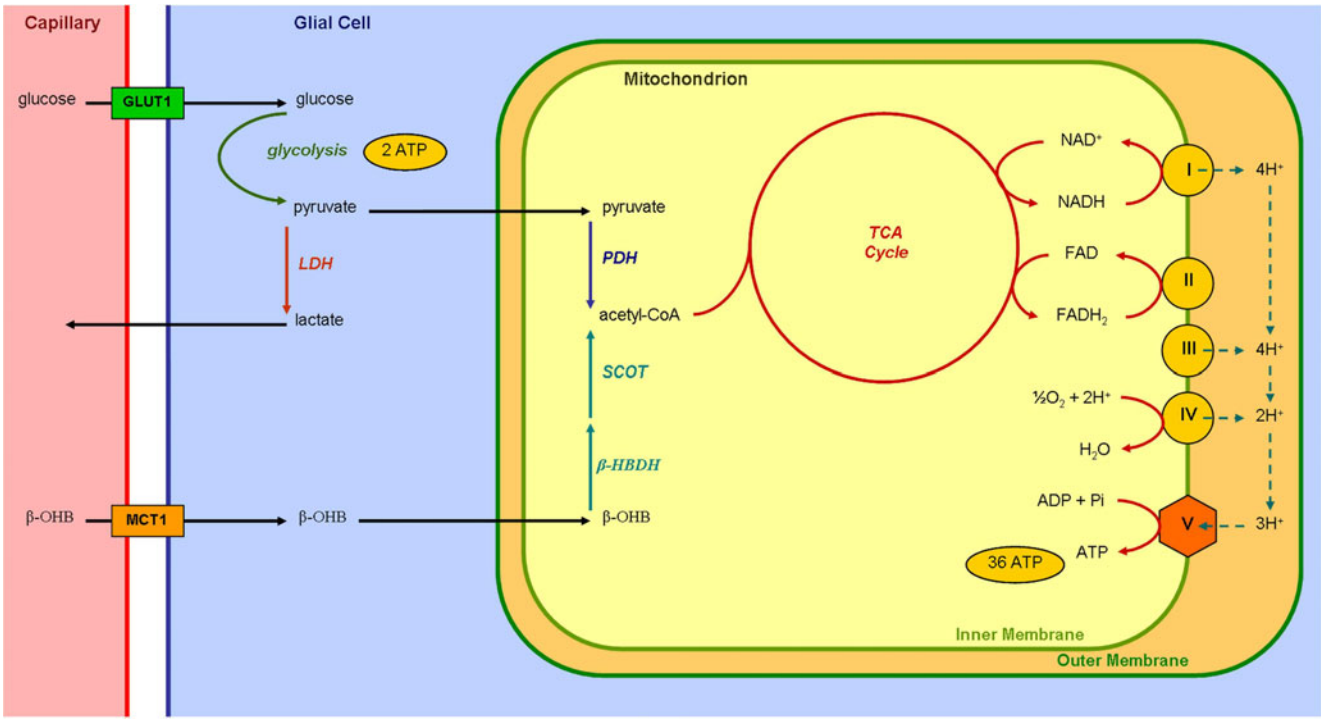

Fig. 1 Pathways of energy metabolism in glial cells. Cytoplasmic glycolysis has a net yield of two ATP per molecule of glucose, whilst acetyl-CoA metabolism, via the TCA cycle and mitochondrial respiratory chain (complexes I–V), requires oxygen and has a net yield of 36 ATP per molecule of acetyl-CoA. Lactate is formed in the absence of oxygen and is recycled into glucose via the Cori cycle in the liver. *GLUT1* glucose transporter 1, *LDH* lactate dehydrogenase, *PDH* pyruvate dehydrogenase, *TCA cycle* tricarboxylic acid cycle, *NAD*$^+$ nicotinamide adenine dinucleotide, *FAD* flavin adenine dinucleotide, *ATP* adenosine triphosphate, *ADP* adenosine diphosphate, *Pi* inorganic phosphate, *β-OHB* β-hydroxybutyrate, *MCT1* monocarboxylate transporter 1, *β-HBDH* β-hydroxybutyrate dehydrogenase, *SCOT* succinyl-CoA-acetoacetate-CoA transferase (modified from figures in [18, 25])

the presence of reduced glucose and elevated β-OHB, presumably due to dysfunction of ketone-derived ATP production [25, 30–32]. This difference in normal glial cell versus glioma cell metabolism has been exploited as a potential therapeutic strategy in two female paediatric cases of malignant glioma (grades III and IV) which were non-responsive to aggressive radiotherapy and chemotherapy [33]. These patients were maintained on a ketogenic diet for 8 weeks, which lowered blood glucose and produced ketosis (elevated circulating ketones) within 7 days. This resulted in marked symptomatic improvement, long-term tumour management (one patient remaining free of disease progression at 12 months with continued consumption of the ketogenic diet) and a 21.8% average reduction in tumour glucose uptake on positron emission tomography [33]. No adverse effects were reported.

Mouse, in vivo, glioma model studies (using implanted mouse and human glioma cell lines) indicate additional anti-inflammatory, anti-angiogenic and pro-apoptotic tumour effects of ketosis induced by dietary glucose restriction [25, 32, 34, 35]. The exact mechanisms underlying these beneficial results remain to be uncovered. Some insight has been provided by glial cell cultures which release pro-inflammatory cytokines in response to lactate, the end product of obligatory glycolytic energy production [36]. This could feasibly be a contributory process to tumour growth, oedema, angiogenesis and apoptosis resistance in the lactate-rich environment of gliomas, which glucose restriction would counter. Furthermore, immunohistochemistry of human CNS tissue shows increased expression of MCT 1 transporters in high-grade gliomas, potentially facilitating increased ketone uptake and making any direct, ketone-induced anti-tumour effects an appealing target for novel therapeutics [22].

Overall, the Warburg hypothesis is supported by the lack of mitochondria-dependent ketone utilisation for energy metabolism. Both point to the presence of dysfunctional mitochondria, which essentially decouple from the normal, cellular energy-generating pathways, thus making glioma dependent on cytosolic glycolysis. Further evidence for this is seen in glioblastoma cell cultures where constitutive activation of the Akt oncogene, a frequent mutation in malignant tumours responsible for overactivity of the cell proliferation pathway, results in a clear shift from normal aerobic respiration to abnormal, persistent aerobic glycolysis for cell survival [37].

Cardiolipin Dysfunction

The mitochondrial membrane has a unique structure due to the phospholipid cardiolipin which is specific to mitochondria and bacteria. Cardiolipin is highly expressed on the inner mitochondrial membrane and at contact points between the inner and outer mitochondrial membranes [38, 39]. It is a dimeric phospholipid with four variable acyl groups, mostly of C_{18} chain length in mammals, whose different combinations give rise to the multitude of cardiolipin molecular species [38, 40, 41]. It is synthesised through the common phospholipid biosynthetic pathway, with the final distinct step, catalysed by the inner mitochondrial membrane-bound enzyme cardiolipin synthase. The end result is the production of cardiolipin within the mitochondrial matrix, which then undergoes further acyl group modification [38, 42].

Cardiolipin has an essential role in mitochondrial function through its interactions with inner mitochondrial membrane proteins. Its different molecular species are closely associated with the quaternary structure of several metabolite carriers and complexes I, III, IV and V of oxidative phosphorylation (Table 2).

As well as incorporation into individual proteins, cardiolipin is required for structural support and maintenance in respirasome supercomplexes, which are the functional units of oxidative phosphorylation formed by an amalgamation of complexes I–IV [49]. Furthermore, mitochondrial structure, membrane stability, production of the proton motive gradient and role in apoptosis (discussed later, page 15) depend on the presence of normal cardiolipin [38, 50].

Loss of mitochondrial function in glioma could be attributed to deficiencies of cardiolipin. This has recently been explored by shotgun lipidomics comparing glial mitochondria of mice highly susceptible to spontaneous gliomas with those of mice refractory to spontaneous tumours. Both strains showed almost equal levels of total cardiolipin; however, the number of molecular species was reduced by ~50% in the susceptible strain even prior to the development of glioma. In addition, the distribution of these molecular species was altered, showing an asymmetrical pattern across the inner mitochondrial membrane, overall leading to an observed reduction in the activity of complexes I, II and III [51]. These data suggest that altered cardiolipin differentiation into molecular species and organisation may be an early component of glioma development and consequently responsible for mitochondrial decoupling from energy production. This is supported by shotgun lipidomics of mitochondria from mouse gliomas which again show fewer cardiolipin molecular species and an associated, significant reduction in the activity of the mitochondrial respiratory chain complexes. Furthermore, the presence of abnormal and poorly developed cardiolipin molecular species was seen [52]. This implies that defective cardiolipin biosynthesis and subsequent failure of acyl group remodelling may play a central part in mitochondrial dysfunction in glioma. Unfortunately, the regulatory mechanisms and pathways for molecular species formation in cardiolipin synthesis are not well understood and require further research.

Oxidative Stress

Normal energy production through aerobic respiration generates reactive oxygen species (ROS) in the mitochondria. At physiological levels, ROS are non-lethal and act as intracellular messengers; however, prolonged excess of ROS results in cellular damage and death by apoptosis [53, 54]. Glial cells contain a pool of glutathione (GSH) and its oxidised form glutathione disulfide (GSSG) which act as a redox buffer system that prevents the buildup of ROS to dangerous levels [55].

In human glioma samples, the levels of GSH/GSSG buffer are significantly lower in the highly proliferative tumour periphery compared to the necrotic centre, even though mitochondrial content is the same [56]. This is important as glucose withdrawal from glioma cell cultures is shown to increase ROS production and induce apoptosis due to insufficient redox buffering [28, 57]. In these glioma

Table 2 Protein interactions of cardiolipin

Protein	Function	Cardiolipin interaction
Complex I	Oxidative phosphorylation	Constitutive to enzymatic activity [43]
Complex III	Oxidative phosphorylation	8 molecules tightly bound [38]
		Constitutive to enzymatic activity [43]
Complex IV	Oxidative phosphorylation	2 molecules in high affinity sites [44]
		Necessary for optimal activity [45]
Complex V	ATP synthesis	4 molecules in high affinity sites [46]
		Essential for normal structure [46]
ADP-ATP carrier	ADP/ATP transport across inner membrane	6 molecules tightly bound [47]
		Necessary for optimal activity [48]

cells, glucose withdrawal is also accompanied by an increased breakdown of fatty acids within the mitochondria, presumably as part of an alternative pathway for generating ATP [57]. However, loss of normal mitochondrial structure, stability and function in glioma is likely to result in abnormalities of the attempted fatty acid metabolism, causing the high output of ROS and lethal oxidative stress. In addition, studies of cardiolipin in rat mitochondria highlight increased levels of ROS as a direct cause of cardiolipin dysfunction through oxidation and, as a result, loss of mitochondrial function [58–60]. This detrimental cycle may be contributing to further mitochondrial damage and decoupling from normal cellular function in glioma (Fig. 2).

Therefore, the proliferating tumour periphery, with its high energy demands and low GSH/GSSG buffer levels, is highly susceptible to apoptosis induced by the metabolic effects of insufficient glucose, once again highlighting the potential of targeting energy metabolism to stop glioma progression.

Apoptosis

Apoptosis is a cascade of biochemical reactions culminating in cellular and nuclear fragmentation, followed by packaging of cellular contents into small vesicles known as apoptotic bodies and subsequent phagocytosis of these vesicles by surrounding cells. This process can be triggered via extrinsic and intrinsic apoptotic pathways (Fig. 3).

The extrinsic apoptotic pathway is activated by the immune system following detection of abnormal surface markers on the cell. There is subsequent release of specific ligands that bind and activate cell surface death receptors

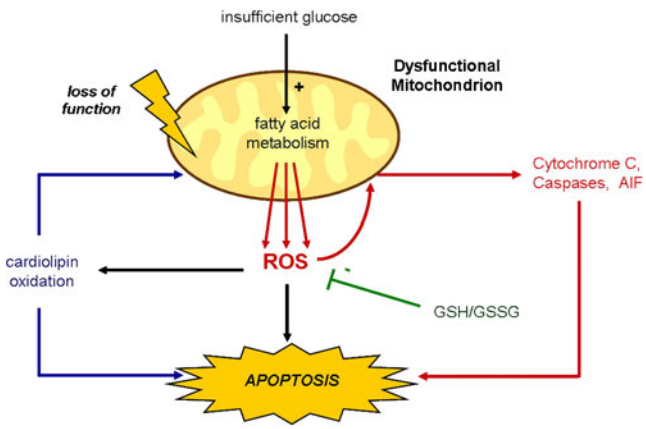

Fig. 2 Effects of metabolic shift from glycolysis to dysfunctional fatty acid metabolism for attempted ATP production due to glucose withdrawal in glioma. *Plus sign* denotes stimulation. *Minus sign* denotes inhibition/buffering. *ROS* reactive oxygen species, *GSH* glutathione, *GSSG* glutathione disulfide

(DR) which include the Fas and TNF receptor families. This results in intracellular signal transduction through the formation of a death-inducing signalling complex, causing activating cleavage of caspases 8 and 10. These in turn cleave caspases 3 and 7, which go on to begin the irreversible apoptotic cascade [61, 62]. In glioma, the extrinsic apoptotic pathway is suppressed, even though its triggers and constituents are present [63, 64]. Administration of the DR-specific TNF-related apoptosis-inducing ligand shows that this may be due to an inherent tumour resistance, with only a minority of glioma cell lines showing susceptibility to apoptosis through this pathway [65]. Further studies of glioma cell lines and human, glioma, tumour samples indicate that this resistance is related to over-activation of the Akt and mammalian target of rapamycin (mTOR) proliferative pathway [66, 67].

The intrinsic apoptotic pathway is triggered by direct damage to the cell from various insults, including hypoxia, oxidative stress, radiotherapy and chemotherapy, all of which can cause influx of Ca^{2+} into the cell, generation of ROS and direct DNA damage. As a result, protein 53 (p53) is activated and subsequently enhances DNA transcription of anti-proliferative and pro-apoptotic genes [6, 15, 61, 62]. The up-regulated pro-apoptotic gene products include p53 upregulated modulator of apoptosis (PUMA), phorbol-12-myristate-13-acetate-induced protein 1 (NOXA) and BCL-2 homology domain 3 proteins (BH3), which interact with the B cell lymphoma-2 (BCL-2) homology family of proteins. The members of this family have a close structural and functional relationship with the mitochondrial membranes and exert either membrane-stabilising or permeability-inducing effects, classifying them into anti-apoptotic and pro-apoptotic, respectively. PUMA, NOXA and BH3 stimulate the pro-apoptotic BCL-2 family members and, together with these, inhibit the anti-apoptotic family members [61, 62, 68]. It is believed that the resulting shift in balance towards apoptosis causes opening of a mitochondrial permeability transition pore (MPTP) through the interaction of several proteins (discussed later, page 14). This pore allows the rapid release of cytochrome C (Cyt C), second mitochondria-derived activator of caspases (SMAC) and Omi from the mitochondrion into the cytosol. Cyt C then binds with apoptotic peptidase activating factor-1, a p53 enhanced gene product, and caspase 9 to form an apoptosome which cleaves caspases 3 and 7 instigating the apoptotic cascade, whilst SMAC and Omi inhibit the function of the inhibitors of apoptosis (IAP) family of proteins which prevent apoptosome action (Fig. 3) [61, 62, 68–70].

Abnormalities of this pathway are frequent in human cancers, including glioma. Inactivating p53 gene mutations are expressed in 30–50% of human glioma tumour samples, which is seen as an early change in low-grade glioma.

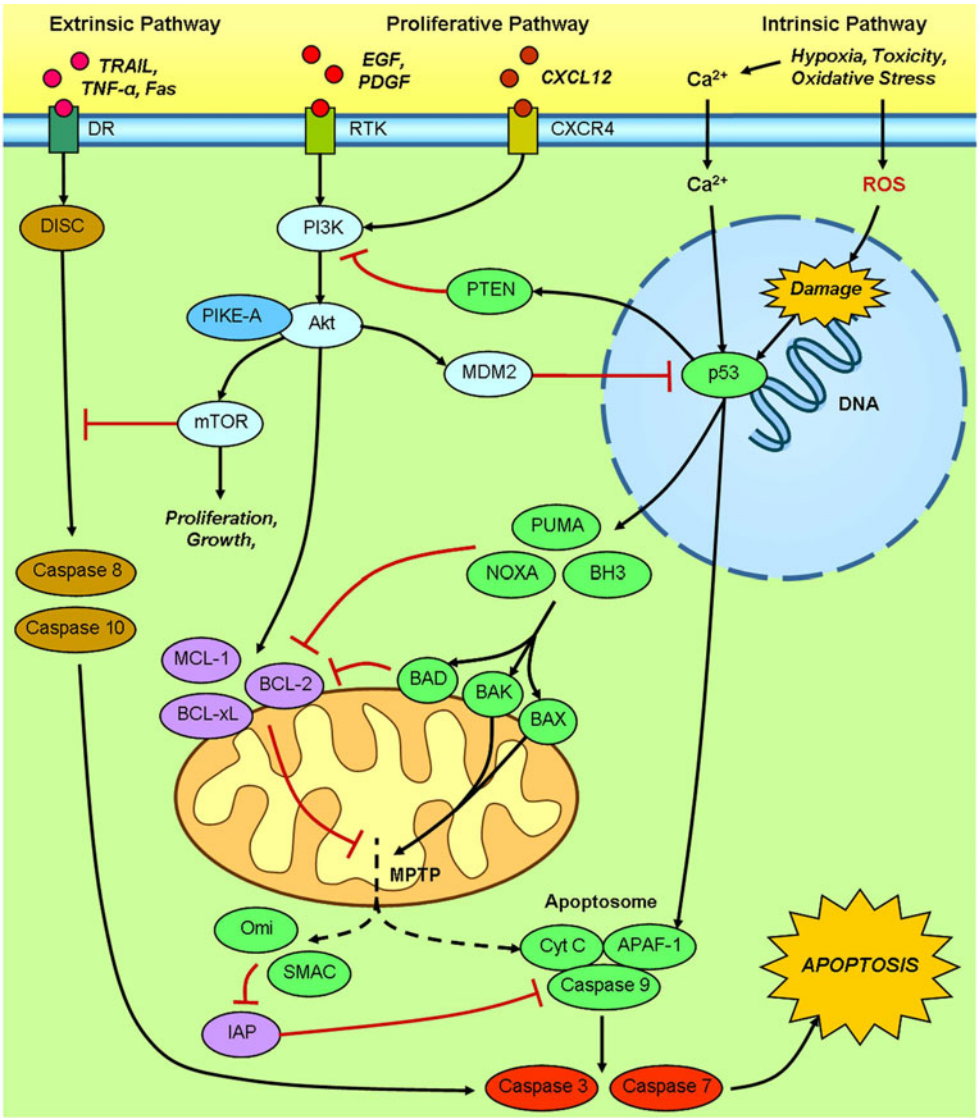

Fig. 3 Extrinsic/intrinsic apoptotic pathways and their interactions. *TRAIL* TNF-related apoptosis-inducing ligand, *TNF-α* tumour necrosis factor-α, *DR* death receptor, *DISC* death-inducing signalling complex, *EGF* epidermal growth factor, *PDGF* platelet-derived growth factor, *CXCL12* chemokine stromal cell-derived factor 12, *RTK* receptor tyrosine kinase, *CXCR4* chemokine receptor 4, *PI3K* phosphoinositide 3-kinase, *PIKE-A* PI3K enhancer activating Akt, *mTOR* mammalian target of rapamycin, *MDM2* murine double minute 2, *PTEN* phosphatase and tensin homolog, *ROS* reactive oxygen species, *DNA* deoxyribonucleic acid, *p53* protein 53, *PUMA* p53 upregulated modulator of apoptosis, *NOXA* phorbol-12-myristate-13-acetate-induced protein 1, *BH3* BCL-2 homology domain 3 protein, *BCL-2* B cell lymphoma-2, *BCL-xL* B cell lymphoma-extra large, *MCL-1* myeloid cell leukaemia sequence 1 (BCL2-related), *BAD* BCL-2-associated death promoter, *BAK* BCL-2 antagonist/killer, *BAX* BCL-2-associated X protein, *MPTP* mitochondrial permeability transition pore, *SMAC* second mitochondria-derived activator of caspases, *IAP* inhibitors of apoptosis, *Cyt C* cytochrome C, *APAF-1* apoptotic peptidase activating factor-1

Perseverance of this mutation to malignant glioma is common and associated with significantly increased angiogenesis and a higher chance of recurrence following resection [71, 72].

The strong association of BCL-2 family members with the mitochondrial membranes makes them possible contributors to mitochondrial dysfunction in glioma. Immunohistochemistry for the anti-apoptotic BCL-2 protein shows that it is highly over-expressed in human glioma cell lines and that this protects the cell from undergoing apoptosis following chemically induced DNA damage [73]. In addition, immunohistochemical studies of human glioma tumour samples show elevated BCL-2 family expression in all glioma cells compared to normal astrocytes, with very highly expressed members found in all grades of malignancy (Table 3) [74, 75].

Paradoxical elevation of the pro-apoptotic BCL-2-associated X protein (BAX) could suggest that over-expression of functional BCL-2 proteins in itself is not the only factor conferring apoptosis resistance. However, mitochondrial membrane dysfunction, which results in ineffective BCL-2 family interactions and subsequent attempted compensation by up-regulation of its members, could also underlie the inherent apoptosis-resistant nature of malignant cancers. If this is the case, then expression of the BCL-2 family could be used as a marker for the degree of mitochondrial dysfunction present.

In view of this, GBM recurrences following resection, radiotherapy and chemotherapy have been shown to contain significantly increased levels of BCL-2, BCL-xL and MCL-1, with decreased BAX compared to the initially resected tumour [75, 76]. Further studies in human malignant glioma cell lines show that high expression of BCL-xL and low expression of BAX is also associated with radiotherapy resistance and that down-regulation of BCL-xL and BCL-2 with antisense complimentary DNA (cDNA) transfection produces chemosensitivity [77–80]. Overall, suggesting that current treatment modalities select out resistant

Table 3 BCL-2 family over-expression in glioma

Protein	Type	Strong positive immune-staining (% of total graded samples)		
		Low-grade astrocytoma (%)	High-grade astrocytoma (%)	Glioblastoma multiforme (%)
BCL-2	Anti-apoptotic	29	67	0
BCL-xL	Anti-apoptotic	63	33	48
MCL-1	Anti-apoptotic	54	33	11
BAX	Pro-apoptotic	54	100	90

tumours, which appear to have a high expression of the mitochondria-associated anti-apoptotic BCL-2 family members, and that targeting this over-expression may provide a novel approach to glioma treatment.

Abnormalities in the intrinsic apoptotic pathway also occur downstream to mitochondria. Over 95% of human GBM samples show increased expression of the anti-apoptotic BCL-2-like 12 (BCL2L12) protein which inhibits caspase 3 through αB-crystallin and caspase 7 directly. Modified rat primary cortical astrocyte cell lines also indicate that BCL2L12 may be responsible for the terminal shift from apoptosis to necrosis, potentially accounting for the highly necrotic nature of gliomas [81]. Finally, IAP family proteins are also constitutively over-expressed in human malignant glioma cell lines. This can, however, be overcome by antisense cDNA transfection and SMAC mimetics which have shown therapeutic potential by increasing sensitivity to radiotherapy and chemotherapy and by potentiation of the extrinsic apoptotic pathway [82–86].

The cell-proliferative pathway is an important modulator of both extrinsic and intrinsic apoptotic pathways. It is triggered by the binding of growth factors to their respective receptor tyrosine kinases (RTKs) found on the cell surface. Activated receptors recruit phosphoinositide 3-kinase (PI3K) which phosphorylates a multitude of signalling proteins, including the protein kinase Akt. This causes activation of Akt, which then phosphorylates further proteins that potentiate the action of anti-apoptotic BCL-2 family members, suppress the intrinsic apoptotic pathway by negative regulation of p53 by murine double minute 2 (MDM2) and inhibit the extrinsic apoptotic pathway, whilst promoting protein synthesis for cell survival, growth and proliferation, by mTOR complexes (Fig. 3) [6, 15, 62, 87].

The constituents of the proliferative pathway are also adversely affected in glioma, producing an overall pro-proliferative and anti-apoptotic stimulus. There is over-expression of RTKs for epidermal growth factor in ~20–40% of high-grade gliomas and for platelet-derived growth factor (PDGF) in ~25% of cases [88–90]. Furthermore, immunohistochemistry has shown the presence of PDGF autocrine and paracrine loops in glioma tumour samples, which suggests that glioma exhibits a malignant ability to promote its own growth [91]. In addition, the proliferative pathway can be triggered by chemokine stromal cell-derived factor 12 (CXCL12) acting on its chemokine receptor (CXCR4), which are both found to be highly expressed in human malignant glioma cell lines and mouse glioma models [92]. Further down the pathway, as mentioned previously, there is often constitutive activation of Akt and enhancement of its anti-apoptotic action by the increased expression of PI3K enhancer activating Akt (PIKE-A) in GBM [37, 93, 94]. Finally, normal inhibition of the proliferative pathway by p53-enhanced transcription of the phosphatase and tensin homolog (PTEN) tumour suppressor gene is disrupted by inactivating PTEN gene mutations in 30–40% of glioblastomas [95].

Mitochondrial Permeability Transition Pore

The release of apoptosis effectors (Cyt C, SMAC and Omi) from mitochondria relies on the permeabilisation of inner and outer mitochondrial membranes. This process occurs through apoptotic-pathway-triggered cleavage of the cytosol expressed, pro-apoptotic BCL-2 family member known as BH3 interacting domain death agonist (BID). The resulting truncated form of the protein (tBID) translocates from the cytosol to the mitochondria where it attracts and activates cytosolic BAX and the outer mitochondrial membrane-bound BCL-2 antagonist/killer (BAK). The currently favoured theory is that recruitment of these pro-apoptotic BCL-2 family members facilitates the opening of a multi-protein pore complex, known as MPTP, found at contact sites between the mitochondrial membranes. This provides a transmembrane channel for the release of Cyt C, SMAC and Omi whilst also causing dissipation of the mitochondrial transmembrane potential and proton gradient (Fig. 4) [96–99].

Cardiolipin is essential for maintaining the normal function of the mitochondrion during induction of apoptosis. Studies using mouse liver isolated mitochondria and liposome mitochondrial membrane models show that cardiolipin is necessary for targeting of tBID to mitochondria and for its subsequent binding, which occurs at the cariolipin-rich contact sites between the inner and outer mitochondrial membranes [100–102]. Interaction of tBID at these sites

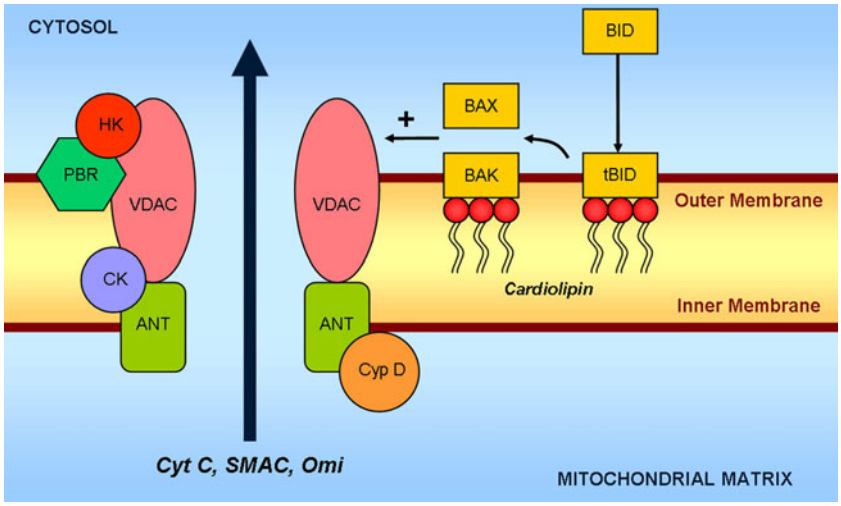

Fig. 4 Structure and interaction of the mitochondrial permeability transition pore (*MPTP*) in apoptosis. *Plus sign* denotes stimulation. *VDAC* voltage-dependent anion channel, *ANT* adenine nucleotide translocator, *PBR* peripheral benzodiazepine receptor, *CK* creatine kinase, *HK* hexokinase, *Cyp D* cyclophillin D, *BID* BH3 interacting domain death agonist, *tBID* truncated BID, *BAX* BCL-2-associated X protein, *BAK* BCL-2 antagonist/killer, *Cyt C* cytochrome C, *SMAC* second mitochondria-derived activator of caspases (modified from [19])

results in the redistribution of cardiolipin on the inner and outer mitochondrial membranes, as observed in haematological cancer cell lines prior to apoptosis [103]. This is likely to alter the structure and function of the mitochondrion to favour membrane permeabilisation. Furthermore, isolated mitochondrial systems suggest that cardiolipin is also required to facilitate the interaction of BAX with the MPTP and result in its opening following pro-apoptotic signalling [98]. Finally, embryonic mouse mitochondria indicate that specific molecular species of cardiolipin are bound to cyt C within the intermembrane space, forming an oxygenase complex. This complex catalyses cardiolipin peroxidation in response to apoptogenic signalling, which induces cyt C and SMAC release [104]. Therefore, it is reasonable to assume that the abnormalities of cardiolipin found in mouse glioma studies of abnormal mitochondrial energy metabolism (described previously, page 8) [51, 52] could also represent an underlying mechanism for mitochondrial dysfunction resulting in intrinsic apoptosis resistance in glioma.

Targeting the MPTP separately from its cardiolipin-dependent activation may provide a useful way of bypassing the mitochondrial dysfunction. Specific ligands to the peripheral benzodiazepine receptor, an over-expressed component of the MPTP in human glioma tumour samples, have shown promise by inducing apoptosis in rat glioma cell lines [105, 106]. In addition, direct MPTP-inducing agents promote apoptosis in human malignant glioma cell lines, suggesting that the pore itself is still functional whilst its activation by pro-apoptotic BCL-2 family members may be interrupted by cardiolipin abnormalities within the mitochondrial membranes [107].

Concluding Remarks/Future Perspectives

This review has highlighted the existence of mitochondrial dysfunction in glioma. Glioma tumour cells generate ATP through glycolosis exclusively, even though the presence of oxygen should allow progression of energy metabolism to

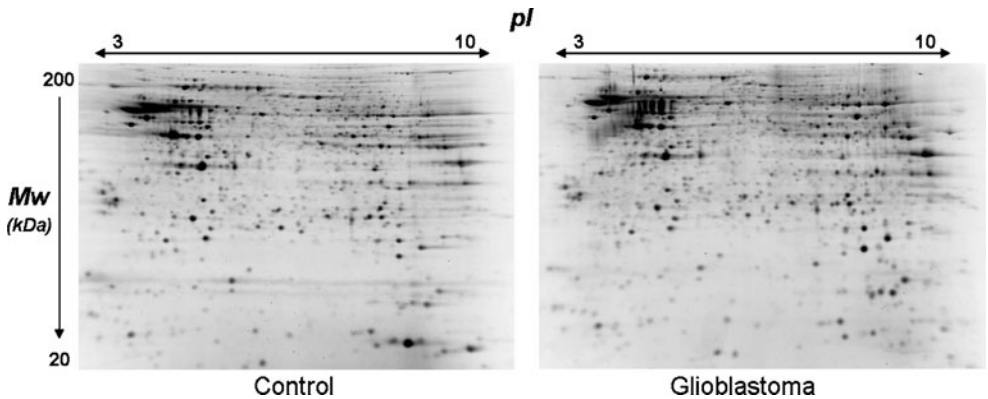

Fig. 5 Representative 2D SDS-PAGE gels from control and glioblastoma mitochondrial fractions. Each *spot* represents an individual protein. Proteins are separated by charge (*x* dimension) and molecular weight (*y* dimension). Eighteen-centimetre nonlinear IPG strips with a p*I* range from 3 to 10 were used. Representative gels of control (*left*) and glioblastoma (*right*) mitochondrial fractions are shown. Alterations in the abundance of various mitochondrial proteins in glioblastomas can be discerned in various quadrants of the gels

mitochondrial aerobic respiration. This decoupling of mitochondria from the energy-generating pathway is described as a fundamental feature in many malignant cancers of different origin [29, 108–110].

In contrast to normal glial cells, gliomas also exhibit an inability to metabolise ketones and fatty acids for energy production following glucose deprivation. Both of these alternative ATP-generating pathways are reliant on mitochondria and their enzyme systems. Additionally, attempted fatty acid metabolism by mitochondria of glioma cells results in the abnormal production of high levels of ROS and subsequent cell death by apoptosis. Therefore, glucose deprivation and high ketogenic diets may provide a tangible therapeutic approach to specifically target glioma tumour cells whilst sparing normal glia from damage.

Several abnormalities of the intrinsic, mitochondria-dependent apoptotic pathway are evident in glioma as well as other malignant tumours [111]. There is a clear over-expression of the BCL-2 homology protein family which is associated with radiotherapy and chemotherapy resistance in different malignant cancers [112–114]. These proteins are closely related to mitochondrial membranes and their interaction mediates mitochondrial release of apoptogenic factors, an interaction which requires cardiolipin, a mitochondrial membrane-specific phospholipid, to function. Abnormalities of cardiolipin are described in glioma cells and are perceived to cause dysfunction of the mitochondrial respiratory chain and energy metabolism. However, bearing in mind the necessity of cardiolipin for pro-apoptotic BCL-2 family members to exert their effects, it is reasonable to speculate that these abnormalities may also potentially account for the interruption of the apoptotic pathway.

In other malignant tumours, oncogene expression and mitochondrial DNA (mtDNA) mutations have been proposed as mechanisms of mitochondrial dysfunction [108–111]. Nevertheless, these mechanisms cannot account for the defective role of mitochondria in apoptosis. In addition, a recent study of mtDNA in chemically induced mouse gliomas showed the absence of any causative mutations [115].

Mitochondrial proteomics is a further approach which can provide a fresh perspective of mitochondrial dysfunction in glioma. Proteomics allows large-scale simultaneous analysis of more than a thousand proteins and is particularly potent in assessing alterations in levels but also in defining protein–protein interactions. Proteomics along with genetic analysis has been employed in glioma with considerable success [116]. Proteomic analysis of mitochondrial fractions from glioma (Fig. 5) may provide additional insight into the dynamics of mitochondrial protein alterations in glioblastomas.

Overall, this review shows that some attempts have been made to explain the precise nature of the mitochondrial abnormalities present in glioma; however, more work is needed to fully explore their mechanisms and provide effective novel therapeutics.

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