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Biophysics of Cell Membrane Lipids in Cancer Drug Resistance: Implications for Drug Transport and Drug Delivery with Nanoparticles

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Abstract

In this review, we focus on the biophysics of cell membrane lipids, particularly when cancers develop acquired drug resistance, and how biophysical changes in resistant cell membrane influence drug transport and nanoparticle-mediated drug delivery. Recent advances in membrane lipid research show the varied roles of lipids in regulating membrane P-glycoprotein function, membrane trafficking, apoptotic pathways, drug transport, and endocytic functions, particularly endocytosis, the primary mechanism of cellular uptake of nanoparticle-based drug delivery systems. Since acquired drug resistance alters lipid biosynthesis, understanding the role of lipids in cell membrane biophysics and its effect on drug transport is critical for developing effective therapeutic and drug delivery approaches to overcoming drug resistance. Here we discuss novel strategies for (a) modulating the biophysical properties of membrane lipids of resistant cells to facilitate drug transport and regain endocytic function and (b) developing effective nanoparticles based on their biophysical interactions with membrane lipids to enhance drug delivery and overcome drug resistance.

Keywords

Endocytosis; P-glycoprotein efflux; Membrane fluidity; Apoptosis; Multidrug resistance; Nanomedicine

1. Introduction

The risk that tumors may acquire resistance to cancer chemotherapy drugs remains a major clinical issue in successfully treating cancer patients [1]. In general, efficacy of cancer chemotherapy is limited by the doses that a patient can tolerate because of significantly greater risk of nonspecific toxicity of these drugs at higher doses [2]. Acquired drug resistance thus could be the effect of subtherapeutic doses to which tumors are exposed, causing cancer cells to adapt to the changing microenvironment [3]. The major known

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mechanisms of drug resistance, such as increased drug efflux via permeability glycoprotein (P-glycoprotein [P-gp]); also known as multidrug resistance [MDR] protein 1) extrusion pumps [4, 5], reduced drug influx, drug entrapment in intracellular vesicles [6, 7], activation of anti-apoptotic pathways, inactivation of apoptotic pathways, and modifications of metabolic pathways [8, 9], can be broadly grouped into two categories: (a) mechanisms that decrease intracellular drug accumulation and (b) mechanisms that alter apoptotic pathways to prevent cancer cell death. In addition, genetic and epigenetic changes that could influence drug interactions with a target gene or could modulate a cancer cell's response to a drug have also been suspected as mechanisms of acquired drug resistance. For example, cancer cells with p53 mutation are resistant to cancer chemotherapy primarily due to the loss of the transcriptional function of wild-type p53. Further, mutant p53 may gain new function in conferring the increases in cell survival, cell growth, and decrease in apoptosis in cells [10, 11]. Similarly, epigenetic changes in cells, such as methylation of DNA or acetylation of histone, can influence the response of anticancer drugs [12, 13]. Resistance to platinum-based cancer chemotherapy is linked to high expression of ERCC1 (Excision repair cross-complementation group 1) activity that facilitates DNA repair [14]. Our recent study has demonstrated that epigenetic changes in breast cancer resistant cells are linked to the altered lipid biosynthesis that directly influenced drug transport and endocytic functions in drug-resistant cells [15]. These examples illustrate different mechanisms of drug resistance.

Significant effort has been devoted to developing nanoparticle (NP)-based drug delivery systems, primarily to circumvent the difficulties of drug transport into cancer cells [16–18]. The widely used approaches involve loading NPs with a combination of drugs that act synergistically by targeting different pathways responsible for drug resistance. Of these, the most common strategies combine a P-gp efflux modulator with anticancer therapy to facilitate intracellular retention of drugs [19, 20] or combine anticancer drugs with agents that promote apoptosis [21, 22]. Recent approaches include the use of anticancer drugs in combination with small interfering RNA or microRNA [23], which can sensitize chemotherapy-resistant cells by silencing the expression of multidrug-resistant genes [24–26]. In addition, combinations of genes and drug therapy have been widely investigated to overcome drug resistance [27, 28].

Although the onset of cancer may have a genetic trigger, significant evidence shows that changes also occur in the lipid profile and biophysical properties of membrane lipids during malignant transformation of cells [29]. The biophysical properties of tumor tissue lipids have been investigated to understand the mechanisms of cancer progression and metastasis, to obtain a prognostic evaluation of the disease state [30], and to monitor response to drug/radiation therapies [31]. It has been reported that anionic phospholipids, which are largely absent from the external leaflet of the plasma membrane of mammalian cells under normal conditions, are exposed during malignant transformation and stress conditions of the tumor microenvironment [32]. However, the role of these changes in the biophysics of membrane lipids, particularly when cancer develops acquired drug resistance to cancer chemotherapy, and how the changes influence drug transport and delivery with NP-based systems has not been investigated.

Recent advances in membrane lipid research show that lipids play a role in the regulation of membrane trafficking and endocytic functions such as endocytosis, the primary mechanism of uptake of NP-based drug delivery systems [33]. There is also convincing evidence that sorting of vesicles and their intracellular trafficking occurs by formation of highly curved transport intermediates [34]. For example, endosomes sort vesicles based on membrane lipid biophysical properties: endocytic vesicles with fluid lipid membranes are sorted toward recycling endosomes, whereas those with rigid membranes are sorted toward late endosomes [35]. From a drug delivery perspective, these developments in membrane biology are very

critical, as most known drug-resistance mechanisms also show alterations in membrane lipids [36, 37], and these alterations may also affect the transport dynamics of drugs or NP-based drug delivery systems. Hence, understanding the role of membrane biophysics in drug resistance is critical for developing effective therapeutic and drug delivery approaches to overcome drug resistance. In this review, we focused primarily on the role of membrane biophysics on drug-resistance mechanisms affecting intracellular drug accumulation, apoptotic signaling, and novel strategies towards developing effective NP-based drug delivery systems based on biophysics of membrane lipids to overcome drug resistance.

2. Role of membrane biophysics on cellular functions affecting drug/nanocarrier accumulation inside cells

The role of membrane lipid composition in signaling and protein functions has been well recognized [38, 39]. In addition, cell apoptosis has also been shown to depend on membrane lipid composition and hence biophysical properties of cell membrane [40]. In other words, various cell membrane functions can be explained on the basis of physical characteristics, i.e., the molecular shape of lipids, known as lipid polymorphism. Based on the distinct molecular shapes of major membrane lipids, they can be divided into three groups [41–43]: cylinder-shaped lipids, cone-shaped lipids, and inverted-cone-shaped lipids. These distinctions in molecular shape arise due to differences in the relative size of each one's headgroup and tail. Major membrane lipids with phosphocholine (PC) and phosphatidyl serine (PS) have a cylindrical shape, as they have headgroups and tails with similar cross-sectional area. Sphingomyelin (SM) has an inverted-cone shape due to its large headgroup; therefore, SM lipids preferentially adopt the lamellar bilayers [44, 45]. Phosphatidyl ethanolamine (PE) and cholesterol display a cone shape because of their small headgroups and prefer nonlamellar bilayers, such as spherical vesicles (Figure 1).

In addition, phospholipids show different degrees of affinity to cholesterol; affinity for cholesterol for different phospholipids is in the order $SM > PS > PC > PE$, suggesting that phospholipid affinity toward cholesterol also depends on the molecular shape of the lipids [46]. For instance, SM, with its inverted cone shape, can protect the larger hydrophobic group of cholesterol better than the cone-shaped phospholipid PE can, therefore, higher levels of SM in the membrane are usually associated with higher cholesterol, whereas an increase in PE results in a lowering of cholesterol. Due to differences in molecular shapes and volumes of lipids, changes in lipid composition can affect the membrane's curvature and permeability (Figure 2). Acyl chain saturation of lipids and fatty acids significantly influences the membrane's biophysical properties, which in turn affect membrane permeability and endocytic functions (Figure 3a & 3b). In addition, acyl chain length of membrane lipids synchronizes with the length of transmembrane proteins. For example, longer transmembrane proteins are associated with lipids with longer acyl chain length and vice versa, which in turn influences the membrane thickness (Figure 3c & 3d).

3. Effect of membrane biophysics on intracellular drug accumulation in resistant cells

From a biophysical perspective, drug-resistance mechanisms affecting intracellular drug accumulation share strong similarities to the mechanisms that control drug or NP transport into cancer cells. In both cases, drug accumulation inside cells depends on the cell membrane's lipid profile and on the interactions of the cell membrane with drugs or NPs. In general, drug-resistant cells prevent intracellular drug accumulation by three different biological processes, namely, reduced drug influx, P-gp efflux, and drug entrapment in intracellular vesicles. Interestingly, all three of these drug-resistance mechanisms have been

reported to have common membrane lipid alterations, i.e., increase in sphingolipids and cholesterol membrane content.

3.1. Membrane biophysics and reduced drug influx

Reduced drug influx is considered to be one of the major factors in low intracellular drug accumulation in drug-resistant cells. Most cancer chemotherapeutics are weak bases with pK values between 7.4 and 8.2 and are lipophilic in neutral form; hence, they are assumed to traverse the cell membrane [47]. Therefore, any decrease in drug influx is generally attributed to alterations in the membrane's biophysical properties. To understand the role of membrane lipids, studies have been deliberately designed to alter the membrane's biophysical properties by growing cells in the presence of anionic phospholipids, saturated or unsaturated fatty acids, or other components affecting membrane properties [48, 49]. The results of these studies have demonstrated that membranes from drug-resistant cells have a different lipid composition than the membranes from the parent drug-sensitive cells. These changes lower the ability of a drug to permeate the membrane by altering membrane fluidity, structural order, lipid packing density, membrane potential or the combination of multiple factors [36, 37]. For instance, a doxorubicin-resistant P388 subline showed a decrease in the membrane's phosphatidylcholine/SM ratio and an increase in membrane order [50, 51]. On the other hand, vinblastine-resistant leukemia T lymphoblast cells that demonstrated higher levels of cholesterol and phospholipids and a 60% increase in protein/lipid ratio in comparison to membranes of sensitive cells [52].

There are very few studies that quantified the effect of lipid bilayer properties on the drug membrane partitioning, binding to P-gp, and efflux by P-gp. Jin et al. have reported significantly higher apparent affinities to P-gp for drugs, actinomycin D (4,000 times) and paclitaxel (100 times) when it is present in P-gp over expressing yeast cell membrane bilayer than with purified P-gp in a detergent layer. The above difference in anticancer drug affinity for P-gp highlights the importance of membrane phase when a drug accesses the P-gp transporter in the membrane [53]. Clay et al. quantified the role of membrane phase in modulating P-gp function, using purified P-gp that is reconstituted into phospholipid bilayers with defined gel to liquid-crystalline melting transitions. Study showed that P-gp has greater affinity to substrates when the lipid bilayer is in the gel phase than in the liquid phase [54]. Overall these studies suggest that P-gp function significantly depends on the lipid composition and hence the biophysical characteristic cell membrane lipid bilayer.

While the change in lipid composition in resistant cells could play a role in drug transport and efflux, it is also possible that *in vivo* the acidic tumor microenvironment could change drugs into ionized form that could reduce their transport across cell membrane. It is known the acidic environment causes cancer cells to become resistant, and studies have shown that proton pump inhibitors sensitize resistant tumors to anticancer drugs [55]. However, the ionic state of the drug in acidic pH in tumor microenvironment may not be relevant when these cell lines isolated from the tumor still show drug resistance under *in vitro* conditions under physiological pH.

Membrane potential has also been reported to affect drug influx. A four-fold lower membrane potential was reported for drug-resistant Friend leukemia cells compared to sensitive cells [56]. This observation was correlated to lower cationic drug accumulation in drug-resistant cells [57]. In some cases, lipid acyl chain mobility, an indicator of membrane fluidity, changes; however, the lipid composition differences are indistinguishable. For example, in Chinese hamster ovary-resistant cells, proton nuclear magnetic resonance (^1H NMR) and gas liquid chromatography studies showed a decrease in the mobility of lipid acyl chains [58]. Thus, these studies in general show an increase in sphingolipids or/and cholesterol content, and membrane order in drug-resistant cells which suggest that these

cells have less permeable cell membrane that significantly reduces diffusion of lipophilic drugs.

In our previous study [59], we isolated lipids from doxorubicin-sensitive (MCF-7) and doxorubicin-resistant (MCF-7/ADR) breast cancer cells to characterize the biophysical properties of their membrane lipids (particularly lipid packing and membrane fluidity) and to understand the effects doxorubicin interaction with cell membrane lipids and its effect on drug transport. The membrane lipids of resistant cells showed significantly altered composition; particularly noticeable was the higher concentration of SM, phosphatidylinositol, cholesterol, and cholesterol esters in the resistant cell membrane lipids than in the sensitive cell membrane lipids. Biophysical characterization of the lipids isolated from resistant and sensitive cells demonstrated that the membrane lipids of resistant cells are more rigid than those of sensitive cells. Doxorubicin showed a strong hydrophobic interaction with resistant cell membrane lipids but significantly less interaction, as well as a different pattern of interaction (i.e., ionic) with sensitive cell membrane lipids. The lipid-doxorubicin interactions appeared to reduce intracellular drug transport via diffusion as the drug becomes trapped in the lipid bilayer (Figure 4). In this regard, biophysical interaction studies with cell membrane lipids might be helpful to designing drugs through drug discovery approaches that can overcome the transport barrier in resistant cells.

3.2. Membrane biophysics and drug sequestration

Drug sequestration is one of the drug-resistance mechanisms whereby drugs are entrapped in intracellular acidic compartments, such as lysosomes, recycling endosomes, and the trans-Golgi network [60–62], which eventually lead the sequestered drug out of cells via exocytosis. In general, sequestration of drugs in various intracellular vesicles has been attributed to the difference in pH between cytoplasm and intracellular vesicles [63]. In drug resistant cells, intracellular vesicles have lower pH compared to the cytoplasmic pH. Anticancer drugs that enter into the cell via endocytosis become protonated in acidic pH, which renders drugs impermeable across the membrane of intracellular vesicles. Sequestration of chemotherapeutic drugs in acidic organelles/vesicles decreases the concentration of drugs in the cytoplasm, thus impairing their efficacy [64, 65]. The significance of this difference in pH was clearly demonstrated in doxorubicin-resistant lung cancer cells, where a small change in cytosolic alkalization (change in pH from 7.0 to 7.4) was shown to trigger an approximately 2000-fold increase in doxorubicin resistance [66]. This resistance was reversed by the administration of verapamil [66], which caused cellular acidification.

The above studies suggest that drug sequestration mechanism is pH-dependent; however, recent reports show the role of cell membrane. In particular, studies have shown alterations in membrane recycling in doxorubicin [67] and cisplatin [68] resistant cells. In doxorubicin-sensitive MCF-7 cells, both the trans-Golgi network and recycling endosome compartment are distributed throughout the cytoplasm which are shown to be altered in doxorubicin-resistant MCF-7 cells, where both the above compartments are perinuclear [64]. Rauch et al. have reviewed the relationship between alkalization of cytoplasm pH in resistant cells and lipid arrangement affecting the membrane's lipid packing and membrane tension, suggesting a close relationship between change in membrane lipid arrangement and drug resistance [69]. Lipid packing density is affected by alterations in average area per lipid, which depends on the competition between lipid headgroup repulsion and hydrophobic attraction [70, 71]. Typically, membrane lipids self-assemble and form thermodynamically stable aggregates (Figure 1). Therefore, any changes in this balance are expected to affect the optimal area per lipid (i.e., their packing) and membrane shape [72, 73]. As the inner leaflet typically consists of negatively charged lipids, such as PS, and these lipids might also be organized in clusters, a slight increase in alkalinity would shield their negative charges and

decrease the electrostatic repulsion between polar groups. Therefore, acidic pH is more likely to be central in abolishing the physical repulsion between lipids, thus decreasing surface tension [74–76], which suggests that the sensitive cells with acidic cytoplasm will tend to have cell membranes with less packing density. The alkaline nature of the cytoplasm in resistant cells facilitates increased packing of lipids, which in turn affects the intracellular accumulation of drugs. Thus the acidic pH of intracellular vesicles and drug resistance may be related to the change in membrane lipid arrangement that could hinder drug diffusion from acidic intracellular vesicles into cytosolic compartment.

3.3. Membrane biophysics and drug efflux by P-gp

Increased drug efflux is the most pronounced and consistent observation in drug-resistant cells. The P-gp efflux system function is believed to be an energy-dependent mechanism [77, 78]. However, in many studies, membrane biophysics, particularly the lipid phase of the plasma membrane, has been shown to play an important role with respect to MDR and P-gp efflux [79, 80]. It is important to understand the significance of the lipid phase with respect to drug resistance, i.e., how the lipid phase facilitates P-gp efflux in cells to confer drug resistance on tumor cells. Generally, lipids can influence membrane proteins at a geometric/steric level by membrane thickness. For example, P-gp which has a relatively long trans-membrane segment, is suited to a thick bilayer (Figure 3c). To accommodate P-gp in the cell membrane, the membrane's hydrophobic region should match with trans-membrane segments of P-gp. Cells seem to achieve this by forming thick lipid rafts. Lipid rafts exist in the liquid-ordered phase state and are rich in cholesterol and sphingolipids [81]. In addition, the lipid raft acyl chains are also in fully extended conformations, thus resulting in a thicker bilayer [82]. From a drug-resistance perspective, the thickness of cell membrane affects the free drug molecule time to traverse the membrane. The trans-bilayer movement rate of the drug seems to affect the ability of P-gp to confer drug resistance on tumor cells (Figure 5a) [83].

ATPase activity, a major factor in cellular drug efflux, has also shown to be influenced by cell membrane lipid composition [84, 85]. For instance, P-gp ATPase activity is shown to be inhibited in membranes without cholesterol or when P-gp is reconstituted into membranes without cholesterol. Since cholesterol is known to modulate membrane fluidity, it may affect P-gp function indirectly. Cholesterol is shown to alter the binding affinity of P-gp for some but not all drugs [86–89].

As mentioned above, lipid bilayer's composition and fluidity affect P-gp function [90]. For instance, vinblastine, verapamil and daunorubicin bind to P-gp with higher affinity in liposomes made from egg PC compared with those composed of brain PS and egg PE. These studies demonstrated that the headgroup, length of the acyl chain, and fluidity of bilayers all affect P-gp's ability to bind to drugs. Shuldes et al. demonstrated the significance of membrane order on the P-gp function in Chinese hamster ovary cells. In this study, authors showed that both a bile salt taurochenodeoxycholate (TCDC) and R-verapamil lowered the membrane order in resistant cells to that in sensitive cells and reversed the resistance to doxorubicin. The bile tauroursodeoxycholate (TUDC) that had no effect on the membrane order in resistant cells did not sensitize the resistant cells [91].

In our studies, breast cancer-resistant cells (MCF-7/ADR) treated with the epigenetic drug decitabine altered cell membrane lipid composition, particularly the levels of SM, which are required to form lipid rafts to accommodate P-gp within the cell membrane [15]. In particular, we showed that, decitabine treated resistant cells have lower SM levels and lower neutral lipid content compared to untreated resistant cells. Lower SM levels, were attributed to higher sphingomyelinase activity in treated cells. Further, comparison of biophysical characteristics of lipid extracts from treated and untreated resistant cells showed that the

lipid extracts from treated resistant cells form fluid membrane than the lipids from untreated cells. Therefore, in our study, the reduced expression of P-gp in decitabine-treated resistant cells is attributed to the change in membrane composition and membrane fluidity. These studies demonstrate the significance of membrane phase state, composition, and fluidity in the expression of P-gp's and its ability to bind to and efflux drugs.

4. Effect of membrane biophysics on apoptosis in drug-resistant cells

Altered or defective apoptosis is one of the hallmarks of drug resistance in cancers [92]. Hence, an insight into apoptotic mechanisms is essential to design therapeutic strategies to overcome drug resistance, particularly those strategies that use apoptotic mechanisms, which are influenced by the unique biophysical properties of lipids involved in the apoptotic pathway [93]. Of particular interest is the involvement of the sphingomyelinase (SMase)/ceramide pathway in apoptosis. The polar headgroup of ceramide, the amide linkage, as well as the hydroxyl groups of sphingosine and the fatty acid chain have extensive hydrogen-bonding capacity. These enhanced interactions between ceramide molecules give rise to in-plane phase separation of ceramide-rich and ceramide-poor microdomains (Figure 6). Several studies have suggested that the ability of ceramide to self-associate and mediate lipid raft reorganization into macrodomains is required for receptor capping and other signaling events leading to apoptosis [94].

Ceramides and SM differ in their affinity for cholesterol [95]. As discussed earlier, SM interacts very tightly with cholesterol, through hydrogen bonding between the C-3 hydroxyl group of cholesterol and the sphingosine moiety of SM, and this interaction serves as the basis for lipid raft formation. Ceramides, on the other hand, have very poor affinity for cholesterol and tend to separate into exclusive ceramide-enriched microdomains. Furthermore, as a hydrophobic molecule, ceramide strongly favors partitioning into bilayers, thus implying that changes in the physical state of the membrane might play a role in ceramide-mediated function [96]. The difference in affinity between SM and ceramide for cholesterol seems to play critical role in ceramide raft generation in apoptosis pathway. Sphingolipid rafts via ceramide generation into macrodomains are considered necessary for signaling and cellular functions as diverse as apoptosis, cell cycle arrest, differentiation, and senescence.

Studies have shown that the ceramide effectively displaces cholesterol from order domain in lipid vesicles. The driving force for sterol displacement by ceramide would be the hydrophobic effect that tends to minimize unfavorable contact between the hydrocarbon groups of the small headgroup lipids [97, 98]. Certain cellular process may be the result of this displacement such as SMase-induced displacement of cholesterol from plasma membranes in cells due to reduction on SM in the membrane [99]. Interestingly, drug-resistant cells seem to utilize the difference in biophysical properties of SM and ceramide effectively to evade apoptosis. Since ceramide is a basic structural unit of sphingolipids, the intracellular concentration of SM or ceramide is regulated by controlling the synthesis or breakdown of SM. For instance, drug-resistant cells maintain low intracellular ceramide levels by either increasing SM synthesis or preventing SM breakdown by regulating SMase intracellular levels. For example, in doxorubicin-resistant breast cancer (T47D) cells, SMase, which hydrolyzes SM to ceramide, is expressed in lower levels than in doxorubicin-sensitive cell lines. Addition of exogenous ceramide increased the doxorubicin sensitivity in these cells, indicating that low SMase levels are essential for maintaining drug resistance [100]. In addition, adriamycin-resistant cells were found to maintain low intracellular levels of ceramide by elevating the transcription of glucosylceramide synthase (GCS), an enzyme that converts ceramide to anti-apoptotic glucosylceramide (glycolipid) [101]. Inhibition of GCS in these cells by using an oligonucleotide antisense strategy restored adriamycin

sensitivity [102]. Furthermore, introduction of GCS into wild-type MCF-7 cells via a retroviral expression system resulted in acquired adriamycin resistance in these cells [103].

5. Effect of membrane biophysics on NP-based drug delivery systems

As discussed above, cell membrane lipid composition and drug-cell membrane lipid interactions are major factors in reduced drug accumulation inside drug-resistant cells. From a drug delivery perspective, this tendency toward accumulation would appear a trivial obstacle to overcome, as the free drug could be encapsulated inside nanocarriers. In addition, NPs would enter into cells by endocytotic vesicles, thereby preventing the encapsulated drug from being recognized by efflux pumps. Upon transport across the cellular membrane, the drug molecules are released from NPs that have been delivered into the cytoplasm. Therefore, one would expect a significant improvement in drug efficacy. However, recent reports in the literature suggest that the ability of existing NPs to overcome drug resistance depends on cell type and carrier system. Of particular interest is the recent study by Kunjachan et al. [104]; the authors investigated different NP formulations, such as liposomes, polymers and micelles, to overcome MDR in four different cell lines (A431, SW620, B16-F10 and CT26) in drug-sensitive and drug-resistant form. Resistance indices (IC_{50} in resistant cells/ IC_{50} in sensitive cells), which indicate the efficacy of a given formulation in overcoming drug resistance, were determined by treating drug-sensitive and drug-resistant cancer cells with increasing concentrations of doxorubicin in free, polymer-bound, micellar and liposomal forms. The study reported a four-fold reduction in the resistance index in A431 cells with polymer-bound doxorubicin. Based on cellular uptake levels of all four formulations, the study concluded that overall efficacy in overcoming MDR with NPs was marginal. Reduced endocytosis was reported in both doxorubicin- [105] and cisplatin-resistant cells [106, 107]. Therefore, the major reason for lack of improvement in overcoming drug resistance with NPs could be attributed to reduced endocytosis. In our studies, Doxil, a liposomal formulation of doxorubicin, was ineffective in inducing toxicity in doxorubicin-resistant, MCF-7/ADR cells [59]. We attributed the inefficacy of Doxil to the rigid nature of the resistant cells' membrane, which impaired the endocytic process that inhibited Doxil uptake (Figure 5b). Sensitive cells with fluid membrane lipids demonstrated dose-dependent uptake of Doxil and demonstrated the cytotoxic effect of the encapsulated doxorubicin [59]. Other investigators have linked reduced endocytosis to altered phospholipid metabolism in drug-resistant cells [15, 59].

In cisplatin-resistant cells, drug resistance was attributed to abnormal morphology of the endocytic recycling compartment (ERC) with respect to its formation and distribution [68]. Here authors demonstrated altered distribution of ERC in drug resistant cells to more rapid sphingomyelin metabolism to ceramide as compared to that in parental drug-sensitive cells. This observation contradicts with the general notion i.e. drug resistant cells require SM to stabilize P-gp in the membrane, and evade apoptosis by controlling the balance between SM and ceramide. It appears that acyl chain lengths of SM and ceramide play significant different roles in their cellular function and trafficking [108, 109]. Longer acyl chain SMs are associated with lipid rafts and P-gp whereas short chain SM are not; they do not partition into ordered domains in model membranes [110]. Further, short chain SMs traffic with recycling endosomes [111] whereas long chain SMs that are associated with lipid rafts and P-gp traffic to the late endosomes [109]. In addition, ceramide function depends on acyl chain length. Ceramides with longer-chain length (to C_{24} or greater) are involved in cellular apoptosis whereas short chain length ceramides (chain length C_6) are not [108]. The different behavior of the long- and short-chain ceramides has been considered to be due to their different molecular geometries that influence their organization in the membrane and hence their physiological effects.

Endocytic processes involve the uptake of membrane lipids and proteins. Generally, it is believed that lipids and lipid-linked proteins enter membrane invaginations passively and thus enter the endocytic compartment. However, recent studies have shown that lipids and lipid-linked proteins are selectively included or excluded in vesicles during intracellular trafficking. For example, lipid acyl chains that prefer to form fluid membrane are shown to be selectively included in recycling vesicles, whereas lipid acyl chains that prefer to form rigid membranes are shown to be included in late-endosome vesicles [35] (Figure 3a vs. Figure 5b). Based on this finding and our observation that the membrane lipid extracts from doxorubicin-resistant breast cancer cells form a rigid membrane, we suggested efficacy of Doxil in sensitive cells to their escape from endocytic vesicles during recycling due to fluid nature of the membrane whereas rigid nature of resistant cells caused Doxil to remain trapped into endocytic vesicles [59]. This was evident from doxorubicin levels that increased exponentially with incubation time in sensitive cells treated with Doxil whereas the drugs levels in resistant cells increased initially but then remained unchanged with incubation time.

Since receptor-mediated uptake is also via endocytosis, and the rigid nature of resistant cell membrane influences the membrane bending that is necessary of endocytic process to take place, we speculate that drug delivery via receptor-targeted NPs will also be impacted when cells develop drug resistance. However, there are few studies that have reported better efficacy with targeted NPs in resistant cells than with non-targeted NPs, primarily attributed due to better drug delivery to the tumor with targeted NPs than with non-targeted NPs [112, 113]. However, there is no direct comparison between improvement in efficacy in resistant and sensitive tumors with targeted NPs as compared to nontargeted NPs. Hence, it is difficult to know whether targeted NPs overcome drug resistance or the improved efficacy is simply the effect of greater tumor drug accumulation with targeted NPs as compared that with nontargeted NPs.

Few studies have shown that NP formulations that overcome drug resistance seem to interact with membrane lipids. Liang et al. [114] demonstrated that metallofullerene NPs that penetrate through plasma membrane reverse drug resistance in cisplatin-resistant PC-3 cells. In this study, NP-plasma membrane interaction has been suggested to restore endocytotic function and increase intracellular cisplatin accumulation. In MCF-7 cells, *trans*-activating transcriptional activator (TAT) peptide-conjugated mesoporous silica NPs [115] were able to overcome drug resistance since the peptide facilitates interactions of NPs with cell membrane lipids [116]. Pluronic block copolymers, which have shown promising results in sensitizing tumors to various anticancer agents, are known to interact with membrane lipids [117, 118]. It is possible that the improved drug efficacy seen with pluronics, TAT-conjugated nanocarriers, polymer-drug conjugates, and metallofullerene NPs is mediated because of modulation of biophysical characteristics of resistant cell membrane upon interaction with nanocarriers. The interactions between nanocarrier surfaces and cell membrane lipids may induce phase separation in the outer membrane of cells, thereby altering the intrinsic intracellular trafficking within the cells.

6. Strategies to improve NP-based drug delivery to overcome drug resistance

Cell membrane lipid composition and consequential biophysical characteristics seem to play a vital role in mechanisms of cancer drug resistance, as well as in achieving drug delivery inside cells. Therefore, it is important to integrate membrane biophysics with drug delivery to develop nanocarriers that can overcome drug resistance. The physical and chemical features of membrane lipids are interlinked and together form the complex biophysical features responsible for various cellular functions. The separation between physical and

chemical events in the membrane is very subtle. For instance, a chemical change due to lipid hydrolysis in the membrane could be manifested as a change in pressure or curvature [119]. Since membrane curvature is a critical factor during endocytic process, any chemical changes that affect the cell membrane's lipid composition would affect that endocytic process. For example, increases in local concentration of lipids that prefer formation of non-bilayer structures could influence membrane curvature, fusion, and fission events critical in endocytosis and exocytic processes [120]. Similarly, a physical change in the membrane may lead to a change in local chemical composition, thereby modulating the biophysical characteristics of the membrane. For example, physical attractive interactions between an NP's surface and the lipid membrane may induce bending or expansion of the membrane that will lead to redistribution of lipids in the membrane bilayer. Thus, one can potentially overcome drug resistance by modulating membrane biophysical characteristics by inducing changes either chemically or physically. As a means of physical method, it has been shown that ultrasound exposure could make resistant cancer cells become more sensitive to anticancer drugs [121]. Ultrasound could alter the membrane lipid arrangement, increasing membrane fluidity that could facilitate drug transport to overcome drug resistance [122]. Similarly, as a means of chemical methods, R-verapamil and linolenic acid have been shown to reverse drug resistance in human bladder carcinoma cell lines by modifying the membrane fluidity [123, 124]. Due to lipophilic nature, R-verapamil can accumulate in the hydrophobic region, the steric effects of isopropyl side chain seem to reduce the van der Waals forces between lipid molecules, resulting in more fluid membranes. In a separate study, Ramu et al. showed that reversal of resistance with verapamil is associated with increased synthesis of PC that increases membrane fluidity [125].

6.1. Modulating biophysical interactions between NP-cell membrane lipids to overcome drug resistance

As stated above, reduced endocytic function or abnormal morphology of ERCs are major issues in drug resistance. Therefore, understanding the relationship between biophysical aspects of the cell membrane in drug-resistance mechanisms and drug delivery processes is critical to overcoming drug resistance. However, the complex and dynamic nature of cell membranes make these investigations difficult to perform in real time in live cells [126]. As a next best means, one can use biomimetic membrane models to understand the role of membrane lipids in cancer drug resistance [15, 59]. We have been investigating nanocarrier-cell membrane interactions and recently have elucidated the role of membrane biophysical characteristics in drug-resistant breast cancer cells [15, 59]. In this study, we demonstrated that the rigid nature of a resistant cell's membrane not only affects uptake of the free drug but also that the endocytic function inhibits drug uptake via nanocarriers. The surfaces of NPs can be designed to enhance their interactions with cell membranes and modify intracellular trafficking of NPs. Recently, using our NP-lipid cell membrane model interactions, we have demonstrated that the attractive interactions between an NP's surface and lipid headgroups influence the cellular uptake of the nanocarrier [116, 127]. In addition, others have shown in theoretical [128] and experimental [129] studies that interactions between nanocarriers and lipid headgroups affect the biophysical properties of the cell membrane, thereby affecting nanocarrier uptake via endocytosis. For instance, the use of cell-penetrating peptides and cationic surfactants has been investigated as an effective means to enhance drug delivery and overcome drug resistance. It is possible that upon interaction between cell-penetrating peptides or cationic surfactant-modified nanocarriers and resistant cell membranes, the outer monolayer phospholipids of the cell membrane can develop lateral inhomogeneities and promote vesiculation of the plasma membrane into the cells. This assumption is further supported by recent studies showing that perturbation of the outer monolayer of the cell membrane with common detergents induces massive endocytosis in cell-membrane lipids with order domains [130, 131]. An interesting observation was that

this endocytic process appears to be independent of the usual endocytic machinery such as dynamins and functional membrane cytoskeleton and other G proteins [130, 131]. Therefore, these studies clearly suggest that nanocarriers modified with cationic surfactants or cell-penetrating peptides might induce endocytosis due to interactions with the cell membrane and thereby enhance intracellular drug uptake.

Our recent study has demonstrated that not only the charge but also the molecular structure of the cationic surfactant at the interface influences the biophysical interactions of surfactant-modified NPs and the lipid monolayer [127]. Our ongoing studies include attempts to understand and characterize the biophysical interactions of different cationic surfactant-modified NPs with lipids isolated from resistant and sensitive cells. These studies will help us understand NP characteristics that are critical in overcoming the transport barrier of resistant cell membranes. In our recent study, we explored biophysical approaches to developing surface modified NPs which preferentially interact with the membrane lipids of malignant *vs.* normal cells (Figure. 7). NPs designed using the above approaches demonstrated improved tumor localization and efficacy with p53 DNA-loaded NPs *in vivo* to inhibit tumor growth and disease progression in a prostate tumor model. In this study, NPs surface modified with a dichain cationic surfactant, DMAB (didodecyldimethylammonium bromide) demonstrated greater interaction with prostate cancer cell membrane lipids, which was 6.7-fold greater than with unmodified NPs and 5.5-fold greater than with endothelial cell membrane lipids. The effect of single-chain cationic surfactant, CTAB (cetyltrimethylammonium bromide) was not the same as dichained surfactant, DMAB, suggesting that the molecular structure of surfactant at the NP interface influence NP-biophysical interactions with membrane lipids [127]. Upon systemic injection, DMAB-modified NPs demonstrated a 4.6-fold increase in tumor accumulation compared to unmodified NPs [132]. The results of this study suggest that characterization of the biophysical interactions between NPs and lipid membranes of tumors or other diseased tissues/organs may be a promising approach for screening and developing targeted delivery systems.

6.2. Modulating biophysical properties with epigenetic drugs to regain endocytic function in drug-resistant cells

Alterations in membrane composition and organization of lipids in resistant cells could arise due to various factors, one of which might be hypermethylation of some or many of the genes involved in cell membrane protein/lipid synthesis [15]. For example, the SMase gene has been reported to be silenced due to hypermethylation in 60% of breast tumors [133]. SMase is an enzyme which hydrolyzes SM (one of the major lipids in cell membrane) to ceramide, a tumor suppressor lipid. The SMase gene is re-expressed by treating the breast cancer cells with the demethylating agent 5-aza-2 deoxy (decitabine) [133].

In a different study, the basal activity of neutral SMase has been reported to be lower in doxorubicin-resistant breast cancer cells. In response to doxorubicin treatment, these cells showed reduced SM hydrolysis, ceramide generation and cell death by the SM-ceramide pathway than in sensitive cells [100]. Therefore, treating breast cancer cells with a demethylating agent could reactivate the silenced SMase, which could hydrolyze the SM to ceramide, which in turn could induce apoptosis. In addition, ceramide can replace cholesterol in the cell membrane [134]. Any decrease in SM and cholesterol levels in the membrane itself alters the biophysical state of the drug-resistant cell membrane from a more rigid to a more fluid nature (similar to doxorubicin-sensitive cells). Thus epigenetic drugs could also be used to modulate biophysical properties of drug-resistant cells [134]. In our recent study, we demonstrated that resistant cells, following treatment with decitabine, showed an increase in membrane lipids that prefer a nonbilayer structure formation and a decrease in lipids that prefer a bilayer formation. These changes in lipid composition have

been shown to alter the membrane's biophysical characteristics and to facilitate doxorubicin transport as well as to help regain endocytic functions (Figure 8) [15]. In this study, we demonstrated significantly enhanced cytotoxicity in doxorubicin-resistant cells pretreated with decitabine, then with doxorubicin or Doxil, which were otherwise ineffective in inducing cytotoxicity in resistant cells.

Recently, we characterized MCF-7/ADR and a number of other drug sensitive breast cancer cell lines using flow cytometry, immunofluorescence, mammosphere formation assay and migration assay, examining their cancer stem cell (CSC) immunophenotypes, presence of CSC proteins, tumorigenicity *in vitro* and migratory rates, respectively. Our results demonstrated that MCF-7/ADR cells uniformly display CSC characteristics yet retain low migratory rate. Furthermore, we demonstrated that MCF-7/ADR cells are selectively sensitive to epigenetic drug, suberoylanilide hydroxamic acid (SAHA), losing drug resistance and changes morphology yet retaining CSC immunophenotypes [135]. Thus there is significant potential to explore epigenetic drugs either alone or in combination with other anticancer drugs to overcome drug resistance.

7. Concluding Statement

There is considerable and growing evidence supporting the major role of membrane lipids in various cellular functions. Changes in lipid biosynthesis in the setting of various disease conditions, including cancer, are known to alter the biophysical properties of the cell membrane, which can directly influence drug transport and endocytic functions. Understanding such changes in membrane lipids and their effects on the biophysical characteristics of the cell membrane during malignant transformation, particularly when cancers develop drug resistance, could open up new opportunities for developing effective agents and drug-delivery systems to treat drug-resistant tumors. However, multiple mechanisms have been postulated for drug resistance, and hence connecting these mechanisms to biophysical changes in membrane lipids and their role in drug delivery remains a critical next step.

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Abbreviations

ADR	Adriamycin-resistant
AFM	Atomic force microscopic
CTAB	Cetyltrimethylammonium bromide
DMAB	Didodecyldimethylammonium bromide
ERC	Endocytic recycling compartment
ERCC1	Excision repair cross-complementation group 1
GCS	Glucosylceramide synthase
HUVECs	Human umbilical vein endothelial cells
MDR	Multidrug resistance
NP	Nanoparticle

P-gp	Permeability glycoprotein
PC	Phosphocholine
PE	Phosphatidyl ethanolamine
PS	Phosphatidyl serine
SM	Sphingomyelin
SMase	Sphingomyelinase
TAT	<i>Trans</i> -activating transcriptional

References

1. Solyanik GI. Multifactorial nature of tumor drug resistance. *Exp. Oncol.* 2010; 32:181–185. [PubMed: 21403614]
2. Lyman GH. Weight-based chemotherapy dosing in obese patients with cancer: back to the future. *J. Oncol. Pract.* 2012; 8:e62–e64. [PubMed: 23181001]
3. Goldie JH, Coldman AJ. The genetic origin of drug resistance in neoplasms: implications for systemic therapy. *Cancer Res.* 1984; 44:3643–3653. [PubMed: 6744284]
4. Szakacs G, Paterson JK, Ludwig JA, Booth-Genthe C, Gottesman MM. Targeting multidrug resistance in cancer. *Nat. Rev. Drug Discov.* 2006; 5:219–234. [PubMed: 16518375]
5. Perez-Tomas R. Multidrug resistance: retrospect and prospects in anti-cancer drug treatment. *Curr. Med. Chem.* 2006; 13:1859–1876. [PubMed: 16842198]
6. Larsen AK, Skladanowski A. Cellular resistance to topoisomerase-targeted drugs: from drug uptake to cell death. *Biochim. Biophys. Acta.* 1998; 1400:257–274. [PubMed: 9748618]
7. Gottesman MM. Mechanisms of cancer drug resistance. *Annu. Rev. Med.* 2002; 53:615–627. [PubMed: 11818492]
8. Shapira A, Livney YD, Broxterman HJ, Assaraf YG. Nanomedicine for targeted cancer therapy: towards the overcoming of drug resistance. *Drug Resist. Updat.* 2011; 14:150–163. [PubMed: 21330184]
9. Qiao L, Wong BC. Targeting apoptosis as an approach for gastrointestinal cancer therapy. *Drug Resist. Updat.* 2009; 12:55–64. [PubMed: 19278896]
10. Knappskog S, Lonning PE. P53 and its molecular basis to chemoresistance in breast cancer. *Expert Opin. Ther. Targets.* 2012; 16(Suppl 1):S23–S30. [PubMed: 22313396]
11. Brosh R, Rotter V. When mutants gain new powers: news from the mutant p53 field. *Nat. Rev. Cancer.* 2009; 9:701–713. [PubMed: 19693097]
12. Lo PK, Sukumar S. Epigenomics and breast cancer. *Pharmacogenomics.* 2008; 9:1879–1902. [PubMed: 19072646]
13. Vijayaraghavalu S, Dermawan JK, Cheriya V, Labhasetwar V. Highly synergistic effect of sequential treatment with epigenetic and anticancer drugs to overcome drug resistance in breast cancer cells is mediated via activation of p21 gene expression leading to G2/M cycle arrest. *Mol. Pharmaceutics.* 2013; 10:337–352.
14. Vilmar A, Sorensen JB. Excision repair cross-complementation group 1 (ERCC1) in platinum-based treatment of non-small cell lung cancer with special emphasis on carboplatin: a review of current literature. *Lung Cancer.* 2009; 64:131–139. [PubMed: 18804893]
15. Vijayaraghavalu S, Peetla C, Lu S, Labhasetwar V. Epigenetic modulation of the biophysical properties of drug-resistant cell lipids to restore drug transport and endocytic functions. *Mol. Pharmaceutics.* 2012; 9:2730–2742.
16. Milane L, Ganesh S, Shah S, Duan ZF, Amiji M. Multi-modal strategies for overcoming tumor drug resistance: hypoxia, the Warburg effect, stem cells, and multifunctional nanotechnology. *J. Control. Release.* 2011; 155:237–247. [PubMed: 21497176]

17. Hu CM, Zhang L. Nanoparticle-based combination therapy toward overcoming drug resistance in cancer. *Biochem. Pharmacol.* 2012; 83:1104–1111. [PubMed: 22285912]
18. Gao Z, Zhang L, Sun Y. Nanotechnology applied to overcome tumor drug resistance. *J. Control. Release.* 2012; 162:45–55. [PubMed: 22698943]
19. Gao J, Feng SS, Guo Y. Nanomedicine against multidrug resistance in cancer treatment. *Nanomedicine (Lond).* 2012; 7:465–468. [PubMed: 22471716]
20. Ozben T. Mechanisms and strategies to overcome multiple drug resistance in cancer. *FEBS Lett.* 2006; 580:2903–2909. [PubMed: 16497299]
21. Fesik SW. Promoting apoptosis as a strategy for cancer drug discovery. *Nat. Rev. Cancer.* 2005; 5:876–885. [PubMed: 16239906]
22. Lefranc F, Facchini V, Kiss R. Proautophagic drugs: a novel means to combat apoptosis-resistant cancers. with a special emphasis on glioblastomas. *Oncologist.* 2007; 12:1395–1403.
23. Chen AM, Zhang M, Wei D, Stueber D, Taratula O, Minko T, He H. Co-delivery of doxorubicin and Bcl-2 siRNA by mesoporous silica nanoparticles enhances the efficacy of chemotherapy in multidrug-resistant cancer cells. *Small.* 2009; 5:2673–2677. [PubMed: 19780069]
24. Yague E, Higgins CF, Raguz S. Complete reversal of multidrug resistance by stable expression of small interfering RNAs targeting MDR1. *Gene Ther.* 2004; 11:1170–1174. [PubMed: 15164094]
25. Huang Y, Anderle P, Bussey KJ, Barbacioru C, Shankavaram U, Dai Z, Reinhold WC, Papp A, Weinstein JN, Sadee W. Membrane transporters and channels: role of the transportome in cancer chemosensitivity and chemoresistance. *Cancer Res.* 2004; 64:4294–4301. [PubMed: 15205344]
26. Wu H, Hait WN, Yang JM. Small interfering RNA-induced suppression of MDR1 (P-glycoprotein) restores sensitivity to multidrug-resistant cancer cells. *Cancer Res.* 2003; 63:1515–1519. [PubMed: 12670898]
27. Dipaola RS, Aisner J. Overcoming bcl-2-and p53-mediated resistance in prostate cancer. *Semin. Oncol.* 1999; 26:112–116. [PubMed: 10190792]
28. Xiang J, Gomez-Navarro J, Arafat W, Liu B, Barker SD, Alvarez RD, Siegal GP, Curiel DT. Proapoptotic treatment with an adenovirus encoding Bax enhances the effect of chemotherapy in ovarian cancer. *J. Gene Med.* 2000; 2:97–106. [PubMed: 10809143]
29. Escriba PV, Gonzalez-Ros JM, Goni FM, Kinnunen PK, Vigh L, Sanchez-Magraner L, Fernandez AM, Busquets X, Horvath I, Barcelo-Coblijn G. Membranes: a meeting point for lipids, proteins and therapies. *J. Cel. Mol. Med.* 2008; 12:829–875.
30. Preetha A, Banerjee R, Huilgol N. Surface activity, lipid profiles and their implications in cervical cancer. *J. Cancer Res. Ther.* 2005; 1:180–186. [PubMed: 17998650]
31. Preetha A, Banerjee R, Huilgol N. Dynamic surface tensiometry of tissues using Langmuir films. *Colloids Surf. B. Biointerfaces.* 2005; 40:35–43. [PubMed: 15620838]
32. Ran S, Downes A, Thorpe PE. Increased exposure of anionic phospholipids on the surface of tumor blood vessels. *Cancer Res.* 2002; 62:6132–6140. [PubMed: 12414638]
33. Ewers H, Helenius A. Lipid-mediated endocytosis. *Cold. Spring Harb. Perspect. Biol.* 2011; 3:a004721. [PubMed: 21576253]
34. McMahon HT, Gallop JL. Membrane curvature and mechanisms of dynamic cell membrane remodelling. *Nature.* 2005; 438:590–596. [PubMed: 16319878]
35. Mukherjee S, Maxfield FR. Role of membrane organization and membrane domains in endocytic lipid trafficking. *Traffic.* 2000; 1:203–211. [PubMed: 11208103]
36. Hendrich AB, Michalak K. Lipids as a target for drugs modulating multidrug resistance of cancer cells. *Curr. Cancer Drug Targets.* 2003; 4:23–30.
37. Pallares-Trujillo J, Lopez-Soriano FJ, Argiles JM. Lipids: A key role in multidrug resistance? (Review). *Int. J. Oncol.* 2000; 16:783–798. [PubMed: 10717249]
38. Phillips R, Ursell T, Wiggins P, Sens P. Emerging roles for lipids in shaping membrane-protein function. *Nature.* 2009; 459:379–385. [PubMed: 19458714]
39. Vigh L, Escriba PV, Sonnleitner A, Sonnleitner M, Piotto S, Maresca B, Horvath I, Harwood JL. The significance of lipid composition for membrane activity: new concepts and ways of assessing function. *Prog. Lipid Res.* 2005; 44:303–344. [PubMed: 16214218]

40. Zhang T, Saghatelian A. Emerging roles of lipids in BCL-2 family-regulated apoptosis. *Biochim. Biophys. Acta*. 2013 [Epub ahead of print].
41. Gruner SM, Cullis PR, Hope MJ, Tilcock CP. Lipid polymorphism: the molecular basis of nonbilayer phases. *Annu. Rev. Biophys. Chem.* 1985; 14:211–238. [PubMed: 3890880]
42. Cullis PR, De Kruijff B. Lipid polymorphism and the functional roles of lipids in biological membranes. *Biochim. Biophys. Acta*. 1979; 559:399–420. [PubMed: 391283]
43. Israelachvili JN, Marcelja S, Horn RG. Physical principles of membrane organization. *Q Rev. Biophys.* 1980; 13:121–200. [PubMed: 7015403]
44. Frolov VA, Shnyrova AV, Zimmerberg J. Lipid polymorphisms and membrane shape. *Cold. Spring Harb. Perspect. Biol.* 2011; 3:a004747. [PubMed: 21646378]
45. Cullis PR, Hope MJ, Tilcock CPS. Lipid polymorphism and the roles of lipids in membranes. *Chem. Phys. Lipids*. 1986; 40:127–144. [PubMed: 3742670]
46. Vandijk PWM. Negatively charged phospholipids and their position in the cholesterol affinity sequence. *Biochim. Biophys. Acta*. 1979; 555:89–101. [PubMed: 476099]
47. Mayer LD, Bally MB, Cullis PR. Uptake of adriamycin into large unilamellar vesicles in response to a pH gradient. *Biochim. Biophys. Acta*. 1986; 857:123–126. [PubMed: 3964703]
48. Saeki T, Shimabuku AM, Ueda K, Komano T. Specific drug binding by purified lipid-reconstituted P-glycoprotein: dependence on the lipid composition. *Biochim. Biophys. Acta*. 1992; 1107:105–110. [PubMed: 1352129]
49. Callaghan R, Stafford A, Epand RM. Increased accumulation of drugs in a multidrug resistant cell-line by alteration of membrane biophysical properties. *Biochim. Biophys. Acta*. 1993; 1175:277–282. [PubMed: 8435444]
50. Ramu A, Glaubiger D, Magrath IT, Joshi A. Plasma membrane lipid structural order in doxorubicin-sensitive and -resistant P388 cells. *Cancer Res.* 1983; 43:5533–5537. [PubMed: 6311408]
51. Ramu A, Glaubiger D, Weintraub H. Differences in lipid composition of doxorubicin-sensitive and -resistant P388 cells. *Cancer Treat. Rep.* 1984; 68:637–641. [PubMed: 6713419]
52. May GL, Wright LC, Dyne M, Mackinnon WB, Fox RM, Mountford CE. Plasma membrane lipid composition of vinblastine sensitive and resistant human leukaemic lymphoblasts. *Int. J. Cancer*. 1988; 42:728–733. [PubMed: 3263327]
53. Jin MS, Oldham ML, Zhang Q, Chen J. Crystal structure of the multidrug transporter P-glycoprotein from *Caenorhabditis elegans*. *Nature*. 1990; 346:566–569. [PubMed: 23000902]
54. Clay AT, Sharom FJ. Lipid bilayer properties control membrane partitioning, binding, and transport of p-glycoprotein substrates. *Biochemistry (Mosc)*. 1992; 52:343–354.
55. De Milito A, Fais S. Tumor acidity, chemoresistance and proton pump inhibitors. *Future Oncol*. 2005; 1:779–786. [PubMed: 16556057]
56. Hasmann M, Valet GK, Tapiero H, Trevorrow K, Lampidis T. Membrane potential differences between adriamycin-sensitive and -resistant cells as measured by flow cytometry. *Biochem. Pharmacol.* 1989; 38:305–312. [PubMed: 2914014]
57. Lampidis TJ, Castello C, Del Giglio A, Pressman BC, Viallet P, Trevorrow KW, Valet GK, Tapiero H, Savaraj N. Relevance of the chemical charge of rhodamine dyes to multiple drug resistance. *Biochem. Pharmacol.* 1989; 38:4267–4271. [PubMed: 2597199]
58. Callaghan R, Van Gorkom LC, Epand RM. A comparison of membrane properties and composition between cell lines selected and transfected for multi-drug resistance. *Br. J. Cancer*. 1992; 66:781–786. [PubMed: 1358166]
59. Peetla C, Bhavre R, Vijayaraghavalu S, Stine A, Kooijman E, Labhasetwar V. Drug resistance in breast cancer cells: biophysical characterization of and doxorubicin interactions with membrane lipids. *Mol. Pharmaceutics*. 2010; 7:2334–2348.
60. Hindenburg AA, Baker MA, Gleyzer E, Stewart VJ, Case N, Taub RN. Effect of verapamil and other agents on the distribution of anthracyclines and on reversal of drug resistance. *Cancer Res.* 1987; 47:1421–1425. [PubMed: 3469017]
61. Meschini S, Molinari A, Calcabrini A, Citro G, Arancia G. Intracellular localization of the antitumour drug adriamycin in living cultured cells: a confocal microscopy study. *J. Microsc.* 1994; 176:204–210. [PubMed: 7869363]

62. Simon SM, Schindler M. Cell biological mechanisms of multidrug resistance in tumors. *Proc. Natl. Acad. Sci. U. S. A.* 1994; 91:3497–3504. [PubMed: 7909602]
63. Simon S, Roy D, Schindler M. Intracellular pH and the control of multidrug resistance. *Proc. Natl. Acad. Sci. U. S. A.* 1994; 91:1128–1132. [PubMed: 8302842]
64. Raghunand N, Martinez-Zaguilan R, Wright SH, Gillies RJ. pH and drug resistance. II. Turnover of acidic vesicles and resistance to weakly basic chemotherapeutic drugs. *Biochem. Pharmacol.* 1999; 57:1047–1058. [PubMed: 10796075]
65. Warren L, Jardillier JC, Ordentlich P. Secretion of lysosomal enzymes by drug-sensitive and multiple drug-resistant cells. *Cancer Res.* 1991; 51:1996–2001. [PubMed: 1672622]
66. Keizer HG, Joenje H. Increased cytosolic pH in multidrug-resistant human lung tumor cells: effect of verapamil. *J. Natl. Cancer Inst.* 1989; 81:706–709. [PubMed: 2565404]
67. Sognier MA, Zhang Y, Eberle RL, Sweet KM, Altenberg GA, Belli JA. Sequestration of doxorubicin in vesicles in a multidrug-resistant cell-line (Lz-100). *Biochem. Pharmacol.* 1994; 48:391–401. [PubMed: 7914406]
68. Liang XJ, Mukherjee S, Shen DW, Maxfield FR, Gottesman MM. Endocytic recycling compartments altered in cisplatin-resistant cancer cells. *Cancer Res.* 2006; 66:2346–2353. [PubMed: 16489040]
69. Rauch C. Toward a mechanical control of drug delivery. On the relationship between Lipinski's 2nd rule and cytosolic pH changes in doxorubicin resistance levels in cancer cells: a comparison to published data. *Eur. Biophys. J.* 2009; 38:829–846. [PubMed: 19296096]
70. Victorov AV, Janes N, Taraschi TF, Hoek JB. Packing constraints and electrostatic surface potentials determine transmembrane asymmetry of phosphatidylethanol. *Biophys. J.* 1997; 72:2588–2598. [PubMed: 9168034]
71. Victorov AV, Taraschi TF, Hoek JB. Phosphatidylethanol as a C-13-NMR probe for reporting packing constraints in phospholipid membranes. *Biochim. Biophys. Acta.* 1996; 1283:151–162. [PubMed: 8809094]
72. Petrache HI, Zemb T, Belloni L, Parsegian VA. Salt screening and specific ion adsorption determine neutral-lipid membrane interactions. *Proc. Natl. Acad. Sci. U. S. A.* 2006; 103:7982–7987. [PubMed: 16702553]
73. Petrache HI, Zemb T, Parsegian VA. Specific salt adsorption, screening of dispersion forces and swelling of zwitterionic bilayers induced by addition salt. *Abstracts of Papers of the American Chemical Society.* 2005; 229:U669–U669.
74. Petelska AD, Figaszewski ZA. Effect of pH on the interfacial tension of bilayer lipid membrane formed from phosphatidylcholine or phosphatidylserine. *Biochim. Biophys. Acta.* 2002; 1561:135–146. [PubMed: 11997114]
75. Petelska AD, Figaszewski ZA. Effect of pH on the interfacial tension of lipid bilayer membrane. *Biophys. J.* 2000; 78:812–817. [PubMed: 10653793]
76. Petelska AD, Figaszewski ZA. pH Effect of the sphingomyelin membrane interfacial tension. *J. Membr. Biol.* 2009; 230:11–19. [PubMed: 19603129]
77. Ambudkar SV, Kim IW, Sauna ZE. The power of the pump: Mechanisms of action of P-glycoprotein (ABCB1). *Eur. J. Pharm. Sci.* 2006; 27:392–400. [PubMed: 16352426]
78. Eckford PD, Sharom FJ. ABC efflux pump-based resistance to chemotherapy drugs. *Chem. Rev.* 2009; 109:2989–3011. [PubMed: 19583429]
79. Ferte J. Analysis of the tangled relationships between P-glycoprotein-mediated multidrug resistance and the lipid phase of the cell membrane. *Eur. J. Biochem.* 2000; 267:277–294. [PubMed: 10632698]
80. Clay AT, Sharom FJ. Lipid bilayer properties control membrane partitioning, binding, and transport of p-glycoprotein substrates. *Biochemistry.* 2013; 52:343–354. [PubMed: 23268645]
81. Simons K, Ikonen E. Functional rafts in cell membranes. *Nature.* 1997; 387:569–572. [PubMed: 9177342]
82. Brown RE. Sphingolipid organization in biomembranes: what physical studies of model membranes reveal. *J. Cell Sci.* 1998; 111(Pt 1):1–9. [PubMed: 9394007]

83. Raviv Y, Pollard HB, Bruggemann EP, Pastan I, Gottesman MM. Photosensitized labeling of a functional multidrug transporter in living drug-resistant tumor cells. *J. Biol. Chem.* 1990; 265:3975–3980. [PubMed: 1968065]
84. Callaghan R, Berridge G, Ferry DR, Higgins CF. The functional purification of P-glycoprotein is dependent on maintenance of a lipid-protein interface. *Biochim. Biophys. Acta.* 1997; 1328:109–124. [PubMed: 9315609]
85. Doige CA, Yu XH, Sharom FJ. The effects of lipids and detergents on ATPase-active P-glycoprotein. *Biochim. Biophys. Acta.* 1993; 1146:65–72. [PubMed: 8095161]
86. Belli S, Elsener PM, Wunderli-Allenspach H, Kramer SD. Cholesterol-mediated activation of P-glycoprotein: distinct effects on basal and drug-induced ATPase activities. *J. Pharm. Sci.* 2009; 98:1905–1918. [PubMed: 18937360]
87. Bucher K, Belli S, Wunderli-Allenspach H, Kramer SD. P-glycoprotein in proteoliposomes with low residual detergent: the effects of cholesterol. *Pharm. Res.* 2007; 24:1993–2004. [PubMed: 17497080]
88. Eckford PD, Sharom FJ. Interaction of the P-glycoprotein multidrug efflux pump with cholesterol: effects on ATPase activity, drug binding and transport. *Biochemistry.* 2008; 47:13686–13698. [PubMed: 19049391]
89. Garrigues A, Escargueil AE, Orlowski S. The multidrug transporter, P-glycoprotein, actively mediates cholesterol redistribution in the cell membrane. *Proc. Natl. Acad. Sci. U. S. A.* 2002; 99:10347–10352. [PubMed: 12145328]
90. Romsicki Y, Sharom FJ. The membrane lipid environment modulates drug interactions with the P-glycoprotein multidrug transporter. *Biochemistry.* 1999; 38:6887–6896. [PubMed: 10346910]
91. Schuldes H, Dolderer JH, Zimmer G, Knobloch J, Bickeboller R, Jonas D, Woodcock BG. Reversal of multidrug resistance and increase in plasma membrane fluidity in CHO cells with R-verapamil and bile salts. *Eur. J. Cancer.* 2001; 37:660–667. [PubMed: 11290442]
92. Wilson TR, Johnston PG, Longley DB. Anti-apoptotic mechanisms of drug resistance in cancer. *Curr. Cancer Drug Targets.* 2009; 9:307–319. [PubMed: 19442051]
93. Hannun YA, Obeid LM. The ceramide-centric universe of lipid-mediated cell regulation: stress encounters of the lipid kind. *J. Biol. Chem.* 2002; 277:25847–25850. [PubMed: 12011103]
94. Kolesnick R. The therapeutic potential of modulating the ceramide/sphingomyelin pathway. *J Clin. Invest.* 2002; 110:3–8. [PubMed: 12093880]
95. Kolesnick RN, Goni FM, Alonso A. Compartmentalization of ceramide signaling: physical foundations and biological effects. *J. Cell. Physiol.* 2000; 184:285–300. [PubMed: 10911359]
96. Veiga MP, Arrondo JL, Goni FM, Alonso A. Ceramides in phospholipid membranes: effects on bilayer stability and transition to nonlamellar phases. *Biophys. J.* 1999; 76:342–350. [PubMed: 9876146]
97. Megha, London E. Ceramide selectively displaces cholesterol from ordered lipid domains (rafts): implications for lipid raft structure and function. *J. Biol. Chem.* 2004; 279:9997–10004. [PubMed: 14699154]
98. Sot J, Ibarguren M, Busto JV, Montes LR, Goni FM, Alonso A. Cholesterol displacement by ceramide in sphingomyelin-containing liquid-ordered domains, and generation of gel regions in giant lipidic vesicles. *FEBS Lett.* 2008; 582:3230–3236. [PubMed: 18755187]
99. Slotte JP, Bierman EL. Depletion of plasma-membrane sphingomyelin rapidly alters the distribution of cholesterol between plasma membranes and intracellular cholesterol pools in cultured fibroblasts. *Biochem. J.* 1988; 250:653–658. [PubMed: 3390137]
100. Gouaze V, Mirault ME, Carpentier S, Salvayre R, Levade T, Andrieu-Abadie N. Glutathione peroxidase-1 overexpression prevents ceramide production and partially inhibits apoptosis in doxorubicin-treated human breast carcinoma cells. *Mol. Pharmacol.* 2001; 60:488–496. [PubMed: 11502879]
101. Kartal Yandim M, Apohan E, Baran Y. Therapeutic potential of targeting ceramide/glucosylceramide pathway in cancer. *Cancer Chemother. Pharmacol.* 2013; 71:13–20. [PubMed: 23073611]

102. Gouaze V, Liu YY, Prickett CS, Yu JY, Giuliano AE, Cabot MC. Glucosylceramide synthase blockade down-regulates P-glycoprotein and resensitizes multidrug-resistant breast cancer cells to anticancer drugs. *Cancer Res.* 2005; 65:3861–3867. [PubMed: 15867385]
103. Liu YY, Han TY, Giuliano AE, Cabot MC. Expression of glucosylceramide synthase, converting ceramide to glucosylceramide, confers adriamycin resistance in human breast cancer cells. *J. Biol. Chem.* 1999; 274:1140–1146. [PubMed: 9873062]
104. Kunjachan S, Blauz A, Mockel D, Theek B, Kiessling F, Etrych T, Ulbrich K, Van Bloois L, Storm G, Bartosz G, Rychlik B, Lammers T. Overcoming cellular multidrug resistance using classical nanomedicine formulations. *Eur. J. Pharm. Sci.* 2012; 45:421–428. [PubMed: 21907796]
105. Takahashi T, Furuchi T, Naganuma A. Endocytic Ark/Prk kinases play a critical role in adriamycin resistance in both yeast and mammalian cells. *Cancer Res.* 2006; 66:11932–11937. [PubMed: 17178891]
106. Shen DW, Su A, Liang XJ, Pai-Panandiker A, Gottesman MM. Reduced expression of small GTPases and hypermethylation of the folate binding protein gene in cisplatin-resistant cells. *Br. J. Cancer.* 2004; 91:270–276. [PubMed: 15199393]
107. Chauhan SS, Liang XJ, Su AW, Pai-Panandiker A, Shen DW, Hanover JA, Gottesman MM. Reduced endocytosis and altered lysosome function in cisplatin-resistant cell lines. *Br. J. Cancer.* 2003; 88:1327–1334. [PubMed: 12698203]
108. Grosch S, Schiffmann S, Geisslinger G. Chain length-specific properties of ceramides. *Prog. Lipid Res.* 2012; 51:50–62. [PubMed: 22133871]
109. Koivusalo M, Jansen M, Somerharju P, Ikonen E. Endocytic trafficking of sphingomyelin depends on its acyl chain length. *Mol. Biol. Cell.* 2007; 18:5113–5123. [PubMed: 17942604]
110. Wang TY, Silvius JR. Different sphingolipids show differential partitioning into sphingolipid/cholesterol-rich domains in lipid bilayers. *Biophys. J.* 2000; 79:1478–1489. [PubMed: 10969009]
111. Hao MM, Mukherjee S, Sun Y, Maxfield FR. Effects of cholesterol depletion and increased lipid unsaturation on the properties of endocytic membranes. *J. Biol. Chem.* 2004; 279:14171–14178. [PubMed: 14734557]
112. Milane L, Duan ZF, Amiji M. Pharmacokinetics and biodistribution of lonidamine/paclitaxel loaded, EGFR-targeted nanoparticles in an orthotopic animal model of multi-drug resistant breast cancer. *Nanomedicine.* 7:435–444. [PubMed: 21220050]
113. Galmarini CM, Warren G, Senanayake MT, Vinogradov SV. Efficient overcoming of drug resistance to anticancer nucleoside analogs by nanodelivery of active phosphorylated drugs. *Int. J. Pharm.* 395:281–289. [PubMed: 20580798]
114. Liang XJ, Meng H, Wang Y, He H, Meng J, Lu J, Wang PC, Zhao Y, Gao X, Sun B, Chen C, Xing G, Shen D, Gottesman MM, Wu Y, Yin JJ, Jia L. Metallofullerene nanoparticles circumvent tumor resistance to cisplatin by reactivating endocytosis. *Proc. Natl. Acad. Sci. U. S. A.* 2010; 107:7449–7454. [PubMed: 20368438]
115. Pan L, Liu J, He Q, Wang L, Shi J. Overcoming multidrug resistance of cancer cells by direct intranuclear drug delivery using TAT-conjugated mesoporous silica nanoparticles. *Biomaterials.* 2013; 34:2719–2730. [PubMed: 23337327]
116. Peetla C, Rao KS, Labhasetwar V. Relevance of biophysical interactions of nanoparticles with a model membrane in predicting cellular uptake: study with TAT peptide-conjugated nanoparticles. *Mol. Pharmaceutics.* 2009; 6:1311–1320.
117. Batrakova EV, Kabanov AV. Pluronic block copolymers: evolution of drug delivery concept from inert nanocarriers to biological response modifiers. *J. Control. Release.* 2008; 130:98–106. [PubMed: 18534704]
118. Batrakova EV, Li S, Brynskikh AM, Sharma AK, Li Y, Boska M, Gong N, Mosley RL, Alakhov VY, Gendelman HE, Kabanov AV. Effects of pluronic and doxorubicin on drug uptake, cellular metabolism, apoptosis and tumor inhibition in animal models of MDR cancers. *J. Control. Release.* 2010; 143:290–301. [PubMed: 20074598]
119. Janmey PA, Kinnunen PK. Biophysical properties of lipids and dynamic membranes. *Trends Cell Biol.* 2006; 16:538–546. [PubMed: 16962778]

120. Vogel V, Sheetz M. Local force and geometry sensing regulate cell functions. *Nat. Rev. Mol. Cell Biol.* 2006; 7:265–275. [PubMed: 16607289]
121. Wu F, Shao ZY, Zhai BJ, Zhao CL, Shen DM. Ultrasound reverses multidrug resistance in human cancer cells by altering gene expression of ABC transporter proteins and Bax protein. *Ultrasound Med Biol.* 37:151–159. [PubMed: 21084157]
122. Yoshida T, Kondo T, Ogawa R, Feril LB Jr, Zhao QL, Watanabe A, Tsukada K. Combination of doxorubicin and low-intensity ultrasound causes a synergistic enhancement in cell killing and an additive enhancement in apoptosis induction in human lymphoma U937 cells. *Cancer Chemother Pharmacol.* 2008; 61:559–567. [PubMed: 17505825]
123. Schuldes H, Dolderer JH, Schoch C, Bickeboller R, Woodcock BG. Cytostatic sensitivity and MDR in bladder carcinoma cells: implications for tumor therapy. *Int. J. Clin. Pharmacol. Ther.* 2000; 38:204–208. [PubMed: 10783830]
124. Dolderer JH, Zimmer G, Woodcock BG, Bockhorn H, Bickeboller R, Schuldes H. Resistance modulation in CHO cells by R-verapamil and bile salts is associated with physical and chemical changes in the cell membrane. *Int. J. Clin. Pharmacol. Ther.* 2000; 38:196–203. [PubMed: 10783829]
125. Ramu A, Ramu N, Rosario LM. Circumvention of multidrug-resistance in P388 cells is associated with a rise in the cellular content of phosphatidylcholine. *Biochem. Pharmacol.* 1991; 41:1455–1461. [PubMed: 2018553]
126. Peetla C, Stine A, Labhasetwar V. Biophysical interactions with model lipid membranes: applications in drug discovery and drug delivery. *Mol. Pharmaceutics.* 2009; 6:1264–1276.
127. Peetla C, Labhasetwar V. Effect of molecular structure of cationic surfactants on biophysical interactions of surfactant-modified nanoparticles with a model membrane and cellular uptake. *Langmuir.* 2009; 25:2369–2377. [PubMed: 19161268]
128. Deserno M, Gelbart WM. Adhesion and wrapping in colloid-vesicle complexes. *J Phys. Chem. B.* 2002; 106:5543–5552.
129. Xu A, Yao M, Xu G, Ying J, Ma W, Li B, Jin Y. A physical model for the size-dependent cellular uptake of nanoparticles modified with cationic surfactants. *Int. J. Nanomedicine.* 2012; 7:3547–3554. [PubMed: 22848178]
130. Hilgemann DW, Fine M. Mechanistic analysis of massive endocytosis in relation to functionally defined surface membrane domains. *J. Gen. Physiol.* 2011; 137:155–172. [PubMed: 21242299]
131. Fine M, Llaguno MC, Lariccia V, Lin MJ, Yaradanakul A, Hilgemann DW. Massive endocytosis driven by lipidic forces originating in the outer plasmalemmal monolayer: a new approach to membrane recycling and lipid domains. *J. Gen. Physiol.* 2011; 137:137–154. [PubMed: 21242300]
132. Sharma B, Peetla C, Adjei IM, Labhasetwar V. Selective biophysical interactions of surface modified nanoparticles with cancer cell lipids improve tumor targeting and gene therapy. *Cancer Lett.* 2013; 334:228–236. [PubMed: 23523612]
133. Demircan B, Dyer LM, Gerace M, Lobenhofer EK, Robertson KD, Brown KD. Comparative epigenomics of human and mouse mammary tumors. *Genes Chromosomes Cancer.* 2009; 48:83–97. [PubMed: 18836996]
134. Slotte JP, Bierman EL. Depletion of plasma-membrane sphingomyelin rapidly alters the distribution of cholesterol between plasma membranes and intracellular cholesterol pools in cultured fibroblasts. *Biochem. J.* 1988; 250:653–658. [PubMed: 3390137]
135. Lu S, Labhasetwar V. Drug Resistant Breast Cancer Cell Line Displays Cancer Stem Cell Phenotype and Responds Sensitively to Epigenetic Drug SAHA. *Drug Deliv. Transl. Res.* 2013; 3:183–194. [PubMed: 23543868]

Figure. 1. Lipid polymorphism and their assembly

Schematic diagram depicting molecular shape-volumes occupied by various membrane lipid types. Cylinder-shaped lipids prefer a flat layer arrangement and therefore form lamellar bilayer structures; inverted-cone-shaped lipids prefer nonlamellar structures such as micelles, hexagonal aggregate structures. SM and cholesterol form ordered domains.

Figure. 2. Effect of lipid composition on cell membrane, endocytic function, and transport
 Schematic diagram depicting the significance of differences in molecular shape-volumes of lipids during endocytosis. **a)** Conical shape of PE facilitates membrane invagination during endocytosis. **b)** High concentration of PE at outer leaflet could alter the membrane lipid arrangement and increase membrane permeability.

Figure. 3. Effect of acyl chain of lipids on cell membrane barrier, endocytic function, and trans-membrane protein organization

a) Phospholipids with unsaturated acyl chains form liquid-disordered domains; a high concentration of these phospholipids can lead to a cell membrane with high permeability for small drug molecules. In the endocytic process, vesicles sort based on the degree of fluidity of the acyl chains, where phospholipid membranes with fluid acyl chains sort towards recycling vesicles. **b)** Phospholipids with saturated acyl chains form ordered membrane domains. A high concentration of these phospholipids can lead to a cell membrane with less permeability for small drug molecules. Phospholipids with saturated acyl chains sort towards late-endosome formation. **c)** Proteins with a long trans-membrane domain are

associated with lipids having long acyl chains. **d)** Proteins with a short trans-membrane domain are associated with lipids having short acyl chains, which in turn affect membrane thickness, therefore the barrier function.

Figure. 4. Biophysics of resistant and sensitive cell membrane lipids, interaction with drugs, and drug transport

a) Doxorubicin strongly interacts with the lipids extracted from doxorubicin-resistant cells (MCF-7/ADR) but not with the lipids extracted from sensitive cells (MCF-7). This disparity is evident from the change in the surface pressure of membrane lipids following interaction with doxorubicin. Resistant cell membrane lipids show an increase in surface pressure, indicating interaction with doxorubicin, whereas there is only an insignificant change in surface pressure of membrane lipids of sensitive cells in the presence of doxorubicin, indicating no or minimal interaction. **b)** Atomic force microscopic (AFM) images show that the lipids isolated from doxorubicin-resistant cells form large and markedly heterogeneous domains compared with lipids from doxorubicin-sensitive cell lipid extracts. Comparison of AFM images of lipids before and after doxorubicin interaction confirms that resistant cell lipid extracts strongly interact with doxorubicin, whereas sensitive cell lipid extracts do not. **c)** Schematic diagram shows that because of changes in the lipid composition and biophysical properties of the cell membrane, doxorubicin is trapped in resistant cell membrane lipids and hence is not transported, whereas because of the fluid nature of sensitive cell membrane lipids, doxorubicin does not become trapped in the membrane lipids and hence is transported. Reproduced with permission from [59].

Figure 5. Biophysics of cell membrane lipids on drug-resistance mechanisms affecting intracellular drug/nanocarrier accumulation

a) Schematic diagram depicting the significance of lipids on P-gp efflux function. Drug-resistant cells are characterized by overexpression of P-gp pump. P-gp's long trans-membrane domain is stabilized by SM with long chains and cholesterol. A long acyl chain increases membrane thickness, which enhances the lipophilic drug residence time during drug diffusion. This long residence time indirectly enhances the probability of drug-P-gp contact, therefore facilitating P-gp efflux. **b)** In endocytosis, lipids with long acyl chains prefer late endosome formation, which may entrap the drug or nanocarrier.

Figure. 6. Significance of sphingomyelin vs. ceramide balance, and ceramide physical properties on apoptosis in drug-resistant cells

Higher levels of sphingomyelin and cholesterol increase the membrane packing and confer drug resistance. Sphingomyelinase induces apoptosis by altering the balance between SM and ceramide. High hydrophobicity of ceramide molecule provides the driving force for the coalescence of ceramide molecules to form submicroscopic patches and then into larger platforms required for downstream signaling during apoptosis.

Figure. 7. Exploiting differences in cell membrane lipid biophysical properties for developing targeted drug delivery systems

a) NPs modified with di-chain cationic surfactant (didodecyldimethylammonium bromide [DMAB]) show significantly greater biophysical interactions with the lipid extracts from prostate cancer cells (PC-3) than with the lipids extracted from human umbilical endothelial cells (HUVECs), which were used as normal cells. **b**) Greater biophysical interactions between DMAB-modified NPs and the lipid extract from PC-3 cells than with lipids extracts from HUVECs correlate with greater uptake of DMAB-modified NP uptake in PC-3 cells than in HUVECs. **c**) Confocal images show greater uptake of DMAB-modified NPs in PC-3 cells than unmodified NPs **d**) DMAB-modified NPs loaded with p53 DNA show greater

efficacy than unmodified NPs in causing regression of tumor growth in a prostate cancer model. Reproduced with permission from [132].

Figure 8. Epigenetic drugs alter cell membrane lipid biophysical properties and drug/nanocarrier accumulation in drug-resistant cells

A) Lipid extracts from drug-resistant breast cancer cells (MCF-7/ADR cells) treated with epigenetic drug, 5-aza-2'-deoxycytidine (decitabine) show significantly lower membrane packing (a) and lower rigidity (b) compared with untreated cell lipid extracts. **B)** Altered biophysical properties of decitabine-treated resistant cells facilitate drug transport with incubation time (a) and with increased doses (c) and regain endocytic function that results in greater drug uptake with Doxil with incubation time (b) and increased doses (d) than in untreated resistant cells. Reproduced with permission from [15].