LDL receptors, caveolae and cholesterol in endothelial cell dysfunction: oxLDL accomplices or victims?

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Abstract

Oxidized low-density lipoproteins (oxLDL) and oxysterols play a key role in the endothelial dysfunction and atherosclerosis development. Loss of vascular endothelium integrity impacts vasomotion, cell growth, adhesiveness and barrier functions. While for some of these disturbances we can give a reasonable explanation from a mechanistic point of view, for many others the involved molecular players are unknown. Caveolae, specific plasma membrane domains, have recently emerged as targets and mediators of oxLDL-induced endothelial cell dysfunction. The current knowledge on oxLDL/caveolae interplay and the associated signal transduction pathways are here reviewed and discussed in light of the possible cross-talk between transducers (from receptors to membrane cholesterol) and/or effectors. A better understanding of how oxLDL interact with endothelial cells (EC) and, in turn, modulate metabolic/signaling pathways in EC is crucial to define their role in atherogenesis and find new targets of intervention.

Introduction

The endothelium is a thin monolayer of cells that covers the blood vessel lumen, creating a barrier, between blood and surrounding tissues, and playing an active role in vascular functioning and homeostasis. Endothelial cells (EC) are involved in the maintenance of vascular tone, blood fluidity, and leukocyte trafficking; they also mediate blood-tissue exchange, and participate in hemostasis and neovascularization, acting as a real organ.

Endothelial dysfunction is a complex event triggered by different agents, such as cytokines and oxidized low-density lipoproteins (oxLDL), that alter the normal state and induce a pro-inflammatory and pro-coagulant phenotype in EC. The occurrence of this condition is considered as a crucial event in the pathogenesis of cardiovascular disease. The expression of cell surface adhesion molecules, such as vascular cell adhesion molecule-1 (VCAM-1), intracellular adhesion molecule-1 (ICAM-1), and endothelial leukocyte adhesion molecule (E-selectin), has been proposed as biomarker of endothelial cell activation. On the other hand, the decreased synthesis of endothelium-derived nitric oxide (NO), which acts as a vasodilator and antithrombotic agent, represents the earliest and one of the most important event involved in endothelial dysfunction (Liao, 2013).

A reduction in NO availability may occur as a consequence of an accelerated degradation of NO under oxidative stress conditions, i.e. in the presence of superoxide anions NO is transformed into peroxynitrite, or due to a decreased endothelial nitric oxide synthase (eNOS) protein expression and/or activity (Förstermann and Münzel, 2006). Direct binding of eNOS to the scaffolding domain of caveolin-1 (Cav-1) is a more recent described mechanism for inactivating eNOS (Chen *et al* ., 2012). Cav-1 is the most abundant protein associated with caveolae which are important mediators of endocytosis, transcytosis, lipid homeostasis, and

signal transduction in EC (Shvets *et al* ., 2014). The endothelium is indeed permeable to small molecules with a diameter below 6 nm and nearly impermeable to macromolecules. Thus, the transport of lipoproteins, including oxLDL, across the cell membrane occurs via transcytosis (Zhang *et al* ., 2018). The transcytosis of low-density lipoproteins (LDL) into the intima can be associated with their modification (e.g., oxidation) that leads to EC dysfunction (Sun*et al* ., 2010). On the other hand, it has been suggested that circulating oxLDL and oxysterols may induce perturbations of membrane cholesterol, thus affecting integrity and dynamics of cholesterol-rich domains such as caveolae (Levitan and Shentu, 2011).

In this paper, the current knowledge on the possible interplay between uptake and transcytosis of LDL/oxLDL, disruption of cholesterol homeostasis, and alteration of caveolae architecture and signaling function is reviewed and discussed considering their implications in oxLDL-induced endothelial cell activation and dysfunction.

LDL oxidation, oxysterol formation and endothelial cell dysfunction

Cholesterol is the most abundant lipid in eukaryotic cells where it is an important component of membranes. It is synthesized within the cells in the endoplasmic reticulum, although this organelle contains only 0.5-5% of the total cell cholesterol (Iuliano, 2011; Lange *et al* ., 1999). LDL are the main carriers of cholesterol and, as such, are important contributors to atherosclerotic lesion progression. Indeed, under physiological conditions, LDL penetrate the intima via transcytosis across EC, but this process is further stimulated by EC dysfunction. Subendothelial accumulation and retention of LDL is an early step in atherogenesis. Skalen and coworkers (2002) demonstrated that the extracellular matrix, mainly consisting of proteoglycans, plays a key role in the retention of LDL. On the other hand, oxidative modification of LDL prevents their interaction with proteoglycans and favors their uptake by macrophages through scavenger receptors, leading to cholesterol accumulation and foam cell formation (Öörni *et al.,* 1997).

The oxidation of LDL is thought to mainly occur in the extracellular matrix underlying the arterial wall because in circulation they are protected by plasma antioxidants (Carmena *et al* ., 1996). Multiple mechanisms mediated by transition metals and enzymes are involved in LDL oxidation. However, at now, the physiological *in vivo* relevant mechanism is still not clear (Yoshida and Kisugi, 2010). Most of the cells present in the arterial intima can promote LDL oxidation *in vitro* by its enzymes, and ROS seem to play an active role. For example, high LDL levels and shear stress can enhance EC-mediated hydrogen peroxide (H_2O_2) production (Zouaoui Boudjeltia *et al* ., 2004). A relevant mechanism leading to oxidation of LDL occurs *via* myeloperoxidase (MPO) secreted by activated phagocytes. This enzyme generates oxLDL by producing hypochlorous acid (HOCl) from H_2O_2 and chloride. Zhang and coworker (2013) speculated that MPO could use the NADPH-derived H_2O_2 in order to produce HOCl, thus promoting the oxidation of LDL.

Oxidized LDL are particularly rich in oxysterols which are 27-atom carbon compounds formed after enzymatic or non-enzymatic cholesterol oxidation *in vivo* . However, oxysterols can also be derived from the diet. The methods of processing, preparation and storage expose the food to air, light or heat leading to the formation of oxysterols (Lordan *et al* ., 2009). Oxysterols circulate in the blood stream in both free and esterified forms, carried by lipoproteins. Interestingly, it has been demonstrated that the incorporation of oxysterols into LDL particles makes LDL more susceptible to oxidation (Vine *et al* ., 1998; Staprans *et al* ., 2003).

Oxysterols are involved in many physiological processes such as cholesterol metabolism, hormone and vitamin D synthesis, and transmembrane signaling as components of membrane microdomains enriched in cholesterol (lipid rafts and caveolae). On the other hand, accumulation of these compounds in tissues and organs has been associated with the progression of several diseases, such as atherosclerosis, neurodegenerative diseases, and cancer (Poli *et al* ., 2013; Voisin *et al* ., 2017). A growing body of evidence suggests that oxysterols and oxLDL play a key role in endothelial dysfunction impairing the formation/production of NO, increasing the formation of ROS and promoting the release of pro-inflammatory cytokines (Lubrano and Balzan, 2014; Maiolino *et al* ., 2013). Furthermore, it has been observed that oxysterols are able to remodel the endothelial layer by inducing endothelial dysfunction followed by cell death (Luchetti *et al* ., 2019; Luchetti *et al* ., 2015).

The role of caveolae and LDL receptors in LDL transcytosis

EC control LDL shuttling across the vessel wall by a process named caveolae-mediated transcytosis. Indeed, the diameter of LDL particles is about 20-30 nm which is much larger than that of gap-junctions (3-6 nm) between adjacent cells in continuous endothelium (Iuliano *et al* ., 2001). Thus, the only way for LDL to cross the endothelium is through transcytosis. In detail, transcytosis may occur *via* fluid phase or receptor-mediated ligand uptake (Fung *et al* ., 2018). The endocytosis of LDL by the LDL receptor (LDLR) has been reported to mediate LDL uptake in the blood-brain barrier, but since this process leads to LDL degradation into the lysosomes, it does not explain the accumulation of LDL in the subendothelium of systemic circulation (Dehouck *et al* ., 1997). Moreover, it has been observed that the LDLR mediated pathway is downregulated at high concentrations of LDL, while an LDLR-independent pathway is enhanced in conditions of hypercholesterolemia (Vasile *et al* ., 1983). Thus, transcytosis in EC of systemic circulation appears to be LDLR-independent and, importantly, requires the presence of caveolae (Figure 1).

Caveolae are specialized plasma membrane subdomains consisting of 50-100 nm invaginations of the apical plasma membrane that detach as vesicles to shuttle their cargo to the basolateral membrane where they fuse and release their content (Figure 1).

Caveolae are present in most cell types but are particularly abundant in EC, adipocytes, fibroblasts, and smooth muscle cells (Chidlow and Sessa, 2010). Like lipid rafts, caveolae are rich in cholesterol, glycosphingolipids and lipid-anchored proteins. Differently from lipid rafts, caveolae are coated with the protein caveolin, a cholesterol binding protein (Sharma *et al* ., 2010). Three caveolin isoforms (Cav-1, -2, 3), which are expressed at different densities in different cell types, have been identified so far. Cav-1 and Cav-2 are the most expressed in EC where caveolae cover up to 40% of the luminal surface of vascular endothelium. In addition, caveolae are involved in signal transduction being equipped with a complete set of effector proteins, from extracellular receptors to intracellular transducers, including heterotrimeric GTP-binding proteins, protein kinase C, endothelin 1 and acetylcholine receptors, ATP-dependent Ca^{2+} pump, and the small GTP-binding protein Ras. Cav-1 acts as a molecular hub able to regulate the signalling of these specific molecules.

Recent studies by Ramirez *et al* . (2019) demonstrated that Cav-1 deletion suppresses atherosclerosis by attenuating LDL transcytosis. In particular, the authors showed that LDL accumulation in atherosclerosis-prone areas was significantly reduced in Cav-1 deficient mice. The number of caveolae and Cav-1 protein levels in the EC luminal plasma membrane may be locally affected by hemodynamic and mechanical stress, thus favouring LDL infiltration in atherosclerosis-prone regions (Boyd *et al* ., 2003; Frank and Lisanti, 2006).

Both scavenger receptor class B type1 (SR-B1) and activin receptor-like kinase 1 (ALK1) receptors, which are localized within caveolae, seem to be involved in LDL loading and subsequent trafficking across the EC. The role of SR-B1 in LDL transcytosis has been investigated by Amstrong*et al.* (2015) who provided evidence that the infiltration of LDL in the subendothelial space is inhibited in SR-B1 deficient mice. More recently, Haung et al. (2019) demonstrated that SR-B1 directly binds LDL and recruits/activates Rac1 that, in turn, is required to sustain SR-B1-mediated LDL uptake. Interestingly, it was reported a higher SR-B1 expression level in atherosclerosis-prone regions of mouse aorta, before lesion formation, and in human atherosclerotic arteries compared with normal arteries. This observation supports the notion that atherosclerosis is favored by increased LDL transcytosis in altered areas of the endothelial barrier rather than by paracellular leak. A second receptor involved in LDL transcytosis has been identified by Kraehling and coworkers (2016) who provided evidence that ALK1 functions as a low affinity receptor for LDL in EC. ALK1 is an EC-restricted transforming growth factor β-type receptor that mediates LDL transcytosis independently of its kinase activity. ALK1 is localized in endothelial caveolae where functionally interacts with Cav-1, and co-localization of the proteins is drastically reduced under conditions of cholesterol depletion from the plasma membrane (Santibanez *et al* ., 2008).

Recently, Gerbod-Giannone *et al* . (2019) demonstrated that LDL endocytosis is affected in EC deficient in Cav-1 or in CD36, suggesting that CD36 may be involved in the transcytosis of native LDL across the endothelium as well. However, data reported by Huang *et al* . (2019) point to a role of CD36 receptor in

LDL uptake but not in transcytosis, while ALK1 and SR-B1 are the only receptors involved in transcytosis, with a predominant role for the latter.

While there are multiple evidences converging on the role of Cav-1, contrasting results are reported in the literature concerning the type of receptors involved in LDL transcytosis. Since LDL uptake and transcytosis are important contributors to atherosclerotic lesion development and receptors represent important pharmacological targets, these discrepancies underly the need to further explore the mechanism(s) involved.

The interplay between oxLDL and caveolae/caveolin and their impact on endothelial dysfunction

Uptake and transcytosis of circulating oxLDL, together with oxidation of LDL in the subendothelium, play an important role in the development of atherosclerosis. As for LDL, these processes seem to involve caveolae-mediated mechanisms. Transcytosis of oxLDL *via* caveolae was suggested by Sun *et al* . (2010) who showed that two caveolae specific inhibitors, filipin and nocodazole, decrease the uptake of oxLDL by human umbilical vein endothelial cells (HUVEC). The lectin-like oxidized LDL receptor 1 (LOX-1) is the major receptor for binding, internalization and degradation of oxLDL in EC, and LOX-1 expression is up-regulated by oxLDL (Sawamura *et al* ., 1997; Li and Mehta, 2000) (Figure 2). Interestingly, also Cav-1 expression is up-regulated by oxLDL, while Cav-1 silencing results in decreased LOX-1 expression upon oxLDL administration, suggesting that caveolin participates in LOX-1 regulation. It is known that the binding of oxLDL to LOX-1 stimulates the development of atherosclerosis through different mechanisms involving: i) activation of MAPK proteins, which causes increased expression of adhesion molecules and chemoattractants; ii) stimulation of NADPH oxidase activity, leading to ROS production, oxidative stress, and consequent reduction of NO levels; and iii) activation of NF-kB signaling pathway, resulting in cytokine and adhesion molecule production as well as increased expression of LOX-1 itself, thus creating a vicious cycle of proinflammatory signaling (Kattoor *et al* ., 2019) (Figure 2).

More recently, the possible role of LOX-1 in oxLDL transcytosis has been questioned by Huang *et al.* (2019) who demonstrated that knockdown of LOX-1 did not alter the uptake of oxLDL in human aortic endothelial cells (HAEC). By contrast, a decrease in oxLDL transfer was observed when the expression of SR-B1 was downregulated, thus suggesting that transcytosis of oxLDL in EC occurs via the SR-B1 receptor rather than LOX-1 (Huang *et al* ., 2019) (Figure 2).

Further investigations are necessary to clarify the molecular pathways responsible for oxLDL uptake and transcytosis and the role of caveolin in these processes.

Caveolae are enriched in free cholesterol and changes in the content of this lipid can affect the morphology and signaling mediated by caveolae. To this regard, Smart *et al* . (1994) described for the first time that cholesterol oxidation results in the translocation of caveolin from plasma membrane to Golgi with a modest reduction in the number of caveolae. Later, Blair *et al.* (1999) were able to show that oxLDL can indeed deplete caveolar cholesterol and induce the transfer of Cav-1 and eNOS to intracellular compartments, thus enabling NO production (Figure 2).

The mechanism through which oxLDL may deplete cholesterol is unknown. It has been hypothesized that oxLDL may act as a cholesterol acceptor to remove cholesterol from cellular membranes rather than loading cells with cholesterol. A higher efflux of cholesterol induced by oxLDL has also been proposed, in a mechanism that involves the binding of oxLDL to CD36 receptors. Finally, a redistribution of cholesterol between membrane-rich and cholesterol-poor domains has been proposed (Shentu*et al* ., 2010). Even though the effects of oxLDL on membrane cholesterol remain elusive and controversial, the effects of oxLDL on EC function impairment are very similar to those observed after experimental-induced cholesterol depletion, suggesting a common mechanism of action (Levitan and Shentu, 2011).

Zhu and coworkers (2005) demonstrated that oxLDL can inhibit the transcription of ATP-binding cassette transporter-1 (ABCA1) in HUVEC cells. This transporter mediates the active efflux of cholesterol and/or phospholipids. The regulation of ABAC1 by oxLDL occurs at the transcriptional level through the inhibition

of endogenous LXR ligand production. The role of caveolin in cholesterol homeostasis is less understood. Overexpression of Cav-1 up-regulated ABCA1 expression and enhanced cholesterol efflux to extracellular effectors (Lin *et al* ., 2007). Conversely, Cav-1 knock down was associated with reduced free cholesterol and increased esterified cholesterol, but it had minimal effects on cellular cholesterol efflux (Frank *et al* ., 2006). Whether oxLDL might affect cholesterol homeostasis by directly interfering with Cav-1 levels is not known. It is well established that Cav-1 is regulated by cellular cholesterol levels (Bist *et al* ., 1997). In line with these observations, caveolin mRNA levels were found up-regulated by free cholesterol, but down-regulated by oxysterols in fibroblast monolayers (Fielding *et al* ., 1997).

Finally, an exchange in free cholesterol between plasma LDL particles and the luminal surface of EC is supposed to occur (Stender, 1982). In this context, oxLDL have been shown to induce an increase in endothelial stiffness by direct incorporation of oxysterols into the endothelial plasma membrane (Figure 2) (Shentu *et al* ., 2012). It has been hypothesized that this event could result in the disruption of the structure of lipid-ordered domains, including caveolae. Moreover, there is evidence that oxysterols interact with Cav-1 (Sleer *et al* ., 2001). These direct oxysterol effects might produce relevant consequences on caveolae-mediated signaling.

Thus, although the molecular mechanisms through which oxLDL lead to endothelial dysfunction must be still clarified, accumulating evidence point to a relevant role of direct/indirect disruption of cholesterol homeostasis/distribution which may in turn affect caveolae function and modulate signaling pathways relevant for the development of atherosclerosis.

oxLDL impact caveolae/Cav -1 signaling

In EC, caveolae sense and transduce hemodynamic changes into biochemical signals to regulate vascular function. Caveolae compartmentalize signaling proteins in the plasma membrane through direct/indirect interactions with Cav-1. These interactions allow to fine-tune the magnitude of signaling cascades.

Within caveolae, Cav-1 functions as scaffolds for several proteins such as eNOS (Shaul, 2003; Williams and Lisanti, 2004) and NADPH oxidase (Patel and Insel, 2009; Chen *et al* ., 2014).

NO is a potent vasodilator and anti-inflammatory mediator produced by a family of enzymes called NOS. Three NOS isoforms have been identified: neuronal NOS (nNOS; NOS1), inducible NOS (iNOS, NOS2) and endothelial NOS (eNOS; NOS3) all of which differ slightly in function and structure. NO is synthesised in vascular EC from the abundant amino acid L-arginine by eNOS. This enzyme is constitutively expressed in EC where its product acts as vasoprotective molecule able to regulate the vascular tone and to attenuate both platelet aggregation and neutrophil-endothelium interaction (Brunner *et al* ., 2003). eNOS has a slow basal activity for NO generation that in EC is enhanced by agonists such as acetylcholine, bradykinin and histamine, which increase intracellular calcium, whereas shear stress and hormones increase eNOS activity independently of changes in intracellular calcium (Chen *et al* ., 2018).

The eNOS isoform is abundantly represented in EC of the vascular intima and it is mainly located in the plasma membrane caveolae where it can also be found also associated with the protein Cav-1. It is important to note that eNOS is also found in the Golgi apparatus, cytosol, cytoskeleton, and even in the nucleus. However, Fulton and coworkers (2002) demonstrated that eNOS is principally active on the plasma membrane.

The interaction between eNOS and Cav-1 has been observed both *in vivo* and *in vitro* . Through colocalization and coimmunoprecipitation experiments it has been shown that binding of eNOS to Cav-1 inhibits the enzyme activity, resulting in reduced NO production (Bucci *et al* ., 2000).

In this context, it has been demonstrated that oxLDL cause selective depletion of cholesterol within the caveolae (Shaul, 2003). This event results in eNOS intracellular redistribution and an attenuated capacity to activate the enzyme (Figure 2). In addition, oxLDL promote the expression of several pro-inflammatory mediators, including iNOS, presumably via the MAPKs/NF-kB pathway. This leads to an unbalance between eNOS and iNOS activity with the production of high amount of NO that works as free radical with

bactericidal and inflammatory function (Figure 2) (Gliozzi *et al* . 2019). The production of high NO concentrations by iNOS causes the generation of high levels of peroxynitrite which has been correlated with apoptosis of EC (Salvemini*et al* ., 2013). Gliozzi and coworkers suggested that this inflammatory condition promotes the nuclear translocation of NF-kB failing the protective mechanisms as autophagy (Gliozzi *et al* . 2019; Mollace *et al* ., 2015). These findings are in line with our previously reported data (Luchetti *et al* ., 2019) showing that high concentrations of secosterol B promotes cell apoptosis via a pathway that involves early phosphorylation of eIF2α and NF-kB activation, suggesting that the adaptive program fails, and the cell activates the apoptotic program. Recently, Potje *et al* . (2019) demonstrated that cholesterol depletion affects the number of caveolae promoting eNOS uncoupling that results in Nox-dependent O_2 production at the expense of NO generation.

One interesting finding is that Cav-1 phosphorylation at Tyr(14), following LPS exposure, favors Cav-1 and Toll-like receptor 4 (TLR4) interaction and, thereby, TLR4 activation of MyD88, leading to NF-ϰB activation and generation of proinflammatory cytokines (Jiao *et al* ., 2013). Notably, the effects observed in LPS-treated cells are mimicked by high-mobility group box 1 (HMGB1), a protein known to accumulate in atherosclerotic lesions and to mediate vascular inflammation. TLR4 activation by HMGB1 in HAEC cells has been demonstrated by Yang *et al.* (2016), as evidenced by expression of its downstream partner MyD88. Treatment with recombinant HMGB1 increased ERK phosphorylation and nuclear translocation of NF-ϰB. Thus, HMGB1-induced activation of TLRs initiates pro-inflammatory signaling pathways and mediates the release of cytokines and chemokines, thus contributing to vascular inflammation and endothelial dysfunction (Jiao et al., 2013) (Figure 2). While there is evidence that oxLDL can promote cytoplasmic relocation and extracellular release of HMGB1 by EC (Zhou*et al* ., 2016; Yu *et al* ., 2012), the role of caveolin in HMGB1-induced TLR4 activation is not clear. Notably, HMGB1 increases endothelial cell Cav-1 and TLR4 protein expression, suggesting that TLR4 and Cav-1 may act together. These proteins colocalize in HUVEC cells and knockdown of TLR4 abrogates Cav-1 induction (Jiang *et al* ., 2014). More recently, Lin et al. (2018), provided evidence that oxLDL promote phosphorylation of Cav-1 in HUVEC and increase oxLDL uptake. Intracellular accumulation of oxLDL induced NF-ϰB activation and HMGB1 translocation from nucleus to cytoplasm resulting in cell apoptosis. NF-ϰB activation also facilitated Cav-1 phosphorylation and HMGB1 expression. Considering that HMGB1 enhances oxLDL uptake through induction of LOX-1 (Lee *et al* ., 2012), it is plausible that a tight crosstalk between HMGB1, TLR4, NF-kB, LOX-1 and caveolin may occur in response to oxLDL.

Only few studies concerning the impact of oxLDL/caveolae interaction on caveolae/Cav-1-mediated signalling are reported in the literature. Therefore, more research focusing on caveolae/Cav-1 and oxLDL signal transduction is urgent in order to better understand the mechanism of atheroma formation.

Conclusions

It is widely accepted that oxLDL play a pivotal role in endothelial dysfunction and atheroma formation; the mechanisms involved are less clear. Recent research suggests that oxLDL may affect caveolae architecture and function in a complex interplay which leads to endothelial cell dysfunction. Caveolae are widely expressed on EC where they act as gatekeeper for LDL infiltration into the intima. Subendothelial accumulation and subsequent oxidation of LDL represent key events in atherogenesis. LDL transcytosis in EC occurs via caveolae, and SR-B1 and ALK1 are the main receptors involved. On the other hand, oxLDL are also present in the circulation from where they can be taken up at the EC interface by LOX-1 receptor, which induces the activation of many signaling pathways, leading to the establishment of a pro-inflammatory and pro-coagulant state in EC. Although SR-B1 has been recently demonstrated to be involved in oxLDL transcytosis, excluding a role for LOX-1, a crosstalk between caveolae and LOX-1 seems to exist. First, LOX-1 activity depends on an intact caveolae system. Second, its activity regulates the expression of the caveolar protein Cav-1 and*vice versa* . On the other hand, caveolae are important for signal transduction as they can concentrate and/or segregate not only receptors but also signalling intermediates. Their function is strictly correlated to their composition rich in cholesterol which allows a certain degree of plasticity. OxLDL and oxysterols have been reported to affect the content and/or the distribution of cholesterol into caveolae. Whether this event results

in an aberrations of signalling cascades which are relevant for the development of atherosclerosis is expected but remains to be established.

Thus, even though multiple evidences point to an interplay between caveolae and oxLDL, as one of the mechanisms underlying oxLDL-induced EC dysfunction, some aspects remain contradictory and the information fragmentary. Future research is expected to further shed light on the connections between these players, focusing on the crosstalk between LDL receptor- and caveolae-mediated signalling and how this may be affected by changes in membrane cholesterol.

Conflict of interest

The authors have no conflicts of interest to declare.

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Figure legends

Figure 1. Physiological transcytosis of circulating LDL*via* caveolae.

Figure 2. Overview of the main interactions occurring between oxLDL and caveolae and their impact on intracellular signaling in EC. LOX-1-mediated oxLDL uptake; oxLDL-induced HMGB1 release and TLR4 activation; ox-LDL transcytosis; oxLDL-mediated membrane cholesterol exchange, depletion, redistribution. Direct evidence , undirect evidence , hypothetical connection .