Mol Neurobiol (2010) 42:89–96
DOI 10.1007/s12035-010-8135-3

Glioma Cell Death: Cell–Cell Interactions and Signalling Networks

H. Anne Leaver · Maria Theresa Rizzo · Ian R. Whittle

Received: 5 April 2010 / Accepted: 5 April 2010 / Published online: 5 May 2010

Abstract The prognosis for patients with malignant gliomas is poor, but improvements may emerge from a better understanding of the pathophysiology of glioma signalling. Recent therapeutic developments have implicated lipid signalling in glioma cell death. Stress signalling in glioma cell death involves mitochondria and endoplasmic reticulum. Lipid mediators also signal via extrinsic pathways in glioma cell proliferation, migration and interaction with endothelial and microglial cells. Glioma cell death and tumour regression have been reported using polyunsaturated fatty acids in animal models, human ex vivo explants, glioma cell preparations and in clinical case reports involving intratumoral infusion. Cell death signalling was associated with generation of reactive oxygen intermediates and mitochondrial and other signalling pathways. In this review, evidence for mitochondrial responses to stress signals, including polyunsaturated fatty acids, peroxidising agents and calcium is presented. Additionally, evidence for interaction of glioma cells with primary brain endothelial cells is described, modulating human glioma peroxidative signalling. Glioma responses to potential therapeutic agents should be analysed in systems reflecting tumour connectivity and CNS structural and functional integrity. Future insights may also be derived from studies of signalling in glioma-derived tumour stem cells.

Keyword Glioma · Cell death signalling · Mitochondria

Abbreviations

AA	arachidonic acid
Akt	serine–threonine protein kinase
CHOP/GADD153	CCAAT/enhancer binding protein homologous transcription factor
CNS	central nervous system
COX	cyclo-oxygenase
$\Delta\Psi m$	transmembrane mitochondrial potential
ER	endoplasmic reticulum
ERK	extracellular signal-regulated kinase
GLA	gamma linolenic acid
GF	growth factors
GFAP	glial fibrillary acidic protein
GRP78/BiP	ER glucose-regulated protein 78
H_2O_2	hydrogen peroxide
iNOS	inducible nitric oxide synthetase
LO	lipoxygenase
MEK-1/2	mitogen-activated protein kinase kinase 1/2
MPT	mitochondrial permeability transition pore
PARP	pol(ADP-ribosyl) polymerase
PBR	peripheral benzodiazepine receptor
PDGF	platelet-derived growth factor
PDR	peripheral benzodiazepine receptor
Plase A2	phospholipase A2
PKAII	type II protein kinase A
PUFA	polyunsaturated fatty acids
roi	reactive oxygen intermediates
TLR	Toll-like receptor-2

H. A. Leaver · I. R. Whittle
Department of Clinical Neurosciences, University of Edinburgh, Edinburgh, UK

H. A. Leaver (✉)
Cell Biology R&D, SNBTS Edinburgh,
21, Ellen's Glen Road,
Edinburgh EH17 7QT, UK
e-mail: hanne.leaver@googlemail.com

M. T. Rizzo
Signal Transduction Laboratory, Methodist Research Institute, Indianapolis, IA, USA

TNF	tumour necrosis factor
TRAIL	TNF-related apoptosis-inducing ligand
roi	reactive oxygen intermediates
VEGFR	vascular endothelial cell growth factor receptor

Introduction

Despite recent advances in treating malignant gliomas, prognosis remains poor. The median current survival in patients with glioblastoma multiforme, the most common, malignant human glioma, is less than 15 months in randomised controlled trials [1]. A better understanding of the complex biology of glioma cells and their interaction with the tumour micro-environment is essential for identification of molecular and signalling targets that can be exploited for therapeutic purposes. Gliomas differ from other tumour types in aetiology and localisation in the lipid-rich tissue of the brain. Glioma cells are unusually resistant to apoptotic cell death, and a central question in glioma treatment is the identity of the survival/death decision points and signals amenable to therapy. In this context, recent studies in glioma have identified signalling pathways that mediate stress responses in glioma cells [2], and stem cell and metabolic studies have shed light on glioma development [3, 4].

This review will evaluate recent signalling pathways of stress signalling and membrane responses [4–29] identifying and characterising their temporal activation and compartmentalisation [4–15, 17–19, 21–24, 26–36] and supporting a role for lipid signalling in glioma cell death [2, 11, 13, 16, 18–21, 33, 37–45]. Current questions concerning cell death decision points will be raised, together with glioma network responses. Earlier events in triggering cell death signalling will be emphasised, rather than downstream, effector signals (caspase signalling is discussed by Simon Brown and Andrew Wyllie in this edition, [46, 47]). Novel concepts defining mitochondrial stress responses in primary glioma cell preparations and their interaction with brain endothelial cells will be presented. It is proposed that advances in therapy will come from understanding glioma signalling in systems which more closely resemble in vivo networks of interconnected cells. Such advances will depend on imaging, glioma stem cell analysis and identification of signal transduction elements leading to glioma cell death. Glioma mitochondrial and lipid metabolism are reviewed by Whittle and Colquhoun in this edition [2, 48] and brain endothelial cell function by Rizzo [49].

Cell Death Signalling in Glioma Pathogenesis

Mitochondrial and lipid metabolics have led to important insights in biology: the chemiosmotic analysis of oxidative phosphorylation [50] demonstrated that links between ion flux and cell signalling extended beyond the central nervous system (CNS). Advances in calcium signalling linked ion flux to phospholipase and membrane metabolism [18, 32, 51–53]. The concept of cell death signalling, initiated by Wyllie and colleagues [47, 54] was developed in seminal studies linking lipid signalling to CNS pathophysiology [30, 55].

Death Signals and Signalling Pathways: Intrinsic and Oxidative Systems In glioma, a range of environmental and pathological signals trigger cell death pathways [Table 1]. Such signals include stress signals, signalling to the intrinsic system, and intracellular reactive oxygen intermediate formation [2, 4, 11, 12, 14, 18, 19, 21, 22, 24, 27, 40], and we have proposed that lipid signalling networks are important in glioma pathophysiology [18, 41, 42, 45]. Salient questions in glioma signalling relate to the complex cellular interactions and signals within glioma tissue, which determine prognostic features for poor outcome. These prognostic properties include glioma proliferation, invasion, vascularisation, necrosis and resistance to radiation and chemotherapy (Fig. 1).

An important question is the extent to which hypoxia in glioma may cause selection of cells resistant to oxidative stress [4, 10, 12]. Signalling elements in oxidative stress in C6 glioma cells include transient activation of transcription factor nuclear factor kappa B and increased c-Jun N-terminal kinase and p38 kinase activity [24]. The intrinsic mitochondrial pathway may play a role in signalling oxidative cell death in rat C6 glioma cells, via the mitochondrial permeability transition pore and depolarisation of the mitochondrial transmembrane potential ($\Delta\Psi m$) [5, 56]. Also, mitochondrial depolarisation, ERK activation and cell death associated with H_2O_2 treatment of the human glioma cell line A172 was inhibitable using catalase and MEK-1/2 inhibitor [22], and we observed mitochondrial depolarisation in response to oxidative stimuli in primary human glioma cell preparations (Fig. 2). Additionally, oxidative stress and reactive oxygen intermediates may act as mediators in radiotherapy [57].

Death Signals and Signalling Pathways: Extrinsic and Facilitative Signals In addition to intrinsic mitochondrial and endoplasmic reticulum (ER) pathways of cell death, extrinsic cell death signalling and sphingolipid signalling have been identified in glioma: TNF-alpha stimulates sphingolipid release and apoptosis [23], and ceramide and TNF-related apoptosis-inducing ligand

Table 1 Pathophysiological stimuli associated with polyunsaturated fatty acid signalling in glioma multiple pathways of cell death are involved, including intrinsic, extrinsic, endoplasmic reticulum (ER) and extrinsic cytokine-associated signalling pathways

Ischaemia, hypoxia,	Proliferation, roi	4, 12, 29
Oxidative stress	NFκB, c-JNK, p38, PARP, ΔΨm	21, 24, 58
	ERK, H_2O_2, Akt, PTEN	2, 11, 22, 49, 56
	Cardiolipin	16, 44, 48
Irradiation	ROS, Bcl-XL, JNK1/2/3	39, 41, 46, 58
ER stress signals	GRP78, ubiquitin, JNK	5, 14, 28
Cytokines	Rodent brain, astrocytes, microglia	9, 36
[TNF, IL-1β,TRAIL]	COX-2, iNOS	13, 23, 65
TLR	Dendritic cell migration	8
PGE2	proliferation, PKA II	33, 34
PUFA	roi, lipoperoxidation	11, 38, 18–20, 29, 41–45
	Glioma regression, MTP, Akt	2, 40
GF, PDGF, melatonin	Progression, PLC-ϒ1	26, 59, 62
HGF, VEGFR	Adhesion, migration, AA, LO	26, 35, 61, 64
PBR	Intrinsic, ΔΨm	60, 62
Heavy metals	Intrinsic, roi, ER	27, 28
Cisplatin	Plase A2	58

(TRAIL) mediate apoptosis in invasive glioma cell preparations [7, 9, 13]. Other mediators which affect invasiveness and proliferative capacity are also associated with lipid signalling: myelin-associated glycoprotein in malignant gliomas contains phophatidylinositol specific phospholipase C, which increases migration and adhesion [50], PGE2 affects proliferation, oncogene expression and COX-2 and microsomal prostaglandin E synthetase-1 regulate migration, growth and apoptosis in human glioma cells [33]. In

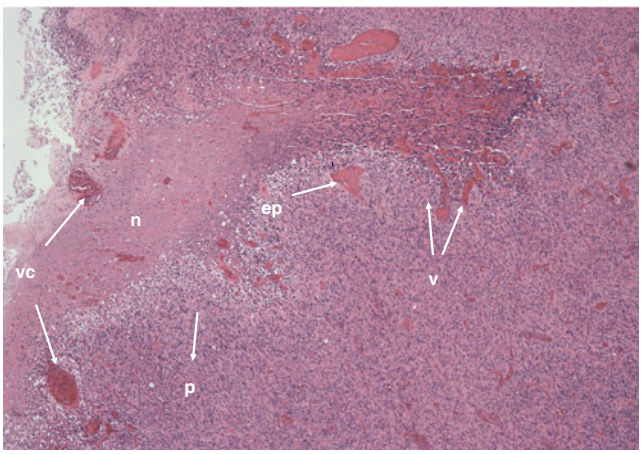

Fig. 1 Signalling of glioma and endothelial cell death and proliferation H&E section through malignant glioma, indicating histological evidence of differences in micro-environment associated with glial and endothelial cell proliferation, differentiation and death. Areas of necrosis (*n*) and intravascular coagulation (*vc*) in the *upper left* portion, border regions with rapid glial cell proliferation (*p*), detected using proliferative markers and evidence of mitosis, endothelial cell proliferation (*ep*), and increased vascularisation (*v*). Regional intratumoural hypoxia and ischemia may select cell clones resistant to hypoxia and with different responses to stress signals

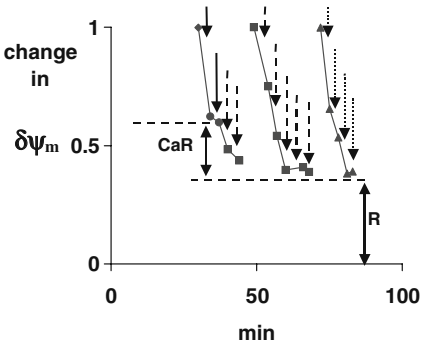

Fig. 2 Effect of calcium ionophore A23187, H_2O_2 and GLA on Δψm in primary glioma cell preparations. Typical experiment in an oligodendroglioma (WHO grade III) glioma cell preparation. Primary human glioma cell preparations were heterogeneous in morphology and in mitochondrial membrane polarisation (Δψm), using JC-1 fluorescence microscopy. The change in Δψm was analysed after exposure to stress stimuli, four to five principal patterns of basal Δψm were detected. The predominant cell types with polarised mitochondria were the large-/medium-sized glial and astroglial cell populations, which varied in different glioma preparations and WHO grades, and which responded to stress stimuli by decreases in mitochondrial polarity. Depolarisation of mitochondria in previously polarised cells was determined by counting 100 cells, whose mitochondria were predominantly *red* (polarised) or *green* (depolarised). The proportion of cells changing from polarised to depolarised is shown on the *y* axis, and the time after loading probe into glioma cells on the *x* axis: Increasing agonist concentrations were added to three samples of glioma cell preparations. Basal Δψm was determined, followed by Δψm in the presence of calcium ionophore A23187, 0.4 and 1.4 μM (*circles*, *solid arrows*) and H_2O_2 (*squares*, *dashed arrows*, 2.5 and 27.5 μM) was then added to the ionophore-treated preparation and further depolarisation, which had been Ca ionophore-resistant (CaR) was detected; H_2O_2 (0.1, 0.35, 14 and 29 μM) or GLA (*triangles*, *dotted arrows*, 10, 34, 133 and 378 μM) were added to two further glioma cell preparations. A residual, peroxidising agonist insensitive cell population (R) was detected in glioma cell preparations, as predicted by the Graeber hypothesis. WHO III and IV tumour mitochondria were more resistant to stress stimuli than WHO II tumours

glioma, it is also necessary to consider cerebral vascular and tumour-associated endothelial cell signalling [26, 47]. Also, gliomas are often located close to crucial neuronal networks which are highly sensitive to neurodegenerative and stress stimuli [5, 7].

Polyunsaturated Fatty Acid Signalling in Glioma Cell Death

Pathophysiological Signals Associated with Polyunsaturated Fatty Acids In glioma, polyunsaturated fatty acid (PUFA) signalling is linked to many pathophysiological stimuli associated with cell death signalling (Table 1). Stimuli associated with PUFA release include ischaemia [29], irradiation [39, 41], heavy metal ions [27, 28, 58] and specific cytokines, including TNF-alpha and TRAIL ligands [9, 23]. Previous work from our laboratory supported a pivotal role of PUFA in glioma cell death. It was proposed that PUFA, as locally released and locally active mediators, may act as network mediators in the pathophysiology of cell death in human primary brain tumours [18]. Therefore, identifying signalling activated early in glioma pathogenesis could provide a more selective and less toxic approach, using PUFA and agents targeting PUFA signalling as pharmacological agents. However, PUFA also plays an important role in neuronal signalling: A close connection between arachidonic acid (AA) and ion flux has been identified, both in recently characterised transient receptor potential and store-operated calcium entry channels and in classical ion channels associated with long-term potentiation and photoreception [32]. This may limit the use of intravenous and dietary agents that affect non-tumour PUFA metabolism.

Exogenous Polyunsaturated Fatty Acids and Glioma Cell Death Signalling In human glioma cell preparations, exogenous PUFA increased development of apoptosis and necrosis [41, 45], and this was also observed in rat and human cell lines [11, 37]. Moreover, in five in vivo and in vitro models of glioma, PUFA [AA and gamma linolenic acid (GLA)] elicited glioma regression and cell death when applied intratumorally at appropriate concentrations [41]. These data suggested the potential to modulate death signalling pathways in glioma cells via alteration of membrane-associated or intracellular content of PUFA. Evidence for selective glioma targeting was obtained, comparing normal brain tissue with tumour, and PUFA signalling pathways via reactive oxygen intermediate (roi) signalling, including changes in basal, compared with PUFA-stimulated roi; increased roi response at higher tumour grade; comparison of normal brain tissue and tumour roi response [11, 19, 41]. The kinetics of intracellular roi generation measured in these studies indicated the balance of anti-oxidant status and the rate of pro-oxidant release within glioma cells. Other studies involving GLA effects on ATP depletion [11], modulation of mitochondrial hexokinase and palmitoyltansferase I and mitochondrial ultrastructure suggested that PUFA had specific effects on tumour mitochondria [2]. Glioma mitochondrial lipid abnormalities may be associated with increased susceptibility to lipoperoxidation and apoptosis [16, 38, 39]. However, both dose and localisation are crucial, as tumour proliferation has been reported at lower doses, and PUFA are involved in normal brain function [25, 41].

Endogenous PUFA and PUFA Metabolites in Glioma Pathogenesis Indirect PUFA involvement in glioma signalling was detected in phospholipase C and growth factor signalling. AA and other PUFA may mediate homeostatic signalling, affecting proliferation, migration and tumour growth [26, 59] via cycloxygenase and PGE2 pathways [33]. There is evidence of PUFA release in proinflammatory signalling [9, 23]. In glioma-derived microvascular endothelial cells, there was also evidence of lipoxygenase stimulating the formation of tubule-like structures [60].

Stress Signalling in Glioma Pathogenesis and Cell Death

The aggressiveness of human glial brain tumours is correlated with the level of tissue hypoxia [4, 12]. These areas of hypoxia were associated with necrosis, which is diagnostic of tumour malignancy, and it has been proposed that extreme anoxic conditions favour the development of cells with heightened resistance to cell death signals [10].

Glioma Stress Signalling via the Intrinsic Pathway of Cell Death Glioma stress signalling has been studied in mitochondria of human primary glioma cell preparations and in primary brain endothelial cells. A typical experiment, in which stress signals were added to glioma cells, is shown in Fig. 1. Glioma cells were exposed to calcium ionophore, hydrogen peroxide (H_2O_2) or GLA. Basal $\Delta\psi m$ and stimulated $\Delta\psi m$ were determined by fluorescence microscopy. Increasing concentrations of stress agonists being added until maximal depolarisation was achieved. Stress signals elicited immediate, dose-dependent depolarisation of glial mitochondria with differing efficacy: calcium ionophore A23187 and elicited partial (20–35%) depolarisation, compared with greater depolarisation in response to H_2O_2 and GLA. A residual, calcium ionophore-insensitive,

H_2O_2-sensitive cell population was detected. Additionally, a residual resistant cell population with high $\Delta\psi m$ was detected after maximal stimulation with oxidative stress stimuli, consistent with the Graeber hypothesis [10]. Also, over longer time periods (4–48 h), other stress signals, such as serum deprivation and anoxia-elicited decreases in glioma cell $\Delta\psi m$.

Glioma and Endothelial Cell Stress Signalling via the Intrinsic Pathway Different responses of brain endothelial cells and other glioma cell types suggest that it is necessary to study signalling in multicellular systems. The stress response of primary glioma and endothelial cells to H_2O_2 resembled that of PUFA, indicating potentially similar roi-associated mechanisms, which may be important in cell death signalling, see also Colquhoun, this edition, [2]. However, the sensitivity of brain endothelial cells to peroxidising agents was higher than that of glial cells. Co-culture of glial and endothelial cells resulted in morphological evidence of cell–cell communication and amplification of the primary stress response.

Stress Signal Transduction in Glioma Stress signalling mediators and systems in glioma are being identified and characterised: H_2O_2-induced apoptosis is mediated by activation of ERK, via growth factor receptor/Ras/Raf/MEK signalling upstream of mitochondrial signalling in human primary glioma cells [22]. Downstream, ER stress activates JNK, ASK, the survival regulator GRP78/BiP, pro-apoptotic factor CHOP/GADD153, ATF6, IRE1α and PERK/eIF2α [14, 17]. Furthermore, peroxidative signal transduction in glioma involves PARP as well as lipid peroxides [21], and many stress signals involving intrinsic mitochondrial pathways have been identified: in rat cortical synaptosomes, lipid peroxidation affected mitochondrial function [15] and neuronal calcium dependent AP-1, caspase and then JNK [5].

Therapeutic Options Associated with Stress Signalling The evidence for roi involvement in cancer and degenerative disease has provoked much debate about the potential therapeutic role of roi in cancer therapy [25]. Many experimental strategies have been developed, but these have not yet translated into therapies: Glioma cells have receptors, such as the peripheral benzodiazepine receptor, closely coupled to mitochondrial intrinsic signalling [60]. Mitochondrial targeting involving gene transfer and stimulation of oxidative metabolism has resulted in glioma regression in vivo and in vitro in a dog model [56]. Networks of interacting pathways and signals are crucial to cell death signalling in glioma; for example, a combination of calcium and ubiquitin signalling was more effective than either pathway alone [14].

Cell Networks: The Role of Functional Plasticity

Understanding the networks regulating cell death is a central problem in glioma pathophysiology. In primary human glioma cell preparations, waves of apoptosis were detected [45], suggesting coordinated pro-apoptotic signals in glioma cells, and in infused C6 implantation gliomas undergoing GLA-induced regression in vivo, apoptosis and necrosis were largely confined to the tumour and did not pass into the surrounding normal tissue [41]. This confirmed in vitro studies and human infusion data, which indicated higher sensitivity of glioma than normal cells to PUFA [19, 38, 40, 42]. There is evidence that coordinated cell–cell signalling is important in glioma cell death, e.g., enhanced immune recognition, involving both increased dendritic cell infiltration and tumour recognition [8]. Additionally, recent advances in stem cell analysis, coupled to in situ imaging, have provided important information on glioma development and neovascularisation.

The Impact of Stem Cell Analysis on Glioma Aetiology and Development Questions about glioma aetiology and development have been investigated using stem cell and genetic analysis [3, 55]. Glioblastomas contain transformed, multipotent, tumour-initiating cancer stem cells, which have radically changed our perspective of glioma pathology. These studies indicate that glioma cells may develop from transformed neural stem cells, but may develop from different precursor types, thus multiple triggers and selection pressures contribute to glioma development. The discovery that new neurones and glia are produced throughout life opens up new approaches to determining the origin and function of CNS tumours. The large astroglial cells expressing GFAP, which responded to stress signals by changes in roi and $\Delta\Psi m$ [19, 20] and Fig. 1, were found to proliferate in vitro and have been proposed as a candidate potential adult neural stem cell [3]. The functional character of these cells includes migratory activity and endothlial cell association when stimulated with appropriate growth factors and microenvironments [26, 33]. New suspension culture approaches [3, 37, 61] have facilitated the selection of these undifferentiated (or de-differentiated) glioma cell populations.

The Impact of Stem Cell Analysis on Glioma-Associated Endothelial Cell Function Microvascular proliferation and dysregulated endothelial cell development is characteristic of high-grade glioma. Endothelial cell signalling is discussed in more detail by Rizzo in this edition [49]. Stem cell research has indicated that the functional capacities of tumour-associated endothelial cells differ from normal endothelial cells in exhibiting greater differentiation capacity [62]. It has been proposed that glioma cells produce

diffusible growth factors that influence endothelial cell growth and neovascularisation [65] and brain endothelial cell migration [26]. Also, glioma-derived microvascular endothelial cells exhibited higher VEGFR and formed intercellular junctions and tubule-like structures [64]. These studies show increased structural connectivity in co-culture. This is reflected in functional evidence of connectivity, for example, in up-regulation of stress responses.

Current Questions in Cell Death Signalling in Glioma

Current knowledge of glioma pathogenesis suggests that diverse pathways and networks are involved in glioma cell death (Table 1). Attention has recently focused on the adaptive processes that gliomas use to evade cell death, with increased understanding of death checkpoints and with stem cell studies defining the functional plasticity of glioma cell interactions. Signalling processes, including ischemic and stress signalling, necrosis and intrinsic and extrinsic pathways, ER and cytokine-associated pathways, all input into critical cell death decision points. These pathways have provided fruitful avenues to investigate novel therapeutic strategies, and the increased sensitivity of mass spectroscopy and imaging techniques has improved our ability to identify transient signalling molecules. But, the cell biology of glioma is still only partially understood, and therapy is less advanced than in many other neuronal pathologies and systemic tumour types, and many basic questions remain to be answered. Some salient questions in glioma signalling relate to glioma cell proliferation and necrosis and endothelial cell dysfunction (Table 2). But, how these signalling mechanisms translate to the prognostic features for glioma malignancy and poor outcome remains a challenge.

Oxidative and Stress Signals and Mitochondria Evidence is accumulating for oxidative and stress signalling pathways as important components in triggering cell death in glioma (Table 2: question 1). Current research in mitochondrial cell death signalling, see Colquhoun [2] and Whittle [48], this edition, and the role of oxidative stress in selecting malignant glioma cells [4, 10] leads to the question as to whether intrinsic signalling and glioma sensitivity to PUFA may lead to useful therapeutic approaches [14, 16, 18, 44] (Table 2: questions 2 and 3); however, anti-angiogenic clinical trials in glioma [1, 25, 66] have achieved limited success and have emphasised the importance of understanding glioma cells and tumour-associated vascular endothelial cells in the context of their signalling networks.

Signalling Networks and Stem Cell Analysis Networks of interacting cells, including vascular endothelial and microglial signalling, provide survival and death signals affecting glioma fate [9, 36, 63]. The role of endothelial cell signalling in glioma pathogenesis is an important area of glioma research (Table 2, question 4). It is unclear whether disorders of endothelial growth and vascularisation are largely reactive to changed growth factor signalling in glial cells or whether endothelial cell signals are necessary in pathogenesis [64–66]. However, in brain endothelial cell studies, novel culture and imaging techniques, together with in vivo imaging are beginning to elucidate transcellular system signalling systems influencing glioma function [2, 3, 36, 63]. These approaches are also useful in determining the role of microglia and the local immune response (Table 2, question 5), where increasing immunogenicity and immune recognition may be important in therapy [8, 9, 36, 66]. Recent stem cell studies have given important information on functional plasticity and signalling networks in glioma (Table 2, question 6). The identification of multipotent glioma stem cells [3], characterised by capacity for self-renewal, multilineage differentiation and changed sensitivity to irradiation and chemotherapy [55], has focussed attention on microenvironmental factors that sustain glioma development and growth.

In conclusion, recent advances in lipid signalling have identified mediators of cell death in glioma, the intrinsic mitochondrial stress response and extrinsic adaptive responses. Effective cell death signalling often involves multiple interacting signal transduction pathways and networks of cells and signals, even when a single anticancer agent, such as PUFA or radiation is used. The brain tumour stem cell hypothesis provides a powerful model, which may

Table 2 Current questions in cell death signalling in glioma

1. Is oxidative stress involved in selecting malignant glioma cells [4, 10, 12]?
2. Is glioma tissue PUFA deficient in specific signalling locations [2, 7, 16, 38]?
3. Is glial cell sensitivity to roi/PUFA/stress signals a potential therapeutic option [11, 18–20, 24, 25, 39, 41–45]?
4. Is neovascularisation necessary for glioma survival [62, 63] and is anti-angiogenic therapy effective in glioma [1, 49, 66]?
5. What is the role of microglia and the resident inflammatory response in glioma cell death signalling and therapy [8, 9, 36, 46, 67]?
6. Will stem cell and epigenetic approaches identify cell death decision disorders in glioma [3, 66, 67]?

elucidate basic processes of glioma development. But, equally important is the identification and characterisation of interacting signals capable of promoting specific and selective glioma cell death.

Acknowledgements We are very grateful to Amarin Neurosciences and to the Chest, Heart and Stroke Association Scotland for financial support, to Simon Brown (Inflammation Research, University of Edinburgh) for cell imaging facilities and to Ujval Anilkumar, Svitlana Loughridge, Viera Muzola, Sarah Bott and Claire Machin for laboratory project work and to Colin Smith (Pathology, University of Edinburgh) for providing neuro-pathology information.

References

1. Anderson E, Grant R, Lewis SC, Whittle IR (2008) Randomised phase III controlled trials of therapy in malignant glioma: where are we after 40 years? Br J Neurosurg 22:339–349
2. Colquhoun A (2010) Lipids, mitochondria and cell death: implications in neuro-oncology. Mol Neurobiol special edition Cell Death Signalling. doi:10.1007/s12035-010-8134-4
3. Piccirillo SGM, Combi R, Cajola L, Patrizi A, Redaelli S, Bentivegna A, Baronchelli S, Maira G, Pollo B, Mangiola A, DiMeco F, Dalprà L, Vescovi AL (2009) Distinct pools of cancer stem-like cells coexist within human glioblastomas and display different tumorigenicity and independent genomic evolution. Oncogene 28:1807–1811
4. Evans SM, Judy KD, Dunphy I, Jenkins WT, Hwang WT, Nelson PT, Lustig RA, Jenkins WT, Hwang WT, Nelson PT, Lustig RA, Jenkins K, Magarelli DP, Hahn SM, Collins RA, Grady MS, Koch CJ (2004) Hypoxia is important in the biology and aggression of human glial brain tumours. Clin Cancer Res 10:8177–8184
5. Camandola S, Poli G, Mattson MP (2000) Lipid peroxidation product, 4-hydroxy NE, increases AP-1 binding through caspase activity in neurones. J Neurochem 74:159–165
6. Colquhoun A (2002) GLA alters the composition of mitochondrial subfractions, decreases outer mitochondrial binding of hexokinase and alters carnitine palitoyltransferase I properties in Walker 256 rat tumour. Biochim Biophys Acta 1583:74–84
7. Coyle JT, Puttfarken P (1993) Oxidative stress, glutamate and neurodegenerative disorders. Science 262:689–695
8. Curtin JF, Liu N, Candolfi M, Xiong W, Assi H, Yagiz K, Edwards MR, Michelsen KS, Kroeger KM, Liu C, Muhammad AKMG, Clark MC, Arditi M, Comin-Anduix B, Ribas A, Lowenstein PR, Castro MG (2009) HMGB1 mediates endogenous TLR2 activation and brain tumour regression. PLoS Med 6:e-1000010
9. Falsig J, Markus L, Leist M (2004) Defined inflammatory states in astrocyte cultures: correlation with susceptibility towards CD95-driven apoptosis. J Neurochem 88:181–193
10. Graeber TG, Osmanian C, Jacks T, Houseman DE, Koch CJ, Lowe SW, Giaccia AJ (1996) Hypoxia-mediated selection of cells with diminished apoptotic potential in solid tumours. Nature; 379:88–91
11. Higuchi Y, Yoshimoto T (2002) AA converts glutathione depletion into necrosis by promoting lipid peroxidation in rat glioma cells. Arch Biochem Biophys 400:133–140
12. Jenkins K, Mararelli DP, Hahn SM, Collins RA, Grady MS, Koch CJ (2004) Hypoxia is important in the biology and aggression of human glial brain tumours. Clin Cancer Res 10:8177–8184
13. Jeremias I, Steiner HH, Benner A, Debatin KM, Herold-Mende C (2004) Cell death induction by betulinic acid, ceramide and TRAIL in primary glioblastoma multiforme cells. Acta Neurochir 146:721–729
14. Kardosh A, Golden EB, Pyrko P, Uddin J, Hofman FM, Chen TC, Louie SG, Petasis NA, Schönthal AH (2008) Aggravated endoplasmic reticulum stress as a basis for enhanced glioblastoma cell killing by bortezomib in combination with celecoxib or its non-coxib analogue, 2,5-dimethyl–celecoxib. Cancer Res 68:843–851
15. Keller JN, Mark RJ, Bruce AJ, Blanc E, Rothstein JD, Uchida K, Waeg G, Mattson MP (1997) 4-Hydoxynonetal, an aldehydic product of lipid peroxidation, impairs glutamate transport and mitochondrial function in synaptosomes. Neuroscience 80:685–696
16. Kiebish MA, Han X, Cheng H, Chuang JH, Seyfried TN. Brain mitochondrial lipid abnormalities in mice susceptible to spontaneous glioma. Lipids;43:951–959
17. Koritzinsky M, Magagnin MG, van den Beucken T, Seigneuric R, Savelkouls K, Dostie J, Pyronnet S, Kaufman RJ, Weppler SA, Voncken JW, Lambin P, Koumenis C, Sonenberg N, Bradly G, Wouters BG (2006) Gene expression during acute and prolonged hypoxia is regulated by distinct mechanisms of translational control. EMBO J 25:1114–1125
18. Leaver HA, Williams JR, CraiG SR, Gregor A, Ironside JW, Whittle IR, Yap PL (1997) Network analysis of arachidonic acid pathophysiology in human phagocytes and primary brain tumours. Ann NY Acad Sci 832:200–214
19. Leaver HA, Williams JR, Gregor A, Ironside JW, Miller EP, Su BH, Prescott RJ, Whittle IR (1999) Dynamics of reactive oxygen intermediate production by human brain tumours: n-6 essential fatty acid effects. Eur J Clin Invest 29:220–231
20. Leaver HA, Williams JR, Smith C, Whittle IR (2004) Intracellular oxidation by human glioma cell populations: effect of arachidonic acid. Prostagl Leukot Essent Fatty Acids 70:449–453
21. Lee YW, Ha MS, Kim YK (2001) H_2O_2-induced cell death in human glioma cells: role of lipid peroxidation and PARP activation. Neurochem Res 26:337–342
22. Lee WC, Choi CH, Cha SH, Oh HY, Kim YK (2005) Role of ERK in hydrogen peroxide induced cell death in human glioma cells. Neurochem Res 30:263–270
23. Malagarie-Canenave S, Andrie-Abadie N, Segul B, Gouaze V, Tardy C, Cuvillier O, Levade T (2002) Sphingolipid signalling: molecular basis and role in TNFα signalling. Rev Mol Med 4:1–15
24. Marangolo M, McGee MM, Tipton KF, Williams DC, Zisterer DM (2009) Oxidative stress induces apoptosis in C6 glioma cells: involvement of MAP kinases and NfκB. Neurotox Res 3:397–409
25. Renschler MF (2004) The emerging role of reactive oxygen species in cancer therapy. Eur J Cancer 40:1934–1940
26. Rush GF, Khan S, Bamisaiye G, Bidwell P, Leaver HA, Rizzo MT (2007) c-jun amin-terminal kinase and mitogen activated protein kinase mediate hepatocyte growth factor-induced migration of brain endothelial cells. Exper Cell Res 313:121–132
27. Watjen W, Beyersman D (2004) Cadmium induced apoptosis in C6 glioma cells: influence of oxidative stress. Biometals 17:65–78
28. Wu Y, Zhang H, Dong Y, Park YM, Ip C (2005) Endoplasmic reticulum stress signal mediators are targets of selenium action. Cancer Res 65:9073–9079
29. Yoshida S, Busto R, Watson BD, Santiso M, Ginsberg MD (2006) Postischemic cerebral lipid peroxidation in vitro. J Neurochem 44:1593–1601
30. Bazán NG (1976) Free arachidonic acid and other lipids in the nervous system during early ischemia and after electroshock. Adv Exp Med Biol 72:317–335
31. Lukiw WJ, Bazan NG (2010) Inflammatory, apoptotic and survival gene signalling in Alzheimer's disease Mol Neurobiol special edition: Cell Death. doi:10.1007/s12035-010-8126-4

32. Meves H (2008) Arachidonic acid and ion channels: an update. Br J Pharmacol 155:4–16
33. Payner T, Leaver HA, Knapp B, Whittle IR, Trifan OC, Miller S, Rizzo MT (2006) Microsomal prostaglandin E synthase-1 regulates human glioma cell growth via prostaglandin E2-dependent activation of type II protein kinase A. Mol Cancer Ther 5:1817–1826
34. Rizzo MT, Pudlo N, Farrell L, Leaver HA (2002) Specificity of arachidonic acid-induced inhibition of growth and activation of c-jun kinases and p38 mitogen-activated protein kinase in hemaptopietic cells. Prostagl, Leucotr Essen Fatty Acids 66:31–40
35. Rizzo MT, Leaver HA, Yu WM, Kovacs RJ (1999) Arachidonic acid induces mobilization of calcium stores and c-jun gene expression: evidence that intracellular calcium release is associated with c-jun. Prostagl Leucotr Essen Fatty Acids 60:187–198
36. Wake H, Moorhouse AJ, Jinno S, Kohsaka S, Nabekura J (2009) Resting microglia directly monitor functional state of synapses in vivo and determine the fate of ischemic terminals. J Neurosci 29:3974–3980
37. Bell HS, Wharton SB, Leaver HA, Whittle IR (1999) Effects of n-6 essential fatty acids on glioma invasion and growth: experimental studies using human and rodent glioma spheroids in collagen gels. J Neurosurg 91:989–998
38. Chan PH, Fishman RA (1982) Alterations in membrane integrity and cellular constituents in neuroblastoma and glioma cells. Brain Res 248:151–157
39. Vartak S, Robbins MEC, Spector AA (1997) Polyunsaturated fatty acids increase the sensitivity of 36B10 rat astrocytoma cells to radiation-induced cell kill. Lipids 32:283–292
40. Das UN (2004) From the bench to the clinic: GLA therapy of human glioma. Prostaglandins Leukot Essent Fatty Acids 70:539–552
41. Leaver HA, Bell HS, Rizzo MT, Ironside JW, Gregor A, Wharton S, Whittle IR (2002) Anti-tumour and pro-apoptotic actions of highly unsaturated fatty acids in glioma. Prostaglandins, Leucot Essent Fatty Acids 66:19–29
42. Leaver HA, Wharton S, Bell HS, Whittle IR (2002) Highly unsaturated fatty acid induced tumour regression in experimental glioma: pharmacodynamics and bioavailability of a novel anti-tumour agent, gamma linolenic acid, in an implantation glioma model: effects on tumour biomass, apoptosis and normal neuronal tissue. Prostagl, Leucotr Essen Fatty Acids 67:283–292
43. Llado V, Gutierrez A, Martinez J, Casas J, Teres S, Higuera M, Galmes A, Saus C, Busquets X, Escriba PV (2008) Minerval induces apoptosis in jurkat and other cancer cells. J Cell Mol Med "postprint"; doi:10.1111/j.1582-4934.2008.00625.x
44. Serini S, Piccioni E, Merendino N, Calviello G (2009) Dietary polyunsaturated fatty acids as inducers of apoptosis: implications for cancer. Apoptosis 14:135–152
45. Williams JR, Leaver HA, Ironside JW, Gregor A, Miller EP, Whittle IR (1998) Apoptosis in human gliomas: actions of arachidonic acid. Prostagl, Leucotr Essen Fatty Acids 58:193–200
46. Zhang J, Shipston MJ, Brown S (2010) A role for potassium permeability in the recognition, clearance and anti-inflammatory effects of apoptotic cells. Mol Neurobiol: special edition Cell Death Signalling. doi:10.1007/s12035-010-8127-3
47. Wyllie AH (2010) Where, O Death, Is Thy Sting? A brief review of apoptosis biology, Molecular Neurobiology special edition Cell Death Signalling. doi:10.1007/s12035-010-8125-5
48. Ordys BB, Launay S, Deighton RF, McCulloch J, Whittle IR (2010) The role of Mitochondria in Glioma pathophysiology. Mol Neurobiol special edition. Cell Death Signalling. doi:10.1007/s12035-010-8133-5
49. Rizzo MT, Leaver A (2010) Brain endothelial cell death: modes, signalling, pathways and relevance to neural development, homeostasis and disease. Mol Neurobiol special edition Cell Death Signalling. doi:10.1007/s12035-010-8132-6
50. Mitchell P (1961) Coupling of phosphorylation to electron and hydrogen transfer by a chemi-osmotic type of mechanism. Nature 191:144–148
51. Michell RH (1975) Inositol lipids and cell surface receptor function. Bichem Biophys Acta 415:81–147
52. Jacobson J, Duchen MR (2002) Mitochondrial oxidative stress and cell death in astrocytes—requirement for stored Ca2+ and sustained opening of the permeability pore. J Cell Sci 115:1175–1188
53. Parnas M, Katz B, Lev S, Tzarfaty V, Dadon D, Shaag G, Metzner H, Yaka R, Minke B (2009) Membrane lipid modulations remove divalent open channel block from TRP-like and NMDA channels. J Neurosci 29:2371–2383
54. Kerr JFR, Wyllie AH, Currie AR (1972) Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics. Br J Cancer 26:239–257
55. Chang CJ, Hsu CC, Yung MC, Chen KY, Tzao C, Wu WF, Chou HY, Lee YY, Lu KH, Chiou SH, Ma HI (2009) Enhanced radiosensitivity and radiation-induced apoptosis in glioma CD133-positive cells by knockdown of SirT1 expression. Biochem Biophys Res Comm 380:236–242
56. Garcia-Escudero V, Gargini R, Izquierdo M (2008) Glioma regression in vitro and in vivo by a suicide combined treatment. Mol Canc Res 6:407–417
57. Horrobin DF (1979) Schizophrenia: reconciliation of the dopamine, prostaglandin, and opioid concepts and the role of the pineal. Lancet 313(8115):529–531
58. Yacoub A, Gupta P, Park MA, Rhamani M, Hamed H, Hanna D, Zhang G, Sarkar D, Lebedeva IV, Emdad L, Koumenis C, Curiel DT, Grant S, Fisher PB, Dent P (2008) Regulation of GST-MDA-7 toxicity in human glioblastoma cells by ERBB1, ERK1/2, PI3K, and JNK1-3 pathway signaling. Mol Cancer Ther 7:314–329
59. Yacoub A, Mitchell C, Hong Y, Gopalkrishnan RV, Su ZZ, Gupta P, Sauane M, Lebedeva IV, Curiel DT, Mahasreshti PJ, Rosenfeld MR, Broaddus WC, James CD, Grant S, Fisher PB, Dent P (2004) MDA-7 regulates cell growth and radiosensitivity in vitro of primary (non-established) human glioma cells. Cancer Biol Ther 8:739–751
60. Ma Y, Yuan R, Fan S, Hu C, Goldberg ID, Laterra JJ, Rosen EM (2006) Identification of genes that modulate sensitivity of U373MG glioblastoma cells to *cis*-platinum. Anticancer Drugs 17:733–751
61. Liao H, Duka T, Teng FYH, Sun L, Bu W, Ahmed S, Tang BL, Xiao Z (2004) Nogo-66 and myelin-associated glycoprotein (MAG) inhibit the adhesion and migration of Nogo-66 receptor expressing human glioma cells. J Neurochem 90:1156–1162
62. Chelli B, Lena A, Vanacore R, Pozzo E, Costa B, Rossi L, Salvetti A, Scatena F, Ceruti S, Abbracchio MP, Gremigni V, Martini C (2004) Peripheral benzodiazepine receptor ligands: mitochondrial transmembrane potential depolarisation and apoptosis in rat C6 glioma. Biochem Pharmacol 68:125–134
63. Bell HS, Whittle IR, Walker M, Leaver HA, Wharton SB (2001) Development of necrosis and apoptosis in glioma: experimental findings using spheroid culture systems. Neuropathol Appl Neurobiol 27:291–304
64. Bian XW, Jiang XF, Chen JH, Bai JS, Dai C, Quing-Liang W, Lu JY, Zhao W, Xin R, Liu MY, Shi JQ, Ji MW (2006) Increased angiogenesis capabilities of endothelial cells from microvessels of malignant human glioma. Int Immunopharm 6:90–99
65. Hurst RD, Fritz IB (1996) Properties of an immortalised vascular endothelial/glioma cell co-culture model on the blood–brain barrier. J Cell Physiol 167:81–88
66. Norden AD, Drappatz J, Wen PY (2008) Novel anti-angiogenic therapies for malignant gliomas. Lancet Neurol 7:1152–1160
67. Zhuang W, Qin Z, Liang Z (2009) The role of autophagy in sensitising malignant glioma cells to radiation therapy. Acta Biochim Biophys Sin 41:341–351