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Forum Editorial

Lipid Rafts and Redox Signaling

PIN-LAN LI[1] and ERICH GULBINS[2]

ABSTRACT

In addition to the amplifying action of enzymes in the cell-signaling cascade, another important mechanism has been shown to amplify the signals massively when ligands bind to their receptors, which is characterized by clustering of membrane lipid microdomains or lipid rafts and formation of various signaling platforms. In this process, many receptor molecules aggregate on stimulation, thereby resulting in a very high density of the receptors and other signaling molecules to form signaling platforms and transmit and amplify the signals from receptor activation. Recent studies have indicated lipid rafts or lipid microdomain platforms may be importantly implicated in redox signaling of a variety of cells in response to agonists or stimuli. In this forum, we collected four original research communications, five review articles, and one news or views report to summarize recent progress in this research area. Information is offered for further understanding of the formation and function of lipid rafts and ceramide-enriched platforms and their roles in redox signaling. We hope that this forum could lead to more studies in this area and enhance our understanding of lipid rafts and redox regulation under physiologic and pathologic conditions. *Antioxid. Redox Signal.* 9, 1411–1415.

LIPID RAFT AND CERAMIDE-ENRICHED SIGNALING PLATFORMS

As a mosaic of different compartments or domains, the biologic membranes can form a number of types of subdomains due to the interaction between membrane components. One type of such subdomains is lipid-enriched microdomains, lipid rafts, which are capable of forming platforms. These platforms have been reported to play an important role in membrane protein sorting and construction of signaling complexes, and in some research fields, they are now taking a center stage in cell signaling, such as redox signaling of death receptors in endothelial cells (19) and apoptotic signaling of lymphocytes and other cells (5). Two types of lipid rafts are identified in the biologic membranes: caveolae and noncaveolae lipid rafts. Because the nature and functional implications of caveoli have been recognized and discussed widely in various transmembrane signaling pathways, and readers are directed to several excellent review articles (13, 16, 18), this caveolae-related signaling mechanism is not further discussed in this forum.

Two major models are commonly cited or accepted currently to describe the nature or behavior of lipid rafts. In the first model, lipid rafts are considered relatively small structures enriched in cholesterol and sphingolipids within which associated proteins are likely to be concentrated (17). In this sphingolipid-enriched lipid raft, the most prevalent component of the sphingolipid fraction in the cell membrane is sphingomyelin (SM), which is composed of a highly hydrophobic ceramide moiety and a hydrophilic phosphorylcholine headgroup. The tight interaction between the cholesterol sterol ring system and the ceramide moiety of SM promotes the lateral association of sphingolipids and cholesterol and thereby the formation of distinct microdomains. In these microdomains, cholesterol exerts a stabilizing role by filling the voids between the large and bulky glycerosphingolipids. It is this cholesterol–SM interaction that determines a transition of these microdomains into a liquid-or-

[1]Department of Pharmacology & Toxicology, Virginia Commonwealth University, Richmond, Virginia.
[2]Department of Molecular Biology, University of Duisburg-Essen, Germany.

dered or even gel-like phase, which is a unique characteristic of lipid rafts. Other domains of the cell membranes primarily exist in a more fluid liquid-disordered phase because of the absence of this cholesterol–SM interaction (5).

Along with this line, recent studies have demonstrated a novel membrane domain type (*i.e.,* ceramide-enriched membrane domains). The biophysical properties of ceramide molecules predict a tight interaction of ceramide molecules with each other, resulting in the formation of stable and tightly packed ceramide-enriched membrane microdomains that spontaneously fuse to form large ceramide-enriched membrane macrodomains or platforms. Although in a broad sense, the ceramide microdomains are also called as lipid rafts, it should be noted that ceramide-enriched membrane platforms might be formed without the existence of classically defined rafts (the small structures enriched in cholesterol, sphingolipids, and associated proteins). Therefore, a concept of ceramide-enriched membrane platforms is often used to describe the signaling mechanism related to these special lipid platforms. Ceramide is generated in the biological membranes either by hydrolysis of sphingomyelin catalyzed by various sphingomyelinases (SMase) or by a *de novo* ceramide synthase pathway. Both sphingomyelinase and *de novo* synthesis–derived ceramides have been shown to be involved in cell signaling. Among SMases, acid SMase (ASMase) has been considered a major enzyme responsible for the formation of ceramide-enriched membrane platforms. The acid SMase localizes within secretory vesicles, which are mobilized on stimulation to fuse with the cell membrane (3, 5). An accompanying study in this forum provides evidence that the ASMase may localize in lysosomal vesicles and that their activation and fusion with the cell membrane are associated with the functional integrity of lysosomes. Disturbance of lysosomal function abolished the formation of ceramide-enriched membrane platforms associated with acid SMase activation (8).

The second model of lipid rafts, the shell hypothesis, emphasizes that the generation of lipid rafts is based on protein–lipid or protein–protein interactions. Based on this model, rafts are constructed of lipid shells, which as small dynamic membrane assemblies are formed by proteins preferentially associated with certain types of lipids. Protein–protein interactions between shell proteins can create larger functional units corresponding to lipid rafts (1). Other nonshell proteins may associate with lipid rafts by additional protein–protein interactions. In addition, an oligomerization of these proteins may create and stabilize large raft domains, forming lipid raft platforms. Therefore, the formation and clustering of lipid rafts are dependent on both protein–lipid interaction and protein–protein interaction (7).

It is clear that the function of lipid rafts is dependent on the formation of macrodomains or platforms, no matter whether they are formed or behave based on sphingomyelin–cholesterol and ceramide–ceramide interactions in the sphingolipid model or protein–protein interactions in the shell protein model. It has been reported that the formation of membrane lipid platforms, in particular, ceramide-enriched membrane platforms, is involved in many signaling pathways, for instance, the signaling induced *via* CD95, CD40, CD20, interleukin-1 receptor, the PAF receptor, CD5, LFA-1, FcγRII, DR5, CD28, and TNF and many stress stimuli, including infection with *Neisseriae gonorrhoeae*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, rhinoviruses, or Sindbis virus (3). It is suggested that these membrane lipid platforms in the cell membrane may be a central motif to reorganize the topology of a given signalosome, which enables various stress stimuli and receptors to transmit a signal into the cells (5).

LIPID RAFTS–MEDIATED REDOX SIGNALING

Redox signaling has emerged as an essential mechanism in the regulation of various cell activities and functions. Despite extensive studies, so far the mechanisms activating or modulating redox signaling are still poorly understood (in particular, the mechanisms linking the redox signaling to the cell responses to various agonists or stimuli). Accumulating evidence exists that membrane lipid rafts and lipid platforms, respectively, may represent the important mechanisms by which redox signals are produced and transmitted in response to various agonists or stimuli (5). In this regard, many studies have shown that lipid rafts or platforms may participate in the signaling of cell apoptosis associated with oxidative stress during activation of various death receptors. It has been well documented that death receptors, in particular, Fas and tumor necrosis factor receptor 1 (TNFR1), are localized in lipid rafts and that the receptors in lipid rafts can interact to stabilize the raft further and allow raft aggregation (*i.e*., clustering) and the recruitment of raftophilic molecules to the complex, producing massive signaling action. The role of ROS or oxidative stress as a downstream mechanism of these death receptors in lipid-raft platforms has been shown. For example, Fas and TNFR1 activation is known to generate ROS in response to stimulation, which has now been shown to be attributed to the production of superoxide ($O_2^{\cdot -}$) because of the formation of lipid raft–derived NADPH oxidase platforms. This lipid raft–associated ROS generation downstream of Fas and TNFR1 may be of high importance in induction of apoptosis or necrosis in many cases (3, 12).

In addition to their role in cell apoptosis, these lipid raft–derived platforms are also involved in the early alterations of cell functions during activation of death receptors, which could be physiological or pathological. It has been reported that various death factors bind to their receptors in individual lipid rafts and subsequently stimulate acid SMase to produce ceramide from sphingomyelin in endothelial cells. Ceramide-enriched membrane platform formation results in aggregation of NADPH oxidase subunits such as gp91^{phox} and p47^{phox} and other proteins and thereby enhances the activity of this enzyme to produce $O_2^{\cdot -}$. $O_2^{\cdot -}$ reacts with NO to decrease NO bioavailability and to produce peroxynitrite ($ONOO^-$). Increased $ONOO^-$ uncouples NOS to produce more $O_2^{\cdot -}$ but less NO. This redox signaling associated with ceramide-enriched membrane platforms contributes to endothelial dysfunction by activation of death receptors before apoptosis of these cells, whereas in phagocytes, similar redox signaling platforms may mediate their physiological phagosomal action (11).

Another important mechanism by which lipid rafts mediate redox signaling is related to aggregation, gating, or activation of many redox-sensing or effector molecules. Among these molecules, a currently identified redox-sensitive protein—tran-

sient receptor protein (TRP)—is particularly interesting. TRPs are a family of calcium-permeable and voltage-independent cation channels. Evidence exists that TRPC3 and TRPC4 localize or relocalize in lipid rafts, and they can form a TRPC3–TRPC4 complex with different properties from their respective homomeric channels, which is redox sensitive. It is likely that these TRP channels may be directly gated or influenced by the formation of lipid-raft platforms, and therefore, their redox-sensing function would be altered. Indeed, the TRPC3 channel activity could be increased by cholesterol loading of the cell membrane when TRPC3 is overexpressed. This increased channel activity may lead to enhanced redox sensitivity of the channels, exerting an important redox regulation or resulting in pathologic actions in different cells (12).

On the other hand, lipid rafts were also demonstrated to mediate the effects of some antioxidant molecules, such as superoxide dismutase (SOD) and thioredoxin (TRX). Included in this forum are two interesting articles that provide convincing evidence for the role of lipid rafts in mediating the actions of TRX on leukocyte–endothelial cell interaction related to redox regulation during inflammation. TRX is a ubiquitous protein with a redox-active disulfide that functions in concert with NADPH and TRX reductase to control the redox state of cysteine residues of different oxidant-targeted proteins. Given the antioxidant role of TRX, the lipid raft–mediated role of TRX in the interaction between leukocytes and endothelial cells may importantly regulate inflammatory responses through counteracting oxidative stress and ROS (6). In addition, TRX can be internalized into the cells through lipid raft–mediated endocytosis. In particular, a TRX mutant, TRX-C35S (with replacement of cysteine 35 by serine), was found to bind rapidly to the cell surface and be internalized into the cells through lipid rafts in the plasma membrane. This indicates that the cysteine at the active site of TRX is important for the internalization and signal transduction of extracellular TRX through lipid rafts (9).

REDOX REGULATION OF LIPID-RAFT SIGNALING

More recently, evidence is increasing that the formation of lipid raft–derived or ceramide-enriched membrane platforms may be altered by redox molecules. Reports indicate that SOD decreased, but $O_2^{\cdot -}$ increased the formation of ceramide-enriched membrane platforms in the membrane of coronary arterial endothelial cells (11). In other studies, H_2O_2 was also found to activate prosurvival signaling pathways, including activation of PI3 kinase/Akt and ERK1/2 by a lipid raft–dependent mechanism. In addition to this direct evidence, ROS were found to influence lipid-raft signalling or function through their actions on many lipid-raft components such as ceramide production, cholesterol, and related raft proteins (3, 12). In this regard, acid SMase as a key enzyme for the formation of ceramide-enriched membrane platforms has been extensively studied. It has been shown that generation of ROS may be involved in activation of the enzyme in response to various stimuli. Pretreatment of neutrophils with the antioxidants *N*-acetylcysteine (NAC) and desferrioxime significantly inhibited the events downstream of acid SMase, such as ceramide generation and CD95 clustering, indicating a requirement of ROS release for acid SMase activation (3, 14).

Although the mechanism mediating the activation of acid SMase is still unclear, a new model is proposed by Gulbins and associates that may summarize the mechanisms by which acid SMase is activated by ROS. Based on this model, the free C-terminal cysteine of acid SMase can be modified and lost by the actions of ROS, whereby a zinc coordination in this enzyme could be altered, leading to activation or inhibition of this enzyme. This model is basically similar to the "cysteine switch" activation mechanism described previously for the matrix metalloproteinase family (20). In an original research communication of this forum, this redox regulation of ceramide-enriched membrane platforms is further confirmed that links to chemotherapy of glioma. It has been demonstrated that transfection of human or murine glioma cells with acid SMase results in a marked sensitization of glioma cells to gemcitabine and doxorubicin, respectively, which was accompanied by an increased activation of acid SMase, elevated ceramide levels and enhanced formation of ceramide-enriched membrane platforms. Scavenging of ROS prevented these events, suggesting that an activation of acid SMase by these therapeutic agents is associated with the actions of ROS (4).

In addition, a new concept is emerging that could be used to define the interactions or amplification of both redox signaling and lipid raft–associated signaling. This concept is characterized by redox-mediated feed forward amplification in lipid platforms. It is proposed that in response to various stimuli, acid SMase is activated to produce ceramide and form ceramide-enriched membrane platforms. In these platforms, NAD(P)H oxidase subunits are aggregated or recruited, and therefore, the enzyme activity increases to produce $O_2^{\cdot -}$. Increased $O_2^{\cdot -}$ could produce various regulatory activities, among which $O_2^{\cdot -}$ and corresponding ROS may enhance acid SMase activity and promote further formation of lipid-raft platforms. This feed forward action constitutes a redox signaling network *via* lipid raft– or ceramide-enriched membrane platforms, which amplifies or fine-controls transmembrane signaling related to redox regulation (11).

IMPLICATIONS OF LIPID RAFT–ASSOCIATED REDOX REGULATION

Given the role of redox molecules in the various responses of cells to physiological or pathological stimuli, the lipid-raft redox signaling network may be involved in a variety of changes in cellular activity or cell function, implicated in the initiation or development of different diseases such as tumor, infection, Alzheimer disease, atherosclerosis, or hypertension. Although numerous studies have revealed the possible role of either redox molecules or lipid rafts in the development of different diseases, only a few reports deal with the functional implications of lipid raft–associated redox regulation or lipid-raft redox-signaling network. This forum summarizes these reports to help readers recognize this important aspect. In addition to the role of lipid raft–associated redox signaling in the regulation of apoptosis and vasodilator responses discussed earlier, other ac-

tions discussed in this forum relate to macrophage reprogramming, foam cell formation, and cell deformability.

It has been indicated that induction of lipid oxidation through ROS can amplify foam cell formation through oxidized low-density lipoprotein (Ox-LDL) uptake and a subsequent clustering of ceramide-enriched lipid domains. In addition, Ox-LDL may affect cell-surface turnover of ceramide-backbone sphingolipids and apoE-mediated uptake by low-density lipoprotein receptor elated protein (LRP) family members. This in turn leads to cell-surface expansion of ceramide-enriched domains and activation of apoE/LRP1/CD1-mediated antigen presentation. High-density lipoprotein (HDL)-mediated lipid efflux, however, disrupts lipid membrane microdomains and prevents foam cell formation. It is concluded that lipid rafts and related oxidative processes play an important role in the formation of macrophage foam cells and thus in the progression of atherosclerosis (15).

Other interesting findings are the contributions of lipid-raft redox regulation in the molecular reprogramming of tissue macrophages related to inflammation and atherosclerosis. This reprogramming is associated with an altered response to subsequent inflammatory stimuli, such as lipopolysaccharide (LPS), leading to enhanced liberation of proinflammatory chemokines and cytokines. Recent studies have shown that the early and direct interaction of oxidants with lipid rafts may result in the mobilization of annexin VI from lipid rafts, leading to the release of Ca^{2+}, which in turn activates Ca^{2+}-dependent kinases and thereby causes further alterations in lipid-raft lipids and eventually lipid-raft proteins. Additional studies demonstrated that oxidant-induced phosphorylation and activation of CaMK II in macrophages require stabile membrane lipid platforms and increased cytosolic Ca^{2+}. It is suggested that alterations of signaling processes in both lipid-raft lipids and proteins may importantly contribute to the reprogramming induced after oxidant exposure (2).

In addition to the role of the lipid-raft redox-signaling network in alterations of macrophage behavior, this signaling network may also be importantly involved in cell deformability, thereby initiating or promoting atherogenesis. It has been indicated that disruption of lipid rafts by oxidants such as Ox-LDL alters the cytoskeletal structure, including the extent of polymerization, stabilization, crosslinking, and membrane association. These molecular alterations may increase force generation by the cytoskeleton, resulting in a stiffening of the cytoskeleton and hence stiffening of the cell and plasma membrane. Increased force generation and increased stiffness may also elevate membrane tension and thereby influence the activity of various mechanosensitive ion channels. Direct evidence suggests that oxLDL could disrupt lipid rafts, resulting in a series of pathological changes in the biomechanical properties of vascular endothelial cells and ultimately inducing endothelial dysfunction and atherogenesis (10).

FUTURE DIRECTIONS

The concept of redox signaling *via* membrane lipid platforms describes the interactions of lipid raft–mediated transmembrane signaling and redox signaling that regulate cellular activities or functions. Although numerous studies exist regarding the mechanism or implications of two individual signaling pathways, evidence is emerging that demonstrates the functioning of a lipid-raft redox complex or network in a variety of cells. It is obvious that many aspects related to this important signaling complex must be clarified. For example, it is imperative to reveal what driving force mediates the formation of this membrane signaling complex and how signaling through these platforms could be specific to agonists and downstream effectors. Although considerable evidence suggests that this lipid raft–associated redox signaling could be linked to a number of cell responses to pathological stimuli and therapeutic compounds, its action under physiological conditions or in various physiological regulations of cell function remains unclear. In addition, much attention is needed to explore the molecular mechanisms by which this lipid-raft redox signaling complex is activated, regulated, and implicated for different diseases. It is believed by all authors of this forum that further studies of this lipid-raft redox signaling complex will provide new insights into the redox regulation of various cell or organ functions and into the pathogenesis or therapy for related diseases.

ABBREVIATIONS

ASMase, acid sphingomyelinase; HDL, high-density lipoprotein; LPS, lipopolysaccharide; LRP, lipoprotein receptor elated protein; NAC, *N*-acetylcysteine; Ox-LDL, oxidized low-density lipoprotein; $O_2^{-\cdot}$, superoxide; $OONO^-$, peroxynitrite; SM, sphingomyelin; SMase, sphingomyelinases; SOD, superoxide dismutase; TNFR1, tumor necrosis factor receptor 1; TRPs, transient receptor proteins; TRX, thioredoxin.

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Address reprint requests to:
Pin-Lan Li, M.D., Ph.D.
Department of Pharmacology and Toxicology Medical College of Virginia
Virginia Commonwealth University
410 North 12th Street
P.O. Box 980613

E-mail: pli@vcu.edu

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