Published in final edited form as:

Cell Signal. 2017 October ; 38: 134–145. doi:10.1016/j.cellsig.2017.06.017.

Sphingolipid Abnormalities in Cancer Multidrug Resistance: Chicken or Egg?

Wing-Kee Lee^{1,2} and Richard N. Kolesnick¹

¹Laboratory of Signal Transduction, Sloan Kettering Institute, Memorial Sloan-Kettering Cancer Center, New York

²Institute for Physiology, Pathophysiology and Toxicology, Centre for Biomedical Research and Training (ZBAF), University of Witten/Herdecke, Witten, Germany

Abstract

The cancer multidrug resistance (MDR) phenotype encompasses a myriad of molecular, genetic and cellular alterations resulting from progressive oncogenic transformation and selection. Drug efflux transporters, in particular the MDR P-glycoprotein ABCB1, play an important role in MDR but cannot confer the complete phenotype alone indicating parallel alterations are prerequisite. Sphingolipids are essential constituents of lipid raft domains and directly participate in functionalization of transmembrane proteins, including providing an optimal lipid microenvironment for multidrug transporters, and are also perturbed in cancer. Here we postulate that increased sphingomyelin content, developing early in some cancers, recruits and functionalizes plasma membrane ABCB1 conferring a state of partial MDR, which is completed by glycosphingolipid disturbance and the appearance of intracellular vesicular ABCB1. In this review, the independent and interdependent roles of sphingolipid alterations and ABCB1 upregulation during the transformation process and resultant conferment of partial and complete MDR phenotypes are discussed.

Keywords

drug resistance; chemotherapy; neoplastic progression; glucosylceramide; lipid rafts; sphingomyelin

1. Introduction

Resistance to chemotherapy remains a hurdle for effective treatment of cancers exhibiting the multidrug resistance (MDR)¹ phenotype, which results from upregulation of broad-range

To whom correspondence should be addressed: Wing-Kee Lee PhD, Institute for Physiology, Pathophysiology and Toxicology, Centre for Biomedical Research and Training (ZBAF), University of Witten/Herdecke, Witten, Germany, Tel: +49 2302 926309, wing-kee.lee@uni-wh.de.

Declaration of Interest: The authors declare no conflicts of interest.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

drug transporters, such as the MDR P-glycoprotein ABCB1. High expression of ABCB1 in tumors is associated with increased cell survival due to efficient extrusion of chemotherapeutic drugs, evasion of apoptosis, as well as increased metastatic potential, resulting in poor prognosis [1-3]. Though drug extrusion is clearly a major contributor to MDR when it is fully developed, the role of drug transporters during the transformation process of normal cells to a tumorigenic phenotype has yet to be clarified. In addition to drug extrusion, MDR transporters can efflux a number of physiologic substrates that might contribute to the MDR phenotype or to development of MDR. A large body of evidence demonstrates that lipids, such as cholesterol, are extruded by MDR transporters to regulate membrane lipid asymmetry, membrane lipid composition, and lipid-mediated signal transduction. Of particular importance is the family of sphingolipids, of which some members are substrates for MDR transporters and are pivotal in balancing cell death and survival.

The capacity of a mutated cell for malignant growth has been attributed to acquisition of specific genetic alterations defined as hallmarks of cancer in two seminal publications by Hanahan and Weinberg [4, 5], including the capability to evade apoptosis, sustain proliferative signaling, and deregulate cellular energetics, that could partly be consequent to perturbed sphingolipid intermediary metabolism coupled with ABCB1.

This review examines the interplay between sphingolipids and MDR transporters with particular focus on the function of P-glycoprotein ABCB1 in the context of development of partial and complete MDR phenotypes during oncogenic transformation.

2. Sphingolipids

In the 19th century, Thudicum discovered a complex family of lipids in the brain whose enigmatic nature led to coining of the name “sphingolipid” to describe their puzzling qualities and impenetrable function. Originally thought to only be structural components in lipid membranes, it is now apparent that sphingolipids are intricately involved in many cellular processes including the calibration of internal and external stress, cell differentiation, cell migration as well as in cell survival that have implications in a multitude of diseases, such as cancer, neurodegenerative disease, depression and inflammation. Interest in sphingolipids was reignited when evidence suggested their role in signal transduction [6, 7]. Fundamental for progress were the discoveries that ceramide functions as a signaling

¹ **Abbreviations:** ABC, ATP binding cassette; ABCB1, ATP-binding cassette subfamily B member 1; Adr, adriamycin; A-SMase, acid sphingomyelinase; Alk-SMase, alkaline sphingomyelinase; BMP, bis (monoacylglycerol)phosphate; BODIPY, boron-dipyrromethene; BCRP, breast cancer related protein; CerS, ceramide synthase; CERT, ceramide transfer protein; COPI, coat protein complex I; CRP, ceramide-rich platform; CsA, cyclosporin A; DEN, diethylnitrosamine; DRM, detergent resistant membrane; ER, endoplasmic reticulum; FRET, fluorescence resonance energy transfer; GCS, glucosylceramide synthase; GEM, glycosphingolipidenriched microdomain; GluCer, glucosylceramide; GD, disialylganglioside; GM, monosialoganglioside; LMP, lysosomal membrane permeabilization; MβCD, methyl-beta-cyclodextrin; MDR, multidrug resistance; MRP, multidrug resistance related protein; NBD, nucleotide binding domain; NF-κB, nuclear factor kappa B; N-SMase, neutral sphingomyelinase; PC, phosphatidylcholine; PDMP, 1-phenyl-2-decanoylamino-3-morpholino-1-propanol; PE, phosphatidylethanolamine; PITX2, paired-like homeodomain transcription factor 2; PKC, protein kinase C; PPMP, N-[(1R,2R)-2-hydroxy-1-(4-morpholinylmethyl)-2-phenylethyl]hexadecanamide hydrochloride; S1P, sphingosine-1-phosphate; SM, sphingomyelin; SMS, sphingomyelin synthase; SNP, single nucleotide polymorphism; SPHK, sphingosine kinase; SPT, serine palmitoyltransferase; TCF, T-cell factor; TM, transmembrane; TMD, transmembrane domain.

molecule, is generated by multiple distinct mechanisms, and is involved in signaling of apoptotic cell death [8-10].

The backbone of sphingolipid family members is the sphingoid base, formed by the condensation of serine and palmitoyl CoA (Fig. 1). At the center is ceramide, comprised of the sphingoid base sphingosine linked to a fatty acid (FA) varying in chain length from C₂ to >C₂₄ via an amide bond. The free hydroxyl head group of ceramide is susceptible to modifications that yield the large majority of simple and complex structures that define this sphingolipid family. Individual sphingolipid metabolites have cognate targets, as well in some cases biophysical effects on membrane structure. Therefore, a dynamic regulation of sphingolipid intermediary metabolism is now recognized to govern the impact of sphingolipid biology on cellular responses.

2.1. Sphingolipid biosynthesis and metabolism

The core sphingolipid signaling pools comprising the triad of sphingomyelin(SM)-ceramide-sphingosine/Sphingosine-1-phosphate (S1P) are modulated through five main mechanisms detailed below (Figure 1).

2.1.1. Sphingomyelin pathway—Sphingomyelin (SM) is by far the most abundant sphingolipid in animal cell membranes. It is localized to the outer membrane leaflet (OML) where it greatly contributes to formation of specialized liquid-ordered domains called lipid rafts (LRs). The transfer of phosphocholine to the head group of the sphingoid base of ceramide by sphingomyelin synthases (SMS) yields SM with different acyl chain lengths, which determine function [11] and membrane biophysical properties [12]. Two isoforms of SMS (*SGMS1* and *SGMS2*) are localized to Golgi. Similar to proteins, SM is trafficked to the plasma membrane (PM) in a subset of specialized Golgi-derived secretory vesicles [13]. Moreover, trafficking processes exhibit dependence on SM [14, 15].

Sphingomyelinase (SMase) enzymes hydrolyze SM into ceramide. Several SMase forms exist defined by their pH optima for enzymatic activity: acid SMase (A-SMase, *SMPD1*), neutral SMase (N-SMase, *SMPD2-4*), and alkaline SMase (Alk-SMase, *ENPP7*). Two gene products are generated from *SMPD1* by proteolytic processing of the full length protein giving rise to a lysosomal A-SMase form and secretory A-SMase form that resides in specialized secretory lysosomes that abut the inner surface of the PM. Secretory A-SMase is released by various cell types in response to physiologic and pathophysiologic stimuli that induce fusion of secretory lysosomes with PM, thereby initiating signal transduction via cell surface SM hydrolysis, and generation of ceramide.

The best studied N-SMase member is N-SMase 2, encoded by *SMPD3*, which is involved in regulating cell growth, apoptosis and cellular stress responses as a molecular basis of diseases, such as inflammation and cancer metastasis [16-18]. The role of Alk-SMase is less well defined though it was recently cloned from rat intestinal mucosa [19, 20], promising advances in elucidation of its biologic import.

2.1.2. De novo ceramide synthesis pathway—Initiation of *de novo* ceramide synthesis occurs in the endoplasmic reticulum (ER) through action of the rate-limiting

enzyme serine palmitoyltransferase (SPT, *SPTLC1-3*), which catalyzes condensation of serine and palmitoyl CoA to form 3-ketosphinganine. Following reduction to sphinganine, ceramide synthases (CerS) convert sphinganine to dihydroceramide by N-acylation of the free amine at the second position of the sphingoid base backbone. Finally, a dihydroceramide desaturase incorporates a *trans* double bond across C4 and C5 of sphinganine converting it to sphingosine to form ceramide [21, 22]. CerS, or longevity assurance genes (LASS), [23, 24], a family of six members in mammals with differing tissue expression, are largely confined to the ER. Each of the CerS1-6 isoforms has a unique tissue expression profile and predilection for a fatty acyl CoA with a specific FA chain length. Thus, depending on the CerS family member, distinct sets of ceramides with varying chain lengths are produced [23].

With few exceptions, naturally occurring mammalian ceramides generally possess acyl chain lengths varying between C₁₆ to C₂₄. The significance of the acyl chain length in determining biologic activity has only recently become apparent (reviewed in [25]), for example, in cell growth [26] and apoptosis [27]. Aside from signaling functions, ceramide species represent important determinants of membrane structure and biophysical properties [28, 29].

2.1.3. Degradation pathway—The product of ceramidase activity, sphingosine, can be phosphorylated to S1P by two sphingosine kinases (SPHK1 and SPHK2). A balance between the pro-apoptotic properties of ceramide and the anti-apoptotic properties of S1P has been termed the ceramide/S1P rheostat and is considered important in balancing cell death and survival in numerous stress situations (reviewed in [30, 31]).

Several forms of ceramidase, dependent on pH optima, have been identified. Acid ceramidase is limited to acidic lysosomal compartments, where it participates in coordinate degradation of complex sphingolipids to sphingosine, whereas the exact subcellular localization of neutral and alkaline ceramidases remains unclear. A handful of reports suggest that neutral ceramidase is present in or at PM [32, 33] and alkaline ceramidases appear mainly localized to ER [34, 35].

2.1.4. Recycling/salvage pathway—Higher order complex sphingolipids can be re-routed to the ceramide pool via the recycling/salvage pathway (reviewed in [36]). During endocytosis, phagocytosis or autophagy, PM SM and glycosphingolipids are internalized and enter the vesicular trafficking compartment. Constitutive degradation of sphingolipids in late endosomes/lysosomes is executed by sequential enzymatic reactions that require the presence of sphingolipid activator proteins culminating in acid ceramidase-dependent degradation of ceramide to free sphingosine. In the recycling/salvage pathway, CerSs are responsible for regenerating ceramide from free sphingosine by re-acylation. Due to CerS localization, the final step most likely takes place in ER, although recent evidence for mitochondrial CerSs has been published [37, 38].

2.1.5. Glycosphingolipid metabolism—Ceramide is delivered from ER to the Golgi by vesicular transport or ceramide transfer protein (CERT). Therein, addition of a simple sugar group by UDP-glucose ceramide glucosyltransferase (UGCG), also known as glucosylceramide synthase (GCS), to the head group of the ceramide molecule yields

glucosylceramide (GluCer), to which additional sugar groups can be added by glycosyltransferases to generate complex glycosphingolipids, such as cerebroside, gangliosides and globosides. GCS localizes to Golgi apparatus where GluCer initially appears on the cytoplasmic surface of Golgi membranes [39], but is re-routed back to the ER where it is flipped to the luminal leaflet and subsequently returned to the Golgi lumen for complex glycosphingolipid generation [40]. Trafficking and exocytosis of glycosphingolipid-containing secretory vesicles releases glycosphingolipids to the PM external leaflet of the lipid bilayer [41]. GluCer can be reverted to ceramide by glucosylceramidase and complex glycosphingolipids can be degraded via the recycling/salvage pathway.

Physiologically, GCS/GluCer aids sorting, targeting and vesicular trafficking of soluble and membrane proteins by maintaining required levels of vesicular glycosphingolipids [42-45]. At the PM surface, glycosphingolipids facilitate LR formation, also known as glycosphingolipid-enriched microdomains (GEMs), which serve as signaling platforms.

2.2. Cellular compartmentalization of sphingolipid metabolism

Under homeostatic condition, alterations in cellular sphingolipid balance do not occur on a global scale. Rather, enzymatic reactions are segregated in various cellular compartments [46]. In PMs, OML SM is hydrolyzed to ceramide by stress-induced secretory A-SMase, [47, 48]. Ceramide, at above a cell-type specific threshold, coalesces to form macrodomains; ceramide-rich platforms (CRPs; ~1-5 microns in length) due to unique hydrophobic and hydrogen bonding properties of this lipid class into which proteins can insert and oligomerize for transmembrane cell stress/death signaling [49-51]. This feature enables calibration of the extent of stress injury to PM by intensity of CRP signal transfer to the cellular interior, often initiating the process of apoptotic death [51-53]. The resulting ceramide can be used by PM SMS to regenerate SM or hydrolyzed by acid ceramidase (which is physically bound to secretory A-SMase), assisting in reduction of CRP-mediated signaling and generating sphingosine/S1P.

In lysosomes, levels of SM/ceramide pools is governed by enzymes with an acidic pH optimum, namely A-SMase and acid ceramidase. In addition, alterations in SM levels may have implications for lysosomal function and stability [54, 55]. Cationic lysosomal A-SMase is normally anchored to bis (monoacylglycero)phosphate (BMP), an anionic lipid found at the intraendo-/lysosomal membrane, through ionic attraction protecting A-SMase from degradation by lysosomal proteases [56, 57], an interaction that can be dissipated by cationic amphiphilic drugs [58]. Consequent to A-SMase degradation, increased SM, and concomitant decreased ceramide, contribute to lowered lysosomal stability and increased susceptibility to lysosomal membrane permeabilization (LMP), culminating in lysosomal cell death [59]. It is also thought that decreased ceramide levels may modify steric conformation of lysosomal membranes resulting in diminished fusion capacity [60-62], inhibition of lysosome-dependent cellular processes, such as autophagy and protein recycling, leading to cell death. These recent studies contrast with earlier studies that reported that lysosomal ceramide accumulation is responsible for cell death initiation [63].

The mechanism by which SM/ceramide balance affects lysosomal membrane stability remains to be clarified.

In mitochondria, ceramide forms pores in the outer membrane that release pro-apoptotic molecules from the intermembrane space [64], coalesces into CRPs for apoptosis signaling [38], or acts as a precursor for S1P and hexadecanal generation to regulate Bak and Bax activation [65]. By compartmentalizing sphingolipids, local changes in sphingolipid pools, rather than on the whole cell level, allow for precision in regulating homeostatic and signaling functions.

2.3. Sphingolipids in lipid rafts

LRs are <100 nm in size and highly enriched in cholesterol, glycosphingolipids and phospholipids with saturated FAs, particularly SM [66]. Due to the immiscibility of cholesterol and glycosphingolipids and the favored interactions between cholesterol and SM, the presence of SM is crucial to LR formation [67, 68]. The high content of saturated FA side chains in sphingolipids results in a compact structure that allows for tight lipid packing. It appears SM first forms complexes with glycosphingolipids, that, in turn, accommodate cholesterol [68]. As a result, and together with the rigid structure of intercalated cholesterol, LRs exist in a liquid-ordered phase that manifests less fluidity than the surrounding membrane bilayer [69, 70]. Further, the closely packed lipids in raft microdomains prevent entry of non-ionic detergents, such as Triton X-100 and Brij-98, into the lipid bilayer at low temperature [71] that can be isolated and studied as “detergent-resistant membranes” (DRMs). However, it should be noted that DRMs do not precisely equate with LRs [72], and it is possible that LRs might form or re-structure during isolation [73]. The original LR hypothesis envisaged favorable lipid-lipid interactions for functional raft formation [74], however, there is mounting evidence for lipid-binding domains of integral and peripheral membrane proteins as the impetus of protein clustering in cholesterol and/or sphingolipid-enriched domains [68, 75]. Here we use the general term LRs to define liquid-ordered domains enriched in cholesterol, saturated phospholipids and sphingolipids, devoid of caveolin and distinct from CRPs.

3. Multidrug resistance

Bacterial resistance to antibiotic drugs was first described after the discovery that penicillin prompted bacteria to develop defense mechanisms culminating in expression of an array of efflux transporters in the outer cell wall. The broad range of substrates used by these transport proteins resulted in coining the term *multidrug resistance* (MDR) as pathogens can limit accumulation of diverse drugs targeted against them [76]. In mammals, MDR efflux transporters operate under physiologic conditions to expel organic metabolites and xenobiotic compounds into excretory tracts. Widespread use of chemotherapeutic drugs resulted in co-option of this program, and the often-reported human form of MDR in tumor cells.

Development of drug-resistant cell lines as an *in vitro* model by exposure to increasing concentrations of cytostatics [77] was crucial for Victor Ling's discovery of a protein that was strongly associated with the MDR phenotype: P-glycoprotein (“altered drug

permeability glycoprotein”), abbreviated to P-gp, gp170, P170 or, according to current nomenclature, ABCB1 (ATP-binding cassette sub-family B member 1). In colchicine-resistant Chinese hamster ovary cells, a membrane glycoprotein, not present in parental cells, was associated with an energy-dependent process that generated an ostensible “permeability barrier” to colchicine toxicity [78]. A year later, this glycoprotein was further characterized and designated P-gp based on its apparent ability to control drug permeation by modulating properties of hydrophobic membrane regions in drug-resistant cells [79]. Since discovery of ABCB1, additional efflux transporters that play a role in MDR have been identified, such as the MDR related proteins (MRPs or ABCCs) [80] or breast cancer related protein (BCRP or ABCG2) [81]. For simplicity, we focus here on ABCB1.

3.1. The MDR P-glycoprotein ABCB1

The canonical MDR protein ABCB1 belongs to the evolutionary-conserved ATP-binding cassette (ABC) superfamily, a large group of transmembrane (TM) transporters that utilize energy to translocate various substrates across membranes. Functional ABC transporters typically consist of at least two TM domains (TMDs) and two nucleotide binding domains (NBDs) [82]. In humans, ABCB1 protein is synthesized from the *MDR1* gene generating a single polypeptide chain consisting of 1,280 amino acids that is folded to form two homologous parts, each consisting of six α -helical membrane-spanning domains and a NBD, joined by a cytoplasmic linker region.

PM ABCB1 can transport a wide range of structurally-unrelated substrates directed towards the extracellular compartment. Efficacy of ABCB1-mediated transport for substrate is limited by size, charge and hydrophobicity: only neutral or cationic compounds ranging from 300-4000 Da can be transported and whilst compounds with high hydrophobicity are favored, amphiphilic compounds are also translocated [82-85]. ABCB1 is also a lipid translocase with broad specificity [86], extruding SM, GluCer, phosphatidylcholine (PC) and phosphatidylethanolamine (PE) in reconstitution experiments [87] as well as ceramide and GluCer from ABCB1-overexpressing cells [88].

ABCB1 expression is predominantly regulated at the transcriptional level. The promoter region of ABCB1 contains sites for multiple transcription factors, including myc, Sp1, AP-1, NF- κ B, TCF (reviewed in [89]), and the more recently-discovered, PITX2 [90]. Since many of these transcription factors are altered by stress signals, ABCB1 is highly susceptible to upregulation during a stress response. ABCB1 expression can also be modified by single nucleotide polymorphisms (SNPs) [91, 92], which play a very important role in the context of cancer therapy, as well as by gene rearrangements [93], mutations [82, 94], epigenetic regulation [95, 96] and post-translational mechanisms [97].

Later studies ascertained that ABCB1 is, in fact, expressed physiologically [98, 99]. ABCB1 is primarily found at important epithelial linings, sites of demarcation between “internal” and “external” environments in the body, as well as at blood-brain and blood-testis barriers. Epithelial cells lining the gut, liver, kidney and pancreas express high levels of ABCB1 in a highly-polarized fashion directing expulsion of physiologic metabolites and xenobiotic compounds into a secretory duct or luminal system for excretion from the body [99]. Similarly, ABCB1 is present at the blood luminal side of capillary endothelial cells that

make up blood-tissue barriers where potential noxious substances are directed into the blood, protecting tissues from damage [100, 101].

Some cancer types harbor intrinsic MDR, most probably due to endogenous expression of drug efflux transport proteins in the tissue of origin, in particular tumors derived from kidney and liver. Other cancer types acquire MDR through prolonged or repeated treatment with chemotherapeutic drugs. The seminal work by Ling and colleagues provided the first molecular connection to MDR and paved the way for development of therapeutic drugs targeting ABCB1. Pharmacologic modulators of ABCB1, now in the third generation, have worked exceptionally well in *in vitro* and rodent models, but unfortunately have not translated well into clinical trials [102]. Their use in humans may be hampered by endogenous expression of ABCB1 where inhibition of physiologic function would lead to drug side effects as well as limit drug accessibility to its targets.

3.1.1. Posttranslational modification of ABCB1—As the original name P-glycoprotein indicates, ABCB1 is heavily glycosylated at three outwardly-directed N-linked recognition sites (Asn 91, 94, 99) [82]. After exportation from the ER as a core-glycosylated ~150 kDa intermediate, ABCB1 is further glycosylated in the Golgi apparatus to the mature ~170 kDa form. N-glycosylation of ABCB1 does not seem to be essential for its transport activity but rather affects stability and trafficking of protein from ER to Golgi apparatus [103]. ABCB1 may be further modified through four phosphorylation sites in the linker region that may affect its degradation [104], transporter drug binding and activity [105-107] as well as expression [107].

3.1.2. Conformation and function of ABCB1—Full length human ABCB1 was cloned in 1986 [108, 109] but the tertiary structure of mouse ABCB1 was first visualized by x-ray crystallography by the group of Geoffrey Chang in 2009 [110]. The α -helical TMDs of each homologous half create a bundle that comes together to form a large internal cavity, which could easily accommodate two compounds [111]. In the resting, or pre-transport, state, ABCB1 is found in a “triangular” conformation where the bundles are in close proximity on the extracellular side and the intracellular NBDs are separated by 30 Å. The substrate binding pocket faces inward and is open to both cytoplasm and inner membrane leaflet (IML) of the lipid bilayer but not to the extracellular compartment or the OML [112]. The predicted substrate binding pocket contains mostly hydrophobic and aromatic residues though there are charged residues present at the bottom of the pocket. Two negatively charged portals of substrate access are created by TMD4/TMD6 and TMD10/TMD12. In the catalytic cycle, ATP binding to the NBDs is stimulated by substrate binding and leads to their dimerization that, in turn, drives the large structural change of ABCB1 from inward to outward facing conformation. The substrate is then released on the opposite side of the membrane through decreased binding affinity, facilitation by ATP hydrolysis, or by the law of mass action. Following hydrolysis of ATP, NBD dimerization is disrupted and ABCB1 returns to the pre-transport state [110]. A number of amino acid residues distributed across TMDs as well as in cytoplasmic loops are pivotal for substrate recognition and mutational studies convincingly evidence that each substrate has a defined amino acid subset for its recognition [113].

3.2. Cellular distribution of ABCB1 expression

By exploiting intrinsic fluorescence of anthracycline drugs doxorubicin or daunorubicin, it was discovered that in MDR cells chemotherapeutics are not only transported across PM, but are also sequestered in intracellular compartments, presumably precluding access to their main nuclear targets (note anthracyclines intercalate into DNA and block transcription via inhibition of topoisomerase II). Numerous studies show ABCB1 locates to acidic vesicular compartments that accumulate lysosomal tracker dyes and/or stain positive for the lysosomal marker LAMP2 [114-117] as well as to acidic Golgi [118, 119], wherein ABCB1 also appears to enhance glycosphingolipid synthesis through its lipid translocase activity [120]. Immunostaining and inhibition of ABCB1 (pharmacologic and genetic) demonstrate intracellular ABCB1 is functional and required for cytosolic drug sequestration. ABCB1 blockers, such as cyclosporin A (CsA), vinblastine and verapamil, prevent lysosomal drug accumulation [115-117] and improve nuclear drug buildup [121].

ABCB1 has also been localized to nuclear envelope in drug-resistant cell lines [122, 123], as well as in brain endothelial and microglial cells [124]. Using isolated nuclei, Calcabrini and colleagues evidenced functional nuclear ABCB1-induced drug efflux from nuclear matrix that was prevented by CsA [122], suggesting an additional mechanism by which ABCB1 might influence nuclear drug accumulation. Though mitochondrial localization of ABCB1 in drug-resistant cell lines has been reported [125, 126], this has been disputed based on possible contamination of mitochondrial membranes with PM [127]. Further, the orientation, and thus the transport direction of mitochondrial ABCB1, is unresolved.

3.3. Hypothetical models for substrate recognition, binding and extrusion by ABCB1

The mechanism behind ABCB1's multidrug specificity has been a conundrum yet to be resolved in its entirety. The ability of ABCB1 to transport a wide variety of structurally unrelated substrates indicates that ABCB1 does not follow simple pharmacophore models determining substrate recognition. Structural and mutational studies reveal potential mechanisms by which ABCB1 may extrude a broad range of substrates [82]. The “vacuum cleaner” model prevails where hydrophobic cationic substrates diffuse into the lipid bilayer, partition into the hydrophobic portion of the IML from which substrates are specifically recognized, drawn out of the membrane and extruded [112, 128, 129]. However, this model is increasingly challenged, as ABCB1 transport kinetics do not follow Michaelis-Menten kinetics, substrate hydrophobicity does not always correlate with transport efficiency, and the broad range of substrates complicates matters regarding the molecular basis of substrate recognition. An alternative hypothesis has been proposed: Rather than targeted substrate extrusion, perhaps ABCB1 continuously oscillates between inward and outward conformations because cytosolic ATP concentrations (3-5 mM) are higher than the K_m of ABCB1 for ATP (0.3-1.0 mM). This results in stochastic extrusion of substrates translocated to the IML by flippase activity [130] or passively distributed into the same membrane locale as the transporter by lateral diffusion [131]. This mechanism is dependent on solubility and lateral diffusibility of substrates within the membrane and frequency of “meetings” between substrate and transporter. Hence elements of physical solubility and diffusibility were introduced to the chemical affinity notion between substrate and transporter, wherein the membrane space surrounding the transporter, into which substrates diffuse, is taken into

consideration to determine apparent substrate “affinity constants”. According to this model, effective ABCB1 inhibitors likely trap ABCB1 in the outward conformation, preventing substrate binding and thereby hindering substrate extrusion. This alternative oscillation model could theoretically complement as well as account for findings that ABCB1 localizes to specialized domains with differing lipid composition in membranes because ABCB1 substrates would be required to distribute into the same lipid domain where ABCB1 is localized, thereby altering apparent substrate affinity constants.

Garnering evidence supports both drug-binding pocket and drug-lipid interaction mechanisms [132, 133]. The partition coefficient of a substrate into the lipid bilayer, which is governed by its chemistry, is an important determinant of ABCB1 affinity and thus transport [134]. Interestingly, in addition to the hydrophobic core of the membrane (i.e. the acyl chain region), ABCB1 seems able to bind substrates from other parts of the IML [132, 135, 136]. Using dipalmitoyl phosphatidylcholine bilayers in molecular dynamics studies, Omote and Al-Shawi [132] showed that ABCB1 substrates verapamil, Hoechst-33342, colchicine, rhodamine 123⁺ and daunorubicin preferentially partition at the cytoplasmic surface (or interfacial region) of the membrane indicating ABCB1 can take up substrates that are outside or partially outside the hydrophobic core. The molecule laterally diffuses to the substrate-binding chamber of ABCB1 for extrusion without direct involvement of the hydrophobic core. Rather, hydrophobic residues in the chamber adsorb the hydrophobic part of the substrate. Taken together, efflux of substrates by ABCB1 is a multistep process involving: 1) initial substrate binding to extracellularly-facing membrane lipids; 2) entry and partitioning into the lipid bilayer or flippase-driven movement into the IML; 3) recognition by ABCB1; 4) uptake from the hydrophobic bilayer core or surface interfacial region, and binding pocket entry; 5) and finally transport to the opposite/extracellular compartment through conformational change of ABCB1 facilitated by ATP hydrolysis.

3.4. Lipid microenvironmental influence on ABCB1 transport activity

Integral membrane proteins are normally dependent on their lipid microenvironment for correct protein folding, topologic presentation and function. LRs provide an optimal lipid composition to facilitate interaction between some membrane proteins and their local environment, functionalizing this protein set for transduction of external signals to the intracellular milieu (reviewed in [137, 138]).

The ABCB1 protein is no exception: Its activity can be modulated by composition of the lipid microenvironment. It localizes not only to conventional LRs [139, 140] but also to non-canonical non-caveolae rafts characterized as Brij-96-insoluble intermediate-density membrane microdomains devoid of markers for caveolar domains and LRs (i.e. caveolin-1, Src kinases, monosialoganglioside (GM₁)) [141]. Two studies report that depleting cholesterol by methyl- β -cyclodextrin (M β CD), thereby collapsing LRs, reduces ABCB1 activity [142, 143]. Fenyvesi et al. [142] showed increased accumulation of the ABCB1 substrate calcein (and hence reduced efflux) by ~30-fold at 5 μ M M β CD, whereas in a more elaborate approach by Troost and colleagues [143], cholesterol depletion reduced ABCB1 transport function, culminating in intracellular accumulation of BODIPY-verapamil to ~60% above control, while cholesterol repletion restored ABCB1 transport.

ABCB1 association with LRs was confirmed by either classical sucrose density gradient DRM isolation [143] or by flow cytometry of DRMs using a fluorescence-coupled ABCB1 antibody [142]. In both cases, decrease in ABCB1 activity was associated with migration of ABCB1 out of LRs by cholesterol depletion, indicating ABCB1 activity dependence on LR localization. However, use of M β CD must be taken with caution as M β CD has other effects, including direct inhibition of ABCB1 catalytic activity [144]. In contrast to these studies, in low-density Triton X-100-resistant membrane domains isolated by a discontinuous sucrose gradient, ABCB1 was found in caveolin-enriched fractions indicating ABCB1 presence in caveolae, a subset of LRs. When purified ABCB1 was reconstituted into liposomes comprising of PC, cholesterol and/or SM, ABCB1 ATP hydrolysis activity was unaffected, implying functions of ABCB1 other than ATP catalysis may be dependent on the lipid microenvironment, including sphingolipids [145]. The role of the other important constituent of LRs, namely, glycosphingolipids, in ABCB1 function has not yet been investigated in depth. In summary, the available information, with some caveats, supports ABCB1 as functional within LRs.

3.5. Do ABCB1 transmembrane domains interact with membrane sphingolipids?

Since membrane lipid composition in the immediate vicinity of ABCB1 can influence its transport activity, ABCB1 could hypothetically localize to LRs or CRPs that form under stress conditions or during transformation. In our own preliminary studies, we observed ABCB1 co-localization with surface ceramide in drug-resistant human renal carcinoma cells (Lee W-K and Kolesnick RN, unpublished observation). Within the predicted TMDs of ABCB1, the amino acid sequence mainly consists of hydrophobic and aromatic residues that facilitate integration into the lipid bilayer. The question arises as to whether these amino acids display a preference for particular lipids to support the idea that ABCB1 might localize to specialized regions of the bilayer, not only within the PM but also within intracellular membranes [115, 146], and whether these lipids modulate ABCB1 transport activity.

Indeed, a number of sphingolipid-interacting domains have been described in proteins (although not TM proteins), such as the cysteine-rich ceramide-binding atypical C1 domain present in a variety of proteins including PKC, Raf family members, and Kinase Suppressor of Ras 1 [147, 148] or the glycolipid and SM-binding V3 loop domain identified in Alzheimer, prion and HIV proteins [149]. However, only one study detailed a potential consensus sphingolipid-binding signature in TM proteins. Based on liposomal FRET studies using C₁₈-SM and the TMD of coat protein complex I (COPI) machinery protein p24, a binding signature was defined where either a β -branched residue (Ile, Thr, Val) or Leu occupies any of the first four positions and an aromatic residue (Phe, Trp, Tyr) occupies the last position [150]. From 768 unique sequence motifs, 13 were overrepresented in a set of 48 candidate mammalian membrane proteins that were mostly localized in the PM but no transport proteins were listed [150]. Furthermore, specificity of the consensus binding sequence for sphingolipids was surprisingly selective: p24 protein only interacted with SM with C₁₈ FA, but not with C₁₄ FA, nor with ceramide or PC. Another COPI protein, p23, which does not contain the SM-binding consensus motif does not harbor sphingolipid interactivity. Hence, Contreras and colleagues [150] identified the C₁₈-SM binding sequence as VXXTLXXIY within the TMDs of various membrane proteins and C₁₈-SM/protein

interactions depended on both sphingolipid head group and backbone. While this sphingolipid-binding sequence was not found in the TMD protein sequence of ABCB1 in its entirety, three TMDs displayed a single mismatch in the final aromatic amino acid residue (instead of Phe/Trp/Tyr, Ile in TMD1, Ala in TMD9, Val in TMD10) as well as a mismatch in the fifth amino acid (Ser instead of Thr/Leu/Val/Ile) in TMD4 by manual sequence analysis. If sphingolipid interaction is primarily dependent on the hydrophobic property of the last amino acid residue, then the mismatch in TMD1 and TMD10 may not affect interaction significantly as Ile and Val are both hydrophobic amino acids with a hydrophobicity index in the same range as aromatic amino acids, whereas for TMD9 the hydrophobicity index of Ala is >50% lower. Further, for TMD4, the polar neutral side chain of Ser differs from Leu, Val and Ile, but is similar to Thr. Since the sphingolipid molecule stretches across the binding sequence, a mismatch in the middle of the sequence may not have such great impact as flanking amino acid residues.

According to the crystal structure of ABCB1, TMD1 and TMD10 belong to one bundle of TMDs and TMD4 and TMD9 belong to the other bundle [110]. The putative SM-binding sequence in ABCB1 is located in opposing leaflets of the lipid bilayer depending on the TMD: In TMD1 and TMD9, it is present in the IML in close proximity to the membrane/cytosol interface whereas in TMD4 and TMD10, it seems to be localized to the OML within the hydrophobic layer. The putative SM-binding sequences in TMD1 and TMD9 are not in the same TMDs as the residues responsible for substrate recognition and binding at the interfacial region, suggesting SM is not directly involved in substrate recognition. But this does not rule out the possibility that it could aid substrate uptake by binding the substrate's hydrophobic portion. Interestingly, TMD4 and TMD10 have important functions for the ABCB1 protein. Firstly, they intertwine with another TMD to stabilize the inward facing conformation of ABCB1. Secondly, they are two of four TMDs that form the entry portals for hydrophobic molecules directly from the membrane. A further interesting observation is that the putative SM-binding sequence in TMD4 and TMD10 is in immediate vicinity of a proline which alters substrate specificity of ABCB1 when mutated [82]. In fact, when this proline is mutated (P223A in TMD4, P866A in TMD10), resistance to colchicine, adriamycin and actinomycin D, but not vinblastine, is reduced by ~75% [151].

Taken together, accumulating evidence points to a preferred lipid microenvironment enriched in sphingolipids, such as SM or possibly ceramide as well as cholesterol for ABCB1 transport activity [145, 152]. Hence, through the presence of multiple putative SM-binding regions in ABCB1 TMDs, it is reasonable to hypothesize that interaction of ABCB1 with sphingolipids and cholesterol creates a specific spatial domain within the PM wherein ABCB1 can optimally bind and transport substrates.

3.6. ABCB1 in oncogenic transformation

ABCB1 upregulation is usually assessed either once cancer is advanced or cell lines have been exposed long-term to chemotherapeutic drugs and in both cases MDR has fully developed making it difficult to judge at which stage ABCB1 expression is initiated. Using the Solt-Farber model of hepatocarcinogenesis, the placental form of glutathione-S-transferase, a known marker for pre-neoplastic and neoplastic lesions in liver, was used to

monitor transformation together with ABCB1 levels by *in situ* hybridization and immunohistochemistry [153]. In early stages of carcinogenesis, ABCB1 expression was low and sporadic. Only later, when carcinoma was identified, did ABCB1 increase indicating ABCB1 associates with late stages of liver carcinogenesis. In contrast, immunohistochemical studies of tissue sections found ABCB1 increased in patients with early stage colorectal cancer [154, 155]. In the Hahn and Weinberg model of tumorigenesis [156], telomerase expression and inactivation of p53/retinoblastoma protein checkpoints are prerequisite for MDR development by doxorubicin. Although most of these studies correlate oncogenic transformation, increased ABCB1 expression and MDR, they do not clarify the exact role of ABCB1, that is, is ABCB1 a cause or consequence, in tumor progression.

3.7. Role of sphingolipids in MDR and ABCB1 expression in cancerous versus non-cancerous cells

Glycosphingolipids are important membrane structural constituents contributing, for instance, to LRs and myelin sheath, but also function as receptors for bacteria and toxins, precursors for signaling molecules such as ceramide, as well as being involved in cell-cell interaction, cell-cell adhesion and cell proliferation [157, 158]. An altered glycosphingolipid profile in cancerous versus non-cancerous cells was observed in cell lines transformed by chemicals or viruses, and impacted cell growth, intercellular recognition and cell adhesivity [159, 160]. Higher levels of polysialylated gangliosides were also reported in human ovarian cancer cell line A2780 and treatment-induced MDR sublines [161]. Approximately 20 years later, glycosylation of ceramide proved to play a significant role in conferring MDR in tumors. Using human breast cancer MCF7 cells resistant to doxorubicin (trade name Adriamycin; MCF7/Adr), Cabot and co-workers discovered that drug-resistant cells had 8 to 10-fold higher capacity to convert the precursor [³H]-palmitic acid to ceramide and further to GluCer, but not to galactosylceramide, than non-drug resistant counterparts [162], confirmed by others in additional drug-resistant cell lines [163]. The fact that GluCer degradation was not reduced in MCF7/Adr cells pointed to increased GluCer synthesis in ABCB1-overexpressing cells. Indeed, increased GCS activity has now been associated with MDR phenotypes across multiple cancer cell lines [164-168] in mouse models [169], and cancer-derived tissues [170-172]. Furthermore, pharmacologic inhibitors of *de novo* ceramide synthesis (fumonisins B₁, L-cycloserine) [162], GCS (PPMP) [162] and ABCB1 (verapamil, tamoxifen, CsA), which appear to inhibit GCS activity at therapeutic concentrations (5 μM) [173], all reduced augmented GluCer levels in MCF7/Adr cells, indicating that both deregulated *de novo* ceramide synthesis and GCS activity regulate increased GluCer associated with MDR. Importantly, drug sensitivity was restored when GCS was inhibited [164, 174] or downregulated [164, 175, 176]. However, not all studies in MDR cells exhibit dependence of drug resistance on GCS/GluCer [177-180]. In addition, pharmacologic inhibitors of GCS, in particular PPMP/PDMP and derivatives, have a number of off-target effects, such as induction of ceramide production [180, 181] or cell cycle arrest [182, 183], that might impact outcome.

Generally, a positive correlative relationship has been observed between GCS/GluCer levels and ABCB1 expression [184-186]. At first, the Cabot group proposed pro-apoptotic ceramide is quashed through conversion to GluCer, thereby resulting in increased cell

survival. Later, they hypothesized that perturbed GCS/GluCer is linked to increased ABCB1 expression and activity. ABCB1 is also a lipid translocase therefore an alternative hypothesis is that increased GCS activity represents consequent counter-regulation to increased GluCer extrusion, and hence diminished intracellular GluCer by MDR cells. Available studies do not distinguish between the different hypotheses.

Interestingly, not only GCS/GluCer is augmented in MDR cells, but there is redistribution of GluCer. Nile-red fluorescent staining for intracellular lipids demonstrated presence of large cytoplasmic neutral lipid droplets in MCF7/Adr cells and incubation with BODIPY-GluCer provided evidence that GluCer is present in these droplets [163]. Moreover, BODIPY-PPMP labelled the extensive Golgi network that was more prominent in MCF7/Adr than MCF7 cells, supporting the notion of increased Golgi GCS/GluCer production in drug-resistant cells. In the same study, both GCS and ABCB1 inhibitors, which also inhibit GCS, prevented GluCer accumulation in lipid droplets, but only ABCB1 inhibitors increased nuclear daunorubicin accumulation, suggesting attenuation of GluCer in cytoplasmic droplets alone is insufficient to reverse MDR. In contrast, in non-cancerous renal proximal tubule cells, blockade of GCS protects against toxic metal-induced cell death [187], implying GluCer acts in a pro-apoptotic manner. In the same study, ceramide/GluCer was extruded by ABCB1, which was upregulated, to protect cells from their cell death-inducing properties. Thus, the role of ABCB1 is consistently pro-survival whether cells are transformed or non-transformed whereas sphingolipid metabolism appears to have distinct roles depending on cell transformation status.

4. Rendering the MDR phenotype and altered sphingolipids: chicken or egg?

Intriguingly, and unexpectedly, heterologous expression of ABCB1 does not always confer the complete MDR phenotype [188, 189]. Though ABCB1 SNPs could be involved [189], it strongly implies that tumor cells need to first undergo a round of transformation steps, such as perturbed sphingolipid metabolism, that “prime” cells for potential subsequent MDR by contributing to upregulation and/or functionalization of MDR drug transporters, of which ABCB1, MRPs, organic cation transporters, and BCRP can be contributors. Here, we propose altered sphingolipid metabolism occurs in early stages of oncogenic transformation, preceding and in parallel to ABCB1 upregulation, as well as acting in concert with ABCB1 in feedforward cooperativity to confer complete MDR.

4.1. Sphingolipid alterations occur in pre-neoplastic cells independent of ABCB1

4.1.1. Altered cellular sphingolipids as an initial event in oncogenic transformation—Rewiring sphingolipid metabolic pathways represents an initial step in oncogenic transformation tailored to altered metabolic needs of the (pre)cancerous cell. While transformational changes associated with cancer often involve genetic mutations, sphingolipid metabolizing enzymes are rarely mutated but rather are regulated by aberrant upstream signaling. Transcriptional [190-195] or post-transcriptional [196] regulation of sphingolipid metabolizing enzymes has been poorly investigated. Despite this, two general lines of evidence support altered sphingolipid intermediary metabolism at an early stage of

oncogenesis: 1) sphingolipid metabolizing enzymes often serve as targets for or are modifiers of oncoproteins and 2) sphingolipids often regulate growth factor receptor signaling [197, 198]. For further details of studies on sphingolipid involvement in cancer, the reader is referred to recent comprehensive reviews [30, 199, 200].

Imbalance of sphingolipids as an early stage event in neoplastic progression has been observed in pre-neoplastic hepatocytes, isolated from rats injected with the carcinogen diethylnitrosamine (DEN), that develop sphingosine-reversible apoptosis resistance and exhibit higher GluCer levels [201] as well as increased SM [202]. In an *in vitro* model for multistage carcinogenesis, whereby cells were exposed to γ -irradiation and PKC stimulator, phorbol 12-myristate 13-acetate, delivery of sphingosine or sphinganine significantly attenuated transformation frequency. As sphingosine was quickly degraded, it was postulated that bioactive metabolites ceramide and SM are responsible [203]. In a mouse ovarian cancer progression model, augmented membrane capacitance, altered cell dielectric properties and lower membrane stiffness (characteristics of advanced cancer cells) were reverted to that of early tumor stage cells by nontoxic sphingosine levels [204, 205]. Finally, sphingolipids lowered incidence of aberrant crypt foci (ACF), which are putative preneoplastic lesions in the colon, suppressed cell proliferation and increased adenoma:carcinoma ratio in colon carcinogen-treated mice when they were fed dairy glycosphingolipids [206] and SM [207, 208] through their diet or *SphK1*, but not *SphK2*, was deleted [209].

4.1.2. Tumor-associated membrane sphingomyelin elevation gives rise to lipid raft expansion—Cancer PMs differ in lipid composition governed by malignancy grade [210]. Phospholipid choline metabolism is ubiquitously perturbed in tumor cells resulting in increased total choline products identifiable in tumor tissue using magnetic resonance spectroscopy [211]. The major sphingolipid in mammalian membranes and driver of LR formation is SM, which is comprised of ceramide and phosphorylcholine, and thus SM levels are also affected by aberrant choline metabolism [212]. In fact, SM is increased in multiple cancer tissues, including prostate [213], thyroid [214, 215], cervical [216], brain [217, 218], liver [219–221], breast [222, 223], as well as in lymph node metastases [224], feces from colorectal cancer [225], and urine from bladder cancer [226] patients. Depressed or unchanged SM levels were also detected in other studies [227–230]. Composition of SM species is also altered. In hepatoma, C16:1 SM was present in higher percentage compared with normal liver tissue [231] while all SM species tested were elevated in cancerous versus adjacent normal breast tissue, though C16:1 SM was not investigated [222]. Furthermore, SM species with a sphinganine base were increased in multiple tumors including hepatoma and nephroma compared with regenerating mouse liver [232]. In addition to deregulation of sphingolipid metabolism and SM accumulation in cancerous cells transforming from healthy, differentiated cells, SM-enriched tumor cells could also be derived from stem cells or rare precursors. However, the cancer stem cell is likely to represent a minor compartment in most solid tumors. Hence, it is unlikely that the tumor stem cell SM content is influencing bulk tumor SM content. Irrespective of the source, accumulation of SM has important consequences for the progression of transformation and MDR.

Importantly, in support of our hypothesis that augmented SM during transformation is prerequisite for ABCB1 functionalization and MDR development, Tang et al. [202] investigated lipid content and composition in livers from a rat model of DEN-induced hepatocarcinoma. SM gradually increased with DEN exposure, already detectable after 2 weeks and reaching maximal levels after 18 weeks but preceding ABCB1 appearance, which occurs in late tumor stages (5-15 months) in a Solt-Farber hepatocarcinogenesis model that also utilizes DEN [153]. Thus SM increase appears during the initial transformation process, rather than as a product of selection.

As SM represents a limiting factor in raft generation, elevated SM would increase density and size of LRs, cementing the liquid-ordered phase across a larger cross section of PMs in transformed cells, and may lay the ground work for optimizing ABCB1 and thus MDR potential.

4.2. Initializing MDR: Spingomyelin recruits ABCB1 to lipid rafts and hinders drug uptake through altered membrane fluidity

4.2.1. Membrane lipid composition of MDR cells—With the exception of one study [233], it is generally agreed that membrane lipid composition is altered in MDR cells. Drug-resistant P388/Adr mouse leukemia cells display increased SM, decreased PC, and no changes in phosphatidylethanolamine (PE) or cholesterol [234], while drug-resistant MCF7/Adr breast cancer cells harbor increased SM and cholesterol, and decreased PC and PE [235]. Unpublished data from our group (Morales A and Kolesnick RN) confirm increased SM levels in P388/Adr cells, as well as in drug-resistant Chinese hamster fibroblast ADX cells compared with their drug-sensitive counterparts. Additional evidence of increased SM associated with drug resistance is reported in leukemia [167, 236, 237] and ovarian cancer [238]. Interestingly, the MDR inhibitors verapamil, tamoxifen and dipyridamole reverse, in part, altered membrane lipid composition, augmenting PC and attenuating SM in P388/Adr cells, but do not affect drug-sensitive P388 cells [239], implying a contribution of ABCB1 in altered membrane lipid composition in addition to that provided by the transformation process. It should be emphasized that total cellular lipids, rather than subcellular compartment lipids, were analyzed in these studies. Aside from SM, the contribution of other sphingolipids to altered membrane lipid composition of MDR cells has not been extensively studied.

As LRs require SM, we posit that dysregulated SM during cancer transformation increases LR size/incidence, which would enhance recruitment of ABCB1 to LRs through interaction of ABCB1 TMDs with the putative SM-binding sequence (see above).

4.2.2. Effect of membrane sphingolipid alterations on ABCB1 and MDR—Increasing SM levels yield a liquid-ordered phase, and thus decreased membrane fluidity. Membrane fluidity is the most widely studied biophysical property studied in MDR cells as efficient drug uptake occurs primarily by passive lateral diffusion through PM or by endocytosis. Victor Ling initially proposed that ABCB1, in addition to conferring drug efflux, decreases fluidity in drug-resistant cells such that passive diffusion of drugs across membrane is impaired [78]. In this context, increased structural order (i.e. decreased

membrane fluidity) was observed in drug-resistant cells using fluorescence polarization of diphenylhexatriene, which distributes selectively into lipid hydrophobic domains, and electron spin resonance [240] or Langmuir isotherm data [235]. Decreased membrane fluidity impairs drug uptake through defective endocytic processes and traps drugs preventing them from reaching their intracellular targets and possibly increasing availability to ABCB1 for efflux. Of note, increased membrane fluidity has also been associated with MDR [241-243], suggesting the MDR phenotype may still be operative in liquid-disordered membranes. Lastly, changes in membrane fluidity seem restricted to PM since ER membrane fluidity did not differ between MDR and non-MDR cells [244].

4.3. Initializing MDR: Perturbed transcriptional regulation of ABCB1 by altered sphingolipids

ABCB1 expression is strongly under transcriptional control, reflected by the large number of transcription factor binding sites in its promoter region [89]. Transcriptional regulators affected by sphingolipids include c-myc, β -catenin, Sp1, NF- κ B, cyclin D1, p53, and p21 [245] (and references therein). Additionally, sphingolipids affect transcription factors through epigenetic regulators. Nuclear S1P regulates transcription of p21 and c-fos by increasing histone acetylation [246] and increased S1P can act through microRNAs to facilitate colon carcinogenesis [247]. Since altered sphingolipid metabolism activates transcription factors that can drive upregulation of ABCB1 [245], sphingolipid abnormalities might thus upregulate additional ABCB1, strengthening the MDR phenotype.

Direct evidence for transcriptional control of ABCB1 by sphingolipids has been demonstrated in MDR cancer cells for GluCer. As described above and in contradistinction to SM elevation, increased GluCer synthesis appears to be the product of MDR function. In this context, inhibition of GCS by ethylenedioxy-P4 prevented ABCB1 upregulation in MDA-MB-435 cells caused by short-term exposure to [3 H]-C₆-ceramide, which is metabolized to C₆-GluCer [185]. However, GluCer is unlikely to be solely responsible for increased ABCB1 expression because the magnitude of GluCer effect did not correlate with extent of ABCB1 upregulation. In subsequent work [175], transient GCS overexpression activated cSrc kinase culminating in nuclear translocation of β -catenin, a known ABCB1 transactivator [154].

Indirect evidence for SM-induced ABCB1 transcriptional upregulation has been reported in the brain of SMS2-deficient mice [248]. In the absence of SMS2, ABCB1 mRNA, protein and transport activity were diminished but how changes of SM levels signal from PM, where SMS2 is normally localized, to the transcriptional machinery is unknown. Hence, through upregulation of ABCB1, sphingolipid changes may participate in a feedforward enhancement of the MDR phenotype albeit data on this topic are incomplete.

4.4. Completion of the MDR phenotype: Engagement of GCS and ABCB1 sphingolipid transport

Accelerated glycosphingolipid synthesis in drug-resistant cell lines resulting from increased GCS activity, which is evidently linked to MDR [162, 164], may detoxify pro-apoptotic ceramide, culminating in GluCer accumulation in cytoplasmic lipid droplets [162, 163].

Such overaccumulation of GluCer is counteracted by the lipid transport activity of ABCB1, which, amongst other lipids, extrudes both GluCer and ceramide [87, 88]. A potential key event in transition from incomplete to complete MDR could lie in disturbed trafficking pathways governing transport of ABCB1 from Golgi to PM. Proteins destined for the PM are sorted and trafficked by the secretory pathway involving the ER, Golgi apparatus and lysosomes. Furthermore, transmembrane proteins are continuously quality controlled and turned over at the PM through endocytic endo/lysosomal, sorting and secretory pathways, which are dependent on glycosphingolipid levels, and where both increases and decreases in raft lipids can be influential [249, 250]. Though classic drug-resistant cells were not employed, ABCB1 trafficking to the apical cell surface is delayed or depleted by GCS inhibitors PDMP or PPMP in hepatocytes [44] and ABCB1 overexpressing cells [251]. Additionally, application of a globoside analog adamantylGb3 disturbed apical ABCB1 trafficking [251], indicating that imbalance of GluCer and higher glycosphingolipids could perturb ABCB1 trafficking and sorting, increase ABCB1 turnover and/or decrease residing time in the PM. The critical amount of GluCer in trafficking and sorting is further exemplified by the GluCer inhibitor NB-DGJ, which could reverse perturbed trafficking of lactosylceramide in macrophages at low concentrations but not at higher concentrations (>25 μ M), despite lowering of GluCer [249]. Analogously to lipid storage diseases, such as Gaucher or Niemann-Pick, wherein endocytic and Golgi sorting pathways are disarrayed by increased GluCer [252-254], accumulation and/or mislocalization of GluCer/higher glycosphingolipids seems to be the cause of perturbed PM turnover of ABCB1 in cancer cells exhibiting altered sphingolipid metabolism and MDR. Consequently, this would give rise to the appearance of intracellularly located ABCB1, further potentiating the cell's total MDR capacity by sequestration of chemotherapeutic drugs that may have escaped the PM. How these multiple potential amplification steps of GCS/GluCer metabolism actually work together to finalize the MDR phenotype will require significant future investigation. Irrespective of this challenge, the evidence indicates that engagement of enhanced GCS/GluCer metabolism by ABCB1 represents a substantive distal and perhaps final step for conferment of a complete MDR phenotype supported by the following lines of evidence: 1) ABCB1 inhibitors lead to GCS inhibition [163] and ceramide accumulation [187]; 2) GCS inhibition reduces ABCB1 expression [175] and restores drug sensitivity [164, 174]; 3) simultaneous downregulation of GCS and ABCB1 maximally reverses drug resistance [255]. Due to the molecular linkage between GCS and ABCB1 [175], in our opinion, the most parsimonious interpretation of this interaction is that of a feedforward loop. Through disruption of sorting and trafficking pathways and/or through enhanced turnover at the PM, aberrant glycosphingolipid biosynthesis in MDR cells appears to be the underlying mechanism for retention of ABCB1-containing vesicles in the cytosolic compartment and completes the transition to the full MDR phenotype.

4.5. Altered sphingolipids as both chicken and egg in MDR development: A potential scenario

In some cells, during initial stages of transformation, altered sphingolipid metabolism (SM synthesis) occurs, an event that is independent of ABCB1 expression/function, which results in GEM/LR increase on the transformed cell surface. Drug selection similarly increases ABCB1 expression independent of SM synthesis, an effect that alone is inadequate to confer

a robust MDR phenotype. If, however, both events occur in the same cell, progressive sphingolipid metabolic restructuring favors ABCB1 upregulation and functionalization in LRs by ABCB1 recruitment via SM-binding domains to confer a partial, yet still, incomplete MDR phenotype. Elevated GCS, a product of evolving MDR function, further disrupts the balance of glycosphingolipid pools, increasing surface LRs and ABCB1 transcription, while simultaneously perturbing Golgi-to-PM directed trafficking of a portion of ABCB1. The net outcome of this set of events is enhanced drug efflux from the fully-activated raft-associated pool of ABCB1 concomitant with drug entry into a cytoplasmic pool of ABCB1-containing vesicles resulting in intracellular sequestration. Ultimately, ongoing activation of these amplification loops and prolonged feedforward cooperativity lead to maintenance of the complete MDR phenotype and intractable drug resistance (Figure 2).

5. Conclusions / Summary / Outlook

Over two decades ago, it was first observed that overexpression of the multidrug resistant P-glycoprotein ABCB1 alone could only confer partial or incomplete MDR, implying additional transformational changes were required. In our proposed hypothesis, independence and interdependence between sphingolipids and ABCB1 are relevant at different stages of oncogenic transformation and for development of the complete MDR phenotype. Initially, SM and ABCB1 increase in parallel. The putative pivotal event in the transition to complete MDR may be in the appearance of intracellular ABCB1 that maximizes MDR capacity. However, the underlying molecular mechanisms that effectuate sphingolipid imbalance and interactions with ABCB1 are for the most part unknown. Future investigations to delineate initiation and transitional events imparted by sphingolipids during MDR development would enrich our understanding to improve chemotherapeutic drug targeting.

Acknowledgments

We would like to acknowledge Dr. Frank Thévenod (Institute for Physiology, Pathophysiology and Toxicology, University of Witten/Herdecke) for valuable discussions and critical reading of the manuscript. W.-K. L was supported by a research exchange fellowship from the Max Kade Foundation, research grants from the Internal Research Funding Program (IFF2016-20, IFF2017-14) and institutional funds from the University of Witten/Herdecke. R.K. was supported by R01CA179067 from the National Institutes of Health and in part through NIH/NCI Cancer Center Support Core Grant P30 CA008748.

Bibliography

1. Gottesman MM, Fojo T, Bates SE. *Nat Rev Cancer*. 2002; 2:48–58. [PubMed: 11902585]
2. Hornicek FJ, Gebhardt MC, Wolfe MW, Kharrazi FD, Takeshita H, Parekh SG, Zurakowski D, Mankin HJ. *Clinical orthopaedics and related research*. 2000:11–17.
3. Landreville S, Agapova OA, Kneass ZT, Salesse C, Harbour JW. *Pigment cell & melanoma research*. 2011; 24:430–437. [PubMed: 21575142]
4. Hanahan D, Weinberg RA. *Cell*. 2000; 100:57–70. [PubMed: 10647931]
5. Hanahan D, Weinberg RA. *Cell*. 2011; 144:646–674. [PubMed: 21376230]
6. Hannun YA, Bell RM. *Science*. 1989; 243:500–507. [PubMed: 2643164]
7. Kolesnick RN. *Progress in lipid research*. 1991; 30:1–38. [PubMed: 1771169]
8. Kolesnick R. *Trends Cell Biol*. 1992; 2:232–236. [PubMed: 14731480]

9. Mathias S, Dressler KA, Kolesnick RN. *Proc Natl Acad Sci U S A*. 1991; 88:10009–10013. [PubMed: 1946418]
10. Obeid LM, Linardic CM, Karolak LA, Hannun YA. *Science*. 1993; 259:1769–1771. [PubMed: 8456305]
11. Koivusalo M, Jansen M, Somerharju P, Ikonen E. *Mol Biol Cell*. 2007; 18:5113–5123. [PubMed: 17942604]
12. Dupuy FG, Maggio B. *The journal of physical chemistry B*. 2014
13. Deng Y, Rivera-Molina FE, Toomre DK, Burd CG. *Proc Natl Acad Sci U S A*. 2016; 113:6677–6682. [PubMed: 27247384]
14. Shakor AB, Taniguchi M, Kitatani K, Hashimoto M, Asano S, Hayashi A, Nomura K, Bielawski J, Bielawska A, Watanabe K, Kobayashi T, Igarashi Y, Umehara H, Takeya H, Okazaki T. *J Biol Chem*. 2011; 286:36053–36062. [PubMed: 21856749]
15. Subathra M, Qureshi A, Luberto C. *PLoS One*. 2011; 6:e23644. [PubMed: 21980337]
16. Wu BX, Clarke CJ, Hannun YA. *Neuromolecular medicine*. 2010; 12:320–330. [PubMed: 20552297]
17. Kosaka N, Iguchi H, Hagiwara K, Yoshioka Y, Takeshita F, Ochiya T. *J Biol Chem*. 2013; 288:10849–10859. [PubMed: 23439645]
18. Rutkute K, Asmis RH, Nikolova-Karakashian MN. *J Lipid Res*. 2007; 48:2443–2452. [PubMed: 17693623]
19. Cheng Y, Nilsson A, Tomquist E, Duan RD. *J Lipid Res*. 2002; 43:316–324. [PubMed: 11861674]
20. Wu J, Cheng Y, Palmberg C, Bergman T, Nilsson A, Duan RD. *Biochim Biophys Acta*. 2005; 1687:94–102. [PubMed: 15708357]
21. Merrill AH Jr, Wang E. *J Biol Chem*. 1986; 261:3764–3769. [PubMed: 3081509]
22. Michel C, van Echten-Deckert G, Rother J, Sandhoff K, Wang E, Merrill AH Jr. *J Biol Chem*. 1997; 272:22432–22437. [PubMed: 9312549]
23. Levy M, Futerman AH. *IUBMB Life*. 2010; 62:347–356. [PubMed: 20222015]
24. Mullen TD, Hannun YA, Obeid LM. *Biochem J*. 2012; 441:789–802. [PubMed: 22248339]
25. Grosch S, Schiffmann S, Geisslinger G. *Progress in lipid research*. 2012; 51:50–62. [PubMed: 22133871]
26. Hartmann D, Lucks J, Fuchs S, Schiffmann S, Schreiber Y, Ferreiros N, Merckens J, Marschalek R, Geisslinger G, Grosch S. *Int J Biochem Cell Biol*. 2012; 44:620–628. [PubMed: 22230369]
27. Senkal CE, Ponnusamy S, Bielawski J, Hannun YA, Ogretmen B, FASEB J. 2010; 24:296–308.
28. Silva LC, Ben David O, Pewzner-Jung Y, Laviad EL, Stiban J, Bandyopadhyay S, Merrill AH Jr, Prieto M, Futerman AH. *J Lipid Res*. 2012; 53:430–436. [PubMed: 22231783]
29. Hartel S, Fanani ML, Maggio B. *Biophys J*. 2005; 88:287–304. [PubMed: 15489298]
30. Newton J, Lima S, Maceyka M, Spiegel S. *Exp Cell Res*. 2015; 333:195–200. [PubMed: 25770011]
31. Proia RL, Hla T. *J Clin Invest*. 2015; 125:1379–1387. [PubMed: 25831442]
32. Hwang YH, Tani M, Nakagawa T, Okino N, Ito M. *Biochem Biophys Res Commun*. 2005; 331:37–42. [PubMed: 15845354]
33. Romiti E, Meacci E, Tanzi G, Becciolini L, Mitsutake S, Farnararo M, Ito M, Bruni P. *FEBS Lett*. 2001; 506:163–168. [PubMed: 11591392]
34. Mao C, Xu R, Bielawska A, Obeid LM. *J Biol Chem*. 2000; 275:6876–6884. [PubMed: 10702247]
35. Mao C, Xu R, Szulc ZM, Bielawski J, Becker KP, Bielawska A, Galadari SH, Hu W, Obeid LM. *J Biol Chem*. 2003; 278:31184–31191. [PubMed: 12783875]
36. Kitatani K, Idkowiak-Baldys J, Hannun YA. *Cell Signal*. 2008; 20:1010–1018. [PubMed: 18191382]
37. Novgorodov SA, Chudakova DA, Wheeler BW, Bielawski J, Kindy MS, Obeid LM, Gudz TI. *J Biol Chem*. 2011; 286:4644–4658. [PubMed: 21148554]
38. Lee H, Rotolo JA, Mesicek J, Penate-Medina T, Rimner A, Liao WC, Yin X, Ragupathi G, Ehleiter D, Gulbins E, Zhai D, Reed JC, Haimovitz-Friedman A, Fuks Z, Kolesnick R. *PLoS One*. 2011; 6:e19783. [PubMed: 21695182]

39. Jeckel D, Karrenbauer A, Burger KN, van Meer G, Wieland F. *J Cell Biol.* 1992; 117:259–267. [PubMed: 1532799]
40. Levine TP. *J Cell Biol.* 2007; 179:11–13. [PubMed: 17923527]
41. Hanada K. *Proceedings of the Japan Academy Series B, Physical and biological sciences.* 2010; 86:426–437.
42. Sprong H, Degroote S, Claessens T, van Drunen J, Oorschot V, Westerink BH, Hirabayashi Y, Klumperman J, van der Sluijs P, van Meer G. *J Cell Biol.* 2001; 155:369–380. [PubMed: 11673476]
43. Tamboli IY, Prager K, Barth E, Heneka M, Sandhoff K, Walter J. *J Biol Chem.* 2005; 280:28110–28117. [PubMed: 15923191]
44. Wojtal KA, de Vries E, Hoekstra D, van Ijzendoorn SC. *Mol Biol Cell.* 2006; 17:3638–3650. [PubMed: 16723498]
45. Melser S, Molino D, Batailler B, Peypelut M, Laloi M, Wattelet-Boyer V, Bellec Y, Faure JD, Moreau P. *Plant cell reports.* 2011; 30:177–193. [PubMed: 21120657]
46. Hannun YA, Obeid LM. *Nat Rev Mol Cell Biol.* 2008; 9:139–150. [PubMed: 18216770]
47. Jenkins RW, Canals D, Idkowiak-Baldys J, Simbari F, Roddy P, Perry DM, Kitatani K, Luberto C, Hannun YA. *J Biol Chem.* 2010; 285:35706–35718. [PubMed: 20807762]
48. Schissel SL, Keesler GA, Schuchman EH, Williams KJ, Tabas I. *J Biol Chem.* 1998; 273:18250–18259. [PubMed: 9660788]
49. Kornhuber J, Rhein C, Muller CP, Muhle C. *Biol Chem.* 2015; 396:707–736. [PubMed: 25803076]
50. Holopainen JM, Subramanian M, Kinnunen PK. *Biochemistry.* 1998; 37:17562–17570. [PubMed: 9860872]
51. Grassme H, Jekle A, Riehle A, Schwarz H, Berger J, Sandhoff K, Kolesnick R, Gulbins E. *J Biol Chem.* 2001; 276:20589–20596. [PubMed: 11279185]
52. Cremesti A, Paris F, Grassme H, Holler N, Tschopp J, Fuks Z, Gulbins E, Kolesnick R. *J Biol Chem.* 2001; 276:23954–23961. [PubMed: 11287428]
53. Grazide S, Maestre N, Veldman RJ, Bezombes C, Maddens S, Levade T, Laurent G, Jaffrezou JP. *FASEB J.* 2002; 16:1685–1687. [PubMed: 12206990]
54. Petersen NH, Olsen OD, Groth-Pedersen L, Ellegaard AM, Bilgin M, Redmer S, Ostfeld MS, Ulanet D, Dovmark TH, Lonborg A, Vindelov SD, Hanahan D, Arenz C, Ejsing CS, Kirkegaard T, Rohde M, Nylandsted J, Jaattela M. *Cancer cell.* 2013; 24:379–393. [PubMed: 24029234]
55. Petersen NH, Kirkegaard T, Olsen OD, Jaattela M. *Cell Cycle.* 2010; 9:2305–2309. [PubMed: 20519957]
56. Linke T, Wilkening G, Lansmann S, Moczall H, Bartelsen O, Weisgerber J, Sandhoff K. *Biol Chem.* 2001; 382:283–290. [PubMed: 11308026]
57. Kolzer M, Werth N, Sandhoff K. *FEBS Lett.* 2004; 559:96–98. [PubMed: 14960314]
58. Hurwitz R, Ferlinz K, Sandhoff K. *Biological chemistry Hoppe-Seyler.* 1994; 375:447–450. [PubMed: 7945993]
59. Aits S, Jaattela M. *J Cell Sci.* 2013; 126:1905–1912. [PubMed: 23720375]
60. Kirkegaard T, Roth AG, Petersen NH, Mahalka AK, Olsen OD, Moilanen I, Zylicz A, Knudsen J, Sandhoff K, Arenz C, Kinnunen PK, Nylandsted J, Jaattela M. *Nature.* 2010; 463:549–553. [PubMed: 20111001]
61. Johansson AC, Appelqvist H, Nilsson C, Kagedal K, Roberg K, Ollinger K. *Apoptosis.* 2010; 15:527–540. [PubMed: 20077016]
62. Utermohlen O, Herz J, Schramm M, Kronke M. *Immunobiology.* 2008; 213:307–314. [PubMed: 18406376]
63. Dumitru CA, Sandalcioglu IE, Wagner M, Weller M, Gulbins E. *Journal of molecular medicine.* 2009; 87:1123–1132. [PubMed: 19763526]
64. Siskind LJ, Kolesnick RN, Colombini M. *Mitochondrion.* 2006; 6:118–125. [PubMed: 16713754]
65. Chipuk JE, McStay GP, Bharti A, Kuwana T, Clarke CJ, Siskind LJ, Obeid LM, Green DR. *Cell.* 2012; 148:988–1000. [PubMed: 22385963]

66. Sezgin E, Levental I, Mayor S, Eggeling C. *Nat Rev Mol Cell Biol.* 2017; 18:361–374. [PubMed: 28356571]
67. Westerlund B, Slotte JP. *Biochim Biophys Acta.* 2009; 1788:194–201. [PubMed: 19073136]
68. Quinn PJ. *Biochim Biophys Acta.* 2014; 1838:1922–1930. [PubMed: 24613791]
69. Barenholz Y. *Subcell Biochem.* 2004; 37:167–215. [PubMed: 15376621]
70. Kaiser HJ, Lingwood D, Levental I, Sampaio JL, Kalvodova L, Rajendran L, Simons K. *Proc Natl Acad Sci U S A.* 2009; 106:16645–16650. [PubMed: 19805351]
71. Schuck S, Honsho M, Ekroos K, Shevchenko A, Simons K. *Proc Natl Acad Sci U S A.* 2003; 100:5795–5800. [PubMed: 12721375]
72. Lagerholm BC, Weinreb GE, Jacobson K, Thompson NL. *Annual review of physical chemistry.* 2005; 56:309–336.
73. Shaw AS. *Nature immunology.* 2006; 7:1139–1142. [PubMed: 17053798]
74. Simons K, Ikonen E. *Nature.* 1997; 387:569–572. [PubMed: 9177342]
75. Lillemeier BF, Pfeiffer JR, Surviladze Z, Wilson BS, Davis MM. *Proc Natl Acad Sci U S A.* 2006; 103:18992–18997. [PubMed: 17146050]
76. Hyde SC, Emsley P, Hartshorn MJ, Mimmack MM, Gileadi U, Pearce SR, Gallagher MP, Gill DR, Hubbard RE, Higgins CF. *Nature.* 1990; 346:362–365. [PubMed: 1973824]
77. Biedler JL, Riehm H. *Cancer Res.* 1970; 30:1174–1184. [PubMed: 5533992]
78. Ling V. *Canadian journal of genetics and cytology. Journal canadien de genetique et de cytologie.* 1975; 17:503–515. [PubMed: 1203765]
79. Juliano RL, Ling V. *Biochim Biophys Acta.* 1976; 455:152–162. [PubMed: 990323]
80. Kruh GD, Zeng H, Rea PA, Liu G, Chen ZS, Lee K, Belinsky MG. *J Bioenerg Biomembr.* 2001; 33:493–501. [PubMed: 11804191]
81. Doyle L, Ross DD. *Oncogene.* 2003; 22:7340–7358. [PubMed: 14576842]
82. Ambudkar SV, Kimchi-Sarfaty C, Sauna ZE, Gottesman MM. *Oncogene.* 2003; 22:7468–7485. [PubMed: 14576852]
83. Chen Y, Simon SM. *J Cell Biol.* 2000; 148:863–870. [PubMed: 10704438]
84. Shapiro AB, Ling V. *Acta physiologica Scandinavica Supplementum.* 1998; 643:227–234. [PubMed: 9789565]
85. Alvarez M, Paull K, Monks A, Hose C, Lee JS, Weinstein J, Grever M, Bates S, Fojo T. *J Clin Invest.* 1995; 95:2205–2214. [PubMed: 7738186]
86. van Helvoort A, Smith AJ, Sprong H, Fritzsche I, Schinkel AH, Borst P, van Meer G. *Cell.* 1996; 87:507–517. [PubMed: 8898203]
87. Eckford PD, Sharom FJ. *Biochem J.* 2005; 389:517–526. [PubMed: 15799713]
88. Lee WK, Torchalski B, Kohistani N, Thévenod F. *Toxicol Sci.* 2011; 121:343–356. [PubMed: 21436125]
89. Scotto KW. *Oncogene.* 2003; 22:7496–7511. [PubMed: 14576854]
90. Lee WK, Chakraborty PK, Thévenod F. *Int J Cancer.* 2013; 133:556–567. [PubMed: 23354914]
91. Sauna ZE, Kim IW, Ambudkar SV. *J Bioenerg Biomembr.* 2007; 39:481–487. [PubMed: 18058211]
92. Wolking S, Schaeffeler E, Lerche H, Schwab M, Nies AT. *Clinical pharmacokinetics.* 2015; 54:709–735. [PubMed: 25860377]
93. Huff LM, Lee JS, Robey RW, Fojo T. *J Biol Chem.* 2006; 281:36501–36509. [PubMed: 16956878]
94. Rund D, Azar I, Shperling O. *Advances in experimental medicine and biology.* 1999; 457:71–75. [PubMed: 10500782]
95. Reed K, Hembruff SL, Sprowl JA, Parissenti AM. *The pharmacogenomics journal.* 2010; 10:489–504. [PubMed: 20125118]
96. Huo H, Magro PG, Pietsch EC, Patel BB, Scotto KW. *Cancer Res.* 2010; 70:8726–8735. [PubMed: 20861184]
97. Yague E, Armesilla AL, Harrison G, Elliott J, Sardini A, Higgins CF, Raguz S. *J Biol Chem.* 2003; 278:10344–10352. [PubMed: 12525496]

98. Fojo AT, Ueda K, Slamon DJ, Poplack DG, Gottesman MM, Pastan I. *Proc Natl Acad Sci U S A*. 1987; 84:265–269. [PubMed: 2432605]
99. Thiebaut F, Tsuruo T, Hamada H, Gottesman MM, Pastan I, Willingham MC. *Proc Natl Acad Sci U S A*. 1987; 84:7735–7738. [PubMed: 2444983]
100. Fromm MF. *Trends Pharmacol Sci*. 2004; 25:423–429. [PubMed: 15276711]
101. Borst P, Schinkel AH. *J Clin Invest*. 2013; 123:4131–4133. [PubMed: 24084745]
102. Leonard GD, Fojo T, Bates SE. *The oncologist*. 2003; 8:411–424. [PubMed: 14530494]
103. Schinkel AH, Kemp S, Dolle M, Rudenko G, Wagenaar E. *J Biol Chem*. 1993; 268:7474–7481. [PubMed: 8096511]
104. Xie Y, Burcu M, Linn DE, Qiu Y, Baer MR. *Mol Pharmacol*. 2010; 78:310–318. [PubMed: 20460432]
105. Henrichs S, Wang B, Fukao Y, Zhu J, Charrier L, Bailly A, Oehring SC, Linnert M, Weiwad M, Endler A, Nanni P, Pollmann S, Mancuso S, Schulz A, Geisler M. *EMBO J*. 2012; 31:2965–2980. [PubMed: 22549467]
106. Bates SE, Lee JS, Dickstein B, Spolyar M, Fojo AT. *Biochemistry*. 1993; 32:9156–9164. [PubMed: 7690250]
107. Katayama K, Yamaguchi M, Noguchi K, Sugimoto Y. *Cancer Lett*. 2014; 345:124–131. [PubMed: 24333728]
108. Chen CJ, Chin JE, Ueda K, Clark DP, Pastan I, Gottesman MM, Roninson IB. *Cell*. 1986; 47:381–389. [PubMed: 2876781]
109. Gros P, Croop J, Housman D. *Cell*. 1986; 47:371–380. [PubMed: 3768958]
110. Aller SG, Yu J, Ward A, Weng Y, Chittaboina S, Zhuo R, Harrell PM, Trinh YT, Zhang Q, Urbatsch IL, Chang G. *Science*. 2009; 323:1718–1722. [PubMed: 19325113]
111. Loo TW, Bartlett MC, Clarke DM. *J Biol Chem*. 2003; 278:39706–39710. [PubMed: 12909621]
112. Chen Y, Pant AC, Simon SM. *Cancer Res*. 2001; 61:7763–7769. [PubMed: 11691790]
113. Loo TW, Clarke DM. *J Biol Chem*. 1993; 268:19965–19972. [PubMed: 8104183]
114. Gervasoni JE Jr, Fields SZ, Krishna S, Baker MA, Rosado M, Thuraisamy K, Hindenburg AA, Taub RN. *Cancer Res*. 1991; 51:4955–4963. [PubMed: 1680024]
115. Shapiro AB, Fox K, Lee P, Yang YD, Ling V. *Int J Cancer*. 1998; 76:857–864. [PubMed: 9626353]
116. Yamagishi T, Sahni S, Sharp DM, Arvind A, Jansson PJ, Richardson DR. *J Biol Chem*. 2013; 288:31761–31771. [PubMed: 24062304]
117. Crivellato E, Candussio L, Rosati AM, Decorti G, Klugmann FB, Mallardi F. *The Histochemical journal*. 1999; 31:635–643. [PubMed: 10576412]
118. Belhoussine R, Morjani H, Millot JM, Sharonov S, Manfait M. *J Histochem Cytochem*. 1998; 46:1369–1376. [PubMed: 9815278]
119. Arancia G, Calcabrini A, Meschini S, Molinari A. *Cytotechnology*. 1998; 27:95–111. [PubMed: 19002786]
120. De Rosa MF, Sillence D, Ackerley C, Lingwood C. *J Biol Chem*. 2004; 279:7867–7876. [PubMed: 14662772]
121. Beretta GL, Zunino F. *Topics in current chemistry*. 2008; 283:1–19. [PubMed: 23605626]
122. Calcabrini A, Meschini S, Stringaro A, Cianfriglia M, Arancia G, Molinari A. *The Histochemical journal*. 2000; 32:599–606. [PubMed: 11202156]
123. Szaflarski W, Sujka-Kordowska P, Januchowski R, Wojtowicz K, Andrzejewska M, Nowicki M, Zabel M. *Biomedicine & pharmacotherapy = Biomedecine & pharmacotherapie*. 2013; 67:497–502. [PubMed: 23602050]
124. Babakhanian K, Bendayan M, Bendayan R. *Biochem Biophys Res Commun*. 2007; 361:301–306. [PubMed: 17651695]
125. Solazzo M, Fantappie O, Lasagna N, Sassoli C, Nosi D, Mazzanti R. *Exp Cell Res*. 2006; 312:4070–4078. [PubMed: 17027968]
126. Munteanu E, Verdier M, Grandjean-Forestier F, Stenger C, Jayat-Vignoles C, Huet S, Robert J, Ratinaud MH. *Biochem Pharmacol*. 2006; 71:1162–1174. [PubMed: 16499877]

127. Paterson JK, Gottesman MM. *Exp Cell Res*. 2007; 313:3100–3105. [PubMed: 17512524]
128. Raviv Y, Pollard HB, Bruggemann EP, Pastan I, Gottesman MM. *J Biol Chem*. 1990; 265:3975–3980. [PubMed: 1968065]
129. Qu Q, Sharom FJ. *Biochemistry*. 2002; 41:4744–4752. [PubMed: 11926837]
130. Eytan GD. *Biomedicine & pharmacotherapy = Biomedecine & pharmacotherapie*. 2005; 59:90–97. [PubMed: 15795101]
131. Rauch C. *Cell biochemistry and biophysics*. 2011; 61:103–113. [PubMed: 21336588]
132. Omote H, Al-Shawi MK. *Biophys J*. 2006; 90:4046–4059. [PubMed: 16565061]
133. Clay AT, Sharom FJ. *Biochemistry*. 2013; 52:343–354. [PubMed: 23268645]
134. Seelig A, Landwojtowicz E. *Eur J Pharm Sci*. 2000; 12:31–40. [PubMed: 11121731]
135. Shapiro AB, Ling V. *Eur J Biochem*. 1997; 250:122–129. [PubMed: 9431999]
136. Shapiro AB, Ling V. *Eur J Biochem*. 1998; 254:181–188. [PubMed: 9652412]
137. Brown DA. *Physiology*. 2006; 21:430–439. [PubMed: 17119156]
138. Simons K, Sampaio JL. *Cold Spring Harbor perspectives in biology*. 2011; 3:a004697. [PubMed: 21628426]
139. Lavie Y, Fiucci G, Liscovitch M. *J Biol Chem*. 1998; 273:32380–32383. [PubMed: 9829965]
140. Klappe K, Hummel I, Hoekstra D, Kok JW. *Chem Phys Lipids*. 2009; 161:57–64. [PubMed: 19651114]
141. Radeva G, Perabo J, Sharom FJ, *FEBS J*. 2005; 272:4924–4937.
142. Fenyvesi F, Fenyvesi E, Szenté L, Goda K, Bacso Z, Bacsakay I, Varadi J, Kiss T, Molnar E, Janaky T, Szabo G Jr, Vecsernyes M. *Eur J Pharm Sci*. 2008; 34:236–242. [PubMed: 18539442]
143. Troost J, Lindenmaier H, Haefeli WE, Weiss J. *Mol Pharmacol*. 2004; 66:1332–1339. [PubMed: 15308763]
144. Eckford PD, Sharom FJ. *Biochemistry*. 2008; 47:13686–13698. [PubMed: 19049391]
145. Modok S, Heyward C, Callaghan R. *J Lipid Res*. 2004; 45:1910–1918. [PubMed: 15258203]
146. Molinari A, Calcabrini A, Meschini S, Stringaro A, Crateri P, Toccaceli L, Marra M, Colone M, Cianfriglia M, Arancia G. *Current protein & peptide science*. 2002; 3:653–670. [PubMed: 12470219]
147. van Blitterswijk WJ. *Biochem J*. 1998; 331(Pt 2):679–680. [PubMed: 9531568]
148. Yin X, Zafrullah M, Lee H, Haimovitz-Friedman A, Fuks Z, Kolesnick R. *Cell Physiol Biochem*. 2009; 24:219–230. [PubMed: 19710537]
149. Mahfoud R, Garmy N, Maresca M, Yahi N, Puigserver A, Fantini J. *J Biol Chem*. 2002; 277:11292–11296. [PubMed: 11792705]
150. Contreras FX, Ernst AM, Haberkant P, Bjorkholm P, Lindahl E, Gonen B, Tischer C, Elofsson A, von Heijne G, Thiele C, Pepperkok R, Wieland F, Brugger B. *Nature*. 2012; 481:525–529. [PubMed: 22230960]
151. Loo TW, Clarke DM. *J Biol Chem*. 1993; 268:3143–3149. [PubMed: 8094081]
152. Aanismaa P, Gatlik-Landwojtowicz E, Seelig A. *Biochemistry*. 2008; 47:10197–10207. [PubMed: 18759452]
153. Nakatsukasa H, Evarts RP, Burt RK, Nagy P, Thorgeirsson SS. *Mol Carcinog*. 1992; 6:190–198. [PubMed: 1359897]
154. Yamada T, Takaoka AS, Naishiro Y, Hayashi R, Maruyama K, Maesawa C, Ochiai A, Hirohashi S. *Cancer Res*. 2000; 60:4761–4766. [PubMed: 10987283]
155. Meijer GA, Schroeijers AB, Flens MJ, Meuwissen SG, van der Valk P, Baak JP, Scheper RJ. *Journal of clinical pathology*. 1999; 52:450–454. [PubMed: 10562814]
156. Hahn WC, Counter CM, Lundberg AS, Beijersbergen RL, Brooks MW, Weinberg RA. *Nature*. 1999; 400:464–468. [PubMed: 10440377]
157. Lingwood, CA. *Cold Spring Harbor perspectives in biology*. 2011. p. 3
158. Schnaar, RL., Suzuki, A., Stanley, P. *Essentials of Glycobiology*. 2. Varki, A. Cummings, RD. Esko, JD. Freeze, HH. Stanley, P. Bertozzi, CR. Hart, GW., Etzler, ME., editors. Cold Spring Harbor (NY); 2009.
159. Hakomori S. *Advances in cancer research*. 1973; 18:265–315. [PubMed: 4587356]

160. Peterson RH, Beutler WJ, Biedler JL. *Biochem Pharmacol.* 1979; 28:579–582. [PubMed: 444244]
161. Mazzoni A, Trave F, Fabbri M, Leto G, Ghidoni R. *Anticancer research.* 1991; 11:2181–2185. [PubMed: 1776860]
162. Lavie Y, Cao H, Bursten SL, Giuliano AE, Cabot MC. *J Biol Chem.* 1996; 271:19530–19536. [PubMed: 8702646]
163. Morjani H, Aouali N, Belhoussine R, Veldman RJ, Levade T, Manfait M. *Int J Cancer.* 2001; 94:157–165. [PubMed: 11668492]
164. Liu YY, Han TY, Giuliano AE, Cabot MC. *FASEB J.* 2001; 15:719–730. [PubMed: 11259390]
165. Liu YY, Yu JY, Yin D, Patwardhan GA, Gupta V, Hirabayashi Y, Holleran WM, Giuliano AE, Jazwinski SM, Gouaze-Andersson V, Consoli DP, Cabot MC. *FASEB J.* 2008; 22:2541–2551. [PubMed: 18245173]
166. Uchida Y, Itoh M, Taguchi Y, Yamaoka S, Umehara H, Ichikawa S, Hirabayashi Y, Holleran WM, Okazaki T. *Cancer Res.* 2004; 64:6271–6279. [PubMed: 15342415]
167. Itoh M, Kitano T, Watanabe M, Kondo T, Yabu T, Taguchi Y, Iwai K, Tashima M, Uchiyama T, Okazaki T. *Clinical cancer research : an official journal of the American Association for Cancer Research.* 2003; 9:415–423. [PubMed: 12538495]
168. Nicholson KM, Quinn DM, Kellett GL, Warr JR. *Br J Cancer.* 1999; 81:423–430. [PubMed: 10507766]
169. Stefanovic M, Tutusaus A, Martinez-Nieto GA, Barcena C, de Gregorio E, Moutinho C, Barbero-Camps E, Villanueva A, Colell A, Mari M, Garcia-Ruiz C, Fernandez-Checa JC, Morales A. *Oncotarget.* 2016; 7:8253–8267. [PubMed: 26811497]
170. Liu J, Zhang X, Liu A, Zhang D, Su Y, Liu Y, You D, Yuan L, Kong X, Wang X, Sun P. *Oncotarget.* 2016; 7:36755–36766. [PubMed: 27191984]
171. Kim JW, Park Y, Roh JL, Cho KJ, Choi SH, Nam SY, Kim SY. *International journal of clinical oncology.* 2016; 21:883–889. [PubMed: 27000845]
172. Zhang C, Lin X, Song Y, Zhang X, Li H, Wang Q. *Chin Med J (Engl).* 2014; 127:3071–3076. [PubMed: 25189947]
173. Lavie Y, Cao H, Volner A, Lucci A, Han TY, Geffen V, Giuliano AE, Cabot MC. *J Biol Chem.* 1997; 272:1682–1687. [PubMed: 8999846]
174. Gerrard G, Butters TD, Ganeshaguru K, Mehta AB. *Eur J Pharmacol.* 2009; 609:34–39. [PubMed: 19285492]
175. Liu YY, Gupta V, Patwardhan GA, Bhinge K, Zhao Y, Bao J, Mehendale H, Cabot MC, Li YT, Jazwinski SM. *Mol Cancer.* 2010; 9:145. [PubMed: 20540746]
176. Liu YY, Han TY, Giuliano AE, Cabot MC. *J Biol Chem.* 1999; 274:1140–1146. [PubMed: 9873062]
177. Prinetti A, Basso L, Appierto V, Villani MG, Valsecchi M, Loberto N, Prioni S, Chigorno V, Cavadini E, Formelli F, Sonnino S. *J Biol Chem.* 2003; 278:5574–5583. [PubMed: 12486134]
178. Tepper AD, Diks SH, van Blitterswijk WJ, Borst J. *J Biol Chem.* 2000; 275:34810–34817. [PubMed: 10945987]
179. Veldman RJ, Mita A, Cuvillier O, Garcia V, Klappe K, Medin JA, Campbell JD, Carpentier S, Kok JW, Levade T. *FASEB J.* 2003; 17:1144–1146. [PubMed: 12692077]
180. Norris-Cervetto E, Callaghan R, Platt FM, Dwek RA, Butters TD. *J Biol Chem.* 2004; 279:40412–40418. [PubMed: 15263008]
181. Bieberich E, Freischutz B, Suzuki M, Yu RK. *J Neurochem.* 1999; 72:1040–1049. [PubMed: 10037475]
182. Rani CS, Abe A, Chang Y, Rosenzweig N, Saltiel AR, Radin NS, Shayman JA. *J Biol Chem.* 1995; 270:2859–2867. [PubMed: 7852361]
183. Dijkhuis AJ, Klappe K, Jacobs S, Kroesen BJ, Kamps W, Sietsma H, Kok JW. *Mol Cancer Ther.* 2006; 5:593–601. [PubMed: 16546973]
184. Gouaze V, Liu YY, Prickett CS, Yu JY, Giuliano AE, Cabot MC. *Cancer Res.* 2005; 65:3861–3867. [PubMed: 15867385]
185. Gouaze-Andersson V, Yu JY, Kreitenberg AJ, Bielawska A, Giuliano AE, Cabot MC. *Biochim Biophys Acta.* 2007; 1771:1407–1417. [PubMed: 18035065]

186. Gutierrez-Iglesias G, Hurtado Y, Palma-Lara I, Lopez-Marure R. *Cancer Chemother Pharmacol.* 2014; 74:809–817. [PubMed: 25113261]
187. Dahdouh F, Raane M, Thevenod F, Lee WK. *Arch Toxicol.* 2014; 88:881–892. [PubMed: 24448832]
188. Huet S, Schott B, Robert J. *Br J Cancer.* 1992; 65:538–544. [PubMed: 1348623]
189. Devine SE, Melera PW. *J Biol Chem.* 1994; 269:6133–6139. [PubMed: 7907088]
190. Azzam R, Hariri F, El-Hachem N, Kamar A, Dbaibo G, Nemer G, Bitar F. *DNA Cell Biol.* 2013; 32:310–319. [PubMed: 23672204]
191. Wang Z, Kirkwood JS, Taylor AW, Stevens JF, Leid M, Ganguli-Indra G, Indra AK. *J Invest Dermatol.* 2013; 133:668–676. [PubMed: 23096701]
192. O'Neill SM, Houck KL, Yun JK, Fox TE, Kester M. *Arch Biochem Biophys.* 2011; 511:31–39. [PubMed: 21530485]
193. Ito H, Yoshida K, Murakami M, Hagiwara K, Sasaki N, Kobayashi M, Takagi A, Kojima T, Sobue S, Suzuki M, Tamiya-Koizumi K, Nakamura M, Banno Y, Nozawa Y, Murate T. *Biochim Biophys Acta.* 2011; 1811:119–128. [PubMed: 21184844]
194. Kilbey A, Terry A, Jenkins A, Borland G, Zhang Q, Wakelam MJ, Cameron ER, Neil JC. *Cancer Res.* 2010; 70:5860–5869. [PubMed: 20587518]
195. Lucki N, Sewer MB. *Biochim Biophys Acta.* 2009; 1791:706–713. [PubMed: 19298866]
196. Tsai YT, Yu RK. *J Neurochem.* 2014; 128:101–110. [PubMed: 24102378]
197. Lebman DA, Spiegel S. *J Lipid Res.* 2008; 49:1388–1394. [PubMed: 18387885]
198. Lopez PH, Schnaar RL. *Current opinion in structural biology.* 2009; 19:549–557. [PubMed: 19608407]
199. Don AS, Lim XY, Couttas TA. *Biomolecules.* 2014; 4:315–353. [PubMed: 24970218]
200. Truman JP, Garcia-Barros M, Obeid LM, Hannun YA. *Biochim Biophys Acta.* 2014; 1841:1174–1188. [PubMed: 24384461]
201. Silins I, Nordstrand M, Hogberg J, Stenius U. *Carcinogenesis.* 2003; 24:1077–1083. [PubMed: 12807752]
202. Tang H, Choy PC, Chen H. *Mol Cell Biochem.* 1992; 109:83–87. [PubMed: 1614422]
203. Borek C, Ong A, Stevens VL, Wang E, Merrill AH Jr. *Proc Natl Acad Sci U S A.* 1991; 88:1953–1957. [PubMed: 2000399]
204. Salmanzadeh A, Elvington ES, Roberts PC, Schmelz EM, Davalos RV. *Integrative biology : quantitative biosciences from nano to macro.* 2013; 5:843–852. [PubMed: 23609351]
205. Babahosseini H, Roberts PC, Schmelz EM, Agah M. *Integrative biology : quantitative biosciences from nano to macro.* 2013; 5:1385–1392. [PubMed: 24056950]
206. Schmelz EM, Sullards MC, Dillehay DL, Merrill AH Jr. *The Journal of nutrition.* 2000; 130:522–527. [PubMed: 10702579]
207. Dillehay DL, Webb SK, Schmelz EM, Merrill AH Jr. *The Journal of nutrition.* 1994; 124:615–620. [PubMed: 8169652]
208. Schmelz EM, Dillehay DL, Webb SK, Reiter A, Adams J, Merrill AH Jr. *Cancer Res.* 1996; 56:4936–4941. [PubMed: 8895747]
209. Kawamori T, Kaneshiro T, Okumura M, Maalouf S, Uflacker A, Bielawski J, Hannun YA, Obeid LM. *FASEB J.* 2009; 23:405–414. [PubMed: 18824518]
210. Campanella R. *Journal of neurosurgical sciences.* 1992; 36:11–25. [PubMed: 1323645]
211. Glunde K, Bhujwalla ZM. *Seminars in oncology.* 2011; 38:26–41. [PubMed: 21362514]
212. Eppler CM, Malewicz B, Jenkin HM, Baumann WJ. *Lipids.* 1987; 22:351–357. [PubMed: 3600211]
213. Narayan P, Dahiya R. *Biomedica biochimica acta.* 1991; 50:1099–1108. [PubMed: 1724598]
214. Das SC, Isichei UP. *Indian journal of experimental biology.* 1989; 27:538–544. [PubMed: 2583743]
215. Moka D, Dietlein M, Raffelt K, Hahn J, Schicha H. *Am J Med.* 2002; 112:634–641. [PubMed: 12034413]

216. Preetha A, Banerjee R, Huilgol N. *Journal of cancer research and therapeutics*. 2005; 1:180–186. [PubMed: 17998650]
217. Ledwozyw A, Lutnicki K. *Acta physiologica Hungarica*. 1992; 79:381–387. [PubMed: 1343190]
218. Poduslo SE, Miller K, Jang Y. *Cancer Res*. 1983; 43:1014–1018. [PubMed: 6825076]
219. Upreti GC, deAntueno RJ, Wood R. *J Natl Cancer Inst*. 1983; 70:567–573. [PubMed: 6572744]
220. Wood R, Upreti GC, deAntueno RJ. *Lipids*. 1986; 21:292–300. [PubMed: 3713448]
221. Krautbauer S, Meier EM, Rein-Fischboeck L, Pohl R, Weiss TS, Sigrüener A, Aslanidis C, Liebisch G, Buechler C. *Biochim Biophys Acta*. 2016; 1861:1767–1774. [PubMed: 27570113]
222. Nagahashi M, Tsuchida J, Moro K, Hasegawa M, Tatsuda K, Woelfel IA, Takabe K, Wakai T. *The Journal of surgical research*. 2016; 204:435–444. [PubMed: 27565080]
223. Abramczyk H, Brozek-Pluska B. *Analytica chimica acta*. 2016; 909:91–100. [PubMed: 26851089]
224. Merchant TE, Diamantis PM, Lauwers G, Haida T, Kasimos JN, Guillem J, Glonek T, Minsky BD. *Cancer*. 1995; 76:1715–1723. [PubMed: 8625039]
225. Sinha R, Ahn J, Sampson JN, Shi J, Yu G, Xiong X, Hayes RB, Goedert JJ. *PLoS One*. 2016; 11:e0152126. [PubMed: 27015276]
226. Wittmann BM, Stirdivant SM, Mitchell MW, Wulff JE, McDunn JE, Li Z, Dennis-Barrie A, Neri BP, Milburn MV, Lotan Y, Wolfert RL. *PLoS One*. 2014; 9:e115870. [PubMed: 25541698]
227. Seijo L, Merchant TE, van der Ven LT, Minsky BD, Glonek T. *Lipids*. 1994; 29:359–364. [PubMed: 8015367]
228. Kuliszkievicz-Janus M, Tuz MA, Kielbinski M, Jazwiec B, Niedoba J, Baczynski S. *Cellular & molecular biology letters*. 2009; 14:35–45. [PubMed: 18839072]
229. Merchant TE, de Graaf PW, Minsky BD, Obertop H, Glonek T. *NMR in biomedicine*. 1993; 6:187–193. [PubMed: 8347452]
230. Merchant TE, Kasimos JN, de Graaf PW, Minsky BD, Gierke LW, Glonek T. *International journal of colorectal disease*. 1991; 6:121–126. [PubMed: 1875121]
231. Merrill AH Jr, Wang E, Wertz PW. *Lipids*. 1986; 21:529–530. [PubMed: 3762323]
232. Dyatlovitskaya EV, Kandyba AG, Kozlov AM, Somova OG. *Biochemistry (Mosc)*. 2001; 66:502–504. [PubMed: 11405884]
233. Callaghan R, van Gorkom LC, Epand RM. *Br J Cancer*. 1992; 66:781–786. [PubMed: 1358166]
234. Ramu A, Glaubiger D, Weintraub H. *Cancer treatment reports*. 1984; 68:637–641. [PubMed: 6713419]
235. Peetla C, Bhawe R, Vijayaraghavalu S, Stine A, Kooijman E, Labhasetwar V. *Mol Pharm*. 2010; 7:2334–2348. [PubMed: 20958074]
236. Lafont E, Kitatani K, Okazaki T, Segui B. *Recent patents on anti-cancer drug discovery*. 2011; 6:324–333. [PubMed: 21762071]
237. Mannechez A, Reungpatthanaphong P, de Certaines JD, Leray G, Le Moyec L. *Cancer Cell Int*. 2005; 5:2. [PubMed: 15703065]
238. Veldman RJ, Klappe K, Hinrichs J, Hummel I, van der Schaaf G, Sietsma H, Kok JW. *FASEB J*. 2002; 16:1111–1113. [PubMed: 12039850]
239. Ramu A, Ramu N, Rosario LM. *Biochem Pharmacol*. 1991; 41:1455–1461. [PubMed: 2018553]
240. Ramu A, Glaubiger D, Magrath IT, Joshi A. *Cancer Res*. 1983; 43:5533–5537. [PubMed: 6311408]
241. Siegfried JA, Kennedy KA, Sartorelli AC, Tritton TR. *J Biol Chem*. 1983; 258:339–343. [PubMed: 6848505]
242. Wheeler C, Rader R, Kessel D. *Biochem Pharmacol*. 1982; 31:2691–2693. [PubMed: 7138564]
243. Montaudon D, Vrignaud P, Londres-Gagliardi D, Robert J. *Cancer Res*. 1986; 46:5602–5605. [PubMed: 3756908]
244. Rintoul DA, Center MS. *Cancer Res*. 1984; 44:4978–4980. [PubMed: 6488160]
245. Patwardhan GA, Liu YY. *Progress in lipid research*. 2011; 50:104–114. [PubMed: 20970453]
246. Hait NC, Allegood J, Maceyka M, Strub GM, Harikumar KB, Singh SK, Luo C, Marmorstein R, Kordula T, Milstien S, Spiegel S. *Science*. 2009; 325:1254–1257. [PubMed: 19729656]

247. Degagne E, Pandurangan A, Bandhuvula P, Kumar A, Eltanawy A, Zhang M, Yoshinaga Y, Nefedov M, de Jong PJ, Fong LG, Young SG, Bittman R, Ahmedi Y, Saba JD. *J Clin Invest*. 2014; 124:5368–5384. [PubMed: 25347472]
248. Zhang Y, Dong J, Zhu X, Wang W, Yang Q. *Biochem Pharmacol*. 2011; 82:287–294. [PubMed: 21554861]
249. Sillence DJ, Puri V, Marks DL, Butters TD, Dwek RA, Pagano RE, Platt FM. *J Lipid Res*. 2002; 43:1837–1845. [PubMed: 12401882]
250. Simons K, Gruenberg J. *Trends Cell Biol*. 2000; 10:459–462. [PubMed: 11050411]
251. De Rosa MF, Ackerley C, Wang B, Ito S, Clarke DM, Lingwood C. *J Biol Chem*. 2008; 283:4501–4511. [PubMed: 18003606]
252. te Vrugte D, Lloyd-Evans E, Veldman RJ, Neville DC, Dwek RA, Platt FM, van Blitterswijk WJ, Sillence DJ. *J Biol Chem*. 2004; 279:26167–26175. [PubMed: 15078881]
253. Pagano RE. *Philosophical transactions of the Royal Society of London. Series B, Biological sciences*. 2003; 358:885–891.
254. Chen CS, Patterson MC, Wheatley CL, O'Brien JF, Pagano RE. *Lancet*. 1999; 354:901–905. [PubMed: 10489949]
255. Zhang X, Li J, Qiu Z, Gao P, Wu X, Zhou G. *Cancer Biol Ther*. 2009; 8:1117–1121. [PubMed: 19502811]

Highlights

- Interdependence of sphingolipids and ABCB1 in multidrug resistance (MDR) is discussed
- Progressive sphingolipid abnormalities occur in multistage development of MDR
- Initial partial MDR phenotype sees increased sphingomyelin and ABCB1 functionalisation
- Glucosylceramide abnormalities perturb trafficking of Golgi secretory vesicles
- Appearance of intracellular vesicular ABCB1 confers complete MDR phenotype

Figure 1. Overview of sphingolipid intermediary metabolism

The backbone of sphingolipid metabolism is the ceramide molecule. De novo synthesis and degradation pathways represent formation of new ceramide molecules and irreversible ceramide removal, respectively. Ceramide can be generated from other sphingolipid moieties, such as sphingomyelin and sphingosine. Ceramide pools can also be replenished by the recycling/salvage pathway that utilizes enzymes in acidic compartments to form free sphingosine and ceramide generation is completed by ceramide synthases in the ER. In turn, ceramide is used as the building block to spawn other sphingolipids, such as glycosphingolipids, sphingosine-1-phosphate and sphingomyelin, through the activity of specific sphingolipid metabolizing enzymes.

SPT, serine palmitoyltransferase; 3-KSR, 3-ketosphinganine reductase; CS, ceramide synthase; CD, ceramidase; SMS, sphingomyelin synthase; SMase, sphingomyelinase; A-SMase, acid sphingomyelinase; GBA, glucosylceramidase beta; A-CD, acid ceramidase; GCS, glucosylceramide synthase; GCase, glucosylceramidase; LCS, lactosylceramide synthase; β -GS, beta-galactosidase; STs, sialyltransferases; GTs, glucosyltransferases; SK, sphingosine kinase; S1PP, sphingosine-1-phosphate phosphatase; S1PL, sphingosine-1-phosphate lyase.

Figure 2. Model of multistage MDR development driven by perturbations in sphingomyelin, glucosylceramide and ABCB1

Cells are targeted by extrinsic tumor promoting signals leading to procurement of genetic modifications that drive imbalances in lipid metabolism and sphingolipid pools, resulting in elevated sphingomyelin (SM). In parallel, signaling pathways are modified resulting in aberrant expression of oncoproteins, some of which transcriptionally upregulate ABCB1. Together, they confer a partial MDR phenotype through lipid raft-mediated ABCB1 functionalization. Amplificatory loops contribute to recruitment of additional cellular adaptations that advance oncogenic transformation. A putative pivotal alteration is a disturbance in glycosphingolipids mediated by ABCB1 resulting in increased glucosylceramide synthase (GCS) and hence glucosylceramide (GluCer) levels, culminating in perturbed ABCB1 trafficking and expansion of drug sequestration capacity through intracellular vesicular ABCB1. The conferment of the complete MDR phenotype is consummated.