Lipid Rafts and Redox Regulation of Cellular Signaling in Cholesterol Induced Atherosclerosis

Betul Catalgol and Nesrin Kartal Ozer*

Department of Biochemistry, Faculty of Medicine, Marmara University, 34668 Haydarpasa, Istanbul, Turkey

Abstract: Redox mediated signaling mechanisms play crucial roles in the pathogenesis of several cardiovascular diseases. Atherosclerosis is one of the most important disorders induced mainly by hypercholesterolemia. Oxidation products and related signaling mechanisms are found within the characteristic biomarkers of atherosclerosis. Several studies have shown that redox signaling via lipid rafts play a significant role in the regulation of pathogenesis of many diseases including atherosclerosis. This review attempts to summarize redox signaling and lipid rafts in hypercholesterolemia induced atherosclerosis.

Keywords: Atherosclerosis, hypercholesterolemia, lipid rafts, reactive species, redox regulation, signaling.

INTRODUCTION

Atherosclerosis, a chronic inflammatory disease which is characterized by the accumulation of plasma lipoproteins that carry cholesterol and triglycerides in the arteries, is one of the major causes of morbidity and mortality worldwide. This accumulation results in the proliferation of certain cell types within the arterial wall. Arterial wall consists of three layers with different cell types and extracellular matrix. The outermost layer, tunica adventitia, includes fibroblasts, type I collagen fibers, elastic network, lymphatic and tiny blood vessels. The middle layer, tunica media, is circularly arranged with smooth muscle cells and in between the smooth muscle layers, elastic network, collagen and proteoglycans take place. The innermost layer, tunica intima, includes single layer endothelial cell lines in the luminal arterial surface. These cells attach on a basement membrane of extracellular matrix and proteoglycans that is bordered by the internal elastic membrane. Endothelial cells form a physical and functional barrier between flowing blood and the stroma of the arterial wall and regulate a wide array of processes including thrombosis, vascular tone, and leukocyte trafficking among others [1].

In the atherosclerotic process, macrophage foam cells are formed with the rapid transformation of phagocytic monocytes penetrated into the subendothelial space and atherogenic lipoproteins like modified low density lipoprotein (LDL) are uptaken by receptor-mediated endocytosis mechanism [2,3]. Following the endocytosis, these cells have an appearance loaded with lipid droplets rich in cholesteryl esters [4]. These foam cells also known as ‘fatty streaks’ and adaptive thickening of the intima are accepted as the main visible lesions at the early stage of the pathogenesis [1,4]. Endothelial dysfunction has been proposed as long-term atherosclerotic lesions which initiates the inflammatory mechanisms and is used as an important diagnostic and prognostic factor [5, 6]. Several biological processes classified as the reasons for advanced lesions in atherosclerosis such as proliferation of intimal smooth muscle cells, accumulation of extracellular matrix components such as collagen, elastic fibers and proteoglycan, and cholesteryl ester and free cholesterol accumulation within the cells and in the surrounding connective tissues [7]. During the progress of the disease, it takes time for the disruption of an atherosclerotic lesion and leading to thrombosis and decrease in oxygen supply to target organs such as heart and brain. As a result of these reduced blood flow, heart attack and stroke are occurred referred to as coronary artery disease and cerebrovascular disease [1].

Enzymatically hydrolyzed LDL (E-LDL) [8], oxidized LDL (ox-LDL) [9] and modified LDL by advanced glycation end products (AGEs) are the types of lipids taken up by macrophages. The term E-LDL is used for proteolytically cleaved apoB and hydrolysed core cholesteryl esters, leading to liposome-like particles present at early stages in atherosclerotic lesions [8, 10]. With LDL oxidation, denatured apoB molecule shows an increase in the platelet-activating factor (PAF)-acyethylhydrolase-like activity with a PLA2-like activity that strips phosphatidylcholine from the ox-LDL surface [11, 12]. Following this, ox-LDL particles aggregate and form polar surface with the remaining phospholipids on the aggregated particles. AGEs, those may cause LDL modification, are formed by nonenzymatic glycation reaction between a reducing sugar and a free amino group on a protein, lipid, or nucleic acid [13].

Cellular uptake of these atherogenic lipids and lipoproteins are mediated by several receptors as summarized in Table 1. LDL receptors are generally shown to be downregulated during cholesterol uptake. Scavenger receptors (SRs) are the most abundant receptors expressed on macrophages and foam cells in atherosclerotic lesions [3]. CD36 takes the most important place in the scavenger receptors playing role in atherosclerotic process. CD36 is a raft associated glycosylated protein with an 88 kDa molecular weight. Studies
showed that CD36 is palmitoylated in cysteine residues of N- and C-terminal of both cytosolic tails and this structure plays important role for the internalization of CD36 in caveolae and lipid membranes. Various ligands such as ox-LDL, apoptotic cells, AGEs bind to the region localized between 155-183 amino acids in the structure of scavenger receptor cluster of differentiation 36 (CD36) [1].

Hyperlipidemia and other cardiovascular risk factors containing age, obesity, hypertension, diabetes mellitus and serum cholesterol are connected with the development and progression of atherosclerotic lesions, plaque rupture, and vascular thrombosis. As an autosomal dominant disorder, familial hypercholesterolemia affects every 1 person in 500 from the general population [1, 3].

**OXIDATION PRODUCTS AND REDOX SIGNALING MECHANISMS IN ATHEROSCLEROSIS**

Free radicals contain unpaired electrons in their outer orbitals and take place in oxidation reactions easily. Free radicals include reactive species (RS) such as reactive oxygen species (ROS) and reactive nitrogen species (RNS). RS damage cellular components such as proteins, lipids, carbohydrates, and nucleic acids [14]. Following the interactions of RS with cellular components, several products are known to be produced. The main investigated products are malondialdehyde (MDA) and 4-hydroxynonenal (HNE) for lipid peroxidation, 8-hydroxydeoxyguanosine for DNA oxidation and protein carbonyls, nitrotyrosines for protein oxidation [15, 16]. In this direction, the term redox signaling defines a process wherein free radicals (particularly reactive oxygen species) and other related species act as messengers in biological systems [17].

Redox signaling process is shown to be involved in the pathogenesis of atherosclerosis besides several different hypotheses [18]. Lipid peroxidation and LDL oxidation induced by RS are the early events in atherosclerotic lesion formation [19-22]. There is now a consensus that atherosclerosis represents protein oxidation process in the vascular wall besides lipid oxidation [1]. Mostly macrophages are thought to be the source of ROS formation in the vessel wall but also other cells like endothelial, smooth muscle and adventitial cells produce ROS in the vessel wall (Fig. 1) [19, 23].

| Class | Members | Location | Function |
|-------|---------|----------|----------|
| A     | Type I MSR-A  | Cell membrane of macrophages | Uptake of Ox-LDL by macrophages, transformation of macrophages into foam cells |
|       | Type II MSR-A | Cell membrane of macrophages |  |
|       | Type III MSR-A MARCO | Cytoplasmic vesicles of macrophages |  |
| B     | CD36     | Monocytes/macrophages, platelets, endothelial cells, adipocytes | Uptake of Ox-LDL by macrophages, transformation of macrophages into foam cells, phagocytosis of apoptotic cells |
|       | SR-BI    | Hepatocytes, steroidogenic cells, epithelial cells, macrophages | HDL receptor mediates the selective uptake of HDL cholesterol |
| C     | SRCL     | Endothelial cells from human umbilical vein and vascular endothelial cells of the heart | Binds galactose and fucose residues |
| D     | CD68/macrosialin | Expressed on endolysosomal compartments and macrophages cell surfaces | Member of lysosomal-associated membrane protein, binds Ox-LDL in vitro, transformation of macrophages into foam cells |
| E     | LOX-1    | Expressed by vascular endothelial cells, macrophages in human and mice | Endocytic uptake and lysosomal degradation of Ox-LDL. Binding to Ox-LDL induces NF-κB activation and inhibits MCP-1 upregulation in endothelial cells. |
| F     | SREC     | Expressed by vascular endothelial cells |  |
| G     | SR-PSOX  | Lipid-laden macrophages in human atherogenic lesions | Recognizes phosphatidylserine and Ox-LDL |
|       | PSR      | Macrophages, fibroblasts, epithelial cells | Phagocytosis of apoptotic cells |
| Others | CD163    | Macrophages | Receptor for hemoglobin–haptoglobin complexes and prevents macrophages from oxidative damage by decreasing heme/iron levels and ROS formation |

MSR, macrophage scavenger receptor; MARCO, macrophage receptor with collagenous structure; SR-BI, Scavenger receptor class B; SRCL, scavenger receptor C-type lectin; SREC, scavenger receptor expressed by endothelial cell-I; SR-PSOX, scavenger receptor for phosphatidylserine and oxidized lipoprotein; PSR, phosphatidylserine receptor.
Lipid Rafts and Redox Regulation in Atherosclerosis

Ox-LDL modulates atherosclerosis biology by cell damage induction, proliferation of smooth muscle cells, foam cell formation, chemotaxis of leukocytes and secretion of inflammatory mediators. Since oxidation of LDL is the main oxidative modification, high plasma levels of native LDL is a risk factor for the progression [24]. Ox-LDL uptake by macrophages is easier compared to non-oxidized LDL. It is known that hypercholesterolemia stimulates ROS formation from smooth muscle cells and this also leads to increased oxidation of LDL [21]. Ox-LDL includes oxidative agents such as aldehyde end products of lipid peroxidation of polyunsaturated fatty acids like HNE, derived from phospholipids, mono-, di-, and triacylglycerols, or cholesteryl esters (CEs), as well as cholesterol oxidation products. These oxidized lipids via their fibrogenic, apoptotic, coagulant, and inflammatory effects, contribute to progression of atherogenic lesions. Oxidation of LDL changes the composition of the particle. In vitro oxidation of LDL can be achieved by copper incubation [25] and incubation with culture medium from 15-lipoxygenase-overexpressing fibroblasts [26]. Oxidation of the lipid moiety of lipoproteins and formation of lipoperoxides transferred to LDL cause oxysterol formation. The unsaturated fatty acyl chains of phospholipids, CEs, and triglycerides are oxidized most readily, and a significant proportion of the unsaturated acyl chains are also oxidized to hydroperoxides, isoprostanes, and more AGEs [27]. Cholesterol and saturated fatty acids react more slowly, and a small proportion of cholesterol is converted to oxysterols, initially 7-hydroperoxycholesterol. Oxysterols, 27-carbon products of cholesterol oxidation, are possible reactive mediators of structural and functional changes of the vascular wall, which are affected by the atherosclerotic process [28]. ApoB, the dominant apoprotein of LDL, which is highly glycosylated, is subject to both direct oxidative modification and reaction with products of lipid oxidation.

Main reactive oxygen species implicated in cardiovascular diseases are superoxide ($\text{O}_2^{-}$), hydroxyl ($\text{OH}^-$) and hydrogen peroxide ($\text{H}_2\text{O}_2$) and reactive nitrogen species are NO and peroxynitrite. While superoxide and hydroxyl radicals are more reactive, hydrogen peroxide is more membrane permeable. As the basic mechanism, these oxygen species are converted to each other by several mechanisms. $\text{O}_2^{-}$ is dismutated nonenzymatically or enzymatically by superoxide dismutase (SOD) to $\text{H}_2\text{O}_2$. Also various enzymes located in the plasma membrane, cytosol, peroxisomes and mitochondria catalyse ROS formation.

Sources of Reactive Oxygen Species

Enzymatic sources of $\text{O}_2^{-}$ include NADPH oxidases, xanthine oxidase, cyclooxygenase, lipoxygenase, cytochrome P-450 enzymes, uncoupled NOs, phagocytic myeloperoxidase system and mitochondrial electron transport chain [29-31].

Mitochondria is an important source of $\text{O}_2^{-}$. In the electron transport chain, mainly respiratory electron carriers in-
duces $O_2^-$ formation. Complex I (containing the flavin mononucleotide [FMN] and iron sulfide [FeS] potential $O_2^-$-producing sites) was, for a long time, considered one of two major sites of $O_2^-$ production. The second major site of mitochondrial $O_2^-$ production is believed to be ubiquinone-Complex III [32]. Ox-LDL was found to induce $O_2^-$ formation in mitochondria [33]. In endothelial cells, 4-HNE was shown to inactivate proteins containing reactive thiols, such as 2-oxoglutarate dehydrogenase and pyruvate dehydrogenase [34], and thereby inhibit complex I-dependent (NADH-linked) respiration [35].

Xanthine Oxidase (XO)

XO is located in the endothelium of various organs and causes $O_2^-$ production during the catalysis of the oxidation of hypoxanthine to urate. Tissue distribution of XO is an important factor of $O_2^-$ induced injury. XO and xanthine dehydrogenase are two forms of the enzyme and in basal conditions dehydrogenase form is present in the tissues. Either through oxidation or by proteolytic cleavage of xanthine dehydrogenase, XO is formed. The levels of plasma-circulating XO and the ability of circulating XO to bind vascular cells of various organs increase during some pathological states such as reperfusion injury, hepatitis, adult respiratory distress syndrome and atherosclerosis [30]. In experimental animals with hypercholesterolemia it is capable of reducing nitric oxide (NO) activity [36]. Additional facts that support the role of xanthine oxidase in the process of atherogenesis are the following: (i) in patients with coronary syndrome the levels of this enzyme were found to be increased-the same applies to NAD(P)H; and (ii) in young asymptomatic patients with familial hypercholesterolemia the increased activity of the enzyme is an early event [37].

NADPH Oxidase

NADPH oxidase, a multiple subunit electron transport system, was discovered in neutrophils where it catalyzes one electron reduction of oxygen to produce $O_2^-$ using NADPH as the electron donor during phagocytosis and plays role in immune protection with its bactericidal activity [31, 38, 39]. This enzyme system plays key role in generating ROS in fibroblasts, vascular smooth muscle cells and endothelial cells besides phagocytic cells. The NADPH oxidase subunits are shown to be present in human blood vessels including atherosclerotic coronary arteries, in veins and mammary arteries with coronary artery disease, which strengthens the importance of the molecular regulation of the enzyme in cardiovascular diseases [6, 40]. NADPH oxidase activity in non-phagocytic cells, such as cardiovascular cells, is acutely increased by diverse pathophysiological stimuli including: (i) G-protein-coupled receptor agonists, e.g. angiotensin II and endothelin-1; (ii) cytokines, e.g. (tumour necrosis factor-α) TNF-α and (transforming growth factor-β) TGF-β; (iii) growth factors, e.g. thrombin, (vascular endothelial growth factor) VEGF and insulin; (iv) ‘metabolic’ factors, e.g. ox-LDL, nonesterified (free) fatty acids and glycated proteins; (v) hypoxia-reoxygenation or ischaemia-reperfusion; and (vi) mechanical stimuli, e.g. oscillatory shear [3].

The phagocytic NADPH oxidase consists of a membrane-associated cytochrome b558 that comprises a large subunit, gp91$^\text{phox}$ (‘phox’ being derived from phagocytic oxidase), and a small one, p22$^\text{phox}$. Besides these, there are at least three cytosolic subunits (p47$^\text{phox}$, p67$^\text{phox}$ and p40$^\text{phox}$) and a low-molecular-weight G protein (Rac1 or Rac2). p47$^\text{phox}$, p67$^\text{phox}$ and gp91$^\text{phox}$ (NOX2) present on phagocytic NADPH oxidase have been identified in vascular cells. However, several studies have confirmed that p22$^\text{phox}$ is present in all NADPH oxidase systems and that this subunit is essential for the functionality of the enzyme. Upon cell stimulation, p47$^\text{phox}$ becomes phosphorylated on multiple sites with several kinases (protein kinase C, protein kinase A, or mitogen activated protein kinase) and the cytosolic subunits form a complex which migrates to the membrane where it binds to the cytochrome b558. Then electrons are transferred from the substrate, NADPH, to $O_2$, leading to $O_2^-$ generation [19, 41]. Phosphorylation and translocation of p47$^\text{phox}$ allows its interaction with p22$^\text{phox}$ and facilitates the binding of p67$^\text{phox}$ to cytochrome b558 [31]. Also another key posttranslational modification involved in oxidase activation is Rac activation.

Several homologues of gp91$^\text{phox}$ have recently been reported to be expressed in nonphagocytic cells. Other members of the NOX family comprise of NOX1,NOX2,NOX3,NOX4 and NOX5, as well as larger and more complex homologues termed DUOX1 and DUOX2 [38]. NOX1 to 5 are 65-kDa core proteins, whereas DUOX 1 and 2 are 175- to 180-kDa proteins that have a domain homologous to gp91$^\text{phox}$ as well as an additional peroxidase domain. Using this new terminology, NOX2 represents the neutrophil gp91$^\text{phox}$. The first homologue of gp91$^\text{phox}$, namely NOX1, was found to have significant proliferative activity and was also therefore known by the alternative term mitogenic oxidase or MOX-1 [30].

Functionally endothelial NADPH oxidase shares some but not all of the characteristics of neutrophil NADPH oxidase. One major difference is that endothelial NADPH oxidase continuously generates a low level of $O_2^-$ even in unstimulated cells, although its activity can be further increased by several agonists. However, neutrophil NADPH oxidase primarily produces $O_2^-$ when the cells are stimulated. In regard to the isoform of NOXs, gp91$^\text{phox}$ (NOX2) has been considered as the major isoform of NOX proteins in vascular endothelial cells [42, 43]. The functional role of this NOX isoform has been confirmed by decrease in phorbol ester-induced $O_2^-$ production and endothelium-dependent relaxation in gp91$^\text{phox}$–/– mice [43]. In addition to gp91$^\text{phox}$, NOX4 mRNA is also detectable in endothelial cells. It appears that NOX4-dependent oxidase functionally contributes to the basal $O_2^-$ production in endothelial cells [44].

Nitric Oxide Synthase (NOS)

Ox-LDL also activates nitric oxide synthase and increases production of NO as a key regulator of vasodilatation. Multiple interactions of NO with oxidizing ligands could lead to either vascular protection or potentiation of inflammatory vascular injury. Ox-LDL increases $O_2^-$ production in endothelial cells and decreases the bioavailability of NO through a process involving lectin-like ox-LDL receptor...
Myeloperoxidases (MPO)

This enzyme uses H$_2$O$_2$ for the production of more powerful oxidative substances by activated phagocytes. This enzyme, through NADPH, leads to the production of hypochlorous acid (HOCl) and its analogs (substances related to enzyme, through NADPH, leads to the production of hypochlorous acid (HOCl) and its analogs (substances related to ONOO$^-$, for example, after inducible nitric oxide synthase (iNOS) expression in inflammation, can be converted to peroxynitrite (ONOO$^-$) and NO$_2$ [45]. In the presence of available O$_2$ radicals, ox-LDL may contribute to ONOO$^-$ formation, which can potentiate inflammatory injury of vascular cells. A role for ONOO$^-$ in initiating lipid oxidation in atherosclerosis has been suggested [46]. The reduction of endothelial-produced NO and O$_2^-$ is able to blunt normal endothelial dysfunction as a result of the decreased endothelial NO production. The increased production of ROS reduces the production and consequently the bioavailability of NO, leading to vascular constriction, platelet aggregation and adhesion of neutrophils to the endothelium [47].

Effects of Reactive Species on Signaling Mechanisms

O$_2^-$ anion, in addition to mediate LDL oxidation, may contribute to the pathogenesis of atherosclerosis in various ways [7]. O$_2^-$ inactivates endogenous vasodilatator, endothelium derived NO, thereby promotes vasoconstriction. Impairment of NO function by O$_2^-$ also results in vascular smooth muscle cell proliferation and migration [58]. It was shown that incubation of vascular smooth muscle cells with H$_2$O$_2$, induced the expression of VEGF, confirming the role of ROS in neovascularization in atherosclerotic plaques and restenotic lesions [59]. Redox molecules including ROS and RNS possess redox potential, play important role in the maintenance of cardia homeostasis by acting through specific signal transduction pathways [60, 61]. Key components in atherogenesis including signaling molecules such as redox sensitive transcription factor NFkB activation and adhesion molecules such as selectins, vascular cell adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule-1 (ICAM-1) and chemokines such as monocyte chemoattractant protein-1 (MCP-1) expressions in the vascular endothelium are known to be increased by RS [58]. Expression of adhesion molecules and MCP-1 are also key steps for the monocyte adhesion and emigration to form macrophages and foam cells [30].

Macropheres colony-stimulating factor (M-CSF) is an important factor regulating the survival, proliferation, differentiation, and chemotaxis of macropheres. During early myeloid differentiation, M-CSF synergizes with other growth factors and interleukin-3 to produce mononuclear phagocyte progenitor cells. After this initial differentiation process, M-CSF by itself can regulate the proliferation and differentiation of mononuclear phagocyte progenitor cells to monocytes, macropheres, and osteoclasts and also supports survival and activity of fully differentiated macropheres. The receptor for M-CSF (M-CSF-1R) is expressed in mononuclear phagocytes and antigen presenting cells (APCs), which can be regarded as a specialized adaptive state rather than a separate lineage. M-CSF was considered as an alternative marker of macropheres, whereas APCs differentiate through the action of granulocyte-macrophage-CSF (GM-CSF), IL-4, and prostaglandin E2 [62]. M-CSF cooperates

MPO also generates HOCl like H$_2$O$_2$ and other ROS, which would be protective. But elevated levels of NO$^-$, for example, after inducible nitric oxide synthase (iNOS) expression in inflammation, can be converted to prooxidant species like peroxynitrite (ONOO$^-$) and NO$_2$ [52]. In the presence of available O$_2$ radicals, ox-LDL may contribute to ONOO$^-$ formation, which can potentiate inflammatory injury of vascular cells. A role for ONOO$^-$ in initiating lipid oxidation in atherosclerosis has been suggested [46]. The reduction of endothelial-produced NO and O$_2^-$ is able to blunt normal endothelial dysfunction as a result of the decreased endothelial NO production. The increased production of ROS reduces the production and consequently the bioavailability of NO, leading to vascular constriction, platelet aggregation and adhesion of neutrophils to the endothelium [47].

Lipooxygenases

Lipooxygenases are a family of iron-containing enzymes that catalyse the dioxygenation of polyunsaturated fatty acids in lipids containing a cis,cis-1,4- pentadiene structure, creating a family of biologically active lipids, such as prostaglandins, thromboxanes and leukotrienes, which participate in inflammatory reactions and increase the permeability of vessels. In experimental models, 15-lipoxygenase was shown to induce LDL oxidation by enzymatic and nonenzymatic reactions. Experimental animals with an absence of the 15-lipoxygenase gene or reduced expression of 5-lipoxygenase are protected from lesions like those found in animals with apolipoprotein E and LDL-receptor deficiency [57]. Clinical data demonstrate that various genotypes of 5-lipoxygenase promoter are found in patients with atherosclerotic lesions or inflammation [21].

(LOX-1). Low levels of NO$^-$ generated by endothelial NOS (eNOS) can terminate lipid radicals and inhibit lipoxygenases, which would be protective. But elevated levels of NO$^-$, for example, after inducible nitric oxide synthase (iNOS) expression in inflammation, can be converted to prooxidant species like peroxynitrite (ONOO$^-$) and NO$_2$ [45]. In the presence of available O$_2$ radicals, ox-LDL may contribute to ONOO$^-$ formation, which can potentiate inflammatory injury of vascular cells. A role for ONOO$^-$ in initiating lipid oxidation in atherosclerosis has been suggested [46]. The reduction of endothelial-produced NO and O$_2^-$ is able to blunt normal endothelial dysfunction as a result of the decreased endothelial NO production. The increased production of ROS reduces the production and consequently the bioavailability of NO, leading to vascular constriction, platelet aggregation and adhesion of neutrophils to the endothelium [47].

High density lipoprotein (HDL) isolated from patients with cardiovascular disease contains elevated levels of 3-chlorotyrosine and 3-nitrotyrosine, which are two characteristic products of MPO, enzyme secreted by macrophages [51]. MPO-dependent oxidation of specific amino acids, mainly tyrosine and methionine residues of apoA-I, impairs its ability to remove excess cellular cholesterol via the ATP-binding cassette transporter A1 (ABCA1) pathway [52]. MPO also generates HOCl like H$_2$O$_2$ and other ROS, which is also secreted by macrophages [53]. Enzymatically active MPO was found in human atherosclerotic lesions [49], and lipoproteins that have been modified by HOCl have been detected in advanced human atherosclerotic plaques. Tyrosylated lipoproteins and HDL are more potent than native HDL in removing cholesterol from lipid-laden fibroblasts and macrophages in vitro. This process does not appear to involve passive cholesterol desorption from the cell-surface membranes [54], which suggests the possibility that tyrosylated HDL promotes reverse cholesterol transport by interacting with ABCA1 in macrophages and perhaps other peripheral tissues more efficiently than native HDL [55, 56]. Enzymatically active MPO and elevated levels of dityrosine, marker for protein oxidation by tyrosyl radicals, have been detected in human atherosclerotic plaques [49].
with the receptor activator of NFκB ligand (RANKL) to regulate the differentiation of mononuclear phagocytes toward osteoclasts [63]. M-CSF also enhances cytotoxicity, ROS (e.g., superoxide radical, peroxy nitrate, hydroxyl radical and hydrogen-peroxide production), as well as phagocytosis, chemotaxis, and cytokine production in monocytes and macrophages [64]. M-CSF-mediated signaling involves many cytoplasmic molecules like c-Src, which is linked with c-Cbl and targets the Vav family members of guanine nucleotide exchange factors (GEFs), which in turn activate Rac-1 as a constituent of activated NADPH-oxidase. Alternatively coregulatory signaling pathways like integrin signaling (e.g., αvβ3, αMβ2) also target Vav. Ox-LDL is directly mitogenic for macrophages and smooth muscle cells, and stimulates the release of MCP-1 and M-CSF from endothelial cells and the production of many inflammatory mediators (e.g., endothelin-1) from other vascular cells and chemotactic for monocytes/APCs and T cells [65].

The effects of ox-LDL on NFκB may be biphasic as concentration dependent. Normally, it activates NFκB and upregulates the expressions of adhesion molecules, tissue factor and LOX-1. In high concentration, ox-LDL inhibits NFκB activation triggered by inflammatory agents such as lipoxigenases and therefore exert immunosuppressive effect [66]. HNEs were shown to activate MAPK in endothelial cells either by directly interacting with PKC or through activation of the EGF receptor [67]. Transcription factor AP-1, plays role in the regulation of TGF-β1, procollagen type 1, platelet-derived growth factor-AA (PDGF-AA), MCP-1, and cyclooxygenase-2 (COX-2) expressions, were activated by HNE [68].

The presence of foam cells in the atherosclerosis process confirms the importance of CD36 scavenger receptors. CD36 has an important role in the intake of ox-LDL by macrophages in the arteria walls and long chain fatty acids into the cells. Following binding of ox-LDL to CD36 receptor, lyn kinase, a src protein tyrosine kinase, is activated. This activation induces mitogen ERK kinase kinase 2 (MEKK2) and c-jun N-terminal kinase (JNK) activation and phagocytosis of proatherogenic ox-LDL [69]. Studies carried out in murine models showed that inhibition of JNK causes a decrease in ox-LDL uptake [70, 71]. CD36, was shown to be upregulated by PKC and PPARγ pathway which are common signaling mechanisms for IL4 and ox-LDL [72]. CD36 scavenger receptor expression was shown to be increased in ox-LDL treated aortic smooth muscle cells in culture [73]. It has been shown as in vivo that hypercholesterolemia increases foam cell formation and atherosclerosis by increasing CD36 mRNA expression and PKC activity in rabbits [74-76].

Fatty acids and their oxidation products activate the nuclear orphan receptors PPARs. These are ligand-activated transcription factors that play an important role in obesity-related metabolic diseases such as high triglyceride/low-HDL syndromes, insulin resistance, and coronary artery disease [77]. PPARs bind, on heterodimerization with RXR, to specific peroxisome proliferator response elements (PPREs) in the promoter of target genes, thus regulating the transcription of these genes. PPARs consist of isoforms α, γ, and δ with distinct expression patterns and biologic activities. PPARs are expressed in atherosclerotic lesions and have been shown to affect transcription of genes in vascular endothelial cells, smooth muscle cells, monocytes, and monocyte-derived macrophages. PPAR-α induces an increase in ROS, which leads to induction of NADPH-oxidase activity in macrophages and results in the generation of LDL species with PPAR-α activation properties [78]. PPAR-γ expression is significantly increased on monocyte-macrophage differentiation, and PPAR-γ protein is present at high levels in monocytes and macrophage-derived foam cells of atherosclerotic lesions [79, 80] and in circulating human monocytes, where its activation increases the expression of macrophage-specific markers, such as CD14 and CD11b, which are constituents of lipid rafts. Treatment of macrophages with ox-LDL in vitro induces mRNA expression of PPAR-γ and LXR-α, a direct transcriptional target of PPAR-γ. Internalization of ox-LDL provides the cell with activators of PPAR-γ, such as the oxidized fatty acids 9- and 13-hydroxyoctadecadienoic acid (9- and 13-HODE), as well as with activators of LXR such as 27- and 25-hydroxycholesterol [81, 82]. PPAR-γ ligands can also be produced locally in atherosclerotic lesions through the oxidation of fatty acids by 12/15 lipooxygenase [83]. Arachidonic acid metabolites derived from the cyclooxygenase and lipooxygenase pathways [e.g., 15-deoxy-Prostaglandin J2 (PGJ2), and 15-hydroxyicosatetraenoic acid (15-HETE)] [82, 84] activate also PPAR-γ. PPAR-γ activators inhibit the expression of MMP-9 in human macrophages [85] and vascular smooth muscle cells [86] and the production of the inflammatory cytokines TNF-α, IL-6, and IL-1β by activated monocytes [87]. The induction of the scavenger receptor CLA-1/SR-BI is inhibited by PPAR-γ in human macrophages [88]. Activation of PPAR-γ has been shown to enhance CD36 expression of macrophages, which may indicate that PPAR-γ could stimulate uptake of ox-LDL and contribute to foam cell formation [82]. These CD36 effects may be compensated through the activation of LXRs, which promotes cholesterol efflux by modulating expression of ABCA1 and apoE [89]. Silverstein and Febbraio [90] showed a decrease in CD36 mRNA expression as a result of inhibition in the transcriptional activity of PPARγ following phosphorylation by TGF-β. Tontonoz et al. [80] confirmed the relation of PKC with PPARγ induction and CD36 expression with the results showing that diacylglycerol and ingenol as PKC activators regulate the mRNA expression of CD36. Additionally rosiglitazone as a PPARγ agonist was shown to increase CD36 expression in macrophages [91]. Leonarduzzi et al. [92] tested the effects of non-oxidized and oxidized cholesterol on monocyte cell differentiation and foam cell formation and found out that while oxysterols stimulated CD36 expression and synthesis in human U937 promonocytes, nonoxidized cholesterol did not exert any effect. When investigated in detail, the upregulation of CD36 was found to be based on the activation of protein kinase Cδ, extracellular signal-regulated kinase 1/2 (ERK1/2) and PPARγ.

When cholesterol acceptors such as HDLs are present, cholesterol efflux from macrophages is accelerated, which prevents foam cell formation. The ATP-binding cassette transporters (ABCs) ABCA1 and ABCG1 facilitate transport of free cholesterol and cholesterol/phospholipid complexes (UC/PL) across cell membranes in cholesterol efflux path-
ways. During this process, ABCA1 promotes nascent discoidal pre-\beta-HDL particle formation from lipid poor apoA-I. In humans and mice, apoA-I is produced primarily in the liver and intestine. Extracellular sources of apoA-I have been shown to increase cholesterol efflux from macrophages in vitro and are considered to be necessary for the activation of cholesterol efflux through the ABCA1 pathway [94]. The Rho family GTPase Cdc42 directly interacts with ABCA1 to control filopodia formation, actin organization, and intracellular lipid transport [95]. Vesicular transport processes involving different interactive proteins like \beta2-syntrophin are involved in cellular lipid homeostasis controlled by ABCA1 [96].

Cell-adhesion molecules such as ICAM-1, present within the endothelium and increases monocyte adhesion, is upregulated by lysophosphatidylcholine formation following phosphatidylcholine degradation. This formation can induce mitochondrial ROS formation through Ca\textsuperscript{2+}-dependent process and leads to the activation of ERK/MAPK pathway. The mechanism is explained as the interaction between the Ca\textsuperscript{2+}-dependent mitochondrial dehydrogenases and complex I [97].

Heme/iron oxidative damage can be promoted by increased heme/iron levels released into the plasma from damaged red blood cells that are removed by binding to hemopexin and haptoglobin and subsequent cellular uptake via CD163 cysteine-rich SRs into monocytes and macrophages. Heme is oxidized and rapidly converted into heme in one portion is removed by hemopexin, but the rest interacts with cell membranes [98] and with circulating LDL and HDL [99]. Accumulation of heme, however, triggers an oxidative-stress response that promotes heme degradation by HO-1 into bilirubin, iron, and CO. Because overexpression of HO-1 has been found to protect animals from atherosclerotic lesions, it is suggested that heme is a risk factor for atherogenesis and that HO-1 can protect cells from oxidative damage [99]. Hemoglobin promotes formation of ROS and catalyzes LDL oxidation and covalent cross-linking of the LDL protein apoB through the globin radical [100].

Tyrosine kinases are known to affect vessels in several ways. In a study, H\textsubscript{2}O\textsubscript{2} was shown to increase the phosphorylation of tyrosine kinases and lead to stronger binding of neutrophil cells on endothelium and alteration of vessel permeability [47].

In advanced lesions, macrophages become apoptotic. Apoptosis is induced with the accumulation of free cholesterol mainly in the endoplasmic reticulum membrane and alters the function of integral endoplasmic reticulum membrane proteins. These chain of events induces the endoplasmic reticulum stress signal transduction pathway also known as the unfolded protein response. Evidence from in vivo studies suggests that this pathway plays important role in atherosclerotic lesions [101].

ANTIOXIDANT SYSTEMS IN ATHEROSCLEROSIS

A redox couple is a molecule or enzyme that switches between reduced and oxidized forms. Two of the most important redox couples are thioredoxin (Trx) and glutathione (GSH). Trx is a small (12 kDa) multifunctional protein carrying two cysteines that reversibly switches from dithiol to disulfide [Trx(\text{SH})\textsubscript{2} to TrxS\textsubscript{2}]. TrxS\textsubscript{2} is generally reduced by NADPH and flavoprotein thioredoxin reductase (TrxR). Trx ubiquitously expressed in all organs including heart and is deeply involved in the protection of cardiomyocytes by its antioxidant, antiapoptotic as well as anti-inflammatory properties. Trx is localized in both the cytosol and the nucleus [18]. Plasma or serum Trx levels can be easily determined by utilizing the ELISA assay [102]. Moreover, in the various cardiovascular disease conditions, the expression level of Trx is altered either in organ or in plasma or in both. The uptake of ox-LDL by macrophages highly induces Trx expression [103]. Highly elevated plasma or serum Trx levels are reported in patients with diabetes mellitus, especially with diabetes mellitus type 2 or with glucose intolerance, and patients with hypertension, hypercholesterolemia, and atherosclerosis, all of which are major risk factors for cardiovascular diseases [103, 104]. By providing electrons, Trx and GSH maintain intracellular proteins in a reduced state. As part of the cellular defence against oxidative stress, expression of different genes of the GSH and Trx systems is increased when cells are exposed to ROS [14]. ROS, RNS, and electrophilic lipids contribute to the posttranslational modification of protein thiols (protein-SH) to form S-nitrosothiols (SNOs). This is a prevalent posttranslational modification involved in redox-based cellular signaling. Under physiologic conditions, protein S-nitrosylation and SNOS provide protection preventing further cellular oxidative and nitrosative stress. Conversely, increased oxidative stress and the resultant dysregulation of NO are implicated in the pathogenesis of cardiovascular diseases [105]. Many intracellular redox-sensitive processes, including synthesis of DNA precursors by ribonucleotide reductase, transcription factor regulation, and cellular growth [106], are modulated by the Trx system, composed of Trx, TrxR, and NADPH. Because the many antioxidant and regulatory roles of cytosolic Trx are dependent on the activity of cytosolic TrxR, this selenoenzyme together with Trx is increasingly being recognized as an essential component for cellular redox control and antioxidant defense [14, 106]. The ubiquitous 55-kDa selenoprotein TrxR1 was found upregulated in human atherosclerotic plaques and expressed in foam cells [107]. TrxR1 mRNA in human monocyte-derived macrophages dose-dependently increases with ox-LDLs, but not with native LDLs.

Specific protein disulfide targets for reduction by Trx are protein disulfidases (PDI) and Trx and is also a specific electron donor for many peroxiredoxins, which are important for the reduction of peroxides and have generated recent interest for their potential to regulate signaling pathways. In macrophage-derived foam cells on ox-LDL stimulation, peroxiredoxin I (Prx I) plays a dual role. As an antioxidant, induction of Prx I during treatment with ox-LDL led to improved cell survival with a decrease in ROS. Additionally, activation of p38/MAPK was dependent on the upregulation of Prx I. Therefore, Prx I in macrophage-derived foam cells could be considered both an antioxidant and a regulator of oxidant-sensitive signal transduction [108].

GSH is a cysteine-containing tripeptide that reversibly forms a homodimer, GSH disulfide (GSSG). The GSH/glutaredoxin (GR) system plays a critical role in pro-
Lipid rafts (LRs) are originally defined as sphingolipid- and cholesterol-rich microdomains in the plasma membrane that play a role in a number of signaling processes involving specific receptors [116]. A growing body of evidence supports the notion that lipid rafts play a crucial role in the redox signaling that regulates the pathophysiology of many degenerative diseases [117]. It is also known that distinct cholesterol- and sphingolipid-rich membrane rafts is importantly involved in transmembrane signaling in a variety of mammalian cells [118, 119]. The formation of LR signaling platforms with aggregation of different signaling molecules may represent one of important mechanisms determining the variety of transmembrane signaling; it also robustly amplifies signals from activated receptors. Among these LR signaling platforms, it is also reported that some large redox signaling molecules are also aggregated or recruited into LR clusters and subsequently produce superoxide and other ROS [120-122]. This type of LR signaling platforms with ROS production has been referred to as LR redox signaling platforms [121]. This LR signaling mechanism has also been reported to play important roles in the normal regulation of many other cell or organ functions and in the development of different pathological conditions of different cells or organs.

LRs are considered to be an important signaling component in the cell membrane [116, 119]. LRs consist of dynamic assemblies of cholesterol and lipids with saturated acyl chains that include sphingolipids and glycosphingolipids in the exoplasmic leaflet of the membrane bilayer. In addition, phospholipids with saturated fatty acids and cholesterol in the inner leaflet are important elements for LRs. By interdigitation and transmembrane proteins, the long fatty acids of sphingolipids in the outer leaflets couple the exoplasmic and cytoplasmic leaflets, which form a very stable and detergent-resistant membrane structure [119, 123]. This stable structure is one of the most basic features of LRs. Different from this stable membrane structure, a large portion of cell membrane lipid (60-80%) is more fluid, as it mostly consists of phospholipids with unsaturated and kinked fatty acid chains, as well as cholesterol. Another interesting feature of these membrane LRs is their capacity of including or excluding proteins to variable extents when cells respond to different physiological or pathological stimuli. Many proteins have been demonstrated to have LR affinity such as glycosylphosphatidylinositol (GPI) anchored proteins, doubly acylated proteins, cholesterol-linked proteins, and some other transmembrane proteins, including ion channels, tyrosine kinases, and different membrane exchangers or transporters [119, 124].

Actually two types of lipid rafts are identified in the biologic membranes: caveolae and noncaveolae lipid rafts. 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 [116]. 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 phosphocholine 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-ordered 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 [125].

Caveolae, 50-100 nm invaginations of the plasma membrane, are a subset of lipid rafts enriched in sphingolipids and cholesterol. The caveolae can selectively sequester membrane-targeted proteins and create a unique signaling microdomain, thereby controlling transmembrane signaling. Caveolae are characterized by the presence of caveolins, which distinguishes caveolae from other lipid raft domains. At least three caveolin isoforms of molecular weights between 22 and 24 kDa have been identified: caveolin 1 and caveolin 2 are abundant in most cell types, while caveolin 3 is specific to muscle cells [126]. Caveolin 1, a substrate for nonreceptor tyrosine kinases including Fyn, Abl, and Src, acts as a scaffolding protein and can be phosphorylated on tyrosine 14 by these kinases in response to external stimuli such as ROS. Such tyrosine phosphorylation activates the downstream signaling targets and thus serves as a crucial step for intracellular signaling occurring within caveolae. Sphingomyelinase (SMase)-dependent cleavage of sphingomyelin resulting in the formation of ceramide appears to play a crucial role in lipid raft formation [127]. Sphingomyelin hydrolysis is catalyzed by SMases that hydrolyze the phosphodiester bond of sphingomyelin, yielding ceramide and phosphocholine. There is convincing evidence that ceramide performs its signaling function from within the lipid rafts,
ordered sphingolipid- and cholesterol-rich lipid domains [128], which can function as an ordered support for receptor-mediated signaling events.

Initiation of intracellular signaling cascades is associated with aggregation or reduction of cell surface receptors through LR clustering in the plasma membrane [120, 129]. These receptors aggregated in LR clusters are many, including T-cell receptor/CD3 complex, B cell receptors, CD2, CD40, CD44, L-selectin, insulin receptors, or integrins, which transfer the signal to transmembrane signaling proteins or proteins in inner leaflets of the cell membrane when they aggregate within LR clusters. This completes the transmembrane signaling process [130, 131]. Recent studies have indicated that several death receptors, including tumor necrosis factor receptor (TNFR), Fas, and DR 4 and 5 produce their detrimental effects through this mechanism [119, 132]. During LR clustering, aggregated receptors or other signaling molecules could be either constitutively located in LRs or translocated by trafficking or recruitment upon stimulation [133, 134]. This dynamic clustering of lipid microdomains may represent a critical common mechanism in transmembrane signal transduction. It has been reported that clustered LRs contain different compositions of proteins, constituting platforms or macrodomains that form a new mixture of molecules, including different signaling molecules and crosslinkers or enzymes [116, 119]. The formation of LR platforms activates, facilitates, or amplifies signal transduction. There is considerable evidence that LR clustering could be formed as a ceramide-enriched membrane platform and that ceramide production or enrichment is through sphingomyelinase (SMase)-catalyzed cleavage of choline from sphingomyelin (SM) in individual LRs [135, 136]. However, ceramide-enriched membrane platforms might also be formed without existence of classically-defined LRs simply through a fusion of several ceramide molecules. These ceramide molecules could come from LRs or other membrane fractions.

These lipid-rich microdomains (lipid rafts) of the cell membrane are central to the understanding of cellular lipid homeostasis and the consequences of lipid loading on cell function. They are ceramide, which is induced by ox-LDL through enhancement of neutral and acidic sphingomyelinase (SMase) activity [137], leads to coalescence of submicroscopic rafts into large ceramide membrane macrodomains [135]. These macrodomains may serve as platforms for protein concentration and oligomerization, transmitting signals across the plasma membrane. Ceramide then activates a variety of diverse protein kinase- and protein phosphatase-dependent signaling pathways, which in most cases suppress cell growth or promote programmed cell death or both [135]. In addition, ceramide is a ligand for apoE, which binds more avidly to ceramide-rich microdomains on sphingomyelinase-treated liposomes [138]. Together with the stimulation of heparan sulfate proteoglycan (HSPG) and low-density lipoprotein receptor eluted protein (LRP)-mediated uptake by macrophages through ceramide and apoE, which is crucial for foam cell formation [139], a function of ceramide-rich microdomains in apoE-dependent metabolism is suggested.

Lipid rafts can be disrupted by cholesterol depletion, whereas cholesterol enrichment stabilizes the formation of lipid rafts [116]. E-LDL preferentially increases cellular cholesterol, whereas ox-LDL increases cellular ceramide content because of a higher mRNA expression of acid and neutral sphingomyelinase (SMase), neutral SMase activation-associated factor, and glucosylceramidase during ox-LDL loading [3]. E-LDL and ox-LDL differentially influence membrane-microdomain formation in human macrophages and thereby differ in their regulation of macrophage effector functions during atherogenesis. Glycosphingolipids, as constituents of lipid rafts and especially ceramides, are ligands for apoE, and apoE binds more avidly to ceramide-rich microdomains on sphingomyelinase-treated liposomes [138]. This indicates a function of ceramide-rich microdomains in apoE-dependent metabolism. The generation of ceramide in the plasma membrane by SMases may stimulate HSPG and low-density lipoprotein receptor-related protein (LRP)-mediated uptake by macrophages, which is catalyzed by apoE, and plays a crucial role in tissue remodeling and foam cell formation [139]. Concerning the relation of atherogenic LDL and lipid rafts in human aortic endothelial cells, ox-LDL causes the disappearance of the lipid-raft marker GM1 from the plasma membrane. Exposure to ox-LDL may result in the disruption or redistribution of lipid rafts, which in turn induces stiffening of the endothelium, an increase in endothelial force generation, and the potential for network formation [140].

A significant amount of eNOS, which generates NO in the endothelium, is found in caveolae. Caveolae are flask-shaped invaginations of the plasma membrane that are coated with the protein caveolin, which can function as a negative regulator of eNOS [141]. They contain proportionately small amounts of phospholipids and large amounts of cholesterol, sphingomyelin, and glycosphingolipids as well as SR-BI and CD36. Ox-LDL causes an efflux of caveolae cholesterol out of the cell and onto ox-LDL, leading to a redistribution of eNOS and caveolin to an intracellular membrane [142]. This requires the presence of ox-LDL binding to CD36, whereas the absence of CD36 protects caveolae from cholesterol depletion and the translocation of eNOS out of caveolae and maintains the ability of acetylcholine to stimulate NO production. Caveolae-localized sphingomyelin may serve as the substrate for the generation of the ceramide that stimulates eNOS. In contrast to ox-LDL and CD36, which remove cholesterol from caveolae, HDL and SR-BI move cholesterol into them. This could serve as an indirect effect on eNOS function and helps to maintain the cholesterol level of caveolae, which allows eNOS to remain associated with caveolae [143].

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 [125]. 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. 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. 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$$^-$ increased the formation of ceramide-enriched membrane platforms in the membrane of coronary arterial endothelial cells [122]. In other studies, H$_2$O$_2$ 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 signaling or function through their actions on many lipid-raft components such as ceramide production, cholesterol, and related raft proteins [144, 145].

It has been indicated that induction of lipid oxidation through ROS can amplify foam cell formation through 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 LRP family members. This in turn leads to cell-surface expansion of ceramide-enriched domains and activation of apoE/LRP1/CD1-mediated antigen presentation. 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 [3].

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 ox-LDL 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 [146].

Mitogen-activated protein kinases and receptor tyrosine kinases were first recognized as residing in caveolin-rich microdomains; certain GPCRs and associated molecules were subsequently shown to be enriched in these domains [147-150]. Besides these proteins, several other proteins are associated to or included in different rafts shown in Table 2 [151-156].

Little is known about how proteins localize to different lipid domains. Different mechanisms for lipid raft targeting have been proposed or described: (i) Proteins may bind to caveolin via a scaffolding domain located near the N-terminus of caveolin-1 and caveolin-3 [157, 158]. Many proteins that bind to caveolin contain a putative caveolin-binding motif (a loosely defined pattern of aromatic and nonaromatic residues) [159]. (ii) N-linked myristoylation (of a glycine residue following the initial methionine) or thio-acylation with palmitate (palmitoylation of cysteine residues) causes partitioning into the lipid-ordered phase of lipid rafts [160-165]. Caveolins function not only as scaffolds that localize signaling proteins, but, in addition, can inhibit numerous enzymes, including AC, eNOS, and several kinases and serine/threonine phosphatases [166-173]. Consistent with the latter findings, data from studies with knockout animal models and from overexpression protocols suggest that caveolins play central roles in regulating signal transduction by various systems [173-177].

M-CSF stimulates raft-associated NADPH oxidase, resulting in ROS formation, which regulates Akt and p38/MAPK, and thus contributes to monocyte/macrophage survival [178]. Superoxide-producing phagocyte NADPH-oxidase consists of a membrane-bound flavocytochrome b558 complex with the subunits gp91phox and p24phox and the cytotoxic factors p47phox, p67phox, and the small GTPase Rac-1, which translocate to the membrane to assemble the active complex after cell activation. Activated Rac-1 stabilizes the NADPH oxidase complex and promotes the production of ROS, which is used for host defense as well as oxidation of LDL (Fig. 2). ROS can also directly activate extracellular signal-regulated kinases (ERK), a member of mitogen-activated protein kinases (MAPKs), which regulate cell proliferation, differentiation, motility, and survival, and the PI3-kinase enzyme complex, creating a bridge between the MAPK and PI3-kinase pathways [179]. In addition, M-CSF can directly induce PI3-kinase activation and phosphatidylidyinositol phosphate formation, resulting in NADPH-oxidase-mediated ROS production, which leads to induced Erk activation and monocyte survival [180].

NADPH oxidase as well as the tyrosine kinases of the Src family (e.g., Lck, Fyn, and Lyn), which are lipid-modified signaling proteins, GPI-linked proteins, and adapter proteins are constituents of raft-membrane microdomains. On receptor binding, immune receptors become raft associated, and additional components of the signaling pathways are recruited to rafts to form signaling complexes. The entry of immune receptors into rafts can regulate cell activation, and raft integrity is crucial for the initiation and maintenance of intracellular signals [181]. It has been shown that superoxide production by NADPH oxidase is inhibited by cholesterol depletion because of impaired translocation of cytosolic protein subunits to the membrane [182]. Formation of lipid rafts in the membrane of coronary endothelial cells induces clustering and activation of reduced NADPH oxidase, thereby forming a redox signaling platform on the cell membrane that mediates transmembrane signaling of death receptors, resulting in endothelial dysfunction [121]. However, the inappropriate or excessive action of the NADPH oxidase system results in chronic inflammatory disorders like atherosclerosis. The sphingolipid ceramide has been reported as one of the critical signaling molecules to mediate the activation of NADH/NADPH oxidase in different cells.
Results demonstrated an induction of ceramide rafts on ox-LDL loading of human macrophages [183], which could be involved in the activation of NADH/NADPH oxidase.

**CONCLUSION**

There is a large body of evidence connecting the effects of oxidative stress and related signaling mechanisms with...
atherogenesis. Redox signaling is shown to play crucial role in several cardiovascular diseases also in hypercholesterolemia induced atherogenesis. Tightly regulated ROS production by a family of NADPH oxidases, which is especially important in redox signaling, are raft associated. Understanding how lipid raft associated redox signaling pathways promote the process of atherosclerosis can be a key factor for clinical approaches.

REFERENCES

[1] Stocker R, Keaney JF Jr. Role of oxidative modifications in atherosclerosis. Physiol Rev 2004; 84(4): 1381-478.
[2] Osterud B, Bjorklid E. Role of monocytes in atherosclerosis. Physiol Rev 2003; 83(4): 1069-112.
[3] Schirrmacher R, Grandi M. Role of redox regulation and lipid rafts in macrophages during Ox-LDL mediated foam cell formation. Anti-oxid Redox Signal 2007; 9(9): 1499-518.
[4] Steinberg D. The LDL modification hypothesis of atherosclerosis: an update. J Lipid Res 2009; 50: S376-S381.
[5] Stocker R, Keaney JF Jr. New insights on oxidative stress in the artery wall. J Thromb Haemost 2005; 3: 1825-34.
[6] Cheung KM, Witztum JL. Mechanisms of superoxide production in human blood vessels: relationship to endothelial dysfunction, clinical and genetic risk factors. J Physiol Pharmacol 2002; 53(4): 515-24.
[7] Pongnitprasert N. Atherosclerosis and NADPH Oxidase. Silpakorn U Sci Tech J 2009; 3(1): 13-24.
[8] Bhakdi S, Dorweiler B, Kirchmann R, et al. On the pathogenesis of atherosclerosis: enzymatic transformation of human low density lipoprotein to an atherogenic mosity. J Exp Med 1995; 182(6): 1959-71.
[9] Ross R. Atherosclerosis--an inflammatory disease. N Engl J Med 1999; 340(2): 115-26.
[10] Seifert PS, Hugo F, Tranum-Jensen J, Zâhringer U, Muhly M, et al. NADPH oxidase activity of vascular smooth muscle cells in vitro. Arterioscler Thromb Vasc Biol 2004; 24: 23-8.
[11] Nordberg J, Arnér ES. Reactive oxygen species, antioxidants, and C-terminal cytoplasmic tails. J Biol Chem 1996; 271: 22315-20.
[12] Nordberg J, Arné S. Reactive oxygen species, antioxidants, and the mammalian thioredoxin system. Free Radic Biol Med 2001; 31(11): 1287-312.
[13] Griffiths HR, Möller L, Bartosz G, Byrne GI. Cellular oxidation of low-density lipoprotein by the sequential action of lipid raft redox signaling platforms in endothelial cells. Annu Rev Pharmacol Toxicol 2003; 43: 180-90.
[14] Parthasarathy S, Barnett J. Phospholipase A2 activity of low density lipoprotein to an atherogenic moiety. Proc Natl Acad Sci USA 1990; 87(24): 9741-5.
[15] Tjoelker LW, Eberhardt C, Unger J, et al. Plasma platelet-activating factor acetylhdrolysis is a secreted phospholipase A2 with a catalytic triad. J Biol Chem 1995; 270(43): 25481-7.
[16] Tao N, Wagner SJ, Lublin DM. CD36 is palmitoylated on both N- and C-terminal cytoplasmic tails. J Biol Chem 1996; 271: 22315-20.
[17] Antoniades C, Tousoulis D, Marinou K, Stefanadis E, Ntarladimas I, Latsios G, Konniari K, Papageorgiou N, Siassos G, Stefanadis C. Effects of lipid profile on forearm hyperemic response in young subjects. Hellenic J Cardiol 2006; 47: 152-7.
[18] Humphries KM, Yoo Y, Zewada LI. Inhibition of NADPH-linked mitochondrial respiration by 4-hydroxy-2-nonenal. Biochemistry 1998; 37(2): 552-7.
[19] STOCKER R, Keaney JF Jr. Regulation of superoxide anion production by NADPH oxidase in monocytes/macrophages contributions to atherosclerosis. Arterioscler Thromb Vasc Biol 2004; 24: 23-8.
[20] Kalayoglu MV, Hoeremen B, LaVerda D, Morrison SG, Morrison RP, Byrne GI. Cellular oxidation of low-density lipoprotein by Chlamydia pneumoniae. J Infect Dis 1999; 180: 780-90.
[21] Gillette-Taylor J, Boullier A, Wittler A, Steinberg D, Quehenberger O. Scavenger receptor class B type I as a receptor for oxidized low density lipoprotein. J Lipid Res 2001; 42(9): 1474-82.
[22] Sigari F, Lee C, Witztum JL, Reaven PD. Fibroblasts that overexpress 15-LOX2 generate bioactive and minimally modified LDL. Arterioscler Thromb Vasc Biol 1997; 17(12): 3639-45.
[23] Tritharanides L, Jessup W, Gifford J, Dean RT. A method for defining the stages of low-density lipoprotein oxidation by the separation of cholesterol- and cholesteryl ester-oxidation products using HPLC. Anal Biochem 1993; 213(1): 79-89.
[24] Leonarduzzi G, Sottero B, Poli G. Oxidized products of cholesterol: dietary and metabolic origin, and proatherosclerotic effects (review). J Nutr Biochem 2002; 13(12): 700-10.
[25] Schnackenberg CG. Physiological and pathophysiological roles of oxygen radicals in the renal microvasculature. Am J Physiol Regul Integr Comp Physiol 2002; 282: R335-R342.
[26] Ray R, Shah AM. NADPH oxidase and endothelial cell function. Clin Science 2005; 109: 217-26.
[27] Dworzakowski R, Anilkumar N, Zhang M, Shah AM. Redox signaling involving NADPH oxidasederived reactive oxygen species. Biochem Soc Trans 2006; 34: 940-6.
[28] Chen YR, Chen CL, Zhang L, Green-Church KB, Zweier JL. Superoxide generation from mitochondrial NADH dehydrogenase induces self-inactivation with specific protein radical formation. J Biol Chem 2005; 280(45): 37339-48.
[29] Zmijewski JW, Landar A, Watanabe N, Dickinson DA, Noguchi N, Darley-Usmar VM. Cell signaling by oxidized lipids and the role of reactive oxygen species in the endothelium. Biochem Soc Trans 2005; 33: 1385-9.
[30] Antoniades C, Tousoulis D, Marinou K, Stefanadis E, Ntarladimas I, Latsios G, Konniari K, Papageorgiou N, Siassos G, Stefanadis C. Effects of lipid profile on forearm hyperemic response in young subjects. Hellenic J Cardiol 2006; 47: 152-7.
[31] Spiekermann S, Landmesser U, Dikalov S, Bredt M, Gamez G, Tatge H, Reepslagner N, Hornig B, Drexler H, Harrison DG. Electron spin resonance characterization of vascular xanthine, oxidized activity in patients with coronary artery disease NAD(P)H, and NADPH, in relation to endothelium-dependent vasodilation. Circulation 2003; 107: 1383-9.
[32] Li JM, Shah AM. ROS generation by nonphagocytic NADPH oxidase: Potential relevance in diabetes nephropathy. J Am Soc Nephrol 2003; 14: S221-S226.
[33] Sin S, Yi F, Li PL. Contribution of lyosomal vesicles to the formation of lipid raft redox signaling platforms in endothelial cells. Antioxid Redox Signal. 2007; 9(9): 1417-26.
[34] Cheng G, Cao Z, Xu X, van Meir EG, and Lambeth JD. Homologs of gp91phox: cloning and tissue expression of Nox3, Nox4, and Nox5. Gene 2001; 269: 131-40.
[35] Forlini F, Brandes RP, Nguyen K, Amidi M, Dehghani F, and Buxis R. A gp91phox containing NADPH oxidase selectively expressed in endothelial cells is a major source of oxygen radical generation in the arterial wall. Circ Res 2008; 103(8): 87-92.
[36] Ago T, Katazono T, Ooboshi H, Iyama T, Han YH, Takada J, Wakisaka M, Ibayashi S, Utsumi H, Iida M. Nox4 as the major
catalytic component of an endothelial NAD(P)H oxidase. Circulation 2004; 109: 227-33.

O’Donnell VB, Freeman BA. Interactions between nitric oxide and lipid oxidation pathways: implications for vascular disease. Circ Res 2001; 88(1): 12-21.

Buttery LD, Springall DR, Chester AH, et al. Inducible nitric oxide synthase is present within human atherosclerotic lesions and promotes the formation and activity of peroxynitrite. Lab Invest 1996; 75(1): 77-85.

Vega S, Scribner WM, Parinandi NL, English D, Garcia JG, Natarajan V. Hydrogen peroxide stimulates tyrosine phosphorylation of focal adhesion kinase in vascular endothelial cells. Am J Physiol 1999; 277: L150-8.

Bergt C, Pennathur S, Fu X, et al. The myeloperoxidase product hypochlorous acid oxidizes HDL in the human artery wall and impairs ABCA1-dependent cholesterol transport. Proc Natl Acad Sci USA 2004; 114: 529-41.

Daughtery A, Dunn JL, Rateri DL, Heinecke JW. Oxidative stress in atherosclerosis. J Clin Invest 1994; 94: 437-44.

Pennathur S, Bergt C, Fu X, et al. HDL atherosclerotic intima and blood of patients with established coronary artery disease contain HDL damaged by reactive nitrogen species. J Biol Chem 2004; 279: 42977-83.

Van Benten BJ, Navab M, Shih D, Fogelman AM, Lusis AJ. The role of high-density lipoproteins in oxidation and inflammation. Trends Cardiovasc Med 2001; 11: 155-61.

Shao B, Oda MN, Bergt C, Fu X, Green PS, Brot N, Oram JF, Heinecke JW. Myeloperoxidase impairs ABCA1-dependent cholesterol efflux through methionine oxidation and site-specific tyrosine chlorination of apolipoprotein A-I. J Biol Chem 2006; 281: 9001-4.

Sugiyama O, Okada Y, Sukhova GK, Virmani R, Heinecke JW, Libby P. Macrophage myeloperoxidase regulation by granulocyte macrophage colony-stimulating factor in human atherosclerosis and implications in acute coronary syndromes. Am J Pathol 2001; 158: 879-91.

Francis GA, Oram JF, Heinecke JW, and Bierman EL. Oxidative stress in human atherosclerotic intima and blood of patients with established coronary artery disease contains HDL damaged by reactive nitrogen species. J Biol Chem 2004; 279: 42977-83.

Francis GA, Mendez AJ, Bierman EL, and Heinecke JW. Oxidative stress in high density lipoprotein by peroxisome proliferator-activated receptor-alpha (PPAR-alpha) expression and reduction of MMP-9 activity in macrophages, leading to the generation of LDL with PPAR-alpha activation properties. Circ Res 2004; 95: 1174-82.

Macdonald DL, Terry TL, Agellon LB, Nation PN, Francis GA. Administration of tyrosyl radicaloxidized HDL inhibits the development of atherosclerosis in apolipoprotein E-deficient mice. Arterioscler Thromb Vasc Biol 2003; 23: 1583-8.

Toussoulis D, Boiger RH, Antoniades C, Stiasno G, Stefanidi E, Stefanidi C. A mechanism of disease: Larginone in coronary atherosclerosis—a clinical perspective. Nat Clin Pract Cardiovasc Med 2007; 4: 274-83.

Dusting GJ, Saldemis S, Jiak F. Mechanism for suppressing NADPH oxidase in the vascular wall. Mem Inst Oswaldo Cruz, Rio de Janeiro 2005; 100 (Suppl. 1): 97-103.

Ruef J, Hu ZY, Yin LY, Wu Y, Hanson SR, Kelly AB, Harker LA, Macdougall DL, Terry TL, Agellon LB, Nation PN, Francis GA. Interactions between nitric oxide and peroxisome proliferator-activated receptor gamma (PPARgamma) expression and reduction of MMP-9 activity in human atherosclerosis—a clinical perspective. Nat Clin Pract Cardiovasc Med 2007; 4: 274-83.
through PPARgamma activation in mononuclear phagocytes in vitro. Am J Pathol 1998a; 153: 17-23.

[86] Marx N, Schonbeck U, Lazar MA, Libby P, Plutzky J. Peroxisome proliferator-activated receptor gamma activators inhibit gene expression and migration in human vascular smooth muscle cells. Circ Res 1999b; 83: 1097-109.

[87] Jiang C, Ting AT, Seed B. PPAR-gamma agonists inhibit production of monocyte inflammatory cytokines. Nature 1998; 391: 82-6.

[88] Chinioti G, Bhangui FG, Gregillo S, Mallat Z, Antonucci M, Poullain P, Chapman J, Frucht JC, Tedgui A, Najib-Frucht J, and Staels B. CLA-1/SR-BI is expressed in atherosclerotic lesion macrophages and regulated by activators of peroxisome proliferator-activated receptors. Circulation 2000; 101: 2411-7.

[89] Venkateswaran A, Laffitte BA, Joseph SB, et al. Control of cellular cholesterol efflux by the nuclear oxygen receptor LXR alpha. Proc Natl Acad Sci USA 2000; 97: 12097-102.

[90] Silverstein RL, Feibraio M. CD36 and atherosclerosis. Curr Opin Lipidol 2000; 11: 483-91.

[91] Zhao M, Liu Y, Wang X, New L, Han J, Brunk UT. Activation of the p38MAPkinase pathway is required for foam cell formation from macrophages exposed to oxidized LDL. APMIS 2002; 110: 458-68.

[92] Leonarduzzi G, Gamba P, Gargiulo S, et al. Oxidation as a crucial reaction for cholesterol to induce tissue degeneration: CD36 overexpression in human monomocytic cells treated with a biologically relevant oxygen mixture. Aging Cell 2008; 7(3): 375-82.

[93] Hara H, Yokoyama S. Interaction of free apolipoproteins with macrophages: formation of high density lipoprotein-like lipoproteins and reduction of cellular cholesterol. J Biol Chem 1991; 266: 3080-6.

[94] Takahashi K, Takeya M, Sakasaita N. Multifunctional roles of macrophages in the development and progression of atherosclerosis in humans and experimental animals. Med Electron Micros 2002; 35: 179-202.

[95] Deiwick W, Orso E, Drobnik W, and Schmitz G. Apolipoprotein AI and HDL(3) inhibit spreading of primary human monocytes through a mechanism that involves cholesterol depletion and regulation of CDC42. Atherosclerosis 2001; 159: 313-24.

[96] Schmitz G, Buechler C. ABCA1: regulation, trafficking and association with heteromeric proteins. Ann Med 2002; 34: 334-47.

[97] Zmijewski JW, Moellerling DR, Le Goffe C, Landar A, Schmitz G, Moellering DR, Le Goffe C, Landar A, Simons K, Toomre D. Lipid rafts and signal transduction. Nat Rev Mol Membr Biol 2006; 23: 49-57.

[98] Morrison JA, Jacobsen DW, Sprecher DL, Robinson K, Khoury P, and Chapman J, Fruchart JC, Tedgui A, Najib-Fruchart J, and Toth PO, Arterioscler Thromb Vasc Biol 2001; 21: 35: 179-203.

[99] Simons K, Ikonen E. Functional rafts in cell membranes. Nature 1997; 387: 569-77.

[100] Wang Y, Qiao M, Meyel JJ, Asmis LM, Asmis R. Molecular mechanism of glutathione-mediated protection from oxidized low-density lipoprotein-induced cell injury in human macrophages: role of glutathione reductase and glutaredoxin. Free Radic Biol Med 2006; 41: 775-85.

[101] Hong J, Shioji K, Nakamura H, Masutani H, Yodoi J, and Tabas I. Apoptosis and plaque destabilization in atherosclerosis: its potential role in antioxidant protection and glutaredoxin, redox-regulating proteins, in pancreatic cancer. Biochem Biophys Acta 2002; 1585: 139-45.

[102] Wang Y, Qiao M, Meyel JJ, Asmis LM, Asmis R. Molecular mechanism of glutathione-mediated protection from oxidized low-density lipoprotein-induced cell injury in human macrophages: role of glutathione reductase and glutaredoxin. Free Radic Biol Med 2006; 41: 775-85.

[103] Gotoh N, Graham A, Nikel E, and Darley-Usmar VM. Inhibition of glutathione synthesis increases the toxicity of oxidized low-density lipoprotein to human monocytes and macrophages. Biochim J 1993; 296: 151-4.

[104] Arner ES, Holmgren A. Physiological functions of thioredoxin and thioredoxin reductase. Eur J Biochem 2000; 267: 6102-9.

[105] Wang Y, Shioji K, Nakamura H, Masutani H, Yodoi J. Redox regulation by thioredoxin in cardiovascular diseases. Antioxid Redox Signal 2003; 5: 795-802.

[106] Shioji K, Nakamura H, Masutani H, Yodoi J. Redox regulation by thioredoxin in cardiovascular diseases. Antioxid Redox Signal 2003; 5: 795-802.
[134] Cheng PC, Dykstra ML, Mitchell RN, Pierce SK. A role for lipid rafts in B cell antigen receptor signaling and antigen targeting. J Exp Med 1999; 190: 1549-60.

[135] Gulbins E, Kolesnick R. Raft receptor in mammalian medicine. Oncogene 2003; 22: 7670-7.

[136] Hoekstra D, Maier O, van der Wouden JM, Slimane TA, van ISC. Membrane dynamics and cell polarity: the role of sphingolipids. J Lipid Res 2003; 44: 869-77.

[137] Kirschfeld R, Claus R, Deigner HP, et al. Modified low density lipoprotein delivers substrate for caveosome formation and stimulates the sphingomyelin-ceramide pathway in human macrophages. FEMS Lett 1997; 405: 55-9.

[138] Morita SY, Nakano M, Sakurai A, Deharu Y, Vertut-Doi A, Hunda T. Formation of caveolae-depleted domains in lipid particles enhances the binding of apolipoprotein E. FEBS Lett 2005; 579: 1759-64.

[139] Morita SY, Kawase M, Sakurai A, et al. Ceramide in lipid particles enhances heparan sulfate proteoglycan and low density lipoprotein receptor-mediated protein uptake by macrophages. J Biol Chem 2004; 279: 24355-61.

[140] Bousset D, Titchenar R, Rothblat GH, Gooch KJ, Levitan I. OxLDL increases endothelial stiffness, force generation, and network formation. J Lipid Res 2006; 47: 715-23.

[141] Feron O, Michel JB, Sase K, Michel T. Dynamic regulation of endothelial nitric oxide synthase: complementary roles of dual acylation and caveolin interactions. Biochemistry 1998; 37: 193-200.

[142] Blair A, Shaul PW, Yuhanna IS, Conrad PA, Smart EJ. Oxidized low density lipoprotein displaces endothelial nitric-oxide synthase (eNOS) from plasmaemmal caveolae and impairs eNOS activation. J Biol Chem 1999; 274: 32512-9.

[143] Chikani G, Zhu W, Smart EJ. Lipids: potential regulators of nitric oxide generation. Am J Physiol Endocrinol Metab 2004; 287: E386-E389.

[144] Dummrit CA, Zhang Y, Li X, Gulbins E. Ceramide: a novel player in reactive oxygen species-induced signaling? Antioxid Redox Signal 2007; 9: 1535-40.

[145] Morgan MJ, Kim YS, Liu Z. Lipid rafts and oxidative stress-induced cell death. Antioxid Redox Signal 2007; 9: 1471-83.

[146] Levitan I, Gooch KJ, Levin I. OxLDL increases endothelial stiffness, force generation, and network formation. J Lipid Res 2006; 47: 715-23.

[147] Feron O, Michel JB, Sase K, Michel T. Dynamic regulation of endothelial nitric oxide synthase: complementary roles of dual acylation and caveolin interactions. Biochemistry 1998; 37: 193-200.

[148] Blair A, Shaul PW, Yuhanna IS, Conrad PA, Smart EJ. Oxidized low density lipoprotein displaces endothelial nitric-oxide synthase (eNOS) from plasmaemmal caveolae and impairs eNOS activation. J Biol Chem 1999; 274: 32512-9.

[149] Chikani G, Zhu W, Smart EJ. Lipids: potential regulators of nitric oxide generation. Am J Physiol Endocrinol Metab 2004; 287: E386-E389.

[150] Dummrit CA, Zhang Y, Li X, Gulbins E. Ceramide: a novel player in reactive oxygen species-induced signaling? Antioxid Redox Signal 2007; 9: 1535-40.

[151] Morgan MJ, Kim YS, Liu Z. Lipid rafts and oxidative stress-induced cell death. Antioxid Redox Signal 2007; 9: 1471-83.

[152] Levitan I, Gooch KJ, Levin I. OxLDL increases endothelial stiffness, force generation, and network formation. J Lipid Res 2006; 47: 715-23.

[153] Feron O, Michel JB, Sase K, Michel T. Dynamic regulation of endothelial nitric oxide synthase: complementary roles of dual acylation and caveolin interactions. Biochemistry 1998; 37: 193-200.

[154] Blair A, Shaul PW, Yuhanna IS, Conrad PA, Smart EJ. Oxidized low density lipoprotein displaces endothelial nitric-oxide synthase (eNOS) from plasmaemmal caveolae and impairs eNOS activation. J Biol Chem 1999; 274: 32512-9.

[155] Chikani G, Zhu W, Smart EJ. Lipids: potential regulators of nitric oxide generation. Am J Physiol Endocrinol Metab 2004; 287: E386-E389.

[156] Dummrit CA, Zhang Y, Li X, Gulbins E. Ceramide: a novel player in reactive oxygen species-induced signaling? Antioxid Redox Signal 2007; 9: 1535-40.

[157] Morgan MJ, Kim YS, Liu Z. Lipid rafts and oxidative stress-induced cell death. Antioxid Redox Signal 2007; 9: 1471-83.

[158] Levitan I, Gooch KJ, Levin I. OxLDL increases endothelial stiffness, force generation, and network formation. J Lipid Res 2006; 47: 715-23.

[159] Feron O, Michel JB, Sase K, Michel T. Dynamic regulation of endothelial nitric oxide synthase: complementary roles of dual acylation and caveolin interactions. Biochemistry 1998; 37: 193-200.

[160] Blair A, Shaul PW, Yuhanna IS, Conrad PA, Smart EJ. Oxidized low density lipoprotein displaces endothelial nitric-oxide synthase (eNOS) from plasmaemmal caveolae and impairs eNOS activation. J Biol Chem 1999; 274: 32512-9.

[161] Shaul PW, Smart EJ, Robinson LJ, et al. Acylation targets endothelial nitric-oxide synthase to plasmaemmal caveolae. J Biol Chem 1996; 271: 6518-22.

[162] Mumbery SM. Reversible palmitoylation of signaling proteins. Curr Opin Cell Biol 1997; 9: 148-54.

[163] Song KS, Sagaiomono C, Galiabbi F, Parenti M, Lisanti MP. Targeting of a G alpha subunit (Gz alpha) and c-Src tyrosine kinase to caveolae membranes: clarifying the role of N-myristoylation. Cell Mol Biol 1997; 43: 293-301.

[164] Galiabbi F, Razani B, Lisanti MP. Emerging themes in lipid rafts and caveolae. Cell 2001; 106: 403-11.

[165] Zacharias DA, Violin JD, Newton AC, Tsien RY. Partitioning of lipid-modified monomeric GFPs into membrane microdomains of live cells. Science 2002; 296: 913-6.

[166] Garcia-Cardena G, Martasek P, Masters BS, et al. Dissecting the interaction between nitric oxide synthase (NOS) and caveolin. Functional significance of the nos caveolin binding domain in vivo. J Biol Chem 1997; 272, 25437-40.

[167] Oka N, Yamamoto M, Schwengcle C, et al. Caveolin interaction with protein kinase C. Isoenzyme-dependent regulation of kinase activity by the caveolin scaffolding domain peptide. J Biol Chem 1999; 274: 33416-21.

[168] Engeljan KA, Chou C, Lin A, et al. Caveolin-mediated regulation of signaling along the p42/44 MAP kinase cascade in vivo. A role for the caveolin-scaffolding domain. FEBS Lett 1998; 428: 205-11.

[169] Feron O, Saldana F, Michel JB, Michel T. The endothelial nitric-oxide synthase-caveolin regulatory cycle. J Biol Chem 1998; 273: 3125-8.

[170] Toya Y, Schwengcle C, Courj T, Lisanti MP, Ishikawa Y. Inhibition of adenylyl cyclase by caveolin peptides. Endocrinol 1998; 139: 2025-31.

[171] Carman CV, Lisanti MP, Benovic JL. Regulation of G protein-coupled receptor kinases by caveolin. J Biol Chem 1999; 274: 8583-84.

[172] Razani B, Lisanti MP. Caveolin-deficient mice: insights into caveolar function human disease. J Clin Invest 2001; 108: 1553-61.

[173] Hnasko R, Lisanti MP. The biology of caveolae: lessons from caveolin knockout mice and implications for human disease. Mol Interact 2003; 3: 445-6.

[174] Razani B, Combs TP, Wang XB, et al. Caveolin-1-deficient mice are lean, resistant to diet-induced obesity, and show hypertiglyceridemia with adipocyte abnormalities. J. Biol. Chem 2002; 277: 8635-47.

[175] Razani B, Wang XB, Engeljan KA, et al. Caveolin-2-deficient mice show evidence of severe pulmonary dysfunction without disruption of caveolae. Mol. Cell. Biol 2002b; 22, 3239-44.

[176] Schubert W, Frank PG, Woodman SE, et al. Microvascular hyperpermeability in caveolin-1 (-/-) knockout mice: treatment with a specific NOSinhbitor, L-NAMe, restores normal microvascular permeability in Cav-1 null mice. J Biol Chem 2002; 277, 40091-8.

[177] Woodman SE, Park DS, Cohen AW, et al. Caveolin-3 knockout mice develop a progressive cardiomyopathy and show hyperactivation of the p42/44 MAP kinase cascade. J Biol Chem 2002; 277: 38988-97.

[178] Wang Y, Zeigler MM, Lam GK, et al. The role of the NADPH oxidase complex, p38 MAPK, and Akt in regulating human monocyte/macrophage survival. Am J Respir Cell Mol Biol 2007; 36: 88-77.
[179] DeLeo FR, Quinn MT. Assembly of the phagocyte NADPH oxidase: molecular interaction of oxidase proteins. J Leukoc Biol 1996; 60: 677-91.

[180] Bhatt NY, Kelley TW, Khramtsov VV, et al. Macrophagecolony-stimulating factor-induced activation of extracellular-regulated kinase involves phosphatidylinositol 3-kinase and reactive oxygen species in human monocytes. J Immunol 2002; 169: 6427-34.

[181] Rajendran L, Simons K. Lipid rafts and membrane dynamics. J Cell Sci 2005; 118: 1099-102.

[182] Vilhardt F, van Deurs B. The phagocyte NADPH oxidase depends on cholesterol-enriched membrane microdomains for assembly. EMBO J 2004; 23: 739-48.

[183] Grandl M, Bared SM, Liebisch G, Werner T, Barlage S, Schmitz G. E-LDL and Ox-LDL differentially regulate ceramide and cholesterol raft microdomains in human Macrophages. Cytometry A 2006; 69: 189-91.