Progress in Lipid Research xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Progress in Lipid Research

journal homepage: www.elsevier.com/locate/plipres

Review

Membrane lipid therapy: Modulation of the cell membrane composition and structure as a molecular base for drug discovery and new disease treatment

Pablo V. Escribá [a], Xavier Busquets [a], Jin-ichi Inokuchi [b,c], Gábor Balogh [d], Zsolt Török [d], Ibolya Horváth [d], John L. Harwood [e,*], László Vígh [d,*]

[a] Department of Biology, University of the Balearic Islands, E-07122 Palma de Mallorca, Spain
[b] Division of Glycopathology, Institute of Molecular Biomembrane and Glycobiology, Tohoku Pharmaceutical University, Sendai, Japan
[c] Core Research for Evolutional Science and Technology, Japan Science and Technology Agency, Saitama, Japan
[d] Institute of Biochemistry, Biological Research Center, Hungarian Academy of Sciences, Szeged, Hungary
[e] School of Biosciences, Cardiff University, Cardiff CF10 3AX, Wales, UK

ARTICLE INFO

Article history:
Received 28 January 2015
Received in revised form 10 April 2015
Accepted 29 April 2015
Available online xxxx

Dedicated to the memory of our late colleague and friend, Professor John E. Halver.

ABSTRACT

Nowadays we understand cell membranes not as a simple double lipid layer but as a collection of complex and dynamic protein–lipid structures and microdomains that serve as functional platforms for interacting signaling lipids and proteins. Membrane lipids and lipid structures participate directly as messengers or regulators of signal transduction. In addition, protein–lipid interactions participate in the localization of signaling protein partners to specific membrane microdomains. Thus, lipid alterations change cell signaling that are associated with a variety of diseases including cancer, obesity, neurodegenerative disorders, cardiovascular pathologies, etc. This article reviews the newly emerging field of membrane lipid therapy which involves the pharmacological regulation of membrane lipid composition and structure for the treatment of diseases. Membrane lipid therapy proposes the use of new molecules specifically designed to modify membrane lipid structures and microdomains as pharmaceutical disease-modifying agents by reversing the malfunction or altering the expression of disease-specific protein or lipid signal cascades. Here, we provide an in-depth analysis of this emerging field, especially its molecular bases and its relevance to the development of innovative therapeutic approaches.

Abbreviations: AA, arachidonic acid; Aβ, fibrillar β-amyloid; ACC, acetyl-CoA carboxylase; Akt, protein kinase B; APP, amyloid precursor protein; ASAH2, neutral ceramidase; BA, benzyl alcohol; BBB, blood–brain-barrier; BMI, body mass index; BPM, bis(monoacylglycero)phosphate; CALM, clathrin assembly lymphoid myeloid domains; CAMKII, calcium/calmodulin-dependent protein kinase II; Cav1, caveolin 1; Cer, ceramide; Chol, cholesterol; CNS, central nervous system; DAG, diacylglycerol; DHA, docosahexaenoic acid; EGFR, endothelial growth factor receptor; ENTH, epsin N-terminal homology domains; EPA, eicosapentaenoic acid; FA, fatty acid; FASN, fatty acid synthase gene; FERM, 4.1 protein-ezrin-radixin-moesin; GEM, glycolipid-enriched membrane microdomain; GLRX, glutaredoxin; GM, monosialodihexosylganglioside; GPCRs, G protein coupled receptors; GSLs, glycosphingolipids; HA, hydroxamic acid; HSF1, heat shock factor 1; Hsp, heat shock proteins; Hsp27, heat shock protein 27; Hsp70, heat shock protein 70; HSR, heat shock response; INSIG1, insulin-induced gene 1; IR, insulin receptor; Ld, liquid disordered microdomains; Lo, liquid ordered microdomains; LDL, low density lipoprotein; LXR, liver X receptor; MAPK, mitogen activated protein kinase; MLT, membrane lipid therapy; MUFA, monounsaturated fatty acids; NMDA, N-methyl-D-aspartate; OLR1, oxidized low-density protein receptor 1; PC, phosphatidylcholine; PDGFR, platelet derived growth factor receptor; PDZ domain, PSD95-Dlg1-zo-1 domains; PE, phosphatidylethanolamine; PH domain, pleckstrin homology domain; PHYH, phytanoyl CoA dioxygenase; PIP2, phosphatidylinositol 4,5-bisphosphate; PI3K, phosphoinositide 3-kinase; PIP3, phosphatidylinositol (3,4,5)-trisphosphate; PKA, protein kinase A; PKC, protein kinase C; PLA2, phospholipase A2; PLC, phospholipase C; PPARs, peroxisome proliferator-activated receptors; PTB domain, phosphotyrosine-binding domain; PUFA, polyunsaturated fatty acid; PYVE domain, Fab-1, YGL023, Vps27, and EEA1 domain; RAR, retinoic acid receptor alpha; REMBRANDT, repository for molecular brain neoplasia data; RXR, retinoid X receptor; S1P, sphingosine-1-phosphate; SCI, spinal cord injury; SGMS1/2, sphingomyelin synthase; SM, sphingomyelin; SMPD2/3, SM phosphodiesterase 2/3 (neutral sphingomyelinases); SNAP23, synaptosomal-associated protein 23; SPC, sphingosylphosphorylcholine; SPHK1/2, sphingosine kinase; SPTLC3, palmitoyltransferase; SREBP1, sterol regulatory element-binding protein 1; TAG, triacylglycerol; TCGA, cancer genome atlas; TNF-α, tumor necrosis factor alpha; UGCG, ceramide glucolsyltransferase; VLDL, very low density lipoprotein.

* Corresponding authors. Tel.: +44 2920874108 (J. Harwood), +36 62432048 (L. Vigh).
E-mail addresses: Harwood@cardiff.ac.uk (J.L. Harwood), vigh@brc.hu (L. Vígh).

http://dx.doi.org/10.1016/j.plipres.2015.04.003
0163-7827/

Contents

1. Introduction

Since the first general structure of cellular membranes was published in the 1970s by Singer and Nicolson [1] numerous studies have expanded upon this to further define its complex structure. In a cell membrane bilayer, hundreds to thousands of different lipid species form a heterogeneous cell boundary with multiple structural and functional properties [2]. The same membrane sequestration strategy that separates the interior of cells from the rest of world is also used for separating the cellular interior into a collection of membrane-bound organelles. The lipid classes that form the different types of cell membranes (Fig. 1) are usually not homogeneously distributed but can form microdomains that act as complex signaling platforms (together with proteins) due to their membrane lipid (structure) preferences. For example, interaction of receptor tyrosine kinases (e.g., EGFR) with Ras, and of Ras with Raf, to propagate proliferation signals into the cell benefits from their common preference for certain membrane microdomains to establish physical productive interactions [3]. Similarly, G protein-coupled receptors (GPCRs) and G proteins exhibit similar membrane lipid environment preferences [2,4,5].

Membrane functions are altered in a wide range of human diseases and this has led to the concept that components of the plasma membrane, for example, specific lipids, enzymes or transcription factors can be targeted to alter its composition and structure [6–8]. This, in turn, would affect the localization and activity of key proteins, or key protein–protein interactions in specific membrane microdomains, and thereby affect signaling cascades. This approach is termed membrane lipid therapy (MLT). Indeed, several studies have now demonstrated the potential of MLT and, although the first clinical trials of rationally designed lipids to regulate the membrane composition and structure to treat cancer and diabetes only began recently (e.g., ClinicalTrials.gov Identifier NCT01792310), other trials using natural lipids were already ongoing (e.g., docosahexaenoic acid (DHA) for Alzheimer's disease: ClinicalTrials.gov Identifier NCT00440050). In this article, the rationale behind targeting the plasma membrane and the different approaches that can be used to modulate its lipid composition, structure and function is provided. Later, we discuss the current state of the art in various therapeutic indications.

2. Molecular bases underlying MLT

A great many cellular functions occur in or around membranes [2], which suggests that changes in the membrane composition and structure could be relevant in the proper functioning of the cells. In the plasma membrane, hundreds of different lipid species can be found. Some of them have a negative charge, which can promote interactions with positively charged amino acids in proteins [4,7]. Other lipids have a small polar head (e.g., phosphatidylethanolamine), allowing docking of bulky protein lipid anchors (e.g., isoprenyl moieties present in Ras: [9]). Other membrane lipids have a bigger polar head and form tightly packed areas where only certain fatty acids (e.g., myristic or palmitic acid) can intercalate to aid proteins (e.g., Gα protein) bind to membrane regions where these lipids are abundant. Therefore, the membrane lipid composition can have a profound role in cell signaling. Changes in the type and abundance of lipids in membranes induce alterations in the propagation of cell messages that can be associated with pathological states or with its therapy. In the following paragraphs we will describe important structural features of membranes and proteins that participate in protein–lipid interactions and can be regulated by MLT drugs.

Thus, it has been seen that a high consumption of saturated or trans-unsaturated fatty acids induce increases in the proportion of

Please cite this article in press as: Escribá PV et al. Membrane lipid therapy: Modulation of the cell membrane composition and structure as a molecular base for drug discovery and new disease treatment. Prog Lipid Res (2015), http://dx.doi.org/10.1016/j.plipres.2015.04.003

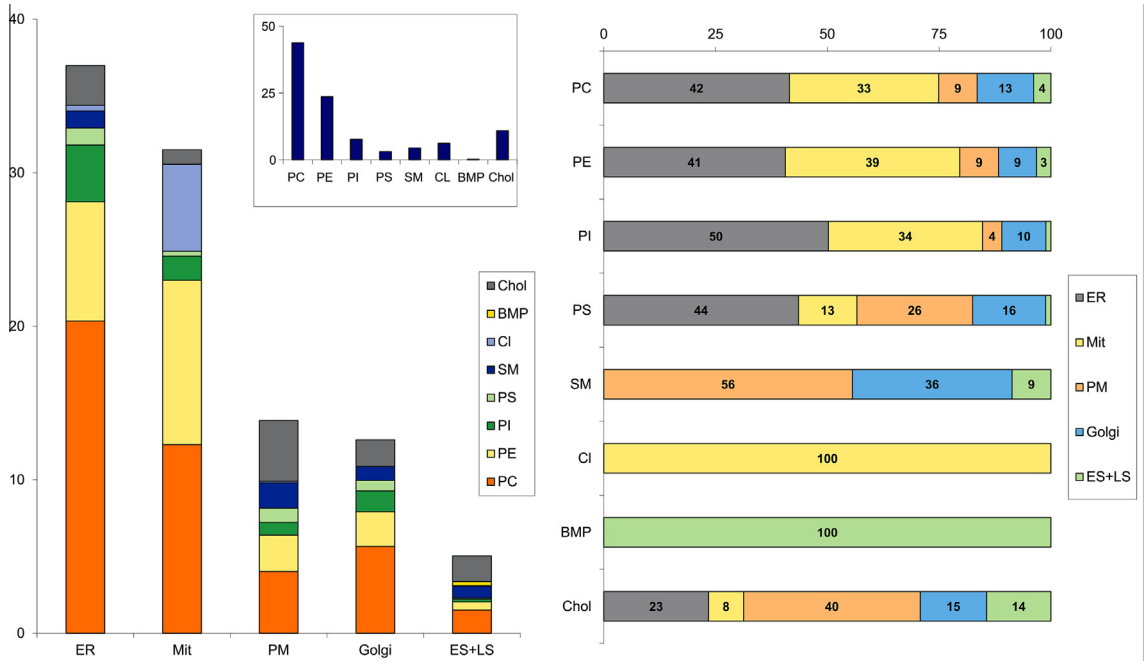

Fig. 1. Lipid composition of biological membranes. (A) Lipid composition of subcellular compartments expressed as mol% of the total cell lipid content. Insert: average lipid composition of a mammalian cell. (B) Distribution of lipids among subcellular compartments expressed as mol% of the total amount of a given lipid class in the whole cell. Data were renormalised by combining previously published data on subcellular lipid compositions [204–206] with surface area of the corresponding subcompartment [207]. Graphical data were converted to numerical values and adjusted to the same average sum. These were then multiplied by the relative surface areas of the corresponding subcompartments (IA). For (B) each lipid class was set to 100%. ER, endoplasmic reticulum; Mit, mitochondria; PM, plasma membrane; ES + LS, Endosomes and Lysosomes; PC, phosphatidylcholine; PE, phosphatidylethanolamine; SM, sphingomyelin; PI, phosphatidylinositol; BMP, bis(monoacylglycero)phosphate; cl, cardiolipin (diphosphatidylglycerol).

saturated and decrease of unsaturated fatty acids in cell membranes [10]. Membrane fatty acid composition changes may, in turn, alter receptor mediated cell signaling (e.g., [11]) and cause, among other health problems, cardiovascular diseases [12].

Different fatty acids can have specific effects on the membrane lipid structure and the formation of membrane microdomains [13,14]. Their influence on the membrane lipid structure can then alter the propagation of signals from G protein-coupled receptors to G proteins, exerting an even greater control on cell signals than receptor agonists and antagonists [8]. Therefore, it is feasible to design therapeutic interventions targeting the membrane lipid composition and structure [5].

2.1. Membrane microdomains:signaling platforms

Lipids are not usually homogeneously distributed in the membrane. They form microdomains that confer specific physico-chemical properties to discrete regions of the bilayer. A variety of raft microdomains with different compositions and biophysical properties have been reported [15]. Sphingomyelin (SM), glycosphingolipids (GSLs) and cholesterol (Chol) are abundant in lipid rafts, and form rigid and liquid ordered (Lo) microdomains. Regions with higher phosphatidylethanolamine (PE) content are more fluid and have a weaker surface pressure because the small polar heads of these lipids produce a lower surface packing density and greater lipid disorder (liquid disordered microdomains, Ld) [16]. The heterogeneous distribution of lipids gives rise to both transient (e.g., lipid rafts, caveolae, coated pits, etc.) or stable (e.g., synaptosomes, tight junctions, brush border, etc.) membrane microdomains, a segregation that is favoured by lipids such as Chol [17,18] and specific proteins [15,17].

Proteins involved in signal transduction interact with distinct membrane microdomains, and the association converts these structures into signaling platforms (termed signalosomes) essential for the cell's physiology. Moreover, they may contain incomplete signaling cascades that can be activated when one further signaling entity (e.g., the receptor) is recruited to the lipid raft [19]. Both physiological and pathological situations can change the cell membrane lipid composition, the biophysical properties of microdomains, and consequently, the proteins they contain and the signals they propagate. In fact, alterations in lipid raft composition and structure have been associated with different pathologies [20,21] and therefore, drug-induced regulation of membrane lipid composition and structure (MLT) can modulate cell signaling, offering potentially effective treatments for a variety of conditions [5,22–24].

2.2. G proteins

G proteins constitute an example of extrinsic membrane proteins whose localization to membrane microdomains and activation is regulated by their interaction with membrane lipids. In the vicinity of GPCRs, there is generally a molar excess of the heterotrimeric (pre-active protein made up of one alpha, beta and gamma subunit, Gαβγ) G proteins to ensure signal amplification. The Gαβγ binding to the Ld regions of the membrane is promoted by (i), the bulky isoprenyl moiety of the Gγ subunit, and (ii) the combined action of PE and the membrane spanning helices of GPCRs to induce a non-lamellar phase propensity [4,9,25]. Upon ligand binding, GPCR-mediated activation of the G protein causes the exchange of GDP for GTP in the Gα subunit, which dissociates from the Gβγ dimer and translocates to lipid rafts due to its greater preference for Lo microdomains [4]. In Lo microdomains, the Gα subunit may activate effector proteins, like adenylyl cyclase, phospholipase C or ion channels among many. By contrast, the Gβγ dimer remains in the Ld microdomains, due to the isoprenyl

Please cite this article in press as: Escribá PV et al. Membrane lipid therapy: Modulation of the cell membrane composition and structure as a molecular base for drug discovery and new disease treatment. Prog Lipid Res (2015), http://dx.doi.org/10.1016/j.plipres.2015.04.003

moiety and a polybasic amino acid domain at the C-terminal region of Gγ, and it either recruits G protein-coupled receptor kinase (GRK) to phosphorylate and inactivate the GPCR, or it activates other effectors [26,27]. Phosphorylated GPCR molecules can be bound by β-arrestin and translocated from the Ld microdomains to clathrin microdomains (coated pits), where the receptor will be internalised by endocytosis. Subsequently, its activity can be restored by phosphatases and its recycling to the plasma membrane [26]. In this context, controlling membrane composition and structure can be used to treat pathologies where GPCRs and/or G proteins are altered.

2.3. Protein kinase C

Upon activation, protein kinase C (PKC) isozymes are translocated to the plasma membrane, where they become activated and phosphorylate a wide variety of protein targets. Recruitment of PKC to Ld membrane microdomains is favoured by: (i) its specific interactions with the H_{II}-prone lipid DAG, and the negatively charged lipids phosphatidylserine (PS) and phosphatidylinositol (PI); and/or (ii), its preference for microdomains with high non-lamellar phase propensity (rich in PE, DAG, etc.) [28]. Moreover, reducing the non-lamellar (H_{II}) phase propensity by the anticancer drug daunorubicin induces PKCα translocation to the cytoplasm, whereas an increase in the non-lamellar phase propensity (produced by adding PE) provokes the recovery of PKCα at the membrane [29].

PKC binding to membranes is not only regulated by lipid structures but also by specific membrane lipids, both factors that define its sub-localization to specific membrane microdomains [28]. Thus, the C1 domain in the N-terminal region of conventional and novel PKC isozymes can bind to the H_{II}-prone lipids, DAG and phorbol esters, and it possibly recognizes non-lamellar phases [30]. By contrast, the C1 domain of atypical PKC isozymes recognizes ceramide (Cer) [30]. The C1 domain of PKCα and β contains a Cys and His rich motif that tightly binds two zinc ions and one DAG or phorbol ester molecule. In addition, Asp residues in the C2 domain (which lies near the C1 domain in the regulatory region of the enzyme) of conventional and novel PKC isozymes participate in the binding of Ca^{2+} ions, which is necessary for the further binding of PS and its cytosol-to-membrane translocation [31,32]. Moreover, basic amino acid residues (e.g., Asn and Arg) participate in the binding of additional acidic phospholipid molecules. The presence (conventional and novel) or absence (atypical) of C2 domains, or the molecular differences found in this motif, explain the different protein–lipid interactions among PKC isozymes and the fine-tuning that underlies their differential membrane localization [33]. For example, a Lys rich cluster in the C2 domain also binds the membrane phospholipid, phosphatidylinositol 4,5-bisphosphate (PIP2), inducing PKCα activation in a manner distinct from that of other membrane lipids [34]. Some studies indicate that the presence of PIP2 determines the localization of the enzyme to the plasma membrane but not to other internal membranes [35]. Therefore, interactions of PKC isozymes with defined lipids or lipid structures in part determine the type of targets that the enzyme will phosphorylate in a given microdomain of the cell's membranes [30].

The C2 domain that is present in PKC and other proteins is not the only lipid-interacting domain that recognizes phosphoinositides. Diverse protein motifs (e.g., pleckstrin homology [PH] domains, FYVE zinc finger domains, PX domains, epsin N-terminal homology [ENTH domains, clathrin assembly lymphoid myeloid [CALM] domains, PSD95-Dlg1-zo-1 [PDZ] domains, phosphotyrosine-binding [PTB] domains, 4.1 protein-ezrin-radixin-moesin [FERM] domains, etc.) can bind different inositol lipids that localize to different cell membranes [36]. The presence of phosphoinositides in specific membrane microdomains facilitates protein docking to these membrane regions and, moreover, some of these protein domains themselves are also directly involved in protein–protein interactions (e.g., PH and PTB domains) [37]. In some cases, these protein domains mediate the binding to various phosphoinositides. Thus, differences in the PH domain sequence produce variations in protein affinity and in the specificity for the various forms of membrane phosphoinositides (such as PIP2, PIP3, etc.) [38]. In other cases, the PI recognition domain has a more defined preference in different proteins, such as the FYVE domain that appears in proteins that regulate vesicular sorting through specific interactions with PI3P [39].

In summary, defined membrane lipid classes, such as acidic phospholipids (e.g., PS or PI), PE or DAG, and membrane lipid structures, such as H_{II}-prone or lamellar-prone (Lo or Ld) bilayers, influence the localization of proteins to membrane microdomains via protein–lipid interactions, facilitating specific protein–protein interactions and their resulting signals [28,29,31,32,34]. Therefore, regulating the membrane lipid composition through pharmaceutical or nutraceutical interventions can serve to normalize signals that have been altered under different (pathological) conditions.

2.4. Sphingomyelin synthase and cell proliferation

Membrane lipid composition can be altered by food intake but is mainly controlled by regulation of the activity of a number of enzymes. While many enzymes are important in the cell's physiology, the relevant role of sphingomyelin synthase (SGMS) has been highlighted recently. This enzyme catalyzes the reversible conversion of PC and Cer into SM and DAG. Recent studies showed that tumor cells, with uncontrolled proliferation have lower levels of SM and higher of PE than non-tumor cells [40]. The ca. 10-fold difference in the PE:SM ratio between proliferating cancer cells and normal cells has been suggested to constitute a switch that would enable certain type of proliferation signaling proteins to bind to the membrane and propagate cell growth messages when the cell has a high PE:SM ratio [41].

2.5. The stress response

Cells and their membranes respond rapidly to various environmental perturbations. It has been demonstrated that subtle membrane alterations are critically involved in the conversion of signals from the environment into the transcriptional activation of stress genes (e.g. heat shock protein (Hsp) genes) [42]. Moreover, the specificity of the stress gene expression can be regulated by the particular occurrence and distribution of membrane microdomains (rafts, caveolae, lipid shells, etc.) that precisely sense biological and physical changes [43,44].

Furthermore, it has also been shown that interactions between specific domains of membranes and certain Hsps remodel the pre-existing architecture and physical order of membranes [45]. This feed-back loop allows interactions which antagonize the heat-induced membrane lipid disorganization and can preserve, at least temporarily, membrane structure and functions during stress. Since highly specific Hsp–lipid interactions are known, these provide a means of targeting Hsps to distinct compartments in the membrane such as lipid rafts which are known to be central to many signaling pathways [6].

Linking membrane microdomain structure and physical states with the regulation of heat shock gene expression, together with the feedback effect of certain Hsps in restoring membrane structure/function, may represent a 'unifying theory' in which membrane microdomains are key players in a new modality of gene expression [42]. This implies a new way of controlling membrane signaling cascades through physical state which consequently

Please cite this article in press as: Escribá PV et al. Membrane lipid therapy: Modulation of the cell membrane composition and structure as a molecular base for drug discovery and new disease treatment. Prog Lipid Res (2015), http://dx.doi.org/10.1016/j.plipres.2015.04.003

has widespread implications for health and disease (see Section 3.1).

2.6. Heterogeneity of membrane composition

The lipid and protein composition of plasma membranes from different cell types is very different. Therefore, the presence or absence of given lipids will differentially affect the biophysical properties of the lipid bilayer and protein activities. Moreover, the individual membrane microdomains in the same cell membrane will be differentially affected by the presence or absence of a given molecule (see below the effect of Cer on Chol-rich lipid rafts). Thus, monounsaturated fatty acid treatments can change the order of Lo and Ld microdomains [40,46]. In addition, the cell is full of organelles and membranous structures with specific lipid and protein compositions. In this scenario, all players can be modulated by MLT approaches. Thus, the overall membrane lipid composition, the composition of membrane microdomains or the composition of internal organelles can be targeted by natural or modified lipids or drugs [47–49]. In this context, fatty acids regulate the biophysical properties of membranes according to their structure. Thus, the cis-monounsaturated fatty acid, oleic acid, induces the formation of nonlamellar phases in model membranes, whereas its saturated (stearic) and trans-monounsaturated (elaidic) analogues do not [13]. The type and abundance of different lipid classes and their fatty acid composition define the types of membrane microdomains that form platforms where signaling partners have productive interactions. This has been used to design synthetic fatty acids that regulate the membrane microdomain organization in a similar fashion to that of natural lipids [14]. This approach has been used to regulate protein–protein interactions in lipid rafts and other membrane microdomains [41,50]. Thus, MLT appears to be an elegant approach to make protein–protein interactions susceptible to drug therapy where they are frequently considered to be a difficult target [51].

In general, membranes are formed by hundreds of different lipid molecular species and they participate in different functions either as single molecules or as structures formed by several molecules. The multiple functions of membranes depend on these lipids, which confer relevant functions to cells. For example, it has been recently reported that the membrane lipid composition constitutes a proliferation switch in tumor cells [47].

3. Molecular bases of targeting the plasma membrane

We describe five different types of regulatory effects in which the plasma membrane is involved (Fig. 2) and can be exploited for therapeutic purposes. These classifications are based on the way the membrane lipid composition is regulated or protein-membrane interactions are controlled.

3.1. Type 1: direct regulation through membrane structure modification

Lipids acquired in diet, or by nutraceutic and pharmaceutic interventions can be incorporated into cell membranes, where they regulate the physico-chemical properties of membranes that, in turn, control the localization and activity of extrinsic membrane proteins (Fig. 2, panel 1). Thus, monomeric Gα proteins prefer lamellar membrane regions with high surface pressure, whereas dimeric or heterotrimeric G proteins prefer nonlamellar-prone microdomains with loose membrane surface packing [4,7]. This fact is due to the presence of a bulky isoprenyl moiety in the Gγ subunit of G protein dimers and trimers that cannot penetrate well into bilayers with dense packing. By contrast, Gα proteins have

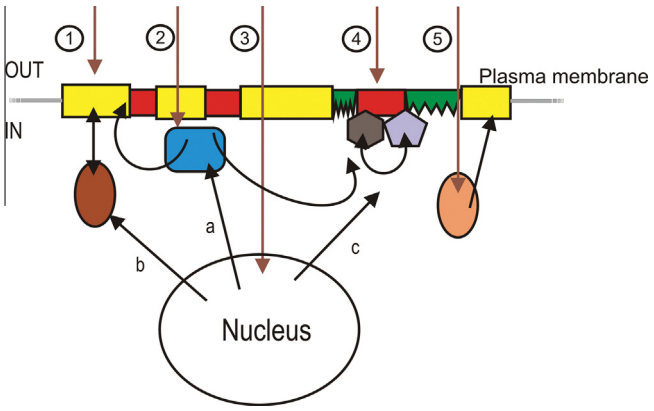

Fig. 2. Mechanisms of action of molecules acting via MLT. The colored squares represent different membrane microdomains with defined compositions and structures. The brown arrows indicate the sites of action (lipid or protein). Numbers 1 through 5 correspond to type-1 to -2 membrane modulation molecular mechanisms of action (MMoA). Type 1 involves direct binding of the compound to the membrane, which alters the interaction of one or more proteins with the membrane (number 1 in this figure). MMoA type 2 involves modification of the activity of an enzyme that changes the composition of the membrane (number 2). MMoA type 3 involves regulation of gene expression that causes changes in the activity of an enzyme that regulates the membrane composition (a), changes in protein–lipid interactions (b) or in protein–protein interactions at the membrane (number 3). Changes in the composition of membrane microdomains or molecules that using other means alter protein–protein interactions at the membrane would be considered type-4 membrane lipid therapy approaches. Finally, protein alterations that affect their interaction with the membrane constitute type-5 membrane lipid therapy approaches (number 5).

fatty acyl moieties that prefer lamellar membrane structures. In this context, the presence of oleic acid but not its trans analogue (elaidic acid) in membranes causes changes in the lipid surface packing that alter G protein-mediated α_2-adrenoceptor signaling with a greater potency than agonist ligands [8]. Moreover, anticancer drugs that regulate the membrane structure also regulate the interaction of G proteins with the membrane and downstream signaling [29,52].

Molecular chaperones [53] mediate the assembly of numerous proteins and degradation of misfolded proteins and are associated with membranes via specific lipid interactions [45,54]. The heat shock response (HSR) can be activated by diverse environmental and physiological stressors that result in the immediate induction of stress genes encoding heat shock proteins (Hsp), molecular chaperones, proteases and other proteins [55]. According to the denatured protein sensor hypothesis, misfolded or aggregated proteins disturbing cell homeostasis may represent a common sensory element [56]. However, membranes also sense environmental changes and, by altering their physical state and microdomain organization, they alter heat shock signals that activate Hsp transcription [42] (Fig. 3).

The formation of isofluid membrane states in response to the application of heat shock, the local anesthetic benzyl alcohol (BA), or other chemical agents results in almost identical increases in Hsp70 expression in B16F10 cells [57]. Importantly, BA induced activation of Hsp expression is not triggered by a protein unfolding signal but rather by membrane hyperfluidization, which is followed by a rapid structural remodeling of the membrane lipids [58–63].

The raft components SM and Chol are also involved in the generation of second messengers in the HSR, and membrane stress caused by heat or chemical membrane fluidization augments the total Cer levels in Jurkat cells [64]. A direct link between heat-induced Cer production and the induction of specific stress proteins has been described in NIH WT-3T3 cells [65]. Application of the exogenous Cer analogue C2-Cer, or increasing

Please cite this article in press as: Escribá PV et al. Membrane lipid therapy: Modulation of the cell membrane composition and structure as a molecular base for drug discovery and new disease treatment. Prog Lipid Res (2015), http://dx.doi.org/10.1016/j.plipres.2015.04.003

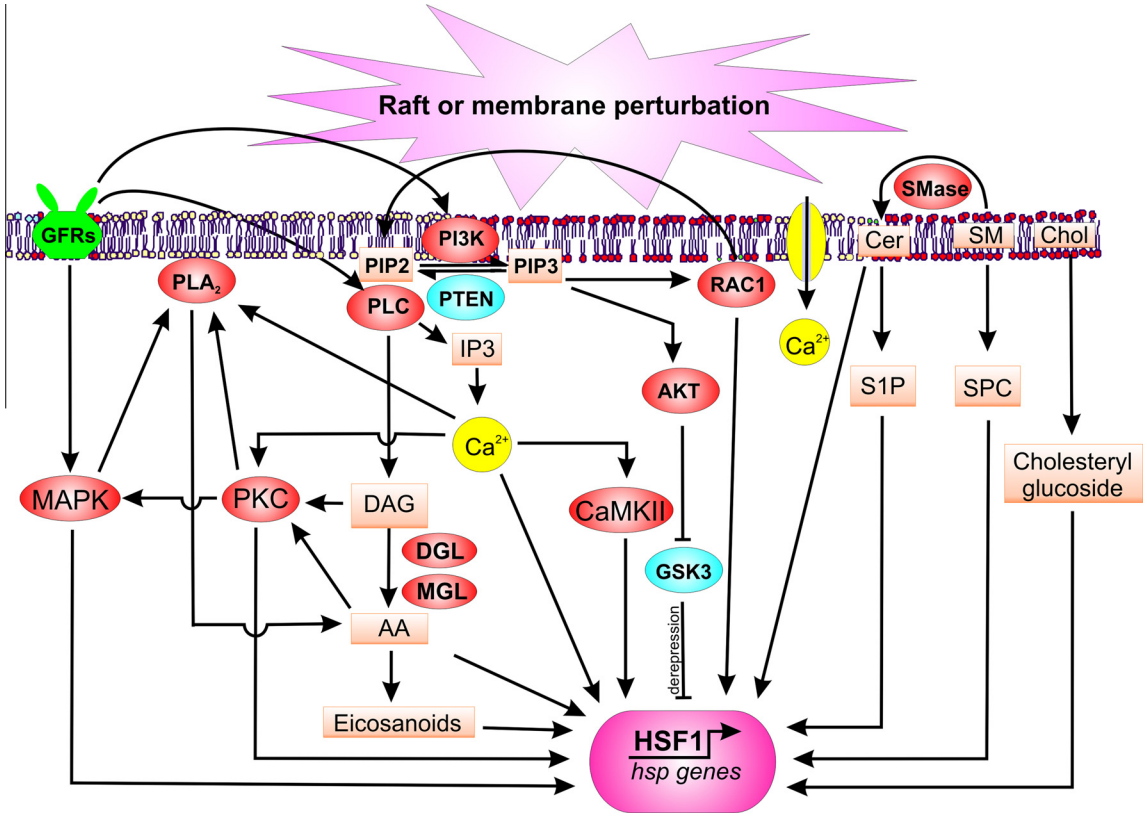

Fig. 3. Overview of surface membrane-controlled signal transduction pathways that potentially control the expression of Hsps via HSF1. AA, arachidonic acid; Akt, protein kinase B; CaMKII, calcium/calmodulin-dependent protein kinase II; Cer, ceramide; Chol, cholesterol; DAG, diacylglycerol; DGL, diacylglycerol lipase; GFR, growth factor receptor; GSK3, glycogen synthase kinase-3; HSF1, heat shock factor 1; IP3, inositol triphosphate; MAPK, mitogen-activated protein kinase; MGL, monoacylglycerol lipase; PI3K, phosphoinositide 3-kinase; PIP2, phosphatidylinositol-4,5-biphosphate; PIP3, phosphatidylinositol-3,4,5-triphosphate; PKC, protein kinase C; PLA2, phospholipase A2; PLC, phospholipase C; PTEN, phosphatase and tensin homologue protein; Rac1, Ras-related C3 botulinum toxin substrate 1; S1P, sphingosine-1-phosphate; SM, sphingomyelin; SMase, sphingomyelinase; SPC, sphingosylphosphorylcholine. The figure is not intended to show the precise localization of all the components. For further details see text.

endogenous intracellular Cer induces the sHsp αβ-crystallin, but not the structurally related Hsp27. Cer has the unique property of fusing membranes and it appears to drive the coalescence of raft microdomains to form large, Cer-enriched membrane platforms that lack Chol [66,67]. Thus, elevated Cer levels rapidly displace Chol from the membrane/lipid-"Chol-raft" to form a "Cer-raft" [68], which might be required for biochemical transfer of stress signals across the plasma membrane. Sphingosylphosphorylcholine (SPC), another sphingolipid metabolite activates Hsp27 via the p38 MAPK pathway in isolated rat cerebral arteries, unlike sphingosine-1-phosphate (S1P) [69]. By contrast, in osteoblast-like MC3T3-E1 cells and aortic smooth muscle A10 cells, S1P stimulates Hsp27 induction via the p38 MAPK and PI3K/Akt pathways [70–72]. In addition, during HSR, Chol can be rapidly converted to cholesteryl glucoside [73]. Indeed, exogenous cholesteryl glucoside rapidly activates the transcription factor HSF1 and induces the synthesis of Hsp70 in fibroblasts [74]. These findings suggest that lipids are important in fine tuning the expression of Hsp chaperones, which may prove beneficial in the treatment of several important diseases, such as cancer, diabetes and various neurodegenerative diseases [44].

A special case of Type 1 MLT is the so-called lipid replacement therapy. A well-known example of this approach is the substitution of membrane lipids in mitochondria. This organelle carries out oxidative phosphorylation to produce energy, so that it is more prone to produce reactive oxygen species that can damage mitochondrial lipids, especially in association with aging [75]. Some studies have demonstrated that therapeutic interventions with lipids and antioxidants can reduce mitochondrial lipid peroxidation and replace damaged lipids, which results in a reduction of fatigue in elderly subjects [76]. Altered mitochondrial membrane lipids affect the activity of important mitochondrial proteins, and treatments with cardiolipin, rich in the polyunsaturated fatty acid linoleic acid, restore the membrane lipid structure and activity of Ca^{2+} channels in mitochondria [77].

3.2. Type 2: regulation of enzymatic activity to alter membrane lipid levels

One example of Type 2 MLT is the effect of hydroxy-C18 unsaturated FAs (hydroxyoleic, hydroxylinoleic, hydroxy-α-linolenic and hydroxy-γ-linolenic acids) on SGMS [40]. The dramatic increases in SM levels following human cancer (lung, glioblastoma multiforme, astrocytoma, leukemia or in cell (A549, U118, SF767, Jurkat and others) treatment with these synthetic FAs (up to 500% following exposure for 72 h) produces significant alterations in the localization of pivotal proteins involved in cell proliferation, such as the translocation of Ras from the plasma membrane to the cytosol [40,41]. Alterations in the activity of enzymes that participate in the metabolism of sphingolipids (such as S1P or Cer) are implicated in many diseases, including cancer [78], suggesting that MLTs may be designed to regulate sphingolipid activity (Fig. 2 panel 2).

Changes in the composition of membranes are also observed in B16F10 cell cultures following thermal stress or benzyl alcohol treatment, which activates certain phospholipases (mainly PLA2 and PLC), and causes loss of polyenes (including the potent HSR modulator arachidonic acid, AA) and concomitant increases in

Please cite this article in press as: Escribá PV et al. Membrane lipid therapy: Modulation of the cell membrane composition and structure as a molecular base for drug discovery and new disease treatment. Prog Lipid Res (2015), http://dx.doi.org/10.1016/j.plipres.2015.04.003

saturated lipid species [58]. Moreover, in CHO cells at elevated, fever-type heat shock temperatures glycosyl-phosphatidylinositol labeled clusters (rafts) disappeared, which was accompanied by an increase in the expression of the small heat shock protein Hsp27 [60]. It is noted that in cell cultures, raft integrity spontaneously recovers at fever-like temperatures [79]. This effect causes redistribution of Chol-rich plasma membrane domains [61,80] and increased membrane lipid packing density in cell cultures [39], with a concomitant change in the localization of relevant signaling proteins. MLT drugs (Bimoclomol, BGP-15) affecting the composition and dynamics of rafts can modulate the expression of certain hsp genes in different cell cultured cells [81–83].

3.3. Type 3: modulation of gene expression that results in changes in membrane lipid structure

Certain DNA-associated phospholipids are found in the nuclear matrix, where their regulatory roles are as yet unknown [84]. Many aspects of lipid metabolism and function in the cytoplasm are recapitulated in the nucleus, and thus, MLT can also target nuclear or other membranes [84]. The nuclear envelope contains the bulk of nuclear lipids, while the nuclear matrix contains enzymes and metabolites necessary for autonomous lipid metabolism in the nucleus [85]. The presence of nuclear lipids and their regulatory effects on numerous nuclear functions have been well documented (e.g., [85]. In this scenario, both the lipid-mediated regulation of nucleic acid function and the control of lipid composition in the nuclear membrane constitute potential activities with which MLT can interact.

Quiescent differentiated cells have high SM levels in the PM, whereas proliferating cells, including cancer cells, exhibit reduced PM SM content [40]. Direct addition of SM to the culture medium does not induce cell cycle arrest, whereas induction of SGMS and the subsequent increase in SM levels does inhibit cell growth and differentiation [40,41]. Interestingly, low nuclear SM has been associated with cell proliferation [86]. Because DNA synthesis is activated when sphingomyelinase is active and SGMS activity is reversed, it could be speculated that part of the actions mediated by MLT could occur in the cell nucleus. In this scenario, MLT may be able to regulate gene expression, thereby modifying lipid composition (Fig. 1). Regulating the expression of genes that alter the lipid composition of the membrane can alter cell activity in various ways. The lipid-binding transcription factors PPARs, RXR, RAR and LXR, can be regulated by synthetic ligands, resulting in the modulation of membrane lipids and cell function [87]. Since lipids control the activity of PPARs and related transcription factors, the activity of these proteins could be regulated by hydrophobic MLT drugs, such as fatty acid analogues, whose partition coefficients are higher than those recommended by the Lipinski's rule of 5 to design or discover drugs. This rule suggests (among other things) that the partition coefficient (log Poctanol/water) should be less than 5 and this may fail for certain MLT molecules, such as modified fatty acids [88].

3.4. Type 4: lipid alterations that affect protein–protein interactions in specific membrane microdomains

The modification of membrane lipids in this scenario affects the productive interaction of 2 signaling partners (Fig. 2 panel 4). Accordingly, the changes in membrane lipid composition and structure may alter a given microdomain or the affinity of one of the proteins involved in the transduction of a specific signal. For example, reductions in the PE-to-SM ratio diminish the RTK–Ras and Ras–Raf interactions at the membrane, provoking a concomitant reduction in MAPK-associated signaling and ultimately, in the proliferation of cancer cells [41].

In a similar fashion, membrane Chol profoundly affects the targeting of the small GTP-binding protein Rac1 to membranes and its interactions with other proteins [89]. The stress-stimulated PI3K-driven conversion of PIP2 to PIP3 has been proposed to activate Rac1 under mild, non-denaturing stress conditions [55], and Rac1 may be involved in mild HS-induced Hsp expression [90]. Accordingly, the redistribution of Chol-rich membrane domains may alter stress responses through Rac1-dependent mechanisms. Indeed, Rac1 inhibition by a specific inhibitor (NSC233766) halves HS-induced *hsp25* expression at fever-like temperatures [79,91]. Clearly, there is a complex network of interconnected pathways that bridge the gap between the cell membrane and HSF-1 mediated modulation of the expression of Hsp chaperons (see Fig. 2).

Stress-induced inositol phosphate signals are generated within minutes and they turnover rapidly [92]. Rapid increases in cytosolic Ca^{2+} levels [57,93] are a prerequisite for *hsp* transcription [94], although in human epidermoid A431 cells heat activates the Na^+–Ca^{2+} exchange system, thereby augmenting $[Ca^{2+}]_i$ while reducing $[Na^+]_i$ [95]. In addition, the HSR is triggered by calcium/calmodulin-dependent protein kinase II (CaMKII) which is activated by increases in $[Ca^{2+}]_i$, the binding of Ca^{2+}/calmodulin and by autophosphorylation [96]. The HSR can also be activated by both AA and PLA_2 [97,98]. Exogenous PLA_2 stimulates AA generation and release, while AA production can be driven by DAG lipase-MAG lipase metabolism of DAG. A positive correlation was recently demonstrated between HS response and cellular AA content [62]. Elevations in $[Ca^{2+}]_i$, PKC phosphorylation and protein–protein interactions can regulate PLA_2 activity [99]. Moreover, the generation of cis-unsaturated FAs by PLA_2 is crucial for the activation of PKC, and may stabilize PKC in an activated state [100,101]. AA may serve to direct Ca^{2+}-sensitive and Ca^{2+}-insensitive PKC isoforms to membrane targets, and mediate feedback modulation of Ca^{2+} signaling [102]. Furthermore, EGFR signaling is transduced by two interconnected pathways, the PLC-PKC and MAPK pathways, which interact at two points. PKC activates MAPK, while MAPK can phosphorylate and activate $cPLA_2$. The AA produced by $cPLA_2$ can act synergistically with DAG to activate PKC [103], and AA can be further metabolized to eicosanoids that in turn mediate signaling via G protein-coupled receptors, and that may also induce HS response [104]. Moreover, AA can act within the cell, or it can cross cell membranes and act on neighboring cells [99]. PIP2 is a substrate of PI3K in the production of PIP3, another lipid with key signaling functions and that plays a major role in the control of cell survival (e.g., stress response), growth and proliferation. The components of the PI3K pathway include upstream regulators of PI3K enzymes (such as EGFR and Ras), PTEN, various Ser/Thr kinases and transcription factors [105]. Several proteins propagate different cellular signals after binding to PIP3, including Akt and Rac1, both of which are important components of membrane-derived stress signal pathways. In addition, membrane depolarization increases Hsp70 expression in cultured skeletal muscle cells, and this effect is critically dependent on Ca^{2+} released from IP_3-sensitive intracellular stores. Furthermore, depolarization-evoked slow Ca^{2+} signals induce PKC-α activation and its translocation to the nucleus, favoring HSF1 phosphorylation leading to increased Hsp70 expression [106].

Gangliosides, sialic acid-containing glycosphingolipids (GSLs), are expressed in a cell-type specific manner and they interact with a variety of molecules on plasma membranes through different non-covalent bonds, such as electrostatic and hydrophobic interactions. Through these interactions, ganglioside family members participate in diverse cell activities by forming dynamic functional complexes in living cell membranes (membrane microdomains or lipid raft) [107]. The expression of cellular gangliosides is influenced by various extracellular stimuli, including inflammatory

Please cite this article in press as: Escribá PV et al. Membrane lipid therapy: Modulation of the cell membrane composition and structure as a molecular base for drug discovery and new disease treatment. Prog Lipid Res (2015), http://dx.doi.org/10.1016/j.plipres.2015.04.003

cytokines. Thus, the presence of gangliosides in membrane microdomains may reflect the characteristics of individual cells in a pathophysiological environment [108].

Insulin is critically dependent on caveolae/microdomains in adipocytes [109,110]. Due to their lower density, these microdomains can be isolated from cell membranes using sucrose gradient centrifugation (after cell disruption with non-ionic detergents at cold temperatures), and they are designated as "detergent-resistant membrane microdomains" (DRMs: [111,112]). Though DRMs are considered as experimental evidence of the existence of lipid rafts and related domains, including caveolae and glycolipid-enriched membrane microdomains (GEM: [111]), detergent-resistant membranes should not be assumed to resemble biological rafts in size, structure, or composition. Functional rafts may not be steady phenomena; they might form, grow, cluster or break up, shrink, and vanish according to functional requirements, regulated by rather subtle changes in the activity of membrane disordering or ordering compounds. However, these and other membrane domains have important functions in cells, such as attracting proteins with which they physically interact to propagate signals. Regulating these domains through interventions acting on GSLs may be of interest for the treatment of certain conditions, such as diabetes (see below).

G protein coupled receptors (GPCRs) constitute the largest family of receptors in humans, and they mediate a large number of cell functions and currently constitute about 40% of all drug targets. Pepducins are lipidated peptides that target the GPCR-G protein interaction aided by a lipid tether, such as palmitic acid in specific membrane microdomains [113]. For example, ATI-2341 targets the first intracellular loop of the chemokine receptor CXCR4 and pepducin-induced activation of this receptor results in blood cell mobilization, which is required prior to autologous bone marrow transplantation [114].

Finally, raft microdomain remodeling by monounsaturated (MUFA) MLT drugs regulates interactions with the Fas death ligand receptor, which clusters in defined membrane regions and oligomerizes in a ligand-free manner to activate the extrinsic caspase cascade, in this way selectively inducing leukemia cell death [115].

3.5. Type 5: direct MLT-drug binding to a protein that alters its membrane binding affinity or that of other signaling proteins

This mechanism of action (Fig. 2 panel 5) could be considered within the scope of conventional chemotherapy, as the drug binds to a protein rather than a lipid. However, the effect can be classed as MLT when the drug's molecular mechanism of action is dependent on protein–lipid interactions (Fig. 2 panel 5). For example, farnesyl transferase and geranylgeranyl transferase inhibitors (e.g., Tipifarnib, which prevents Ras farnesylation) impair the binding of Ras and related proteins to membranes. This compound is currently used in various anti-cancer combination therapy protocols, and it prevents Ras from interacting with upstream (e.g., EGFR) and downstream (e.g., Raf) signaling proteins [116]. Thus, this type of drug indirectly inhibits the activity of the Ras-MAPK pathway by impairing Ras binding to the membrane and its subsequent interactions with other signaling partners [116]. Impairment of Ras binding to the plasma membrane has been associated with inhibition of cancer cell proliferation and subsequent induction of differentiation and death [41].

4. Therapeutic areas where MLT has been developed

In the following sections, therapies that modulate the lipid membrane that are currently under investigation are discussed.

4.1. Oncology

In the early 1980s, the anticancer drugs anthracyclines were shown to kill cancer cells by a direct interaction with the plasma membrane [117]. Agarose–doxorubicin complexes that cannot enter the cancer cells kill them [117] by modulating the membrane lipid structure, and subsequently altering the localization and activity of important signaling proteins [29]. Based on this pioneering study [29], several drugs were designed to regulate the membrane lipid structure with the aim of abolishing the off-target interactions of anthracyclines and improving their efficacy and toxicity profiles [8,52,101,115].

This research led to the rational design of 2-hydroxyoleic acid (Minerval) [101], which currently is in phase I/II clinical trials for glioma. Its presence in membranes causes order reduction in lamellar disordered regions (Ld, non-raft domains) [14,40,41]. In addition, it specifically activates SGMS, inducing a marked increase in SM levels in human cancer cells that enhances the order of the ordered lamellar domains [40] (Lo, lipid rafts). Minerval activates SGMS and induces dramatic increases in its product, SM, accompanied by massive reductions in the levels of its substrate PE. Treatment with Minerval causes normalization of membrane levels of PE and SM, and increases ca. 10-fold the SM:PE ratio [40]. By contrast, Minerval does not significantly alter membrane lipid composition in non-tumor cells, because the enzyme product (SM) is found in high levels and its substrates (PC and PE) are limiting. Thus, the changes induced in the lipid composition of cancer cells appear to act as a functional switch that changes the cell status from proliferating to quiescent. This compound selectively induces the translocation of Ras from the membrane to the cytoplasm thus giving rise to MAP kinase pathway inactivation that causes cell cycle arrest in cancer cells followed by cell death [41,101,115].

Propofol-docosahexanoic acid (P-DHA) is another interesting molecule with an MLT-related mechanism of action. This molecule is a hybrid compound comprised of the anesthetic propofol covalently bound to DHA [118]. The therapeutic activity of this compound in vitro is unsurprising as both propofol and DHA alone exert anticancer effects. Potent anti-cancer effects have also been described recently for analogues of P-DHA, particularly for the treatment of breast cancer [119]. Another MLT-based anticancer drug is the alkyl lysophospholipid analogue edelfosine. This compound and its analogues alter the membrane lipid raft microdomain structure, initiating a series of molecular events that result in the induction of apoptosis in cancer cells [120]. However, due to its toxicity and modest efficacy, edelfosine could not complete all clinical phases and be authorized for marketing for the treatment of cancer.

Interestingly, the oncogenic antigen-519, a molecular marker found in breast cancer patients with poor prognosis, has been identified as FA Synthase (FAS) [121]. The potential use of FAS inhibitors is currently under investigation, and siRNA and chemical inhibitors of this target (e.g., C75, (−)-epigallocatechin-3-gallate) selectively induce cell growth inhibition and apoptosis of cancer but not normal cells in vitro [121,122]. Another chemical FAS inhibitor, orlistat (marketed under the trade name Xenical by Roche in most countries, and over-the-counter as Alli by GlaxoSmithKline in the UK and US), blocks growth and induces cell death in breast cancer cells when administered concurrently with the monoclonal antibody trastuzumab [123].

The alterations in lipid composition and lipid metabolism are beginning to be associated with tumorigenesis and cancer patient survival. For example, some lipid metabolism genes, such as, OLR1 and GLRX are upregulated in breast and prostate cancer tissues [124]. In this context, proteins related to fatty acid biosynthesis and lipid metabolism regulation, such as, ACC, FASN, INSIG1,

Please cite this article in press as: Escribá PV et al. Membrane lipid therapy: Modulation of the cell membrane composition and structure as a molecular base for drug discovery and new disease treatment. Prog Lipid Res (2015), http://dx.doi.org/10.1016/j.plipres.2015.04.003

Table 1
Number of glioma (brain cancer) patients and expression of selected lipid genes.

Gene	Down (*n*, %)	Intermediate (*n*, %)	Up (*n*, %)
ASAH2	115 (33.5%)	224 (65.3%)	4 (1.2%)
FASN	101 (29.4%)	234 (68.2%)	8 (2.3%)
SMPD2	0 (0%)	288 (84.0%)	55 (16.0%)
SMPD3	74 (21.6%)	269 (78.4%)	0 (0%)
PHYH	30 (8.7%)	311 (90.7%)	2 (0.6%)
SPTLC3	15 (4.4%)	197 (57.4%)	131 (38.2%)
SGMS1	84 (24.5%)	258 (75.2%)	1 (0.3%)
SGMS2	16 (4.7%)	254 (74.1%)	73 (21.3%)
SPHK1	6 (1.7%)	283 (82.5%)	54 (15.7%)
SPHK2	20 (5.8%)	292 (85.1%)	31 (9.0%)
UGCG	21 (6.1%)	283 (82.5%)	39 (11.4%)

Analysis of the expression of 11 representative genes related to lipid metabolism and from the data of gene expression arrays from 343 patients with glioma taken from the REMBRANDT database (Glioma Molecular Diagnostic Initiative) [128]. Genes are listed with standard abbreviations. Down: Gene (mRNA) expression twofold below average. Intermediate: average expression. Up: twofold expression above average. The altered expression of these genes was found to be highly associated with changes in the median survival of the patent population and in ca. 90% of these glioma patients it was observed at least one alteration of the above genes. Adapted from [47].

and SREBP1 are highly expressed in breast cancer tumors and associated with low patient survival [125] while colorectal carcinoma risk has been associated with hepatic lipase polymorphisms [126]. Analysis of the Cancer Genome Atlas (TCGA) database for 38 ovarian cancer samples demonstrated 39 differentially expressed lipid genes in ovarian cancer tissue compared to normal ovarian tissues [127].

Very recently the REMBRANDT database was analyzed to explore the potential role of lipid metabolism in the prognosis of individuals with brain cancer [128,129]. It was found that at least twofold down-regulation of SMGS1 lowered very significantly the median of survival of patients with glioblastoma multiforme (a type of brain cancer): from approx 18 months in patients with intermediate expression to approx 10 months in patients with SGMS1 downregulation [128]. Similar data were found in patients with glioma [47]. Moreover, the 10-year survival of glioblastoma multiforme patients with low SGMS1 expression was approx 10-fold lower than that of patients with normal SGMS1 expression. These data indicate the possible relevance of SM and other lipid metabolism genes in the malignant transformation in the various types of cancer studied so far [40,41]. Moreover, the highly frequent alterations in genes related to lipid metabolism induce a regulation in the median glioma patient survival from 22.6 months to 14.6 months ([47] and Table 1), further supporting the relevance of membrane lipids in cell physiology and glioma etiopathology. Similarly, tumors overexpressing fatty acid synthase (FAS), the enzyme responsible for de novo synthesis of fatty acids, display aggressive behavior compared to those tumors with normal FAS levels, suggesting that FAS overexpression confers a selective growth advantage. Glioblastoma multiforme patients overexpressing FASN have a reduced median survival (approx 12 months) with respect to patients with intermediate FASN expression (approx 28 months) [47,128].

4.2. Metabolic diseases

4.2.1. Obesity

Administration or consumption of fats rich in the cis-MUFA, oleic acid (ω-9), is associated with lower body mass index (BMI) values [130]. Moreover, in rats with ad libitum access to food, a daily supplement of olive oil (in which oleic acid constitutes about 70–80% of all FAs) induces body weight reductions [131]. By contrast, the trans isomer of oleic acid, elaidic acid does not produce body weight decrease. Oleic and elaidic acid have the same chemical composition, but their different molecular structure causes divergent effects in the lipid bilayer structure [13], which has been shown to be associated with their opposite impact on protein–lipid interactions and human health [132]. In this context, the consumption of saturated (or trans-MUFA) fats has been linked with obesity and related health problems. Moreover, the saturated FA palmitic acid is a known inducer of endoplasmic reticulum (ER) stress and cell death [133]. Indeed, lipstatin, a potent natural inhibitor of pancreatic lipases and its derivative orlistat, a FAS (enzyme that mainly produces palmitate) inhibitor and lipid absorption blocker, are effective in the treatment of obesity [134].

4.2.2. Diabetes

Numerous studies demonstrate the association between the type of dietary fats consumed and the development of diabetes, which is placing a growing burden on medical care services for persons over 65 [135]. The ratio of saturated-to-unsaturated FAs in erythrocyte membranes of diabetic patients is greater than that observed in healthy controls (0.78 and 0.72, respectively) [136], which indicates the close relationship between lipids and diabetes. High oleic acid intake improves the glycemic status of these patients and it also reduces the levels of saturated FAs, while increasing those of unsaturated FAs, resulting in a reduction in the saturated-to-unsaturated FA ratio from 0.78 to 0.66. Moreover, it has been seen that concomitant with this effect, the levels of various G proteins in these elderly diabetics are significantly regulated in blood cells [136]. In this scenario, treatments with unsaturated FA derivatives have been shown to reduce glycemia in rats [137], while other molecules that regulate lipid turnover and metabolism (e.g., orlistat, see above) prevent type 2 diabetes. This control of the membrane lipid composition in animals and humans after treatment with natural oleic acid or synthetic analogues of this FA has been seen to be associated with changes in the membrane lipid surface packing (nonlamellar H_{II} phase propensity), which regulates the interaction of some G proteins (relevant in metabolic signaling) with the membrane [8,13,136,137].

Membrane alterations affect signaling pathways from membrane lipids to hsp genes and Hsps themselves play fundamental roles in the etiology of several diseases, including type 2 diabetes [2]. Typically, the decreased expression of stress proteins in patients with type 2 diabetes correlates with reduced insulin sensitivity, while activation of Hsp70 by heat therapy improves clinical parameters [138,139]. The non-proteotoxic lipid-interacting hydroxamic acid (HA) and its derivatives physiologically restore the HS protein response, representing a new class of MLT pharmaceuticals [140]. At the molecular level HA derivatives are broad-spectrum, multi-target compounds that stabilize membranes and remodel their lipid rafts [79,81]. The HA derivative BGP-15, currently being tested in clinical trials for the treatment of diabetes (Table 1), remodels Chol-enriched lipid platforms in a similar manner to that observed following non-lethal heat priming or membrane stress [61,79]. BGP-15 also induces Hsp chaperone expression through the Rac1 signaling cascade, in accordance with the effects of Chol on the membrane-targeting of Rac1 (Fig. 3) [91].

Gangliosides (composed of a glycosphingolipid with one or more sialic acids linked on the sugar chain) modulate a variety of cellular processes (Fig. 4). TNFα produces a striking increase in cellular ganglioside GM3 levels in mouse 3T3-L1 adipocytes in a state of insulin resistance, and in the adipose tissues of obese/diabetic rodents, including Zucker fa/fa rats and ob/ob mice [141]. Treatment of adipocytes with TNFα revealed that the increased GM3 levels result in the elimination of insulin receptors (IRs) from DRMs (while caveolin and flotillin are retained), effectively disrupting insulin metabolic signaling [142]. In agreement with these

Please cite this article in press as: Escribá PV et al. Membrane lipid therapy: Modulation of the cell membrane composition and structure as a molecular base for drug discovery and new disease treatment. Prog Lipid Res (2015), http://dx.doi.org/10.1016/j.plipres.2015.04.003

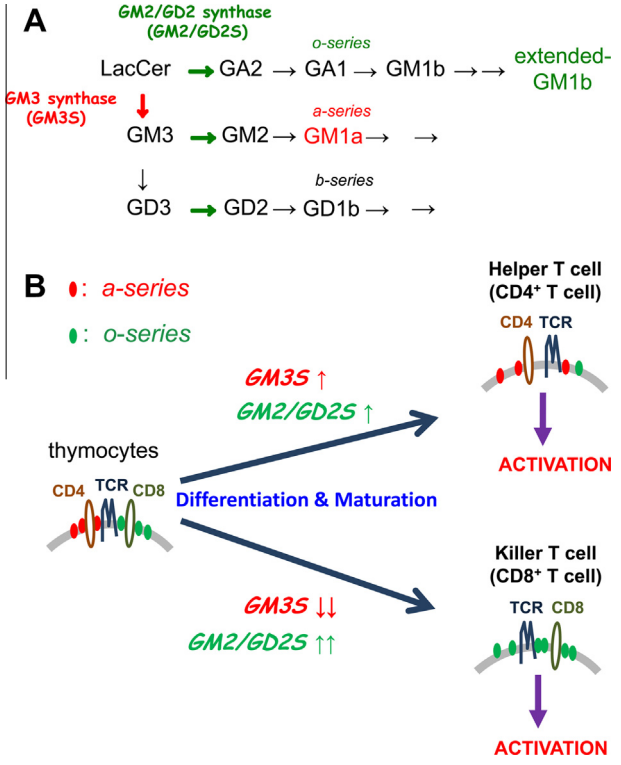

Fig. 4. Functional repertoire through ganglioside selection. (A) The core ganglioside biosynthetic pathway. (B) Distinct differences between lipid rafts in individual T cell subsets. Repertoire selection from immature thymocytes (CD4+ and CD8+ double positive) to mature single positive T cell subpopulations is accompanied by selective ganglioside expression. Gene expression patterns and ganglioside analysis confirm that CD4+ T cells dominantly express a-series gangliosides due to GM3S upregulation while CD8+ T cells carry o-series gangliosides due to the downregulation of GM3S and the upregulation of GM2/GD2S expression. These observations suggest that each T cell subset contains unique rafts composed of different ganglioside species, and that these rafts serve distinct functions during different intracellular events following receptor-mediated stimulation. This ganglioside selection process may be crucial for the formation of distinct and functional lipid rafts in mature T cells.

findings, insulin signaling is enhanced in mice lacking GM3 synthase [143]. IRs form complexes with Cav1 and GM3 independently, and in GM3-enriched membranes IR mobility is increased by its dissociation from Cav1. As insulin metabolic signal transduction in adipocytes is critically dependent on caveolae [109,110], a new pathological feature of insulin resistance in adipocytes has been proposed, whereby the dissociation of the IR-Cav1 complex occurs due to IR-GM3 interactions in DRM microdomains [144] (Fig. 4). Thus, novel therapeutic interventions aimed at inhibiting GM3 biosynthesis may prove beneficial for the treatment of metabolic diseases, including type 2 diabetes [108] (See Table 2).

Many receptor tyrosine kinases are localized in lipid rafts, including growth factors EGFRs, PDGFRs, and insulin receptor (IR), and all three of these receptor types carry a caveolin binding motif in the cytoplasmic region [145]. Although the localization of these receptors in caveolae is reportedly interrupted by elevated levels of endogenous gangliosides, the precise mechanism underlying this phenomenon has not been determined yet [146]. Interestingly, like IRs, some of these growth factor receptors contain basic amino acids just above their transmembrane domains, driving spatial proximity to GEM [144].

4.2.3. Hypercholesterolemia

Hyperlipidemia is a major risk factor for atherosclerosis and cardiovascular disease, including coronary heart disease. Thus,

Table 2
Physiological features of the metabolic syndrome.

Organ/tissue	Cellular characteristics
Pancreas	β-cell growth and survival **R** Glucose sensing **R**
Brain	Appetite reduction **R** Increased sympathetic tone **S**
Liver	High free FAs and triacylglycerol secretion **S** Reduced glucose production **R** High lipoprotein uptake **R**
Fat	High triacylglycerol synthesis **S** Reduced lipolysis **R**
Macrophages	Increased fat infiltration **S** Increased survival **R**
Myocardium	High glucose oxidation **R** Decreased free fatty acid oxidation **R**
Arteries	Low plaque formation **R**

R, insulin resistant; **S**, insulin sensitive.
Adapted from [208].

hypercholesterolemia is a risk factor for atherosclerosis, stroke, infarction, etc. [147]. The impact of high Chol on health is underscored by widespread use of Lipitor (atorvastatin), which is the best-selling drug of all time [148]. In addition, high Chol levels and alterations in Chol metabolism have been consistently associated with the development of Alzheimer's disease (see Section 4.4.1).

Chol is one of the main constituents of cell membranes, where it plays structural and functional roles, contributing to the formation of membrane microdomains (e.g., lipid rafts), which act as signaling platforms [4,149]. Nevertheless, high LDL/VLDL-associated Chol constitutes a major risk factor for the development of atheroma plaques that decrease the size of the lumen of blood vessels thus increasing the likelihood of ischemic thrombi that cause atherosclerosis, stroke and myocardial infarction [147,150]. In addition, high plasma Chol levels are associated with increased levels of Chol in the plasma membranes of cardiovascular and other cells, resulting in altered membrane structure and cell signaling [151].

4.2.4. Metabolic syndrome

Metabolic syndrome is defined by the simultaneous occurrence of various risk factors that collectively increase the probability of developing type 2 diabetes mellitus and cardiovascular disease [151,152]. High intake of saturated and ω-6 FAs together with low intake of oleic acid and ω-3 FAs promotes the development of metabolic syndrome, while the inverse combination prevents its occurrence [153]. These data indicate that lipid derivatives, particularly FA-derived drugs, may play an important role in the treatment of metabolic syndrome. Because 2-hydroxyoleic acid efficaciously controls most symptoms associated with this condition, it represents a serious candidate for monotherapy of metabolic syndrome [131,132,154,155].

4.3. Cardiovascular diseases: hypertension

Human erythrocyte cell membranes of hypertensive individuals contain more Chol, Chol esters and TAGs, and less phospholipids than normotensive subjects, in association with alterations in G protein activity [156]. In contrast, high oleic acid (MUFA) intake

is associated with reduced blood pressure [157]. Moreover, the synthetic hydroxy fatty acid, 2-hydroxyoleic acid (MUFA), induces greater reductions in systolic blood pressure in hypertensive rats than oleic acid [132,154]. This dramatic reduction in blood pressure is mediated by the regulatory effects of membrane lipids, which increase adenylyl cyclase activity, activate PKA and reduce of Rho kinase expression [154].

Long chain ω-3 FA intake also provoke a reduction in blood pressure [158], while diets deficient in these FAs are associated with hypertension. Thus, dietary supplementation with α-linolenic acid (18:3 ω-3) reduces high blood pressure [159]. In this context, the ω-3 FAs, EPA and DHA, also induce blood pressure reductions and protection against myocardial infarction and ischemic stroke [160]. As described for MUFAs, polyunsaturated FA (PUFA) intake influences the composition of the cell membrane, which in turn regulates its structural properties and controls the activity of membrane signaling proteins [161–163]. Finally, unsaturated FAs have been shown to have cardioprotective effects ([164] and references therein). Thus, 2-hydroxyoleic acid controls the transient outward K^+ current (Ito) and the cytosolic Ca^{2+} transient levels in isolated cardiomyocytes, which has been associated with its normotensive effect [164]. By contrast, saturated FA and/or Chol intake have negative effects on blood pressure [153].

Inflammatory responses are inhibited by 2-hydroxyarachidonate [165], which may prevent neuronal death of brain penumbra area neurons after stroke, indicating its potential therapeutic use after post-ischemic events [137]. Various unsaturated FA analogues have also been shown to prevent atherosclerosis and improve cardiovascular health in general [137]. Similarly, HA derivatives may be useful for the treatment of atrial fibrillation [166] due to their heat-stress-like effect, which influences the abundance of Chol-microdomains.

4.4. Neurodegenerative disorders

After adipose tissue, the central nervous system (CNS) represents the largest location of lipids in the body. The myelin sheath of neuronal axons is formed by glial cells and consists of a succession of lipid bilayers. Certain neurodegenerative disorders are associated with demyelinization or significant alterations in membrane lipids. These facts and the capacity of lipid drugs to interact with myelin and to cross the blood–brain-barrier (BBB) suggest that MLT may play a major role in the treatment of CNS disorders.

4.4.1. Alzheimer's disease

Alzheimer's disease is the most common neurodegenerative disease resulting in progressive dementia in the elderly (about 60–70% of all cases) [167]. The pathophysiology of Alzheimer's disease is characterized by the loss of neurons and synapses, together with the accumulation of extracellular deposits of fibrillar β-amyloid (Aβ), known as senile plaques, and intraneuronal neurofibrillary tangles that are generated by the abnormal hyperphosphorylation of tau protein [168]. However, Bapineuzumab (Pfizer, ClinicalTrials.gov identifier NCT00663026), an antibody directed against Aβ plaques, failed to induce improvements in a phase III trial, which along with other findings suggests that these alterations may be downstream of more fundamental molecular/-cellular events that perhaps involve membrane lipid alterations. In agreement with this suggestion, the efficacy of acetylcholinesterase inhibitors and NMDA blockers appears to be limited.

DHA is the most abundant FA in neuronal membranes in the cerebral cortex gray matter (30–40% of all FAs esterified to membrane phospholipids) and its decline is associated with age, and with the loss of memory and learning that accompanies Alzheimer's disease [169,170]. Numerous epidemiological studies have demonstrated an inverse association between Alzheimer's disease risk and ω-3 PUFA dietary intake [171]. The biophysical properties of DHA indicate that changes in its abundance may cause alterations in amyloid precursor protein (APP) proteolysis, receptor mediated signaling and Tau protein phosphorylation. With the aim of reversing the structural and functional alterations induced by DHA loss, 2-hydroxy-DHA (LP226A1, Lipopharma) was designed, and tested in an animal model of severe Alzheimer's disease (5XFAD mice). A 4-months treatment with this synthetic FA increased neurogenesis and restored cognitive scores in the radial maze test to control values [48,172]. In this context, γ-secretase cleavage occurs in an APP site located in the middle of the membrane, suggesting that the lipid environment may influence the production of amyloidogenic or non-amyloidogenic peptides, and that it may therefore be involved in Alzheimer's disease pathogenesis [173]. In addition, there is mounting evidence implicating Chol in the pathogenesis of Alzheimer's disease [174]. In fact, the regulation of Chol turnover by means of statins has been shown to reduce amyloid load [175,176]. Accordingly, biotech/pharma companies are currently developing statin derivatives to control Chol with a view to preventing or treating mild to moderate Alzheimer's disease (e.g., ClinicalTrials.gov Identifier NCT00024531).

4.4.2. Spinal cord injury and other neurological conditions

A neurotrophic lipid factor formed by binding oleic acid to albumin in vitro induces significant motor recovery (~40%) in rats with spinal cord injury (SCI) [177] ameliorating both spasticity and pain. By contrast, elaidic acid, which is the trans isomer of oleic acid, causes no significant improvements in paralysis, spasticity or pain. Interestingly, oleic acid induces significant changes in the membrane structure, whereas elaidic acid has no such effect [13], indicating that the action of the former is mediated by structural effects. Similarly, LPA181, formed by the oleic acid analog, hydroxyoleic acid, and albumin, is more efficacious than the latter inducing motor recovery of ~80%, and ameliorating spasticity and pain [178].

HA and its derivatives may be useful for the treatment of broad range of neurological and neuromuscular diseases, including amyotrophic lateral sclerosis [179], Huntington's disease [180] and muscular dystrophy [181].

Finally, ω-3 and ω-6 FA abnormalities have been implicated in the development of psychiatric diseases, such as schizophrenia and bipolar disorders [182,183], so that lipids may also be directly relevant to certain psychiatric disorders.

4.5. Other pathologies

HA derivatives have been shown to have the potential to ameliorate diverse acute and chronic conditions such as retinopathy [184], nephropathy [185], wound healing [186], acetaminophen liver toxicity [187], chemotherapeutic neuropathy [188], intracranial hemorrhage [189], atrial fibrillation [190], sunburn [191], ischemia reperfusion [192], vascular hypertension damage [193], myocardial infarction [194], mercuric nephropathy [195], brain hypoxia [196], pancreatitis [197], and ethanol intoxication [198].

Furthermore, MLT principles are being used to develop new therapeutic drugs to treat infectious diseases. As the lipid composition of bacterial lipid bilayers greatly differs from that of eukaryotic cells, it is possible to rationally design antimicrobial peptides that specifically form pores in the prokaryotic envelope without affecting the host, therefore overcoming resistance against other conventional antibiotics [199,200]. In addition to the treatment of bacterial infections, MLT approaches (i.e., edelfosine) have been designed to treat eukaryotic parasite infections, such as leishmaniasis [201]. Alkyl lysophospholipid derivatives (e.g., miltefosine) are also currently under development (phase II) for the treatment of

Please cite this article in press as: Escribá PV et al. Membrane lipid therapy: Modulation of the cell membrane composition and structure as a molecular base for drug discovery and new disease treatment. Prog Lipid Res (2015), http://dx.doi.org/10.1016/j.plipres.2015.04.003

antihistamine-resistant urticaria (ClinicalTrials.gov identifier NCT01170949). Moreover, these molecules have proven efficacy for the treatment of rheumatoid arthritis, atopic dermatitis, cutaneous leishmaniasis, psoriasis and allergy [23,202].

AA is a relatively abundant FA in cells and it is a molecule that can act as a precursor of inflammatory mediators (e.g., prostaglandins, leukotrienes, thromboxanes). The COX/LOX inhibitor LP204A1 (AA analogue) readily crosses the BBB and prevents neuronal death in the penumbra area, in which inflammatory processes provoke the death of many neurons hours or days after a stroke [137,165,203].

5. Future directions and conclusions

In summary, MLT has emerged as a novel and innovative therapeutic concept that facilitates the design/discovery of new molecules. Molecules developed using this strategy target the membrane lipid boundary of cells and/or internal organelles, where many cellular functions occur. The development of such new drugs is aided by the identification of the factors regulating membrane lipid structures, and their roles in cell signaling and pathophysiological processes, and such information has allowed and will facilitate the design and discovery of novel molecules for the treatment of important diseases. This new knowledge may result in molecular modifications to improve the already demonstrated efficacy of MLT compounds or overcome certain limitations associated with their use.

The great potential of therapies that modulate the plasma membrane requires further contributions, such as new animal model of disease based on known lipid alterations, and the study of lipid alterations in existing animal models with the aim of evaluating MLT candidates. In addition, a deeper knowledge of membrane lipid structure and its effects on protein localization, protein–protein and –lipid interactions, cell signaling and pathophysiology, would be necessary to discover or design new membrane-interacting drugs. Given the current state of the art of this field, oncology, neurodegenerative disorders and cardiovascular diseases appear to be areas where treatments with drugs targeted at the membrane could be highly efficacious.

Acknowledgements

This work was supported in part by Grants BIO2013-49006 (Ministerio de Economía y Competitividad), Grups Competitius (Govern de les Illes Balears), the Marathon Foundation, by the Hungarian National Development Agency (TAMOP-4.1.1.C-13/1/KONV-2014-0001) and the Hungarian Basic Research Fund (OTKA, NK100857, NN111006, ANN112372). This work was also supported by research grants for a Grant-in-Aid for Scientific Research (B) and for Scientific Research on Innovative Areas (No. 23110002) from MEXT, Japan.

References

[1] Singer SJ, Nicolson GL. The fluid mosaic model of the structure of cell membranes. Science 1972;175:720–31.
[2] Escribá PV, González-Ros JM, Goñi FM, Kinnunen PK, Vigh L, Sánchez-Magraner L, et al. Membranes: a meeting point for lipids, proteins and therapies. J Cell Mol Med 2008;12:829–75.
[3] Mineo C, James GL, Smart EJ, Anderson RG. Localization of epidermal growth factor-stimulated Ras/Raf-1 interaction to caveolae membrane. J Biol Chem 1996;271:11930–5.
[4] Vögler O, Casas J, Capó D, Nagy T, Borchert G, Martorell G, et al. The Gβγ dimer drives the interaction of heterotrimeric Gi proteins with nonlamellar membrane structures. J Biol Chem 2004;279:36540–5.
[5] Escribá PV. Membrane-lipid therapy: a new approach in molecular medicine. Trends Mol Med 2006;12:34–43.
[6] Vigh L, Horváth I, Maresca B, Harwood JL. Can the stress protein response be controlled by "membrane-lipid therapy"? Trends Biochem Sci 2007;32:357–63.
[7] Escribá PV, Ozaita A, Ribas C, Miralles A, Fodor E, Farkas T, et al. Role of lipid polymorphism in G protein–membrane interactions: nonlamellar-prone phospholipids and peripheral protein binding to membranes. Proc Natl Acad Sci U S A 1997;94:11375–80.
[8] Yang Q, Alemany R, Casas J, Kitajka K, Lanier SM, Escribá PV. Influence of the membrane lipid structure on signal processing via G protein-coupled receptors. Mol Pharmacol 2005;68:210–7.
[9] Barceló F, Prades J, Encinar JA, Funari SS, Vögler O, González-Ros JM, et al. Interaction of the C-terminal region of the Gγ protein with model membranes. Biophys J 2007;93:2530–41.
[10] O'Connell KA, Dabkowski ER, de Fatima Galvao T, Xu W, Daneault C, de Rosiers C, et al. Dietary saturated fat and docosahexaenoic acid differentially effect cardiac mitochondrial phospholipid fatty acyl composition and Ca^{2+} uptake, without altering permeability transition or left ventricular function. Physiol Rep 2013;1:e00009.
[11] Gawrisch K, Soubias O, Mihailescu M. Insights from biophysical studies on the role of polyunsaturated fatty acids for function of G-protein coupled membrane receptors. Prostaglandins Leukot Essent Fatty Acids 2008;79:131–4.
[12] Michas G, Micha R, Zampelas A. Dietary fats and cardiovascular disease: putting together the pieces of a complicated puzzle. Atherosclerosis 2014;234:320–8.
[13] Funari S, Barceló F, Escribá PV. Effects of oleic acid and its congeners, elaidic and stearic acids, on the structural properties of phosphatidylethanolamine membranes. J Lipid Res 2003;44:567–75.
[14] Ibarguren M, López DJ, Escribá PV. The effect of natural and synthetic fatty acids on membrane structure, microdomain organization, cellular functions and human health. Biochim Biophys Acta 2014;1838:1518–28.
[15] Lindner R, Naim HY. Domains in biological membranes. Exp Cell Res 2009;315:2871–8.
[16] Ibarguren M, López DJ, Encinar JA, González-Ros JM, Busquets X, Escribá PV. Partitioning of Lo/Ld membrane microdomains induced by the fluidifying effect of 2-hydroxylated fatty acid derivatives: implications in their therapeutic molecular mechanisms of action. Biochim Biophys Acta 2013;1828:2553–63.
[17] Epand RM. Proteins and cholesterol-rich domains. Biochim Biophys Acta 2008;1778:1576–82.
[18] Mabrey S, Mateo PL, Sturtevant JM. High-sensitivity scanning calorimetric study of mixtures of cholesterol with dimyristoyl- and dipalmitoylphosphatidylcholines. Biochemistry 1978;17:2464–8.
[19] Pike LJ. Lipid rafts: bringing order to chaos. J Lipid Res 2003;44:655–67.
[20] Michel V, Bakovic M. Lipid rafts in health and disease. Biol Cell 2007;99:129–40.
[21] Marin R, Rojo JA, Fabelo N, Fernandez CE, Diaz M. Lipid raft disarrangement as a result of neuropathological progresses: a novel strategy for early diagnosis? Neuroscience 2013;245:26–39.
[22] Mollinedo F, de la Iglesia-Vicente J, Gajate C, Estella-Hermoso de Mendoza A, Villa-Pulgarin JA, Campanero MA, et al. Lipid raft-targeted therapy in multiple myeloma. Oncogene 2010;29:3748–57.
[23] Baumer W, Wlaz P, Jennings G, Rundfeldt C. The putative lipid raft modulator miltefosine displays immunomodulatory action in T-cell dependent dermal inflammation models. Eur J Pharmacol 2010;628:226–32.
[24] Dölle S, Hoser D, Rasche C, Loddenkemper C, Maurer M, Zuberbier T, et al. Long-term reduction in local inflammation by a lipid raft molecule in atopic dermatitis. Allergy 2010;65:1158–65.
[25] Prades J, Encinar JA, Funari SS, González-Ros JM, Escribá PV, Barceló F. Interaction of transmembrane-spanning segments of the α_2-adrenergic receptor with model membranes. Mol Membr Biol 2009;26:265–78.
[26] Ribas C, Penela P, Murga C, Salcedo A, García-Hoz C, Jurado-Pueyo M, et al. The G protein-coupled receptor kinase (GRK) interactome: role of GRKs in GPCR regulation and signaling. Biochim Biophys Acta 2007;1768:913–22.
[27] Escribá PV, Wedegaertner PB, Goñi FM, Vögler O. Lipid–protein interactions in GPCR associated signaling. Biochim Biophys Acta 2007;1768:836–52.
[28] Goñi FM, Alonso A. Structure and functional properties of diacylglycerols in membranes. Prog Lipid Res 1999;38:1–48.
[29] Escribá PV, Sastre M, García-Sevilla JA. Disruption of cellular signaling pathways by daunomycin through destabilization of nonlamellar membrane structures. Proc Natl Acad Sci U S A 1995;92:7595–9.
[30] Corbalán-García S, Gómez-Fernández JC. Protein kinase C regulatory domains: the art of decoding many different signals in membranes. Biochim Biophys Acta 2006;1761:633–54.
[31] Lee MH, Bell RM. Phospholipid functional groups involved in protein kinase C activation, phorbol ester binding, and binding to mixed micelles. J Biol Chem 1989;264:14797–805.
[32] Verdaguer N, Corbalan-Garcia S, Ochoa W, Fita I, Gomez-Fernandez JC. Ca^{2+} bridges the C2 membrane-binding domain of protein kinase Cα directly to phosphatidylserine. EMBO J 1999;18:6329–38.
[33] Ochoa WF, Corbalán-García S, Eritja R, Rodríguez-Alfaro JA, Gómez-Fernández JC, Fita I, et al. Additional binding sites for anionic phospholipids and calcium ions in the crystal structures of complexes of the C2 domain of protein kinase C. J Mol Biol 2002;320:277–91.
[34] Corbalán-García S, García-García J, Rodríguez-Alfaro J, Gomez-Fernandez J. A new phosphatidylinositol 4,5-bisphosphate binding site located in the C2 domain of protein kinase Cα. J Biol Chem 2003;278:4972–80.

Please cite this article in press as: Escribá PV et al. Membrane lipid therapy: Modulation of the cell membrane composition and structure as a molecular base for drug discovery and new disease treatment. Prog Lipid Res (2015), http://dx.doi.org/10.1016/j.plipres.2015.04.003

[35] Evans JH, Murray D, Leslie CC, Falke JJ. Specific translocation of protein kinase Cα to the plasma membrane requires both Ca^{2+} and PIP2 recognition by its C2 domain. Mol Biol Cell 2006;17:56–66.
[36] Balla T. Inositol-lipid binding motifs: signal integrators through protein–lipid and protein–protein interactions. J Cell Sci 2005;118:2093–104.
[37] Lodowski DT, Pitcher JA, Capel WD, Lefkowitz RJ, Tesmer JJ. Keeping G proteins at bay: a complex between G protein-coupled receptor kinase 2 and Gβγ. Science 2003;300:1256–62.
[38] Cozier GE, Carlton J, Bouyoucef D, Cullen PJ. Membrane targeting by pleckstrin homology domains. Curr Top Microbiol Immunol 2004;282:49–88.
[39] Burd CG, Emr SD. Phosphatidylinositol 3-phosphate signaling mediated by specific binding to RING FYVE domains. Mol Cell 1998;2:157–62.
[40] Barceló-Coblijn G, Martin ML, de Almeida RF, Noguera-Salvà MA, Marcilla-Etxenike A, Guardiola-Serrano F, et al. Sphingomyelin and sphingomyelin synthase (SMS) in the malignant transformation of glioma cells and in 2-hydroxyoleic acid therapy. Proc Natl Acad Sci U S A 2011;108:19569–74.
[41] Terés S, Lladó V, Higuera M, Barceló-Coblijn G, Martin ML, Noguera-Salvà MA, et al. 2-Hydroxyoleate, a nontoxic membrane binding anticancer drug, induces glioma cell differentiation and autophagy. Proc Natl Acad Sci U S A 2012;109:8489–94.
[42] Vígh L, Maresca B, Harwood JL. Does the membrane's physical state control the expression of heat shock and other genes? Trends Biochem Sci 1998;23:369–74.
[43] Vígh L, Escribá PV, Sonnleitner A, Sonnleitner M, Piotto S, Maresca B, et al. The significance of lipid composition for membrane activity: new concepts and ways of assessing function. Prog Lipid Res 2005;44:303–44.
[44] Vígh L, Török Z, Balogh G, Glatz A, Piotto S, Horváth I. Membrane regulated stress response: a theoretical and practical approach. Adv Exp Med Biol 2007;594:114–31.
[45] Horváth I, Multhoff G, Sonnleitner A, Vígh L. Membrane-associated stress proteins: more than simply chaperones. Biochim Biophys Acta 2008;1778:1653–64.
[46] Martin ML, Barceló-Coblijn G, de Almeida RF, Noguera-Salvà MA, Terés S, Higuera M, et al. The role of membrane fatty acid remodeling in the antitumor mechanism of action of 2-hydroxyoleic acid. Biochim Biophys Acta 2013;1828:1405–13.
[47] Lladó V, López DJ, Ibarguren M, Alonso M, Soriano JB, Escribá PV, et al. Regulation of the cancer cell membrane lipid composition by NaCHOleate: effects on cell signaling and therapeutical relevance in glioma. Biochim Biophys Acta 2014;1838:1619–27.
[48] Torres M, Price SL, Fiol-Deroque MA, Marcilla-Etxenike A, Ahyayauch H, Barceló-Coblijn G, et al. Membrane lipid modifications and therapeutic effects mediated by hydroxydocosahexaenoic acid on Alzheimer's disease. Biochim Biophys Acta 2014;1838:1680–92.
[49] Nicolson GL, Ash ME. Lipid replacement therapy: a natural medicine approach to replacing damaged lipids in cellular membranes and organelles and restoring function. Biochim Biophys Acta 2014;1838:1657–79.
[50] Cox AD, Fesik SW, Kimmelman AC, Luo J, Der CJ. Drugging the undruggable RAS: mission possible? Nat Rev Drug Discov 2014;13:828–51.
[51] Moreira RA, Mendanha SA, Hansen D, Alonso A. Interaction of miltefosine with the lipid and protein components of the erythrocyte membrane. J Pharm Sci 2013;102:1661–9.
[52] Escribá PV, Morales P, Smith A. Membrane phospholipid reorganization differentially regulates metallothionein and heme oxygenase by heme-hemopexin. DNA Cell Biol 2002;21:355–64.
[53] Hartl FU, Bracher A, Hayer-Hartl M. Molecular chaperones in protein folding and proteostasis. Nature 2011;475:324–32.
[54] Horváth I, Vígh L. Cell biology: stability in times of stress. Nature 2010;463:436–8.
[55] Kültz D. Molecular and evolutionary basis of the cellular stress response. Annu Rev Physiol 2005;67:225–57.
[56] Morimoto RI. Regulation of the heat shock transcriptional response: cross talk between a family of heat shock factors, molecular chaperones, and negative regulators. Genes Dev 1998;12:3788–96.
[57] Balogh G, Horváth I, Nagy E, Zs Hoyk, Benkő S, Bensaude O, et al. The hyperfluidization of mammalian cell membranes acts as a signal to initiate the heat shock protein response. FEBS J 2005;272:6077–86.
[58] Balogh G, Péter M, Liebisch G, Horváth I, Török Z, Nagy E, et al. Lipidomics reveals membrane lipid remodelling and release of potential lipid mediators during early stress responses in a murine melanoma cell line. Biochim Biophys Acta 2010;1801:1036–47.
[59] Balogh G, Maulucci G, Gombos I, Horváth I, Török Z, Péter M, et al. Heat stress causes spatially-distinct membrane re-modelling in K562 leukemia cells. PLoS One 2011;6:e21182.
[60] Brameshuber M, Weghuber J, Ruprecht V, Gombos I, Horváth I, Vígh L, et al. Imaging of mobile long-lived nanoplatforms in the live cell plasma membrane. J Biol Chem 2010;285:41765–71.
[61] Nagy E, Balogi Z, Gombos I, Akerfelt M, Björkbom A, Balogh G, et al. Hyperfluidization-coupled membrane microdomain reorganization is linked to activation of the heat shock response in a murine melanoma cell line. Proc Natl Acad Sci U S A 2007;104:7945–50.
[62] Péter M, Balogh G, Gombos I, Liebisch G, Horváth I, Török Z, et al. Nutritional lipid supply can control the heat shock response of B16 melanoma cells in culture. Mol Membr Biol 2012;29:274–89.
[63] Balogh G, Péter M, Glatz A, Gombos I, Török Z, Horváth I, et al. Key role of lipids in heat stress management. FEBS Lett 2013;587:1970–80.
[64] Moulin M, Carpentier S, Levade T, Arrigo A. Potential roles of membrane fluidity and ceramide in hyperthermia and alcohol stimulation of TRAIL apoptosis. Apoptosis 2007;12:1703–20.
[65] Chang Y, Abe A, Shayman JA. Ceramide formation during heat shock: a potential mediator of alpha B-crystallin transcription. Proc Natl Acad Sci U S A 1995;92:12275–9.
[66] Grassmé H, Riethmüller J, Gulbins E. Biological aspects of ceramide-enriched membrane domains. Prog Lipid Res 2007;46:161–70.
[67] Gulbins E, Kolesnick R. Raft ceramide in molecular medicine. Oncogene 2003;22:7070–7.
[68] Patra SK. Dissecting lipid raft facilitated cell signaling pathways in cancer. Biochim Biophys Acta 2008;1785:182–206.
[69] Mathieson FA, Nixon GF. Sphingolipids differentially regulate mitogen-activated protein kinases and intracellular Ca^{2+} in vascular smooth muscle: effects on CREB activation. Br J Pharmacol 2006;147:351–9.
[70] Kozawa O, Niwa M, Matsuno H, Tokuda H, Miwa M, Ito H, et al. Sphingosine 1-phosphate induces heat shock protein 27 via p38 mitogen-activated protein kinase activation in osteoblasts. J Bone Miner Res 1999;14:1761–7.
[71] Kozawa O, Tanabe K, Ito H, Matsuno H, Niwa M, Kato K, et al. Sphingosine 1-phosphate regulates heat shock protein 27 induction by a p38 MAP kinase-dependent mechanism in aortic smooth muscle cells. Exp Cell Res 1999;250:376–80.
[72] Takai S, Tokuda H, Matsushima-Nishiwaki R, Hanai Y, Kato K, Kozawa O. Phosphatidylinositol 3-kinase/Akt plays a role in sphingosine 1-phosphate-stimulated HSP27 induction in osteoblasts. J Cell Biochem 2006;98:1249–56.
[73] Kunimoto S, Kobayashi T, Kobayashi S, Murakami-Murofushi K. Expression of cholesteryl glucoside by heat shock in human fibroblasts. Cell Stress Chaperones 2000;5:3–7.
[74] Kunimoto S, Murofushi W, Kai H, Ishida Y, Uchiyama A, Kobayashi T, et al. Steryl glucoside is a lipid mediator in stress-responsive signal transduction. Cell Struct Funct 2002;27:157–62.
[75] Shigenaga MK, Hagen TM, Ames BN. Oxidative damage and mitochondrial decay in aging. Proc Natl Acad Sci U S A 1994;91:10771–8.
[76] Nicolson GL, Ellithrope R. Lipid replacement and antioxidant nutritional therapy for restoring mitochondrial function and reducing fatigue in chronic fatigue syndrome and other fatiguing illnesses. J Chron Fatigue Syndr 2006;13:57–68.
[77] Sparagna GC, Lesnefsky EJ. Cardiolipin remodeling in the heart. J Cardiovasc Pharmacol 2009;53:209–301.
[78] Furuya H, Shimizu Y, Kawamori T. Sphingolipids in cancer. Cancer Metastasis Rev 2011;30:567–76.
[79] Gombos I, Crul T, Piotto S, Güngör B, Török Z, Balogh G, et al. Membrane-lipid therapy in operation: The HSP co-inducer BGP-15 activates stress signal transduction pathways by remodeling plasma membrane rafts. PLoS One 2011;6:e28818.
[80] Csoboz B, Balogh GE, Kusz E, Gombos I, Péter M, Crul T, et al. Membrane fluidity matters: hyperthermia from the aspects of lipids and membranes. Int J Hyperthermia 2013;29:491–9.
[81] Török Z, Tsvetkova NM, Balogh G, Horváth I, Nagy E, Pénzes Z, et al. Heat shock protein coinducers with no effect on protein denaturation specifically modulate the membrane lipid phase. Proc Natl Acad Sci U S A 2003;100:3131–6.
[82] Török Z, Crul T, Maresca B, Schütz GJ, Viana F, Dindia L, et al. Plasma membranes as heat stress sensors: from lipid-controlled molecular switches to therapeutic applications. Biochim Biophys Acta 2014;1838:1594–618.
[83] Crul T, Tóth N, Török Z, Balogh G, Gombos I, Gallyas F, et al. Hydroximic acid derivatives: pleiotrophic Hsp co-inducers restoring homeostasis and robustness. Curr Pharm Des 2013;19:309–46.
[84] Irvine RF. Nuclear lipid signalling. Nat Rev Mol Cell Biol 2003;4:349–60.
[85] Ledeen RW, Wu G. Nuclear sphingolipids: metabolism and signaling. J Lipid Res 2008;49:1176–86.
[86] Albi E, Lazzarini A, Lazzarini R, Floridi A, Damaskopoulou E, Curcio F, et al. Nucler lipid microdomain as place of interaction between sphingomyelin and DNA during liver regeneration. Int J Mol Sci 2013;14:6529–41.
[87] Weiss K, Mihály J, Liebisch G, Marosvölgyi T, Schmitz G, Decsi T, et al. Effect of synthetic ligands of PPAR α, β/δ, γ, RAR, RXR and LXR on the fatty acid composition of phospholipids in mice. Lipids 2011;46:1013–20.
[88] Lipinski CA. Lead- and drug-like compounds: the rule-of-five revolution. Drug Discov Today Technol 2004;1:337–41.
[89] del Pozo MA, Alderson NB, Kiosses WB, Chiang HH, Anderson RG, Schwarth MA. Integrins regulate Rac targeting by internalization of membrane domains. Science 2004;303:839–42.
[90] Han SI, Oh SY, Woo SH, Kim KH, Kim J-H, Kim HD, et al. Implication of a small GTPase Rac1 in the activation of c-jun N-terminal kinase and heat shock factor in response to heat shock. J Biol Chem 2001;276:1889–95.
[91] Gungor B, Gombos I, Crul T, Ayaydin F, Szabó L, Török Z, et al. Rac1 participates in thermally induced alterations of the cytoskeleton, cell morphology and lipid rafts, and regulates the expression of heat shock proteins in B16F10 melanoma cells. PLoS One 2014;9:e89136.
[92] Calderwood SK, Stevenson MA, Hahn GM. Heat stress stimulates inositol trisphosphate release and phosphorylation of phosphoinositides in CHO and Balb C 3T3 cells. J Cell Physiol 1987;130:369–76.
[93] Kiang JG, Tsokos GC. Heat shock protein 70 kDa: molecular biology, biochemistry and physiology. Pharmacol Ther 1998;80:183–201.
[94] Price BD, Calderwood SK. Ca^{2+} is essential for multistep activation of the heat shock factor in permeabilized cells. Mol Cell Biol 1991;11:3365–8.

Please cite this article in press as: Escribá PV et al. Membrane lipid therapy: Modulation of the cell membrane composition and structure as a molecular base for drug discovery and new disease treatment. Prog Lipid Res (2015), http://dx.doi.org/10.1016/j.plipres.2015.04.003

[95] Kiang JG, Koenig ML, Smallridge RC. Heat shock increases cytosolic free Ca^{2+} concentration via Na^{+}–Ca^{2+} exchange in human epidermoid A 431 cells. Am J Physiol 1992;263(1 Pt 1):C30–8.
[96] Holmberg CI, Hietakangas V, Mikhailov A, Rantanen JO, Kallio M, Meinander A, et al. Phosphorylation of serine 230 promotes inducible transcriptional activity of heat shock factor 1. EMBO J 2001;20:3800–10.
[97] Jurivich DA, Sistonen L, Sarge KD, Morimoto RI. Arachidonate is a potent modulator of human heat shock gene transcription. Proc Natl Acad Sci U S A 1994;91:2280–4.
[98] Jurivich DA, Pangas S, Qiu L, Welk JF. Phospholipase A2 triggers the first phase of the thermal stress response and exhibits cell-type specificity. J Immunol 1996;157:1669–77.
[99] van Rossum DB, Patterson RL. PKC and PLA2: probing the complexities of the calcium network. Cell Calcium 2009;45:535–45.
[100] Huang ZH, Hii CS, Rathjen DA, Poulos A, Murray AW, Ferrante A. N-6 and n-3 polyunsaturated fatty acids stimulate translocation of protein kinase Ca, -bI, -bII and -e and enhance agonist-induced NADPH oxidase in macrophages. Biochem J 1997;325:553–7.
[101] Martínez J, Vögler O, Casas J, Barceló F, Alemany R, Prades J, et al. Membrane structure modulation, protein kinase C alpha activation, and anticancer activity of minerval. Mol Pharmacol 2005;67:531–40.
[102] O'Flaherty JT, Chadwell BA, Kearns MW, Sergeant S, Daniel LW. Protein kinases C translocation responses to low concentrations of arachidonic acid. J Biol Chem 2001;276:24743–50.
[103] Bhalla US, Iyengar R. Emergent properties of networks of biological signaling pathways. Science 1999;283:381–7.
[104] Santoro MG. Heat shock factors and the control of the stress response. Biochem Pharmacol 2000;59:55–63.
[105] Bunney TD, Katan M. Phosphoinositide signalling in cancer: beyond PI3K and PTEN. Nat Rev Cancer 2010;10:342–52.
[106] Jorquera G, Juretić N, Jaimovich E, Riveros N. Membrane depolarization induces calcium-dependent upregulation of Hsp70 and Hmox-1 in skeletal muscle cells. Am J Physiol Cell Physiol 2009;297:C581–90.
[107] Inokuchi J. Membrane microdomains and insulin resistance. FEBS Lett 2010;584:1864–71.
[108] Inokuchi J. Physiopathological function of hematoside (GM3 ganglioside). Proc Jpn Acad Ser B Phys Biol Sci 2011;87:179–98.
[109] Bickel PE. Lipid rafts and insulin signaling. Am J Physiol Endocrinol Metab 2002;282:E1–E10.
[110] Cohen AW, Combs TP, Scherer PE, Lisanti MP. Role of caveolin and caveolae in insulin signaling and diabetes. Am J Physiol Endocrinol Metab 2003;285:E1151–60.
[111] Hakomori SI. Cell adhesion/recognition and signal transduction through glycosphingolipid microdomain. Glycoconj J 2000;17:143–51.
[112] Simons K, Toomre D. Lipid rafts and signal transduction. Nat Rev Mol Cell Biol 2000;1:31–9.
[113] Covic L, Gresser AL, Talavera J, Swift S, Kuliopulos A. Activation and inhibition of G protein-coupled receptors by cell-penetrating membrane-tethered peptides. Proc Natl Acad Sci U S A 2002;99:643–8.
[114] Tchernychev B, Ren Y, Sachdev P, Janz JM, Haggis L, O'Shea A, et al. Discovery of a CXCR4 agonist pepducin that mobilizes bone marrow hematopoietic cells. Proc Natl Acad Sci U S A 2010;107:22255–9.
[115] Lladó V, Gutierrez A, Martínez J, Casas J, Terés S, Higuera M, et al. Minerval induces apoptosis in Jurkat and other cancer cells. J Cell Mol Med 2010;14:659–70.
[116] Widemann BC, Arceci RJ, Jayaprakash N, Fox E, Zannikos P, Goodspeed W, et al. Phase 1 trial and pharmacokinetic study of the farnesyl transferase inhibitor tipifarnib in children and adolescents with refractory leukemias: a report from the Children's Oncology Group. Pediatr Blood Cancer 2011;56:226–33.
[117] Triton TR, Yee G. The anticancer agent Adriamycin can be actively cytotoxic without entering cells. Science 1982;217:248–50.
[118] Siddiqui RA, Zerouga M, Wu M, Castillo A, Harvey K, Zaloga GP, et al. Anticancer properties of propofol-docosahexanoate and propofol-eicosapentanoate on breast cancer cells. Breast Cancer Res 2005;7:R645–54.
[119] Harvey K, Xu Z, Whitley P, Davisson VJ, Siddiqui RA. Characterization of anticancer properties of 2,6-diisopropylphenol-docosahexaenoate and analogues in breast cancer cells. Bioorg Med Chem 2010;18:1866–74.
[120] Mollinedo F, de la Iglesia-Vicente J, Gajate C, Estella-Hermoso de Mendoza A, Villa-Pulgarin JA, Campanero MA, et al. Lipid raft-targeted therapy in multiple myeloma. Oncogene 2010;29:3748–57.
[121] Kuhajda FP, Jenner K, Wood FD, Hennigar RA, Jacobs LB, Dick JD, et al. Fatty acid synthesis: a potential selective target for antineoplastic therapy. Proc Natl Acad Sci U S A 1994;91:6379–83.
[122] De Schrijver E, Brusselmans K, Heyns W, Verhoeven G, Swinnen JV. RNA interference-mediated silencing of the fatty acid synthase gene attenuates growth and induces morphological changes and apoptosis of LNCaP prostate cancer cells. Cancer Res 2003;63:3799–804.
[123] Menendez JA, Vellon L, Lupu R. Antitumoral actions of the anti-obesity drug orlistat (Xenical™) in breast cancer cells: blockade of cell cycle progression, promotion of apoptotic cell death and PEA3-mediated transcriptional repression of Her2/neu (erbB-2) oncogene. Ann Oncol 2005;16:1253–67.
[124] Hirsch HA, Iliopoulos D, Joshi A, Zhang Y, Jaeger SA, Bulyk M, et al. A transcriptional signature and common gene networks link cancer with lipid metabolism and diverse human diseases. Cancer Cell 2010;17:348–61.
[125] Hilvo M, Denkert C, Lehtinen L, Müller B, Brockmöller S, Seppänen-Laakso T, et al. Novel theranostic opportunities offered by characterization of altered membrane lipid metabolism in breast cancer progression. Cancer Res 2011;71:3236–45.
[126] Crous-Bou M, Rennert G, Salazar R, Rodriguez-Moranta F, Rennert HS, Lejbkowicz F, et al. Genetic polymorphisms in fatty acid metabolism genes and colorectal cancer. Mutagenesis 2012;27:169–76.
[127] Ying H, Lv J, Ying T, Jin S, Shao J, Wang L, et al. Gene–gene interaction network analysis of ovarian cancer using TCGA data. J Ovarian Res 2013;6:88.
[128] REMBRANDT Homepage. Institute, N. C., <http://rembrandt.nci.nih.gov2005> [accessed Dec 15th 2014].
[129] Madhavan S, Zenklusen JC, Kotliarov Y, Sahni H, Fine HA, Buetow K. Rembrandt: helping personalized medicine become a reality through integrative translational research. Mol Cancer Res 2009;7:157–67.
[130] López-Miranda J, Pérez-Jiménez F, Ros E, De Caterina R, Badimón L, Covas MI, et al. Olive oil and health: summary of the II international conference on olive oil and health consensus report, Jaén and Córdoba (Spain) 2008. Nutr Metab Cardiovasc Dis 2008;20:284–94.
[131] Vögler O, López-Bellan A, Alemany R, Tofé S, González M, Quevedo J, et al. Structure–effect relation of C18 long-chain fatty acids in the reduction of body weight in rats. Int J Obes 2008;32:464–73.
[132] Alemany R, Terés S, Baamonde C, Benet M, Vögler O, Escribá PV. 2-Hydroxyoleic acid: a new hypotensive molecule. Hypertension 2004;43:249–54.
[133] Marcilla-Etxenike A, Martín ML, Noguera-Salvà MA, García-Verdugo JM, Soriano-Navarro M, Dey I, et al. 2-Hydroxyoleic acid induces ER stress and autophagy in various human glioma cell lines. PLoS One 2012;7:e48235.
[134] Torgerson J, Hauptman J, Boldrin MN, Sjöström L. XENical in the prevention of diabetes in obese subjects (XENDOS) study: a randomized study of orlistat as an adjunct to lifestyle changes for the prevention of type 2 diabetes in obese patients. Diabetes Care 2004;27:155–61.
[135] Sloan FA, Bethel MA, Ruiz Jr D, Shea AM, Feinglos MN. The growing burden of diabetes mellitus in the US elderly population. Arch Intern Med 2008;168:192–9.
[136] Perona JS, Vögler O, Sánchez-Domínguez JM, Montero E, Escribá PV, Ruiz-Gutierrez V. Consumption of virgin olive oil influences membrane lipid composition and regulates intracellular signaling in elderly adults with type 2 diabetes mellitus. J Gerontol A Biol Sci Med Sci 2007;62:256–63.
[137] Escribá PV, Busquets X, Terés S, Barceló-Coblijn G, LLadó V, Marcilla-Etxenike A, et al. Use of derivatives of polyunsaturated fatty acids as medicaments. European patent 2012/EP2409963(A1).
[138] Chung J, Nguyen AK, Henstridge DC, Holmes AG, Chan MH, Mesa JL, et al. HSP72 protects against obesity-induced insulin resistance. Proc Natl Acad Sci U S A 2008;105:1739–44.
[139] Hooper PL, Hooper JJ. Loss of defense against stress: diabetes and heat shock proteins. Diabetes Technol Ther 2005;7:204–8.
[140] Vígh L, Literáti PN, Horváth I, Török Z, Balogh G, Glatz A, et al. Bimoclomol: a nontoxic, hydroxylamine derivative with stress protein-inducing activity and cytoprotective effects. Nat Med 1997;3:1150–4.
[141] Tagami S, Inokuchi Ji J, Kabayama K, Yoshimura H, Kitamura F, Uemura S, et al. Ganglioside GM3 participates in the pathological conditions of insulin resistance. J Biol Chem 2002;277:3085–92.
[142] Kabayama K, Sato T, Kitamura F, Uemura S, Kang BW, Igarashi Y, et al. TNFalpha-induced insulin resistance in adipocytes as a membrane microdomain disorder: involvement of ganglioside GM3. Glycobiology 2005;15:21–9.
[143] Yamashita T, Hashiramoto A, Haluzik M, Mizukami H, Beck S, Norton A, et al. Enhanced insulin sensitivity in mice lacking ganglioside GM3. Proc Natl Acad Sci U S A 2003;100:3445–9.
[144] Kabayama K, Sato T, Saito K, Loberto N, Prinetti A, Sonnino S, et al. Dissociation of the insulin receptor and caveolin-1 complex by ganglioside GM3 in the state of insulin resistance. Proc Natl Acad Sci U S A 2007;104:13678–83.
[145] Couet J, Li S, Okamoto T, Ikezu T, Lisanti MP. Identification of peptide and protein ligands for the caveolin-scaffolding domain. Implications for the interaction of caveolin with caveolae-associated proteins. J Biol Chem 1997;272:6525–33.
[146] Inokuchi J, Kabayama K. Modulation of growth factor receptors in membrane microdomains. Trends Glycosci Glycotech 2008;20:353–71.
[147] Lewis SJ. Lipid lowering therapy: Who can benefit? Vasc Health Risk Manag 2010;7:525–34.
[148] News in Brief, End of the Lipitor era. Nat Rev Drug Discov 2011;10:889.
[149] Hoeckstra D, Maier O, van der Wouden JM, Slimane TA, van Jzendoorn SC. Membrane dynamics and cell polarity: the role of sphingolipids. J Lipid Res 2003;44:869–77.
[150] Neal RC, Jones PH. Complementary therapy to target LDL cholesterol: the role of the ezetimibe/simvastatin combination. Vasc Health Risk Manag 2006;2:31–8.
[151] Trapani L, Segatto M, Pallottini V. Regulation and deregulation of cholesterol homeostasis: the liver as a metabolic "power station". World J Hepatol 2012;4:184–90.
[152] Albertini KG, Zimmet P, Shaw J. Metabolic syndrome. A new worldwide definition. A consensus statement from the international Diabetes Federation. Diabet Med 2006;23:469–80.
[153] Kremmyda LS, Tvrzicka E, Stankova B, Zak A. Fatty acids as biocompounds: their role in human metabolism, health and disease. A review. Part 2: fatty

Please cite this article in press as: Escribá PV et al. Membrane lipid therapy: Modulation of the cell membrane composition and structure as a molecular base for drug discovery and new disease treatment. Prog Lipid Res (2015), http://dx.doi.org/10.1016/j.plipres.2015.04.003

acid physiological roles and applications. Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub 2011;155:195–218.
[154] Alemany R, Vögler O, Terés S, Egea C, Baamonde C, Barceló F, et al. Antihypertensive action of 2-hydroxyoleic acid in SHRs via modulation of the protein kinase A pathway and Rho kinase. J Lipid Res 2006;47:1762–70.
[155] Escribá PV, Xaubet XB, Barcelo-Coblijn G, Canellas VL, Martinez RA, Jiménez ST, et al. Alpha-derivatives of cis-monounsaturated fatty acids for use as medicines. Patent US 2011/0294883 A1.
[156] Escribá PV, Sánchez-Dominguez JM, Alemany R, Perona JS, Ruiz-Gutiérrez V. Alteration of lipids, G proteins, and PKC in cell membranes of elderly hypertensives. Hypertension 2003;41:176–82.
[157] Terés S, Barceló-Coblijn G, Benet M, Alvarez R, Bressani R, Halver JE, et al. Oleic acid content is responsible for the reduction in blood pressure induced by olive oil. Proc Natl Acad Sci U S A 2008;105:13811–6.
[158] Liu JC, Conklin SM, Manuck SB, Yao JK, Muldoon MF. Long-chain omega-3 fatty acids and blood pressure. Am J Hypertens 2011;24:1121–6.
[159] Begg DP, Sinclair AJ, Stahl LA, Premaratna SD, Hafandi A, Jois M, et al. Hypertension induced by omega-3 polyunsaturated fatty acid deficiency is alleviated by alpha-linolenic acid regardless of dietary source. Hypertens Res 2010;33:808–13.
[160] Mori TA. Omega-3 fatty acids and hypertension in humans. Clin Exp Pharmacol Physiol 2006;33:842–6.
[161] Calder PC, Yagoob P. Omega-3 polyunsaturated fatty acids and human health outcomes. BioFactors 2009;35:266–72.
[162] Wassall SR, Stillwell W. Polyunsaturated fatty acid–cholesterol interactions: domain formation in membranes. Biochim Biophys Acta 2009;1788:24–32.
[163] Gawrisch K, Soubias O, Mihailescu M. Insights from biophysical studies on the role of polyunsaturated fatty acids for function of G-protein coupled membrane receptors. Prostaglandins Leukot Essent Fatty Acids 2008;79:131–4.
[164] Borchert GH, Giggey M, Kolar F, Wong TM, Backx PH, Escirá PV. 2-Hydroxyoleic acid affects cardiomyocyte $[Ca^{2+}]_i$ transient and contractility in a region-dependent manner. Am J Physiol Heart Circ Physiol 2008;294:H1948–55.
[165] Lopez DH, Fiol-deRoque MA, Noguera-Salvà MA, Terés S, Campana F, Piotto S, et al. 2-Hydroxy arachidonic acid: a new non-steroidal anti-inflammatory drug. PLoS One 2013;8:e72052.
[166] Zhang D, Ke L, Mackovicova K, Van Der Want JJ, Sibon OC, Tanguay RM, et al. Effects of different small HSPB members on contractile dysfunction and structural changes in a *Drosophila melanogaster* model for Atrial Fibrillation. J Mol Cell Cardiol 2011;51:381–9.
[167] Schaeffer EL, Figueiro M, Gattaz WF. Insights into Alzheimer disease pathogenesis from studies in transgenic animal models. Clinics 2011;66:45–54.
[168] Zhang C, McNeil E, Dressler L, Siman R. Long-lasting impairment in hippocampal neurogenesis associated with amyloid deposition in a knock-in mouse model of familial Alzheimer's disease. Exp Neurol 2007;204:77–87.
[169] Söderberg M, Edlund C, Kristensson K, Dallner G. Fatty acid composition of brain phospholipids in aging and in Alzheimer's disease. Lipids 1991;26:421–5.
[170] Favrelère S, Stadelmann-Ingrand S, Huguet F, De Javel D, Piriou A, Tallineau C, et al. Age-related changes in ethanolamine glycerophospholipid fatty acid levels in rat frontal cortex and hippocampus. Neurobiol Aging 2000;21:653–60.
[171] Hashimoto M, Hossain S. Neuroprotective and ameliorative actions of polyunsaturated fatty acids against neuronal diseases: beneficial effects of docosahexaenoic acid on cognitive decline in Alzheimer's disease. J Pharmacol Sci 2011;116:150–62.
[172] Fiol-deRoque MA, Gutierrez-Lanza R, Terés S, Torres M, Barceló P, Rial RV, et al. Cognitive recovery and restoration of cell proliferation in the dentate gyrus in the 5XFAD transgenic mice model of Alzheimer's disease following 2-hydroxy-DHA treatment. Biogerontology 2013;14:763–75.
[173] Grziwa B, Grimm MO, Masters CL, Beyreuther K, Hartmann T, Lichtenthaler SF. The transmembrane domain of the amyloid precursor protein in microsomal membranes is on both sides shorter than predicted. J Biol Chem 2003;278:6803–8.
[174] Simons M, Keller P, De Strooper B, Beyreuther K, Dotti CG, Simons K. Cholesterol depletion inhibits the generation of beta-amyloid in hippocampal neurons. Proc Natl Acad Sci U S A 1998;95:6460–4.
[175] Fassbender K, Simons M, Bergmann C, Stroick M, Lutjohann D, Keller P, et al. Simvastatin strongly reduces levels of Alzheimer's disease beta-amyloid peptides Aβ 42 and Abeta 40 in vitro and in vivo. Proc Natl Acad Sci U S A 2001;98:5856–61.
[176] Simons M, Schwärzler F, Lütjohann D, von Bergmann K, Beyreuther K, Dichgans J, et al. Treatment with simvastatin in normocholesterolemic patients with Alzheimer's disease: a 26-week randomized, placebo controlled, double-blind trial. Ann Neurol 2002;52:346–50.
[177] Avila-Martin G, Galán-Arriero I, Gómez-Soriano J, Taylor J. Treatment of rat spinal cord injury with the neurotrophic factor albumin-oleic acid: translational application for paralysis, spasticity and pain. PLoS One 2011;6:e26107.
[178] Avila-Martin G, Galan-Arriero I, Ferrer-Donato A, Busquets X, Gomez-Soriano J, Escribá PV, et al. Oral 2-hydroxyoleic acid inhibits reflex hypersensitivity and open-field induced anxiety after spared nerve injury. Eur J Pain 2015;19:111–22.
[179] Kieran D, Kalmar B, Dick JR, Riddoch-Contreras J, Burnstock G, Greensmith L. Treatment with arimoclomol, a coinducer of heat shock proteins, delays disease progression in ALS mice. Nat Med 2004;10:402–5.
[180] Haldimann P, Muriset M, Vígh L, Goloubinoff P. The novel hydroxylamine derivative NG-094 suppresses polyglutamine protein toxicity in *Caenorhabditis elegans*. J Biol Chem 2011;286:18784–94.
[181] Gehrig SM, van der Poel C, Sayer TA, Schertzer JD, Henstridge DC, Church JE, et al. Hsp72 preserves muscle function and slows progression of severe muscular dystrophy. Nature 2012;484:394–8.
[182] Condray R, Yao JK. Cognition, dopamine and bioactive lipids in schizophrenia. Front Biosci 2011;1:298–330.
[183] Balanzá-Martínez V, Fries GR, Colpo GD, Silveira PP, Portella AK, Tabarés-Seisdedos R, et al. Therapeutic use of omega-3 fatty acids in bipolar disorder. Expert Rev Neurother 2011;11:1029–47.
[184] Bíró K, Pálhalmi J, Tóth AJ, Kukorelli T, Juhász G. Bimoclomol improves early electrophysiological signs of retinopathy in diabetic rats. NeuroReport 1998;9:2029–33.
[185] Racz I, Tory K, Gallyas F, et al. BGP-15 – a novel poly(ADPribose) polymerase inhibitor – protects against nephrotoxicity of cisplatin without compromising its antitumor activity. Biochem Pharmacol 2002;63:1099–111.
[186] Vígh L, Literáti PN, Horváth I, et al. Bimoclomol: a nontoxic, hydroxylamine derivative with stress protein-inducing activity and cytoprotective effects. Nat Med 1997;3:1150–4.
[187] Nagy G, Szarka A, Lotz G, et al. BGP-15 inhibits caspase independent programmed cell death in acetaminophen-induced liver injury. Toxicol Appl Pharmacol 2010;243:96–103.
[188] Bárdos G, Móricz K, Jaszlits L, et al. BGP-15, a hydroximic acid derivative, protects against cisplatin- or taxol-induced peripheral neuropathy in rats. Toxicol Appl Pharmacol 2003;190:9–16.
[189] Erdö F, Erdö SL. Bimoclomol protects against vascular consequences of experimental subarachnoid hemorrhage in rats. Brain Res Bull 1998;45:163–6.
[190] Sapra G, Tham YK, Cemerlang N, Matsumoto A, Kiriazis H, Bernardo BC, et al. Small-molecule BGP-15 protects against heart failure and atrial fibrillation in mice. Nat Commun 2014;5:5705. http://dx.doi.org/10.1038/ncomms6705.
[191] Farkas B, Magyarlaki M, Csete B, et al. Reduction of acute photodamage in skin by topical application of a novel PARP inhibitor. Biochem Pharmacol 2002;63:921–32.
[192] Szabados E, Literati-Nagy P, Farkas B, Sumegi B. BGP-15, a nicotinic amidoxime derivate protecting heart from ischemia reperfusion injury through modulation of poly(ADP-ribose) polymerase. Biochem Pharmacol 2000;59:937–45.
[193] Jednákovits A, Kurucz I, Nánási PP. Effect of subchronic bimoclomol treatment on vascular responsiveness and heat shock protein production in spontaneously hypertensive rats. Life Sci 2000;67:1791–7.
[194] Lubbers NL, Polakowski JS, Wegner CD, et al. Oral bimoclomol elevates heat shock protein 70 and reduces myocardial infarct size in rats. Eur J Pharmacol 2002;435:79–83.
[195] Stacchiotti A, Borsani E, Ricci F, et al. Bimoclomol ameliorates mercuric chloride nephrotoxicity through recruitment of stress proteins. Toxicol Lett 2006;166:168–77.
[196] Xu K, Sun X, Erokwu BO, Cernak I, Lamanna JC. A heat-shock protein co-inducer treatment improves behavioral performance in rats exposed to hypoxia. Adv Exp Med Biol 2011;701:313–8.
[197] Rakonczay Z, Iványi B, Varga I, et al. Nontoxic heat shock protein coinducer BRX-220 protects against acute pancreatitis in rats. Free Radic Biol Med 2002;32:1283–92.
[198] Toth ME, Gonda S, Vigh L, Santha M. Neuroprotective effect of small heat shock protein, Hsp27, after acute and chronic alcohol administration. Cell Stress Chaperones 2010;15:807–17.
[199] Teixeira V, Feio MJ, Bastos M. Role of lipids in the interaction of antimicrobial peptides with membranes. Prog Lipid Res 2012;51:149–77.
[200] Kim JK, Lee SA, Shin S, Lee JY, Jeong KW, Nan YH, et al. Structural flexibility and the positive charges are the key factors in bacterial cell selectivity and membrane penetration of peptoid-substituted analog of Piscidin 1. Biochim Biophys Acta 2010;1798:1913–25.
[201] Varela-M RE, Villa-Pulgarin JA, Yepes E, Müller I, Modolell M, Muñoz DL, et al. In vitro and in vivo efficacy of ether lipid edelfosine against Leishmania spp. and SbV-resistant parasites. PLoS Negl Trop Dis 2012;6:e1612.
[202] Maurer M, Magerl M, Metz M, Weller K, Siebenhaar F. Miltefosine: a novel treatment option for mast cell-mediated disease. J Dermatolog Treat 2013;24:244–9.
[203] Luheshi NM, Kovács KJ, Lopez-Castejón G, Brough D, Denes A. Interleukin-1alfa expression precedes IL-1beta after ischemic brain injury and is localised to areas of focal neuronal loss and penumbral tissues. J Neuroinflammation 2011;8:186.
[204] van Meer G, de Kroon AI. Lipid map of the mammalian cell. J Cell Sci 2011;124:5–8.
[205] van Meer G, Voelker DR, Feigenson GW. Membrane lipids: where they are and how they behave. Nat Rev Mol Cell Biol 2008;9:112–24.
[206] Horvath SE, Daum G. Lipids of mitochondria. Prog Lipid Res 2013;52:590–614.
[207] Griffiths G, Back R, Marsh M. A quantitative analysis of the endocytic pathway in baby hamster kidney cells. J Cell Biol 1989;109:2703–20.
[208] Rask-Madsen C, Kahn CR. Tissue-specific insulin signaling, metabolic syndrome and cardiovascular disease. Arterioscler Thromb Vasc Biol 2012;32:2052–9.

Please cite this article in press as: Escribá PV et al. Membrane lipid therapy: Modulation of the cell membrane composition and structure as a molecular base for drug discovery and new disease treatment. Prog Lipid Res (2015), http://dx.doi.org/10.1016/j.plipres.2015.04.003