

REVIEW

Unraveling the role of membrane microdomains during microbial infections

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Abstract Infectious diseases pose major socioeconomic and health-related threats to millions of people across the globe. Strategies to combat infectious diseases derive from our understanding of the complex interactions between the host and specific bacterial, viral, and fungal pathogens. Lipid rafts are membrane microdomains that play important role in life cycle of microbes. Interaction of microbial pathogens with host membrane rafts influences not only their initial colonization but also their spread and the induction of inflammation. Therefore, intervention strategies aimed at modulating the assembly of membrane rafts and/or regulating raft-directed signaling pathways are attractive approaches for the management of infectious diseases. The current review discusses the latest advances in terms of techniques used to study the role of membrane microdomains in various pathological conditions and provides updated information regarding the role of membrane rafts during bacterial, viral and fungal infections.

Keywords Lipid rafts · Microdomains · Bacteria · Virus · Fungus · Signaling · Caveolae · Cholesterol

Introduction

The cell, the basic unit of life, is separated from its surroundings by a selectively permeable cell membrane. Receptors and channels within cell membranes mediate interactions between environmental factors and cells. Early concepts of cell membranes envisioned a thin film of lipoidal material coating water-soluble proteins and surrounding the living cell. This model, which allowed for selective membrane permeability, was superseded by the fluid mosaic model of Singer and Nicholson (Singer and Nicholson 1972). According to the fluid mosaic model, cell membranes are two-dimensional liquid organelles with random distribution of phospholipids and proteins in no specific organization pattern. Since introduction of this concept, there have been revolutionary changes regarding the concept of the plasma membrane and this has been an area of intense research. For example, the existence of detergent-resistant sphingolipid-rich domains was demonstrated by Yu et al. (1973) and asymmetric distribution of phospholipids in the plasma membrane of erythrocytes were demonstrated by van Meer and coworkers (van Meer et al. 1980, 1987). Further, Simons and Ikonen (1997) (Simons and Ikonen 1997) proposed a glycosphingolipid/cholesterol raft model based on the formation of lateral lipid assemblies in an unsaturated glycerophospholipid environment. Since then, several reports have shown

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that the plasma membrane is actually more mosaic than fluid (Pike 2003; Engelman 2005; Vieira et al. 2010), and that the lipids are not randomly distributed. This has led to the current model in which the plasma membrane is patchy, with segregated portions that are distinct in structure and function and may vary in thickness and composition (Pike 2003; Garcia-Marcos et al. 2006; Pike 2009). The formation of rafts is thought to be mediated by attractive forces between sphingolipids with saturated hydrocarbon chains and cholesterol. This model is based on the fact that formation of lipid rafts cannot be based only on lipid-protein interactions as it also depends on lipid-lipid associations, which includes phase separation coupled with lateral associations. These associations involve formation of hydrogen bonds, electrostatic forces, van der Waals forces, hydrophobic, and hydrophilic interactions (Pike 2009; Riethmuller et al. 2006; Nicolson 2014). In a seminal talk given at the Keystone Symposium on Lipid Rafts and Cell Function (2006) in Steamboat Springs, CO, lipid rafts were defined as small (10–200 nm), heterogeneous, highly dynamic, and sterol- and sphingolipid-rich domains that compartmentalize cellular processes (Pike 2006). At this Keynote Symposium, it was also suggested that small rafts can sometimes be stabilized to form larger platforms through protein-protein and protein-lipid interactions.

Lipid rafts are enriched in sphingolipids like sphingomyelin and cholesterol and have a preponderance of saturated acyl chains (Mishra and Joshi 2007; Lingwood and Simons 2010). Membrane proteins are attached via glycosylphosphatidylinositol (GPI) anchors to the lipid raft entities. The signaling proteins which reside or translocate to the rafts include Ras, nitric oxide synthase (NOS), phosphatidylinositol 3-kinase (PI3K), tyrosine kinases including Fyn and Src, insulin, and epidermal growth factors to name a few. These membrane domains/rafts exert their actions by separating or concentrating specific membrane proteins and lipids within microdomains, which were earlier thought to serve as platforms in the trans-Golgi network for apical membrane sorting and as foci for the recruitment and concentration of signaling molecules at the plasma membrane (Alonso and Millan 2001). The receptors and stimuli that induce the formation of rafts include cluster of differentiation (CD)5, CD20, CD40, CD95, bacterial and viral infections, as well as exposure to UV light, γ -irradiation, and chemical agents (Bollinger et al. 2005). Numerous proteins involved in cell signaling

have been identified in caveolae and lipid rafts (planar), suggesting that these structures function as signal transduction moieties. Depletion of membrane cholesterol through the use of cholesterol-binding drugs or by blocking cellular cholesterol biosynthesis disrupts the formation and function of both lipid rafts and caveolae, indicating that these membrane domains are involved in a wide range of biological processes. The role of lipid rafts in bringing together specific set of proteins in response to stimulus has been extensively documented in the literature (Simons and Toomre 2000; Lafont and van der Goot 2005). Further, lipid rafts can also include or exclude proteins depending on their modifications. For example, palmitoylation increases the affinity of proteins for rafts, but not the ability of proteins to associate with rafts (Melkonian et al. 1999). Another example is monomeric transmembrane proteins which only reside in rafts for time periods until they are crosslinked or oligomerized (Harder et al. 1998). The distribution of lipids between the lipid bilayer has also been shown to play an important role in membrane function (Simons and Toomre 2000).

Caveolins and flotillins constitute a group of proteins that are enriched within lipid rafts (Hansen and Nichols 2009; Bauer and Pelkmans 2006; Yao et al. 2009). Caveolin-1, a palmitoylated membrane protein, is an essential component of a major subclass of rafts known as caveolae (flask-shaped invaginations of the plasma membrane), which causes rafts to polymerize (Rothberg et al. 1992; Anderson 1998). In contrast, planar rafts have flotillins as essential components (Giri et al. 2007; Zhao et al. 2011). Three caveolin isoforms transcribed from different genes have been identified so far which include caveolin-1, caveolin-2, and caveolin-3. The role of caveolin-1 and caveolin-3 in the formation of caveolae is well documented; however, the role of caveolin-2 is unclear (Sowa et al. 2008; Drab et al. 2001; Razani et al. 2001). Caveolin-1 mediates activation of insulin receptor- β and regulation of the angiotensin/TGF- β pathways suggesting a role in diabetes (Yamamoto et al. 1998) and pulmonary and cardiac fibrosis, (Wang et al. 2006; Cohen et al. 2003), respectively. On the other hand, caveolin-3 defects are associated with muscular dystrophy (autosomal dominant limb girdle muscular dystrophy, idiopathic hyperCKemia, rippling muscle disease, and distal myopathy) (Minetti et al. 1998; Betz et al. 2001; Tateyama et al. 2002). The flotillin family includes flotillin-1 and flotillin-2, which are important for signaling, endocytosis, and interactions of

rafts with the cytoskeleton. Functional roles of flotillins have also been documented in polarization and chemotaxis of neutrophils in response to chemo attractants in vivo (Ludwig et al. 2010; Rossy et al. 2009). Both types of raft entities are also enriched in cholesterol and glycosphingolipids (Simons and Ikonen 1997; Korade and Kenworthy 2008; Wang 2014). The outer leaflet consists of sphingolipids, phosphatidylcholine, and sphingomyelin, whereas the inner leaflet consists of phosphatidylinositol, phosphatidylethanolamine, and phosphatidylserine with cholesterol being found in both leaflets (Mishra and Joshi 2007). Enrichment of phospholipids with saturated fatty acids allows close packing of lipids within rafts, and as a result lipid rafts are more complex and less fluid than the surrounding membrane.

Meanwhile, the formation of rafts rich in ceramide following exposure to external stimuli/stress has also received significant attention (Hannun and Obeid 2002) (Tables 1 and 2). Ceramide can be generated by de novo synthesis or hydrolysis of sphingomyelin by sphingomyelinases (Gault et al. 2010; Silva et al. 2009). Ceramide is composed of sphingosine and a fatty acid chain, and is found in higher concentrations within the cell membrane. An increasing body of literature has shown ceramide (sphingolipid), as an important molecule that regulates a diverse array of cellular processes including differentiation, immune responses, apoptosis, growth arrest, and senescence (Korade and Kenworthy 2008; Caliceti et al. 2012; Arana et al. 2010; DiNitto et al. 2003; Castro et al. 2009; Megha and London 2004; Yu et al. 2005). The role of sphingomyelinase and ceramide has been implicated in pathological conditions including pulmonary failure and erythrocyte apoptosis caused by platelet activating factor (Goggel et al. 2004; Lang et al. 2005). Furthermore, ceramide is also involved in the fusion of endosomes with lysosomes

during the internalization of microbial pathogens into mammalian cells (Bollinger et al. 2005; Anes et al. 2003; Gulbins et al. 2004; Miller et al. 2012; Hartlova et al. 2010; Heung et al. 2006). Conversion of sphingomyelin into ceramide plays an important role in modulation of the membrane structure, which results in membrane vesiculation, fission/fusion and vesicular trafficking and contributes to cellular signaling (Devaux et al. 2008). Interestingly, rafts on one leaflet of the membrane can induce localized changes in the other leaflet of the bilayer, and they can potentially serve as the link between signals from outside the cell to those produced inside the cell (van Meer et al. 2008). In addition, ceramide has been shown to form organized, large channels traversing the mitochondrial outer membrane, which leads to the egress of proteins from the intermembrane space. Ceramide may affect the permeability of the mitochondrial outer membrane and the release of cytochrome C (Siskind et al. 2002). Thus, in the process of apoptosis, the breakdown of plasma membrane sphingomyelin to ceramide results in disorganization of rafts and may regulate apoptotic body formation. High ceramide levels in the lipid rafts increases the size of these platforms due to fusion, and reorganized ceramide-rich rafts retain/restrict proteins differently than other rafts (Spiegel and Milstien 2003). Ceramide-activated proteins act as secondary messengers and they are directly involved in activation of protein phosphatases PP1 and PP2, isoforms of protein kinase C, cathepsin D, and phospholipase A2, other signaling pathway components, although the precise mechanism of activation is unknown yet. Thus, formation of ceramide may serve different functions at distinct locations in the cell, and therefore, the effects of ceramide rafts are still under investigation (Silva et al. 2009; Zheng et al. 2006).

Table 1 Involvement of ceramide rafts in bacterial infections

S.No	Bacteria	Receptor	Model used	Reference
1	<i>Pseudomonas aeruginosa</i>	CD95 receptor	(HNEpC)	(Grassme et al. 2003b; Grassmé et al. 2001)
2	<i>Neisseria gonorrhoea</i>	CD66 receptor	Human epithelial cells and primary fibroblasts	(Hauck et al. 2000; Grassmé et al. 1997)
3	<i>Staphylococcus aureus</i>	GPI-anchored proteins	Human 293 T cells	(Esen et al. 2001; Sinha et al. 1999)
4	<i>Mycobacterium avium</i>	GPI-anchored proteins CR3 and CR4,	Murine macrophage	(Maldonado-García et al. 2004; Irani and Maslow 2005)

Table 2 Involvement of ceramide rafts in viral infections

S.No	Viruses	Receptor	Model used	References
1	Rhinovirus	ICAM-1	Hela epithelial cells and WI-38 fibroblasts	(Greve et al. 1989; Staunton et al. 1989; Arruda et al. 1994)
2	Dengue virus	ICAM, Heat shock proteins (HSP)-HSP90 and 70, Fcy receptor	Human myelomonocyte cell line U937	(Reyes-del Valle et al. 2005; H Puerta-Guardo et al. 2010; Takahashi and Suzuki 2011b)
3	Hepatitis C virus (HCV)	CD81, SR-BI	Huh-7 cells	(Kapadia et al. 2007)
4	HIV-1 (human immunodeficiency virus)	Chemokine receptors- CXCR4/CCR5 and CD4	CD4+ cells, human embryonic kidney 293 T cells, H9 leukemic T cells and Peripheral blood mononuclear cells (PBMC)	(Dragic et al. 1996; Hill and Littman 1996; Ostrowski et al. 1998)
5	Influenza virus	Sialic acid and GPI-anchored proteins	GM 95 cells, mouse B16 melanoma cells	(Suzuki et al. 2000; Matrosovich et al. 2006)
6	Ebola virus	GPI- anchored protein and folate receptor (FR α)	Human 293 T cells	(Takahashi and Suzuki 2011b; Chan et al. 2001)
7	Measles virus	CD46 and signaling lymphocyte-activation molecule (SLAM)	T and B cells, CD46-transgenic mice, HeLa cells, and the established murine lung and kidney cultures	(Dhiman et al. 2004; Blixenkron-Møller et al. 1998)

Techniques used to study the formation and functions of lipid rafts

Ordered and tightly packed acyl chains in lipid raft domains render them the ability to withstand disruption by non-ionic detergents, whereas the low protein/lipid ratio gives them much lower density than other solubilized membrane proteins. Utilizing this property, lipid rafts were originally isolated by sucrose density gradient ultracentrifugation from cellular extracts prepared in 1% Triton-X (Mishra and Joshi 2007; Ostrom and Liu 2007). Several other detergents have subsequently been used to isolate rafts. An alternative approach to study the role of lipid rafts or the association of certain components with rafts is treating them with cholesterol-sequestering agents or chelators like methyl- β -cyclodextrin to disrupt/deplete lipid raft constituents. Moreover, exogenous unsaturated fatty acids, gangliosides or cholesterol can also be used to destabilize raft assembly (Ostrom and Liu 2007; Brown 2006). The drawback of using a cholesterol-sequestering agent or chelator is the possible induction of structural or metabolic changes. Therefore, these may not be appropriate options to determine raft-associated cellular changes. Alternatively, genetic approaches such as small interfering RNA (siRNA) or gene knockout animals are widely used to disrupt planar rafts or caveolae (Pelkmans et al.

2004; Maguy et al. 2006). These studies address the physiological importance of membrane microdomains and help to determine their importance in signal transduction and bacterial/viral entry into cells (Zhong et al. 2008).

Recent use of more advanced and non-disruptive techniques have significantly advanced our understanding about these highly dynamic and fragile rafts. These include fluorescence correlation spectroscopy (FCS), single-molecule tracking microscopy (Kusumi et al. 2014; Parton and del Pozo 2013; Gambin et al. 2014), the use of a 2-dimethylamino-6-lauroyl naphthalene (laurdan) labeling probe for studying lateral organization of membranes using conventional confocal microscopy (Dodes Traian et al. 2012), and fluorescence resonance energy transfer (FRET) for determining the formation and influence of lipid rafts (Silvius and Nabi 2006). The sensitivity of the FCS technique for the determination of phase separation using free standing artificial lipid bilayers was demonstrated in earlier reports (Korlach et al. 1999; Bacia et al. 2004). This technique utilizes excitation and detection as in scanning microscopy to determine temporal fluorescence fluctuations due to the diffusion of individual fluorescent molecules. The auto-correlation curve generated from these fluctuations is used to determine the mobility (diffusion coefficients) of particles and can be used to monitor a single molecule

to hundreds of molecules (Schutz et al. 2000; Ha 2001a, b). Further advancement in fluorescence microscopy techniques came with single molecule imaging (SMI) or single molecule tracking (SMT). This technique provides the ability to monitor collisions, interactions, binding, dissociation, organization, concentration, and disengagement of molecules in living cells with some limitations (Kusumi et al. 2014; Kusumi et al. 2005a; 2005b). Therefore, SMT, which is performed using high resolution photon detector(s) to track only one molecule or to simultaneously image thousands of molecules, has significantly advanced understanding of the three-tiered architecture of plasma membrane (Hinner and Johnsson 2010). In conjugation with FRET, SMT can also be used to detect activation of single molecules. The combination of these two techniques was first used to assess the activation of the G protein Ras (fluorescently labeled), which functions downstream of receptor-type tyrosine kinases, by detecting its binding the fluorescent GTP (energy acceptor) (Murakoshi et al. 2004). Moreover, a fluorescent membrane probe, Laurdan, can be used to determine the extent of water penetration into the lipid bilayer enabling assessment of lipid packing and membrane fluidity. The photophysical properties of laurdan determine its spectral sensitivity in polar solvents and leads to a shift of its emission spectra in loosely packed membranes with higher water penetrability (Dodes Traian et al. 2012; Kwiatek et al. 2013). The studies conducted using these techniques not only support the existence of rafts in live cells, but electron paramagnetic resonance studies and single molecule tracking methods have been used to determine the half-life of rafts and to assess their interactions with various proteins, respectively (Swamy et al. 2006). As a result, it is now clear that the half-life of rafts is in the range of 10^2 ns, which is not only faster than the rate of many enzyme reactions, but also less than the half-life of interactions between several proteins known to be involved in signal transduction (Swamy et al. 2006). Thus, there was imminent need for improving techniques for isolation of membrane rafts and for exploring the functional changes involved in signaling. In this regard, a recent study by Doughty et al. used secondary ion mass spectrometry (Nano SIMS) technology to determine the subcellular localization of hopanoid lipids in bacteria using stable-isotope labeling (Doughty et al. 2014). The SIMS technique, however, uses primary ions like Cs^+ or O^- to bombard the surface of sample resulting in generation of secondary ions that are then measured by mass spectrometry.

Images are formed from the collection of mass spectra data from several spots on the surface. This technique can also be utilized to determine isotope ratios with high precision and used to visualize labeled sample regions (Altelaar et al. 2006; Grignon 2007). Furthermore, advances in microscopy techniques utilizing fluorescent labels or genetically-encoded probes and the mini-singlet-oxygen generator (miniSOG) to visualize the sub-cellular localization of proteins and lipids by electron microscopy moved the field forward significantly (Huang et al. 2009a). Additionally, new super-resolution microscopy techniques such as 3D-structured illumination microscopy (SIM), photoactivated localization microscopy (PALM), stochastic optical reconstruction (STORM), and stimulated emission depletion microscopy (STED) allow the investigation of lipid rafts at a level that was previously unattainable (Huang et al. 2009a). Among these, SIM is an ultra-resolution light microscopy technique that utilizes patterned illumination from a coherent light source to observe structures below the resolution limit of light microscopy by generating difference/beat frequencies termed *Moiré fringes*. This technique can be used with any conventional fluorophore and cell preparation (Kusumi et al. 2005a, b, 2014). On the other hand, PALM method is based on serial photoactivation or photoswitching and subsequent bleaching of several randomly scattered fluorescent proteins. This method provides a significant edge over the limitations of electron microscopy and conventional fluorescent microscopy to quantify protein aggregates or view cluster sizes that lie below diffraction limits. Utilizing total internal reflection fluorescence geometry (TIRF) in PALM further improves the detection range of fluorescent molecules. In order to investigate lipid rafts, another important technique which can be utilized is STORM imaging. This method involves several imaging cycles where a fraction of fluorophores are optically resolved in the field of view during each cycle, resulting in a highly accurate determination of individual active fluorophores (Rust et al. 2006). Yet another important technique in lipid rafts research is the STED technique, which uses non-linear fluorophore responses to improve the resolution and acquire images below the diffraction limit (Kusumi et al. 2014; Egging et al. 2013; Sahl and Moerner 2013). Both PALM and STORM techniques, on the contrary utilize mathematical modeling to reconstruct the sub-diffraction limit from multiple sets of diffraction-limited images (Rust et al. 2006; Sengupta et al. 2011; Hess et al. 2006). To summarize, the utilization of novel

tools has helped generate deeper insight into the functional aspects of lipid rafts, which will help direct future studies and therapeutic intervention strategies targeting lipid rafts and lipid raft-associated proteins.

Lipid rafts and bacterial infections

Lipid rafts have been shown to play an important role during bacterial infections. Evidence of the ruffling of membrane lipids and proteins during pathogen engulfment and requirement for cholesterol during pathogen invasion substantiates this claim. Further, the requirement of raft-associated receptors on epithelial cells, including CD55 on the apical membrane and CD44 on the basolateral membrane, has been reported for bacterial entry (Selvarangan et al. 2000; Peiffer et al. 1998; Stuart et al. 2002a; Oliferenko et al. 1999). Several strains of bacteria invade phagocytes and non-phagocytic cells through the endocytic pathway in order to avoid the host defense mechanism (Simons and Gruenberg 2000; Steinberg and Grinstein 2008; Gruenberg and van der Goot 2006). Pathogens entering the host cells through the endosome-lysosome pathway involving clathrin-coated pits have the ability to avoid fusion or acidification following fusion with lysosomes to prevent degradation. Recent evidence suggests that these endocytic entry mechanisms are directed by the structure of lipid rafts, which are internalized via clathrin-dependent pathways. After endocytosis lipid rafts can form caveosomes and fuse with the Golgi complex or endoplasmic reticulum, or alternatively, form intracellular vesicles and remain within the organelle. The entry of pathogens via lipid rafts promotes intracellular survival and dissemination within the host. Raft-mediated entry of *Escherichia coli* (*E. coli*), *Shigella flexneri*, *Salmonella thyphymurium*, *Mycobacterium* spp., *Chlamydia* spp., *Ehrlichia chaffeensis*, *Anaplasma phagocytophilum*, *Campylobacter jejuni* (Lafont and van der Goot 2005; Manes et al. 2003a), *Listeria monocytogenes* (Seveau et al. 2004), *Mycoplasma fermentas* (Yavlovich et al. 2006), and *Sphingomonas* spp. (Ammendolia et al. 2004) have been studied extensively to find out the role of lipid rafts in the pathogenesis of the bacterial infections. In this regard, role of molecular motors kinesin and cytoplasmic dynein has also been investigated in terms of trafficking of pathogens inside cells towards lysosomal degradation. Molecular motors are important in the transport of organelle cargoes along microtubules in the cell. This transport driven by molecular motors, either

unidirectional or bidirectional is regulated through their post-translational modifications, interactions with effector molecules or autoinhibition. Movement of early phagosomes along microtubules is bidirectional manner, and of late phagosomes is unidirectional which promotes its fusion with lysosomes (Grover et al. 2016). In a recent study, Rai et al. demonstrated that the mobility of early and late phagosomes correlates with the appearance of lipid rafts and clustering of dynein motors in phagosome membrane. The geometrical reorganization allows dyneins within cluster generate cooperative force on a single microtubule and results in rapid directed transport of phagosome which probably promotes phagosome and lysosome fusion leading to pathogen degradation. Disruption of clustering of small lipid microdomains by several pathogens can help in survival of pathogen inside the host by inhibition of phagosome motion and phagosome-lysosome fusion (Rai et al. 2016). Interestingly, lysosome function can also be regulated by cholesterol. Further, Deng and coworkers demonstrated that cholesterol depletion can also disrupt lysosome membrane permeability (Deng et al. 2009). In this line of thought, mycobacteria was shown to exploit lipid rafts to enhance their adaptive fitness and protection from intracellular degradation by lysosomal pathways. Mycobacteria induce recruitment of tryptophan aspartate-containing coat protein (TACO), which associates with the phagosomal membrane in a cholesterol-dependent way and prevents its fusion with lysosomes (Gatfield and Pieters 2000a). A report by Wang and coworkers demonstrate that depletion of lipid rafts by methyl- β -cyclodextrin resulted in reduced survival and virulence of *Porphyromonas gingivalis*, a periodontal pathogen in the host. Interestingly, lipid rafts disruption was shown to promote the colocalization of internalized *P. gingivalis* with lysosome (Wang and Hajishengallis 2008). While *Chlamydia trachomatis*, a common sexually transmitted pathogen also use lipid rafts to disguise the phagosome as a vesicles which are derived from Golgi apparatus. These phagosomes are transported to Golgi apparatus to capture sphingolipid-rich vesicles to disguise host defense system (Norkin et al. 2001). These studies clearly indicate an important role of lipid rafts in pathogen survival beyond entry. Therefore, it is important to understand the mechanism(s) of invasion used by pathogens in order to design the successful strategy for regulating disease pathogenesis.

Mechanisms of bacterial entry in cells Caveolae, Clathrin-coated pits and phagocytosis all provide entry

pathways to toxins, and fall within the size range of 60–100 nm, respectively (Conner and Schmid 2003; Rollason et al. 2007). Of these, clathrin-coated pits are considered raft-free, while the caveolae pathway has been considered to be raft-mediated. Interestingly, Shiga toxin and the protective antibody against anthrax toxin both associate with rafts on the cell surface and are subsequently internalized via clathrin-coated pits (Kurzychalia 2003; Sandvig and van Deurs 2002a, b). Association of caveolae with *E. coli*-containing phagosomes has been reported during its cellular entry (Jermy 2010; Zaas et al. 2005), which reflects that caveolae may provide signaling platforms required for bacterial engulfment.

Signaling pathways regulated during bacterial infections The entry of bacteria through lipid rafts leads to changes in signaling pathways including autophagy, alterations in inflammatory cytokine production, and induction of apoptosis (Fig. 1). Some bacteria like *Salmonella typhimurium* and *S. flexneri*, modulate host signaling by secreting bacterial invasion effector proteins which associate with membrane rafts (Manes et al. 2003b; Lafont et al. 2002; Lacalle et al. 2002). Further, while

cellular entry of certain toxins and bacteria have been found to occur through raft-containing pathways, the step(s) required may differ depending on the pathogens.

Besides utilizing rafts to generate phagosomes as a survival mechanism within non-professional and professional phagocytic cells (Gatfield and Pieters 2000b; Kim et al. 2002; Ferrari et al. 1999; Watarai et al. 2001), bacteria like *L. monocytogenes*, an intracellular bacterium, have been shown to augment raft-mediated signaling thereby leading to increased cytokine production at the site of infection (Seveau et al. 2004; Coconnier et al. 2000; Parihar et al. 2013; Gekara et al. 2010; Barbuddhe and Chakraborty 2008; Kayal and Charbit 2006). Moreover, recent studies have shown impaired immune responses and reduced survival of *caveolin-1*-deficient mice in response to pulmonary challenge with *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* (Guo et al. 2012; Gadjeva et al. 2010). Further, it was observed that knocking down *caveolin-1* resulted in reduced bacterial clearance and an increase in production of pro-inflammatory cytokines in *Klebsiella*-challenged lung epithelial MLE-12 cells (Guo et al. 2012). Lipid rafts have thus been proposed to modulate bacterial internalization, which may impact host repair mechanisms, a critical

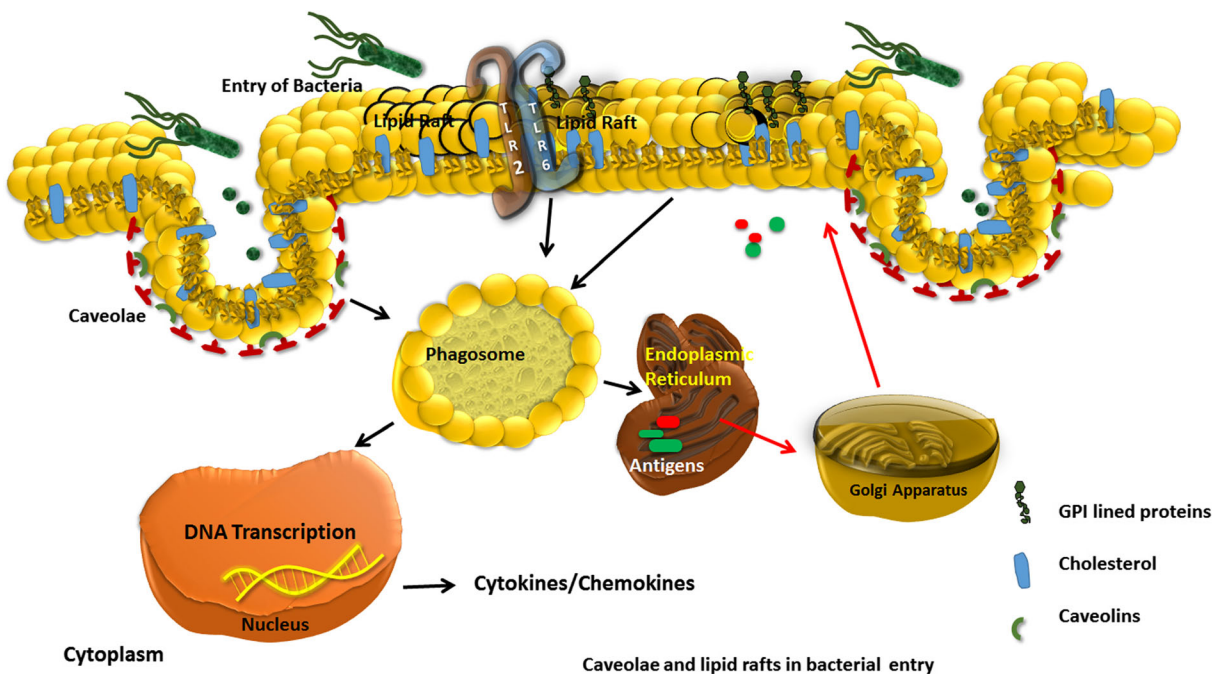


Fig. 1 Role of caveolae and lipid rafts in bacterial entry. Bacteria usually enters the cell through endocytic pathway and survives by avoiding fusion with lysosome. The entry is mediated through caveolae or lipid rafts and can be targeted to a number of different

intracellular compartments. Bacteria that enter in a lipid raft-dependent manner trigger signaling pathways including induction of apoptosis and cytokine production

phenomenon for host defense against the possibility of DNA damage caused by *K. pneumoniae* infection (Huang et al. 2013). It was reported that lungs from *caveolin-1* deficient mice showed suppressed NF- κ B activation in response to intraperitoneal injection of lipopolysaccharide, an effect believed to be mediated via endothelial NO synthase (eNOS) (Garrean et al. 2006) (Fig. 2). *P. aeruginosa*, on the contrary, induces the formation of ceramide-rich rafts, which not only promote host-pathogen interactions but also induces apoptosis of infected cells (Grassme et al. 2003a). Together, these studies suggest that lipid rafts play critical roles not only in bacterial entry, but also in influencing the host inflammatory responses critical for promoting or clearing infection.

Bacterial membrane microdomains In addition to the role of host cell membrane domains during infections, bacterial proteins appear to recognize specific sites on their own membranes as well. Recently Lopez and Kolter described the existence of functional microdomains in bacterial membranes that harbor homologs of Flotillin-1 (eukaryotic lipid raft protein) and are rich in polyisoprenoids and other signaling and transport proteins (Lopez 2015; Lopez and Kolter 2010). Bacterial membrane proteins contained in lipid rafts function similarly to those found in eukaryotes and are involved in cell signaling pathways (Barak and Muchova 2013). Furthermore, as discussed by Barak and Muchova, differential aspects of a bacterial membrane's physical characteristics, specifically, a negative curvature (concave) or a positive curvature (convex), are recognized by bacterial proteins. Specifically, membranes with a convex shape are linked with asymmetric cell division. Yet another physical characteristic that is important for the organization/localization of proteins in bacterial membranes is the transmembrane electric potential (Barak and Muchova 2013). As utilized for study of eukaryotic lipid rafts, the procedure used to analyze bacterial rafts is based on their ability to resist disaggregation by non-ionic detergents followed by separation by sucrose gradient. Studies conducted by Lopez and coworkers demonstrate that the detergent-resistant membranes (DRM) of *S. aureus* involves the proteins required for biofilm formation, virulence, and signaling (Lopez and Kolter 2010). Moreover, all of these processes can be inhibited by blocking the formation of lipid rafts with the use of small molecules (Bramkamp and Lopez 2015). Zaragozic acid (ZA), a known inhibitor of squalene synthase, which is involved in the formation of polyisoprenoids, inhibits the formation of lipid rafts resulting in impaired biofilm

formation. In a recent study, Somani and coworkers demonstrated constitutive expression of FlotP, a Flotillin-1 homolog in *Bacillus anthracis*. The importance of rafts in the growth, membrane fluidity, and virulence of *B. anthracis* was demonstrated by studies conducted using ZA (Somani et al. 2016). Evidence of detergent soluble and detergent-resistant membrane fractions in *Bacillus halodurans* and *Bacillus subtilis* and the presence of flotillin-1 like protein (FloT) in the cytoplasmic membrane of the latter was also provided in recent studies (Donovan and Bramkamp 2009; Zhang et al. 2005). The membranes microdomains of bacterium *Borrelia burgdorferi*, which causes Lyme disease, have also been shown to possess properties similar to eukaryotic lipid rafts. Likewise, *B. burgdorferi* domains favor selective incorporation of molecules which are membrane anchored via saturated acyl chains but not those having unsaturated acyl chains (LaRocca et al. 2013). The other interesting observation reported in this study suggests that any changes in *B. burgdorferi* membrane morphology and integrity may affect the physical connection between flagella and the outer membranes. This may result in loss of flat wave morphology, which changes the membrane permeability leading to loss of flagellar subunits followed by death of spirochetes due to increased sensitivity to secondary osmotic lysis. These events confirm the functional importance of membrane microdomains in *B. burgdorferi* (LaRocca et al. 2013).

Bacterial membrane microdomains were discovered relatively recently, and therefore, increased understanding is still needed. Future studies need to focus on determining the structural components of bacterial rafts and their biological role in different bacterial species. Further, since the perturbation of bacterial microdomains can affect several physiological processes associated with pathogenesis, this field of study also may open new avenues for the control of bacterial infections.

Lipid rafts and viral infections

Viruses are classified into four basic types based on their outer structure. These include the following: enveloped DNA viruses, enveloped RNA viruses, non-enveloped DNA viruses, and non-enveloped RNA viruses. In addition, the general virus cycle of infection is typically divided into four stages: entry, translation, replication, and assembly/release (Fig. 3). The early stage of viral entry into the host cell involves the binding of the virus to one or

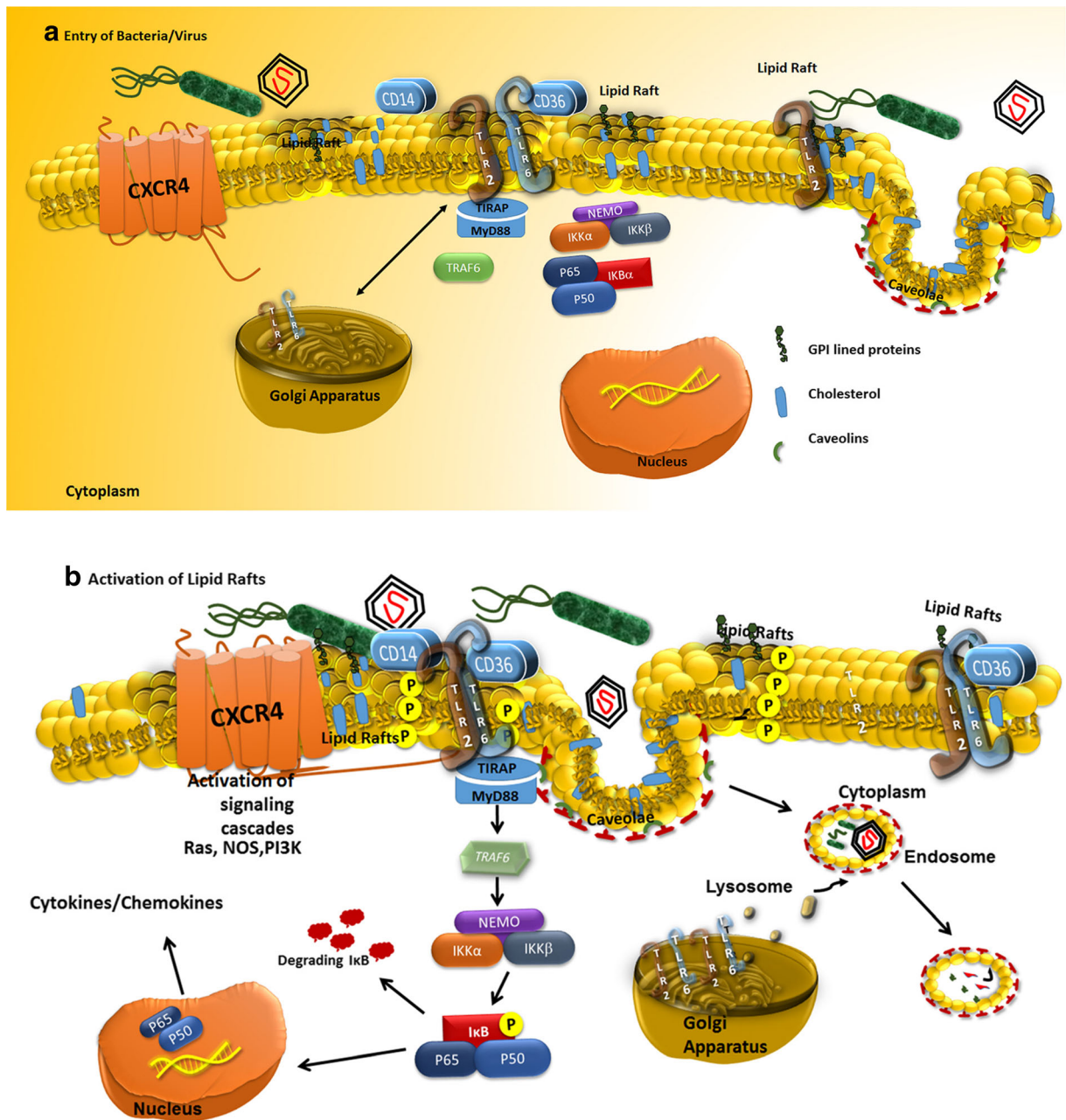


Fig. 2 Bacterial entry and interaction with receptor molecules. **a** Entry of bacteria into a host cell involves specific interactions of bacterial PAMPs (e.g., LPS and lipopeptides) with receptor molecules on cell surface, leading to conformational changes in rafts. **b** Upon stimulation by bacterial lipopeptides, TLR2 forms heterodimers with TLR6 in lipid raft domains, while CD14 and CD36 (cluster of differentiation) act as adaptor molecules for ligand

transfer onto TLR2/6 heterodimers. Following ligand stimulation TIRAP binds to TLR2 through TIR domains and recruits adaptor protein MyD88, which then activates of TNF receptor associated factor 6 (TRAF6). This pathway induces the activation of NF-κB pathway leading to production and secretion of pro-inflammatory cytokines

more cell-surface receptors followed by entry into the cell (Ono and Freed 2005). Study of the localization of viral structural proteins and the effects of use of

cholesterol/raft disrupting agents on the replication of several viruses have demonstrated the role of membrane microdomains in viral cycle.

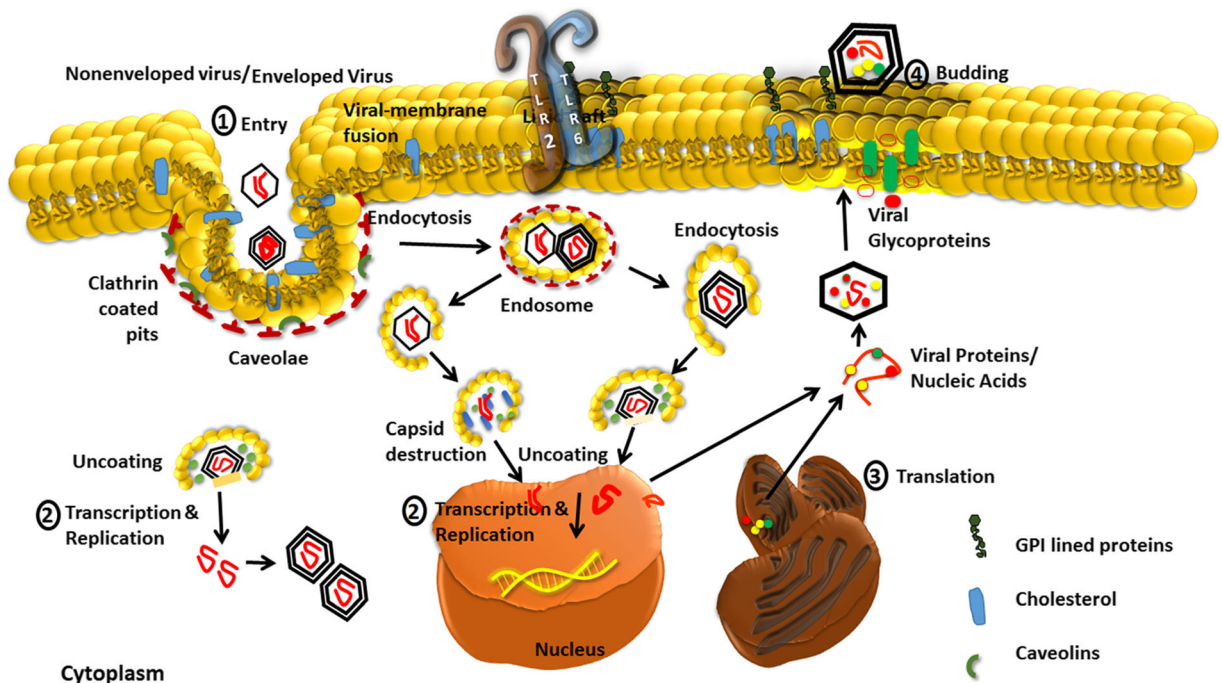


Fig. 3 Entry of virus through lipid rafts. Enveloped viruses enter the cells through direct fusion between the viral membrane and cell-surface receptor or through endocytic-mediated pathway, whereas in non-enveloped viruses are translocated directly into cytoplasm by destruction of the viral capsid. Transcription and replication process of viral genome in non-enveloped viruses takes

place in the nucleus but for enveloped viruses it takes place both in nucleus and cytoplasm. After assembly the viral proteins, viral glycoprotein's and infectious particles of viruses are released from cells via budding process at intracellular membranes (e.g., the ER) and at the plasma membrane which disrupts the humoral and cellular responses of the cell

Viruses generally enter cells through one of two basic mechanisms: (1) enveloped RNA viruses from the *Rhabdoviridae*, *Flaviviridae*, *Orthomyxoviridae*, and *Togaviridae* families and enveloped DNA viruses from the *Papovaviridae* and *Adenoviridae* families enter through endocytic vesicles and (2) through translocation or penetration of viral particles directly into the cytoplasm, by fusion of the viral envelope at the plasma membrane, or through destruction of the viral capsid as in the case of viruses from the *Paramyxoviridae* family (Takahashi and Suzuki 2011a). In contrast, viruses from the *Herpesviridae* family utilize both pathways (Takahashi and Suzuki 2011a). Moreover, most viral genomes of enveloped RNA viruses replicate and are transcribed in the cytosol, while those of enveloped DNA viruses replicate in the nucleus. Progeny viruses subsequently bud and are released from the cell membrane.

Involvement of surface receptors in viral entry There is plethora of information regarding the involvement of cell-surface receptors localized within lipid rafts in facilitating viral entry. In brief, facilitation of HIV-1 entry into

the cell by binding to the CD4 receptor causes conformational changes in the surface glycoprotein gp120 resulting in its interaction with CXCR4 or CCR5. These events lead to conformational changes in the transmembrane glycoprotein gp41 leading to unmasking of its fusogenic domain (Fantini et al. 1997; Hammache et al. 1998; Hug et al. 2000). While glycosyl phosphatidylinositol (GPI) anchored folate receptor- α (FR α) localized in membrane rafts is required for the entry of ebola and marburg viruses to enter host cells. The EBV human herpes virus infects B lymphocytes via the human complement receptor type 2 (CR2 aka CD21), which exists as a complex with CD19 and palmitoylated CD81 in lipid rafts. This CD19/21/81 complex functions to prolong the stability of, and signaling via, the B cell antigen receptor (Fingerroth et al. 1984; Cherukuri et al. 2004; Birkenbach et al. 1992). While discussion about all the related studies is beyond the scope of this article, most of the work in this area is referenced in Table 3.

Mechanisms of viral entry in cells Following the attachment of non-enveloped viruses to cell-surface receptors,

Table 3 Receptors involved in the entry process of viruses

S.No	Family	Viruses	Mode of entry	Receptor	Model Used	References
1	<i>Herpesviridae</i>	Human herpes virus-6 (HHV- 6)	Membrane rafts	CD46	HSB-2 cells	(Tang et al. 2008)
2	<i>Herpesviridae</i>	Human herpes virus-8	Clathrin-dependent pathway	PI3K,RhoA-GTPase and $\alpha 3 \beta 1$ integrins	HMVEC-d and BCBL-1 cells, HFF cells, 293 cells, HMVEC-d, BJAB, BCBL-1, CHO-B2	(Raghu et al. 2007; Akula et al. 2002; Naramatt et al. 2003)
3	<i>Herpesviridae</i>	Murine cytomegalovirus (MCMV)	Clathrin-dependent/caveolae/raft-dependent pathway	Early endosomal marker EEA-1	Primary mouse embryonic fibroblasts (MEF), COS-7 and HeLa cells	(Sharp et al. 2009; Low et al. 2016)
4	<i>Herpesviridae</i>	Lymphotrophic herpes virus saimiri (HVS)	Clathrin-dependent pathway/raft-dependent pathway	T cell receptor, CD4	293 T cells, Jurkat T cells	(Lee et al. 2004; Cho et al. 2006)
5	<i>Herpesviridae</i>	Epstein-Bar virus (EBV)	Endocytic pathway and By Direct fusion with cell-surface membrane	C3d Complement Receptor (CR2), dynamin, caveolin-1	B cell lines, B95-8, Mutu I and Mutu III	(Nemerow et al. 1985; Nanbo et al. 2013)
7	<i>Herpesviridae</i>	Herpes simplex virus	Endocytosis	Nectin1, heparan sulfate	HCJE cells	(Akhtar et al. 2008; Shukla and Spear 2001)
8	<i>Poxviridae</i>	Vaccinia virus	Cholesterol-mediated raft entry	GM1	BSC-40 cells	(Chung et al. 2005)
9	<i>Hepadnaviridae</i>	Hepatitis B virus	Clathrin-dependent endocytosis	Sodium/bile acid co transporting Peptide(SLC10A1)	HuS-E/2 cells	(Xiao et al. 2013; Huang et al. 2012)
10	<i>Adenoviridae</i>	Simian virus 40	Clathrin and caveolae-dependent and independent pathway	MHC class I and ganglioside GM1	HuH7, African green monkey kidney fibroblasts, CV-1 cells	(Norkin et al. 2002b; Dammm et al. 2005; Tsi et al. 2003b; Anderson et al. 1998b)
11	<i>Papovaviridae</i>	Bovine papillomavirus	Clathrin-dependent endocytosis and caveolae-dependent pathway	Glycosaminoglycan	293 cells	(Laniosz et al. 2008)
12	<i>Papovaviridae</i>	Human papillomavirus	Clathrin-dependent endocytosis and caveolae-independent pathway	alpha(6) integrin, heparansulphate proteoglycans and $\alpha 6$ integrin	HaCaT (epithelium), HeLa (cervix), C33A (cervix), Caski (cervix), T98G (glioblastoma), SK-N-SH (neuroblastoma), OVCAR-4 (ovary), K562 (leukemia), and T47D (breast), and one monkey kidney cell line Cos-7	(Androutsopoulos et al. 2015; Yoon et al. 2001)
14	<i>Retroviridae</i>	Human T lymphotropic virus 1 (HTLV-1)	Membrane rafts and utilizes Glucose transporter (Ghut 1)	Chemokine receptors-CXCR4/CCR5 and CD4	K562,MT2 Cell lines, COS, and HEK 293 T cells	(Pique et al. 1993; Jones et al. 2006)
15	<i>Retroviridae</i>	Human immunodeficiency virus (HIV)	Caveolin-dependent raft and endocytosis pathway	Chemokine receptors-CXCR4/CCR5 and GPI-anchored proteins, CD4	Human embryonic kidney 293 T cells, H9 leukemic T cells and peripheral blood mononuclear cells (PBMC), T cell	(Liao et al. 2001b; Nguyen and Hildreth 2000; Nguyen and Taub 2002b; Alfsen et al. 2005; Leung et al. 2008b; Popik et al. 2002)

Table 3 (continued)

S.No	Family	Viruses	Mode of entry	Receptor	Model Used	References
16	<i>Rhabdoviridae</i>	Vesicular stomatitis virus (VSV)	Endocytic pathway/lipid-raft-mediated entry	GPI-anchored proteins	lymphoma cell line, Jurakat, HeLa cells	(Patel et al. 2009)
17	<i>Filoviridae</i>	Ebola virus	Clathrin-dependent and caveolae-dependent endocytosis	GPI-anchored protein	Human 293 T cells	(Bavari et al. 2002b)
18	<i>Filoviridae</i>	Japanese encephalitis virus (JEV)	Clathrin-dependent and caveolae-dependent endocytosis	GPI-anchored protein-Src/PI3K and lipid raft-mediated entry	Mouse neural stem cell line C17.2, Huh7, BHK-21, and African green monkey kidney Vero E6 cells, astrocyte cell culture	(Das et al. 2010b; Chen et al. 2011a; Zhu et al. 2012)
19	<i>Filoviridae</i>	Dengue virus (DEN)	Lipid-raft-mediated entry	ICAM, heat shock proteins (HSP)-HSP90 and 70, GPI-anchored proteins	Human myelomonocyte cell line U937 and association of dengue virus NS1 protein with lipid rafts	(Reyes-del Valle et al. 2005; Noisakran et al. 2008)
20	<i>Paramyxoviridae</i>	Respiratory syncytial virus (RSV)	Caveolin-mediated endocytosis	GPI-anchored proteins	Hep 2 cells and vero C 1008 cells	(McDonald et al. 2004; Brown et al. 2002c)
21	<i>Arenaviridae</i>	Lymphocytic choriomeningitis virus (LCMV)	Clathrin/caveolin-raft-dependent pathway	GPI-anchored proteins	HeLa cells and vero cells	(Quirin et al. 2008)
22	<i>Coronaviridae</i>	SARS-CoV, corona virus	Clathrin-independent and caveolae-independent pathway	ACE 2 receptor	Vero E6 cells and HEK 293 T cells	(Li et al. 2007; Wang et al. 2008)
23	<i>Flaviviridae</i>	Human hepatitis C virus (HCV)	Endocytosis	CD81 receptor	Huh-7 cells, HOS, CDR5 cells, HeLa and 293 T cell line	(Zhang et al. 2004)
24	<i>Orthomyxoviridae</i>	Influenza virus	Clathrin-independent endocytosis, caveolae-independent endocytosis	GPI-anchored proteins	HeLa-T4 cells, MDCK cells (Madin-Darby canine kidney)	(Cheong et al. 1999; Zhang et al. 2000)
25	<i>Orthomyxoviridae</i>	Measles virus	Raft-mediated	GPI-anchored proteins, Receptors of H protein-Complement regulatory molecule CD46	Human B-lymphoblastoid cell line	(Manié et al. 2000)
26	<i>Togaviridae</i>	Sindbis virus	Cholesterol-mediated pathway	GPI-anchored proteins	BHK-21 cells	(Ng et al. 2008)
27	<i>Togaviridae</i>	Semliki forest virus	Cholesterol-mediated pathway	GPI-anchored proteins	BHK-21 cells	(Lu and Kielian 2000)
28	<i>Rhabdoviridae</i>	Rabies virus	Endocytosis	GPI-anchored proteins	BHK-cells	(Whitt et al. 1991)
29	<i>Picornaviridae</i>	Echo virus 1	Caveolae-dependent and membrane raft-dependent endocytosis	$\alpha 2\beta 1$ integrins and DAF receptors(CD55)	Green monkey kidney cell line	(Xing et al. 2004)
31	<i>Picornaviridae</i>	Enterovirus	Caveolae-dependent and membranerraft-dependent endocytosis	DAF receptors	HT29 cells	(Stuart et al. 2002b)
32	<i>Picornaviridae</i>	Rhino virus	Lipid rafts	ICAM-1	Human bronchial epithelial cells and HeLa cells	(Bentley et al. 2007b)

Table 3 (continued)

S.No	Family	Viruses	Mode of entry	Receptor	Model Used	References
33	<i>Picornaviridae</i>	Coxsackie virus	Clathrin-independent; caveolae/raft-dependent endocytic pathway	DAF (decay accelerating factor) CD55, MHC class I, and integrins family	Hela cells, and GMK cells	(Triantafyllou et al. 2002; Triantafyllou and Triantafyllou 2003)
34	<i>Picornaviridae</i>	Rotavirus	Endocytosis pathway	Integrins, $\alpha2\beta1$ $\alpha v\beta3$ and hsc 70 dynamin	MA104 cells	(Gutiérrez et al. 2010)
35	<i>Picornaviridae</i>	Poliovirus	Receptor-mediated endocytosis	Poliovirus receptor (CD155)	African green monkey kidney cells	(Ohka et al. 2004)
36	<i>Reoviridae</i>	Blue tongue virus	Clathrin-dependent and lipid raft-mediated	SNARE receptor-VP5 (soluble N-ethylmaleimide-sensitive fusion attachment protein receptor)	BHK cells (baby hamster kidney cell line) and HeLa cells	(Bhattacharya and Roy 2008b)

the bound virions can be internalized through a process mediated by rafts involving membrane invaginations and intracytoplasmic vesiculation (Ono and Freed 2005; Takahashi and Suzuki 2011a; Suzuki and Suzuki 2006). This type of viral entry, also termed atypical endocytosis, has been observed for the SV40 (*Papovaviridae* family) virus. Entry of SV40 into target cells is mediated through an association of major histocompatibility complex-1 (MHC-1) with caveolae (Stang et al. 1997; Anderson et al. 1998a; Norkin et al. 2002a; Pelkmans et al. 2001) or the GM1 ganglioside receptor in lipid rafts (Tsai et al. 2003a; Pelkmans 2005). Virus-incorporated caveolae then undergo budding, and the caveolae carries the virus to the endoplasmic reticulum (ER) along cellular microtubules.

Non-enveloped DNA viruses also enter into cells through typical endocytosis utilizing clathrin-dependent or clathrin-independent and caveolae/raft-dependent pathways followed by release of their viral DNA genomes into the cytoplasm by viral capsid destruction (Ono 2010). Reports suggest that *Coxsackievirus*, which causes aseptic meningitis with symptoms resembling a respiratory disease in addition to flaccid paralysis and chronic myocarditis, utilizes the cellular coxsackievirus and adenovirus receptor for its entry via membrane rafts (Triantafyllou and Triantafyllou 2003; 2004). In addition, several enteroviruses, which cause nerve paralysis, cerebral meningitis, anathema and respiratory symptoms, also utilize caveolae-dependent or raft-dependent endocytosis mechanisms for cellular entry (Stuart et al. 2002a; Marjomaki et al. 2002; Upla et al. 2004; Pietiainen et al. 2005). In contrast, entry of rhinovirus is mediated by ceramide-rich rafts (Grassme et al. 2005).

Enveloped viruses also use rafts during the internalization and fusion process. The entry of enveloped viruses involves virus attachment followed by close apposition of the virus and plasma membranes. The two membranes then fuse to deliver the virus' genomic RNA into the host cells, which requires conversion of the virus-encoded envelope glycoprotein (Env) from its native state to its fusion-activated form (Manes et al. 2003a; Fantini et al. 2002; Chazal and Gerlier 2003; Brown et al. 2002a)

Virus assembly The later stages of the virus infection cycle involve the assembly of viral components into virions and maturation into infectious particles. Viruses use lipid rafts as a platform because their structure offers

an efficient system for concentrating all the viral proteins and glycoproteins required for the assembly of new virions (Ono and Freed 2005; Suzuki and Suzuki 2006; Das et al. 2010a). There is abundant evidence to support the idea that membrane lipids are not randomly incorporated into the viral envelope. Further, it has also been suggested that viral glycoproteins determine the site of virus assembly and budding (Vieira et al. 2010; Garoff and Simons 1974; Garoff et al. 1974; Allison et al. 1995; Vennema et al. 1996; Bruss 2004). Moreover, in polarized epithelial cells, the viral glycoproteins contain sorting signals or motifs and are directed to the specific site where assembly and budding will occur (Vieira et al. 2010; Nayak et al. 2004). Finally, the lipid composition of the influenza virus family is affected by the affinity of the haemagglutinin and neuraminidase glycoproteins for these lipids, while some reports also suggest that the influenza virus buds from raft domains (Vieira et al. 2010; Chazal and Gerlier 2003; Nayak et al. 2004). HIV-1 is also enclosed in a lipid envelope enriched in cholesterol and sphingolipids, suggesting specific membrane localization is required for assembly (Vieira et al. 2010; Aloia et al. 1993; Campbell et al. 2001; Raulin 2002). With similar methods of assembly and budding within membrane rafts, many other viruses, including influenza virus, measles virus, Ebola virus, and possibly Sendai virus, also use lipid rafts as assembly platforms (Vieira et al. 2010; Luo et al. 2008). In this regard, it was also suggested that the RSV (respiratory syncytial virus) assembles within lipid rafts where viral proteins colocalize with caveolin-1 (Vieira et al. 2010; Brown et al. 2002a, b). Taken together, these structures then have the ability to regulate budding; however, the mechanism by which the lipid raft can favor the budding and/or fission process has yet to be explored in detail (Vieira et al. 2010; Nayak et al. 2004; Luo et al. 2008).

For enveloped viruses, the infectious particles are released from the cell via the budding process. Furthermore, the budding of new virions from the raft allows the exclusion or inclusion of specific host cell membrane proteins in the virus particle, which can disrupt cellular and/or humoral immune responses to the virus (Suzuki and Suzuki 2006; Das et al. 2010a). Non-enveloped viruses are released from the infected cell by disrupting the plasma membrane, whereas enveloped viruses contain a host cell-derived lipid bilayer, which is acquired during budding. Only cytolytic viruses lead to disruption of cell integrity, while other viruses are released in a non-destructive manner. In this context,

studies demonstrated that ebola and marburg viruses use viral glycoprotein-bearing rafts for budding from host cells. Specifically, VP40 (ebola virus matrix protein) oligomerizes with rafts and plays an important role in assembly and budding (Bavari et al. 2002a), while glycoprotein complexes composed of gp120 and gp41 along with membrane rafts are used for HIV-1 assembly (Bhattacharya et al. 2004; Ono et al. 2005; Ono et al. 2007; Leung et al. 2008a). Koshizuka's group demonstrated the association of HSV-1 UL11 (protein involved in virion maturation) with lipid rafts (Koshizuka et al. 2007). Among non-enveloped viruses, the association of viral protein VP4 and NSP4-purified virions with lipid rafts have been studied for rotavirus (Sapin et al. 2002; Cuadras and Greenberg 2003) while an outer capsid protein important for virus assembly, VP5, was observed to co-purify with lipid raft entities during bluetongue virus infection. The association of VP5 with rafts was shown to depend on membrane docking domain similar to what occurs with the soluble N-ethylmaleimide-sensitive fusion attachment protein receptor (SNARE) family (Bhattacharya and Roy 2008a). Although rafts are involved in virus assembly, we have to keep in mind that only a fraction of viral proteins are found associated with rafts. However, this could be due to the poor biochemical characterization of raft subsets or to the transient nature of the association (Vieira et al. 2010).

Signaling pathways regulated during viral infections Since membrane rafts are involved in the entry, assembly, and budding processes of several viruses, it is not surprising that cellular signaling pathways are also affected during viral infections. Lee et al. reported the anti-apoptotic role of PI3K signaling in response to dengue and japanese encephalitis virus (JEV) infections in human lung carcinoma and murine neuroblastoma cell lines (Lee et al. 2005). However, using microglial cells, Chen and coworkers demonstrated the induction of the Src/Ras±/Raf/ERK/NF-κB pathways leading to induction of TNF-α and IL-1β in response to challenge with JEV (Chen et al. 2011). This is important because Src-mediated signaling induced by JEV is known to play a critical role in neuronal cell death. Earlier studies revealed the role of lipid rafts in the activation of Src, Ras, Raf, ERK, and NF-κB, which all contribute to JEV-induced TNF-α and IL-1β production (McCubrey et al. 2007; Wu et al. 2015). Furthermore, involvement of lipid rafts in Src-mediated signaling pathways upstream of the activation of Raf/

ERK/NF- κ B has been reported in response to several stimuli (Roux and Blenis 2004). These studies provide substantial evidence that lipid rafts play an important role in Src/Ras/Raf/ERK/ NF- κ B signaling during JEV infection, thereby influencing host immune responses. In addition, Bentley and coworkers (Bentley et al. 2007a) also demonstrated that rhinovirus (RV) activates PI3K and Akt, which leads to IL-8 expression. Further, they observed that RV colocalizes with Src, PI3K, and Akt in membrane rafts. These and several related findings demonstrate that lipid raft-mediated signaling pathways are regulated during viral infections and play critical role in disease pathogenesis (Bentley et al. 2007a; Silva et al. 2011; Ebihara et al. 2008; Huang et al. 2009b; Fang et al. 2010) (Fig. 2).

Lipid rafts and fungal infections

Information regarding the role of lipid rafts in fungal infections is evolving; however, initial studies suggest that rafts facilitate fungal infections by regulating hyphal growth and biofilm formation, and by concentrating virulence factors (VFs) (Vieira et al. 2010; Farnoud et al. 2015).

Role of the host rafts during fungal infection and modulation of host signaling The connection of fungal infection with lipid rafts has not been extensively explored; however, evidences for modulation in host cell signaling pathways by mycopathogens has been demonstrated. Paracoccidioidomycosis is one of common fungal disease with high prevalence in South and Central America and is caused by *Paracoccidioides brasiliensis*. Infection with *P. brasiliensis* promotes the aggregation of lipid rafts in epithelial cells, which then support fungal adhesion and activation of Src kinases (Fig. 4). Localization of Ganglioside (GM1) in the host membrane microdomains allows *P. brasiliensis*-epithelial cell contact and fungal adhesion, which was found to be inhibited by methyl- β -cyclodextrin (M β CD) (Maza et al. 2008). Moreover, invasion by *Candida albicans* or *P. brasiliensis* leads to the activation of host cell tyrosine kinases which can be regulated by tyrosine kinase inhibitors (Belanger et al. 2002; Monteiro da Silva et al. 2007). Further, hyphal growth and biofilm formation facilitate invasive growth of *Cryptococcus neoformans* into the central nervous system; for which the pathogen

needs to travel across the blood-brain barrier composed of human brain microvascular endothelial cells (HBMECs). CD44 is the *C. neoformans* receptor believed to be anchored in lipid rafts on the surface of HBMECs during infection. It is known that in *caveolin-1* knockdown HBMECs, GM1 and CD44 protein become randomly distributed and clustered into irregular-islands on the cell surface (Long et al. 2012). This suggests an important structural role of caveolin-1 in *C. neoformans* invasion. Activated PKC α , can directly interact with caveolin-1, on the plasma membrane, and is required for *C. neoformans* invasion into HBMEC (Jong et al. 2008; Oka et al. 1997). Finally, the microsporidium parasite, *Encephalitozoon cuniculi*, which affects the nervous system, as well as the respiratory and digestive tracts, has been shown to localize to lipid rafts. While residing in a parasitophorous vacuole, this pathogen is surrounded by host cell lipids; which could be labeled with DiI16 (1,1'-dihexadecyl-3,3,3',3'-tetramethylindocarbocyanine), a marker for lipid rafts, and DiO (3,3'-dilinoleylloxycarbocyanine), a marker for non-raft membrane domains. These results suggest that rafts contribute to the formation of parasitophorous vacuole membrane (Ronnebaumer et al. 2008) and also that mycopathogens may also infect cells in a raft-dependent manner (Vieira et al. 2010).

Role of fungal rafts in virulence and drug resistance The primary raft-forming lipids in *C. albicans*, a dimorphic opportunistic pathogen, include ergosterol and glycosphingolipids, while the transporter of N-acetylglucosamine protein (Ngt1), an efflux pump and drug transporter protein (CaCdr1p), GPI-anchored protein (Gas1p), and proton pump protein (Pma 1) are the major proteins associated with these rafts (Martin and Konopka 2004; Simonetti et al. 1974; Pasrija et al. 2008). *C. albicans* rafts are capable of forming biofilms creating a protected niche for microorganism against antibiotics and a source of persistent infection. The efficiency of biofilm formation can be significantly reduced after treatment with myriocin and aureobacidin suggesting an important role of sphingolipids (lipid rafts) in biofilms formation (Martin and Konopka 2004; Lattif et al. 2011). Furthermore, displacement of these proteins from membrane by disruption of ergosterol or the sphingolipid biosynthesis pathway negatively influences the drug resistance of fungi and suggests the importance of raft assembly for localization of these proteins (Wang et al. 2012). Similarly, when CaCdr1p is

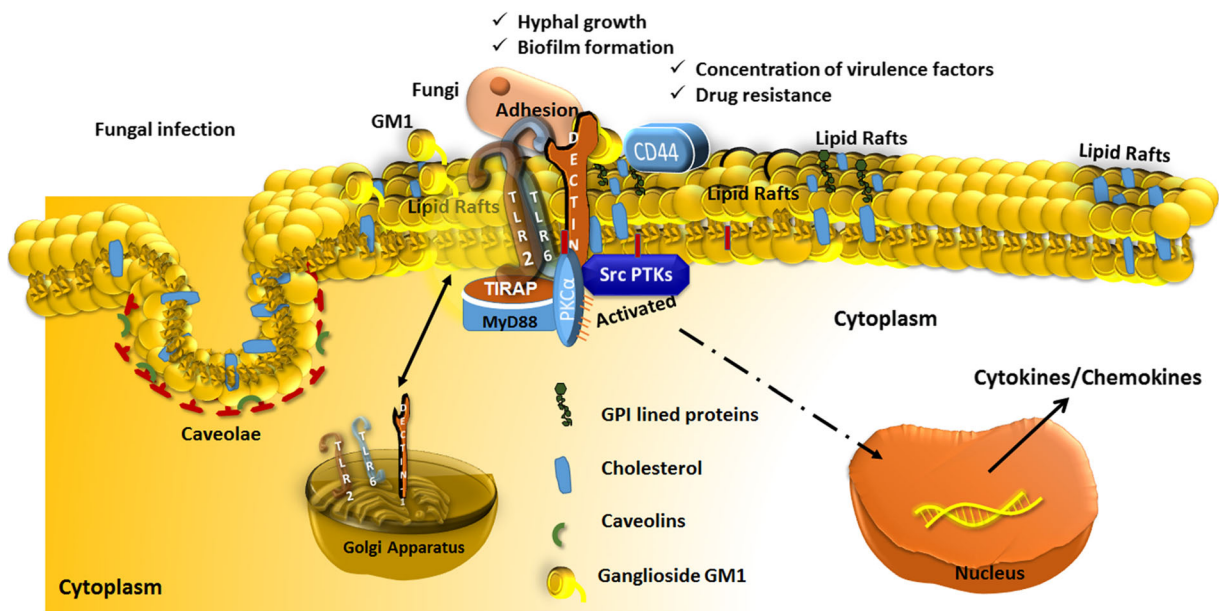


Fig. 4 Fungal infection in association with lipid rafts and host cell signaling. Fungal infection promotes the aggregation of lipid rafts in epithelial cells. Localization of ganglioside (GM1) in the host membrane microdomains allows cell contact and fungal adhesion. Fungal adhesion leads to activation of PKC α and Src protein

tyrosine kinases leading to downstream signaling events in the host cells. Lipid rafts facilitate fungal infections by regulating hyphal growth, biofilm formation, concentrating virulence factors, and drug resistance

depleted from DRMs, *Saccharomyces cerevisiae* becomes prone to methotrexate and fluconazole (Pasrija et al. 2008). Furthermore, ergosterol depletion has also been shown to impair phagocytosis of *Histoplasma capsulatum*, which may result from the release of proteins with anti-phagocytic function (Tagliari et al. 2012). Although, secretion of anti-phagocytic proteins has been demonstrated in case of *C. neoformans*, the direct link with ergosterol depletion and secretion of these proteins during *H. capsulatum* infection has not yet been established (Tagliari et al. 2012; Luberto et al. 2003). While studying chitosan-resistant and chitosan-sensitive filamentous fungi, Palma-Guerrero and coworkers demonstrated the importance of fungal rafts in drug resistance. They identified an *Neisseria crassa* desaturase mutant with chitosan resistance and high membrane rigidity. The polymeric compound chitosan exerts its fungicidal effect by permeabilizing plasma membrane of filamentous fungi (Palma-Guerrero et al. 2010). Additionally, the role of detergent-resistant membranes in fungi for concentrating VFs-phospholipase B1 and Cu/Zn superoxide dismutase; and proton pump protein Pma1 was demonstrated by Siafakas et al. and Holyoak groups, respectively (Siafakas et al. 2006; Holyoak et al. 1996). Several reports have demonstrated the functional

role of these VFs in evading hosts immune responses and Pma1 in the pathogenicity of *C. neoformans* (Farnoud et al. 2014).

Perspectives

There is abundance of literature defining the role of lipid rafts in several pathological conditions including Alzheimer's disease, Prion disease (Campbell et al. 2001; Peters et al. 2003; Liao et al. 2001a; Nguyen and Taub 2002a; Liao et al. 2003), abnormalities associated with the cardiovascular system such as hypertension and cardiac hypertrophy (Fantini et al. 2002; Peters et al. 2003; Liao et al. 2001a; Golde and Eckman 2001) and during bacterial and viral infections. Therefore, understanding the regulation and formation/composition of rafts is imperative for the design of therapeutic strategies for prevention and cure of related pathologies, as well as to aid in patient management. For example, the use of statins in cancer chemotherapy has been suggested as these drugs have been shown to alter endothelial cell function and inflammatory responses (Mo and Elson 2004). However, the effect of statins is not directly

mediated through cholesterol, but is rather through their effect on isoprenoids, which share initial biochemical synthetic steps with cholesterol and play important roles in anchoring several GTPases in the membrane (Vaughan 2003). Similar effects including the activation of GTPase were reported in cholesterol depletion and repletion models used to study the raft function in cell culture models (Pierini et al. 2003; Nagao et al. 2007). In this regard, a membrane-tethering inhibitor of β -secretase that targets endosomes was designed for treatment of Alzheimer's disease (Rajendran et al. 2008). This same idea may be employed for development of small lipophilic inhibitors to target raft-anchored proteins that are activated in membrane compartments.

Earlier reports have also demonstrated that modulating the structure/composition of rafts can lead to inhibition of the infection cycle of HIV-1, which enters the host cell through rafts, and can regulate raft-mediated signaling pathways. SP01A, a widely used inhibitor of HIV-1 infection, affects cholesterol synthesis and regulates the cholesterol content of rafts thereby preventing HIV-1 fusion with CD4+ cells. Likewise, the use of anti-raft drugs shows clear promise as an alternative to antibiotics to help reduce the threat of drug-resistant bacteria. Further, targeting lipid raft formation in bacteria using small molecules has also been shown to reduce their virulence by regulating diverse processes including exoprotease production, biofilm formation, and attachment (Kong et al. 2006; Liu et al. 2008). However, careful assessment of the strategies utilized should be made while targeting cholesterol in the rodent models, as there exists quantitative and qualitative difference in its balance as compared to humans. Further, high concentrations of cholesterol at several anatomical sites would also impair the ability to uniformly target cholesterol for pathological conditions that effect various tissues/organs (Dietschy and Turley 2002).

Given that there is a unique assembly of proteins and lipids during the formation of signaling/raft domains in response to infection with various pathogens, alteration of the composition of lipid rafts through nutritional intervention may be other interesting approach to combat pathogens and modulate the inflammatory responses. This strategy has received wide acceptance in the recent years and growing evidence suggests that consumption of n-3 long-chain polyunsaturated fatty acids (LCPUFAs) can reduce the risk of cardiovascular disease and breast cancer. In this context, n-3 LCPUFA has been shown to alter the partitioning of proteins and

composition of rafts including the reduction of cholesterol (Hashimoto et al. 1999) and caveolae (Ma et al. 2004), and has been shown to regulate Akt and NF- κ B activation (Schley et al. 2007). In addition, n-3 LCPUFA induces p38MAPK and EGFR phosphorylation, which leads to increased apoptosis in tumor cells (Schley et al. 2007; Tikhomirov and Carpenter 2004). An extensive report was published by Turk and Chapkin (Turk and Chapkin 2013) describing the regulation of lipid raft size, composition, and associated signaling mechanisms by n-3 LCPUFA. These findings underscore the need to further assess the therapeutic potential of n-3 LCPUFA in disease states mediated by lipid raft-associated processes.

Conclusions

With the majority of the scientific community focusing on DNA, RNA, and proteins to understand the pathogenesis of disease, the role of lipids has remained relatively unexplored. However, recent advances in the field of membrane microdomains/lipid rafts research has opened new avenues to investigate alternative therapeutic intervention strategies for many diseases. Although there is abundant literature suggesting important role of lipid rafts in bacterial, viral, and fungal infections, several questions still remain to be answered in order to define and target raft-mediated responses. Thus, impairment of bacterial and viral entry and propagation in the host in response to deviations/alterations in functional membrane microdomains warrants further study in order to better assess their role in bacterial and viral infections. Further, the involvement of structural components in terms of regulating rafts size, the concentration of lipids in microdomains, the specificity of protein and lipid clustering, and rafts scaffolding during disease pathogenesis are areas requiring additional focus and potentially, even more sophisticated techniques. Addressing these particular aspects of functional membrane microdomains will lead to a better understanding of disease mechanisms and potentially more effective treatment strategies including cell-based or personalized medicine approaches. The former approach is based on the concept of delivering therapeutic agents/drugs to specific cell types using nano-particle based delivery of drugs to the plasma membrane of cells for raft-dependent intracellular trafficking (Partlow et al. 2008). With regard to personalized medicine

approaches, lipidomics monitoring can provide a description of the concentration, structure, and function of complete sets of lipids as well as information regarding the interaction of lipids with proteins and other metabolites. This information can be utilized to design a personalized strategy to restore membrane balance using lipids and cofactors from nutritional sources (Escriba 2006). In addition, recent reports have suggested increased individual disease susceptibility to several diseases due to the limitations of several therapeutic proteins like growth factors and antibodies to reach the cytoplasm of diseased cells. These limitations can be overcome through covalent modification with glycolipid structures (GPI anchors) resulting in directed lipid raft-mediated signaling. Furthermore, research and clinical studies using anticancer drugs that target lipid messengers or their carriers are ongoing. These drugs are designed to disrupt crosstalk between cells within the tumor microenvironment and cancer cells (Muller 2011). Future studies aiming to reduce the generation and secretion of exosomes, which possess the bioactive lipids found in the extracellular space in tumor environment, have also been proposed by the experts in cancer research. These new approaches hold promise and the potential to offer novel therapeutic targets with translation potential for future intervention studies.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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