Mol Neurobiol (2010) 42:76–88
DOI 10.1007/s12035-010-8134-4

Lipids, Mitochondria and Cell Death: Implications in Neuro-oncology

Alison Colquhoun

Received: 5 April 2010 / Accepted: 5 April 2010 / Published online: 29 April 2010

Abstract Polyunsaturated fatty acids (PUFAs) are known to inhibit cell proliferation of many tumour types both in vitro and in vivo. Their capacity to interfere with cell proliferation has been linked to their induction of reactive oxygen species (ROS) production in tumour tissues leading to cell death through apoptosis. However, the exact mechanisms of action of PUFAs are far from clear, particularly in brain tumours. The loss of bound hexokinase from the mitochondrial voltage-dependent anion channel has been directly related to loss of protection from apoptosis, and PUFAs can induce this loss of bound hexokinase in tumour cells. Tumour cells overexpressing Akt activity, including gliomas, are sensitised to ROS damage by the Akt protein and may be good targets for chemotherapeutic agents, which produce ROS, such as PUFAs. Cardiolipin peroxidation may be an initial event in the release of cytochrome *c* from the mitochondria, and enriching cardiolipin with PUFA acyl chains may lead to increased peroxidation and therefore an increase in apoptosis. A better understanding of the metabolism of fatty acids and eicosanoids in primary brain tumours such as gliomas and their influence on energy balance will be fundamental to the possible targeting of mitochondria in tumour treatment.

Keyword Lipids · Mitochondria · Cell death · Neuro-oncology

A. Colquhoun (✉)
Laboratory of Tumour Cell Metabolism,
Department of Cell and Developmental Biology,
Biomedical Sciences Institute, University of São Paulo,
Avenida Prof. Lineu Prestes, 1524,
CEP 05508-900 São Paulo, SP, Brazil
e-mail: alison@usp.br

Introduction

Gliomas are the most common form of central nervous system tumour, and the high-grade, malignant gliomas (World Health Organization grades III and IV) are notoriously difficult to treat. They exhibit a highly aggressive phenotype with invasive behaviour, which permits rapid spread throughout the brain, although they rarely form metastases [1]. Long-term survival rates for malignant glioma patients have improved very little with the advent of impressive improvements in both brain tumour diagnosis and surgery. Thus, despite many treatment options including surgical debulking, radiotherapy, and more recently chemotherapy, patient prognosis remains dismal and advances in novel treatment strategies are as urgently needed.

Polyunsaturated fatty acids (PUFAs) are known to inhibit cell proliferation of many different tumour types both in vitro and in vivo. Their capacity to interfere with cell proliferation has been linked to their induction of reactive oxygen species (ROS) production in tumour tissues leading to cell death, but the exact mechanisms of action are far from clear, particularly in brain tumours including gliomas. This review provides a brief summary of the effects of PUFAs upon mitochondrial metabolism, cell proliferation and apoptosis and raises questions about the mechanisms by which PUFAs exert their antitumour effects on gliomas in experimental models.

Fatty Acid Effects on Tumour Cell Proliferation

PUFAs have been found to inhibit the cell proliferation of many different tumour types in vitro. The earliest studies appeared in the 1980s and many additional reports have

confirmed and extended this general finding during the past 25 years [2–5]. Initially, research was directed towards tumour cell lines such as breast and colon cancer for which epidemiological data suggested a link between dietary fat intake and cancer incidence. Over time, many other tumour types were found to be sensitive to the inhibitory effects of fatty acids, and relationships were identified among the number of carbon atoms and the number of double bonds present in the fatty acids and their respective tumour inhibitory properties. The position of the double bonds within the fatty acid carbon chain was also found to cause differences in the effects on tumour cell proliferation [5–8]. While the n-3 PUFAs had strong inhibitory effects on tumour cell proliferation, the n-6 PUFA gamma-linolenic acid (GLA) was often found to be one of the most inhibitory and most selective fatty acids in studies comparing tumour cells with normal cells in vitro [5, 9]. The susceptibility of individual fatty acids to peroxidation was also identified as an important factor in their ability to inhibit tumour cell proliferation [4–6, 9–13].

The epidemiological data have made a strong case for an increased risk of cancer development in populations consuming occidental diets rich in saturated fatty acids. In addition to the saturated nature of the majority of the fatty acids, the small quantity of PUFAs present in the diet is typically of the n-6 family. Thus, the relative paucity of dietary n-3 PUFAs versus n-6 PUFAs has been advocated as one of the risk factors for cancer development. Further research has pointed to changes in breast cancer incidence in populations with traditionally high dietary ingestion of n-3 PUFAs when members of the population have moved to other regions of the world and adopted local dietary habits [14–17].

In vivo studies have also shown significant effects of PUFAs upon tumour development and progression in animal models. Many of the animal models have followed (1) tumour development after exposure to carcinogenic stimuli, (2) progression of established tumours or (3) development, progression and eventual metastasis of tumours, each in the presence of different types of PUFA. Early studies in mouse models of tumour growth found that supplementation of an essential fatty acid-deficient diet with the n-6 PUFA linoleic acid (LA) stimulated tumour growth. In contrast, the n-6 PUFA arachidonic acid (AA) had no significant effect on tumour growth rates. Most of the animal studies have classified the n-6 PUFA LA as a stimulator of tumour growth in in vivo models [18–21].

The picture is quite different when animal tumours are exposed to n-3 PUFAs, with most studies finding marked inhibition of tumour growth in the presence of these PUFAs. Initial work in this field tested the effects of n-3 PUFAs in the complex mixtures of PUFAs found in fish oils. Hepatoma growth was significantly reduced when animals were fed diets enriched with fish oil. Similar findings were reported for breast and prostate tumour growth rates [22–28]. In a mammary tumour model in mice dietary supplementation with a fish oil, supplement containing 12% docosahexaenoic acid (DHA) and 18% eicosapentaenoic acid (EPA) also reduced tumour growth. More recent protocols have used highly purified EPA or DHA in models of colon, breast and prostate tumour development after exposure to carcinogenic compounds [14, 25]. In both cases, the n-3 PUFAs significantly reduced the incidence and development of tumours in these models. The importance of fatty acids during metastatic spread has also been highlighted in studies were n-3 PUFAs have been found to decrease the number of metastatic foci in a breast cancer model [26]. In brain tumours, studies comparing dietary fat intake and brain tumour incidence are scarce and have not found convincing epidemiological evidence of a significant association between, for example, gliomas and dietary PUFAs [29].

Effects of Fatty Acids on Mitochondrial Metabolism

The utilisation of fatty acids for energy production via the mitochondrial beta-oxidation pathway depends upon the availability of fatty acyl CoAs within the mitochondrial matrix. The provision of fatty acyl CoAs for beta-oxidation relies on the concerted activity of carnitine palmitoyltransferase I (CPT I), the carnitine-acylcarnitine translocase (CACT) and carnitine palmitoyltransferase II (CPT II) in order to pass from the cytoplasm to the mitochondrial matrix. In tumour cells, fatty acid oxidation rates are often reduced in comparison with the normal tissue of origin, and it is, therefore, often stated in the literature that tumour cells do not oxidise fatty acids. This idea is propagated by the lower quantity of mitochondria often found in tumour versus normal tissue and by the common tendency of tumour cells to utilise glucose as their principal energy source via the glycolytic pathway [30, 31]. However, the evidence for a lack of fatty acid oxidation by tumour cells was scarce, and when CPT activities and fatty acid oxidation rates of various tumour cells were analysed, it became apparent that many different tumour types do in fact retain fatty acid oxidative capacity, which may be utilised during specific periods of tumour growth [32–35]. The tumour cell's ability to oxidise fatty acids was found to vary considerably depending on the fatty acid, which is related to the specificity of CPT I for substrate [35, 36].

With this in mind, studies in the author's laboratory tested the effects of PUFAs on the capacity of tumour cells to oxidise fatty acids and found significant effects of several fatty acids. Both GLA and AA caused approximately 60% inhibition of tumour cell CPT I activity, while

oleic and LA had no effect on CPT I activity. Both GLA and AA also caused approximately 50% inhibition of [1-^{14}C]-palmitate oxidation to $^{14}CO_2$ [37]. These effects were blocked by the inhibition of cyclooxygenase with indomethacin suggesting a role for prostaglandins (PGs) in this effect. Indeed, addition of PGE_1 and PGE_2 to the tumour cells also caused a significant inhibition of both CPT I activity and [1-^{14}C]-palmitate oxidation to $^{14}CO_2$. The alterations in fatty acid oxidation within the tumour cells could potentially cause disturbances in other pathways of fatty acid metabolism including triacylglycerol and cholesterol synthesis, membrane biosynthesis and eicosanoid synthesis amongst others. These changes in fatty acid metabolism could lead to selective inhibition of tumour cell proliferation, and in the cell lines tested, this was found to be the case. In the same study, a potential positive control was used in the form of the colon cancer cell-line HT29 whose proliferation was known to be unaltered by PGE_2 [38]. Supporting the hypothesis that altered CPT I could result in altered proliferation while lack of inhibition would result in unchanged proliferation, the HT29 cells exposed to GLA, AA, PGE_1 or PGE_2 showed no alterations in CPT I activity and no alterations in proliferation [37]. Later reports found that HT29 DNA fragmentation, indicating apoptosis, was unaffected by various PUFAs with the exception of DHA, which was a strong inhibitor of cell proliferation in this cell line [39].

In vivo studies using the Walker 256 carcinosarcoma model in the rat reported that modification of the fatty composition of the diet with n-3 fatty acids caused significant changes in mitochondrial fatty acid composition. The activity of the mitochondrial acyl CoA synthase was also significantly increased in the tumours from the PUFA-rich diet [36, 40]. These changes in mitochondrial metabolism were accompanied by a significant decrease in mitochondrial membrane potential (MMP). Further studies using the same animal model found that GLA also caused a marked increase in mitochondrial acyl CoA synthase activity, and again, MMP was decreased by the PUFA diet [40].

A similar 5.5% GLA diet was subsequently found to cause a 61% inhibition of CPT I activity in the Walker 256 carcinosarcoma, which was accompanied by both a decrease in the malonyl CoA sensitivity of the enzyme and a decrease in its affinity for palmitoyl CoA substrate [36]. The separation of the mitochondria into outer membrane, contact sites and inner membrane fractions provided detailed information on the variations in susceptibility of individual biological membranes to dietary alterations in fatty acid composition. The outer membrane was highly susceptible to dietary influence on fatty acid composition, with the contact sites less so and the inner membrane fraction more refractory to changes enforced by the diet.

Another important change that was found in these treated tumours was the large decrease in mitochondrially bound hexokinase (HK) activity present in the tumours from 67% (control tumour) to 39% (GLA tumour) of the total detectable HK activity [36]. Despite this large change in localisation, the total HK activity was itself unchanged. Tumour cells very often have a large part of their HK I and/or II enzyme bound to the mitochondria, which is believed to improve energy production by placing the first step of glycolysis very close to the mitochondrial source of ATP [41–43]. Such a change in the intracellular localisation of this important enzyme could lead to a situation where intramitochondrial ATP synthesis becomes less efficiently coupled to cytoplasmic glucose-6-phosphate generation. In turn, this reduced efficiency could alter the rate of glycolysis and alter the energy balance within the rapidly proliferating tumour cells. This study provided evidence that fatty acids could influence the capacity of HK to bind to the mitochondrial outer membrane protein voltage dependent-anion channel (VDAC). Long-chain fatty acids have been reported to inhibit heart HK (type I HK), which is also known to bind to mitochondria [44]. It was proposed that high intracellular concentrations of fatty acids may serve as an additional control over HK binding and function in the heart, and this is a possibility that may be extrapolated to tumour HK in some cases where high-intracellular fatty acid concentrations may be achieved. The loss of bound HK from the VDAC has recently been directly related to loss of protection from apoptosis provided by this HK–VDAC interaction [45]. The capacity of HK I and HK II to interact with VDAC points to the role of these proteins in promoting tumour cell survival through binding to VDAC, which in turn inhibits the release of cytochrome *c* from the mitochondrial intermembrane space [42, 43, 46, 47]. These recent reports on HK–VDAC interaction and apoptosis further support the previous data from our laboratory linking loss of HK from mitochondria as having an important role in the subsequent loss of MMP and apoptosis described later in this review [48–52].

The susceptibility of individual fatty acids to peroxidation was previously identified as an important factor in their ability to inhibit tumour cell proliferation [5, 6, 9–12]. Studies in the Walker 256 rat carcinosarcoma cell line and in bladder tumour cells found that GLA or EPA caused significantly increased thiobarbituric acid reactive substances (TBARS) formation, indicative of lipid peroxidation, and also increased oxidation of dihydroethidium, indicative of ROS generation such as superoxide [40, 48]. The superoxide and lipid peroxide production pointed to altered mitochondrial function [53], and when mitochondrial enzyme activities were determined, both NADH cytochrome *c* oxidoreductase (complex I + III) and cytochrome *c* oxidase (complex IV) activities were greatly reduced by the presence

of PUFAs [49]. Together with reduced electron transport capacity, the mitochondrial fraction was seen to lose its cytochrome *c* content in a controlled manner, which did not involve generalised mitochondrial rupture [49]. The loss of cytochrome *c* would directly alter the mitochondrial ability to generate ATP and in addition serves as a stimulus for cell death. The n-6 PUFA AA has been reported to uncouple mitochondrial respiration by the selective inhibition of complex I and complex III and to cause an increase in ROS production by the mitochondria [54].

The reduced mitochondrial function was accompanied by an increase in glucose utilisation and lactate production by the tumour cells [49, 55]. This change in metabolic activity would compensate to some extent the oxidative deficit produced by the presence of PUFAs and was reflected in the reduction of intracellular ATP by between 205 and 27% depending on the PUFA used. Despite metabolic adaptations made by the tumour cells, PUFAs led to reduced MMP suggestive of the opening of the mitochondrial permeability transition (MPT) pore [47, 52, 53]. Oxidative stress was reported to directly influence the occurrence of MPT and cytochrome *c* release [56–58]. It has been proposed that when mitochondrial complex I or II activity is inhibited, cancer cells may compensate with an increased flux through glycolysis to generate sufficient ATP to sustain the apoptotic pathway and cause cell death [59].

The increased acyl CoA found in the PUFA exposed cells may also be relevant to the inhibition of mitochondrial metabolism and the induction of apoptosis, as acyl CoAs are known to inhibit the mitochondrial adenine nucleotide transporter (ANT). This protein is present at contact sites and is one of the components of the MPT pore [47, 52, 53]. It is possible that increased intracellular acyl CoA concentrations in the microenvironment of the mitochondrial membrane, due to increased substrate availability and increased enzyme activity [40, 48], could inhibit the ANT and compromise energy metabolism. While this has not been directly proven, it is an important factor that could drastically alter mitochondrial energy metabolism and organelle integrity [47]. A significant reduction in mitochondrial oxidation would also lead to a reduction in citrate production and efflux from the mitochondria, which would interfere directly with the activity of ATP-citrate lyase and fatty acid synthesis in the rapidly proliferating tumour cells [46, 60].

Interestingly, in HT29 colon, tumour cells LA and AA caused modifications in total phospholipid and mitochondrial cardiolipin fractions, which were accompanied by decreases in MMP [61]. In Morris hepatoma cells, cardiolipin composition was significantly altered by LA, while in Walker 256 cells, GLA and EPA altered cardiolipin composition [49]. EPA also significantly altered the cardiolipin fatty acyl chain composition in bladder tumour cells [55, 62], and in both the Walker 256 cells and bladder tumour cells, MMP was significantly reduced. The peroxidation of cardiolipin has been proposed to be an initial event in the release of cytochrome *c* from the mitochondria, and enriching cardiolipin with PUFA acyl chains may lead to increased peroxidation and will be addressed later in this review [55, 63].

Fatty Acids and Mitochondrial Ultrastructure

Alterations in the fatty acid composition of the diet are known to influence the mitochondrial composition and their metabolism as described in the previous section. These findings raised the question of whether changes in mitochondrial metabolism may be related to actual ultrastructural changes induced by PUFAs. Initial studies found that in the Walker 256 carcinosarcoma model, a high-GLA-containing diet caused an increase in mitochondrial area and volume, while a low-GLA-containing diet caused no difference from a control diet [48]. These studies were extended in order to investigate the relationship between the alterations in mitochondrial metabolism and the organelle ultrastructure in vivo. Particular attention was paid to alterations in ultrastructure previously considered to be related to alterations in intracellular metabolic state, including the quantity of cytoplasmic lipid droplets, the number of mitochondrial matrix granules and the number of mitochondrial membrane contact sites [36]. A strong relationship has been proposed among the number of contact sites and matrix granules and the metabolic activity of the mitochondria [64, 65].

Quantitative biochemical analysis of the incorporation of GLA into various lipid fractions found that while neither phospholipid nor cholesterol fractions were altered in quantity, the triacylglycerol fraction almost doubled in size in the GLA dietary group [36]. Ultrastructural analysis of the lipid droplet fraction confirmed the biochemical data showing a doubling of the number of lipid droplets per total cell area and a doubling of the lipid droplet area per unit cell area. In this study, the mitochondrial area and volume were also increased by GLA as previously found in [48]. However, the number of mitochondria per unit cell area remained unchanged indicating that GLA did not cause mitochondrial proliferation. GLA exposure caused a significant reduction in the number of matrix granules to approximately half of those present in the control tumour. The surface density of mitochondrial cristae was reduced to almost half that of the control tumour, which would in itself reduce the physical space available for membrane insertion of the enzymes involved in the respiratory chain and may also in part explain the reduction in complex I, III and IV activity in PUFA-treated tumour cells [36, 49]. Changes in

cristae structure have been linked to changes in cytochrome *c* localisation within the mitochondria from the cristae to the intermembrane space, which may facilitate cytochrome *c* release during the induction of cell death [47].

This finding was accompanied by a 66% decrease in mitochondrial membrane contact sites in the GLA-exposed tumours [36]. Such a large difference in contact site number may have a dramatic effect on mitochondrial function as many tumours are already depleted of contact sites when compared with their normal tissues of origin [66]. Thus, a further reduction caused by GLA exposure may push the mitochondria to the limit of their capacity to function with insufficient contact sites available for essential metabolic activities. In earlier studies, fatty acids were also linked to a decrease in the formation of contact sites, while essential fatty acid deficiency can produce megamitochondria, proving that fatty acids can significantly alter mitochondrial ultrastructure [64, 67].

The large change in contact site number may be directly related to the significant decrease in CPT I activity previously mentioned, as CPT I activity, at least in rat liver, has been found to have differing kinetic properties when located at the outer mitochondrial membrane or at contact sites [68–70]. Taking into consideration the large decrease in contact sites in the GLA-exposed tumours, most of the CPT I enzyme was relocated to the outer membrane fraction rather than the contact site fraction. This change in localisation would mean that CPT I would (1) be exposed to a different fatty acyl chain composition in the outer membrane in comparison with the composition of the contact sites and (2) be exposed to an altered outer membrane composition in the GLA diet in comparison with the control diet outer membrane. Thus, the loss of contact sites causes a direct effect on the enzyme by altering its membrane environment through both altered location and altered compositions.

Fatty Acid Induction of Cell Death

In vivo studies in the Walker 256 carcinosarcoma model found that cell cycle changes occurred in the presence of n-3 PUFA-rich diets leading to a decrease in cells in the G1 phase and an increase in cells in the G2-M phase [40]. As previously mentioned, these changes were associated with an increase in TBARS content in the tumour cells, and a large decrease in cell proliferation and an increase in the apoptotic index were also found. Interestingly, in this model, EPA caused a higher rate of TBARS production along with a greater inhibition of tumour growth in vivo than the more unsaturated DHA. Both GLA and AA inhibited the cell cycle in various other tumour cells causing reductions in S-phase and induction of apoptosis [71–73]. DHA has also been reported to exert similar effects in leukaemia cells with S-phase cycle arrest, increased p21 expression and increased pro-caspase-3 cleavage leading to increased apoptotic rate [74, 75].

Further studies using the same Walker 256 tumour model found that GLA produced a dose-dependent inhibition of tumour growth with an almost 50% inhibition in the high-GLA group, which consumed a diet containing 5.5% GLA. Once again, the apoptotic index within the tumour tissue was significantly increased by the PUFA-rich diet [48]. Corroborating the fact that more cells were apoptotic, the nuclear size and shape factor of GLA exposed tumours was significantly reduced as would be expected in cells with pyknotic and apoptotic nuclei present [36].

As previously mentioned, a significant change in the intracellular localisation of mitochondrially bound HK could lead to a situation where intramitochondrial ATP synthesis becomes less efficiently coupled to cytoplasmic glucose-6-phosphate generation. In turn, this reduced efficiency could alter the rate of glycolysis and alter the energy balance within the rapidly proliferating tumour cells, thereby producing a signal for the induction of apoptosis due to reduced ATP production. The decreased MMP found in GLA-exposed tumours may be related to changes in the MPT pore induced by the alterations in mitochondrial HK binding [76–79]. In a recent study, mitochondrial pore-targeted drugs caused MPT and subsequently apoptosis in temozolamide-resistant glioma cells [79].

An article published around the time of Colquhoun [36] reported that mitochondrially bound HK was a modulator of early apoptotic events through the serine–threonine protein kinase, PKB/Akt [76]. Akt stimulates the binding of HK to the mitochondria, thereby increasing the capacity for coupling mitochondrial ATP synthesis to the first step of the glycolytic pathway catalysed by HK [46]. Recent work on Akt has shown that the Akt protein has an Achilles' heel. Normally, Akt is able to inhibit apoptosis induced by many different apoptotic stimuli. However, Akt is unable to protect against ROS-mediated apoptosis and actually sensitises cells to this form of cell death [80]. Tumour cells overexpressing Akt activity, often due to PTEN mutation, may therefore be good targets for chemotherapeutic agents that produce ROS, which would permit the exploitation of this aspect of Akt to increase the tumour cell kill rate. The increase in ROS typically present in PUFA-exposed tumour cells could be particularly effective in tumours overexpressing Akt.

Another finding that suggests a link between metabolic changes in fatty acid-treated tumour cells and the induction of apoptosis comes from the previously mentioned studies involving CPT I. The suggestion that CPT I could be involved in apoptosis was originally proposed by Paumen et al. [81, 82]. These data showed the capacity of the anti-

apoptotic protein Bcl2 to bind to CPT I in a yeast two-hybrid system. In interleukin-3-dependent cells undergoing apoptosis after interleukin-3 deprivation, CPT I expression was increased. This article raised the possibility that an increase in CPT I offered protection from palmitate-induced apoptosis by improving the clearance of long-chain acyl CoAs from the cytoplasm [81, 82]. In cardiomyocytes, a reduction in CPT I in the presence of high concentrations of palmitate was associated with a decrease in MMP and an increase in apoptosis [83]. Studies in the author's laboratory found that PUFAs caused inhibition of CPT I, inhibition of fatty acid oxidation and increased cytoplasmic acyl CoA and triacylglycerol content in both in vitro and in vivo tumour models and identified a strong correlation among the inhibition of CPT I, the inhibition of cell proliferation and the induction of apoptosis in a human larynx tumour cell line [84]. In this cell line, the PUFA effects on CPT I activity found at 6 hours after exposure were correlated with the apoptotic events seen at 12 and 24 hours after exposure, suggesting that the inhibition of mitochondrial CPT I and fatty acid oxidation may lead to the induction of apoptosis in certain tumour cells.

The altered composition of cardiolipin found in several tumour cells exposed to PUFAs has been proposed to increase its likelihood of suffering peroxidation [49, 55]. Recent studies have shown that cardiolipin peroxidation is an important initiating factor in the induction of some forms of cell death [59, 85]. The release of cytochrome *c* from the mitochondria in PUFA-treated cells involves the peroxidation of cardiolipin in order to release cytochrome *c* after which apoptosis can be induced via the remainder of the intrinsic mitochondrial pathway. The increased ROS typically found in PUFA-treated cells would tend to increase the probability of oxidising cardiolipin. In PUFA-treated Walker 256 cells, bladder tumour cells and larynx tumour cells caspase-3 were activated after cytochrome *c* release from the mitochondria, and this was followed by DNA fragmentation readily detected by in situ TUNEL - terminal deoxynucleotidyl transferase mediated dUTP nick-end labelling assays and FACS - fluorescence-activated cell sorting analysis. These changes were accompanied by significant loss of MMP of up to 50% of control cell values [49, 55]. Interestingly, complexes I, III, IV and V of the mitochondrial respiratory chain require cardiolipin for optimal function; complexes III, IV and V contain cardiolipin in their quaternary structure and cardiolipin is also required by several mitochondrial substrate carriers including the phosphate carrier, the ANT and the CACT [85]. The changes found in cardiolipin fatty acyl composition, which increase its tendency for peroxidation, could lead to deficiencies in functional cardiolipin within the tumour cell and may in part explain the reductions in mitochondrial respiratory capacity. The involvement of cardiolipin with the CACT is intriguing as cardiolipin is also associated with changes in CPT I activity [86]. It is possible that increased peroxidation of cardiolipin in the PUFA-treated tumour cells is partially responsible for the reduction in CPT I activity and fatty acid oxidation previously mentioned [36, 37]. Recent studies have also raised the question of whether interactions between the pro-apoptotic Bcl2 family members and peroxidised cardiolipin may regulate and/or stimulate cytochrome *c* release and suggest that the manipulation of cardiolipin peroxidation may be a target for the sensitisation of tumour cells to apoptosis [85]. For further reading on PUFAs and their ability to induce apoptosis, an excellent recent review is recommended [87].

Implications for Neuro-oncology

Two of the earliest studies linking fatty acids to inhibition of tumour cell growth involved neuroblastoma cells and glioma cells [4, 88]. Since then, the n-6 PUFAs GLA and AA have been shown to inhibit glioma cell proliferation, inducing ROS production and leading to cell death [89]. Brain tumours are often deficient in essential fatty acids containing far more saturated than unsaturated fatty acids in their cell membranes, which may reduce their intracellular production of ROS [13, 90, 91]. In a group of human gliomas including oligoastrocytomas, anaplastic astrocytomas and glioblastoma multiforme, AA and GLA were found to stimulate tumour cell peroxidation and apoptosis [89, 92, 93]. Normal brain cells were less susceptible to ROS production and apoptosis in the presence of PUFAs, and necrotic subpopulations of tumour cells also produced less ROS than other regions of the tumour tissue.

Several human studies have tested the effects of GLA on glioma progression, and although the design of these studies was less than ideal, the data suggest that GLA has inhibitory effects on glioma progression in vivo. In these studies, doses of up to 1 mg GLA/day were used for up to 20 days, and the dosage was well tolerated in the majority of patients, some of which showed signs of disease stabilisation during the studies [94–98].

Further in vitro studies have shown that GLA significantly inhibited glioma cell proliferation in three-dimensional spheroids grown in collagen gels and stimulated a dose-dependent increase in apoptosis in the same model [99]. In the C6 cell line, both GLA and EPA were reported to inhibit cell migration on a plastic substrate, while DHA was found to have no effect at similar concentrations [100] (unpublished data). Although migratory tumour cells are considered to be non-proliferative, glioma cells can rapidly stop migration, proceed through cell division and enter into a migratory phase again [101]. With this in mind, mitosis was evaluated in the migratory field and found to be significantly reduced in the presence of either GLA or EPA [100].

More recent studies in the author's laboratory have attempted to identify some of the molecular mechanisms involved in the effects of GLA upon glioma cells both in vitro and in vivo. The in vitro studies found that, as expected, GLA caused a significant decrease in cell proliferation. From the experimental findings, it was apparent that the reduced proliferative rate was accompanied by a reduction in the expression of the protein E2F1, which is required for the entry of cells into the S-phase of the cell cycle and by a reduction in the number of cells in the S-phase when analysed by FACS [102]. In HT29 colon tumour cells, DHA reduced the ability of E2F1 to bind to DNA suggesting reduced activation of E2F1 by DHA and resulted in reduced S-phase [103]. The effects of GLA could only be partially reverted by the natural antioxidant alpha-tocopherol suggesting that ROS production is only partially responsible for GLA-induced changes in cell proliferation in the C6 glioma cell in vitro [102].

GLA caused significant alterations in the expression of the pro-apoptotic protein Bax and the anti-apoptotic protein Bcl2, which are both involved in the control of the intrinsic mitochondrial apoptosis pathway [51, 104, 105]. However, at the concentration of GLA used in this study, C6 cells did not enter into apoptosis even though MMP is reduced [100, 102]. However, at higher concentrations, GLA does induce cell death in these cells (unpublished data). Although the increase in Bax expression would suggest a possible increase in apoptosis [53], in neuroblastoma cells Bax can associate with the DNA repair protein Ku70, thereby blocking Bax's pro-apoptotic function [106, 107]. Ku70 can also function as a physiological inhibitor of Bax-induced apoptosis. Ku70/Ku80 heterodimers represent the regulatory subunit of the DNA-dependent protein kinase (DNA-PKc) which plays an important role in DNA damage repair. In GLA-treated C6 cells, Ku80 expression is significantly reduced which may leave more Ku70 protein free in the cytoplasm where it could bind to Bax and annul its potentially pro-apoptotic effects. Interestingly, Ku proteins are related to hyperproliferation in tumour cells, and inhibition of their expression may lead to decreased cell proliferation as they are implicated in the maintenance of the proliferating cell nuclear antigen (PCNA) on chromatin and can associate with E2F1 [107–109]. The oxidative stress produced by GLA may also be responsible for the reduced Ku80 levels, as oxidative stress in other studies was reported to cause nuclear loss of Ku proteins [110]. The large reduction in Ku80 protein expression could be directly related to impaired DNA replication resulting in cell cycle changes and the micronucleus formation seen in these GLA-exposed cells. Indeed RNAi studies blocking production of Ku80 were reported to cause cell cycle block in G1, reduced S-phase and decreased PCNA expression [111]. GLA was shown to reduce PCNA expression in HeLa cells, and this was associated with possible loss of DNA repair capacity [73]. Recent studies have shown that blocking or reduction of Ku protein function can increase tumour cell sensitivity to both radiotherapy and chemotherapy [112–114].

GLA and other PUFAs are known to increase the sensitivity of tumour cells to both radiotherapy and chemotherapy, although the mechanisms behind these effects are poorly understood [9, 115–119]. Thus, the fact that GLA alters DNA repair protein Ku80 expression in glioma cells may be involved in its capacity to improve tumour cell responses to chemotherapy and radiotherapy and even improve the multi-drug resistance of tumour cells [120, 121]. Of relevance to this is the finding that in C6 glioma cells, the improved sensitivity to radiotherapy did not appear to be directly related to increased ROS generation [121]. It is also possible that changes in energy metabolism and ATP synthesis may impede the activity of ATP-dependent multi-drug resistant proteins so often active in tumour cells [122].

The in vivo studies from Leaver et al. [121, 123] have tested the efficacy of varying doses of GLA applied directly into the tumour bed via osmotic pumps using the orthotopic C6 glioma model. Normal brain morphology was well preserved with no difference in reactive changes at the infusion site between the control and GLA-treated tumours. In tumours exposed to 2mM GLA for 7 days of continuous infusion at 1 μl/h, the tumour size was less than 50% of the phosphate-buffered saline control-treated tumours and the Ki67 labelling index in the GLA-treated tumours was significantly decreased [123].

In a recently published study from the author's laboratory, GLA was applied directly into the tumour bed via osmotic pumps using the same orthotopic C6 glioma model used by Leaver et al. [121, 123, 124]. The infusion rate was reduced to 0.5 μl/h, and the treatment consisted of 5 mM GLA infusion for a total of 14 days with the expectation that a higher dose for an extended period of time would improve tumour response to GLA [124]. The average tumour area of the GLA treated tumours was reduced by 75% in comparison with the control cerebrospinal fluid-treated tumours, and this was accompanied by a change in tumour fatty acid composition. The expression of proteins involved in cell cycle control and apoptosis were significantly changed by GLA treatment including cyclin D1, p53 and p27 expression, and these changes were concomitant with decreased cell proliferation, decreased bromodeoxyuridine incorporation in the S-phase of the cell cycle and increased apoptosis. The possible role of increased p53 expression in altered mitochondrial energy metabolism should also be taken into consideration in this in vivo model as another potential mechanism for reducing cell proliferation [60, 125–127].

Together with the changes in cell proliferation and apoptosis, GLA was found to decrease the expression of proteins involved in angiogenesis including vascular endothelial growth factor (VEGF), ERK1/2, VEGF receptor Flt1 and the matrix metalloproteinase MMP2 and to decrease the microvessel density of the tumour. The findings from this particular study point to several areas in which GLA exerts either direct or indirect effects on tumour growth [124]. Pancreatic tumour cells treated with DHA were also found to have reduced ERK1/2 expression along with increased expression of p27, and in mammary tumours, DHA caused a decrease in proteosomal degradation of p27 [128, 129]. Exposure of HT29 colon cancer tumours to n-3 PUFAs in vivo also caused decreased expression of VEGF, decreased ERK1/2 phosphorylation and decreased microvessel density, leading to decreased tumour growth [130].

Particularly important in these glioma studies is the identification of a dual action of GLA both inhibiting tumour cell proliferation and angiogenesis in vivo [123, 124]. Previous studies reported that GLA altered the expression of proteins involved in endothelial junction formation such as VE-cadherin and occludin and interfered with endothelial tube formation [131–133].

A better understanding of the metabolism of fatty acids and eicosanoids in brain tumours and their influence on energy balance will be fundamental to the possible targeting of mitochondria in tumour treatment [134–136]. A recent review article stated that "the mitocan class of drugs includes compounds that affects such mitochondria-associated activities as hexokinase inhibition, activation of the mitochondrial permeability transition pore, inhibition of bcl2 anti-apoptotic proteins and blocking of the electron transport/ respiratory chain" [59]. Through the author's own and many other studies, GLA can readily be placed in this category, and it is reasonable to suggest that the use of combination therapies with complimentary targets and modes of action together with PUFAs such as GLA may lead to gains in treatment efficacy in gliomas. Fig. 1 contains a summary of the ideas and data presented in this review.

Conclusions and Future Directions

It is clear from the data reviewed in the preceding sections that PUFAs exert significant inhibitory effects on tumour cell proliferation. This is in part related to the increased production of ROS, which occurs when the tumour cells are exposed to PUFAs. The role of CPT I in the function of normal astrocytes is poorly understood and even less is known of its importance in gliomas, leaving many questions unanswered regarding PUFA effects on fatty acid oxidation and eicosanoid metabolism in gliomas and is an area that deserves investigation [136–140]. HK binding to mitochondria and its involvement in the inhibition of apoptosis through interactions with VDAC, as well as the possibility of inhibiting ANT through increased acyl CoA concentrations, are also areas of interest for future glioma research [141–143]. The question remains whether PUFAs are able to alter glioma HK binding to mitochondria and whether this may be an important target for exploitation when taking into account the additional involvement of HK in the activity of Akt, a protein often activated in gliomas due to mutation of PTEN. The ability of PUFAs to increase tumour sensitivity to both radiotherapy and chemotherapy also merits further attention in gliomas, as it may provide a means to improve tumour response to conventional treatment options. Research into the targeting of mitochondrial

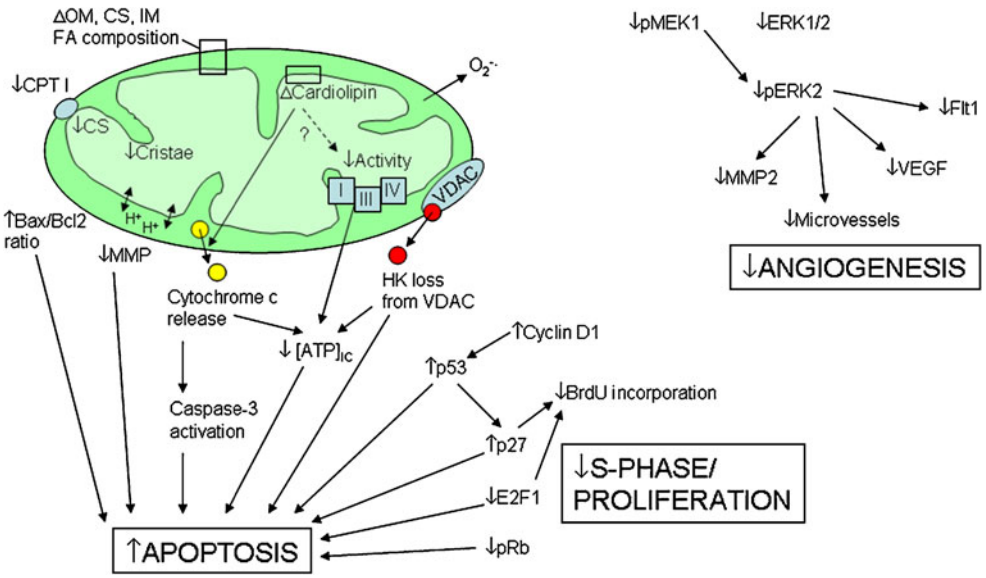

Fig. 1 Effects of PUFAs on tumour metabolism. *OM* outer mitochondrial membrane; *CS* mitochondrial membrane contact site; *IM* mitochondrial inner membrane; *FA* fatty acid; *CPT I* carnitine palmitoyltransferase I; *MMP* mitochondrial membrane potential ; *I, III, IV* mitochondrial respiratory complexes I, III and IV; *VDAC* voltage-dependent anion channel; *HK*; *BrdU* bromodeoxyuridine; *MMP2* matrix metalloprotease 2; *VEGF* vascular endothelial growth factor; *Flt1* VEGF receptor Flt1; $O_2^{\cdot-}$ superoxide

metabolism in an attempt to direct tumour cells towards apoptotic and, more recently, autophagic cell death is gaining momentum in the literature and may provide novel strategies for the treatment of primary brain tumours such as gliomas [51, 52, 59, 79, 135, 144, 145].

References

1. Louis DN, Ohgaki H, Wiestler OD, Cavenee WK (2007) WHO classification of tumours of the central nervous system, 4th edn. IARC, France
2. Booyens J, Engelbrecht P, le Roux S, Louwrens CC, Van der Merwe CF, Katzeff IE (1984) Some effects of the essential fatty acids linoleic acid and alpha-linolenic acid and of their metabolites gamma-linolenic acid, arachidonic acid, eicosapentaenoic acid, docosahexaenoic acid, and of prostaglandins A1 and E1 on the proliferation of human osteogenic sarcoma cells in culture. Prostaglandins Leukot Med 15(1):15–33
3. Bégin ME, Ells G, Das UN, Horrobin DF (1986) Differential killing of human carcinoma cells supplemented with n-3 and n-6 polyunsaturated fatty acids. J Natl Cancer Inst 77(5): 1053–1062
4. Fujiwara F, Todo S, Imashuku S (1986) Antitumor effect of gamma-linolenic acido n cultured human neuroblastoma cells. Prostaglandins Leukot Med 23(2–3):311–320
5. Bégin ME, Ells G, Horrobin DF (1988) Polyunsaturated fatty acid-induced cytotoxicity against tumor cells and its relationship to lipid peroxidation. J Natl Cancer Inst 80(3):188–194
6. Das UN, Huang YS, Begin ME, Ells G, Horrobin DF (1987) Uptake and distribution of *cis*-unsaturated fatty acids and their effect on free radical generation in normal and tumor cells in vitro. Free Radic Biol Med 3:9–14
7. Colquhoun A, Curi R (1998) Effects of saturated and polyunsaturated fatty acids on human tumor cell proliferation. Gen Pharmac 30:191–194
8. Hawkins RA, Sangster K, Arends MJ (1998) Apoptotic death of pancreatic cancer cells induced by polyunsaturated fatty acids varies with double bond number and involves an oxidative mechanism. J Pathol 185(1):61–70
9. Vartak S, McCaw R, Davis CS, Robbins ME, Spector AA (1998) Gamma-linolenic acid (GLA) is cytotoxic to 36B10 malignant rat astrocytoma cells but not to 'normal' rat astrocytes. Br J Cancer 77(10):1612–1620
10. Das UN, Begin ME, Ells G, Huang YS, Horrobin DF (1987) Polyunsaturated fatty acids augment free radical generation in tumor cells in vitro. B B Res Com 145:15–24
11. Das UN (1991) Tumoricidal action of cis-unsaturated fatty acids and their relationship to free radicals and lipid peroxidation. Cancer Letts 56:235–243
12. Vartak S, Robbins ME, Spector AA (1999) The selective cytotoxicity of gamma-linolenic acid (GLA) is associated with increased oxidative stress. Adv Exp Med Biol 469:493–498
13. Das UN (2002) A radical approach to cancer. Med Sci Monit 8 (4):RA79–RA92
14. Rose DP, Connolly JM (1999) Omega-3 fatty acids as cancer chemopreventive agents. Pharmacol Ther 83(3):217–244
15. Nkondjock A, Shatenstein B, Maisonneuve P, Ghadirian P (2003) Specific fatty acids and human colorectal cancer: an overview. Cancer Detect Prev 27(1):55–66
16. Simopoulos AP (2001) The Mediterranean diets: what is so special about the diet of Greece? The scientific evidence. J Nutr 131(11 Suppl):3065S–3073S
17. Ziegler RG, Hoover RN, Pike MC, Hildesheim A, Nomura AM, West DW, Wu-Williams AH, Kolonel LN, Horn-Ross PL, Rosenthal JF, Hyer MB (1993) Migration patterns and breast cancer risk in Asian-American women. J Natl Cancer Inst 85 (22):1819–1827
18. Hillyard LA, Abraham S (1979) Effect of dietary polyunsaturated fatty acids on growth of mammary adenocarcinomas in mice and rats. Cancer Res 39(11):4430–4437
19. Abraham S, Hillyard LA (1983) Effect of dietary 18-carbon fatty acids on growth of transplantable mammary adenocarcinomas in mice. J Natl Cancer Inst 71(3):601–605
20. Welsch CW (1992) Relationship between dietary fat and experimental mammary tumorigenesis: a review and critique. Cancer Res 52(7 Suppl):2040s–2048s, 1
21. Sauer LA, Blask DE, Dauchy RT (2007) Dietary factors and growth and metabolism in experimental tumors. J Nutr Biochem 18(10):637–649
22. Rose DP, Connolly JM (1993) Effects of dietary omega-3 fatty acids on human breast cancer growth and metastases in nude mice. J Natl Cancer Inst 85(21):1743–1747
23. Cohen LA, Chen-Backlund JY, Sepkovic DW, Sugie S (1993) Effect of varying proportions of dietary menhaden and corn oil on experimental rat mammary tumor promotion. Lipids 28 (5):449–456
24. Gonzalez MJ, Schemmel RA, Dugan L Jr, Gray JI, Welsch CW (1993) Dietary fish oil inhibits human breast carcinoma growth: a function of increased lipid peroxidation. Lipids 28(9):827–832
25. Chapkin RS, McMurray DN, Lupton JR (2007) Colon cancer, fatty acids and anti-inflammatory compounds. Curr Opin Gastroenterol 23(1):48–54
26. Rose DP, Connolly JM, Liu XH (1997) Fatty acid regulation of breast cancer cell growth and invasion. Adv Exp Med Biol 422:47–55
27. Colquhoun A, Miyake JA, Benadiba M (2009) Fatty acids, eicosanoids and cancer. Nutritional Therapy and Metabolism 27 (3):105–112
28. Kobayashi N, Barnard RJ, Henning SM, Elashoff D, Reddy ST, Cohen P, Leung P, Hong-Gonzalez J, Freedland SJ, Said J, Gui D, Seeram NP, Popoviciu LM, Bagga D, Heber D, Glaspy JA, Aronson WJ (2006) Effect of altering dietary omega-6/omega-3 fatty acid ratios on prostate cancer membrane composition, cyclooxygenase-2, and prostaglandin E2. Clin Cancer Res 12 (15):4662–4670
29. Nathoo N, Barnett GH, Golubic M (2004) The eicosanoid cascade: possible role in gliomas and meningiomas. J Clin Pathol 57(1):6–13
30. Gillies RJ, Gatenby RA (2007) Adaptive landscapes and emergent phenotypes: why do cancers have high glycolysis? J Bioenerg Biomembr 39:251–257
31. Herrmann PC, Herrmann EC (2007) Oxygen metabolism and a potential role for cytochrome *c* oxidase in the Warburg effect. J Bioenerg Biomembr 39:247–250
32. Prip-Buus C, Bouthillier-Voisin AC, Kohl C, Demaugre F, Girard J, Pegorier JP (1992) Evidence for an impaired long-chain fatty acid oxidation and ketogenesis in Fao hepatoma cells. Eur J Biochem 209(1):291–298
33. Colquhoun A, Curi R (1995) Human and rat tumour cells possess mitochondrial carnitine palmitoyltransferase I and II: effects of insulin. Biochem Mol Biol Int 37:599–605
34. Colquhoun A, Curi R (1996) Immunodetection of rat Walker 256 tumour mitochondrial carnitine palmitoyltransferase I and II: evidence for the control of CPT II expression by insulin. Biochem Mol Biol Int 38:171–174
35. Colquhoun A, Curi R (1997) Metabolic fate and effects of saturated and unsaturated fatty acids in Hep2 human larynx tumor cells. Biochem Mol Biol Int 41:597–607

36. Colquhoun A (2002) Gamma-linolenic acid alters the composition of mitochondrial membrane subfractions, decreases outer mitochondrial membrane binding of hexokinase and alters carnitine palmitoyltransferase I properties in the Walker 256 rat tumour. Biochim Biophys Acta 1583(1):74–84
37. Colquhoun A, de Mello FE, Curi R (1998) Regulation of tumour cell fatty acid oxidation by n-6 polyunsaturated fatty acids. Biochem Mol Biol Int 44(1):143–150
38. Qiao L, Kozoni V, Tsioulias GJ, Koutsos MI, Hanif R, Shiff SJ, Rigas B (1995) Selected eicosanoids increase the proliferation rate of human colon carcinoma cell lines and mouse colonocytes in vivo. Biochim Biophys Acta 1258(2):215–223
39. Chen ZY, Istfan NW (2000) Docosahexaenoic acid is a potent inducer of apoptosis in HT29 colon cancer cells. Prostaglandins Leukot Essent Fat Acids 63(5):301–308
40. Colquhoun A, Ramos KL, Schumacher RI (2001) Eicosapentaenoic acid and docosahexaenoic acid effects on tumour mitochondrial metabolism, acyl CoA metabolism and cell proliferation. Cell Biochem Func 19:97–105
41. Bustamante E, Pedersen PL (1977) High aerobic glycolysis of rat hepatoma cells in culture: role of mitochondrial hexokinase. Proc Natl Acad Sci U S A 74(9):3735–3739
42. Pedersen PL (2007) Warburg, me and Hexokinase 2: multiple discoveries of key molecular events underlying one of cancers' most common phenotypes, the "Warburg Effect", i.e., elevated glycolysis in the presence of oxygen. J Bioenerg Biomembr 39 (3):211–222
43. Mathupala SP, Ko YH, Pedersen PL (2008) Hexokinase-2 bound to mitochondria: cancer's stygian link to the "Warburg Effect" and a pivotal target for effective therapy. Semin Cancer Biol 19 (1):17–24
44. Stewart JM, Blakely JA (2000) Long chain fatty acids inhibit and medium chain fatty acids activate mammalian cardiac hexokinase. BiochemBiophys Acta 1484:278–286
45. Arzoine L, Zilberberg N, Ben-Romano R, Shoshan-Barmatz V (2009) Voltage-dependent anion channel 1-based peptides interact with hexokinase to prevent its anti-apoptotic activity. J Biol Chem 284(6):3946–3955
46. Brooks Robey R, Hay N (2009) Is Akt the "Warburg kinase"—Akt-energy interactions and oncogenesis. Semin Cancer Biol 19 (1):25–31
47. Kumarswamy R, Chandna S (2009) Putative partners in Bax mediated cytochrome-*c* release: ANT, CypD, VDAC or none of them? Mitochondrion 9:1–8
48. Colquhoun A, Schumacher RI (2001) Modifications in mitochondrial metabolism and ultrastructure and their relationship to tumour growth inhibition by gamma-linolenic acid. Mol Cell Biochem 218(1–2):13–20
49. Colquhoun A, Schumacher RI (2001) gamma-Linolenic acid and eicosapentaenoic acid induce modifications in mitochondrial metabolism, reactive oxygen species generation, lipid peroxidation and apoptosis in Walker 256 rat carcinosarcoma cells. Biochim Biophys Acta 1533(3):207–219
50. Bartrons R, Caro J (2007) Hypoxia, glucose metabolism and the Warburg's effect. J Bioenerg Biomembr 39(3):223–229
51. Gogvadze V, Orrenius S, Zhivotovsky B (2009) Mitochondria as targets for chemotherapy. Apoptosis 14:624–640
52. Gogvadze V, Orrenius S, Zhivotovsky B (2009) Mitochondria as targets for cancer chemotherapy. Semin Cancer Biol 19(1):57–66
53. Ott M, Gogvadze V, Orrenius S, Zhivotovsky B (2007) Mitochondria, oxidative stress and cell death. Apoptosis 12 (5):913–922
54. Cocco T, Di Paola M, Papa S, Lorusso M (1999) Arachidonic acid interaction with the mitochondrial electron transport chain promotes reactive oxygen species generation. Free Radic Biol Med 27(1–2):51–59
55. Colquhoun A (2009) Mechanisms of action of eicosapentaenoic acid in bladder cancer cells in vitro: alterations in mitochondrial metabolism, reactive oxygen species generation and apoptosis induction. J Urol 181(4):1885–1893
56. Kantrow SP, Tatro LG, Piantadosi CA (2000) Oxidative stress and adenine nucleotide control of mitochondrial permeability transition. Free Radic Biol Med 28(2):251–260
57. Gendron MC, Schrantz N, Métivier D, Kroemer G, Maciorowska Z, Sureau F, Koester S, Petit PX (2001) Oxidation of pyridine nucleotides during Fas- and ceramide-induced apoptosis in Jurkat cells: correlation with changes in mitochondria, glutathione depletion, intracellular acidification and caspase 3 activation. Biochem J 353(Pt 2):357–367
58. Cao Y, Pearman AT, Zimmerman GA, McIntyre TM, Prescott SM (2000) Intracellular unesterified arachidonic acid signals apoptosis. Proc Natl Acad Sci U S A 97(21):11280–11285
59. Neuzil J, Dyason JC, Freeman R, Dong LF, Prochazka L, Wang XF, Scheffler I (2007) Ralph SJ Mitocans as anti-cancer agents targeting mitochondria: lessons from studies with vitamin E analogues, inhibitors of complex II. J Bioenerg Biomembr 39 (1):65–72
60. Jones RG, Thompson CB (2009) Tumor suppressor and cell metabolism: a recipe for cancer growth. Genes Dev 23:537–548
61. Watkins SM, Carter LC, German JB (1998) Docosahexaenoic acid accumulates in cardiolipin and enhances HT-29 cell oxidant production. J Lipid Res 39(8):1583–1588
62. Gonzalez B, Iturralde M, Alava MA, Anel A, Piñeiro A (2000) Metabolism of n -9, n -6 and n -3 fatty acids in hepatoma Morris 7777 cells. Preferential accumulation of linoleic acid in cardiolipin. Prostaglandins Leukot Essent Fat Acids 62(5):299–306
63. Nomura K, Imai H, Koumura T, Kobayashi T, Nakagawa Y (2000) Mitochondrial phospholipid hydroperoxide glutathione peroxidase inhibits the release of cytochrome *c* from mitochondria by suppressing the peroxidation of cardiolipin in hypoglycaemia-induced apoptosis. Biochem J 351(Pt 1):183–193
64. Brdiczka D (1991) Contact sites between mitochondrial envelope membranes. Structure and function in energy- and protein-transfer. Biochim Biophys Acta 1071(3):291–312
65. Jacob WA, Bakker A, Hertsens RC, Biermans W (1994) Mitochondrial matrix granules: their behavior during changing metabolic situations and their relationship to contact sites between inner and outer mitochondrial membranes. Microsc Res Tech 27(4):307–318
66. Denis-Pouxviel C, Riesinger I, Bühler C, Brdiczka D, Murat JC (1987) Regulation of mitochondrial hexokinase in cultured HT 29 human cancer cells. Na ultrastructural and biochemical study. Biochim Biophys Acta 902(3):335–348
67. Kryvi H, Aarsland A, Berge RK (1990) Morphologic effects of sulfur-substituted fatty acids on rat hepatocytes with special reference to proliferation of peroxisomes and mitochondria. J Struct Biol 103(3):257–265
68. Fraser F, Zammit VA (1998) Enrichment of carnitine palmitoyltransferases I and II in the contact sites of rat liver mitochondria. Biochem J 329(Pt 2):225–229
69. Fraser F, Padovese R, Zammit VA (2001) Distinct kinetics of carnitine palmitoyltransferase I in contact sites and outer membranes of rat liver mitochondria. J Biol Chem 276 (23):20182–20185
70. Hoppel CL, Kerner J, Turkaly P, Tandler B (2001) Distinct kinetics of carnitine palmitoyltransferase I in contact sites and outer membranes of rat liver mitochondria. Arch Biochem Biophys 392:321–325

71. Seegers JC, de Kock M, Lottering ML, Grobler CJ, van Papendorp DH, Shou Y, Habbersett R, Lehnert BE (1997) Effects of gamma-linolenic acid and arachidonic acid on cell cycle progression and apoptosis induction in normal and transformed cells. Prostaglandins Leukot Essent Fat Acids 56(4):271–280
72. Seegers JC, Lottering ML, Panzer A, Bianchi P, Stark JH (1998) Comparative anti-mitotic effects of lithium gamma-linolenate, gamma-linolenic acid and arachidonic acid, on transformed and embryonic cells. Prostaglandins Leukot Essent Fat Acids 59 (4):285–291
73. de Kock M, Lottering ML, Grobler CJ, Viljoen TC, le Roux M, Seegers JC (1996) The induction of apoptosis in human cervical carcinoma (HeLa) cells by gamma-linolenic acid. Prostaglandins Leukot Essent Fat Acids 55(6):403–411
74. Siddiqui RA, Jenski LJ, Neff K, Harvey K, Kovacs RJ, Stillwell W, Siddiqui EA (2001) Docosahexaenoic acid induces apoptosis in Jurkat cells by a protein phosphatase-mediated process. Biochim Biophys Acta 1499(3):265–275
75. Siddiqui RA, Jenski LJ, Harvey KA, Wiesehan JD, Stillwell W, Zaloga GP (2003) Cell-cycle arrest in Jurkat leukaemic cells: a possible role for docosahexaenoic acid. Biochem J 371(Pt 2):621–629
76. Gottlob K, Majewski N, Kennedy S, Kandel E, Robey RB, Hay N (2001) Inhibition of early apoptotic events by Akt/PKB is dependent on the first committed step of glycolysis and mitochondrial hexokinase. Genes Dev 15(11):1406–1418
77. Crompton M (1999) The mitochondrial permeability transition pore and its role in cell death. Biochem J 341(Pt 2):233–249
78. Desagher S, Martinou JC (2000) Mitochondria as the central control point of apoptosis. Trends Cell Biol 10(9):369–377
79. Lena A, Rechichi M, Salvetti A, Bartoli B, Vecchio D, Scarcelli V, Amoroso R, Benvenuti L, Gagliardi R, Gremigni V, Rossi L (2009) Drugs targeting the mitochondrial pore act as citotoxic and cytostatic agents in temozolomide-resistant glioma cells. J Transl Med 7:13
80. Nogueira V, Park Y, Chen CC, Xu PZ, Chen ML, Tonic I, Unterman T, Hay N (2008) Akt determines replicative senescence and oxidative or oncogenic premature senescence and sensitizes cells to oxidative apoptosis. Cancer Cell 14(6):458–470
81. Paumen MB, Ishida Y, Muramatsu M, Yamamoto M, Honjo T (1997) Inhibition of carnitine palmitoyltransferase I augments sphingolipid synthesis and palmitate-induced apoptosis. J Biol Chem 272(6):3324–3329
82. Paumen MB, Ishida Y, Han H, Muramatsu M, Eguchi Y, Tsujimoto Y, Honjo T (1997) Direct interaction of the mitochondrial membrane protein carnitine palmitoyltransferase I with Bcl-2. Biochem Biophys Res Commun 231(3): 523–525
83. Sparagna GC, Hickson-Bick DL, Buja LM, McMillin JB (2000) A metabolic role for mitochondria in palmitate-induced cardiac myocyte apoptosis. Am J Physiol Heart Circ Physiol 279(5): H2124–H2132
84. Colquhoun A (1998) Induction of apoptosis by polyunsaturated fatty acids and its relationship to fatty acid inhibition of carnitine palmitoyltransferase I activity in Hep2 cells. Biochem Mol Biol Int 45(2):331–336
85. Gonzalvez F, Gottlieb E (2007) Cardiolipin: setting the beat of apoptosis. Apotosis 12:877–885
86. Mynatt RL, Greenhaw JJ, Cook GA (1994) Cholate extracts of mitochondrial outer membranes increase inhibition by malonyl-CoA of carnitine palmitoyltransferase-I by a mechanism involving phospholipids. Biochem J 299(Pt 3):761–767
87. Serini S, Piccioni E, Merendino N, Calviello G (2009) Dietary polyunsaturated fatty acids as inducers of apoptosis: implications for cancer. Apoptosis 14(2):135–152
88. Liepkalns VA, Icard-Liepkalns C, Cornwell DG (1982) Regulation of cell division in a human glioma cell clone by arachidonic acid and alpha-tocopherolquinone. Cancer Lett 15(2):173–178
89. Williams JR, Leaver HA, Ironside JW, Miller EP, Whittle IR, Gregor A (1998) Apoptosis in human primary brain tumours: actions of arachidonic acid. Prostaglandins Leukot Essent Fat Acids 58(3):193–200
90. Martin DD, Robbins ME, Spector AA, Wen BC, Hussey DH (1996) The fatty acid composition of human gliomas differs from that found in nonmalignant brain tissue. Lipids 31(12):1283–1288
91. Kokoglu E, Tüter Y, Yazici Z, Sandikci KS, Sönmez H, Ulakoğlu EZ, Ozyurt E (1998) Profiles of the fatty acids in the plasma membrane of human brain tumors. Cancer Biochem Biophys 16(4):301–312
92. Leaver HA, Williams JR, Ironside JW, Miller EP, Gregor A, Su BH, Prescott RJ, Whittle IR (1999) Dynamics of reactive oxygen intermediate production in human glioma: n-6 essential fatty acid effects. Eur J Clin Invest 29(3):220–231, Comment in: Eur J Clin Invest 1999 Mar; 29(3):185-8
93. Leaver HA, Williams JR, Smith C, Whittle IR (2004) Intracellular oxidation by human glioma cell populations: effect of arachidonic acid. Prostaglandins Leukot Essent Fat Acids 70 (5):449–453
94. Naidu MR, Das UN, Kishan A (1992) Intratumoral gamma-linoleic acid therapy of human gliomas. Prostaglandins Leukot Essent Fat Acids 45(3):181–184
95. Das UN, Prasad VV, Reddy DR (1995) Local application of gamma-linolenic acid in the treatment of human gliomas. Cancer Lett 94(2):147–155
96. Bakshi A, Mukherjee D, Bakshi A, Banerji AK, Das UN (2003) Gamma-linolenic acid therapy of human gliomas. Nutrition 19 (4):305–309, Comment on: Nutrition. 2003 Apr; 19(4):386-8
97. Das UN (2004) From bench to the clinic: gamma-linolenic acid therapy of human gliomas. Prostaglandins Leukot Essent Fat Acids 70(6):539–552
98. Das UN (2007) Gamma-linolenic acid therapy of human glioma-a review of in vitro, in vivo, and clinical studies. Med Sci Monit 13(7):RA119–RA131
99. Bell HS, Wharton SB, Leaver HA, Whittle IR (1999) Effects of N-6 essential fatty acids on glioma invasion and growth: experimental studies with glioma spheroids in collagen gels. J Neurosurg 91(6):989–996
100. Ramos KL, Colquhoun A (2003) Protective role of glucose-6-phosphate dehydrogenase activity in the metabolic response of C6 rat glioma cells to polyunsaturated fatty acid exposure. Glia 43(2):149–166
101. Farin A, Suzuki SO, Weiker M, Goldman JE, Bruce JN, Canoll P (2006) Transplanted glioma cells migrate and proliferate on host brain vasculature: a dynamic analysis. Glia 53(8):799–808
102. Benadiba M, Miyake JA, Colquhoun A (2009) Gamma-linolenic acid alters Ku80, E2F1, and bax expression and induces micronucleus formation in C6 glioma cells in vitro. IUBMB Life 61(3):244–251
103. Chen ZY, Istfan NW (2001) Docosahexaenoic acid, a major constituent of fish oil diets, prevents activation of cyclin-dependent kinases and S-phase entry by serum stimulation in HT29 cells. Prostaglandins Leukot Essent Fat Acids 64(1):67–73
104. Kim R, Emi M, Tanabe K (2005) Role of mitochondria as the gardens of cell death. Cancer Chemother Pharmacol 57(5): 545–553

105. Susnow N, Zeng L, Margineantu D, Hockenbery DM (2009) Bcl-2 family proteins as regulators of oxidative stress. Semin Cancer Biol 19(1):42–49
106. Subramanian C, Opipari AW Jr, Bian X, Castle VP, Kwok RP (2005) Ku70 acetylation mediates neuroblastoma cell death induced by histone deacetylase inhibitors. Proc Natl Acad Sci U S A 102:4842–4847
107. Gullo C, Au M, Feng G, Teoh G (2006) The biology of Ku and its potential oncogenic role in cancer. Biochim Biophys Acta 1765:223–234
108. Pedley J, Pettit A, Parsons PG (1998) Inhibition of Ku autoantigen binding activity to the E2F motif after ultraviolet B irradiation of melanocytic cells. Melanoma Res 8(6):471–481
109. Park SJ, Ciccone SL, Freie B, Kurimasa A, Chen DJ, Li GC, Clapp DW, Lee SH (2004) A positive role for the Ku complex in DNA replication following strand break damage in mammals. J Biol Chem 279(7):6046–6055
110. Song JY, Lim JW, Kim H, Kim KH (2003) Role of NF-kappaB and DNA repair protein Ku on apoptosis in pancreatic acinar cells. Ann N Y Acad Sci 1010:259–263
111. Rampakakis E, Di Paola D, Zannis-Hadjopoulos M (2008) Ku is involved in cell growth, DNA replication and G1-S transition. J Cell Sci 121(Pt 5):590–600
112. Yang QS, Gu JL, DU LQ, Jia LL, Qin LL, Wang Y, Fan FY (2008) ShRNA-mediated Ku80 gene silencing inhibits cell proliferation and sensitizes to gamma-radiation and mitomycin c-induced apoptosis in esophageal squamous cell carcinoma lines. J Radiat Res (Tokyo). 2008 Apr 9
113. Groesser T, Chun E, Rydberg B (2007) Relative biological effectiveness of high-energy iron ions for micronucleus formation at low doses. Radiat Res 168(6):675–682
114. Zhang F, Zhang T, Gu ZP, Zhou YA, Han Y, Li XF, Wang XP, Cheng QS, Mei QB (2008) Enhancement of radiosensitivity by roscovitine pretreatment in human non-small cell lung cancer A549 cells. J Radiat Res (Tokyo) 49(5):541–548
115. Kinsella JE, Black JM (1993) Effects of polyunsaturated fatty acids on the efficacy of antineoplastic agents toward L5178Y lymphoma cells. Biochem Pharmacol 45(9):1881–1887
116. Vartak S, Robbins ME, Spector AA (1997) Polyunsaturated fatty acids increase the sensitivity of 36B10 rat astrocytoma cells to radiation-induced cell kill. Lipids 32(3):283–292
117. Germain E, Chajès V, Cognault S, Lhuillery C, Bougnoux P (1998) Enhancement of doxorubicin cytotoxicity by polyunsaturated fatty acids in the human breast tumor cell line MDA-MB-231: relationship to lipid peroxidation. Int J Cancer 75(4): 578–583
118. Madhavi N, Das UN (1994) Effect of n-6 and n-3 fatty acids on the survival of vincristine sensitive and resistant human cervical carcinoma cells in vitro. Cancer Lett 84(1):31–41
119. Kenny FS, Pinder SE, Ellis IO, Gee JM, Nicholson RI, Bryce RP, Robertson JF (2000) Gamma linolenic acid with tamoxifen as primary therapy in breast cancer. Int J Cancer 85(5):643–648
120. Das UN, Madhavi N, Sravan Kumar G, Padma M, Sangeetha P (1998) Can tumour cell drug resistance be reversed by essential fatty acids and their metabolites? Prostaglandins Leukot Essent Fat Acids 58(1):39–54
121. Leaver HA, Bell HS, Rizzo MT, Ironside JW, Gregor A, Wharton SB, Whittle IR (2002) Antitumour and pro-apoptotic actions of highly unsaturated fatty acids in glioma. Prostaglandins Leukot Essent Fat Acids 66(1):19–29
122. Godinot C, de Laplanche E, Hervouet E, Simonnet H (2007) Actuality of Warburg's views in our understanding of renal cancer metabolism. J Bioenerg Biomembr 39(3):235–241
123. Leaver HA, Wharton SB, Bell HS, Leaver-Yap IM, Whittle IR (2002) Highly unsaturated fatty acid induced tumour regression in glioma pharmacodynamics and bioavailability of gamma linolenic acid in an implantation glioma model: effects on tumour biomass, apoptosis and neuronal tissue histology. Prostaglandins Leukot Essent Fat Acids 67(5):283–292
124. Miyake JA, Benadiba M, Colquhoun A (2009) Gamma-linolenic acid inhibits both tumour cell cycle progression and angiogenesis in the orthotopic C6 glioma model through changes in VEGF, Flt1, ERK1/2, MMP2, cyclin D1, pRb, p53 and p27 protein expression. Lipids Health Dis 8:8
125. Ma W, Sung HJ, Park JY, Matoba S, Hwang PM (2007) A pivotal role for p53: balancing aerobic respiration and glycolysis. J Bioenerg Biomembr 39(3):243–246
126. Frezza C, Gottlieb E (2009) Mitochondria in cancer: not just innocent bystanders. Semin Cancer Biol 19(1):4–11
127. Olovnikov IA, Kravchenko JE, Chumakov PM (2009) Homeostatic functions of metabolism and antioxidant defense. Semin Cancer Biol 19(1):32–41
128. Khan NA, Nishimura K, Aires V, Yamashita T, Oaxaca-Castillo D, Kashigawa K, Igarashi K (2006) Docosahexaenoic acid inhibits cancer cell growth via p27Kip1, CDK2, ERK1/ERK2, and retinoblastoma phosphorylation. J Lipid Res 47(10):2306–2313
129. Schley PD, Jijon HB, Robinson LE, Field CJ (2005) Mechanisms of omega-3 fatty acid-induced growth inhibition in MDA-MB-231 human breast cancer cells. Breast Cancer Res Treat 92:187–195
130. Calviello G, Palozza P, Piccioni E, Maggiano N, Frattucci A, Franceschelli P, Bartoli GM (1998) Dietary supplementation with eicosapentaenoic and docosahexaenoic acid inhibits growth of Morris hepatocarcinoma 3924A in rats: effects on proliferation and apoptosis. Int J Cancer 75(5):699–705
131. Cai J, Jiang WG, Mansel RE (1999) Inhibition of the expression of VE-cadherin/catenin complex by gamma linolenic acid in human vascular endothelial cells, and its impact on angiogenesis. Biochem Biophys Res Commun 258(1):113–8, 29
132. Jiang WG, Bryce RP, Horrobin DF (1998) Essential fatty acids: molecular and cellular basis of their anti-cancer action and clinical implications. Crit Rev Oncol Hematol 27(3):179–209
133. Yamagata K, Tagami M, Takenaga F, Yamori Y, Nara Y, Itoh S (2003) Fatty acids induce tight junctions to form in brain capillary endothelial cells. Neuroscience 116:649–656
134. Schiazza L, Lamari F, Foglietti MJ, Hainque B, Bernard M, Beaudeux JL (2008) Métabolisme énergétique cellulaire du tissue cérébral: spécificités métaboliques des tumeurs gliales. Ann Biol Clin 66(2):131–141
135. Pilkington GJ, Parker K, Murray SA (2008) Approaches to mitochondrially mediated cancer therapy. Sem Cancer Biol 18:226–235
136. Auestad N, Korsak RA, Morrow JW, Edmond J (1991) Fatty acid oxidation and ketogenesis by astrocytes in primary culture. J Neurochem 56(4):1376–1386
137. Blázquez C, Sánchez C, Velasco G, Guzmán M (1998) Role of carnitine palmitoyltransferase I in the control of ketogenesis in primary cultures of rat astrocytes. J Neurochem 71(4):1597–1606
138. Blázquez C, Woods A, de Ceballos ML, Carling D, Guzmán M (1999) The AMP-activated protein kinase is involved in the regulation of ketone body production by astrocytes. J Neurochem 73(4):1674–1682
139. Blázquez C, Sánchez C, Daza A, Galve-Roperh I, Guzmán M (1999) The stimulation of ketogenesis by cannabinoids in

cultured astrocytes defines carnitine palmitoyltransferase I as a new ceramide-activated enzyme. J Neurochem 72(4):1759–1768
140. Hertz L, Peng L, Dienel GA (2007) Energy metabolism in astrocytes: high rate of oxidative metabolism and spatiotemporal dependence on glycolysis/glycogenolysis. J Cereb Blood Flow Metab 27(2):219–249, Epub 2006 Jul 12
141. Oudard S, Miccoli L, Beurdeley-Thomas A, Dutrillaux B, Poupon MF (2004) Homophilic anchorage of brain-hexokinase to mitochondria-porins revealed by specific-peptide antibody cross recognition. Bull Cancer 91(6):E184–E200
142. Abu-Hamad S, Zaid H, Israelson A, Nahon E, Shoshan-Barmatz V (2008) Hexokinase-I protection against apoptotic cell death is mediated via interaction with the voltage-dependent anion channel-1: mapping the site of binding. J Biol Chem 283 (19):13482–13490
143. Lewandrowski U, Sickmann A, Cesaro L, Brunati AM, Toninello A, Salvi M (2008) Identification of new tyrosine phosphorylated proteins in rat brain mitochondria. FEBS Lett 582(7):1104–1110
144. Jou MJ (2008) Pathophysiological and pharmacological implications of mitochondria-targeted reactive oxygen species generation in astrocytes. Adv Drug Deliv Rev 60(13-14): 1512–1526
145. Hamm-Alvarez S, Cadenas E (2008) Mitochondrial medicine and mitochondrion-based therapeutics. Adv Drug Deliv Rev 60 (13-14):1437–1438