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Lipid Rafts, Endoplasmic Reticulum and Mitochondria in the Antitumor Action of the Alkylphospholipid Analog Edelfosine

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Abstract: The so-called alkylphospholipid analogs (APLs) constitute a family of synthetic antitumor compounds that target cell membranes. The ether phospholipid edelfosine has been considered the long-standing prototype of these antitumor agents and promotes apoptosis in tumor cells by a rather selective way, while sparing normal cells. Increasing evidence suggests that edelfosine-induced apoptosis involves a number of subcellular structures in tumor cells, including plasma membrane lipid rafts, endoplasmic reticulum (ER) and mitochondria. Edelfosine has been shown to accumulate in plasma membrane lipid rafts, ER and mitochondria in different tumor cells in a cell type-dependent way. Edelfosine induces apoptosis in several hematopoietic cancer cells by recruiting death receptor and downstream apoptotic signaling molecules into lipid rafts and displacing survival signaling molecules from these membrane domains. However, *in vitro* and *in vivo* evidences suggest that edelfosine-induced apoptosis in solid tumor cells is mediated through an ER stress response. Both raft- and ER-mediated proapoptotic responses require a mitochondrial-related step to eventually promote cell death, and overexpression of Bcl-2 or Bcl-x_L prevents edelfosine-induced apoptosis. Edelfosine can also interact with mitochondria leading to an increase in mitochondrial membrane permeability and loss of mitochondrial membrane potential. Edelfosine treatment also induced a redistribution of lipid rafts from the plasma membrane to mitochondria, suggesting a raft-mediated link between plasma membrane and mitochondria. The involvement of lipid rafts, ER and mitochondria in the apoptotic response induced by edelfosine may provide new avenues for targeting cancer cells as well as new opportunities for cancer therapy.

Keywords: Apoptosis, CASMER, death receptor, edelfosine, endoplasmic reticulum stress, ether phospholipid, lipid rafts, mitochondria.

INTRODUCTION

The first so-called alkylphospholipid analogs (APLs), also known as alkyl-lysophospholipid analogs (ALPs) or synthetic antitumor lipids (ATLs), were initially synthesized about fifty years ago as metabolically stable analogs of 2-lysophosphatidylcholine (1-acyl-*sn*-glycero-3-phosphocholine, LPC; Fig. **1**), where ester bonds at C1 and C2 of the glycerol backbone of phosphocholine were replaced by ether linkages [1, 2]. Although the initial purpose for the synthesis of those compounds was the search for immune modulators, it later turned out that some of these ether lipids exerted antitumor activities [2-4]. Among the distinct compounds synthesized at the time, it turned out that the ether phospholipid 1-*O*-octadecyl-2-*O*-methyl-*rac*-glycero-3-phosphocholine (edelfosine, ET-18-OCH_3) (Fig. **1**) showed the highest antitumor activity [1, 2]. Additional APLs include (Fig. **1**): a) miltefosine (hexadecylphosphocholine), the minimal structural requirement for the antitumor activity of APLs [5], which has been approved since 1998 in several European countries as a topical cytostatic drug, marketed under the trade name of Miltex® (6% miltefosine solution; Zentaris/Asta Medica, Frankfurt am Main, Germany) as a palliative treatment for cutaneous metastases from breast cancer [6]; b) erucyl-phosphocholine (ErPC, [13Z]-docos-13-en-1-yl 2-(trimethylammonio)ethyl phosphate), an APL-derivative with a 22 carbon atom chain and a *cis*-13, 14 double bond, shows distinctive reduced haemolytic activity, thereby allowing intravenous injection, and holds promise for the treatment of human brain tumors [7]; c) perifosine (D21266, octadecyl-[1,1-dimethyl-piperidino-4-yl] phosphate), identified in 1997 as a promising new orally-active APL compound with potential antineoplastic activity [8], and currently in clinical trials [7].

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Edelfosine has been widely considered the prototype molecule of APLs, and much of the current knowledge on the biological activities and mechanisms of action of APLs derive from studies on edelfosine. In 1993, two independent studies showed that edelfosine induced apoptosis in cancer cells [9, 10], and later on it was found that this ether phospholipid promoted selectively apoptosis in cancer cells, while sparing normal cells [11, 12]. Edelfosine-induced cell death shows all the typical ultrastructural and biochemical hallmarks of apoptosis, including blebbing, chromatin condensation, nuclear membrane breakdown, extensive vacuolation, internucleosomal DNA degradation, caspase activation, and loss of mitochondrial transmembrane potential [11, 12].

Accumulative evidence has shown that edelfosine exerts its destructive actions on tumor cells by interfering with cell membrane phospholipid metabolism and with different processes occurring at the cell membrane [2, 7, 13-15]. Although APLs act mainly through targeting cell membranes, edelfosine also affects gene expression, likely as a consequence of its action on cell membranes. This effect on gene expression should also be taken into account to fully understand the manifold effects triggered by this ether lipid. Incubation of a number of human leukemic cells, including acute T-lymphoblastic leukemia Jurkat cells, acute myeloid HL-60 leukemia cells or leukemic monocyte lymphoma U937 cells, with edelfosine led to a remarkable increase in the steady-state mRNA levels of c-*jun* proto-oncogene [16]. The levels of *jun* B and *jun* D mRNA proto-oncogenes were also dramatically upregulated following edelfosine treatment, whereas expression of c-*fos* was weakly induced [16]. These increases in c-*fos* and c-*jun* mRNA levels were associated with the activation of the AP-1 transcription factor after addition of edelfosine to human leukemic cells, as assessed by an enhanced binding activity of transcription factor AP-1 to its cognate DNA sequence as well as by stimulation of transcription from an AP-1 enhancer element [16]. Thus, edelfosine can affect gene expression by modulating the activity of transcription factor AP-1. Edelfosine induces c-*jun* upregulation and persistent c-Jun N-terminal kinase (JNK) activation, and these actions seem to be involved in its antitumor action [17, 18].

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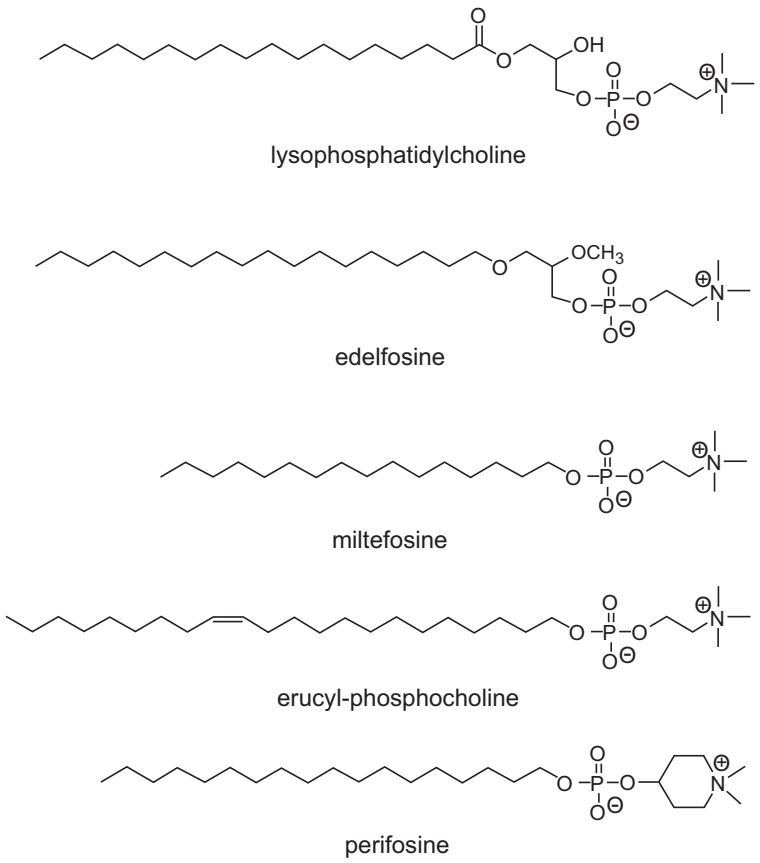

Fig. (1). Chemical structures of LPC and synthetic APLs.

Membrane-located signaling events might be envisaged to be involved in the effect of edelfosine on gene expression. In this regard, phospholipase D activity has been shown to play a role in the activation of transcription factor AP-1 [19]. Edelfosine has also been reported to inhibit the activation of the transcription factor nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) induced by 12-*O*-tetradecanoylphorbol-13-acetate (TPA), but not by tumor necrosis factor-α (TNF-α)- or interleukin-1α (IL-1α)-induced NF-κB activation [20].

There is increasing and compelling evidence to suggest that APLs act mainly through targeting different subcellular structures at the membrane level [21-24]. As mentioned above, a remarkable and pivotal trait of edelfosine antitumor action is its selective proapoptotic activity against tumor cells, whereas normal non-transformed cells, save activated T-cells [25], are spared [11, 21, 26-29]. On these grounds, it is important to unravel the mechanism of action of this family of antitumor drugs that show promise in the treatment of cancer. The present review discusses new developments in the mechanism of action of these APLs, focusing in edelfosine, by targeting several subcellular structures, which set novel frameworks in both apoptosis regulation and tumor therapy, thus opening new avenues for cancer treatment.

MECHANISM OF ACTION OF EDELFOSINE INVOLVING THE CELL MEMBRANE

Effects of APLs on Proteins Located or Acting at the Membrane Level

Because of their chemical structure, APLs are expected to insert into the lipid bilayer of the plasma membrane through their long apolar hydrocarbon chain. Edelfosine is a surface-active amphiphile, containing both lipophilic and hydrophilic moieties, with critical micellar concentrations at 3.5 μM and 19 μM in water [30]. Upon interaction with phospholipid vesicles, edelfosine exhibits a weak detergent activity, causing release of vesicle contents to a low extent (<5%), and a small proportion of lipid solubilization [30]. The weak detergent properties of edelfosine can be related to its very low critical micellar concentrations. Its high affinity for lipid monolayers combined with low lytic properties support the use of edelfosine as a putative clinical drug [30]. Edelfosine affects several biophysical parameters on model lipid membranes, similarly to a number of structurally-related asymmetric phospholipids, but in contrast to these latter structurally-related compounds, edelfosine shows a potent antitumor activity [31]. This suggests that other factors, such as the presence of specific proteins and the interaction of proteins with membranes, might be implicated in the anticancer action of edelfosine [31]. The ready incorporation of edelfosine into membranes may provide an explanation for the effect of edelfosine on membrane protein function as well as on protein membrane composition, affecting the interaction of proteins with other proteins or lipids at the membrane. In this regard, edelfosine and additional APLs have been shown to affect the function of a number of proteins that act at the cell membrane (Table **1**).

Edelfosine has been reported to reduce the number of epidermal growth factor receptor (EGFR) sites without affecting the affinity of the receptors in human breast cancer cell lines [32], and this reduction in the epidermal growth factor (EGF) binding capacity was suggested to be related to the edelfosine-induced inhibition of the growth of hormone-dependent breast cancer cells. Edelfosine had no effect on the binding of EGF to its receptors, their activation, or p21-Ras activation [33]. However, edelfosine has been shown to interfere with the association of Raf-1 with membranes, resulting in a decrease in Raf-1 kinase activity in membranes of edelfosine–treated cells [34]. Activation of the

Table 1. Effect of APLs on signaling and metabolic proteins that act at the membrane level.

Protein	APL	Effect	Process	Reference
PI-PLC	Edelfosine	Inhibition	---	[37]
PLC-β1	Edelfosine	Inhibition	Inhibition of the association of PLC-β1 with its activator $G\alpha_{q/11}$	[36]
PLD	Edelfosine, ilmofosine	Inhibition	Inhibition of phorbol ester-stimulated PLD activity	[39]
CCT	Edelfosine, miltefosine	Inhibition	---	[64-67]
Ras	Edelfosine	Inhibition	Inhibition of the association of Ras and Raf-1	[34]
Akt	Perifosine, miltefosine, edelfosine	Inhibition	Decrease in the plasma membrane localization of phosphorylated Akt; perturbation of membrane translocation of pleckstrin (PH) domain-containing molecules; displacement of Akt from lipid rafts	[43-45, 175]
Fas/CD95	Edelfosine, perifosine	Activation	Recruitment of Fas/CD95 and downstream signaling molecules in lipid rafts	[21, 22, 27-29, 124]

mitogen-activated protein kinase (MAPK) pathway subsequent to growth factor stimulation requires the recruitment of Raf-1 from the cytosol to the membrane, a process mediated by the interaction of Raf-1 with activated Ras. Edelfosine had no direct effect on MAPK or Raf-1 kinase activity, but a correlation between edelfosine accumulation, inhibition of cell proliferation, Raf-1 association with the membrane, and MAPK activation was established, thus suggesting that inhibition of the MAPK cascade by edelfosine as a result of its effect on Raf-1 activation may be involved in the inhibition of cell proliferation by edelfosine [33]. Edelfosine has been suggested to associate with Raf-1 in the cytosol to interfere in the interaction of Raf-1 with activated Ras, thereby reducing the levels of Raf-1 that are translocated to the membrane for activation, and suggesting Raf-1 as a molecular target of edelfosine [34]. In cells that were able to take up the drug but were insensitive to edelfosine, high levels of membrane Raf-1 were detected, and these levels did not change significantly after incubating the cells with the ether lipid; suggesting that edelfosine-induced reduction of Raf-1 levels in drug-sensitive cancer cells is due to inhibition of Raf-1 translocation [35].

Edelfosine alters the physical association of phospholipase C (PLC)-β1 with its direct activator $G\alpha_{q/11}$ [36]. Edelfosine has also been reported to be a potent inhibitor of phosphatidylinositol (PI)-selective PLC, but a weak inhibitor of phosphatidylcholine (PC)-selective PLC and phospholipase D (PLD) [37]. This aspect is of interest as PLC-β1 is more highly expressed in small-cell lung carcinoma (SCLC) than in non-small-cell lung carcinoma (NSCLC), and NSCLC cell lines are significantly more sensitive to the antiproliferative effects of edelfosine compared with the SCLC cell lines [36]. NSCLC cell lines (NCI-H157, NCI-H520, NCI-H522) exhibited G_2/M arrest, significant apoptosis and some degree of JNK activation in response to drug treatment, whereas none of the SCLC cell lines assayed showed edelfosine-induced G_2/M arrest or significant apoptosis [38].

The APLs edelfosine and 1-*S*-hexadecylthio-2-methoxymethyl-2-deoxy-*rac*-glycero-3-phosphocholine (BM41.440; ilmofosine) have been found to inhibit phorbol ester-stimulated PLD [39]. However, there is a certain controversy on this issue as other reports show no inhibition of PLD by edelfosine [37, 40]. On the other hand, *Saccharomyces cerevisiae* yeast with deleted *SPO14* gene, which encodes the sole yeast PC hydrolyzing PLD, showed increased sensitivity to edelfosine compared to its wild counterpart [41]. This sensitivity was rescued by the wild-type Spo14p protein, but not by a catalitically inactive point mutant ($Spo14p^{K\rightarrow H}$) or a mutant protein lacking 150 amino acids in its NH_2 terminus [41], that is enzymatically active but mislocalized [42]. Thus, an enzymatically active and properly localized PLD is required to alleviate cytotoxicity to edelfosine in yeast. On these grounds, phosphatidic acid generated from phosphatidylcholine hydrolysis by Spo14p seems to regulate susceptibility to edelfosine [41].

Edelfosine, perifosine and miltefosine have been reported to inhibit phosphatidylinositol 3-kinase (PI3K)-Akt/protein kinase B (PKB) survival pathway [43], likely through inhibition of the plasma membrane recruitment of the survival serine-threonine kinase Akt *via* its pleckstrin homology (PH) domain [44, 45]. Unlike previous ATP-competitive inhibitors, perifosine has been shown to prevent the recruitment of Akt to the plasma membrane by disrupting membrane microdomains essential to growth factor signaling and Akt activation and/or by displacing the natural ligands, PI(4,5)P2 and PI(3,4,5)P3 (generated by PI3K activity) from the PH domain of Akt [44-46], thus Akt cannot adopt the favorable conformation for its activation through phosphorylation at Thr308 and Ser473 [47]. Only the myristoylated form of Akt, which bypasses the requirement for PH domain-mediated membrane recruitment, abrogated perifosine-mediated decrease of Akt phosphorylation and cell growth inhibition [44]. Perifosine does not directly affect PI3K, phosphoinositide-dependent kinase 1 or Akt activity at concentrations inhibiting Akt phosphorylation and membrane localization [44]. Perifosine decreased the plasma membrane localization of Akt, and this was relieved by myristoylated-Akt along with relief of downstream drug effect on induction of p21(WAF1/CIP1) [44]. In this regard, a number of rationally designed phosphatidylinositol ether lipid analogs, some of them structurally related to edelfosine, but containing inositol instead of choline, inhibit Akt by targeting the PH domain, thus inhibiting growth factor-induced translocation of Akt to the plasma membrane [48]. In addition, newly synthesized cyclopentanecarboxylate-substituted alkylphosphocholines have been recently found to exhibit inhibition of Akt phosphorylation and cytotoxicity against several human cancer cell lines, including lung cancer A549, breast carcinoma MCF-7 and gastric carcinoma KATO III cells [49]. PI3K/Akt signaling is a major factor in the tight regulation of Fas/CD95 activation, inhibiting the triggering of this cell death receptor [50-52]. The function of class I PI3Ks is to convert phosphatidylinositol-4,5-bisphosphate (PI(4,5)P2) to phosphatidylinositol-3,4,5-trisphosphate at the inner leaflet of the plasma membrane [53]. This PI(3,4,5)P3 generation is in turn tightly controlled and its production is prevented by the action of several phosphatidylinositol phosphatases (PTEN, SHIP1, and SHIP2). The production of PI(3,4,5)P3 leads to the binding and recruitment to the inner leaflet of the plasma membrane of enzymes containing PH domains, such as Akt. Once being recruited at the cell surface, Akt is activated through its phosphorylation on Thr308 and Ser473, and then phosphorylates a variety of substrates involved in pleiotropic cell functions, including cell growth, proliferation and survival, what it makes of this PI3K/Akt signaling route a major target in cancer therapy [53-63].

One of the first targets in the action of APLs was ascribed to the inhibition of CTP:phosphocholine cytidyltransferase (CCT) with the subsequent inhibition of PC synthesis [64-68]. Edelfosine and

several additional APLs inhibit PC synthesis [64-68], and this effect seems to be a rather universal action of APLs. PC biosynthesis occurs predominantly *via* the Kennedy or CDP-choline pathway [69] (Fig. **2**). This route involves the choline uptake by the cell, followed by its phosphorylation by choline kinase, and then CCT catalyzes the transfer of choline from phosphocholine to cytidine 5'-diphosphate choline (CDP-choline), which is subsequently used by choline phosphotransferase along with diacylglycerol to form PC [70-72]. CCT is a key enzyme controlling PC biosynthesis, acting as the rate-limiting and regulatory step [70-72], and it has long been shown to be required for cell survival in mammalian cells [73, 74]. CCT resides in the nucleus and cytoplasmic compartments, and translocates to the ER when activated [75-78]. Edelfosine and miltefosine have been reported to inhibit *de novo* PC synthesis at the CCT step, leading to mitotic arrest and cell death in a number of tumor cells [64-68]. Overexpression of CCT, corresponding to CCTα isoform, prevented edelfosine-induced apoptosis in HeLa cells [67]. Edelfosine and miltefosine mimic LPC, a physiological regulator of CCT activity, inhibiting CDP-choline formation and CCT activity by competitive inhibition with respect to lipid activators [64-66]. Addition of exogenous LPC, an alternative precursor for PC formation through its acylation to generate PC [79], prevents apoptosis induction by either edelfosine [65] and miltefosine [64], suggesting that inhibition of PC synthesis is involved in APL-induced apoptosis. Thus, the uptake and subsequent acylation of exogenously added LPC circumvented the requirement of CCT activity by providing an alternate route to PC synthesis, and prevented edelfosine- and miltefosine-induced apoptosis [64, 65, 68]. However, a number of findings have challenged the association of PC inhibition with the antitumor action of edelfosine, including: a) induction of apoptosis by edelfosine is very rapid in HL-60 cells, whereas changes in PC metabolism take place at longer incubation times [80]; b) there is a lack of correlation between PC inhibition and edelfosine sensitivity in different cell lines [80-82]; c) edelfosine inhibits PC synthesis in drug-resistant Bcl-x_L-trasfected HeLa cells similarly to wild-type sensitive HeLa cells [24]; d) similar inhibition patterns of PC synthesis are observed in both wild-type sensitive cells and drug-resistant $bax^{-/-}/bak^{-/-}$ cells [24].

Because PC is the major lipid component in mammalian membranes, accounting for more than 50% of cell membrane phospholipids and more than 30% of total cellular lipid content, and cells must double their phospholipid mass to form daughter cells during cell division, we can envisage that a major consequence of APL-induced PC biosynthesis inhibition is the blockade of cell cycle. The G_1 phase of the cell cycle is characterized as having a high rate of PC turnover, both degradation and resynthesis [83]. The rate of PC degradation decreases by an order of magnitude in S phase, and thereby a net accumulation of phospholipid is a periodic event associated with the S phase of the cell cycle. On the other hand, the G_2 and M phases are characterized by the cessation of phospholipid metabolism [83]. In agreement with this, the key regulatory enzyme in PC biosynthesis, CCT, is phosphorylated on its carboxy-terminal domain at the G_2/M phase, thus leading to enzyme inhibition [84, 85]. Gene transfer-mediated overexpression of Bcl-x_L prevents apoptosis in edelfosine-treated HeLa cells, but the ether lipid still inhibits PC synthesis and cells accumulate in G_2/M phase [24]. Likewise, $bax^{-/-}bak^{-/-}$ double knockout cells are resistant to edelfosine, accumulate in G_2/M phase following edelfosine treatment, and PC synthesis is weakly and similarly inhibited in either wild-type and $bax^{-/-}bak^{-/-}$ double knockout cells [24]. In addition, edelfosine arrests cell cycle in G_2/M phase by inhibiting cytokinesis in a number of cancer cells [86, 87] (Gajate, G. and Mollinedo, F., unpublished results). Thus, it is worthy to note that although PC synthesis occurs at the S phase, the whole process of nuclear division is not apparently affected, and only cytokinesis is inhibited in edelfosine-treated cells. Furthermore, miltefosine-induced apoptosis in CHO cells was found to differ substantially from the apoptosis observed in CHO-MT58 cells that contain a genetic defect in CCT and PC synthesis, as it was (i) not accompanied by a large decrease in the amount of PC and diacylglycerol (DAG), (ii) not preceded by induction of the proapoptotic protein GADD153/CHOP, and (iii) not dependent on the synthesis of new proteins. Furthermore, LPC as well as lysophosphatidylethanolamine (LPE) could antagonize the apoptosis induced by miltefosine in CHO-MT58 cells, even though only LPC was able to rescue the effect of the drug on PC synthesis [88]. On these grounds, it is unlikely that inhibition of PC synthesis is the major process involved in APL-induced apoptosis. Thus, it might be envisaged that APL-induced apoptosis is more dependent on a general effect on membrane structure than in a specific inhibition of PC synthesis.

The putative involvement of ceramide in APL-induced apoptosis is a subject of debate. While miltefosine has been

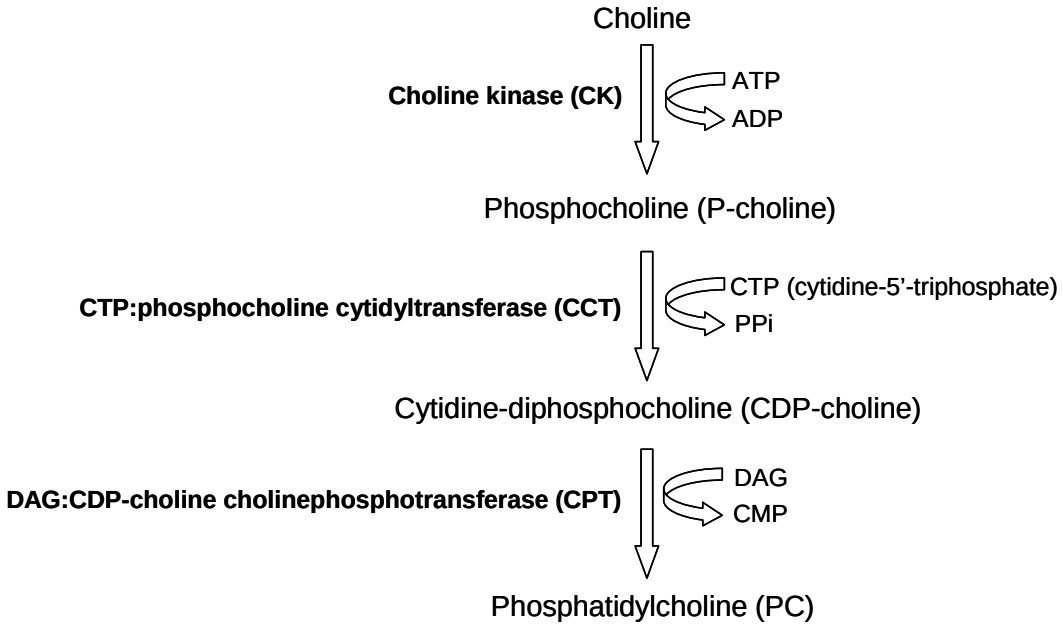

Fig. (2). Kennedy pathway of PC biosynthesis.

reported to increase ceramide cellular content [89], edelfosine failed to induce significant elevations in endogenous ceramide levels in drug-sensistive tumor cells [21].

LIPID RAFTS AND DEATH RECEPTOR FAS/CD95 IN THE MECHANISM OF ACTION OF EDELFOSINE

Interestingly, edelfosine prompts a potent aggregation and capping of Fas/CD95 death receptor during the triggering of edelfosine-induced apoptosis [26], which turned out to co-localize with aggregates or platforms of specific membrane microdomains enriched in cholesterol and sphingolipids named "lipid rafts" [22].

Cholesterol is of major importance in raft organization and acts as the dynamic "glue" that holds the raft together [90-93]. Cholesterol is composed of a four-ring structure with an aliphatic tail that can pack tightly with the lipid acyl chains of sphingolipids to create a compacted region of condensed bilayer leading to a liquid-ordered state [94, 95]. The presence of saturated hydrocarbon chains in sphingolipids allows for cholesterol to be tightly intercalated, leading to the presence of distinct liquid-ordered phases, membrane rafts, dispersed in the liquid-disordered matrix, and thereby more fluid, lipid bilayer. This rather unique lipid composition makes lipid rafts insoluble in cold non-ionic detergents, a property that has been extensively used to isolate these membrane domains followed by gradient centrifugation [96-98]. Current evidence indicates that lipid rafts are membrane microdomains of reduced fluidity that serve as foci for recruitment and concentration of signaling molecules at the plasma membrane, where signaling molecules assemble and function, and thus they have been implicated in signal transduction from cell surface receptors [90, 94, 99, 100]. Interestingly, edelfosine was found to interact with cholesterol and affects cholesterol-enriched model membranes [101, 102].

The co-clustering of rafts and Fas/CD95 induced by edelfosine unveiled a new molecular process regulating apoptosis, thus involving for the first time membrane rafts in Fas/CD95-mediated apoptosis and cancer chemotherapy [22]. Co-aggregation of Fas/CD95 and lipid rafts was assessed by both confocal microscopy (Fig. **3**) and isolation of membrane rafts through sucrose gradient centrifugation [22]. Disruption of rafts following pretreatment with methyl-β-cyclodextrin (MCD), that depletes cholesterol from the cell membrane, inhibited both edelfosine-induced Fas/CD95 clustering and apoptosis [22]. Subsequent studies showed that adaptor protein Fas-associated death domain-containing protein (FADD) and procaspase-8, which together with Fas/CD95 form the so-called "death-inducing signaling complex" (DISC), were also recruited in lipid rafts following edelfosine treatment [21]. A major role of lipid rafts in Fas/CD95-mediated apoptosis was further supported by the finding that overexpression of membrane sphingomyelin, a major component of lipid rafts, through transfection with sphingomyelin synthase 1 (SMS1), a gene responsible for sphingomyelin synthesis, enhanced Fas/CD95-mediated apoptosis through increasing DISC formation, activation of caspases, efficient translocation of Fas/CD95 into lipid rafts, and subsequent Fas clustering [103]. Fas/CD95 is palmitoylated and this post-translational modification of Fas/CD95 is essential for its redistribution into lipid rafts and its association to actin cytoskeleton [104], which plays a critical role in the

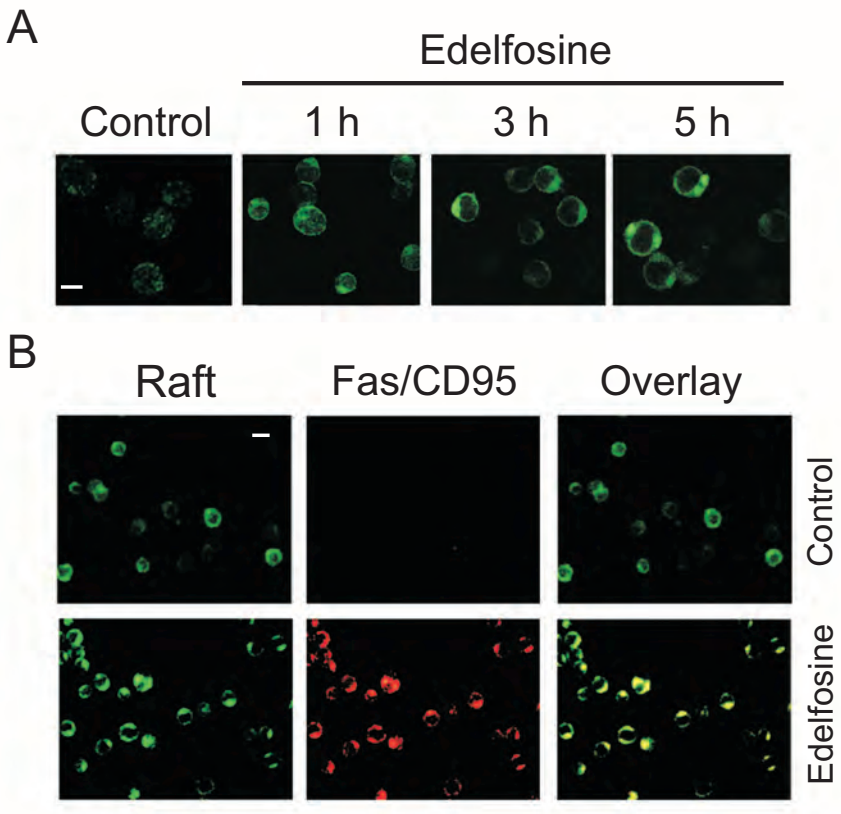

Fig. (3). Co-clustering of membrane rafts and Fas/CD95 in edelfosine-treated leukemic cells. (**A**) Time-course of the effect of antitumor phospholipid ether edelfosine on aggregation of membrane rafts. T-cell leukemic Jurkat cells were either untreated (Control) or treated with 10 μM edelfosine for the times indicated. Cells were then stained with fluorescein isothiocyanate-labeled cholera toxin B subunit (FITC-CTx) used as a raft marker, and analyzed by confocal microscopy. *Bar*, 7 μm. (**B**) Co-localization of membrane rafts (Raft) and Fas/CD95 in edelfosine-treated Jurkat cells. Cells were either untreated (Control) or treated with 10 μM edelfosine for 3 h, and processed for confocal microscopy using FITC-CTx (green fluorescence for lipid rafts) and anti-Fas/CD95 monoclonal antibody, followed by CY3-conjugated anti-mouse antibody (red fluorescence for Fas/CD95). Areas of co-localization between membrane rafts and Fas/CD95 in the overlay panels are yellow. *Bar*, 10 μm. Data from ref. 22.

internalization of the death receptor, a necessary step for the death signal transmission [105].

Following the first studies with edelfosine on Fas/CD95 and lipid rafts, it turned out that Fas/CD95 recruitment into lipid rafts was not exclusive to edelfosine treatment. An increasing number of chemotherapeutic agents have been reported to recruit Fas/CD95 in rafts, including resveratrol [106-108], cisplatin [109], aplidin [110], avicin D [111], rituximab [112], anandamide [113], and MS-275 [114]. In addition, the APL perifosine has also been found to be an efficient inducer of Fas/CD95 into lipid rafts in multiple myeloma cells [27].

DISC FORMATION IN RAFTS FOLLOWING EDELFOSINE TREATMENT

Unlike other cell surface receptors that have intrinsic enzymatic activities, such as kinase activity, cell death receptors depend on protein-protein interactions. Physiological activation of Fas/CD95 by its natural ligand FasL/CD95L leads to the assembly of the DISC [115], containing Fas/CD95, FADD and procaspase-8, which interact each other through the death domain (DD) present in Fas and FADD and the death effector domain (DED) present in FADD and procaspase-8, thus forming a core stoichiometry of a 5Fas/CD95:5FADD complex [116-118]. DISC formation leads to procaspase-8 transactivation that releases mature caspase-8, thus initiating apoptosis through a subsequent caspase activation cascade [119, 120] or through cleavage of BH3-interacting domain death agonist (Bid) into truncated Bid (tBid) [121, 122], which then triggers the apoptotic mitochondrial signaling pathway.

Edelfosine and perifosine have been shown to translocate Fas/CD95 as well as additional downstream signaling molecules, such as the DISC constituents FADD and procaspase-8, into rafts in hematopoietic cancer cells (Table **2**), thus facilitating the triggering of apoptosis [27, 123]. In addition, electron microscopy evidence and co-immunoprecipitation assays provided compelling evidence for the formation of DISC complex into plasma membrane rafts in hematopoietic cancer cells upon edelfosine treatment [27, 124] (Fig. **4**). In addition, downstream apoptotic signaling molecules, including procaspase-10, JNK and Bid were also translocated into membrane rafts of cancer cells following edelfosine treatment [21, 27, 125, 126] (Table **2**). Persistent JNK activation is associated with apoptosis [17, 127, 128], and Bid acts as a connector between Fas/CD95-mediated extrinsic signaling and the mitochondrial-dependent intrinsic pathway of apoptosis [121]. This may explain the dependence of edelfosine-mediated apoptosis on both JNK and mitochondrial signaling [12, 17, 27, 129]. This redistribution of death receptors and downstream signaling molecules into lipid rafts does not require protein synthesis, and therefore it is achieved from the pre-existing protein pool [21]. On these grounds it can be envisaged that the concentration of Fas/CD95, together with FADD and procaspase-8, in rafts from a highly dispersed distribution could facilitate the formation of DISC and downstream signaling, and it may represent a general mode of regulating Fas/CD95 activation.

EDELFOSINE-MEDIATED PROAPOPTOTIC ACTION IS INDEPENDENT OF FASL/CD95L AND EXERTS ITS ACTION FROM WITHIN THE CELL

Subsequent studies to the edelfosine-induced translocation of Fas/CD95 in rafts [22] showed that this receptor was also recruited into lipid rafts following activation with its physiological ligand FasL/CD95L [130, 131]. Some antitumor drugs, such as doxorubicin, methotrexate, cytarabine, and bleomycin have been shown to promote *de novo* FasL/CD95L and kill Fas/CD95-expressing tumor cells *via* a suicide or fratricide Fas/CD95-FasL/CD95L interaction [132-134]. This apoptotic signal was abrogated by pretreating the cells with blocking anti-Fas/CD95 or anti-FasL/CD95L antibodies, thus interfering with Fas/CD95 receptor/ligand interaction [132-134]. However, we found that the involvement of Fas/CD95 in edelfosine-induced apoptosis was independent of FasL/CD95L [21, 26, 123, 135]. Edelfosine did not upregulate FasL/CD95L expression in cancer cells [26], and its proapoptotic action on tumor cells was not inhibited by pretreatment with blocking anti-Fas/CD95 antibodies that otherwise prevented FasL/CD95L-induced apoptosis [26]. The fact that the translocation and capping of Fas/CD95 into membrane rafts following edelfosine treatment was independent of the receptor interaction with its natural ligand FasL/CD95L [22, 26], indicated that Fas/CD95 aggregation and activation could be modulated pharmacologically. Thus, edelfosine might be suggested

Table 2. Effect of APLs on the location of proteins in lipid rafts.

APL	Protein	Change in Raft Location*	Cell Type	Technique**	Reference
Edelfosine/perifosine	Fas/CD95	R	JK, MM cells (primary culture cells, MM-144), JVM-2, EHEB, HL-60, CEM	DRMGC, IEM, IFM	[21, 22, 27-29, 124, 139, 215]
Edelfosine/perifosine	DR4	R	MM-144	DRMGC, IFM	[27, 28]
Edelfosine/perifosine	DR5	R	MM-144	DRMGC, IFM	[27, 28]
Edelfosine/perifosine	FADD	R	JK, MM-144	DRMGC, IEM	[21, 27, 28]
Edelfosine/perifosine	Procaspase-8	R	JK, MM-144	DRMGC, IEM	[21, 27]
Edelfosine	Procaspase-9	R	MM-144	DRMGC	[126]
Edelfosine	Procaspase-10	R	JK	DRMGC	[21]
Edelfosine/perifosine	Bid	R	MM-144	DRMGC	[21, 27]
Edelfosine	JNK	R	JK	DRMGC	[21, 125]
Edelfosine	Hsp90	R	JK	DRMGC	[125]
Edelfosine	Cytochrome *c*	R	MM144	DRMGC	[126]
Edelfosine	APAF-1	R	MM144	DRMGC	[126]
Edelfosine	Akt	D	Z-138, JVM-2, Jeko-1	DRMGC	[175]
Edelfosine/perifosine/miltefosine	Pma1p	D	*S. cerevisiae*	DRMGC	[155, 178, 179]

*D, displacement from rafts; R, recruitment into rafts.

**IFM, immunofluorescence microscopy; DRMGC, non-ionic detergent-resistant membrane (DRM) fractions at 4ºC followed by flotation in density gradient centrifugation; IEM, immunogold electron microscopy.

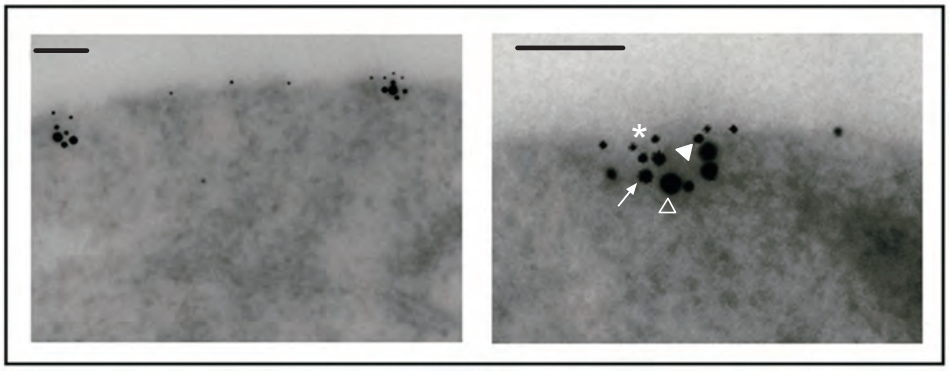

Fig. (4). Electron microscopic evidence for Fas/CD95-DISC clustering in lipid rafts in edelfosine-treated leukemic cells. Sections of edelfosine-treated human acute T-cell leukemia Jurkat cells were labeled with the raft marker ganglioside GM1 using cholera toxin B subunit (6-nm gold, asterisk), anti-Fas/CD95 antibody (10-nm gold, closed arrowhead), anti-FADD antibody (15-nm gold, arrow), and anti-procaspase-8 antibody (20-nm gold, open arrowhead). Lipid rafts are labelled on the external face of the membrane, whereas DISC components are located in the internal face of raft-enriched membrane domains. Bar, 400 nm. Data from ref. 124.

to mimic to some extent the action of the natural ligand FasL/CD95L on promoting death receptor recruitment in lipid rafts. However, there was a noticeable difference in the action of both molecules, namely FasL/CD95 was acting from outside the cell membrane whereas edelfosine acted from within the cell [21, 26]. Thus, Fas/CD95-deficient cells were spared following edelfosine treatment, but became sensitive after bestowing Fas/CD95 expression by gene transfer, and provided that the ether lipid was taken up by the target cell [21, 26, 27]. Deletion of the Fas/CD95 57 C-terminal amino acids that included part of the Fas/CD95 cytoplasmic death domain prevented apoptosis [21, 27]. Fas/CD95-deficient cells that were unable to incorporate the drug were spared, but turned into sensitive ones after cell transfection with wild-type human Fas/CD95 and drug microinjection [21]. Silencing of Fas/CD95 by RNA interference, transfection with a FADD dominant-negative mutant that blocks Fas/CD95 signaling, and specific inhibition of caspase-8 prevented the apoptotic response triggered by edelfosine, hence demonstrating the functional role of Fas/CD95 signaling in drug-induced apoptosis [124]. Taking together, the above findings indicate that Fas/CD95 plays a major role in edelfosine-mediated apoptosis and that edelfosine must be inside the cell to trigger cell death through intracellular activation of Fas/CD95 [14, 21]. Whether edelfosine triggers Fas/CD95 activation by direct interaction with the cytoplasmic part of the death receptor or through an indirect process remains to be elucidated. Preliminary computational docking studies have led to the proposal of a putative interaction of edelfosine with the intracellular Fas/CD95 death domain [14]. Because edelfosine is preferentially taken up by cancer cells [11, 26-29], and once inside the cell, edelfosine triggers Fas/CD95 activation leading to apoptosis, this mechanism represents the first selective activation of Fas/CD95 in tumor cells [21]. The intracellular activation of Fas/CD95 is an attractive way to target cancer cells from within the cell, thus avoiding the deleterious systemic activation of Fas/CD95 death receptor in normal cells, especially in liver [14, 21]. The clinical usefulness of exogenous activation of Fas/CD95 as well as of the other major death receptors, namely TNF and TRAIL receptors, by their respective ligands or agonists may be hampered by toxic side effects. Systemic administration of TNF causes a severe inflammatory response syndrome resembling septic shock [136], administration of agonistic antibody to Fas/CD95 in mice is lethal because of liver failure through massive hepatocyte apoptosis [137], and TRAIL induces apoptosis of human hepatocytes [138]. In contrast, both human and rat hepatocytes are spared after edelfosine treatment [21]. This is due to the fact that edelfosine does not activate Fas/CD95 extracellularly, but from inside the cell in a FasL/CD95L-independent manner, and the ether lipid is taken up preferentially by tumor cells, sparing normal cells. Thus, edelfosine takes advantage of an apparently selective drug uptake in tumor cells and of a general Fas/CD95-mediated apoptotic signaling [11, 21, 26, 27].

PI3K SIGNALING AS MAJOR REGULATOR OF FAS/CD95 LOCALIZATION IN LIPID RAFTS

A number of signaling processes have been suggested as the underlying bases for the above described redistribution of Fas/CD95 into lipid rafts, leading to the elimination of leukemia cells [21, 22, 27, 110, 123, 124]. One of these processes involves the PI3K-Akt signaling pathway, which leads to Akt activation [139]. Legembre's group has shown that inhibition of PI3K signaling triggers the rapid formation of Fas/CD95 clusters and the redistribution of Fas/CD95 into lipid rafts [139]. In addition, edelfosine was shown to down-modulate PI3K signaling in T leukemia cell lines, leading to the induction of a Fas/CD95-mediated apoptotic signal, and the formation of micrometer range clusters of Fas/CD95 into large raft platforms [139]. Edelfosine was very efficient at killing the human T-cell acute lymphoblastic leukemia cell lines CEM and Jurkat as well as activated peripheral blood T lymphocytes, which show a strong PI3K activation [25, 139], while it was ineffective at eliminating both the human T-cell lymphoma H9 cell line and resting naïve peripheral blood lymphocytes, which display a weak PI3K activation [25, 139]. The human B-lymphoblastoid EBV-transformed SKW6.4 cell line that shows an intermediate PI3K activation exhibited also an intermediate sensititivity to edelfosine [139]. The leukemic T-cell line Jurkat does not express the lipid phosphatases Src homology 2 domain-containing inositol-5-phospahatase (SHIP) and the phosphatase and tensin homologue deleted on chromosome 10 (PTEN), which normally counterbalance the PI3K-mediated generation of PI(3,4,5)P3 and hence the level of Akt phosphorylation [140]. Thus, because the APL perifosine has been reported to be unable to inhibit directly PI3K or Akt activity [44], the edelfosine-induced decrease in Akt phosphorylation detected in Jurkat cells can be associated to an indirect down-regulation of the PI3K activity or to the abrogation of the PI(3,4,5)P3 docking action [139]. These data suggest that edelfosine-mediated inhibition of PI3K leads to Fas/CD95-raft co-clustering and eventually to cell killing. As stated above, an outstanding trait of edelfosine proapoptotic action is the intracellular activation of Fas/CD95, that is Fas/CD95 clustering and engagement, which is rendered from inside the cell after the drug is incorporated into the cell [21, 26]. In this regard, the above data point out that an intracellular stimulus (PI3K activation) is able to control the aggregation level of the trasmembrane receptor Fas/CD95. Thus, activated PI3K signaling pathway prevents the

formation of Fas/CD95 clusters in rafts. Because the antiapoptotic PI3K signal is found constitutively activated in numerous types of tumors and cancer cell lines, including Jurkat cells, this could explain the absence of Fas/CD95 in the rafts from untreated Jurkat cells, and the recruitment of Fas/CD95 into rafts [21, 22], paralleled to PI3K inhibition [139], upon edelfosine treatment. On the other hand, activation of PI3K after ligation of CD3 in Th2 cells has been reported to block Fas/CD95 aggregation and subsequent caspase-8 cleavage, by modulating lateral diffusion of CD95 and blocking the formation of Fas/CD95 aggregates, through the effects of PI3K upon actin cytoskeleton [50, 51]. Actin cytoskeleton could inhibit lateral diffusion of Fas/CD95, and after cytochalasin D treatment, a well-characterized agent that promotes actin depolymerization, the lateral diffusion of Fas/CD95 was increased favoring Fas/CD95 aggregation and the ensuing caspase-8 processing towards apoptosis [50]. On these grounds it could be envisaged that one of the actions of PI3K signaling in promoting survival might be the inhibition of Fas/CD95 clustering required to trigger downstream apoptosis signaling.

LIPID RAFTS IN THE PROAPOPTOTIC ACTION OF APLs IN HEMATOPOIETIC CANCER CELLS

We have found that hematopoietic cancer cells, including human cell lines and patient-derived cancer cells from acute myeloid leukemia, acute lymphoid leukemia, chronic lymphocytic leukemia (CLL), multiple myeloma, and mantle cell lymphoma, are especially sensitive to the proapoptotic action of APLs, edelfosine being the most potent APL in promoting apoptosis [11, 27-29, 124]. Edelfosine and perifosine were shown to promote apoptosis in multiple myeloma, CLL and mantle cell lymphoma through the recruitment of Fas/CD95 and TRAIL receptors DR4 (death receptor 4) and DR5 (death receptor 5) together with downstream signaling molecules to lipid rafts [27-29]. Furthermore, the concentration of death receptors in lipid rafts following APL treatment rendered multiple myeloma cells more sensitive to the action of death receptor ligands [27]. Thus, edelfosine and perifosine could be not only effective in the killing of multiple myeloma cells, but also they might be promising drugs in combination therapy, in particular with TRAIL, which shows antimyeloma activity [141-143]. *In vivo* assays in tumor xenograft animal models for multiple myeloma, CLL and mantle cell lymphoma showed a potent antitumor activity of edelfosine oral administration [28, 29]. In addition, tissue distribution studies showed that edelfosine accumulated preferentially in the tumor [28, 29]. Following edelfosine oral administration in non-tumor-bearing mice, a rather wide drug distribution pattern to several tissues, including lung, spleen, intestine, liver and kidney, was observed [144]. However, when severe combined immune deficiency (SCID) mice were injected with human mantle cell lymphoma, CLL, or multiple myeloma xenografts, the edelfosine distribution pattern was dramatically altered with a major APL accumulation in the tumor, thus supporting a rather selective tumor targeting for edelfosine [28, 29]. In this regard, we found that edelfosine and perifosine killed malignant multiple myeloma cells while spared normal cells derived from the same patients [27].

In addition, the small tumors isolated from the above edelfosine-treated mice were poorly vascularized, supporting previous studies showing an antiangiogenic effect of edelfosine [145]. Furthermore, no apparent damage in the distinct organs analyzed following necropsy in the above *in vivo* studies was detected [28, 29]. These data are in agreement with the previous *in vitro* reports showing selective induction of apoptosis in cancer cells while sparing normal cells [11, 21, 27], and with the lack of significant toxicity in rats by using histologic, functional and biochemical parameters [146]. A decrease in tumor cell cholesterol, a major raft component, inhibited the *in vivo* antimyeloma action of edelfosine and reduced drug uptake by the tumor [28]. These data provide the proof-of-principle and rationale for further clinical evaluation of edelfosine and for a cholesterol-rich raft-targeted therapy, for which edelfosine could be its first and leading drug, in order to improve patient outcome in cancer [28].

Expression of a death-domain mutated Fas/CD95 (Fas/CD95 Q257K) in Jurkat cells, previously reported to reduce Fas/CD95-mediated apoptotic signaling [147], partially inhibited the proapoptotic activity of edelfosine [139]. As stated above, silencing of Fas/CD95 by RNA interference, transfection with a FADD dominant-negative mutant that blocks Fas/CD95 signaling, and specific inhibition of caspase-8 prevented the apoptotic response triggered by edelfosine in Jurkat cells [124]. Taking together, these data support the functional role of DISC in edelfosine-induced apoptosis. However, van Blitterswijk *et al*. [148] have found that edelfosine-induced apoptosis in mouse S49.1 lymphoma cells was independent of Fas/CD95 engagement, as assessed by the lack of inhibition by retroviral transduction with dominant-negative FADD or with $cFLIP_L$, which inhibits caspase-8 [149], or treatment with the caspase-8 inhibitor z-IETD-fmk (benzyloxycarbonyl-Ile-Glu-Thr-Asp-fluoromethylketone). This apparent discrepancy could be explained by the use of different cell types. There are two types of cells regarding how they activate Fas/CD95-mediated apoptosis [150]. In type I cells, induction of apoptosis was accompanied by activation of large amounts of caspase-8 by the DISC, whereas in type II cells DISC formation and caspase-8 activation were strongly reduced and apoptosis occurred following the involvement of mitochondria [150], being blocked by overexpression of Bcl-2 or Bcl-x_L [151]. In the so-called type I cells (such as JY, HuT78, SKW6, H9, S49), Fas/CD95 is located in lipid rafts [152, 153] and DISC formation is fast and efficient, with high amounts of active caspase-8 formed, which activates directly the effector caspase-3. In type II cells (such as CEM, Jurkat, multiple myeloma cells) Fas/CD95 is largely located outside rafts and redistributes into rafts upon ligation [22, 27, 152, 153], so DISC formation is delayed and insufficient to activate caspase-3 directly. Induction of apoptosis in these type II cells need an amplification pathway *via* mitochondria, which can be inhibited by overexpression of Bcl-2 or Bcl-x_L [151]. We have found that edelfosine-induced apoptosis require the involvement of mitochondria and overexpression of Bcl-2 or Bcl-x_L blocked cell death [11, 12, 27], thus highlighting the essential role of mitochondria in edelfosine-induced and raft-mediated cell death.

EDELFOSINE ACCUMULATES IN LIPID RAFTS

Edelfosine accumulates in cholesterol- and sphingolipid-rich lipid rafts [21, 28, 68, 102, 124, 154-156]. Lipid rafts are membrane microdomains of reduced fluidity consisting of dynamic assemblies of cholesterol and sphingolipids that may serve as foci for the recruitment of distinct molecules at the plasma membrane [90, 94, 98, 99]. As stated above, edelfosine shows a high affinity for cholesterol and for cholesterol-enriched membranes such as lipid rafts [101, 102]. The molecular geometry of the lipid molecules dictates the structure of the lipid aggregates [157]. The affinity of edelfosine for cholesterol can be explained, at least in part, by complementarity of the molecular geometrics of sterols and edelfosine [101]. Geometry compensation by combination of "cone-shape" sterols and "inverted cone-shape" edelfosine leads to a more stabe bilayer [101]. Edelfosine and miltefosine by themselves do not form sterol-rich domains in fluid model membranes, in contrast to the two chain ether lipid 1,2-*O*-dihexadecyl-*sn*-glycero-3-phosphocholine, but all these three lipids stabilize palmitoyl-sphingomyelin/sterol-rich domains [158]. Recent data by using different membrane model systems indicate that edelfosine increases fluidity in membrane raft domains, abolishing the fluidity buffering effect of cholesterol [102]. Langmuir monolayer studies showed that the fluidizing effect of edelfosine on model membranes is highly cholesterol-dependent [159]. Additional studies have suggested a strong interaction between edelfosine and ganglioside GM1 [160, 161], which is a major constituent of lipid rafts [162-166] and changes in ganglioside metabolism have been associated with tumor progression [167-170]. Interestingly, edelfosine

increased the thickness and fluidity of model membranes for lipid rafts [102], and these actions might affect protein composition. ^{2}H-nuclear magnetic resonance (^{2}H NMR) assays with 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC)/sphingomyelin (SM)/cholesterol model lipid membranes showed that edelfosine increased the gel phase so that the fluidity buffering effect of cholesterol was abolished [102].

APLs AND CLUSTER OF APOPTOTIC SIGNALING MOLECULE-ENRICHED RAFTS (CASMER) FORMATION

A series of studies have shown that not only Fas/CD95 together with FADD and procaspase-8 are recruited into lipid rafts, forming the so-called DISC, but additional downstream apoptotic signaling molecules, including procaspase-10, JNK, and Bid are also translocated into membrane rafts following edelfosine treatment [21, 110] (Table **2**). Additional chemotherapeutic drugs [123], including the APL perifosine and the marine anticancer drug aplidin [27, 110], are also effective in recruiting DISC complex together with downstream signaling molecules into lipid rafts. Thus, membrane rafts could serve, in addition to generate a high local concentration of Fas/CD95, as platforms for coupling adaptor and effector proteins involved in Fas/CD95 signaling, and to facilitate and amplify signaling processes by local assembly of various cross-interacting signaling molecules. This is particularly critical in Fas/CD95-mediated signal transduction as the initial signaling events depend on protein-protein interactions.

We recently coined the term CASMER (Fig. **5**) as an acronym of "cluster of apoptotic signaling molecule-enriched rafts" [110, 126, 171, 172] to refer to the emerging concept of "liquid-ordered" plasma membrane raft platforms where death receptors together with downstream apoptotic signaling molecules are recruited [171, 172]. The efficiency in promoting CASMER formation, as well as CASMER protein composition, would depend on the cell type and the triggering stimulus [171, 172]. A primary CASMER would require the structure of DISC to be functionally active in apoptosis [171, 172], but the addition of downstream molecules would lead to a more effective proapoptotic signaling. Ultrastructural, genetic and pharmacologic approaches have shown the formation of DISC in aggregated rafts, that is forming a CASMER, and its role in edelfosine-induced apoptosis [27, 124]. CASMER represents a novel raft-based supramolecular entity, which seems to play a critical role in the regulation of apoptosis, acting as death-

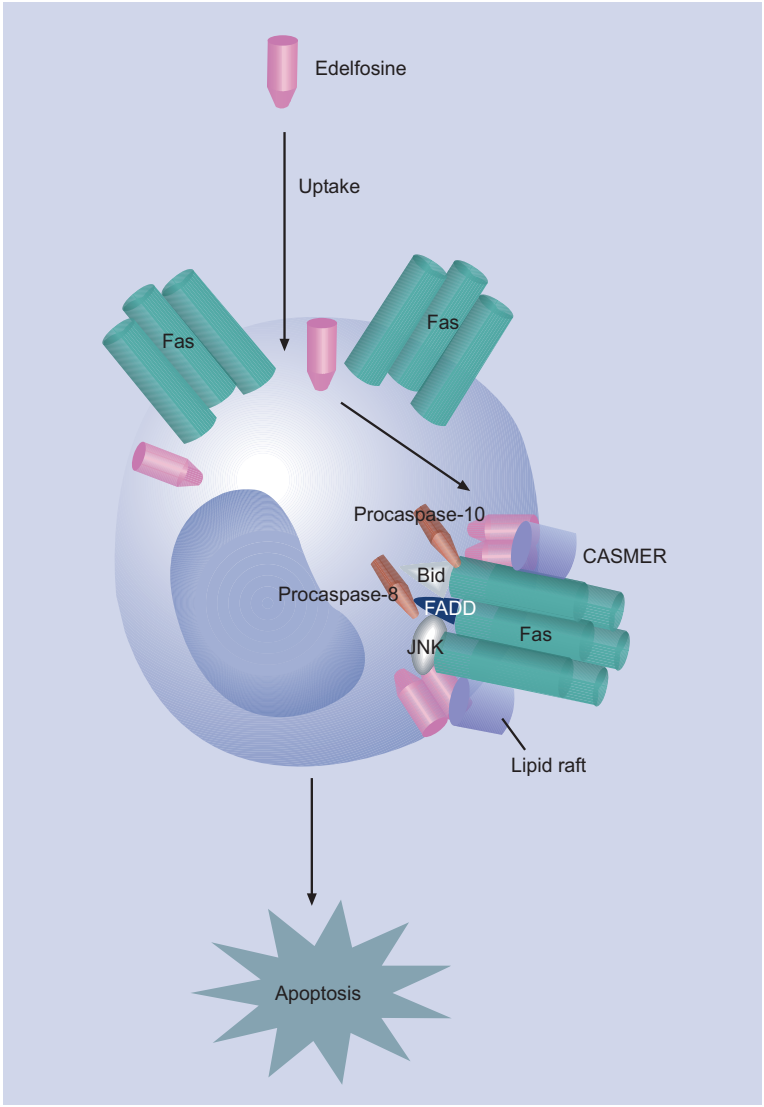

Fig. (5). CASMER formation in edelfosine-induced apoptosis. Edelfosine is taken up by cancer cells and accumulates in lipid rafts, promoting aggregation of raft membrane domains and the recruitment and clustering of Fas/CD95 death receptor and downstream signaling molecules in these membrane raft domains, leading to the generation of CASMER. Reproduced from Future Oncology, vol. 6, nº 5, pages 811-821, with permission of Future Medicine Ltd (Ref. 172). ©Future Science Group 2010.

promoting scaffolds where death receptors and downstream signaling molecules are brought together, thus facilitating protein-protein interactions and the transmission of apoptotic signals [171, 172]. On these grounds, CASMER may reduce the apoptotic signal threshold by concentrating apoptotic molecules in a reduced membrane region and stabilizing protein/protein interactions, and thereby, this CASMER formation would favor the transmission of apoptotic signals [171, 173, 174].

MODULATION OF SURVIVAL AND APOPTOTIC SIGNALING IN RAFT PLATFORMS BY EDELFOSINE

A major consequence of the concept of CASMER is that the apoptosis machinery can be compartmentalized to favor the required interaction of the apoptotic molecules, and hence facilitating the triggering of a potent cell death response.

Novel data suggest that apoptosis/survival signaling balance might be modulated by a redistribution and local accumulation of apoptotic molecules in lipid rafts, keeping apoptotic (Fas/CD95, FADD, caspase-8, JNK) and survival (ERK, Akt) signaling molecules apart from each other [125, 175]. In this context, edelfosine treatment in T-cell leukemia Jurkat cells recruits heat shock protein 90 (Hsp90) and JNK in lipid rafts (Table **2**), thus preserving sustained JNK activation [125]. The molecular chaperone Hsp90 is overexpressed in many tumor cells and keeps the correct folding and function of a wide number of client proteins promoting cancer cell growth, such as HER-2/Erb2, Akt, Raf-1, Bcr-Abl, and mutated p53 [176]. Interestingly, edelfosine treatment seems to set Hsp90 apart from their normal client survival proteins, and instead it is recruited to apoptotic molecule-enriched rafts or CASMERs [125], hence turning Hsp90 into a stabilizer of apoptotic signaling. Thus, JNK became a novel Hsp90 client protein when both molecules were concentrated in rafts [125]. The sorting and concentration of apoptotic molecules in lipid rafts, being separated from survival signaling molecules, is suggested to promote a dramatic local change in the apoptosis/survival signaling ratio in a specific subcellular structure and restricted area that eventually triggers a cell death response. In this regard, segregation of insulin-like growth factor-1 receptor (IGF-1R) in and out of lipid rafts may dynamically regulate the pro- and anti-apoptotic effects of IGF-1 induced by TNF superfamily members in colon carcinoma cells [177]. Very recently, we have found that edelfosine treatment in mantle cell lymphoma cells displaced survival PI3K/Akt signaling from lipid rafts, whereas Fas/CD95 was recruited into the membrane domains, thus inducing cell death [175].

RAFT REORGANIZATION, TRAFFICKING AND INTRACELLULAR ACIDIFICATION IN EDELFOSINE ACTION IN YEAST

Similarly to what has been described above in the induction of cell death by displacing Akt from rafts in cancer cells, edelfosine leads to cell death in *Saccharomyces cerevisiae* by displacement of Pma1p, a critical yeast survival protein, from lipid rafts [155, 178, 179]. The three APLs edelfosine, perifosine and miltefosine have been recently found to displace the essential proton pump Pma1p from rafts in *S. cerevisiae*, inducing its internalization into the vacuole, thus suggesting that this process is a hallmark of APL cytotoxicity in yeast [178, 179]. The use of radioactive and synthetic fluorescent edelfosine analogs allowed us to localize edelfosine in yeast plasma membrane rafts and the endoplasmic reticulum (ER) [178]. Despite both edelfosine and Pma1p were initially located at membrane rafts, the internalization route followed by the ether lipid to the ER, which does not seem to be critical for drug cytotoxicity in yeast, differed from that of Pma1p to the vacuole [178, 179]. After performing chemogenomic screens in the *S. cerevisiae* gene-deletion strain collection, we found that mutants affecting endocytosis, vesicle sorting, or trafficking to the vacuole, including the retromer and endosomal sorting complex required for transport (ESCRT) complexes, prevented Pma1p internalization and were edelfosine-resistant [178]. Thus, these data suggest that edelfosine-induced cytotoxicity involves raft reorganization and retromer- and ESCRT-mediated vesicular transport and degradation of essential raft proteins, such as Pma1p, leading to cell death in yeast. Pma1p is a key regulator of pH homeostasis in yeast [180], and the edelfosine-induced displacement of Pma1p from lipid rafts leads to intracellular acidification in wild-type yeast [179]. This alteration in pH homeostasis emerges as a major contributor to yeast sensitivity toward edelfosine [179]. In this regard, it is worth to note that edelfosine has been previously reported to inhibit Na^+/H^+ exchanger leading to cytoplasmic acidification in the breast cancer-derived cell line MCF-7 [181]. Edelfosine cytotoxicity in yeast was circumvented by inactivating genes that then result in the recycling of internalized cell-surface proteins back to the plasma membrane [178]. Interestingly, edelfosine also triggers the internalization of ergosterol (the yeast counterpart of mammalian cholesterol) from the plasma membrane by a route different from that of Pma1p, and fluorescence microscopy evidence suggests that sterol internalization may precede Pma1p exit from the plasma membrane [179]. In addition to the proton-pump Pma1p, edelfosine treatment also induces the internalization of nutrient H^+-symporters Can1p and Fur4p, but not the structural eisosome protein Sur7p [179]. The arginine transporter Can1p and the uracil transporter Fur4p are associated with lipid rafts in *S. cerevisiae* [180], and therefore edelfosine affects raft-located proteins involved in ion homeostasis and intracellular pH, like Pma1p, and H^+-symporters that play a major role in the regulation of cell survival or death in yeast. Thus, cytotoxicity of APLs depends mainly on the changes they induce in plasma membrane raft-located proteins. In yeast, these changes lead to the internalization and subsequent degradation of raft proteins [178]. As stated above, in mammalian cancer cells, the anticancer activity of APLs would highly depend on either recruitment of proteins in lipid rafts that leads to the activation of a proapoptotic signaling, or to the displacement of survival proteins or signaling molecules from rafts, thus leading to their inactivation, which eventually favors a cell death outcome. Taking into account the evidence gathered from yeast and mammalian assays, it could be envisaged that raft reorganization is critical in the action of edelfosine and other APLs. Thus, lipid rafts serve as a potential pharmacological target in the treatment of cancer as well as in the regulation of survival/cell death signaling routes.

MECHANISM OF ACTION OF EDELFOSINE INVOLVING ER

Edelfosine Accumulates in the ER in Solid Tumor Cells

A significant difference in the time-course to induce cell death in hematopoietic cancer cells and solid tumor cells was soon detected [11]. Edelfosine induces rapid apoptosis in human hematopoietic cancer cells, including acute T-cell leukemia Jurkat and Peer cells, acute myeloid leukemia HL-60 cells, leukemic monocyte lymphoma U937 cells as well as multiple myeloma, CLL and mantle lymphoma cell lines, but promotes a rather late apoptotic response, preceded by G_2/M arrest, in human solid tumor cells, such as cervix epithelioid carcinoma HeLa cells, lung carcinoma A549 cells, and pancreatic adenocarcinoma BxPC-3, Capan-2, CFPAC-1 and HuP-T4 cell lines [17, 27-29, 129, 182]. Both types of hematopoietic and solid tumor cells incorporate similar amounts of edelfosine, but at different subcellular locations. By using different fluorescent edelfosine analogs (Table **3** and Fig. **6**), we found a major accumulation of these edelfosine analogs at the plasma membrane lipid rafts in blood cancer cells and at the ER in solid tumor cells [24, 27-29, 124, 129, 182], thus suggesting that edelfosine targets two different subcellular structures in a cell type-dependent manner.

Table 3. Subcellular localization of APLs in eukaryotic cells.

APL	Subcellular Organelle	Cell Type	Technique	Reference
[^{3}H]edelfosine	Lipid rafts	Mouse S49.1 lymphoma cells and human epidermal carcinoma KB cells	Raft isolation by sucrose gradients and radioactivity counting	[68, 156, 216]
[^{3}H]edelfosine	Lipid rafts	Human acute T cell leukemia Jurkat cells and multiple myeloma MM144 cells	Raft isolation by sucrose gradients and radioactivity counting	[28, 102, 124]
[^{14}C]perifosine	Lipid rafts	Mouse S49.1 lymphoma cells and human epidermal carcinoma KB cells	Raft isolation by sucrose gradients and radioactivity counting	[156, 216]
PTE-ET (fluorescent edelfosine analog)	Lipid rafts	Human acute T cell leukemia Jurkat and Peer cells	Fluorescence microscopy	[21, 102, 182]
PTE-ET (fluorescent edelfosine analog)	Lipid rafts	Multiple myeloma MM144 cells	Fluorescence microscopy	[28]
[^{3}H]edelfosine	Lipid rafts	*Saccharomyces cerevisiae* yeast	Raft isolation by sucrose gradients and radioactivity counting	[178]
PTE-ET (fluorescent edelfosine analog)	ER	Human cervix epitheloid carcinoma HeLa and lung carcinoma A549 cells	Fluorescence microscopy	[24, 182]
Et-BDP-ET (fluorescent edelfosine analog)	ER	Human pancreatic adenocarcinoma cell lines HuP-T4 and Capan-2 cells HuP-T4 and Capan-2 cells	Confocal fluorescence microscopy	[129]
PTE-ET, PTRI-ET, Et-BDP-ET, YN-BDP-ET (fluorescent edelfosine analogs)	ER	*Saccharomyces cerevisiae* yeast	Fluorescence microscopy	[178]
PTE-ET, PTRI-ET, Et-BDP-ET, YN-BDP-ET (fluorescent edelfosine analogs)	Mitochondria	Human cervix epitheloid carcinoma HeLa	Fluorescence and confocal microscopy	[23]
Polyfosine (fluorescent edelfosine analog)	Mitochondria	COS7	Two-photon-excited and epifluorescence microcopy	[190]
Polyfosine (fluorescent edelfosine analog)	ER, nuclear envelope and plasma membrane	COS7	Two-photon-excited and epifluorescence microcopy	[190]

Et-BDP-ET, 1-*O*-(11'-(6''-ethyl-1'',3'',5'',7''-tetramethyl-4'',4''-difluoro-4''-bora-3a'',4a''-diaza-*s*-indacen-2''-yl)undecyl)-2-*O*-methyl-*rac*-glycero-3-phosphocholine; polyfosine, *sn*-1-*O*-(c16:5-alkyl)-*sn*-2-*O*-methyl-glycerophosphocholine; PTE-ET, *all*-(*E*)-1-*O*-(15'-phenylpentadeca-8',10',12',14'-tetraenyl)-2-*O*-methyl-*rac*-glycero-3-phosphocholine; PTRI-ET, *all*-(*E*)-1-O-(15'-phenylpentadeca-10',12',14'-trien-8'-ynyl)-2-*O*-methyl-*rac*-glycero-3-phosphocholine; Yn-BDP-ET, 1-*O*-(13'-(1'',3'',5'',7''-tetramethyl-4'',4''-difluoro-4''-bora-3a'',4a''-diaza-*s*-indacen-2''-yl)tridec-12'-ynyl)-2-*O*-methyl-*rac*-glycero-3-phosphocholine.

EDELFOSINE INDUCES ER STRESS RESPONSE IN SOLID TUMOR CANCER CELLS

The ER is an organelle responsible for critical cell functions, including protein and lipid biosynthesis, post-translational protein modification, cellular calcium store, and can modulate cell death. Disturbance in ER functionality leads to a series of events collectively termed as ER stress, which initially triggers the so-called unfolded protein response (UPR) to restore normal ER function. However, a persistent ER stress can turn the cytoprotective functions of UPR into a cell death promoting mechanism, by triggering ER-dependent apoptotic cascades that include the activation of several molecules, such as CHOP (C/EBP homologus protein)/growth arrest and DNA damage-inducible gene 153 (GADD153) or JNK, which further push the cell down the path of death. Three major pathways of ER stress-induced apoptosis are known, namely: (1) upregulation of the transcription factor CHOP/GADD153 [183]; (2) JNK activation [184]; (3) activation of caspase-12 in murine systems or caspase-4 in human cells [185].

The accumulation of edelfosine in the ER of solid tumor cancer cells leads to a potent ER response characterized by: inhibition of protein synthesis, CHOP/GADD153 upregulation, eIF2α phosphorylation, and activation of Bax, caspase-4, and JNK [24, 129]. Inhibition of JNK prevents edelfosine-induced apoptosis [17, 24, 129], and ASK1 overexpression enhanced both edelfosine-induced JNK activation and apoptosis [24], indicating that ASK1/JNK signaling plays a major role in edelfosine-induced apoptosis. Edelfosine upregulated *sXBP1*, an UPR key modulator, during the ER stress response, but activation of this cytoprotective response was unable to prevent cell death [129]. Another UPR hallmark, Grp78/BiP, is not upregulated by edelfosine treatment [24, 129]. The balance between Grp78/BiP (cytoprotective) and CHOP/GAD153 (proapoptotic) expression may lead to either cell survival or cell death in an ER stress response. This balance is tipped towards an ER stress-induced cell death following edelfosine treatment, as the drug upregulates CHOP/GAD153, whereas Grp78/BiP protein expression is not affected [24, 129]. This ER stress response in edelfosine-treated cancer cells is detected both *in vitro* and *in vivo*, in a xenograft animal model [129]. CHOP/GAD153 upregulation was visualized in tumors isolated from edelfosine-treated SCID mice bearing pancreatic cancer xenografts, together with an increase in apoptotic index [129].

We also found that mitochondria were critical in edelfosine-induced cell death initiated by ER stress, and transfection-enforced expression of Bcl-x_L, which localizes specifically in mitochondria, prevented edelfosine-induced apoptosis in solid tumor cell lines [24, 129]. The above action of edelfosine on PC synthesis inhibition seems to be one of the multiple actions that the ether lipid exerts at the ER. Edelfosine accumulation in the membrane of ER in several cancer cells triggers a whole set of actions that account for the different phenotypes and features detected upon drug addition, including inhibition of PC and protein synthesis, changes in intracellular free Ca^{2+} concentration and ER stress [24, 129].

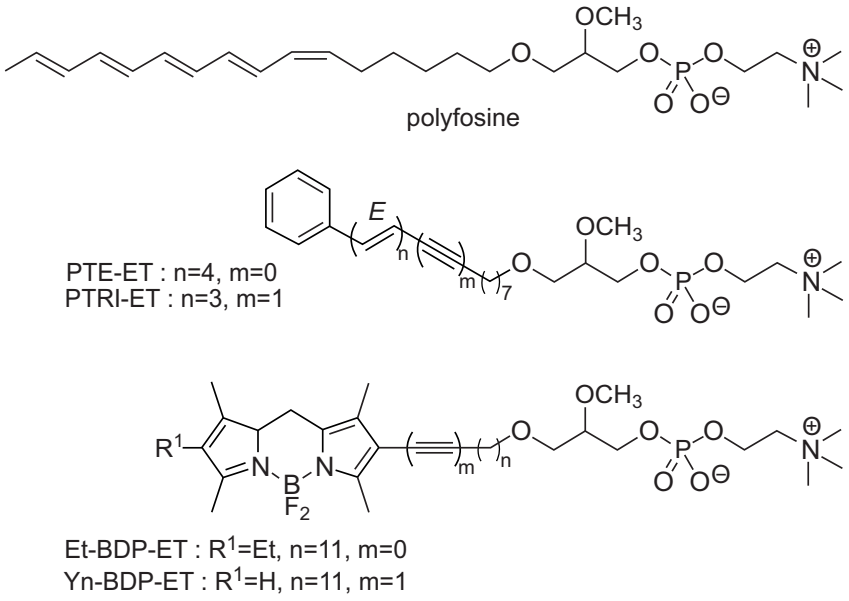

Fig. (6). Chemical structures of fluorescent edelfosine analogs.

MECHANISM OF ACTION OF EDELFOSINE INVOLVING MITOCHONDRIA

All Apoptotic Signals Induced by Edelfosine Converge on Mitochondria as a Critical Step in the Induction of Cell Death

As stated above edelfosine can accumulate in either the plasma membrane (lipid rafts) or in the ER, triggering apoptotic signaling cascades from each subcellular location. However, all signals derived from either plasma membrane rafts or ER converge on mitochondria to proceed to apoptosis as the final outcome. Overexpression of Bcl-2 or Bcl-x_L, protecting mitochondria, totally blocks the apoptotic response induced by the ether lipid edelfosine in cancer cells [11, 12, 24, 27, 129]. Edelfosine induces apoptosis through the loss of mitochondrial transmembrane potential, promotes the release of cytochrome *c* from mitochondria, and activates caspase-9, while all these effects are suppressed by Bcl-2 or Bcl-x_L overexpression [12, 27, 129]. Thus, these data unveil the key involvement of mitochondria in the cell death response triggered by edelfosine, and indicate that mitochondria behave as the critical subcellular organelles that eventually dictate the final outcome of the cell following edelfosine treatment.

Interestingly, edelfosine acts on Bid and BAP31, two molecules that link the extrinsic pathway of apoptosis and the ER, respectively, with mitochondria. Bid acts as a bridge between Fas/CD95 signaling and mitochondria [121, 122]. BAP31 was cleaved into the p20 fragment upon edelfosine treatment [24, 129]. BAP31 is an integral membrane protein of the ER regulating ER-mediated apoptosis through its caspase-8-mediated cleavage into a 20-kDa fragment, which directs proapoptotic signals between the ER and mitochondria [186-188]. Treatment of cancer cells with edelfosine leads to Bid and BAP31 cleavage, thus suggesting a crosstalk between lipid rafts, ER and mitochondria in the induction of apoptosis by this ether lipid. Edelfosine recruits Bid to lipid rafts and cleaves it into the p18 fragment, thus connecting the cell death receptor extrinsic pathway of apoptosis with mitochondria [27, 126]. In this regard, lipid rafts have been shown to act as scaffolds where extrinsic and intrinsic apoptotic signaling pathways concentrate following edelfosine action [126] (Table **2**). Furthermore, edelfosine has been shown to promote a redistribution of lipid rafts from the plasma membrane to mitochondria, suggesting a raft-mediated link between plasma membrane and mitochondria [23].

EDELFOSINE LOCALIZATION IN MITOCHONDRIA

We have recently found that a series of fluorescent edelfosine analogs, containing the conjugated *all-(E)*-phenyltetraene or *all-(E)*-phenyltrienyne blue-emitting chromophores (PTE-ET and PTRI-ET, respectively), with several double bonds in the C1 aliphatic chain of edelfosine, and a 2-substituted borondifluorodipirromethene group (BODIPY or BDP) attached to and aligned with the alkyl chain of the ether-lipid (green-emitting Et-BDP-ET and Yn-BDP-ET) [21, 23, 178, 189] (Fig. **6**), localize in mitochondria as assessed by confocal laser microscopy [23] (Table **3**). Likewise, polyfosine, a fluorescent analog of edelfosine (*sn*-1-*O*-(c16:5-alkyl)-*sn*-2-*O*-methyl-glycero-phosphocholine) (Fig. **6**), also localized in mitochondria in COS7 cells [190] (Table **3**). Polyfosine staining was also compatible with ER and nuclear envelope in addition to mitochondria, while hardly any staining could be detected in lysosomes [190]. Images shown in this latter work showed a major localization of polyfosine fluorescence close to the nuclear envelope that might represent or be linked to ER [190]. Furthermore, acylation of fluorescent polyene lyso-ether phosphatidylcholine (polyene-lyso-ePC; *sn*-1-*O*-(c16:5-alkyl)-*sn*-2-lyso-glycerophosphocholine) in COS7 cells led to cell-derived fluorescent polyene-ePC [190], like natural glycerophospholipids that often contain unsaturated fatty acids, most prominently arachidonic acid [191]. As a matter of fact, arachidonic (20:4) was the predominant acylation partner in the newly formed polyene-ePC, followed by other endogenous unsaturated fatty acids, such as oleate (18:1), linoleate (18:2), and palmitoleate (16:1) as well as saturated fatty acids, such as palmitate (16:0) and myristate (14:0), and this polyene-ePC mainly stained the mitochondria, nuclear envelope and ER [190]. In addition, edelfosine induces swelling in isolated mitochondria, indicating an increase in mitochondrial membrane permeability [23]. Edelfosine and perifosine inhibit mitochondrial respiration and decrease transmembrane electric potential in isolated hepatic mitochondria [192], further demonstrating the direct actions of APLs on mitochondria. This interaction between edelfosine and mitochondria might explain the involvement of mitochondria in edelfosine-mediated apoptosis [11, 12, 27]. Interestingly, raft disruption inhibited the mitochondrial localization of edelfosine as well as edelfosine-induced swelling in isolated mitochondria, which was independent of the generation of reactive oxygen species [23]. Thus, it might be envisaged that edelfosine could accumulate in

mitochondrial lipid rafts and the interaction between edelfosine and mitochondria might eventually underlie the antitumor action of this ether lipid. The presence of raft-like domains in mitochondria remains a controversial issue [193-195]. However, increasing evidence supports their presence in this organelle. Mitochondria have been reported to contain ganglioside GD3- and cholesterol-containing lipid raft-like areas that accommodate voltage-dependent anion channel protein 1 [196, 197]. Despite mitochondria are considered cholesterol-poor organelles, ranging from 0.5-3% of the content found in plasma membrane [198], the mitochondrial pore-forming complex has been shown to contain cholesterol [199], and an appropriate cholesterol level in mitochondrial raft-like domains seems to be crucial for the proper function of mitochondria during apoptosis [196]. Depletion of cholesterol impairs mitochondrial bioenergetics and decreases calcium-induced swelling [200].

The inverted cone shape of edelfosine [101] is likely to generate membrane curvature if a high local concentration is met [201], as that found in the cell membrane [102], and this local change can lead to membrane deformation and certain degree of alteration that may facilitate vesicle budding and fusion, as well as the recruitment or displacement of proteins. Thus, APLs might influence the distribution and function of proteins in the membrane. In this regard, edelfosine induces changes in the lipid and protein composition of rafts [21, 22, 27, 28, 102, 123, 124, 126, 155], and membrane destabilization in liposome model systems by formation of interdigitated structures, micelles and small vesicles [31]. Physical membrane destabilization and loss of mitochondrial integrity, such as that observed by the insertion of lysophosphatidylcholine species in mitochondria, leads to the release of mitochondrial apoptotogenic factors like cytochorome *c* [202], and eventually to apoptosis [203].

It is interesting to note the physical interaction between ER and mitochondria [204], and that the transport of phospholipids between membranes of the ER and mitochondria occurs at specialized fractions of the ER tightly associated with mitochondria [205, 206], which are termed mitochondria-associated membranes [207-211]. The protein complex referred to as ER-mitochondria encounter structure (ERMES) complex [212, 213], composed of both ER and mitochondrial transmembrane proteins, is located at the interface of the two organelles and serves to zipper them together. This complex, that provides a tethering force between the ER and the mitochondria, seems to play a critical role in the lipid movement and metabolism between the ER and mitochondria [214].

CONCLUSIONS AND PERSPECTIVES

Recent evidence indicates that edelfosine can accumulate in three different subcellular structures, namely plasma membrane lipid rafts, ER and mitochondria. Edelfosine accumulates in lipid rafts and induces a reorganization of the protein and lipid composition of membrane rafts [21, 22, 27-29, 102, 124, 155]. This redistribution of proteins induced by edelfosine in lipid rafts leads to: a) recruitment of apoptotic signaling molecules, including death receptors and downstream signaling, into rafts [21, 22, 27, 124, 125], forming the so-called CASMERs, as novel supramolecular entitities that modulate the triggering of apoptosis [110, 126, 171, 172]; and b) displacement of survival signaling molecules from rafts [155, 175, 178, 179]. This reorganization of lipid raft protein composition prompts apoptosis in tumor cells. The location and effects of edelfosine in lipid rafts opens a new avenue in cancer chemotherapy unveiling a raft-mediated signaling in cell death regulation that could be modulated pharmacologically, independently of death receptor ligands [21, 22, 27, 110, 123, 135, 174, 175]. Perifosine treatment also recruited death receptors and downstream signaling into rafts [27], thus suggesting that this raft-mediated response might be a general feature of APLs. Interestingly, accumulation of edelfosine in the ER leads to ER stress and apoptosis [24, 129]. This edelfosine-mediated ER response was detected both *in vitro* and *in vivo*, and demonstrates that ER is a novel target for edelfosine [24, 129]. A direct interaction of edelfosine with mitochondria has also been found that eventually leads to mitochondrial dysfunction and apoptosis [23]. Edelfosine promoted a redistribution of lipid rafts from the plasma membrane to mitochondria, suggesting a raft-mediated link between plasma membrane and mitochondria [23].

Thus, recent studies on edelfosine unveil a new framework in cancer chemotherapy that involves a link between lipid rafts, ER and mitochondria in the mechanism of action of an antitumor drug, thus opening new avenues for cancer treatment. Organelles are interdependent units and they must establish physical links to communicate and exchange metabolites and constituents in eukaryotic cells. Even though inter-organelle communication is still poorly understood, organelle interaction opens an exciting field of research in trafficking and regulation of both novel biosynthesized as well as exogenous molecules, and therefore this gives us new insights to design and understand the mechanism of action of novel agents in cancer chemotherapy. The reported interaction between ER and mitochondria [204], and the fact that phospholipids can be transported between membranes of the ER and mitochondria through the presence of ERMES complex at the interface of the two organelles [212, 213], which is involved in the lipid movement between the ER and mitochondria [214], might be of special importance in the studies reported and discussed in this review. This interaction between ER and mitochondria is in good agreement with the localization of edelfosine in both ER and mitochondria, and suggests that edelfosine might be an interesting molecule to yield further insight on these organelle interactions, providing new opportunities to understand the physiological and pharmacological relevance of ER-mitochondria junctions. Membrane targeting by the prototypic APL edelfosine might unveil a fascinating network of communication between plasma membrane and organelle membranes to control cellular growth or death decisions. In addition the new data on animal models demonstrate that edelfosine is a potent and rather selective antitumor agent against a number of different hematologic and solid tumors [28, 29, 129]. These results suggest that lipid rafts and specific subcellular organelles (ER and mitochondria) might be promising targets for therapeutic intervention, thus setting the basis for a new and exciting avenue in cancer therapy.

CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflict of interest.

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LIST OF ABBREVIATIONS

ALP	=	alkyl-lysophospholipid analog
APL	=	alkylphospholipid analog
ATLs	=	synthetic antitumor lipids
Bid	=	BH3-interacting domain death agonist

CASMER = cluster of apoptotic signaling molecule-enriched rafts
CCT = CTP:phosphocholine cytidyltransferase
CHOP = C/EBP homologous protein)/growth arrest
CLL = chronic lymphocytic leukemia
DAG = diacylglycerol
DED = death effector domain
DD = death domain
DISC = death-inducing signaling complex
EGF = epidermal growth factor
EGFR = epidermal growth factor receptor
ER = endoplasmic reticulum
ERMES = ER-mitochondria encounter structure
ErPC = [13Z]-docos-13-en-1-yl 2-(trimethylammonio) ethyl phosphate (erucylphosphocholine)
ESCRT = endosomal sorting complex required for transport
ET-18-OCH_3 = 1-*O*-octadecyl-2-*O*-methyl-*rac*-glycero-3-phosphocholine (edelfosine)
FADD = Fas-associated death domain-containing protein
FasL/CD95L = Fas ligand/CD95 ligand
GADD153 = growth arrest and DNA damage-inducible gene 153
Hsp90 = heat shock protein 90
IGF-1R = insulin-like growth factor-1 receptor
IL-1α = interleukin-1α
JNK = c-Jun N-terminal kinase
LPC = 2-lysophosphatidylcholine (1-acyl-*sn*-glycero-3-phosophocholine)
LPE = lysophosphatidylethanolamine
MAPK = mitogen-activated protein kinase
MCD = methyl-β-cyclodextrin
NF-κB = nuclear factor kappa-light-chain-enhancer of activated B cells
NSCLC = non-small-cell lung carcinoma
PC = phosphatidylcholine
PH = pleckstrin homology
PI = phosphatidylinositol
PI(3,4,5)P3 = phosphatidylinositol (3,4,5)-trisphosphate
PI(4,5)P2 = phosphatidylinositol (3,4)-bisphosphate
PI3K = phosphatidylinositol 3-kinase
PKB = protein kinase B
PLC = phospholipase C
PLD = phospholipase D
PTEN = phosphatase and tensin homologue deleted on chromosome 10
tBid = truncated Bid
TNF-α = tumor necrosis factor-α
TPA = 12-*O*-tetradecanoylphorbol-13-acetate
TRAIL = TNF-related apoptosis-inducing ligand
SCID = severe combined immune deficiency
SCLC = small-cell lung carcinoma
SHIP = Src homology 2 domain-containing inositol-5-phosphatase
SMS1 = sphingomyelin synthase 1
UPR = unfolded protein response

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