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Anti-tumor activities of lipids and lipid analogues and their development as potential anticancer drugs

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ABSTRACT

Lipids have the potential for development as anticancer agents. Endogenous membrane lipids, such as ceramides and certain saturated fatty acids, have been found to modulate the viability of tumor cells. In addition, many tumors over-express cyclooxygenase, lipoxygenase or cytochrome P450 enzymes that mediate the biotransformation of ω−6 polyunsaturated fatty acids (PUFAs) to potent eicosanoid regulators of tumor cell proliferation and cell death. In contrast, several analogous products from the biotransformation of ω−3 PUFAs impair particular tumorigenic pathways. For example, the ω−3 17,18-epoxide of eicosapentaenoic acid activates anti-proliferative and proapoptotic signaling cascades in tumor cells and the lipoxygenase-derived resolvins are effective inhibitors of inflammatory pathways that may drive tumor expansion. However, the development of potential anti-cancer drugs based on these molecules is complex, with in vivo stability a major issue. Nevertheless, recent successes with the antitumor alkyl phospholipids, which are synthetic analogues of naturally-occurring membrane phospholipid esters, have provided the impetus for development of further molecules. The alkyl phospholipids have been tested against a range of cancers and show considerable activity against skin cancers and certain leukemias. Very recently, it has been shown that combination strategies, in which alkyl phospholipids are used in conjunction with established anticancer agents, are promising new therapeutic approaches. In future, the evaluation of new lipid-based molecules in single-agent and combination treatments may also be assessed. This could provide a range of important treatment options in the management of advanced and metastatic cancer.

Contents

Abbreviations: Akt, protein kinase B; ALP, alkyl phospholipid; CDK, cyclin-dependent kinase; COX, cyclooxygenase; CYP, cytochrome P450; DHA, docosahexaenoic acid; DISC, death-inducing signaling complex; EET, epoxyeicosatrienoic acid; EPA, eicosapentaenoic acid; ERK, extracellular signal-regulated kinase; FAK, focal adhesion kinase; HETE, hydroxyeicosatetraenoic acid; HPETE, hydroperoxyeicosatetraenoic acid; LOX, lipoxygenase; LT, leukotriene; MAPK, mitogen-activated protein kinase; PG, prostaglandin; PI3K, phosphoinositide 3-kinase; PPAR, peroxisome proliferator-activated receptor; PUFA, polyunsaturated fatty acid; ROS, reactive oxygen species; TTA, tetradecylthioacetic acid; TRAIL, TNF-related apoptosis-inducing ligand; VEGF, vascular endothelial growth factor; WM, Waldenström's macroglobulinemia.

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1. Introduction

In recent years cancer drug development has undergone fundamental changes in which there has been a shift away from non-specific cytotoxic agents in favor of more selective agents that target dysregulated pathways in cancer cells. By this approach a number of molecules that inhibit tyrosine and other kinases that are overactive in tumor cells have already become clinically indispensible. For example, imatinib targets the bcr-abl and c-kit tyrosine kinases that are aberrantly expressed in acute myelogenous leukemia and gastrointestinal stromal tumors, and sorafenib targets the Raf/MEK/ERK- and vascular endothelial growth factor (VEGF) receptor-linked cascades that are over-expressed in renal and hepatocellular carcinomas (Heinrich et al., 2003; Druker et al., 2006; Escudier et al., 2007; Llovet et al., 2008). In general, these agents are much better tolerated and exhibit fewer adverse effects than the more established, but non-specific, cytotoxic anticancer drugs. However, there is an ongoing need for the development of further well-tolerated targeted molecules to provide additional options in cancer chemotherapy. Whether used as single agents or in combination with other anticancer drugs, such agents can be used to develop novel cancer treatment regimen, especially in advanced disease.

There is accumulating evidence that many lipids and lipid analogues are critical regulators of tumorigenesis. Much of this information has emerged from investigations that have been undertaken in vitro in tumor cells or in vivo in experimental animals after dietary conditioning and using tumor cell xenografts. The exploitation of such molecules in cancer therapy is at an early stage, but some show considerable promise. In considering which lipid-based molecules might be developed it is important to derive mechanistic information that underpins their anticancer actions. However, there are also particular issues that arise with lipid-based drugs. Although the biological properties of certain molecules have appeared promising, and could be captured in novel cancer therapeutics, relatively few have made it through the drug development process because of chemical instability, rapid metabolism in vivo and, in some cases, the incidence of side effects. For example, a number of synthetic prostaglandin (PG) analogues have previously been developed as potential antiulcer, antihypertensive and fertility control agents (Collins & Djuric, 1993). Some have reached advanced trials or have even entered clinical use, but their application has been limited somewhat by adverse effects that are often extensions of the activity of the corresponding naturally-occurring prostanoids. Nevertheless, particularly in the area of cancer chemotherapy, several lipid-based agents have emerged that offer promise as effective antitumor agents. This review focuses on the roles of lipids and their analogues in the regulation of tumorigenesis. Existing lipid-based agents that are used in cancer chemotherapy and others that have the potential for development as clinically useful molecules are also discussed.

2. The control of cell growth and cell death

2.1. The cell cycle regulates cell proliferation and mitogenesis

An appreciation of the mechanisms by which lipids and their metabolites regulate tumorigenic processes requires background information on the growth and dissemination of cancer cells. Cancer is a multistage process in which cells develop the capacity for unregulated proliferation, become resistant to proapoptotic stresses that kill normal cells, and acquire the ability to migrate to adjacent and distant tissues to establish secondary metastases.

The cell cycle describes the sequence of events between successive rounds of mitosis by which cells proliferate. Most mammalian cells are quiescent in G_0 phase but may re-enter the cell cycle in G_1 phase in response to mitogenic stimulation (Zetterberg & Larsson, 1985). During mitogenesis cyclins and their associated cyclin-dependent kinases (CDKs) are activated in a coordinated fashion to regulate gene transcription and cell replication. The activities of cyclin/CDK complexes are also modulated by interactions with antiproliferative CDK-inhibitors, including $p21^{Cip1}$, $p27^{Kip1}$ and the INK4 proteins ($p16^{INK4a}$, $p15^{INK4b}$, $p18^{INK4c}$ and $p19^{INK4d}$) (Malumbres & Barbacid, 2001). DNA synthesis occurs in S-phase, which is followed by G_2 phase, in which the cell prepares for mitosis (M phase). Cell cycle regulatory genes are subject to mutation in cancers and the amplification or dysregulation of cyclins, CDKs and CDK-inhibitors is common (Vermeulen et al., 2003). Over-activation of cyclin–CDK complexes results in unregulated gene transcription and increased rates of mitogenesis (Williams & Stoeber, 2012).

2.2. Signaling pathways and cell proliferation

Several signaling cascades have important roles in the regulation of cell growth and survival. The proliferative extracellular signal-regulated kinase (ERK), which is a member of the mitogen-activated protein kinases (MAPKs), is activated by growth factors, hormones and chemokines that are ligands for the corresponding growth factor, cytokine and chemokine receptors (Tilton et al., 2000; Roberts & Der, 2007; Fig. 1A). Mitogenic stimuli trigger the translocation of activated ERK from the cytoplasm to the nucleus, which then stimulates the formation of active cyclin D1–CDK4/6 complexes (Chambard et al., 2007).

The phosphoinositide 3-kinase (PI3K)/protein kinase B (Akt) pathway is also activated by growth factors and hormones and promotes cell survival (Fig. 1A). Indeed, full induction of cyclin D1 by mitogens requires the participation of both PI3K/Akt and ERK pathways (Sherr & Roberts, 1999). Other downstream targets for PI3K/Akt include kinases, such as glycogen synthase kinase-3, and transcription factors, like the NF-κB/IκB complex (Reddy et al., 2000). Akt regulates cell proliferation by targeting the CDK-inhibitors $p21^{Cip1}$ and $p27^{Kip1}$, and cell survival by direct inhibition of pro-apoptotic mediators like Bad, Bim and procaspase-9.

NF-κB is normally present in the cell cytoplasm as an inactive complex bound to inhibitory proteins of the IκB family, but IκB may be dissociated by a variety of stimuli, including infection, proinflammatory cytokines, mitogens and growth factors, and reactive oxygen species (ROS) (Viatour et al., 2005; Gloire et al., 2006). Dissociation of IκB activates NF-κB and modulates cell proliferation and survival by activating the expression of cyclin D1 and the anti-apoptotic bcl-xL and bcl-2 (Guttridge et al., 1999; Piva et al., 2006; Fig. 1A).

2.3. Cell death pathways

The most studied mechanism of programmed cell death is apoptosis, which occurs along the so-called intrinsic and extrinsic pathways. The intrinsic, or mitochondrial, pathway is activated by intracellular stress signals from DNA-damaging chemicals and ROS (Fig. 1B). These stimuli increase mitochondrial membrane permeability by modifying the interplay between Bcl-2 family proteins that interact with mitochondrial membrane voltage-dependent anion channels (Shimizu et al., 2000). Bcl-2 proteins have either proapoptotic (eg Bak, Bax, or Bok) or anti-apoptotic roles (eg Bcl-2, Bcl-XL, or Mcl-1); the BH3-only proteins (eg Bid, Bim, or Puma) also modulate pro- and anti-apoptotic Bcl-2 protein interactions. Apoptotic stimuli shift the balance between these proteins and promote mitochondrial membrane destabilization, cytochrome c release into the cytoplasm and activation of executioner caspases that cleave cytoplasmic and nuclear macromolecules and produce the morphologic features of apoptosis, like DNA fragmentation (Degterev et al., 2003).

ROS are not only mediators of damage to cell macromolecules but also modulate signal transduction. Major sources of ROS are mitochondrial complexes that mediate oxidative phosphorylation and enzymes, such as cyclooxygenases (COX), cytochromes P450 (CYP), lipoxygenases (LOX), and NADPH- and xanthine oxidases that operate

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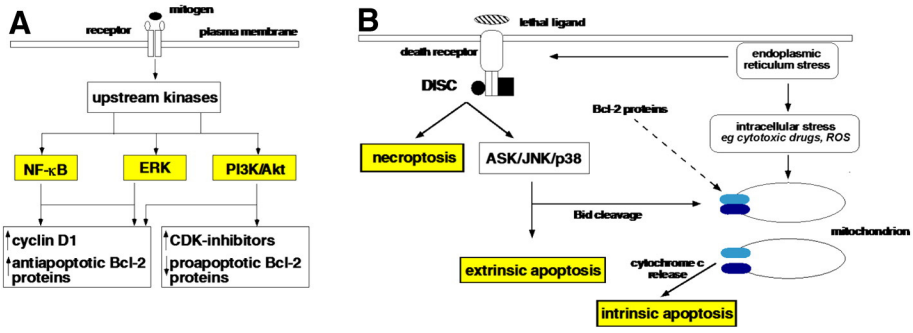

Fig. 1. (**A**) Overview of pro-tumorigenic NF-κB, ERK and PI3K/Akt signaling cascades that are activated by membrane receptors for growth factors, chemokines and cytokines that promote tumor expansion. The functional kinases are activated along canonical pathways involving upstream kinases. Downstream targets include transcription factors and proteins that regulate the cell cycle and cell death, including cyclin D1, CDK-inhibitors and pro- and anti-apoptotic Bcl-2 proteins. (**B**) Inter-relationships between intrinsic and extrinsic apoptotic mechanisms and necroptosis. Intracellular stresses from a number of sources, including cytotoxic drugs and reactive oxygen species (ROS), promote intrinsic apoptosis due to a shift in Bcl-2 protein composition, cytochrome c release and caspase activation. Death receptor ligands activate extrinsic apoptosis and necroptosis by recruiting adaptor and mediator proteins to intracellular death-inducing signaling complexes (DISCs), the composition of which determines the mode of cell killing. The resultant ASK/JNK/p38 MAPK activation also promotes Bid cleavage, which disrupts mitochondrial Bcl-2 protein interactions and activates intrinsic apoptotic pathways.

by radical-dependent mechanisms (Glickman & Klinman, 1996; Murray, 1999; Rouzer & Marnett, 2003). Interference with coupled proton and electron flow between mitochondrial complexes increases ROS production (Brand & Nicholls, 2011). Reactive lipid hydroperoxides are also formed when COX and other enzymes act on unsaturated fatty acids and mediate protein damage. ROS also activate the proliferative EGFR/Raf/MEK/ERK signaling pathway (Wu et al., 2008) by inhibiting protein phosphatases that otherwise terminate signaling (Klann & Thiels, 1999).

The extrinsic pathway of apoptosis transduces signals from TNFα, FasL and TNF-related apoptosis-inducing ligand (TRAIL) via their cognate death receptors (TNFα-receptor-1/2, CD95 (APO-1/Fas) and TRAIL-receptors 1/2; Fig. 1B). Following ligand activation, death receptors recruit adaptor proteins, the MAPK kinase kinase ASK1, procaspases and other modulatory proteins to their cytoplasmic death domains to form death-inducing signaling complexes (DISCs). ASK1 is upstream from the Jun-N-terminal kinase (JNK) and p38 MAPKs that mediate apoptosis and cell cycle arrest (Tobiume et al., 2001). Although the extrinsic and intrinsic pathways are distinct, they converge on the proapoptotic BH3-only proteins, that induce mitochondrial permeabilization (Sarosiek et al., 2013).

Unlike apoptosis, necrosis was previously considered to be a passive form of cell death in response to pathogens or toxins that promote massive ATP depletion. Necrotic cell death is characterized by breakdown of the plasma membrane, the release of cellular contents and a proinflammatory response. This contrasts with the ordered dismantling of the cell that occurs in apoptosis. However, it has emerged recently that programmed necrosis, or necroptosis, is activated by death receptor ligands and also involves assembly of intracellular DISCs (Fig. 1B), but is caspase-independent (Christofferson & Yuan, 2010). The signals that determine which proteins remain within the DISC, and so determine whether apoptosis or necroptosis ensues, are not completely clear, but could relate to ATP availability.

2.4. Defects in proliferative and death pathways in cancer cells

High constitutive levels of the ERK, PI3K/Akt and NF-κB proliferative and prosurvival pathways are frequently observed in human cancers, due to molecular alterations in genes that encode key pathway intermediates or to upstream activation mediated by mutations or amplification of cell-surface receptors (Schubbert et al., 2007; Courtney et al., 2010). In normal cells, protein phosphatases modulate signal duration by controlling the dephosphorylation of phosphoprotein signaling intermediates; these may also be dysregulated in cancer cells (Klann & Thiels, 1999). Deregulated ERK, PI3K/Akt and NF-κB signaling in cancer cells promotes uncontrolled growth and survival, and oncogenic transformation and progression.

Tumors of small diameter are adequately oxygenated by diffusion, but tumor expansion requires a substantial new blood supply to deliver oxygen and nutrients (Gimbrone et al., 1972; Folkman, 1990). The hypoxic environment in solid and growing tumors promotes the activation of pro-angiogenic genes, such as VEGF. Angiogenesis not only facilitates the vascularity of tumors, but also promotes the metastatic potential of tumor cells (Kleiner & Stetler-Stevenson, 1999). It is now also clear that pro-inflammatory signals within the tumor microenvironment, due in part to over-activity of NF-κB signaling, contributes to the invasive and angiogenic phenotype that promotes metastasis (Rajput & Wilber, 2010).

3. Lipids and fatty acids

3.1. Esterified and free fatty acids in cells

Triglyceride and phospholipid esters have important biological functions in energy storage and as membrane structural components. Triglycerides possess a glycerol backbone, with each of the three hydroxyl groups esterified to fatty acid residues. The major pathway of triglyceride synthesis begins with glycerol-3-phosphate that undergoes three esterifications mediated by acyltransferases (Athenstaedt & Daum, 2006). Phospholipids are also acylglycerols but possess hydrophilic substituents at the 3-position (Fagone & Jackowski, 2009). Phospholipids are designated according to this substituent, eg phosphatidylcholine is derived from choline and is the major phospholipid found in mammalian cell membranes, while other phospholipids are phosphatidylethanolamine, phosphatidylserine, phosphatidylinositol and cardiolipin. Hormone-sensitive lipases hydrolyze triglycerides that are stored in adipocytes, and produce free fatty acids that can undergo oxidation to produce a large number of high energy ATP molecules (Athenstaedt & Daum, 2006).

Free fatty acids may be esterified in cell membrane phospholipids that may undergo hydrolysis by phospholipases to generate bioactive lipid mediators, including diacylglycerol, phosphatidic acid, lysophosphatidic acid and polyunsaturated fatty acids (PUFAs) (Park et al., 2012). Esterified fatty acids may be saturated (having no carbon–carbon double bonds, such as palmitic acid (16:0)), monounsaturated (having one double bond, such as oleic acid (18:1 ω−9)) or polyunsaturated (having multiple carbon–carbon double bonds, such as arachidonic acid (20:4 ω−6)). The double bonds in naturally occurring unsaturated fatty acids are primarily in the *cis* configuration. Trivial names for lipids such as arachidonic acid are commonly used, although the more systematic nomenclature is favored, eg of the form

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C:D, where C is the number of carbon atoms in the fatty acid chain and D is the number of double bonds in the fatty acid; thus arachidonic acid is 20:4. In PUFAs the location of the double bond in relation to the terminal methyl carbon is designated as n or ω. Arachidonic acid is classified as an n−6 or ω−6 PUFA, because the most distal olefinic bond is six carbons from the terminal methyl group.

The properties of cell membranes are significantly altered by the degree of unsaturation of their component fatty acids. While unsaturated fatty acids have fewer degrees of freedom than saturated fatty acids, the potential energy barrier for carbon–carbon single bond rotation in unsaturated chains is lower (Feller et al., 2002). Acyl chain flexibility therefore increases with unsaturation due to rapid isomerization through different conformational states (Feller & Gawrisch, 2005). Docosahexaenoic acid (DHA; 22:6 ω−3), for example, can occupy its full conformational space in tens of nanoseconds (Soubias & Gawrisch, 2007). Thus, compared with less saturated bilayers, PUFA-rich membranes are thinner and have greater fluidity, corresponding to a lower density of component molecules (Salmon et al., 1987; Rajamoorthi et al., 2005). Significant differences in the flexibility and fatty acid spatial distribution of ω−3 and ω−6 PUFA-containing bilayers also exist due to the longer saturated chain regions present in ω−6 PUFAs (Eldho et al., 2003; Rajamoorthi et al., 2005). Alterations to membrane properties brought about by fatty acid composition influence important cellular processes, including the function of integral membranes and lipid microdomain formation.

3.2. Fatty acid biotransformation: roles of enzymes and regulation of cell viability by eicosanoid metabolites

Phospholipase A_2-dependent hydrolysis of membrane phospholipid esters releases free fatty acids within the cell (Park et al., 2012). Liberated PUFAs are substrates for three families of enzymes: the COX enzymes that produce PGs, prostacyclin and thromboxanes, the LOX enzymes that produce hydroxyeicosatetraenoic acids (HETEs), lipoxins and leukotrienes (LTs), and the CYP monooxygenases that generate epoxides and atypical HETEs (Spector & York, 1985; Oates et al., 1988; Oliw, 1994). These PUFA metabolites alter cell viability by modulating ERK, PI3K/Akt and NF-κB signal transduction pathways, that are implicated in tumorigenesis because they control cell proliferation, survival, angiogenesis and metastasis.

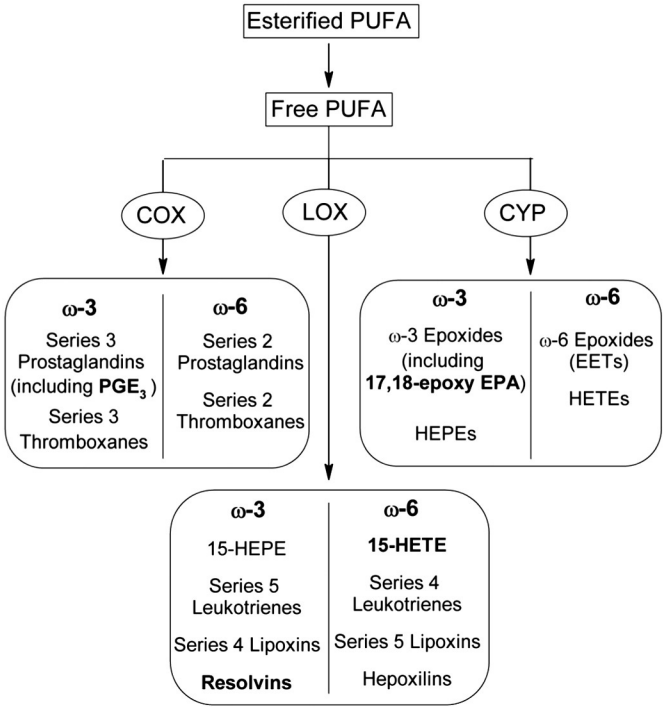

Fig. 2. Biotransformation of PUFA by COX, LOX and CYP enzymes to metabolites that modulate tumorigenesis. The bolded products — PGE_3, 17,18-epoxy-EPA and resolvins — are metabolites of the ω−3 PUFA eicosapentaenoic acid (EPA) that have anti-cancer properties that can be adapted in drug development strategies.

COX enzymes catalyze the formation of the prostanoid precursor PGH_2 from ω−6 arachidonic acid that is converted to the range of series-2 PGs (PGD_2, PGE_2, $PGF_{2\alpha}$ and PGI_2) and thromboxane by specific synthases; the analogous series-3 prostanoids are produced by the action of COX on the ω−3 PUFA eicosapentaenoic acid (20:5 ω−3) (Fig. 2). Although the preferred substrate is arachidonic acid, COX enzymes can also accommodate other ω−3 and ω−6 PUFA as substrates, but with varying efficiencies (Vecchio et al., 2010); such reactions also generate products that are structurally similar to the PGs derived from arachidonic acid, but that have quite distinct biological activities (Wada et al., 2007; Siddiqui et al., 2008).

PGs bind to specialized cell surface receptors: EP1–EP4 are receptors for PGE_2, DP1 and DP2 accommodate PGD_2 and the FP and IP receptors bind $PGF_{2\alpha}$ and PGI_2, respectively (Breyer, 2001; Breyer et al., 2001). Some PGs also bind nuclear peroxisome proliferator-activated receptors (PPARs) that activate DNA response elements in the promoter regions of target genes linked to inflammation, cell proliferation, apoptosis and differentiation (Alaynick, 2008). For example, PGI_2 can transactivate $PPAR_\delta$, and the dehydration product of PGD_2, 15d-PGJ_2, is a natural ligand for $PPAR_\gamma$ (Elrod & Sun, 2008).

LOX enzymes are a family of non-heme iron-containing dioxygenases that catalyze hydrogen atom abstraction from the *bis*-allylic carbons in arachidonic acid, which is followed by radical rearrangement and oxygen addition to generate hydroperoxyeicosatetraenoic acids (HPETEs). LOX isoforms are normally expressed in leukocytes (5-LOX), platelets (12-LOX) and endothelial/epithelial cells (15-LOX; Shappell et al., 1999). 5-LOX converts arachidonic acid into 5-HPETE whereas 12- and 15-LOX generate the 12- and 15-HPETE isomers, respectively (Funk, 2001). HPETEs either undergo reduction to the alcohols, termed HETEs (Fig. 2), or are conjugated with glutathione and converted to the cysteinyl-leukotrienes (Oates et al., 1988). Leukotrienes (LTs) exert their biological functions via LTB_4 receptors (BLT1, BLT2) or the two G-protein-coupled receptors CysLT1 and CysLT2 (Haeggström & Funk, 2011).

While CYPs are most studied for their roles in drug and xenobiotic oxidations, they also have physiologically important roles in the biotransformation of fatty acids and other lipophilic endobiotics. CYP2J2, CYP2C8 and CYP3A4 convert the ω−6 arachidonic acid to four enantiomeric epoxyeicosatrienoic acids (or EETs; Fig. 2): 5,6-EET, 8,9-EET, 11,12-EET, and 14,15-EET (Roman, 2002; Fig. 2, Fig. 3). Arachidonic acid is also oxidized by the CYP4A/4F ω-hydroxylases to several HETEs (Fig. 2), the principal being the proinflammatory 20-HETE (Roman, 2002). These CYPs also oxidize the ω−3 PUFA eicosapentaenoic acid (EPA) to five epoxides — four of which are analogues of the corresponding ω−6 EETs (the 5,6-, 8,9-, 11,12-, and 14,15-EPA-epoxides) but the fifth is ω−3 17,18-epoxy-EPA that is formed at the ω-3 olefinic bond that is absent in ω−6 arachidonic acid (Fer et al., 2008; Fig. 3).

Many CYPs are modulated by xenobiotic exposure, proinflammatory cytokines and dietary nutrients, and in a range of disease states, including cancer and hepatic cirrhosis (Murray et al., 1987; Murray, 1991; Morgan, 1997). Thus, hepatic CYPs 2C8 and 3A4 are inhibited or induced by exposure to coadministered drugs, which can give rise to pharmacokinetic drug interactions and could also modulate EET formation (Guengerich, 2006; Crettol et al., 2010). In contrast, CYP2J2 is inhibited by terfenadine derivatives and 17-octadecynoic acid (Jiang et al., 2007; Chen et al., 2009) and is subject to altered regulation in hypoxia and by antioxidant chemicals (Marden et al., 2003; Lee & Murray, 2010). In normal tissues EETs modulate hormone and ion channel activity and have been found to regulate proliferation, apoptosis and angiogenesis in tumor cells by activating the proliferative EGFR/Raf/MEK/ERK and

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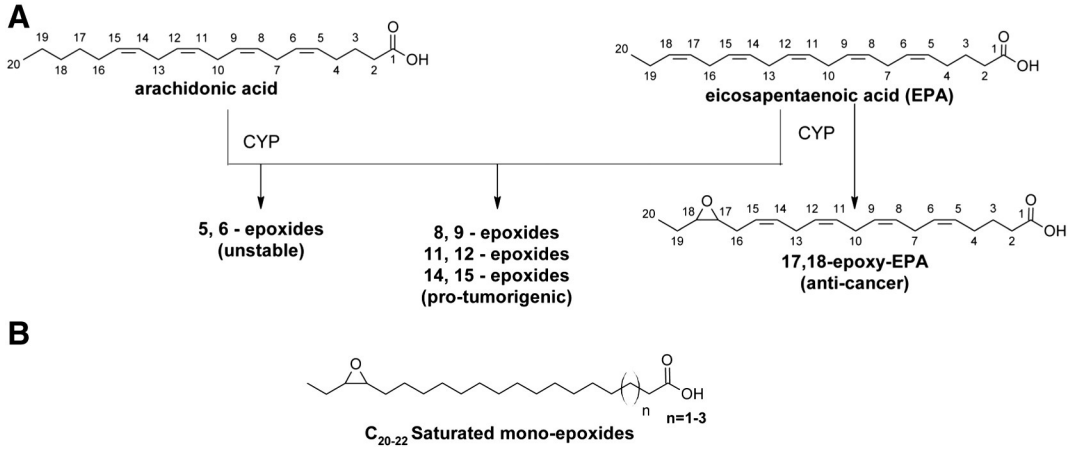

Fig. 3. (**A**) CYP-dependent oxidation of the olefinic bonds in the ω−6 and ω−3 PUFAs arachidonic acid and eicosapentaenoic acid (EPA) to epoxides. While the 5,6-epoxides are chemically unstable, the 8,9-, 11,12- and 14,15-epoxides formed from both PUFAs increase cell proliferation and inhibit apoptosis. The 17,18-epoxide of EPA selectively inhibits proliferation and activates apoptosis. (**B**) General structure of the synthetic ω−3 monoepoxides of the C20–C22 long-chain saturated fatty acids that exhibit anti-tumor activity in breast cancer cells.

anti-apoptotic PI3K/Akt cascades (Jiang et al., 2005; Chen et al., 2009; Panigrahy et al., 2012). Evidence has also been provided for signaling through the Jak-STAT and PPAR pathways (Chen et al., 2001; Pozzi et al., 2010). Important target genes include the cell cycle regulators cyclin D1 and the CDK inhibitor $p27^{Kip1}$ (Potente et al., 2002; Cui et al., 2011). EETs promote angiogenesis by up-regulating endothelial nitric oxide synthase and activating endothelial cell proliferation via ERK and PI3K/Akt pathways (Pozzi et al., 2005).

3.3. Lipid rafts and the regulation of cell viability

Membrane bilayers consist of phospholipids with esterified fatty acids chains that are packed tightly with cholesterol and sphingolipids. Within membranes lipid rafts are small (10–200 nm), dynamic, heterogeneous and detergent-resistant microdomains (Simons & Toomre, 2000). Originally controversial, rafts are now implicated in many physiological and pathophysiological processes including apoptosis, cell signaling, viral entry and neurodegeneration (Simons & Toomre, 2000; Hancock, 2006; Adamson & Freed, 2010). Integrins and growth factor receptors, and associated intracellular signaling molecules are enriched in rafts that can be regarded as sites for the initiation of signaling pathways. The focal adhesion kinase (FAK) is activated and localized to lipid rafts by integrins, which detect adhesion to the extracellular matrix in adherent cell types (Xia et al., 2004; Baillat et al., 2008). Many signaling complexes are protected from non-raft membrane phosphatases that could otherwise attenuate signaling. A switch between raft and non-raft localization of signaling components may be an important mechanism for regulation of signaling activity.

Lipid rafts in the outer leaflet of the plasma membrane are cholesterol and sphingolipid rich. Sphingomyelin, which is composed of a hydrophobic ceramide moiety and a hydrophilic phosphorylcholine group, is the most common sphingolipid in the plasma membrane. Ceramides are amides of fatty acids and sphingosine and are constituents of biological membranes. The enrichment of ceramide in lipid rafts causes fusion of raft microdomains into larger and more stable platforms (Silva et al., 2009). Raft-associated death receptors may activate the lysosomal acidic sphingomyelinase and the release of ceramide from membrane sphingomyelin (Sessler et al., 2013). Ceramides are now recognized for their signaling roles in the regulation of cell proliferation, differentiation, and cell death. The hydrolysis of sphingomyelin to ceramide is catalyzed by sphingomyelinases while de novo synthesis is mediated by multiple ceramide synthases that produce endogenous ceramides with a range of fatty-acid chain lengths; longer-chain ceramides are pro-apoptotic.

Ceramide accumulation in cells occurs after treatment with anticancer agents or saturated fatty acids, such as palmitic acid (Merrill & Jones, 1990). Direct addition of C2-ceramide (~1 μM) altered the mitochondrial transmembrane potential ($\Delta\Psi$) by forming channels or by targeting Bcl-2 proteins, which released cytochrome c and activated caspase-3 (Garcia-Ruiz et al., 1997). These proapoptotic actions of ceramide are mediated by p38 and JNK MAPKs (Chen et al., 2008). Ceramide can also be cleaved by ceramidase which terminates the apoptotic actions of long-chain ceramides and is over-expressed in cancer cells (Seelan et al., 2000).

4. Fatty acid biotransformation enzymes in cancer and dysregulation of metabolite signaling

4.1. COX-2 and PG over-production

Fatty acid biotransformation enzymes are frequently dysregulated in cancers. While COX-1 is constitutively expressed in many tissues, COX-2 is increased by proinflammatory stimuli and growth factors (Oates et al., 1988). Over-expression of COX-2 protein is related to tumor size, grade and proliferation and to upregulation of tumorigenic factors such as VEGF and chemokine receptors (Wang & Dubois, 2010). In tumors over-expression of COX-2 also increases the biotransformation of fatty acids to PGs (Wang & Dubois, 2010). Similar to COX-2, the downstream PGE_2 synthase mPGES-1 is also upregulated during inflammatory conditions (Murakami et al., 2000). PGE_2 promotes the growth of colon and breast cancer cells and angiogenesis by activation of the EGFR, protein kinase A and PI3K/Akt pathways (Di Popolo et al., 2000; Tortora et al., 2003). Moreover, disruption of the EP2 and EP3 receptors decreased the multiplicity and size of intestinal polyps, extent of angiogenesis, and VEGF expression in mice that were genetically susceptible to intestinal polyp development (Sonoshita et al., 2001).

Other PUFA-metabolizing enzymes may also be over-expressed in a range of cancers. Immunochemical analysis of prostate tissue showed that thromboxane synthase expression is low in normal differentiated luminal or secretory cells, significantly increased in poorly differentiated or advanced tumors, and markedly increased in invasive tumors (Nie et al., 2004). Thromboxane synthase is also associated with a poor prognosis in other tumor types and its inhibition induces cell death in vitro in lung, bladder and colorectal cancer cells (Sakai et al., 2006; Moussa et al., 2008; Leung et al., 2009, 2010; Cathcart et al., 2011).

4.2. LOX metabolites in tumorigenesis

LOX enzymes have also been implicated in tumor development. 5-LOX and 12-LOX are protumorigenic, while 15-LOX inhibits carcinogenesis (Pidgeon et al., 2007). 5-LOX and 12-LOX are inducible by pro-

inflammatory stimuli, and are often over-expressed in tumor cell lines (Avis et al., 1996, 2001; Hong et al., 1999) and cancers (Avis et al., 1996; Gupta et al., 2001). Histological analysis of human adenoma samples found that 5-LOX expression was strongly correlated with polyp size and intra-epithelial neoplasia. Consistent with these findings the addition of 5-HETE to breast and prostate cancer cells in vitro enhanced proliferation (Avis et al., 2001; Moretti et al., 2004) and 5-LOX inhibition induced apoptosis in LNCaP and PC3 prostate cancer cells; thus, 5-LOX metabolites maintain tumor cell viability (Ghosh & Myers, 1998). Cell death was prevented by direct addition of 5-HETE and its analogues (Ghosh & Myers, 1998). Similarly, in breast cancer cells the inhibition of 5-LOX caused G_1 cell cycle arrest, decreased growth, and increased apoptosis, as reflected by the down-regulation of bcl-2 and up-regulation of bax (Avis et al., 2001).

12-LOX expression was increased in advanced prostate cancer (Gao et al., 1995) with existing data suggesting that 12-LOX derived eicosanoids are proliferative and pro-angiogenic. 12-LOX inhibition in human AGS and MKN-28 gastric cancer cells (Wong et al., 2001) and PC3 and DU-145 prostate cancer cell lines (Pidgeon et al., 2002) was found to decrease PI3K/Akt survival signaling and to activate caspase-3/7 and apoptosis.

There are two isoforms of 15-LOX, with 15-LOX-1 more highly expressed in malignant than normal human prostate tissue and 15-LOX-2 expression decreased in breast cancer and colorectal adenomas (Shureiqi et al., 2005; Jiang et al., 2006). Over-expression of 15-LOX-1 accelerated the growth of prostate cancer cells (Kelavkar et al., 2001), while 15-LOX-2 had the opposite effect (Bhatia et al., 2003). These differential effects of 15-LOX isoforms have been attributed to differences in fatty acid substrate preference: 15-LOX-1 and 15-LOX-2 accommodate linoleic acid (18:2 ω−6) and arachidonic acid, respectively. Linoleic acid is converted to 13*S*-hydroxyoctadecadienoic acid, which enhances growth factor-stimulated prostate cancer cell proliferation, while arachidonic acid is converted to the anti-proliferative 15-HETE (Hsi et al., 2002).

4.3. CYP-mediated EETs in tumorigenesis

CYP2J2 is over-expressed in many invasive human cancers and CYP2J2-derived EETs have been implicated in driving tumor cell migration in nude mice carrying human breast cancer cell xenografts (Jiang et al., 2005). EETs are also proliferative and proangiogenic in cerebral capillary endothelial cells in vitro and stimulated endothelial tube formation and angiogenesis in a Matrigel plug in vivo (Munzenmaier & Harder, 2000; Zhang & Harder, 2002). Similarly, transfection of tumor cells with CYP2J2 enhanced proliferation and prevented apoptosis (Jiang et al., 2005). Interestingly, it is now emerging that individual EET enantiomers may activate angiogenesis by different mechanisms. 11,12-EET stimulates vessel formation by activating the EGF receptor and sphingosine kinase-1 (Michaelis et al., 2003; Yan et al., 2008) while 14,15-EET acts via PI3K/Akt signaling and Src-dependent STAT-3-mediated VEGF expression (Zhang et al., 2006; Cheranov et al., 2008).

CYP2J2 inhibitors decreased EET production in tumor cells, which then attenuated EGFR/ERK and PI3K/Akt signaling, prevented proliferation and decreased their ability to adhere, invade, and migrate. The metastatic behavior of human MDA-MB-435 breast cancer cells in a murine xenograft model tumor growth was repressed, lung metastases were decreased, and TUNEL and Annexin V staining were decreased (Chen et al., 2009).

One of the alternate EET synthases – CYP3A4 – has also been associated with tumorigenesis. In a breast cancer tissue microarray study CYP3A4 was expressed in ~80% of breast cancers and was related to decreased overall survival in breast cancer (Murray et al., 2010). CYP3A4 expression was associated with the presence of nodal metastases (Haas et al., 2006). It is feasible that multiple CYP epoxygenases may contribute to tumorigenesis in different tumors depending on their relative expression.

5. Anticancer strategies based on lipid-derived metabolites and signaling

5.1. Inhibition of enzymes that produce pro-tumorigenic metabolites

Inhibition of COX-2 activity is an attractive strategy for the prevention of tumorigenesis and has been shown to be effective in colon, lung and prostate cells in vitro (Kamijo et al., 2001; Nagatsuka et al., 2002) and in xenografted nude mice in vivo (Nagatsuka et al., 2002). Clinical trials have tested the value of selective COX-2 inhibitors in anticancer strategies (Papadimitrakopoulou et al., 2008; Antonarakis et al., 2009), but their association with adverse cardiovascular risk detracts from their use (Bresalier et al., 2005). Whether this approach is clinically viable with better tolerated drugs is presently unclear. Recently a group of novel ω−3 monounsaturated fatty acids was found to inhibit the in vitro proliferation and migration of breast cancer cells that over-expressed COX-2 (Cui et al., 2012). These lipid-based agents were well coordinated in the active site of the enzyme and decreased PGE_2 formation. If lipid-based molecules of this type could be developed, the original clinical strategy might be revived.

The combination of the COX-2 and 5-LOX inhibitors celecoxib and zileuton, as an adjunct to chemotherapy has been tested in 134 patients with advanced non-small cell lung carcinoma. Although results did not demonstrate overall clinical benefit, potential advantages of celecoxib plus chemotherapy appeared possible in patients with moderate to high COX-2 expression (Edelman et al., 2008). To date there have been no attempts to inhibit CYPs to decrease EET production in tumors. This strategy would be complicated by the high likelihood of pharmacokinetic drug–drug interactions. It may be possible, however, to identify selective inhibitors of PUFA biotransformation enzymes for future use, perhaps in combination with established anticancer agents.

5.2. Synthetic antitumor alkyl phospholipids (ALPs)

5.2.1. ALPs decrease tumor cell viability

Edelfosine (Fig. 4) was the first synthetic ALP analogue evaluated as a potential anticancer agent, followed by ilmofosine that has a thioether moiety in place of the methoxy substituent (Fig. 4). Further structural modification to remove the glyceryl nucleus produced the alkylphosphocholine analogue miltefosine, and replacement of the choline moiety with a piperidine system produced perifosine (Fig. 4). More recently, two other molecules, erucylphosphocholine and its homocholine analogue erufosine, have been developed that possess a longer 22-carbon chain and a ω−9-*cis*-double bond (Fig. 4). These structural developments have enhanced the selectivity of the agents for cancer cells over normal cells and improved their metabolic stability (Mollinedo et al., 1997; Ruiter et al., 1999; Gajate et al., 2004).

The cellular uptake of ALPs is dependent on lipid rafts (van der Luit et al., 2007). ALPs elicit a number of antitumor actions in cells, including interference with membrane lipid raft function, impaired PI3K/Akt survival signaling, inhibition of phosphatidylcholine synthesis, generation of ROS and activation of endoplasmic reticulum stress (Gajate et al., 2012; Fig. 5). Thus, the evidence to date supports multiple potential mechanisms in the mode of action. ALPs decrease the viability of tumor cells in several ways. They promote cell cycle arrest in G2/M phase by inducing the CDK-inhibitor $p21^{Cip1}$ and inhibit proliferative ERK and PI3K/Akt signaling, possibly by interfering with the membrane association of Raf-1, leading to a decrease in Raf-1 kinase activity (Samadder & Arthur, 1999; Elrod et al., 2007; Kumar et al., 2009).

By modulating lipid raft composition ALPs enhance the recruitment of the death receptor Fas/CD95, which activates apoptosis in a ligand-independent manner. Fas/CD95 was found to be essential for apoptosis because ALPs were ineffective in Fas/CD95-deficient cells and retroviral transduction of Fas/CD95 restored sensitivity (Gajate & Mollinedo, 2007). Further recruitment of FADD, procaspase-8, TRAIL-R1, TRAIL-R2 and Bid into lipid rafts promoted apoptotic DISC formation (Gajate &

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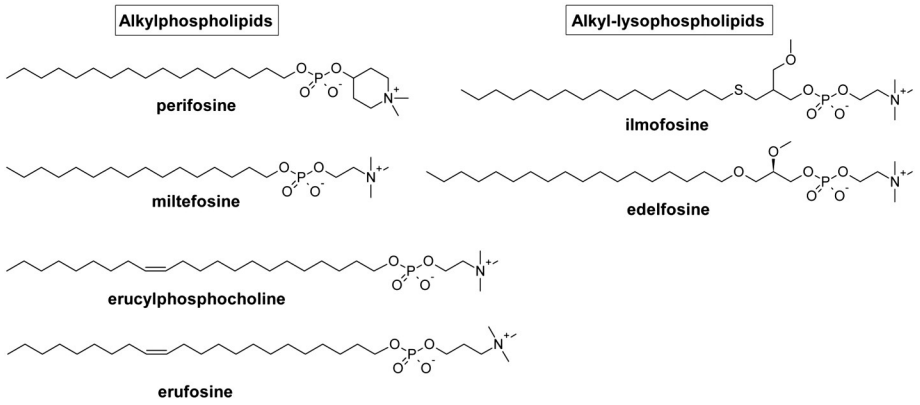

Fig. 4. Structures of anti-tumor alkylphospholipids and alkyl-lysophospholipids that have been evaluated, or are continuing to be evaluated, in clinical studies.

Mollinedo, 2007). This mechanism operated in a range of cell lines including HL-60 promyelocytic leukemia, HEL erythroblast leukemia and Jurkat cells (Gajate et al., 2009). Raft disruption by cholesterol depletion abrogated edelfosine uptake (Mollinedo et al., 2011).

5.2.2. ALPs activate tumor cell apoptosis

As mentioned, there is evidence for additional antitumor actions of ALPs. It has been suggested that edelfosine redistributes lipid rafts from plasma membrane to the mitochondrion and modulates mitochondrial phosphocholine content, which alters membrane permeability, induces swelling of the organelle and activates apoptosis (Mollinedo et al., 2011). Indeed, the relative potencies of ALPs to induce apoptosis in S49 cells were related to the capacity to inhibit the enzyme CTP: phosphocholine cytidylyltransferase that participates in phosphatidylcholine synthesis (van der Luit et al., 2007). It has been proposed that continuous phosphatidylcholine synthesis is essential for cell survival and that a lack of phosphatidylcholine blocks the downstream synthesis of membrane lipids.

Other apoptotic mechanisms have also been proposed to account for the actions of ALPs in tumor cells. In some cell types ALPs activate ROS

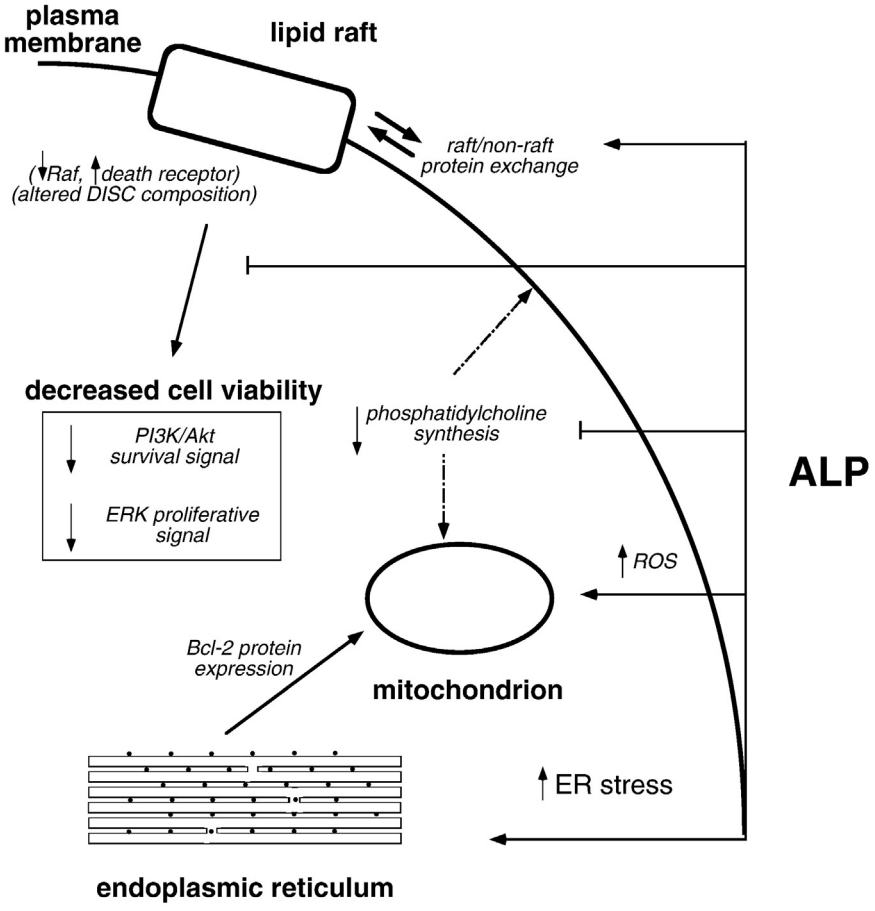

Fig. 5. Alkylphospholipids (ALPs) decrease cancer cell viability by multiple mechanisms. Disruption of lipid rafts on the plasma membrane, and possibly also the mitochondrial membrane, modulates the distribution of death receptors and Raf and other kinases between raft and non-raft locations. ALPs also impair phosphatidylcholine synthesis and activate reactive oxygen species (ROS) production and endoplasmic reticulum (ER) stress that may promote apoptotic cell death. Disruption of PI3K/Akt survival signaling and proliferative ERK signaling may also contribute to the decrease in cell viability produced by ALPs.

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production, which oxidizes thioredoxin, and enables its dissociation from the N-terminus of ASK1 (Matsukawa et al., 2004). ASK1 then activates downstream proapoptotic JNK/p38 MAPK signaling, which cleaves Bid, disrupts the mitochondrial membrane, enhances cytochrome c release and promotes cell death (Nieto-Miguel et al., 2006; Gajate & Mollinedo, 2007; Fig. 5). Overexpression of the antiapoptotic Bcl-2 proteins Bcl-XL or Bcl-2 prevented ALP-induced cytochrome c release from the mitochondrion in multiple myeloma cells (Gajate & Mollinedo, 2007).

APLs also induce endoplasmic reticulum stress, possibly as a consequence of impaired phosphatidylcholine production, and which could be an alternate mechanism of ASK1–JNK activation (Nieto-Miguel et al., 2007). Consistent with this possibility, phosphatidylcholine depletion has been shown separately to induce the endoplasmic reticulum stress-related pro-apoptotic transcription factor CHOP/GADD153 (van der Sanden et al., 2003), that may in turn activate the pro-apoptotic Bcl-2 family members Bax and Bak and the BH3-only protein Bim. It has been suggested that the sensitivity of tumor cells to APLs may relate to differential activation of the profile of pro- and anti-apoptotic Bcl-2 proteins (Mollinedo et al., 1997).

5.2.3. Clinical use of ALPs

Although well tolerated in preclinical studies the systemic clinical use of synthetic ALPs has been restricted somewhat by their hemolytic potential and gastrointestinal toxicity (Berdel et al., 1987). Other important toxicities reported in clinical studies include fever, myalgia, arthritis and pain. Most studies to date have evaluated ALPs as single agents (Table 1). Topical application of miltefosine as a 6% solution or ointment has been found to have activity in the treatment of skin-metastasized breast cancers and cutaneous lymphomas. Thus, in 100 patients across five studies of varying duration, 18 patients experienced complete remission over the study period, 25 a partial remission and the disease was stabilized in 36 (Dummer et al., 1993; Terwogt et al., 1999; Clive et al., 1999; Smorenburg et al., 2000; Dumontet et al., 2006). The

Table 1
Summary of clinical evaluations of alkyl phospholipids.

Drug	Patient group [patients (major tumor types)]	Comments	Reference
Single agent			
Edelfosine	16 (7 NSCLC)[a]	PR[b] in 2 patients	Berdel et al., 1987
Ilmofosine	53 (12 lung adenocarcinoma, 15 malignant melanoma, 10 colon adenocarcinoma)	PR in 3 patients	Herrmann et al., 1987
Ilmofosine	15 — only 10 evaluable (6 CRC)	Minor response in 1	von Mehren et al., 1995
Ilmofosine	15 — only 14 evaluable; NSCLC	No activity	Woolley et al., 1996
Ilmofosine	39 — only 36 evaluable (21 CRC)	Pharmacokinetic study	Giantonio et al., 2004
Miltefosine	54 (31 colorectal, 13 NSCLC, 10 head and neck)	Dose-finding study	Verweij et al., 1992
Miltefosine	15; cutaneous lymphoma	6% ointment, topical application, CR in 5, PR in 5, stable disease in 3	Dummer et al., 1993
Miltefosine	34; metastatic CRC	PR in 1 patient, stable disease in 3	Planting et al., 1993
Miltefosine	19; head and neck	Limited activity	Verweij et al., 1993a
Miltefosine	21; sarcoma	Limited activity	Verweij et al., 1993b
Miltefosine	34 — only 30 evaluable; skin metastasized breast cancer	6% solution, topical application CR in 7, PR in 6, stable disease in 10	Terwogt et al., 1999
Miltefosine	25; cutaneous metastasized breast cancer	CR in 1, PR in 2, minor responses in 6, stable disease in 11	Clive et al., 1999
Miltefosine	20 — only 18 evaluable	6% solution, topical application PR in 4, stable disease in 7	Smorenburg et al., 2000
Miltefosine	12; cutaneous T-cell lymphoma	6% solution, topical application CR in 5, PR in 2, stable disease in 5	Dumontet et al., 2006
Perifosine	22 (11 CRC)	Pharmacokinetic study	Crul et al., 2002
Perifosine	42 (19 CRC, 6 renal)	PR in 1, stable disease in several patients	Van Ummersen et al., 2004
Perifosine	18 — only 17 evaluable; metastatic melanoma	No responses observed	Ernst et al., 2005
Perifosine	19; prostate cancer	Minimal activity	Posadas et al., 2005
Perifosine	19 — only 18 evaluable; head and neck	Minimal activity	Argiris et al., 2006
Perifosine	23 — only 22 evaluable; sarcoma	PR in 1, stable disease in 5	Bailey et al., 2006
Perifosine	17 — only 15 evaluable; sarcoma	Stable disease in 4	Knowling et al., 2006
Perifosine	25 — only 24 evaluable; prostate cancer	Based on PSA reduction 20% had modest response	Chee et al., 2007
Perifosine	10; pancreatic adenocarcinoma	Stable disease in 1	Marsh et al., 2007
Perifosine	18 — only 17 evaluable; advanced breast cancer	Stable disease in 3	Leighl et al., 2008
Perifosine	36 (7 lung, 5 melanoma, 4 gastrointestinal)	No activity	Unger et al., 2010
Perifosine	37; Waldenstrom's macroglobulinemia	PR in 4, minimal response in 9; stable disease in 20	Ghobrial et al., 2010
Perifosine	64; renal cell carcinoma	PR in 6; stable disease in 27	Cho et al., 2012
Perifosine	16; chronic lymphocytic leukemia	PR in 1; stable disease in 6	Friedman et al., 2014
Combination treatments with perifosine			
Radiation	21 (17 NSCLC)	CR in 2, PR in 5, stable disease in 10 Further study warranted	Vink et al., 2006
Capecitabine	34; metastatic CRC 26 capecitabine alone	CR in 1, PR in 6, stable disease in 19; overall 70% versus 34% in capecitabine alone	Bendell et al., 2011; [c]
Bortezomib/dexamethasone	84 — only 73 evaluable; relapsed/refractory multiple myeloma	Overall response rate of 41%	Richardson et al., 2011; [c]
Lenalidomide/dexamethasone	32 — only 30 evaluable; relapsed/refractory multiple myeloma	CR in 4, PR in 11, minimal response in 7, stable disease in 6	Jakubowiak et al., 2012
Docetaxel	21; ovarian cancer	PR in 1, stable disease in 3	Fu et al., 2012
7-Hydroxy-staurosporine	13; relapsed/refractory leukemias	No activity	Gojo et al., 2013
Sorafenib	36; lymphoproliferative diseases (25 with Hodgkin lymphoma)	PR in 8, stable disease in 15[d] (PR in 7, stable disease in 8)	Guidetti et al., 2014

[a] NSCLC, non-small cell lung cancer; CRC, colorectal cancer.
[b] PR, partial response; CR, complete response.
[c] Subsequent phase III trials did not demonstrate clinical benefits (Bendell et al., 2012; Richardson et al., 2013)
[d] 40 began with single-agent perifosine; 4 achieved PR; the remaining 36 received sorafenib/perifosine.

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systemic activity of miltefosine against sarcomas, colorectal cancers, and head and neck cancers, however, was not as promising, although individual patients were able to tolerate the drug, and some experienced partial remissions for limited periods (Planting et al., 1993; Verweij et al., 1993a; Verweij et al., 1993b).

Perifosine has found clinical application in the treatment of Waldenström's macroglobulinemia (WM), which is a rare lymphoproliferative disorder characterized by the infiltration of bone marrow with lymphoplasmacytic cells. Existing therapies for WM include cytotoxic agents like chlorambucil and rituximab that have overall response rates of 30–40% and median response durations of 0.5–1 year (Dimopoulos et al., 1995) but, in a phase II trial, single-agent perifosine induced at least a median response in 35% of patients with relapsed or refractory disease and a median progression-free survival of over 1 year (Ghobrial et al., 2010; Table 1). Dose reductions were required in 43% of patients who received perifosine because of neutropenia, gastrointestinal symptoms, or arthritis.

The activity of perifosine is related to the inhibition of prosurvival PI3K/Akt activity, which decreased proliferation, increased apoptosis, and inhibited the migration and adhesion of WM cells in vitro and, in an in vivo subcutaneous xenograft model, the drug prevented homing of WM tumor cells to the bone marrow microenvironment (Leleu et al., 2007). Gene expression profiling and immunohistochemistry of WM samples from patients before and after perifosine treatment identified differentially-expressed genes. Immunochemical analysis revealed a decrease in pGSK3/β protein in most patient samples and a number of PI3K/Akt and NF-κB-regulated genes were down-regulated. Perifosine initially showed some promise in small scale clinical evaluations against a wide range of tumors, at least in occasional patients (Van Ummersen et al., 2004; Bailey et al., 2006; Cho et al., 2012). However, subsequent phase I and phase II studies with single-agent perifosine have been disappointing. Recently the use of perifosine in the treatment of patients with chronic lymphogenous leukemia appeared promising at 3 months, but was only sustained in one patient after 6 months (Friedman et al., 2014; Table 1). Other ALPs have also exhibited limited activity against non-small cell lung cancers, lung adenomas, malignant melanomas and adrenal adenomas, among others (Berdel et al., 1987; Herrmann et al., 1987).

5.2.4. Development of drug combinations containing ALPs

The use of anticancer drug combinations involving perifosine is promising (Table 1). In recent studies perifosine enhanced the antineoplastic effect of lenalidomide and dexamethasone in multiple myeloma (Jakubowiak et al., 2012) and bortezomib, with or without dexamethasone, in the same disease (Richardson et al., 2011). However, a phase III trial with the latter combination showed no clinical benefit, although overall survival may have been extended somewhat in the perifosine arm of the trial (Richardson et al., 2013). Due to slow recruitment and the absence of clear benefit the study has been terminated (Figg et al., 2014). Earlier studies also identified promising activity of perifosine in conjunction with capecitabine for metastatic colorectal cancer (Bendell et al., 2011). A follow-up randomized phase III trial again found that overall or progression-free survival was not increased by the drug combination, at least in the refractory colorectal cancer setting (Bendell et al., 2012). These disappointing findings in myeloma and colorectal cancer could be attributed to the dosing schedules that were selected; further studies with different regimen may be warranted.

In vitro synergism was observed in cells that were treated with the combination of perifosine and the multikinase inhibitor sorafenib (Locatelli et al., 2013). In in vivo xenograft studies there was a reduction in tumor burden, increased survival, and enhanced tumor cell killing produced by this combination compared with single agents (Locatelli et al., 2013). A recent study also evaluated the combination in patients with lymphoproliferative diseases (Guidetti et al., 2014; Table 1). Patients who had received single-agent perifosine for relapsed and refractory lymphoproliferative diseases, and who achieved less than a partial response, received the combination therapy until the disease progressed or toxicity was unacceptable. Initial results supported a clinical response in 7 of 25 patients with Hodgkin lymphoma. Also promising, 4 of 8 patients with chronic lymphocytic leukemia responded to perifosine alone (Guidetti et al., 2014). The clinical responses observed in the patients with relapsed and refractory Hodgkin lymphoma suggest that this subgroup could serve as target population for new studies (Guidetti et al., 2014).

Recent preclinical studies have identified several additional perifosine-containing drug combinations of potential value. Treatment of human acute myeloid leukemia cells with perifosine and the cyclin-dependent kinase inhibitor SNS-032 enhanced cell death compared to treatment with either agent alone — most likely due to a decrease in PI3K/Akt survival signaling by perifosine (Meng et al., 2013). Combination of perifosine with the mTOR inhibitor CCI-779 produced cell cycle arrest and growth inhibition in a number of human cancer cell lines (Pitter et al., 2011). These preclinical data suggest that inhibition of the PI3K/Akt/mTOR pathway at two points in the cascade may produce more optimal effects, and this is being assessed clinically (https://clinicaltrials.gov; accessed December 12, 2014). NCT02238496 is a Phase II study that is assessing the efficacy of the combination of perifosine and the mTOR inhibitor temsirolimus in recurrent or progressive malignant glioma. Two other active trials are also assessing this combination but are not recruiting patients: NCT01051557 (a Phase I/II study to assess efficacy and safety in recurrent and progressive malignant glioma) and NCT01049841 (a Phase I study of different dose schedules in recurrent pediatric solid tumors). Two further trials that are aimed at assessing single-agent perifosine in recurrent and progressive malignant glioma (NCT00590954) and recurrent pediatric solid tumors (NCT00776867) are also active but not recruiting.

Apart from drug combinations, earlier preclinical studies identified promising activity of perifosine in combination with radiation for non-small cell lung cancer (Vink et al., 2006; Table 1), brainstem gliomas (Becher et al, 2010) and prostate cancer cells in vitro and in vivo (Gao et al., 2011). Beneficial effects have also been noted with the other ALPs erucylphosphocholine and erufosine in human astrocytoma and glioblastoma cell lines in combination with radiation (Rübel et al., 2006). Complete and sustained regression of squamous cell carcinoma xenografts occurred after combined treatment of radiation and perifosine (Vink et al., 2006). Short-term treatment with erufosine produced only a transient decrease in the growth of the T98G glioblastoma tumors that was enhanced by repeated application (Henke et al., 2012). These data suggest that in vivo efficacy may depend on an extended treatment schedule.

Together, it appears that combination approaches involving the better tolerated ALPs and conventional cytotoxic or targeted agents, or concurrent radiation, hold significant promise. Experimental studies also suggest that the clinical application of such combinations could also be useful in the management of some drug-resistant tumors. However, optimal schedules do not yet appear to have been identified.

5.3. Other lipid-derived molecules with the potential for development as anti-cancer agents

5.3.1. Anticancer mechanisms of ω−3 PUFAs

Evidence is accumulating that intake of ω−3 PUFA can decrease cancer risk. From population studies the incidence of breast, prostate and colon cancers is lower in populations that have high dietary intakes of oily fish, such as the Japanese (Hardman, 2002). Moreover, as fish intake by these groups decreases, and is replaced by western diets high in ω−6 PUFA, there is an increase in tumor incidence. Indeed, in Japanese women who migrated to North America, the incidence of breast cancer reportedly increased within a single generation (Hardman, 2002). Direct evidence for a relationship between tissue

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levels of ω−3 PUFA and cancer risk has also been obtained. In one study, the incidence of breast cancer was found to be lowest in women with the highest ratio of ω−3/ω−6 PUFA in breast adipose tissue (Maillard et al., 2002).

Findings from experimental studies have largely supported the population studies. The principal long chain ω−3 PUFAs DHA and EPA were anti-proliferative and decreased the viability of cells from a range of tumor types, including colon, prostate and hepatocellular carcinomas (Cerella et al., 2010). Dietary supplementation with ω−3 PUFA decreased the development of human breast and colorectal tumors that had been implanted in nude mice, including decreased tumor volume, decreased microvessel density and decreased VEGF expression (Tevar et al., 2002).

There are a number of possible mechanisms by which ω−3 PUFA decrease tumorigenesis, including direct effects on ROS production to promote apoptosis, alterations in the conversion of ω−6 PUFA to protumorigenic eicosanoid metabolites, altered regulation of PUFA biotransformation enzymes and the conversion of ω−3 PUFA to metabolites that have anticancer activity (Fig. 2). In the non-ionized state medium and long-chain fatty acids readily penetrate the mitochondrial membrane (McLaughlin & Dilger, 1980; Gutknecht, 1988; Kamp & Hamilton, 1992). Free fatty acids, including saturated fatty acids, uncouple oxidative phosphorylation, release protons into the matrix space and efflux fatty acid anions via the adenine nucleotide translocator (Wojtczak & Schonfeld, 1993). This not only dissipates mitochondrial $\Delta\Psi$, impairs electron transport and decreases ATP production, but may also activate apoptosis by promoting the release of cytochrome c. Enhanced radical production depletes cellular glutathione and other antioxidants, which stimulates apoptosis. There is evidence that ω−3 PUFA promote apoptosis by directly modulating mitochondrial ROS production and, in addition, ω−3 PUFA themselves are susceptible to peroxidation which also depletes glutathione and promotes apoptosis (Barrera, 2012).

There is also evidence that the anti-tumor actions of ω−3 PUFA are mediated in part by preventing the actions of protumorigenic ω−6 PUFA metabolites. Thus, ERK phosphorylation and HIF-1α protein over-expression in colon cancer cells and in xenografts in nude mice were inhibited by ω−3 PUFA (Calviello et al., 2004). In colorectal cells EPA decreased COX-2 expression and PGE_2 formation and increased the formation of its EPA-derived analogue PGE_3, which antagonized PGE_2/EP4-dependent pro-tumorigenic signaling (Hawcroft et al., 2010). Decreased growth of prostate and breast cancer cell xenografts in nude mice by intake of fish oil or ω−3 PUFA has also been associated with decreased PGE_2 production (Karmali et al., 1987; Rose & Cohen, 1988; Rose & Connolly, 1997; Berquin et al., 2007). By up-regulating the CDK-inhibitors $p21^{Cip1}$ and $p27^{Kip1}$ DHA decreased cell cycle progression, which could account in part for its antiproliferative actions (Narayanan et al., 2003). Suppression of COX-2 could be mediated by inhibition of NF-κB, which decreases the expression of anti-apoptotic Bcl-2 family proteins and reactivates apoptosis (Schwartz et al., 1999). ω−3 PUFA supplementation also decreased the synthesis of the pro-inflammatory 5-LOX-mediated metabolite LTA_4 and inhibited the release of the cytokines IL-1β and TNF-α (Taccone-Gallucci et al., 2006). Together these findings are consistent with the impairment of pro-tumorigenic signaling mechanisms by ω−3 PUFA.

Long chain ω−3 PUFAs have been found to modulate lipid raft composition in MDA-MB-231 breast cancer cells and to alter signaling by raft-associated proteins. Thus, EPA and DHA modulated the phosphorylation of EGFR that influences tumor cell growth (Schley et al., 2007). ω−3 PUFAs also decreased the activity of the oncogenes ras and AP-1 downstream from the EGFR, which inhibited mitosis (Hardman, 2002). It has also been shown that ω−3 PUFA decrease membrane expression of the chemokine receptor CXCR4 in MDA-MB-231 breast cancer cells, which decreases their migration potential (Altenburg & Siddiqui, 2009). This appears to be due to the incorporation of ω−3 PUFAs into the cell membrane, which disrupts the cholesterol-rich lipid rafts that are required for CXCR4 dimerization and signaling through NF-κB (Wang et al., 2006).

5.3.2. Anti-tumorigenic ω−3 PUFA metabolites

Apart from indirect effects on ω−6 PUFA biotransformation, there is also evidence that certain ω−3 PUFA metabolites exert antitumor actions in their own right. Eicosanoid metabolites derived from ω−3 PUFAs have diminished pro-inflammatory, proliferative, invasive and pro-angiogenic actions compared to those formed from ω−6 PUFA (Abou-el-ela et al., 1989; Rose & Connolly, 2000; Hardman, 2002). The anti-angiogenic activities of EPA in human endothelial cells, including decreased invasion and endothelial tube formation, have been attributed to COX-2-derived PGE_3 and possibly other metabolites (Fig. 2); PGE_3 directly suppressed the induction of the pro-angiogenic mediator angiopoietin-2 by VEGF (Szymczak et al., 2008). The underlying mechanisms by which certain ω−3 PUFA metabolites regulate angiogenesis and related processes have not been completely clarified, but could involve altered prostanoid receptor signaling. Thus, ω−3 EPA-derived eicosanoids activate prostanoid receptors, but less efficiently than the corresponding ω−6 arachidonic acid-derived products (Wada et al., 2007).

CYP-mediated epoxides of ω−3 PUFAs have also been shown to exert growth suppressing and anticancer effects (Fig. 2). The 17,18-epoxide of EPA, but not its regioisomers, decreased endothelial cell proliferation and activated apoptosis (Cui et al., 2011; Fig. 3A). This led to cell cycle arrest by activation of the growth suppressing p38 MAPK and subsequent down-regulation of cyclin D1 (Cui et al., 2011). Zhang et al. (2013) showed recently that DHA epoxides exerted anticancer effects by suppressing VEGF-mediated angiogenesis. Inhibition of angiogenesis resulted in a decrease in primary tumor growth and metastasis in vitro.

Certain LOX-dependent metabolites may also exhibit anti-tumor activity including the antiproliferative arachidonic acid metabolite 15-HETE and the resolvins (Fig. 2), which are LOX-mediated metabolites formed from ω−3 EPA and DHA (the E-series resolvins and protectins, respectively) (Haeggström & Funk, 2011). More complex eicosanoids may also be formed in dual biotransformation reactions. Thus, DHA is converted to 17*S*-hydroxy-DHA by 15-LOX, then to 7*S*-hydroperoxy,17*S*-hydroxy-DHA by 5-LOX and on to resolvin D1 (7*S*,8*R*,17*S*-trihydroxydocosa-4*Z*,9*E*,11*E*,13*Z*,15*E*,19*Z*-hexaenoic acid) after epoxidation, which could involve CYPs. Similarly, 4*S*-hydroperoxy,17*S*-hydroxy-DHA is another LOX-generated product from 17*S*-hydroxy-DHA that also undergoes epoxidation to produce resolvins D3 and D4. These resolvins exhibit anti-inflammatory properties in vivo when administered either intravenously or orally (Dangi et al., 2009).

5.3.3. Adaptation of anti-tumor ω−3 PUFA epoxides in cancer drug development

Certain ω−3 PUFA epoxides hold promise as a novel group of potential anticancer agents. Very recently a small series of synthetic ω−3 epoxides of C20–C22 long chain saturated fatty acids was evaluated for their anti-proliferative and pro-apoptotic actions in human breast cancer cells (Dyari et al., 2014; Fig. 3B). These were developed from the naturally occurring ω−3 17,18-epoxy-EPA by removal of the additional olefinic bonds, because of the potential for oxidation to the isomeric epoxides that stimulated proliferation and inhibited apoptosis. The synthetic ω−3 epoxy-fatty acids impaired the viability of MDA-MB-231 cells (decreased ATP production, increased caspase-3 activity and increased annexin V/propidium iodide staining) and, to a lesser extent, MDA-MB-468, MCF-7 and T-47D breast cancer cells. Activity was dependent on the ability to activate JNK signaling because pharmacological inhibitors and silencing of JNK impaired apoptosis. Decreased proliferation was associated with down-regulation of cyclin D1, and led to failure to complete the cell cycle. The epoxides themselves are unlikely to be suitable for in vivo application, however, due to low

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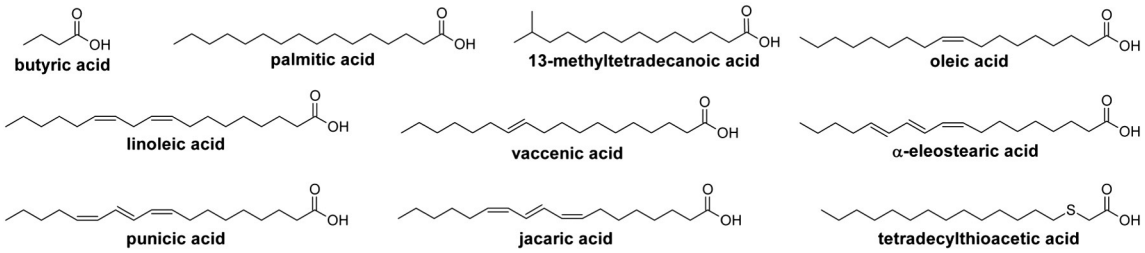

Fig. 6. Structures of saturated fatty acids (butyric acid, palmitic acid and 13-methyltetradecanoic acid), unsaturated fatty acids (oleic acid, linoleic acid, vaccenic acid, α-eleostearic acid, punicic acid and jacaric acid) and the thiofatty acid tetradecylthioacetic acid that exhibit antiproliferative, proapoptotic or antimetastatic properties against tumor cells and xenografts.

stability: epoxide hydrolase converts the epoxides to the inactive diols (Inceoglu et al., 2008). A potentially valuable approach is the co-administration of soluble epoxide hydrolase inhibitors, such as *trans*-4-[4-(3-adamantan-1-yl-ureido)-cyclohexyloxy]-benzoic acid, that enabled retention of the in vivo activity of DHA 19,20-epoxide against tumor growth in a lung carcinoma model (Zhang et al., 2013).

5.4. Other fatty acids with the potential for application in anti-cancer strategies

Several studies have found that naturally occurring fatty acids from a range of sources, including saturated and certain unsaturated analogues, activate apoptosis and impair ATP production. Some of these lipid-based agents have the potential for application in future cancer treatments.

Butyric acid and similar short-chain saturated fatty acids were found to induce cell cycle arrest in G0/G1 and G2/M phases (Heerdt et al., 1997; Fig. 6; Table 2). In COLO 205 and HT-29 colorectal cells butyrate also upregulated the death receptor TNF-R1, activated Bid, released cytochrome c from the mitochondrion and activated executioner caspases to induce apoptosis. Longer-chain palmitic acid (C16:0; Fig. 6; Table 2) decreased mitochondrial $\Delta\Psi$ and effected cytochrome c release, which induced the proteolysis of poly-ADP ribose polymerase and the fragmentation of DNA (de Pablo et al., 1999). Mitochondrial uncoupling is greatest with C12–C16 saturated and longer *cis*-unsaturated fatty acids (Korshunov et al., 1998; Bernardi et al., 2002). However, there may be additional mechanisms by which free fatty acids decrease tumor cell viability, including the activation of extrinsic apoptosis, inhibition of signaling pathways to induce cell cycle arrest, and upregulation of the tumor suppressor p53 and the CDK-inhibitor $p21^{Cip1}$ (Emenaker et al., 2001; Fauser et al., 2011).

13-Methyltetradecanoic acid (Fig. 6; Table 2) is an iso-C_{15} branched-chain saturated fatty acid that disrupts mitochondria and activates apoptosis in tumor cells (Yang et al., 2000; Wongtangtintharn et al., 2005; Lin et al., 2012; Table 2). In human bladder cancer cells, this fatty acid altered the balance between Bcl-2 proteins, activated pro-apoptotic p38 and JNK MAPKs and inhibited prosurvival PI3K/Akt (Lin et al., 2012). In vivo growth of xenografted prostate and hepatocarcinoma-derived cells into nude mice was also inhibited by 13-methyltetradecanoic acid. Apoptosis was induced without evidence of major toxicity, which suggests that 13-methyltetradecanoic acid could have value as a single- or combination-agent in human cancer chemotherapy (Yang et al., 2000).

In breast cancer the monounsaturated oleic acid (18:1, ω−9; Fig. 6) reportedly exerts anti-tumorigenic effects by suppressing human EGFR-2 (Colomer & Menendez, 2006); this is in accord with the reported health benefits of the Mediterranean diet, which contains large quantities of oleic acid. However, conflicting reports have also appeared. Thus, oleic acid also mediates the production of arachidonic acid, which is converted to eicosanoid metabolites that activate FAK phosphorylation and drive MDA-MB-231 breast cancer cell migration (Navarro-Tito et al., 2010). If the important factor is the intermediary conversion to fatty acids like arachidonic acid that generates tumorigenic products then replacement with a monounsaturated fatty acid may be useful. Synthetic longer chain ω−3 monounsaturated fatty acids (C19–C22) were found recently to decrease the proliferation of MDA-MB-231 breast cancer cells by inhibiting PGE_2 formation (Cui et al., 2012). These agents also decreased the invasive behavior of breast cancer cells and increased apoptosis. The molecules were well coordinated within the active site of COX-2 via interactions with Arg120, Tyr355 and a number of hydrophobic residues. Because COX-2 is responsible for the aggressive characteristics of many mammary tumors it is feasible

Table 2
Overview of potential anti-tumor actions of fatty acids.

Fatty acid derivative	Antitumor activity	References
Butyric acid	Dissipation of mitochondrial membrane potential ($\Delta\Psi$)	Heerdt et al., 1998
	Caspase-3 activation	Milovic et al., 2000
Palmitic acid	↓ Mitochondrial $\Delta\Psi$, cytochrome c release	Merrill & Jones, 1990
		Schlame et al., 2000
		Ostrander et al., 2001
13-Methyltetradecanoic acid	Mitochondrial disruption, caspase-3 activation	Yang et al., 2000
		Wongtangtintharn et al., 2005
		Lin et al., 2012
Jacaric acid	↑ROS,[a] caspase-3 activation	Shinohara et al., 2012
	PARP cleavage	Gasmi & Sanderson, 2013
α-Eleostearic acid	↓Mitochondrial $\Delta\Psi$, ↑lipid peroxidation	Tsuzuki et al., 2004
	↑DNA fragmentation	Grossmann et al., 2009
Vaccenic acid	↑DNA fragmentation, Caspase activation	Miller et al., 2003
Punicic acid	↓Mitochondrial $\Delta\Psi$, ↑lipid peroxidation	Grossmann et al., 2010
	↑DNA fragmentation	Gasmi & Sanderson, 2010
Tetradecylthioacetic acid	Caspase-3 activation, PARP cleavage	Tronstad et al., 2001, 2003

[a] ROS, reactive oxygen species; PARP, poly (ADP-ribose) polymerase.

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that the synthetic fatty acids could have clinical utility by inhibiting the formation of protumorigenic PGE_2.

Other monounsaturated fatty acids, such as vaccenic acid (11-*trans*-18:1 ω−7) and punicic acid (18:3 ω−5; Fig. 6; Table 2) have been found to decrease mitochondrial permeability, impair proliferation and deplete cytosolic glutathione (Table 2); these findings are consistent with activation of the intrinsic pathway of apoptosis by ROS (Miller et al., 2003; Grossmann et al., 2010). Apart from monounsaturated fatty acids, certain atypical PUFAs also hold promise. Jacaric acid (Fig. 6; Table 2) from jacaranda is an isomer of linolenic acid (Fig. 6) and has a conjugated triene system that exerts anti-tumor actions in vitro and in nude mice carrying DLD-1 cell xenografts in vivo (Shinohara et al., 2012). Similarly, α-eleostearic acid (9-*cis*-11–*trans*-13–*trans* 18:3; Fig. 6; Table 2) from bitter gourd was relatively effective in increasing lipid peroxidation, decreasing $\Delta\Psi$ and releasing cytochrome c from the mitochondrion in MDA-MB-231 cells in vitro. In vivo, tumor cell-induced vessel formation was suppressed in mice that were administered α-eleostearic acid (50 and 100 mg/kg/day; Tsuzuki & Kawakami, 2008). α-Eleostearic acid also inhibited capillary network formation and migration by human umbilical vein endothelial cells. The underlying mechanism was decreased angiogenesis by down-regulation of VEGF-receptors 1 and 2, activation of PPAR-γ and induction of apoptosis.

6. Strategies to enhance the stability of eicosanoids and other lipid-derived agents

Because eicosanoids are autacoid signaling molecules that are generated near the site of action, and are rapidly metabolized after eliciting a biological response, their chemical and metabolic instability has hindered their development as useful drugs. However, their potential value has motivated research on strategies to inhibit their metabolism and improve stability. To date this has led to numerous agents with improved in vivo activity and several clinically approved drugs. Some of these strategies are also applicable to development of stabilized fatty acids, as both compound classes share metabolic routes including β- and ω-oxidation. Fatty acids present their own set of challenges to drug design and development. While ligand-based design approaches are straight forward with prior knowledge of the drug target, the flexibility inherent in fatty acid chains can confound pharmacophore and pseudo-receptor modeling as the identification of optimal low-energy and biologically active conformations may be difficult and time consuming (Cui et al., 2012). However as the ALPs demonstrate, development of efficacious fatty acid-based drugs in still achievable without identification of the target. In this section the major pathways of eicosanoid and fatty acid degradation are elaborated in relation to the strategies used to circumvent them.

6.1. C15-hydroxyl oxidation

Oxidation of the 15-hydroxyl group in PGs, prostacyclins and lipoxins is mediated by C_{15}-hydroxyprostaglandin dehydrogenase and is the major metabolic route for these eicosanoids (Collins & Djuric, 1993). Oxidation or inversion of stereochemistry at this moiety renders these compounds biologically inactive (Collins & Djuric, 1993; Duffy & Guiry, 2010). Inhibition of this process was first achieved by addition of either one C15 methyl (Table 3, entry 1) or two C16 methyl groups (Table 3, entry 2) to PGE_2 (Bundy et al., 1971; Robert et al., 1976). The resulting compounds were orally active and exhibited longer durations of action than the parent. Methylation has since become a common strategy to stabilize the C15-hydroxyl group, appearing in a number of PG and lipoxin A_4 analogues (Collins & Djuric, 1993; Serhan et al., 1995).

Inclusion of bulky groups adjacent to the C15 hydroxyl has also proved a valuable strategy to prevent degradation. PGE_2 analogues bearing phenoxy groups at the C16 position, including sulprostone (Table 3, entry 3), were not metabolized by C_{15}-hydroxyprostaglandin dehydrogenase and possessed enhanced in vivo activity compared to alkyl analogues (Schaaf et al., 1981). Cyclohexyl (Table 3, entry 4) and phenoxy-substituted lipoxin A_4 analogues were similarly resistant to C15 oxidation as were the C15 methylated analogues (Serhan et al., 1995). It is therefore not surprising that phenyloxy, substituted phenoxy, cyclopentyl and cyclohexyl groups are common structural motifs in stable eicosanoid analogues (Collins & Djuric, 1993).

6.2. β-Oxidation

β-Oxidation is a major pathway for the metabolism of fatty acids and eicosanoids (Diczfalusy et al., 1991) that occurs primarily in mitochondria, and is a chain-shortening process in which fatty acids are broken down into acetyl-CoA units that are processed in the citric acid cycle to generate energy. Following transport to the mitochondrion, the first step in β-oxidation is dehydrogenation between the α and β carbons adjacent to the carboxylic acid. The resulting olefin is hydrated, oxidized and cleaved to remove a 2 carbon unit from the lipid chain in order to generate acetyl-CoA.

A common strategy to prevent β-oxidation is insertion of a heteroatom into the β-position of the fatty acid chain. The heteroatom – typically oxygen or sulfur – prevents the first step of β-oxidation, acetyl CoA dehydrogenase mediated carbon-carbon double bond formation. An early and successful application of this strategy can be found in the development of stable prostacyclin analogues. Iloprost, a stabilized prostacyclin analogue, was found to have a short half-life ($t_{1/2}$ = 20–30 min) due to rapid metabolism, primarily through β-oxidation (Hildebrand et al., 1990). However, cicaprost (Table 3, entry 5), which contains a 3-oxo group, has a longer half-life of 1–2 h and its clearance was only ~20% of that of iloprost (Hildebrand et al., 1989); additionally, ~80% of the dose was excreted unchanged, which is consistent with improved metabolic stability. Further β-oxidation-resistant ethers were prepared, including the prostacyclin analogue 3-oxa-iloprost (Stürzebecher et al., 1986), and stable analogues of PGE_2 (Elworthy et al., 2004) (Table 3, entry 6) and lipoxin (Table 3, entry 7) (Guilford et al., 2004). In general the biological activities of the parent compound were conserved in this approach and β-oxidation was prevented, but not always with a corresponding improvement in pharmacokinetic profile.

Introduction of cycloalkyl, phenyl (Elworthy et al., 2004; Zhao et al., 2007) and heterocyclic (Elworthy et al., 2004; Kambe et al., 2012) groups adjacent to the carboxylic acid have also been used to block β-oxidation. A prostanoid derivative bearing a cyclobutylene adjacent to the carboxylic acid group (Table 3, entry 8) had a six-fold improvement in half-life following oral administration, as well as greater in vitro biological activity (Matsumura et al., 1995). In an attempt to prevent β-oxidation of PGE_2, the α- and β-carbons were replaced with a thiophene ring, which extended the half-life (Cameron et al., 2006). Although detailed metabolic data have not yet been provided, biological activity is generally well retained in these compounds.

Tetradecylthioacetic acid (TTA; Fig. 6) is an anticancer lipid that has a sulfur atom at the C3 position in the carbon chain which prevents β-oxidation (Hvattum et al., 1991). TTA decreased proliferation and induced apoptosis in a range of tumor cell lines in vitro and in vivo (Tronstad et al., 2003; Iversen et al., 2006). Long-chain 3-thia-fatty acids uncouple oxidative phosphorylation and dissipate the mitochondrial $\Delta\Psi$ due to direct interactions with the adenine nucleotide translocator and which leads to decreased ATP production (Wieckowski & Wojtczak, 1998). TTA also stimulates mitochondrial ROS production, which depletes glutathione and renders mitochondria susceptible to further damage (Tronstad et al., 2001, 2003). Release of mitochondrial cytochrome c enhanced caspase-3 activation and PARP

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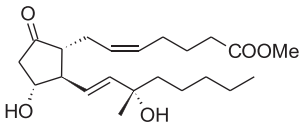
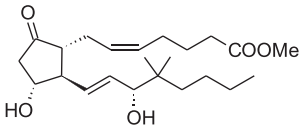
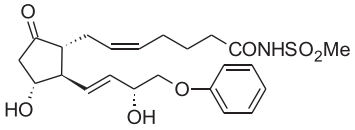
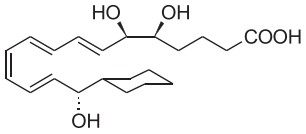
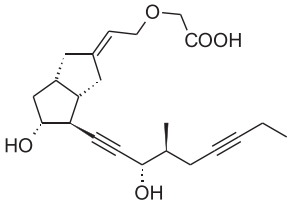
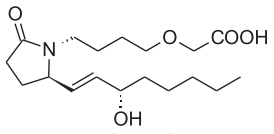
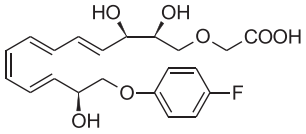
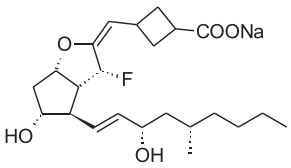
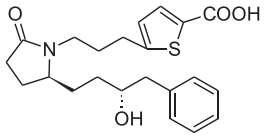
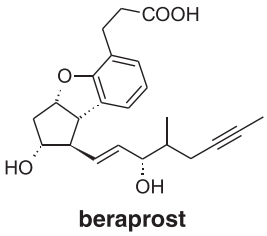
cleavage. A diet containing TTA increased the vascularization of colon cancer xenografts in mice and improved the survival of mice with leukemia xenografts (Jensen et al., 2007).

Amelioration of β-oxidation has also been achieved by bioisosteric replacement of the carboxylate group. Incorporation of a methane sulfonamide isosteric group into sulprostone (Table 3, entry 3), a PGE_2

Table 3
Structural modifications in prostanoid and fatty acid metabolites that have enhanced stability.

Entry	Structure	Parent	Metabolic liability	Modification	Outcome	Reference
1		PGE_2	Oxidation of 15-hydroxyl group	C15-methylation	Orally active, ↑ duration of action, 30-fold ↑ in potency in vivo	Robert et al., 1976
2		PGE_2	Oxidation of 15-hydroxyl group	C16-dimethylation	Orally active, ↑ duration of action, 50-fold ↑ in potency in vivo	Robert et al., 1976
3	 **sulprostone**	PGE_2	15-Hydroxyl oxidation and β-oxidation	Terminal phenoxy group and isosteric sulfonamide	Not substrate for C15-hydroxyprostaglandin dehydrogenase, 30-fold ↑ in potency in vivo	Schaaf et al., 1981
4		LXA_4	15-Hydroxyl oxidation	Terminal cyclohexyl group	↑ Resistance to C15-hydroxyprostaglandin dehydrogenase	Serhan et al., 1995
5	 **cicaprost**	prostacyclin	β-Oxidation and cyclic enol ether hydrolysis	3-Oxo group and enol ether oxygen to methylene conversion	↑ Half-life, ↓ clearance, 80% of dose excreted unchanged	Hildebrand et al., 1989
6		PGE_2	β-Oxidation and hydroxyl cyclopentanone degradation	3-Oxo group and hydroxyl cyclopentanone to lactam conversion	↓ Clearance but no improvement in half-life relative to alkyl analogue.	Elworthy et al., 2004
7		LXA_4	β-Oxidation	3-Oxo group	Prevention of β-oxidation but no improvement in pharmacokinetics	Guilford et al., 2004
8		prostacyclin	β-Oxidation and cyclic enol ether hydrolysis	Cycloalkyl group adjacent to COOH and ring electron withdrawing group	6-Fold increase in duration of action relative to iloprost	Matsumura et al., 1995
9		PGE_2	β-Oxidation and hydroxyl cyclopentanone degradation	Thiophene ring adjacent to COOH and hydroxyl cyclopentanone to lactam conversion	↑ Half-life	Cameron et al., 2006
10	 **beraprost**	prostacyclin	Cyclic enol ether hydrolysis	Benzoannelation	Orally active, ↑ half-life	Melian & Goa, 2002

(continued on next page)

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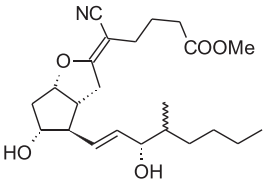
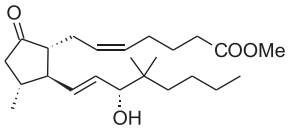
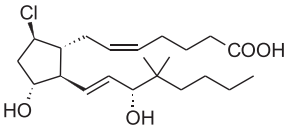
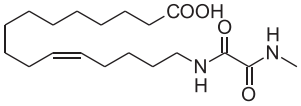
Table 3 (*continued*)

Entry	Structure	Parent	Metabolic liability	Modification	Outcome	Reference
11	 **nileprost**	prostacyclin	15-Hydroxyl oxidation and cyclic enol ether hydrolysis	C16 methylation and 5-cyano group	↑ Acid stability, ↑ half-life	Krause et al., 1983
12	 **trimoprostil**	PGE_2	15-Hydroxyl oxidation and hydroxyl cyclopentanone degradation	C16-dimethylation and hydroxyl to methyl conversion	↑ Half-life	Wills et al., 1986
13	 **nocloprost**	PGE_2	15-Hydroxyl oxidation and hydroxyl cyclopentanone degradation	C16-dimethylation and carbonyl oxygen to chloro conversion	↑ Stability, orally available	Tüber et al., 1993
14		17,18-epoxy EPA	Epoxide hydrolysis	Epoxide to oxamide conversion	↑ Stability	Falck et al., 2011

analogue, dramatically improved in vivo activity and was attributed to β-oxidation resistance (Jacob & Shulgin, 1981). Other potential carboxylate isosteres include tetrazole and alkyl phosphinic acid (Soper et al., 2001).

6.3. ω-Oxidation

ω-Oxidation is a CYP-mediated pathway in which the carbon at the ω-end of the fatty acid chain is oxidized to a hydroxyl group that enables further oxidation by alcohol and aldehyde dehydrogenases (Roman, 2002). This can be considered as activation of the lipophilic carbon chain in fatty acids to facilitate β-oxidation at either end of the molecule. Replacement of the terminal ω-alkyl chain with aromatic (Carpio et al., 1987; Serhan et al., 1995) and cycloalkyl (Serhan et al., 1995) groups is a straightforward strategy to prevent ω-oxidation while retaining biological activity (Collins & Djuric, 1993). These substituents also inhibit C15-hydroxyl group oxidation, which effectively prevents two metabolic pathways simultaneously.

6.4. Rapid metabolism

The ring systems of prostanoids and related lipid mediators are sites of chemical and metabolic degradation. The cyclic enol ether moiety of prostacyclin is chemically and metabolically labile to hydrolysis ($t_{1/2} = 5$ min at pH 7.4) (Whittaker et al., 1976), preventing its clinical application. One successful stabilization strategy has been replacement of the ether oxygen with a methylene group. This modification has been widely used and has produced clinically approved drugs, such as carbacyclin, iloprost and cicaprost (Table 3, entry 5). Other heteroatoms, such as sulfur (Nicolaou et al., 1977) and nitrogen (Bundy & Baldwin, 1978), have also been used as oxygen replacements. Incorporation of the double bond into an aromatic system, to produce benzoannelated analogues, has resulted in stable and functional prostacyclin analogues, such as beraprost (Table 3, entry 10) (Melian & Goa, 2002). Reduction of the olefinic bond also produced more stable prostacyclin analogues that retained activity (Fraga et al., 1996). The introduction of electron-withdrawing groups has been used to stabilize enol ethers against acid hydrolysis; an example is incorporation of a 5-cyano group at the distal vinyl carbon of nileprost (Table 3, entry 11) (Krause et al., 1983) or fluorination of the bicyclic ring system (Table 3, entry 8) (Fried et al., 1980; Matsumura et al., 1995).

Stabilization of the labile hydroxyl cyclopentanone ring to produce PGE_2 analogues has been an important step towards the clinical utilization of this class of compound. Removal or substitution of either the hydroxy or carbonyl oxygen has been used to stabilize the ring against degradation. For example, the hydroxyl-to-methyl and carbonyl oxygen-to-chloro substitutions found in trimoprostil (Table 3, entry 12) (Wills et al., 1986) and nocloprost (Table 3, entry 13) (Tüber et al., 1993), respectively, produced PGE_2 analogues with acceptable pharmacokinetic profiles. Robust PGE_2-type analogues have also been prepared through substitution of the native ring system with heterocycles. Thus, γ-lactam (Table 3, entries 6 and 9) (Elworthy et al., 2004; Cameron et al., 2006; Kambe et al., 2012) and pyrazolidinone (Zhao et al., 2007) systems have been used to improve chemical and metabolic stability and to produce analogues with higher EP receptor subtype selectivity. Stable analogues of PGE_3, the COX-2/PGE synthase-derived product of ω−3 EPA biotransformation, could have value in anticancer drug development. Modifications at C15 and C16, the ω-carbon, prevention of β-oxidation, heteroatom replacement, benzoannelation and ring modification (Fig. 7) could now be undertaken to attempt to capture the anti-tumorigenic properties of the eicosanoid.

6.5. Epoxide isosteres

Bioisosteric replacement of the epoxide group has been successfully employed to produce metabolically robust epoxide mimics. Isosteric groups that most effectively retained the biological activity of the parent epoxide include ethers (Falck et al., 2003; Imig et al., 2010), ureas (Falck et al., 2009), oxamides (Falck et al., 2009, 2011), and amides (Falck et al., 2009, 2011). In metabolic studies conducted in rat liver homogenates it is noteworthy that ω−3 17,18-epoxy-EPA was ~80% metabolized over 30 min, while a partially saturated analogue bearing an oxamide isosteric group remained largely intact (Table 3, entry 14).

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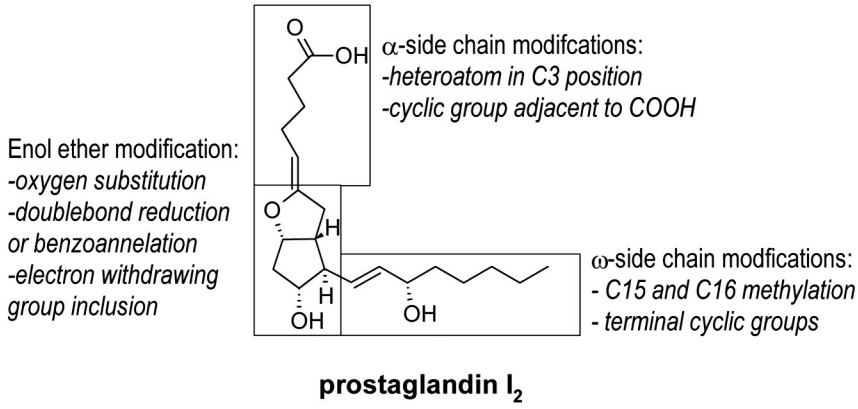

Fig. 7. Summary of synthetic strategies that may be used to increase PG metabolite stability against metabolic degradation, using prostacyclin as the template.

Such analogues of ω−3 epoxyfatty acids, including urea, amide and carbamate isosteres, were assessed recently for their anticancer activity (Dyari et al, 2014). However, these proved to be much less effective than their epoxide counterparts as anti-proliferative and pro-apoptotic agents against human breast cancer cell lines. Further structural modification is now warranted to assess whether alternate isosteres could be effective.

6.6. Development of anticancer strategies based on ω−3 PUFA and their metabolites

In addition to the beneficial direct activities of ω−3 PUFA and certain metabolites, such agents could be tested in combination with established anticancer agents. Fish oil supplementation enhances the efficacy of doxorubicin against MCF-7 breast carcinoma xenografts in nude mice (Hardman et al., 2001) and doxorubicin in combination with dietary DHA supplementation shrank mammary tumors in rats (Colas et al., 2006). The mechanism may involve enhanced lipid peroxidation and ROS production because antioxidants, such as α-tocopherol, abolished the augmentation effect (Colas et al., 2005). Integration of DHA and related PUFAs into membrane phospholipids could increase the susceptibility of tumor cells to free radicals and cytotoxicity induced by drugs such as the anthracyclines. There may be further mechanisms by which ω−3 PUFA metabolites, including epoxides, may contribute to anticancer effects, such as altered raft signaling and ceramide accumulation. These mechanisms may contribute to the beneficial therapeutic effects of drug combinations, such as has been found recently with antitumor ALPs. Thus, by combining classical chemotherapy with targeted therapy, it may be possible to enhance toxicity while lowering the effective concentrations of classical chemotherapeutics necessary for effective elimination of the particular tumor.

7. Summary and conclusions

Evidence is increasing that endogenous lipids are important regulators of proliferative and cell death mechanisms in cancer cells. The adaptation of lipid-based agents into clinical strategies may represent new approaches for the treatment of drug-resistant and advanced tumors. The most intensively studied group of lipid-based agents has been the ALPs. Unlike the majority of conventional anti-cancer agents that target tumor cell DNA or dysregulated signaling cascades, the ALPs act by several potential mechanisms, including the modulation of tumor cell membrane function and interference with phospholipid homeostasis (Fig. 5). This promotes intracellular stresses in tumor cells and activates apoptosis. In other cases intracellular lipid metabolites have been shown to promote cancer cell growth and survival after treatment with cytotoxic agents. Thus, PGE_2 and EETs are protumorigenic metabolites formed in cells by biotransformation of the ω−6 PUFA arachidonic acid. This has led to clinical and experimental approaches to modulate tumor expansion by inhibiting the formation of pro-tumorigenic lipid mediators. However, to the present these strategies have only been partially successful in part because of the toxicity of the inhibitory drugs, especially COX inhibitors. However, better tolerated agents may enable the revival of this strategy. For example, lipid-based synthetic monounsaturated fatty acids may be useful as inhibitors of COX enzymes in tumors that generate proliferative and anti-apoptotic PGs. Alternately, inhibition of downstream signaling mechanisms that promote tumor growth and survival could facilitate new anticancer strategies. Such an approach is now being tested in clinical studies that combine conventional anticancer agents with ALPs like perifosine that act in part by inhibiting the PI3K/Akt prosurvival cascade. For these strategies to be effective greater understanding of important protumorigenic metabolites and how they modulate cancer development is essential.

There are additional classes of lipids that have the potential for further development as anticancer molecules. These include certain saturated fatty acids and ceramides. In studies to date these agents have been found to be well tolerated and to produce minimal toxicity in non-target cells and/or preclinical animal models. The ceramides are particularly interesting because they are released when tumor cells are treated with conventional anticancer agents, or inhibitors. If the release of proapoptotic ceramides could be enhanced by treatments without the non-target toxicity associated with conventional cytotoxic agents, this could be a useful clinical approach.

Additionally there are medicinal chemistry strategies that offer promise in adapting the anticancer activity of naturally-occurring molecule including lipid metabolites, such as the ω−3 PUFA derived PGE_3, epoxides and resolvins. These agents have valuable anticancer actions but these are short-lived in cells due to rapid enzymic degradation. Chemical modification aims to capture the beneficial anti-tumor activities of the molecules. At present these approaches are only in their infancy and there are few examples of the application of these strategies, but studies to date have been promising and have shown in vivo activity. Further experimental studies are now required to optimize these potential lead molecules in cells and experimental animals with the aim of achieving clinical application.

Conflict of interest statement

The authors declare no conflicts of interest.

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