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Lipid analogues as potential drugs for the regulation of mitochondrial cell death

Michael Murray\textsuperscript{1}, Herryawan Ryadi Eziwar Dyari\textsuperscript{1}, Sarah E. Allison\textsuperscript{1} and Tristan Rawling\textsuperscript{2}

\textsuperscript{1}Pharmacogenomics and Drug Development Group, Discipline of Pharmacology, University of Sydney, NSW 2006, Australia, and \textsuperscript{2}School of Pharmacy, Graduate School of Health, University of Technology, Sydney, PO Box 123, Broadway NSW 2007, Australia.

Address for correspondence: Dr Michael Murray

Pharmacogenomics and Drug Development Group,

Discipline of Pharmacology,

Medical Foundation Building, Room 105,

University of Sydney,

NSW 2006, Australia

Tel: (61-2-9036-3259)

Fax (61-2-9036-3244)

Email: michael.murray@sydney.edu.au

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Abstract

The mitochondrion has fundamental roles in the production of energy as ATP, the regulation of cell viability and apoptosis, and the biosynthesis of major structural and regulatory molecules, such as lipids. During ATP production reactive oxygen species are generated that alter the intracellular redox state and activate apoptosis. Mitochondrial dysfunction is a well recognized component of the pathogenesis of diseases such as cancer. Understanding mitochondrial function, and how this is dysregulated in disease, offers the opportunity for the development of drug molecules to specifically target such defects. Altered energy metabolism in cancer, in which ATP production occurs largely by glycolysis, rather than by oxidative phosphorylation, is attributable in part to the upregulation of cell survival signaling cascades. These pathways also regulate the balance between pro- and anti-apoptotic factors that may determine the rate of cell death and proliferation. A number of anticancer drugs have been developed that target these factors and one of the most promising groups of agents in this regard are the lipid-based molecules that act directly or indirectly at the mitochondrion. These molecules have emerged in part from an understanding of the mitochondrial actions of naturally occurring fatty acids. Some of these agents have already entered clinical trials because they specifically target known mitochondrial defects in the cancer cell.

Keywords  N-acylethanolamines, cancer cell, ether phospholipids, fatty acid biotransformation, free fatty acids, intrinsic pathway of apoptosis, mitochondrial ATP production, polyunsaturated fatty acid epoxides, reactive oxygen species

Abbreviations  Caspase, cysteine-aspartic protease; CLA, conjugated linoleic acid; COX, cyclooxygenase, CYP, cytochrome P450; EET, epoxyeicosatrienoic acid; EGFR, epidermal growth factor receptor; ERK, extracellular signal-regulated kinase; HETE, hydroxyeicosatetraenoic acid; JNK, Jun-N-terminal kinase; LOX, lipoxygenase; MAP kinase, mitogen-activated protein kinase; PI3K, phosphatidylinositol 3’ kinase; PG, prostaglandin; PUFA, polyunsaturated fatty acid; ROS, reactive oxygen species; TTA, tetradecylthioacetic acid
Mitochondrial function: Introduction

The mitochondrion has a number of critical homeostatic functions, including the production of energy as ATP, the control of apoptotic cell death and viability, and the biosynthesis of molecules such as steroids and lipids that perform regulatory and structural roles in cells (Kroemer et al., 2007). Mitochondria are also the major source of reactive oxygen species (ROS) that determine the intracellular redox state and modulate cell proliferation and apoptosis. It is increasingly recognized that disease processes, such as tumourigenesis and the metabolic syndrome, are associated with mitochondrial dysfunction (Kroemer et al., 2007). A detailed understanding of how the regulation of mitochondrial function is altered during cancer progression may provide opportunities for drug design strategies that target underlying defects in disease.

Mitochondrial energy production and altered ATP generation in cancer cells

The role of the mitochondrion in energy metabolism is well established and glucose is the primary fuel molecule utilized by the cell in ATP production (Kroemer et al., 2007). The initial glycolytic step occurs in the cytoplasm of the cell and generates pyruvate that enters the mitochondrion where it is converted to citrate; together these two reactions produce four molecules of ATP from each molecule of glucose. In addition, four large multi-protein respiratory complexes in the inner mitochondrial membrane work in concert to generate a much larger number of ATPs by oxidative phosphorylation (~28-32 per glucose molecule). Simultaneously this builds up the proton gradient across the inner and outer mitochondrial membranes that drives many mitochondrial processes.

Fats and proteins may also be utilized by the mitochondrion to produce ATP (Kroemer et al., 2007). Triglyceride esters in adipose tissue are hydrolysed to free fatty acids that undergo mitochondrial β-oxidation to acetyl-CoA units that are then able to enter the citric acid cycle and generate ATP. The oxidative deamination of amino acids produces up to 15% of total metabolic energy in animals. In the initial phase, amino acids are converted to a series of intermediary
molecules - pyruvate, α-ketoglutarate, succinyl-CoA, fumarate, oxaloacetate, acetyl-CoA and acetoacetate – that may then enter the citric acid cycle and generate ATP.

Whereas normal cells generate much of their ATP by oxidative phosphorylation, aggressive cancer cells exhibit pronounced bioenergetic differences and overproduce lactate even under normoxic conditions by “aerobic glycolysis” (Rossignol et al., 2004). This occurs because most of the pyruvate formed by glycolysis is unable to enter the mitochondrion, and is instead converted to lactate by cytosolic lactate dehydrogenase.

**Mitochondria and ROS production: activation of the intrinsic pathway of apoptosis**

The ROS H$_2$O$_2$, superoxide (O$_2^-$), and hydroxyl radical (OH$^-$) are generated by mitochondrial respiratory complexes during uncoupled substrate turnover in the process of ATP formation (Cadenas and Davies, 2000; Hanahan and Weinberg, 2011). Premature leakage of electrons from respiratory complexes, rather than coupled transfer during ATP synthesis, leads to superoxide formation (Skulachev, 1998; Di Paola and Lorusso, 2006; Pike et al., 2011). The mitochondrion is not only the main site of ROS generation it is also their primary target. The generation of ROS has several consequences in cells, including direct peroxidative damage to membranes and modification of DNA bases that may initiate mutagenesis (Larsen et al., 2005; Nathan and Cunningham-Bussel, 2013). ROS also modulate cellular redox homeostasis (Higdon et al., 2012) and decrease the concentration of thiol-containing species, such as glutathione, that are cytoprotective (Mari et al., 2009). Indeed, glutathione depletion decreases the integrity and activities of mitochondrial respiratory complexes, which compromises ATP production and cell viability (Merad-Boudia et al., 1998). More recently it has been recognized that ROS are also able to activate the Jun-N-terminal kinase (JNK; Trachootham et al., 2006) and p38 mitogen-activated protein (MAP) kinase (Ito et al., 2006) signaling pathways that may trigger apoptotic cell death.

Apoptosis is a coordinated cell-death program that is important in normal tissues (Fulda and Debatin, 2006). There are two major pathways of apoptosis: the extrinsic and intrinsic pathways. In
the extrinsic pathway tumour necrosis factor family receptors at the plasma membrane are activated by FasL and related ligands and modulate intracellular signaling pathways leading to cell deletion. The intrinsic, or mitochondrial, pathway of apoptosis is activated intracellularly by ROS, the inhibition of pro-survival signaling cascades, or by major cellular stresses, including DNA damage from exposure to cytotoxic anticancer drugs.

The relative ratio of pro- and anti-apoptotic Bcl-2 family proteins is a determinant of the response of cells to apoptotic stimuli (Fulda and Debatin, 2006). Thus, pro-apoptotic Bcl-2 proteins, such as Bax, Bak and Bid, form dimers that destabilize the outer mitochondrial membrane to apoptotic stimuli by forming channels that allow mitochondrial factors to exit, whereas anti-apoptotic members such as Bcl-2, Bcl-XL and Bcl-w stabilize the membrane. Thus, cytochrome c, Smac/Diablo or HtrA2/Omi are released to the cytosol, which triggers the commitment step of the mitochondrial apoptotic cascade (Kluck et al., 1997; Yang et al., 2003). The intrinsic and extrinsic apoptotic pathways converge on the executioner cysteine-aspartic acid proteases (caspases)-3 and 7, which lyse a number of cellular protein targets, including the DNA-repair protein poly (ADP-ribose) polymerase; inactivation of the latter promotes apoptosis (Fulda and Debatin, 2006). However, release of Smac/Diablo or HtrA2/Omi from the mitochrondrion does not directly activate caspase; rather these factors block the action of the protein inhibitor of apoptosis that normally acts to suppress caspases. Finally, there is also a caspase-independent mitochondrial pathway in which apoptosis-inducing factor and endonuclease G are released to the cytosol (Susin et al., 1999). The translocation of these factors leads to DNA fragmentation which is the hallmark of apoptosis.

Mitochondrial permeability is controlled by the permeability transition pore complex which regulates the flow of small molecules across the mitochondrial membrane. Pore opening occurs in response to elevated Ca^{2+} concentrations, increased ROS and depleted adenine nucleotides (Kroemer et al., 1997). Permeability transition pore opening in turn dissipates the proton gradient, uncouples oxidative phosphorylation and decreases ATP formation. In consequence, antioxidant molecules such as glutathione exit the mitochondrion which further decreases the capacity to
detoxify locally generated ROS (Mari et al., 2009). The release of cytochrome c from the mitochondrion sets in train the activation of apoptotic death.

The permeability transition pore is a complex arrangement of proteins including the adenine nucleotide translocator, which exchanges ATP and ADP (Marzo et al., 1998a; Brenner et al., 2000), the voltage-dependent anion channel (also termed porin), the soluble mitochondrial matrix protein cyclophilin D (Woodfield et al., 1998), a number of Bcl-2 family proteins (Marzo et al., 1998b; Shimizu et al., 1999), the peripheral benzodiazepine receptor and several proteins that regulate energy metabolism (e.g., hexokinase II and creatine kinase) (Marzo et al., 1998a). Several genes encoding components of the mitochondrial permeability transition pore including the peripheral benzodiazepine receptor, the associated protein Prax-1, and the energy-metabolising enzyme creatine kinase, are overexpressed in some tumours (Kanazawa et al., 1998; Venturini et al., 1998; Galiegue et al., 1999).

While the pro-apoptotic Bax may be down-regulated in cancer cells (Brimmell et al., 1998), Bcl-2 or its anti-apoptotic homologues are frequently overexpressed (Kroemer et al., 1997). This shifts the balance in the cancer cell toward the prevention of apoptosis. Thus, an important adaptation is that the phosphatidylinositol 3-kinase (PI3K)/Akt signaling cascade inhibits pro-apoptotic Bcl-2 factors and positively regulates the anti-apoptotic factor Bcl-2 (Skorski et al., 1997; Pugazhenthi et al., 2000). As described above, this shift in Bcl-2 factor composition stabilizes the outer mitochondrial membrane. PI3K/Akt has additional survival actions in tumour cells in that it is able to impair signaling by the pro-apoptotic JNK MAP kinase, enhance glucose uptake by transporters and activate glycolytic enzymes, such as hexokinase (Kroemer et al., 1997; Skorski et al., 1997; Pugazhenthi et al., 2000). These adaptations shift the capacity for energy production toward the glycolytic pathway that characterises the cancer cell.

**Biosynthetic roles of the mitochondrion in normal and cancer cells**
Some important steps in lipid metabolism occur in the mitochondrion. While fatty acid synthesis occurs primarily in the cytoplasm, β-oxidation to produce ATP occurs in mitochondria. However, the mitochondrion also has a central role in the biosynthesis of phospholipids and triglycerides. The first step in the phospholipid synthesis pathway is the esterification of α-glycerol phosphate by acyl-CoA to produce lysophosphatidic and phosphatidic acids (Zborowski and Wojtczak, 1969; Bremer et al., 1976). Some phospholipids, such as phosphatidylcholine, phosphatidylserine and phosphatidylinositol, are synthesized in other organelles and transported to the mitochondrion where further biotransformation occurs. Cardiolipin, a characteristic phospholipid of the inner mitochondrial membrane, is synthesized within this organelle (Hostetler and van den Bosch, 1972).

In mammals, fatty acids are activated to acyl-CoAs on the outer mitochondrial membrane before entering the glycerolipid biosynthetic pathway via glycerol-3-phosphate acyltransferase. Highly proliferative cancer cells have an increased requirement for lipids for the assembly of cell membranes (Samudio et al., 2009; Zaugg et al., 2011). Increased expression of fatty acid synthase in tumour cells promotes formation of long-chain fatty acids and confers a growth advantage (Sabine et al., 1967; Ookhtens et al., 1984). Indeed, fatty acid synthase inhibition decreases the rate of cancer cell proliferation and promotes apoptosis, as reflected by increased caspase-3 activation, down-regulation of anti-apoptotic proteins and the release of cytochrome c (Pizer et al., 1998; De Schrijver et al., 2003).

**Lipid synthesis and biotransformation in cells**

Fatty acids perform structural roles in cells by modulating membrane fluidity and the arrangement of receptors and other proteins in plasma membrane lipid rafts (Spector and Yorek 1985). Naturally occurring long-chain fatty acids of 18-22 carbons in length are stored in cell membranes as triglycerides or phospholipids. Triglycerides are composed of three fatty acid residues connected to the hydroxyl groups of glycerol via ester linkages (Figure 1a).
Phosphoglycerides are the major phospholipid class, and are comprised of five components: two fatty acids, glycerol, a phosphate and an alcohol (Figure 1b). Subclasses are designated according to the alcohol group; thus phosphatidylcholines contain the choline group (Figure 1c). Other common alcohols include serine, ethanolamine, and inositol. Phosphatidates share this same basic structure but lack the alcohol group, and are intermediates in the biosynthesis of many phosphoglycerides. The glycerol group may also be substituted with sphingosine (2-amino-4-octadecene-1,3-diol), giving rise to sphingomyelins.

Constituent fatty acids in membranes are either saturated (contain no carbon-carbon double bonds, as exemplified by palmitic acid (C16:0)), mono-unsaturated (contain only one olefinic bond; such as oleic acid (18:1 n−9) or polyunsaturated (PUFAs, that possess multiple olefinic bonds, typified by ω-6 arachidonic acid (20:4 n-6) and ω-3 eicosapentaenoic acid (20:5 n-3); Figure 1d). The double bonds in naturally occurring unsaturated fatty acids are primarily in the cis configuration, which kinks the carbon chain and imparts greater membrane fluidity.

The carbon atoms of fatty acid chains are numbered starting from the carboxyl group and the carbon at the distal end of the molecule is the ω-carbon. Monounsaturated fatty acids and PUFA are classified by the position of the olefinic bond that is furthest from the carboxylate, and this is indicated in the lipid number notation. Thus, arachidonic acid (20:4 n-6) is a C20 fatty acid that has four olefinic bonds, with the double bond furthest from the carboxylate located six bonds from the ω-carbon (to produce a ω-6 olefinic bond).

Release of fatty acids from their esterified phospholipid forms in cell membranes is mediated by phospholipase A2. Free PUFAs are substrates for cyclooxygenase (COX), lipoxygenase (LO) and cytochrome P450 (CYP) enzymes that generate multiple metabolites that have diverse homeostatic functions (Figure 2; Spector and Yorek 1985; Oates et al., 1988; Oliw, 1994; Chen et al., 1995; Murray, 1999; Marden et al., 2003). Together, these enzymes produce prostaglandins, thromboxanes, fatty acid peroxides and their downstream leukotrienes, hydroxyfatty
acids and epoxides. In comparison, saturated fatty acids primarily undergo CYP-dependent oxidation to the corresponding ω- and ω-1 hydroxy acids.

Apart from free fatty acids there is evidence that certain lipids may be present at increased levels in cancer cells. Thus, phosphocholine derivatives are increased in cancer cells and solid tumours (Ackerstaff et al., 2003) and plasma triglycerides are reportedly higher in women with invasive breast cancers (Goodwin et al., 1997). Some important enzymes involved in fatty acid biotransformation are also differentially expressed in cancers. Over-expression of COX and CYP enzymes promotes the formation of certain prostaglandin and epoxide metabolites that influence the fate of cells, including the rate of proliferation and the inhibition of apoptosis (Tsujii et al., 1997; Jiang et al., 2005). Moreover PUFA-derived metabolites modulate signaling pathways such as the extracellular signal-regulated kinase (ERK) and PI3K/Akt that have been implicated in tumourigenesis. As discussed below, inhibition of the formation of these PUFA metabolites modulates apoptosis and other aspects of tumour growth (Chen et al., 2009). Moreover, inhibition of the enzymes that mediate PUFA biotransformation has proven successful in the prevention of experimental tumour progression in vitro and in vivo (Koehne and DuBois, 2004; Chen et al., 2009). However, at present there is a deficiency of inhibitory agents that are well tolerated over the prolonged treatment periods required during anticancer chemotherapy.

The potential of lipids as anticancer agents that act at the mitochondrion

Fatty acids are thought to uncouple oxidative phosphorylation and decrease ATP production by facilitating the leakage of protons across the lipid mitochondrial membrane. Uncoupling is greatest with C12–C16 saturated and longer cis-unsaturated fatty acids (Korshunov et al., 1998; Bernardi et al., 2002). For example, 5 µM laurate (C12-saturated) effectively inhibited H₂O₂ production by mitochondria (Korshunov et al., 1998). Free fatty acids also impair electron transport and activate apoptosis by releasing cytochrome c from the inner mitochondrial membrane. There is increasing evidence that several classes of naturally occurring and synthetic lipids have the potential
for development as anticancer agents that mediate their effects, at least in part, at the mitochondrion. However, some of these lipid-mediated anti-mitochondrial actions may be indirect, by modulating intracellular signaling pathways.

\textit{(a) Naturally occurring free fatty acids and synthetic analogues}

(i) Saturated fatty acids

In the non-ionised state medium and long-chain fatty acids readily penetrate the mitochondrial membrane (McLaughlin and Dilger, 1980; Gutknecht, 1988; Kamp and Hamilton, 1992). Butyric acid and similar short-chain fatty acids induce cell cycle arrest and apoptosis by dissipating the mitochondrial membrane potential ($\Delta \Psi$), leading to growth arrest and apoptosis in colon carcinoma cells \textit{in vitro}, as evidenced by activation of caspase-3 (Heerdt \textit{et al.}, 1997). In a small structure-activity study, the C4-butyric acid inhibited the growth of the human colonic adenocarcinoma cell lines HT-29, Colo-320, and SW-948 ($IC_{50}$s 0.55-2.28 mM), while C3-propionic acid was less potent and C5-valeric and C2-acetic acids were ineffective (Figure 3, Table 1; Milovic \textit{et al.}, 2000). The apoptotic mechanism of butyric acid in colon cancer cells is mediated at least in part by inducing overexpression of the pro-apoptotic protein bak, which alters the bak/bcl-2 ratio (Hague \textit{et al.}, 1997; Ruemmele \textit{et al.}, 1999), but not by modulating ROS or ATP production (Heerdt \textit{et al.}, 1997). Bromo-analogues of butyric and propionic acids ($IC_{50}$s 0.13-0.39 mM) were several-fold more potent pro-apoptotic agents than butyrate, but also produced some cytotoxicity, so cautious development of these molecules as potential mitochondrially-targeted agents is warranted (Milovic \textit{et al.}, 2000).

Longer-chain saturated fatty acids also act at the mitochondrion to activate apoptosis. Palmitic acid (C16:0) decreased $\Delta \Psi$ and effected cytochrome c release, which induced the proteolysis of poly-ADP ribose polymerase and the fragmentation of DNA (de Pablo \textit{et al.}, 1999). This may also be a process of endogenous importance because long chain fatty acids can be generated intracellularly by activation of phospholipase A$_2$. In addition, such free fatty acids may
also accumulate in mitochondria following exposure of cells to stimuli such as oxidative stress or increased Ca$^{2+}$ concentrations (Broekemeier and Pfeiffer, 1995).

13-Methyltetradecanoic acid is an iso-C$_{15}$ branched-chain saturated fatty acid that has been found to disrupt mitochondrial integrity and induce apoptosis in a wide range of cancer cell lines (Figure 3, Table 1; Yang et al., 2000; Wongtangtintharn et al., 2005; Lin et al., 2012). Induction of apoptosis by 13-methyltetradecanoic acid was rapid and was detected after only 2 hours of treatment over the concentration range 0.04-0.35 mM (Lin et al., 2012). In some cell types apoptosis appeared to be caspase-independent pathway but, in human bladder cancer cells, 13-methyltetradecanoic acid down-regulated Bcl-2, up-regulated Bax, promoted mitochondrial dysfunction and cytochrome c release, and activated caspases (Lin et al., 2012). 13-Methyltetradecanoic acid also inhibited the PI3K/Akt survival cascade and activated the pro-apoptotic p38 and JNK MAP kinase pathways (Lin et al., 2012).

In vivo growth of the prostate carcinoma cell line DU 145 and hepatocarcinoma-derived LCI-D35 cells after orthotopic implantation of tumour xenografts into nude mice was also inhibited by 13-methyltetradecanoic acid (35-105 mg/kg/day). Apoptosis was induced without evidence of major toxicity, which suggests that 13-methyltetradecanoic acid could be a potential candidate for chemotherapy of human cancers (Yang et al., 2000).

(ii) Unsaturated fatty acids

Naturally occurring PUFAs also modulate cell proliferation and apoptosis. The $\omega$-3 PUFA eicosapentaenoic acid (20:5 n-3) is incorporated into mitochondrial phospholipids, which has a number of consequences for mitochondrial function, including decreased mitochondrial membrane potential and ATP production, increased ROS generation and increased apoptosis (Colquhoun, 2009). $\omega$-6 PUFA also have the potential to activate apoptosis in human cancer cell lines by promoting lipid peroxidation (Cao et al., 2000).

Conjugated linoleic acid (CLA) is a mixture of geometric isomers of linoleic acid (Figure 3) that decreases the viability of various cancer cell types, including those of the skin, forestomach,
mammary gland and colon. CLA (32 µM), but not linoleic acid, inhibited growth of rat mammary epithelial cell organoids that was mediated both by a decrease in DNA synthesis and increased apoptosis (Ip et al., 1999). In vivo activity was also noted and feeding CLA to rats that harboured premalignant lesions induced apoptosis in a mammary tumour cell line, as determined by a decrease in expression of the anti-apoptotic bel-2 (Table 1; Ip et al., 2000). Cho et al. (2003) showed that CLA inhibited the proliferation of HT-29 human colorectal cancer cell line by activating apoptosis, due in part to inhibition of PI3K/Akt signalling. Similarly, the 10-trans,12-cis CLA isomer (5 µM) inhibited the proliferation of Caco-2 colon carcinoma cells, and enhanced apoptosis in premalignant lesions, but not in normal cells (Kim et al., 2002).

Other types of naturally-occurring conjugated fatty acids that have pro-apoptotic actions include conjugated linolenic acids such as α-eleostearic acid (9-cis,11-trans,13-trans-18:3) from bitter gourd oil and calendic acid (8-trans,10-trans-12-cis-18:3) from pot marigold. α-Eleostearic acid was quite potent relative to CLA isomers against tumour cells in vitro (Tsuzuki et al., 2004). α-Eleostearic acid (40 µM) promoted lipid peroxidation and apoptosis in MDA-MB-231-derived cell lines as evidenced by a loss of mitochondrial membrane potential and the release of apoptotic factors from the mitochondrion. When treated with α-eleostearic acid in the presence of α-tocotrienol (20 µM), growth inhibition and apoptosis did not occur, thus providing further support for involvement of ROS-mediated lipid peroxidation in the apoptotic mechanism (Grossmann et al., 2009).

The monounsaturated fatty acid vaccenic acid (11-trans-18:1 n-7) decreased cell growth, induced DNA fragmentation and depleted cytosolic glutathione levels; these findings are consistent with activation of the intrinsic pathway of apoptosis by lipid peroxides (Miller et al., 2003). Punicic acid (18:3 n-5) is an ω-5 long chain PUFA found in pomegranate seed oil. In MDA-MB-231 and MDA-ERα7 cells punicic acid disrupted the mitochondrial membrane potential and induced apoptosis, apparently also by a prooxidant mechanism (Grossmann et al., 2010). DNA
fragmentation was observed after 24 h treatment of cells at concentrations in the range 10-100 µM (Gasmi and Sanderson, 2010).

Jacaric acid is a linolenic acid isomer obtained from jacaranda that has a conjugated triene system and elicits potent antitumour effects both in vitro and in vivo in nude mice into which DLD-1 cells had been xeno-transplanted (Shinohara et al., 2012). When compared with natural conjugated linolenic acids in DLD-1 adenocarcinoma cells the antitumour effects of jacaric acid were most potent and correlated with increased ROS production. Thus, jacaric acid induced concentration- and time-dependent LNCaP cell death in part through activation of intrinsic apoptotic pathways, resulting in cleavage of caspase-3, -8 and -9, modulation of pro- and anti-apoptotic Bcl-2 family of proteins and increased cleavage of poly (ADP-ribose) polymerase-1 (Gasmi and Sanderson, 2013).

Several dietary C18 unsaturated fatty acids have been tested for cytotoxicity and induction of apoptosis in human prostate cancer cells. These included three octadecatrienoic geometric isomers (α- and β-calendic and catalpic acids) and two mono-unsaturated C18 fatty acids (trans- and cis-vaccenic acid) in addition to jacaric and punicic acids (Figure 3, Table 1). Jacaric acid and four of its octadecatrienoic acid geometric isomers selectively induced apoptosis in both hormone-dependent (LNCaP) and hormone-independent (PC-3) human prostate cancer cells when tested at concentrations around 10 µM, without affecting the viability of normal human prostate epithelial cells (Gasmi and Sanderson, 2013). Together these findings suggest that some of the pro-apoptotic actions of antitumour fatty acids may be cell type-specific.

From the foregoing it is evident that most studies to date have focused on ROS generation by unsaturated fatty acids as the mechanism of their pro-apoptotic action. However, there may be additional, more selective, mechanisms that could be developed in synthetic anticancer fatty acids. In a recent study, a series of n-3 monounsaturated fatty acids of chain length C16-C22 was synthesized and evaluated in MDA-MB-468 breast cancer cells that stably overexpressed COX-2 (Cui et al., 2012). This reflects the situation that may operate in many human cancers in which
COX-2 is upregulated. The longer chain C19-C22 analogues were found to inhibit proliferation and activate apoptosis; C16-C18 analogues were less active. PGE\(_2\) formation was decreased by the C19-C22 analogues, consistent with COX-2 inhibition, which was supported by molecular modeling that revealed effective interactions with specific amino acid residues in the COX-2 active site. Strategies of this type, in which potential fatty acid drugs target a biotransformation enzyme present in tumours, may be worthy of further consideration. Such agents may enable approaches based on COX-2 inhibition to be retained, perhaps without the toxicity associated with conventional non-steroidal anti-inflammatory drugs.

(iii) Fatty acid analogues

Tetradecylthioacetic acid (TTA) is a saturated fatty acid that has a sulphur atom inserted at the C3 position in the carbon chain. TTA (0.2-0.5 mM) decreased proliferation and induced apoptosis in a diverse range of tumour cell lines \textit{in vitro} and \textit{in vivo} (Tronstad \textit{et al.}, 2003; Iversen \textit{et al.}, 2006). Long-chain 3-thia-fatty acids in general also uncouple oxidative phosphorylation and dissipate the mitochondrial membrane potential (\(\Delta\Psi\)) by direct interaction with the adenine nucleotide translocator to open the mitochondrial permeability transition pore, which leads to decreased ATP production (Wieckowski and Wojtczak, 1998). In accord with this mechanism, TTA stimulates mitochondrial ROS production (Tronstad \textit{et al.}, 2001), leading to glutathione depletion which renders mitochondria susceptible to further damage (Tronstad \textit{et al.}, 2003). Activation of apoptosis was indicated by the release of mitochondrial cytochrome c that enhanced caspase-3 activation and poly (ADP ribose) polymerase cleavage.

TTA is resistant to mitochondrial \(\beta\)-oxidation and, compared with naturally occurring saturated fatty acids, is degraded relatively slowly to dicarboxylic acids by microsomal oxidation at the \(\omega\)-carbon and sulphur atoms and by peroxisomal \(\beta\)-oxidation (Hvattum \textit{et al.}, 1991). This property could be useful in development of TTA and analogues as drugs since these molecules are likely to have superior durations of action \textit{in vivo}. Indeed, a diet containing TTA increased the
vascularisation of colon cancer xenografts in mice and improved the survival of mice with leukemia xenografts (Jensen et al., 2007).

Jasmonates are plant hormones that structurally resemble fatty acid esters (Figure 3). These agents interact directly with mitochondria in cancer cells to detach hexokinase-II from its location on the voltage-dependent anion channel in the mitochondrial permeability transition pore (Goldin et al., 2008). Indeed, the susceptibility in cancer cells to these molecules is dependent on the association of hexokinase with the mitochondrion. Pro-apoptotic mitochondrial actions of low millimolar concentrations of jasmonates include membrane depolarization, mitochondrial swelling, cytochrome c release and cell death; interestingly the agents were inactive in normal cells (Table 1; Fingrut and Flescher, 2002; Rotem et al., 2005).

Correlations have been reported between methyl jasmonate cytotoxicity in a range of cell types and the extent of ATP depletion (Goldin et al., 2007). Glucose protected against this loss of ATP, whereas the glycolysis inhibitor, 2-deoxyglucose, synergised with methyl jasmonate (Fingrut et al., 2005; Heyfets and Flescher, 2007). Similar effects have been elicited by other hexokinase-detaching agents, such as hypericin and clotrimazole, that also deplete ATP and decrease cell viability (Miccoli et al., 1998; Machida et al., 2006). These findings are consistent with the functional importance of hexokinase in glycolysis, which is a major pathway of ATP production in cancer cells.

Taken together, a number of studies have found that endogenous fatty acids, including saturated and certain unsaturated analogues, activate apoptosis via the mitochondrial intrinsic pathway and impair ATP production. Frequently this may be due to increased ROS production which alters mitochondrial membrane potential, but the example of jasmonate, involving hexokinase detachment, illustrates the potential for further development of mitochondrially-targeted fatty acids that selectively disrupt energy metabolism in cancer cells.

(b) Fatty acid metabolites
Several PUFA-derived metabolites have been shown to modulate signaling pathways that are implicated in tumourigenesis. Thus, inhibition of the enzymic formation of these fatty acid metabolites has been found to decrease cancer cell viability. Important metabolites that have emerged in this regard include COX-mediated PGE$_2$, CYP-dependent epoxyeicosatrienoic acids (EETs) and certain LOX-mediated hydroxyeicosatetraenoic acids that inhibit apoptosis and enhance proliferation (Avis et al., 2001; Koehne and DuBois, 2004; Chen et al., 2009). An understanding of the involvement of fatty acid metabolites in the pathogenesis of cancer could open new avenues for the production of new and safer therapeutic and chemopreventive agents.

Certain fatty acid biotransformation enzymes have been detected at high level in human cancers. Thus, COX-2 and CYP2J2 are over-expressed in many invasive human cancers (Tsujii et al., 1997; Jiang et al., 2005). COX-2-derived PGE$_2$, LOX metabolites and CYP2J2-derived EETs are implicated in aggressive tumour behavior (Figure 4, Table 1; Avis et al., 2001; Koehne and DuBois, 2004; Hoque et al., 2005; Jiang et al., 2005). Mechanistic information is available for some of these metabolites. Thus, PGE$_2$ and EETs enhance tumour cell proliferation and survival by activating the proliferative epidermal growth factor receptor (EGFR)/ERK MAP kinase and anti-apoptotic PI3K/Akt signaling pathways (Chen et al., 2009).

Inhibition of 5-LOX by MK886 induces apoptosis in both hormone-responsive (LNCaP) and hormone-unresponsive (PC3) prostate cancer cells (Ghosh and Myers, 1998). An immediate and sustained rise in cytosolic calcium is followed by mitochondrial uncoupling and cytochrome c release (Maccarrone et al., 2001). Soon after treatment cells underwent the mitochondrial permeability transition, followed by other apoptotic events, including externalization of phosphatidylserine and degradation of DNA (Ghosh and Myers, 1998). Cell death was prevented by direct addition of 5-HETE and analogues such as 5-HETE-lactone and 5-oxoeicosatetraenoic acid (50-500 nM; Ghosh and Myers, 1998). In breast cancer cells inhibition of 5-LOX, but not COX, reduced growth, increased apoptosis, down-regulated bcl-2, up-regulated bax, and caused G$_1$ cell
cycle arrest (Avis et al., 2001). Lipid peroxidation and the depletion of mitochondrial glutathione have been linked to the activation of apoptotic Bcl-2 proteins in these cells.

While the inhibition of biotransformation enzymes that generate lipid metabolites may be beneficial in the prevention of cancer progression, there is also increasing evidence emerging that ω-3 PUFAs undergo biotransformation to metabolites that inhibit carcinogenesis. Inhibition of protein kinase C by the parent ω-3 PUFAs eicosapentaenoic and docosahexaenoic acids activates apoptosis by down-regulating Bcl-2 (Denys et al., 2005); COX-2-derived ω-3 PGE₃ was recently implicated in anti-cancer actions of ω-3 PUFAs (Szymczak et al., 2008). Epoxides of ω-3 PUFAs have also been shown to exert growth suppressing and anticancer effects. The 17,18-epoxide of eicosapentaenoic acid (ω-3-epoxy-eicosapentaenoic acid), but not other regio-isomeric epoxides of eicosapentaenoic acid, was shown to inhibit cell proliferation. At physiologically relevant concentrations, ω-3-epoxy-eicosapentaenoic acid induced apoptosis and cell cycle arrest of brain microvascular endothelial bEND.3 cells through activation of growth suppressing p38 MAP kinase and subsequent down-regulation of cyclin D1 (Cui et al., 2011). More recently, Zhang et al. (2013) showed that epoxygenase-mediated metabolites of the C22 ω-3 fatty acid docosahexaenoic acid exerted anticancer effects by suppressing vascular endothelial growth factor-mediated angiogenesis. Inhibition of angiogenesis resulted in a decrease in primary tumour growth and metastasis in vitro. By co-administration with t-AUCB, a soluble epoxide hydrolase inhibitor, the 19,20-epoxide was active in vivo, reducing tumour growth in the Lewis lung carcinoma model (Zhang et al., 2013). These actions were opposite to those of ω-6 arachidonic acid-derived EETs. However, at present, whether PUFA epoxides also interact directly with the mitochondrion, in addition to their indirect actions mediated by signaling cascades, to elicit these actions is unclear.

(c) N-acyllipids

(i) ceramides

Ceramides are a class of N-acylated sphingoid bases (Figure 4) found at high concentration in cell membranes and cytosolic organelle membranes and act as intracellular second messengers in
the regulation of growth, differentiation, and apoptosis. Ceramide accumulation occurs after treatment of cells with apoptotic agents including chemotherapeutic agents, or after treatment with saturated fatty acids such as palmitic acid (Merrill and Jones, 1990). Direct addition of ~1 μM ceramide to mitochondria and ceramide accumulation in cells both produced changes in the mitochondrial transmembrane potential by forming channels or targeting Bcl-2 family members that leads to translocation of cytochrome c from mitochondria to the cytoplasm, and caspase-3 activation (Garcia-Ruiz et al., 1997). Because respiratory complex III is inhibited by C2-ceramide the proximal effects of ceramide in cells are mediated at least in part at the mitochondrion (Gudz et al., 2007). Inhibition of p38 and JNK MAP kinases decreased ceramide-induced apoptosis by preventing the loss of mitochondrial transmembrane potential and inhibition of caspase activation (Chen et al., 2008). This suggests that ceramides may operate by both direct and indirect mechanisms to exert apoptotic actions at the mitochondrion.

In tumour cells the cell-permeable shorter-chain exogenous C2- and C6-ceramide analogs, but not the longer-chain, naturally-occurring C16-ceramide, activated intrinsic apoptotic events at concentrations ≥ 10 μM, including caspase-3 activation, poly (ADP-ribose) polymerase degradation, and mitochondrial cytochrome c release (Fillet et al., 2003; Flowers et al., 2012). C6-ceramide increased ROS levels in MDA-MB-231 cells, shifted the Bax:Bcl-2 ratio and depolarized the mitochondrial membrane (Flowers et al., 2012). Thus, analogues containing fatty acids of medium chain length may be particularly suited to development as putative anticancer agents that target the mitochondrion.

(ii) N-Acylethanolamines
Anandamide (Figure 4) and other N-acylethanolamines reportedly promote apoptosis and/or inhibit cell proliferation (Table 1; Schwarz et al., 1994; De Petrocellis et al., 1998; Maccarrone et al., 2000; Sarker et al., 2000). These molecules appear to act in part by increasing the inner mitochondrial membrane permeability at concentrations up to 100 μM (Epps et al., 1982). Anandamide and N-oleylethanolamine exerted protonophoric effects in mitochondria due to
dissipation of the transmembrane potential and opening of the permeability transition pore (Wasilewski et al., 2004). Long-chain N-acylethanolamines, including anandamide, accumulate in mammalian tissues under a variety of pathological conditions (Schmid et al., 2002). Indeed they have been detected at levels up to 500 nmol/g of infarcted canine myocardium. They have also been shown to inhibit the growth of various cancer cell lines in vitro. Cancer tissues usually contain substantially higher concentrations of these lipids than adjacent benign tissue, when normalized to wet weight, since most tumours also contained higher levels of phospholipids.

Despite these apparent pro-apoptotic effects of N-acylethanolamines in cancer cells there is also evidence for protective effects in cells. The long-chain N-palmitoyl- and N-stearoylethanolamine (100 µM) inhibited lipid peroxidation in hepatic mitochondria, which is consistent with membrane protective properties (Gulaya et al., 1998). The beneficial effect of N-acylethanolamines on cell survival when oxygen availability is low depends in part on the inhibition of lipid oxidation. The effect of different N-acylethanolamines on lipid peroxidation seems to be dependent on the length of acyl chain as was found for other effects of N-acylethanolamines in the cell (Gulaya et al., 1993). These findings highlight the need for clarification of the relationships between chain length in N-acylethanolamines and their cellular actions prior to development of analogues as potential anticancer drugs.

Very recently novel fatty acid derivatives of isopropylaminopropanol containing C16:0 or C18:1 substituents were prepared and were found to be effective against the growth of hepatoma cells in vitro (IC₅₀s ~5-10 µM) and in vivo xenografts when dosed at 25 mg/kg (Cao et al., 2013). These agents inhibited the activity of multiple kinases, including the prosurvival Akt, which increased caspase and poly (ADP-ribose) polymerase cleavage. It will now be of interest to explore in greater detail how these agents modulate mitochondrial activity to elicit anticancer actions.

(d) Phospholipid derivatives

(i) Cardiolipin
Cardiolipin is a structurally complex diphosphatidylglycerol lipid that is synthesised by the mitochondrion (Figure 4). Mitochondrial respiratory complexes have been shown to require cardiolipin for full function. Palmitic acid decreased the levels of cardiolipin, which is responsible for insertion and retention of cytochrome c in the inner membrane of the mitochondrion (Schlame et al., 2000; Ostrander et al., 2001). Decreased cardiolipin and altered mitochondrial function mediate palmitate-induced breast cancer cell death by promoting ROS production and release of cytochrome c by permeabilization of the outer membrane (Petrosillo et al., 2003); overexpression of the anti-apoptotic Bcl-2 family members Bcl-xL and Bcl-w blocked apoptosis (Kuwana et al., 2002).

(ii) ether phospholipids

The ether phospholipids are a promising class of antitumour lipids that act in part at the mitochondrion. Ether phospholipids have one or more glycerol carbons bonded to an alkyl chain via an ether linkage, as opposed to the usual ester linkage. Ether phospholipids include miltefosine, ilmofosine, perifosine, edelfosine and erucylphosphocholine (Figure 4).

The novel alkylphospholipid analog perifosine is a PI3K/Akt inhibitor (Table 1; Kondapaka et al., 2003) that inhibits growth at low micromolar concentrations and activates the intrinsic pathway of apoptosis in cells as evidenced by increased caspase activity and cleavage of poly(ADP-ribose) polymerase. Activated caspase-8 cleaves Bid which migrates to mitochondria and induces cytochrome c release (Chiarini et al., 2008). Interestingly, these agents are effective in rapidly proliferating cancer cells, but not quiescent normal cells.

Clinical evaluation of perifosine has been conducted, or is continuing, in patients with cancers of the endometrium, breast, prostate, bladder and other tissues. In 2010 perifosine was evaluated in phase II trials for metastatic colon cancer and extended the time taken for tumour progression. However, in 2013, it was announced that the drug failed trials in patients with relapsed or relapsed/refractory multiple myeloma (http://www.aezsinc.com/en/page.php?p=60&q=550, accessed June 28, 2013). When used in combination with the multikinase inhibitor sorafenib, perifosine induced intrinsic apoptosis in cells and antitumour effects in NOD/SCID mice with
Hodgkin lymphoma cell line xenografts (Locatelli et al., 2013). In cell lines the combination treatment inhibited MAP kinase, activated PI3K/Akt phosphorylation and suppressed growth; synergistic induction of mitochondrial dysfunction and cell death was noted. In in vivo xenograft studies there was a reduction in tumour burden, increased survival time, increased apoptosis and necrosis in perifosine/sorafenib-treated animals compared with single agents. Subsequently, treatment of human leukemia T cells with the PI3K/Akt inhibitor perifosine and etoposide also effected synergistic induction of apoptosis by dual activation of intrinsic and extrinsic pathways (Nyåkern et al., 2006). This combination produced a two-fold increase in caspase-8 activation, and a marked increase in caspase-9, caspase-3, and poly(ADP-ribose) polymerase cleavage, as well as increased Bim, Bid and Bcl-XL expression. Etoposide and perifosine induced leukemic cell death in part by inactivation of the PI3K/Akt pathway that increased mitochondrial dysfunction. However, it is of considerable interest that ether phospholipids proved to be highly effective when used in combination with other anticancer agents.

Edelfosine induces changes in mitochondrial membrane permeability and inhibits mitochondrial respiration (Burgeiro et al., 2013). Edelfosine, miltefosine, erucylphosphocholine and perifosine all activate the JNK MAP kinase pathway which induces apoptosis (Ruiter et al., 1999; Nieto-Miguel et al., 2006). The mechanism involves direct activation of apoptosis by phosphorylation of the anti-apoptotic Bcl-XL (Kharbanda et al., 2000; Aoki et al., 2002). Edelfosine also disrupts the mitochondrial transmembrane potential, apparently by altering mitochondrial membrane phosphocholine content (Vrablic et al., 2001), promotes the cleavage of caspase-3 and poly (ADP-ribose) polymerase, and enhances production of ROS in human leukaemic T cells (Table 1; Cabaner et al., 1999; Gajate et al., 2000). Edelfosine-induced apoptosis is blocked by Bcl-2 (Mollinedo et al., 1997; Ruiter et al., 2003; Hideshima et al., 2006).

New fluorescent edelfosine analogs retained the pro-apoptotic activity of the parent, and colocalized with mitochondria (Mollinedo et al., 2011). These agents induced the swelling of isolated mitochondria, consistent with an increase in mitochondrial membrane permeability. Free
radical scavengers did not affect swelling, suggesting that ROS do not contribute. It was suggested that edelfosine promoted a redistribution of lipid rafts from plasma membrane to mitochondria (Mollinedo et al., 2011). In summary, ether phospholipids have potential for development as a novel class of anticancer agents that act in part by altering mitochondrial function.

**Challenges in the development of lipid molecules as drugs**

Fatty acids differ from typical drugs in that there is a large degree of molecular flexibility inherent in fatty acid alkyl chains. Although fewer than 10 rotatable bonds is seen as desirable for adequate oral bioavailability and membrane permeation (Veber et al., 2002), fatty acids possess satisfactory pharmacokinetic profiles. For example, medium and long chain dietary fatty acids are readily absorbed and distributed throughout all tissues of the body including the brain. Moreover the ether phospholipid edelfosine is orally active despite possessing 27 rotatable bonds. The favorable pharmacokinetic profiles of fatty acids may be in part due to the facilitation of uptake and transport (Ramirez et al., 2001), including binding to serum albumin and incorporation into triglycerides, that distribute these essential nutrients throughout the body. However, the flexibility of fatty acids does present challenges in drug design, particularly in ligand-based approaches where the structure of the drug target is unknown. Conformational analysis is a critical step in pharmacophore and pseudo-receptor modeling, and the high number of rotatable bonds can make identification of low-energy and bioactive conformations difficult and time-consuming. When the structure of the drug target is known ligand docking approaches are straightforward, as exemplified by our recent modeling of the interactions of a series of novel ω-3 monounsaturated fatty acids in the COX-2 active site (Cui et al., 2012).

As mentioned earlier, fatty acids are subject to numerous metabolic processes mediated by COX, LOX and CYP enzymes that can present challenges for the development of lipid-derived drugs that have acceptable in vivo stability. Furthermore, lipid-derived mediators (e.g. prostaglandins, epoxides, resolvins and others) frequently possess labile functional groups that are
also readily metabolized. Improvement of the metabolic as well as chemical stability of lipid-based
drugs, particularly those derived from prostaglandins (Collins and Djuric 1993; Das et al., 2007)
and lipoxin (Duffy and Guiry 2010), has received much attention and a number of strategies have
been developed and successfully employed in drug discovery settings. These strategies commonly
involve addition of functional groups to block particular metabolic processes, or bioisosteric
replacement of labile functional groups with more robust equivalents. For example, incorporation of
a heteroatom in alkyl chains at the position β to the carboxylate functionality can improve the
duration of action in vivo, as shown by the prostaglandin analogue cicaprost (Hildebrandt et al.,
1989). These approaches have led to the development of numerous marketed drugs, which clearly
demonstrate the potential of lipids as drugs and as lead compounds in drug discovery.

**Development of new mitochondrial targeted inhibitors in cancer chemotherapy**

Evidence is increasing that fatty acids and other lipids act in part by modulation of
mitochondrial function. These actions may be direct, such as the detachment of hexokinase from the
cancer cell mitochondrial transition pore complex or by uncoupling of respiratory complexes that
produce ATP. Alternately, some lipid agents may act indirectly by increasing ROS activity in cells
or by interfering with cell signaling pathways, to perturb the balance of pro- and anti-apoptotic bel-2
proteins that regulate mitochondrial membrane stability. It should be noted, however, that
mitochondrial actions of certain lipid-based molecules may operate alongside non-mitochondrial
mechanisms, including altered membrane raft composition or altered gene regulation.

A point of major interest that has emerged from cellular studies is that fatty acid derivatives
often exhibit activity against cancer cells, but not normal cells. This appears to be a property that
augers well for new anti-cancer drug development based on lipids. The available information does
not provide a full understanding for this selectivity because the targeted pathways may be present in
both cell types. It will now be of major importance to define in detail the defects present in
mitochondria of cancer cells so that new drugs may be developed that have optimal potency with fewer off-target actions.
Acknowledgements

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Table 1: Naturally occurring and synthetic fatty acids and other lipids: mitochondrial actions of potential value in cancer chemotherapy

<table>
<thead>
<tr>
<th>Fatty acid derivative</th>
<th>Mitochondrial actions</th>
<th>Pathways effected</th>
<th>Function</th>
<th>References</th>
</tr>
</thead>
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<tr>
<td>Butyric acid</td>
<td>Dissipation of the mitochondrial membrane potential (ΔΨ)</td>
<td>Caspase-3 activation</td>
<td>↑Apoptosis</td>
<td>Heerdt et al. 1998</td>
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<td></td>
<td></td>
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<td>Growth arrest</td>
<td>Milovic et al. 2000</td>
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<td>Bromo- analogues of</td>
<td>↑ROS, DNA damage</td>
<td></td>
<td>↑Apoptosis</td>
<td>Milovic et al. 2000</td>
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<tr>
<td>butyric and propionic acids</td>
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<td>Palmitic acid</td>
<td>↓Mitochondrial membrane ΔΨ, cytochrome c release</td>
<td></td>
<td>↑Apoptosis</td>
<td>Merrill and Jones, 1990</td>
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<td></td>
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<td>↑Cell death</td>
<td>Schlame et al., 2000</td>
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<td>Ostrander et al., 2001</td>
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<td>13-Methyltetradecanoic acid</td>
<td>Disrupted mitochondrial integrity and caused dysfunction, cytochrome c release</td>
<td>↑Bax, ↓Bcl-2</td>
<td>↑Apoptosis</td>
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<td>Caspase-3 activation</td>
<td>Growth inhibition</td>
<td>Wongtangtintharn et al., 2005,</td>
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<td>↓pAkt</td>
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<td>Arachidonic acid (AA)-derived PGE₂</td>
<td>↑EGFR/ERK MAP kinase</td>
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<td>↑Survival</td>
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<td>↑PI3K/Akt</td>
<td>↑Proliferation</td>
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<td>Acid</td>
<td>ATP glycolysis, ROS, mitochondrial membrane ΔΨ</td>
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<td>Apoptosis</td>
<td>Mitochondrial membrane ΔΨ, DNA fragmentation, lipid peroxidation</td>
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<td>Eicosapentaenoic acid (EPA)</td>
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<td>Epidic trans-epoxides</td>
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<td>Protein kinase C inhibition, ↑Bcl-2, caspase-3, caspase-8 and 9</td>
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<td>Docosahexaenoic acid (DHA)</td>
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<td>Protein kinase C inhibition, ↓Bcl-2, PI3K/Akt, Poly (ADP-ribose) polymerase cleavage</td>
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<td>Conjugated linoleic acid (CLA)</td>
<td>↓DNA synthesis</td>
<td>↓Bcl-2, ↓PI3K/Akt</td>
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<td>Jacaric acid</td>
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<td>α-Eleostearic acid</td>
<td>↓Mitochondrial membrane ΔΨ, DNA fragmentation, lipid peroxidation</td>
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<td>Vaccenic acid</td>
<td>↑DNA fragmentation</td>
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<td>Effect on Mitochondria</td>
<td>Effect on Apoptosis</td>
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<td>Disrupted the mitochondrial membrane $\Delta \Psi$, ↑DNA fragmentation, ↑lipid peroxidation</td>
<td>Caspase-3 and -9 activation, ↓Bcl-2 (decreased Bcl-2:Bax ratio), ↓pAkt</td>
<td>↑Apoptosis, ↓Cytosolic glutathione, ↑Cell death, Grossmann <em>et al.</em>, 2010, Gasmi and Sanderson, 2010</td>
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<td>Compound</td>
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<td>Epoxyeicosatrienoic acids (EETs)</td>
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<td>HETEs and downstream leukotrienes</td>
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<td>Cytochrome c release,</td>
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<td>Anandamide and N-acyl ethanolamines</td>
<td>↑Permeability of inner mitochondrial membrane, ↑↓lipid peroxidation*, energetic and permeability transition alterations, Ca^{2+} overload and PTP modulation</td>
<td>↑↓Apoptosis</td>
<td>Epps et al., 1982</td>
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<td>Cardiolipin</td>
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<td>↑Cytochrome c release, mitochondrial oxidative phosphorylation disruption, induction of permeability transition</td>
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<td>Growth inhibition</td>
<td>Mollinedo et al., 2011</td>
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*dependent on fatty acid chain length and saturation
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Figure legends

Figure 1. Chemical structures of fatty acids, triglycerides and phospholipids. (a) general structure of triglycerides; (b) general structure of phospholipids; (c) phosphatidylcholine; (d) important dietary fatty acids with carbon numbering.

Figure 2. PUFA biotransformation pathways. PUFA are released from their esterified form in cell membranes to free fatty acids. The enzymatic actions of cyclooxygenase (COX), lipoxygenase (LOX) and cytochrome P450 (CYP) signaling molecules with diverse homeostatic actions.
Figure 3. Naturally occurring fatty acids with anticancer activity.

Figure 4. Chemical structures of important fatty acid metabolites and phospholipids, and general structures of ceramides and sphingomyelins.
prostaglandin E2

5-HETE

17,18-epoxy-EPA

anandamide

cardiolipin;  
R¹-R⁴=fatty acid

sphingomyelins;  
R¹=fatty acid  
R²=choline/ethanolamine

perifosine

edelfosine