Review

The Lectin-Like Oxidized Low-Density Lipoprotein Receptor-1 and its Soluble Form: Cardiovascular Implications

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The lectin-like oxidized low density lipoprotein receptor-1 (LOX-1) is a multiligand receptor, whose repertoire of ligands includes oxidized low-density lipoprotein, advanced glycation endproducts, platelets, neutrophils, apoptotic/aged cells and bacteria. Sustained expression of LOX-1 by critical target cells, including endothelial cells, smooth muscle cells and macrophages in proximity to these ligands, sets the stage for chronic cellular activation and tissue damage suggesting the interaction of cellular LOX-1 with its ligands to contribute to the formation and development of atherosclerotic plaques. Studies with transgenic and knockout mouse models have elucidated in part the role of LOX-1 in the pathogenesis of atherosclerosis and cardiac remodeling.

Recently, a circulating soluble form of LOX-1 (sLOX-1), corresponding solely to its extracellular domain, has been identified in human serum. Circulating levels of sLOX-1 are increased in inflammatory and atherosclerotic conditions and are associated with acute coronary syndrome, with the severity of coronary artery disease, and with serum biomarkers for oxidative stress and inflammation, suggesting that they could be a useful marker for vascular injury. However, many interesting questions have not yet been answered and in this review, we provide an updated overview of the literature on this receptor and on likely future directions.

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Introduction

Atherosclerosis is a multifactorial disease of the vessel wall involving lipid accumulation, thrombogenic components, cell death and inflammatory responses in the arterial wall, causing heart disease and stroke[1)]. Modification of low-density lipoprotein (LDL) in the arterial wall, particularly by oxidation, is crucial to the cellular uptake of LDL in the early stages of atherosclerotic plaque development[2)].

Oxidized LDL (oxLDL) is involved in the very early critical steps of atherogenesis, such as endothelial injury, expression of adhesion molecules, and leukocyte recruitment and retention, as well as the formation of foam cells and thrombi (for a review see[2)]). Macrophages internalize and degrade oxLDL mainly through scavenger receptors, and one crucial step in the initiation and progression of atherosclerosis is the unregulated uptake of oxLDL through these receptors, leading to foam cell formation[2)]. Another receptor, named lectin-like oxLDL receptor-1 (LOX-1), is expressed in all cell types involved in the atherosclerotic lesion, i.e. endothelial cells (ECs), macrophages, and smooth muscle cells (SMCs)[3)]. LOX-1, a membrane glycoprotein that binds oxLDL and acetylated LDL (AcLDL) but not native LDL[4, 5)], differs from the macrophage scavenger receptors because it contributes to the atherosclerotic process mainly through receptor-mediated signaling mechanisms[6)].

However, LOX-1 is a multiligand receptor that can also recognize multiple classes of ligands, such as activated platelets, neutrophils, apoptotic/aged cells

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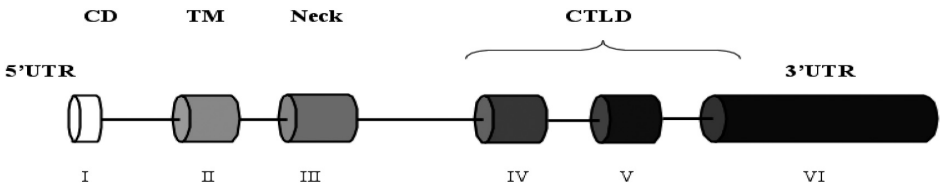

Fig. 1. Schematic model of the human OLR1 gene.

The OLR1 gene spans more than 7000 base pairs (bp) and consists of 6 exons (as indicated) interrupted by 5 introns. The upper names indicates the domain structure of the LOX-1 protein encoded by OLR1 mRNA: 5'UTR: 5'-untranslated region; CD: cytoplasmic domain; TM: transmembrane domain; Neck: neck domain; 3'UTR: 3'-untranslated region; CTLD: C type lectin-like domain.

and bacteria[7-10], implying versatile physiological functions. This review specifically focuses on the structure, regulation and ligands of LOX-1 and highlights the mechanisms by which LOX-1 activation contributes to atherosclerosis.

The activation of LOX-1 triggers intracellular signaling, production of superoxide radicals and gene expression induced by the redox-sensitive transcription factor NF-κB which plays a key role in these processes[11]. In 2000, the same research group that revealed the presence of LOX-1 in vascular tissue[12] discovered the soluble form of this membrane receptor, named soluble LOX-1 (sLOX-1), showing that it derived from membrane-bound LOX-1 itself[13].

The crucial role of LOX-1 in the atherosclerotic process[14] highlighted this receptor as a logical and attractive candidate for therapeutic intervention to limit vascular damage and its long-term consequences, although several key questions have not yet been answered. Here we provide a critical review of the recent literature on this receptor and likely future directions.

LOX-1 Gene: Structure, Polymorphism and Alternative Transcripts

Gene Structure

LOX-1 is encoded by the oxLDL receptor 1 (OLR1) gene, located in the natural killer gene complex (NKC) on chromosome 12 at p12-p13, which also contains several other families of lectin-like genes, including the CD94 and NKG2 NK receptor genes[15]. The region telomeric of CD94 contains in addition to the LOX-1 gene, the novel human DECTIN-1 and the CLEC-1 and CLEC-2 genes within about 100 kb. Sequence similarities and chromosomal arrangement suggest that these genes form a separate subfamily of lectin-like genes within the NKC encoding receptors with important immune and/or scavenger functions in monocytes, dendritic cells and EC[16, 17].

The OLR1 gene spans more than 7000 base pairs (bp), and consists of 6 exons interrupted by 5 introns; exons 1−5 range from 102 to 246 bp, whereas exon 6 is relatively long, being 1722 bp (**Fig. 1**). Exon 1 encodes the 5'-untranslated region (UTR) and cytoplasmic domain, exon 2 encodes the remainder of the cytoplasmic domain and the transmembrane domain, exon 3 encodes the neck domain, and exons 4, 5 and 6 encode the lectin-like domain and the 3'-UTR of LOX-1 protein[15, 18] (**Fig. 1**) (as better described in the section "protein structure of LOX-1"). The 5' promoter/enhancer region (about 2500 bp) of the human OLR1 gene was also studied, and TATA and CAAT boxes were found in the proximal part of the 5'-flanking region. Computer-based analysis identified a wide variety of potential transcription factor-binding sites, including sites for the STAT family and NF-interleukin (IL)-6[15, 19].

The transcriptional repressor octamer-1-binding site within the promoter region plays an important role in the human LOX-1 promoter trans-activation in response to oxLDL. Furthermore, the promoter region between nucleotides-2131 and -2247, which includes an active NF-κB-binding site, is required for angiotensin Ⅱ (Ang Ⅱ)-induced transactivation of the human LOX-1 promoter[20].

Gene Polymorphisms and Splicing Variants

Some studies have shown common genetic variation in the OLR1 gene to be associated with the risk of coronary artery disease (CAD). Three common LOX-1 single nucleotide polymorphisms (SNPs) have been identified in intron 4 (G→A), intron 5 (T→G) and 3'UTR (T→C)[21]. Contemporaneously, the involvement of LOX-1 in atherosclerosis and acute myocardial infarction (AMI) was confirmed by defining OLR1 genetic variation in an association study of intragenic SNPs[22]. Seven SNPs were identified of

which six were within intron 4, intron 5, and the 3' UTR. These six polymorphisms were in complete linkage disequilibrium behaving as a single SNP block, including exon 5.

It was observed that the LOX-1/3'UTR SNP genotype and allele frequencies differed significantly between the control and the AMI groups in which the subjects with the T/T or C/T genotype were at higher risk of developing AMI[22]. Despite the positive results on OLR1 polymorphisms in CAD or AMI subjects[22-24], other similar studies were unable to confirm these putative genetic risks[24-26]. In particular, regarding a functional SNP, the G→C transition at position 501 in exon 4 which produces a single amino acid change (K167N) in the ligand-binding domain and affects markedly LOX-1 receptor activity[27], different conclusions have been reported[22-26].

Furthermore, it has been explored whether the SNPs could give rise to a functional product by examining the existence of messenger RNA (mRNA) isoforms as a consequence of alternative splicing[28]. A reproducible pattern of alternative splicing in the OLR1 gene around exon 5, with 2 resulting OLR1 transcripts in the RNA fractions, was identified: one of these products corresponded to the full-length transcript, while the other (named LOXIN) lacked exon 5. The LOXIN spliced mRNA has a stop codon in the open reading frame that leads to a premature termination of the translation product and generates a protein that lacks 2/3 of the lectin-like domain and is unable to bind oxLDL[28].

Experiments both *in vivo* and *in vitro* indicate that the splice variant LOXIN is expressed at a similar level to the full-length receptor LOX-1, and their relative transcription is modulated by the presence of SNPs[28]. Macrophages from subjects carrying the "non-risk" haplotype expressed more LOXIN and so fewer cells underwent apoptosis on oxLDL induction[28]. Therefore, higher levels of LOXIN protect cells from LOX-1-induced apoptosis. Also LOXIN tends to dimerize, therefore its protective role may be due to inactivation of the LOX-1 receptor via the formation of a heterodimer[28]. Molecular LOX-1/LOXIN interaction and the formation of non-functional hetero-oligomers have been recently confirmed[29], suggesting hetero-oligomerization between naturally occurring isoforms of LOX-1 to represent a general model for the regulation of LOX-1 function by its variants.

It is difficult to draw definite conclusions from these conflicting studies; multiple large, well-matched cohorts of cases and controls will be required to achieve valid progress in the genetic analysis of atherosclerosis and other complex human diseases indicating mostly the need for caution in the interpretation of genetic associations.

Protein Structure of LOX-1

LOX-1 has a molecular weight of 50 kDa and is a type II membrane protein belonging to the C-type lectin family[12]. LOX-1 is synthesized as a 40-kDa precursor protein, subsequently glycosylated at four potential N-linked glycosylation sites in the extracellular C-terminal domain, and processed into a 48-kDa mature form within 40 min[30].

LOX-1 consists of four domains: a short N-terminal cytoplasmic domain, a single transmembrane domain, a connecting stalk region (neck) domain, and a lectin-like extracellular domain at the C-terminus which binds oxLDL[12] (**Fig. 2**). The extracellular part of LOX-1 comprises an 82-residue neck and a ligand-binding domain. A coiled-coil structure is located in the C-terminal part of the neck, and is in dynamic equilibrium among multiple conformational states. This chimeric structural property of the neck region may enable LOX-1 to cluster at the cell surface, where it exists as a homodimer to recognize oxLDL[31, 32]. One-third of the N-terminal neck is less structured than the remainder of the protein and is highly sensitive to cleavage by a variety of proteases[32]. Both positively charged and non-charged hydrophilic residues are involved in ligand binding, suggesting that ligand recognition of LOX-1 depends on more complex conformational interactions, rather than merely electrostatic interaction between positively charged residues and negatively charged ligands[33].

LOX-1 also exists as a soluble form (sLOX-1), corresponding to its extracellular domain only[13]. Two soluble forms of similar molecular weight (approximately 35 kDa) were identified in the supernatant of bovine aortic EC[13]. In tumor necrosis factor-α (TNF-α)-activated cells, cell-surface expression of LOX-1 precedes sLOX-1 release[13] which is inhibited by serine protease inhibitors, particularly by PMSF[13]. Nevertheless, it has been recently shown that overexpression of ADAM10, a member of the disintegrin and metalloproteinase family, enhanced the cleavage of sLOX-1 while its inhibition suppressed it, suggesting the effect of PMSF on sLOX-1 production to be indirect[34]. Purification and sequencing of bovine sLOX-1 identified the two cleavage sites between Arg(86)-Ser(87) and Lys(89)-Ser(90), which were located in the membrane proximal extracellular domain of LOX-1[13].

It has been further shown that IL-18 stimulated the shedding of LOX-1 and subsequent release of

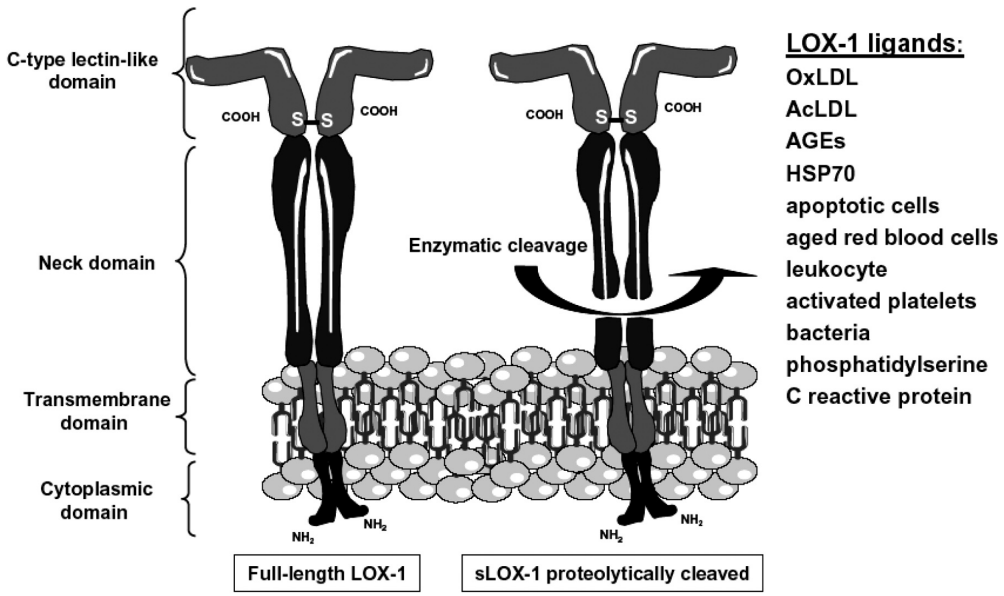

Fig. 2. Structure of the full-length LOX-1 and its soluble form.

The extracellular region of the primary structure of LOX-1 consists of a C-type lectin-like domain at the C-terminus, followed by a connecting stalk region (neck domain). The full-length LOX-1, in addition to the extracellular domain, displays a single transmembrane spanning region and a short N-terminal cytosolic tail. As illustrated, LOX-1 exists as a disulfide-linked homodimer at cysteine 140 on the cell surface. The circulating form derives from enzymatic cleavage at two potential sites of the full-length cell-surface receptor, between Arg(86)-Ser(87) or Lys(89)-Ser(90) residues located in the membrane proximal extracellular domain, and consists only of a part of the extracellular domain of the receptor (a portion of the neck and the entire C-terminal domain). The ligands of LOX-1 are shown on the right side of the figure.

sLOX-1[34]. A circulating soluble form of LOX-1, corresponding solely to its extracellular domain, has been identified in human serum, as we will discuss in detail afterwards.

Regulation of LOX-1 Expression

The LOX-1 promoter is constitutively active only at a low level though its expression can be induced by oxLDL[35, 36], fluid shear stress[37], Ang Ⅱ[20], proinflammatory cytokines[4, 19, 38], lipopolysaccharide[38], phorbol 12-myristate 13-acetate (PMA)[4], oxidants[39], fluid shear stress, heparin-binding epidermal growth factor (EGF)-like growth factor (HB-EGF)[40] and others (**Table 1**).

In ECs, glycoxidized LDL and oxLDL significantly increase LOX-1 expression and the production of sLOX-1, although the effect of glycoxidized LDL is greater than that of oxLDL[41]. In addition in the same cells, advanced glycation endproducts (AGEs), C reactive protein (CRP), high glucose and IL-6 induce expression of LOX-1[42-44].

Furthermore, lysophosphatidylcholine, the main phospholipid component of oxLDL, as well as super-

Table 1. Stimuli that regulate the expression of LOX-1

Lipopolysaccharide[38]
Tumor necrosis factor-α[4, 19, 38]
Phorbol 12-myristate 13-acetate[4]
Fluid shear stress[37, 38]
OxLDL[35, 36]
Lysophosphatidylcholine[36, 45]
Angiotensin Ⅱ[20]
Transforming growth factor-β[47]
High glucose concentration[42-44, 52]
Norepinephrine[50]
Endothelin-1[50]
Oxidant species[39]
Homocysteine[39]
C reactive protein[43, 44]
HB-EGF[40]
IL-6[43, 44], IL-1α[19], IL-1β[19]
Remnant-like lipoprotein particles[49]
Advanced glycation endproducts[42]
Glycoxidized LDL[41]

oxide anions, hydrogen peroxide and homocysteine up-regulate the expression of LOX-1 mRNA[36, 39, 45]. Also endothelin-1 (ET-1) stimulates endothelial LOX-1 expression in a receptor-mediated manner[46]. Transforming growth factor-β, which plays a crucial role in vascular remodeling and the pathogenesis of atherosclerosis, induces the expression of LOX-1 in both EC and SMCs[47]. In this latter cell type, the mediators of the inflammatory acute phase response (IL-1α, IL-1β and TNF-α) regulate the expression of LOX-1[19]. In addition, in SMCs, LOX-1 acts as a receptor for remnant-like lipoprotein particles (RLPs) that up-regulate its expression and induce cell migration, suggesting a proatherogenic role, especially in the settings of postprandial hyperlipidemia, diabetes and metabolic syndrome[48, 49].

LOX-1 expression in cardiac myocytes, as well as in vessel walls of failing rat hearts *in vivo*, is induced by neurohormonal factors activated in heart failure such as norepinephrine and ET-1[50]. In this regard, since increased protease activity is a key feature of plaque instability that may achieve an enhancement of sLOX-1 release, cardiac myocytes, may be another source of sLOX-1[50].

In macrophages, the expression of LOX-1 can be up-regulated by TNF-α and high glucose concentrations[51, 52].

In summary, a number of proinflammatory and proatherothrombotic factors are therefore able to regulate the expression of this receptor, suggesting LOX-1 to have an important role in amplifying local inflammatory responses during atherosclerotic development.

LOX-1 as a Multi-Ligand Receptor with High Specificity for OxLDL: is it a Multifunctional Environmental Sensor or an Endocytic Receptor?

LOX-1 has interesting specificity because it binds oxLDL and with lower affinity, AcLDL, while it does not bind native LDL[5, 53, 54]. In spite of this specificity, LOX-1 recognizes multiple ligands including hypochlorite-modified high density lipoprotein[55], aged red blood cells, apoptotic cells[7], leukocytes[56], activated platelets, bacteria[8, 9], phosphatidylserine[57] and AGEs[58] suggesting a versatility of function for LOX-1 under pathophysiological conditions *in vivo* (**Fig. 2**). AGEs are well-known ligands for receptor for AGEs (RAGE) but also for other scavenger receptors, including LOX-1, whose binding was effectively suppressed by an anti-LOX-1 antibody[58]. Moreover, LOX-1 has been identified as an additional receptor for endocytic uptake of heat shock protein 70 (HSP70), chaperoned peptides and CRP[59-61]. As LOX-1 recognizes and binds activated platelets, exposure of LOX-1 on the activated platelet surface assists thrombosis as demonstrated by the accumulation of LOX-1 protein at sites of thrombi in atherosclerotic plaques[62]. Its broad range of ligands in part overlaps with that of other scavenger receptors and toll-like receptors, and implicates LOX-1 in innate immunity, although its precise role in this immunological context has yet to be determined.

The negative charge on ligands is crucial to recognition by LOX-1. In fact, substitution of basic with neutral amino acids greatly decreases the binding capacity of LOX-1. The crystal structure of LOX-1 revealed the ligand-binding interface to be hydrophobic except for the basic spine, which is composed of arginine residues[5]. Experiments with mutated LOX-1 revealed that the substitution of a single amino acid in the basic spine, resulted in the greatest loss of affinity for AcLDL[5].

The ligand-binding domain of LOX-1 has a homodimeric structure with an intermolecular disulfide bond[5]. A single amino acid substitution in the interface caused a severe reduction in binding activity, suggesting the correct arrangement of the dimer to be crucial for binding to oxLDL. Site-directed mutagenesis indicated that cysteine 140 has a key role in the formation of disulfide-linked human LOX-1 dimers[31]. LOX-1 assembles on the cell surface as at least a hexamer comprising three homodimeric LOX-1 molecules in binding to oxLDL[31]. Comparing the size of the dimer surface of LOX-1 (70 Å) with the diameter of LDL (250 Å), it seems plausible that LOX-1 binds to oxLDL as an assembly protein[5]. Furthermore, amphipathic α-helices on LDL can act as multiple binding sites for LOX-1 assembly on the cell surface[5]. It is reasonable to think that circulating plasma sLOX-1 is still able to bind oxLDL; however, since the amount of sLOX-1 in circulation is very low, it does not affect oxLDL plasma levels and so could not act as a decoy for oxLDL.

Macrophages use scavenger receptors to internalize and degrade modified LDL, producing foam cells. LOX-1 was detected on human and mouse macrophages, but not on monocytes[51, 63] and the expression of LOX-1 can be induced in human monocytes after macrophage-like differentiation *in vitro*[63]. In macrophages, LOX-1 expression appears to be regulated differently from CD36 and SR-A expression[64]. Based upon studies with gene-knockout (KO) mice, CD36 and SR-A play crucial roles in macrophage apoptosis and determining the complexity of atherosclerotic lesions[65]. LOX-1 may play significant roles when macrophages

are stimulated by atherogenic lipids or proinflammatory stimuli in atherosclerotic plaques[66].

Recently, using LOX-1 KO mice, it has been shown that there was no difference in uptake or degradation of oxLDL in macrophages from wild-type and LOX-1 KO mice and no difference in the rate of clearance of oxLDL from plasma *in vivo*[66]. However, when expression of LOX-1 was induced with lysophosphatidylcholine, oxLDL uptake and degradation increased 2-fold in wild-type macrophages but did not change in LOX-1 KO macrophages, suggesting that in proinflammatory conditions in which macrophage LOX-1 expression can increase, the oxLDL uptake could substantially increase[66]. Recently it has been shown that LOX-1 is constitutively internalized from the plasma membrane and thus likely to mediate oxLDL trafficking in vascular tissues[67].

Given the many disparities between studies *in vitro* and *in vivo*, it remains likely that this receptor is a multifunctional sensor but also an endocytic receptor. However, how it can facilitate intracellular signaling during trafficking of the receptor-ligand complex along the endocytic pathway is not known. Alternatively, it may be that there is diversity of function depending on cell and tissue type, and availability of ligands.

Signal Transduction Pathways Triggered by Ligand-LOX-1 Interaction

LOX-1 is expressed in most cell types relevant to the development of atherosclerotic plaques (i.e., ECs, SMCs, macrophages), and the interaction of LOX-1 with its ligands modifies the cell phenotype in a proatherogenic sense, so that the cells become dysfunctional and more prone to death[68] (**Table 2**).

The identification of LOX-1 as the major receptor for oxLDL in ECs has provided a new clue about the mechanisms by which oxLDL are involved in vessel wall injury.

Most information about LOX-1 signal transduction pathways on EC comes from the activation or inhibition of LOX-1 by oxLDL and LOX-1 antibody respectively. OxLDL has dual effects on cultured cells depending on its concentration and the exposure time: at a lower concentration (from 5 to 10 μg/mL) and shorter exposure time it induces proliferation, whereas at a higher concentration (from 50 to 300 μg/mL) and longer exposure time it induces apoptosis in ECs, macrophages, and SMCs[69], and even necrosis[70, 71] (**Fig. 3**). Low concentrations of oxLDL cause the activation of protein kinaseC-β (PKC-β)[72], generate low levels of reactive oxygen species (ROS) and promote angiogenesis through an increase in vascular endothelial growth factor (VEGF) via LOX-1-mediated redox-sensitive signaling pathways[73, 74]. OxLDL induces ROS production via NADPH oxidase[75, 76] and downstream alterations include activation of p38 mitogen-activated protein kinase (p38MAPK), a component of oxidant stress-sensitive signaling pathways, phosphatidylinositol-3-kinase, and extracellular-signal-regulated kinase (ERK1/2), which leads to activation of the transcription factor NF-κB[35, 77, 78] (**Fig. 3**). OxLDL also represses DNA binding of the transcriptional regulator nuclear factor 1, diminishes mRNA levels of monooxygenase cytochrome P450 and decreases production of endothelial-derived hyperpolarization factor, a key regulator of vascular tone[75, 76] (**Fig. 3**). Furthermore, oxLDL induces the endothelial expression of plasminogen activator inhibitor-1, implicated in atherothrombosis, through ERK1/2 activation[79]. Furthermore, the binding of oxLDL to LOX-1 decreases intracellular NO levels through increased production of O^{2-}, which could inactivate NO itself[73, 80].

OxLDL treatment enhances the expression of vascular cell adhesion molecule 1 (VCAM-1), intercellular cell adhesion molecule 1 (ICAM-1), monocyte chemoattractant protein 1 (MCP-1) and selectins, leading to monocyte attachment and activation[81]. The notion that oxLDL mediates these effects via LOX-1 is borne out by the finding that antisense

Table 2. Cellular effects of ligand-LOX-1 interaction on atherogenesis

Endothelial cells
Alteration of vascular tone[75]
Increased intracellular oxidative stress[73]
Induction of apoptosis[35]
Induction of proliferation and angiogenesis by increasing VEGF[§] expression[70, 71, 74]
Increased expression of adhesion molecules (VCAM-1[†], ICAM-1[††], Selectins)[81]
Increased expression of monocyte chemoattractant protein-1[81]
Induction of plasminogen activator inhibitor-1[79]
Reduction of endothelial nitric oxide synthase[72, 83]
Release of matrix metalloproteinases[72, 83]
Smooth muscle cells
Induction of apoptosis[86]
Monocytes
Induction of monocyte adhesion and activation[44]
Increased oxLDL uptake and foam cell formation[52]

[§]Vascular endothelial growth factor; [†]Vascular cell adhesion molecule-1; [††]Intercellular cell adhesion molecule-1.

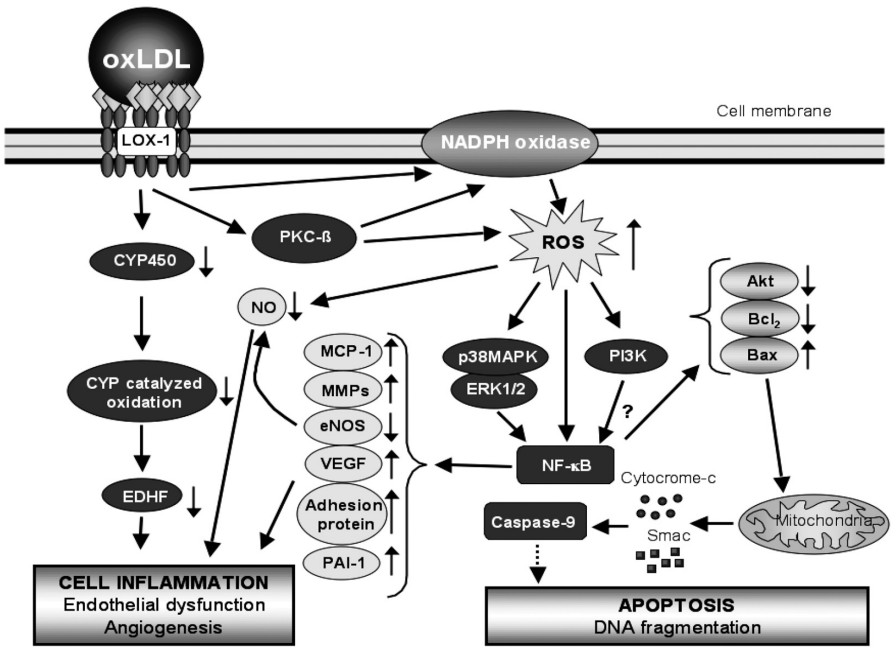

Fig. 3. Signal transduction pathways triggered by LOX-1 activation.

oxLDL-LOX-1 interaction may lead to endothelial dysfunction and/or apoptosis. Low concentrations of oxLDL induce the generation of ROS by activation of NADPH oxidase and PKC-β. ROS activate the ERK1/2-MAPK and PI3K pathways, leading to NF-κB's nuclear translocation. Consequently, a reduction in eNOS expression and NO's release, an increase in adhesion molecule (VCAM-1, ICAM-1 and selectins) expression, and enhanced release of MCP-1 and MMPs, lead to endothelial dysfunction. The activation of LOX-1 could also decrease cytochrome P450 (CYP450) protein levels resulting in diminished levels of endothelial-derived hyperpolarization factor (EDHF). High concentrations of oxLDL cause diminished Akt gene transcription and an enhanced bax/bcl-2 ratio leading to apoptosis via the release of cytochrome c and Smac from mitochondria to the cytoplasmic compartment, and through activation of caspase-9.

LOX-1 mRNA decreased LOX-1 expression and subsequent adhesion molecule expression[81]. However, recently it has been shown that LOX-1 is involved in redox-sensitive, Akt/eNOS and Ca^{2+} signaling pathways caused by the attachment of monocyte to EC and the blockade of LOX-1 by antibody or small interfering RNA inhibited the monocyte adhesion-triggered signaling pathway in ECs independent of the oxLDL-LOX-1 axis[82].

The binding of oxLDL to LOX-1 induces a reduction in endothelial nitric oxide synthase (eNOS) expression, increases levels of matrix metalloproteinase (MMP) 1, MMP 3 and MMP 9 and collagenase activity, but does not increase the expression of tissue inhibitors of MMPs[72, 83]. Recently, a study provided evidence that in ECs, the membrane type 1-MMP (MT1-MMP), which is involved in atherogenesis, colocalizes and forms a complex with LOX-1[84]. Since the blockade of LOX-1 or MT1-MMP prevented the oxLDL-induced RhoA-dependent downregulation of eNOS protein expression, Rac1-mediated NADPH oxidase activity, and generation of ROS, the LOX-1-MT1-MMP axis may play a crucial role in the small GTPase RhoA and Rac1 activation signaling pathways[84]. Recently, from a cDNA microarray analysis performed in a EC line over-expressing LOX-1 and treated with oxLDL, LOX-1-dependent changes in gene expression associated with inflammation, and the induction of mRNA expression for a number of cytokines, including IL-8, cell adhesion, and signal transduction, were confirmed[85]. In addition, a promoter analysis of the genes that changed following oxLDL-mediated LOX-1 activation identified EGR1 and CREB as potential novel transcription factors that function downstream of LOX-1[85].

In SMCs, oxLDL at high concentrations induces apoptosis by increasing the Bax/Bcl-2 ratio[86], and in EC, oxLDL decreases the expression of the antiapoptotic protein Bcl-2, induces the release of cytochrome C and Smac from mitochondria into the cytoplas-

mic compartment, and activates caspase-9[87] (**Fig. 3**). Decreased eNOS and Bcl-2 expression, together with induction of MMP collagenase activity, resulted in EC injury, dysfunction, and apoptosis (**Fig. 3**). The pro-apoptotic effects of oxLDL are mediated by its receptor LOX-1, because pretreatment of ECs with anti-sense-LOX-1, but not sense-LOX-1, blocked these effects[87].

Other evidence has shown that CRP also binds to endothelial LOX-1 resulting in the adhesion of monocyte to ECs and increases oxLDL uptake, so contributing to endothelial dysfunction[44, 88].

In cardiac myocytes, activation of LOX-1 by oxLDL induced apoptosis via activation of p38MAPK, which could be blocked by an antioxidant catalase[50].

In chondrocytes, the binding of oxLDL with LOX-1 markedly increased VEGF mRNA expression and protein release through activation of peroxisome proliferator-activated receptor-γ (PPAR-γ), suggesting that LOX-1 signal transduction pathways also lead to PPAR-γ activation[89].

Lessons from Animal Studies

Several animal models have been developed to dissect the contribution of LOX-1 to the pathogenesis of vasculopathy in pro-atherogenic settings including hypertension, hyperlipidemia, diabetes and mainly atherosclerosis[90]. Its expression is dramatically up-regulated in the aorta in hypertensive rats, suggesting a potential role for LOX-1 in the pathogenesis of hypertension[38]. In addition, LOX-1 overexpression in hypertensive animal models is localized to vascular ECs, which is compatible with the hypothesis that LOX-1 is related to endothelial dysfunction in hypertension[39, 91].

In rats with streptozotocin-induced diabetes, LOX-1 expression was markedly up-regulated in ECs, especially in bifurcations of artery branches from the aorta[43]. In rats with ischemia-reperfusion (I/R) injury, LOX-1 expression was up-regulated, which through p38MAPK activation increased the expression of MMP-1 and adhesion molecules[92]. Furthermore, increased LOX-1 expression in I/R was associated with lipid peroxidation and apoptosis, a large infarcted area, and a loss of left ventricular function[93].

The generation of transgenic and KO mice has elucidated the pathogenic role of LOX-1 in atherosclerosis. LOX-1 KO mice fed a high-cholesterol diet show decreased oxLDL-binding to the aortic endothelium and a preservation of endothelium-dependent vasorelaxation after treatment with oxLDL[94]. The cross-breeding of LOX-1 KO mice with LDL receptor-deficient (LDLr KO) mice led to a reduction in atherogenesis compared with LDLr KO mice. Proinflammatory signals, the expression of NF-κB and inflammatory markers were increased in LDLr KO mice, but not in double KO mice. Furthermore, LOX-1 KO mice had greater levels of eNOS while in the LDLr KO mice, there was a marked reduction in eNOS expression and its restoration with LOX-1 deletion[94]. Thus, the deletion of LOX-1 sustains endothelial function leading to a reduction in atherogenesis in association with a reduction in proinflammatory and pro-oxidant signals. Conversely, LOX-1 transgenic mice overexpressing LOX-1 but lacking apoE displayed accelerated intramyocardial vasculopathy, and a 10-fold increase in atheroma-like lesion area compared with non-transgenic littermates after 3-weeks on a high-fat diet[95].

Wild-type mice subjected to I/R developed a marked decrease in left ventricular systolic pressure and an increase in left ventricular end-diastolic pressure following I/R, and the degree of change was much less in the LOX-1 KO mice, indicating preservation of left ventricular function with LOX-1 deletion[96]. There was evidence for marked oxidative stress (NADPH oxidase expression, malondialdehyde and 8-isoprostane) and increase of collagen deposition, fibronectin and osteopontin expression following I/R in wild-type mice, but much less so in LOX-1 KO mice[96].

Furthermore, I/R in LOX-1 KO mice resulted in a significant decrease in myocardial injury as well as in the accumulation of inflammatory cells in I/R myocardium and lipid peroxidation[97]. The phosphorylation of p38MAPK and PKB/Akt-1, as well as inducible NOS, was enhanced during I/R in wild-type mice, but much less so in LOX-1 KO mice. These findings provide convincing evidence that LOX-1 is a key modulator of cardiac remodeling which starts immediately following I/R, and its effect is mediated by pro-oxidant signals[97].

Many studies have investigated the relationship between LOX-1 expression and plaque instability.

LOX-1 is extensively expressed in the new blood vessels in the core of human carotid advanced atherosclerotic lesions and colocalized with apoptotic cells, which are present mostly in the rupture-prone regions of atherosclerotic plaques[6, 98].

In hypercholesterolemic rabbits, LOX-1 expression was more prominent in atherosclerotic plaques with a thinner fibromuscular cap and was localized to the macrophage-rich lipid core[99]. Moreover, in the same areas, LOX-1 expression was positively correlated with tissue factor expression and apoptosis, suggesting

the involvement of LOX-1 in the destabilization and rupture of atherosclerotic lesions and the subsequent formation of thrombi[100]. Since LOX-1 could be implicated in vascular cell dysfunction related to plaque instability, it has been investigated as a potential target for an imaging tracer of atherosclerosis. In a recent study[101], AMI-prone Watanabe heritable hyperlipidemic rabbits and control rabbits were injected intravenously with (99m)Tc-LOX-1-mAb. Imaging clearly visualized the atherosclerotic aortas; the level of (99m)Tc-LOX-1-mAb accumulation in unstable plaques was higher than that in neointimal lesions or other, more stable lesions and therefore nuclear imaging of LOX-1 expression with this tracer may be a useful means of identifying atheromas at high risk of rupture. However, in the absence of suitable animal models of vulnerable plaques, we are far from suggesting that LOX-1 is involved in the destabilization of atherosclerotic plaques.

From Bench to Bedside: from LOX-1 to sLOX-1

Several clinical studies have investigated the significance of serum sLOX-1 concentrations in several pathological conditions, particularly vascular disease and diabetes.

The first clinical study to highlight sLOX-1 as a marker in cardiovascular disease dates back to 2005[102]. In this study, serum sLOX-1 levels were elevated in acute syndrome coronary (ACS). Serum sLOX-1 levels did not show any correlation with troponin-T or creatine phosphokinase levels, suggesting that sLOX-1 is not a marker for cardiac necrosis or injury[102]. A clinical study carried out on patients with ACS reported an association of serum sLOX-1 levels with the severity of CAD and urinary 8-isoprostane levels and an inverse correlation with extracellular superoxide dismutase levels suggesting that increased serum sLOX-1 levels reflect enhanced oxidative stress in vascular walls[103].

In type 2 diabetic patients, serum sLOX-1 levels were higher compared with controls[42]. Serum sLOX-1 decreased after improvements in glycemic control and the magnitude of this reduction correlated with the improvement in hemoglobin A1c and AGEs but not with the reduction in oxLDL[42]. Recently, we reported that sLOX-1 levels were increased in CAD patients and associated with the severity of CAD and inflammatory markers[104]. Other recent data showed that obese women have higher sLOX-1 levels, which may reflect increased LOX-1 expression in adipose tissue[105].

Recently, a likely relationship between LOX-1 gene polymorphisms and circulating sLOX-1 levels has been reported. The 3'UTR-T allele was associated with lower sLOX-1 levels in healthy older subjects suggesting LOX-1 gene variation to be important in the regulation of sLOX-1 levels in plasma[106].

Since association studies aiming to link metabolic and cardiovascular disease with sLOX-1 are far from conclusive due to over or misinterpretation of associations and supposed correlations, the clinical relevance of these observations needs to be assessed in extended clinical trials.

Drug Modulation of LOX-1 Expression

Several drugs commonly used in the treatment of type 2 diabetes, hypertension and hypercholesterolemia, appear to inhibit LOX-1 expression[107].

In hypertensive rats, and in cultured glomerular cells, not only antihypertensive drugs but also antioxidants suppressed LOX-1 overexpression[108]. PPARγ activators inhibited TNF-α- and PMA-induced LOX-1 mRNA overexpression in cultured ECs, but PPARα activators did not. In addition, thiazolidinedione pretreatment suppressed the renal LOX-1 expression induced by intraperitoneal TNF-α in mice[91], thus, the anti-atherogenic effect of PPARγ ligands appears to be at least partially mediated through LOX-1. Furthermore, in cultured ECs the PPARγ ligand pioglitazone inhibited LOX-1 expression and monocyte adhesion in a fashion similar to an antisense probe to LOX-1 mRNA[109]. It is not excluded that the inhibitory effect of PPARγ ligands on LOX-1 expression may be exerted through their antioxidant effect[91]. In SMCs, LOX-1 expression induced by IL-1β was decreased by the PPARγ activators as well[19].

Treatment with losartan, an antagonist of Ang Ⅱ, attenuated aortic intimal proliferation in rabbits on a high-cholesterol diet and markedly decreased LOX-1 expression[110]. Reinioside C, the main component of Polygala fallax Hemsl, which has putative antihyperlipidemic properties, inhibited oxLDL-induced LOX-1 mRNA and protein expression[111]. Mulberry leaf aqueous extracts had potential antioxidative effects, and inhibited LOX-1 m-RNA and protein expression induced by TNF-α and lipopolysaccharide, through inhibitionof NF-κB[112].

Metformin, a hypoglycemic agent, at therapeutically relevant concentrations reduced the expression of both RAGE and LOX-1 in a dose-dependent manner, with an associated reduction in intracellular ROS[113]. In aortas of streptozotocin-treated rats, the dietary supplement taurine improved vascular endothelial dysfunction, an effect possibly associated with down-

regulation of LOX-1 expression via antioxidative activity[114].

A number of studies have shown that 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors (statins) reduced total LDL cholesterol levels and exerted a cardioprotective effect.

Pretreatment of ECs with simvastatin or atorvastatin reduced the oxLDL-induced expression of LOX-1 as well as adhesion molecules. Both statins inhibited the activation of NF-κB induced by oxLDL[81]; the effect of statins on LOX-1 expression is associated with an increase in PKB activity[115].

The same treatments reduced the oxLDL-induced downregulation of eNOS expression and activation of MAPK[83]. The inhibitory effect on LOX-1 and subsequently MAPK activity provides a potential mechanism of beneficial effects of statins beyond the lowering of cholesterol levels.

In the aorta of hypercholesterolemic rabbits, benidipine, a dihydropyridine-type calcium channel blocker, significantly prevented the up-regulation of VCAM-1 mRNA expression and tended to inhibit LOX-1 mRNA expression, while pravastatin significantly prevented the up-regulation of both VCAM-1 and LOX-1 mRNA expression[116]. Moreover, aspirin and salicylate (but not indomethacin) reduced oxLDL-mediated LOX-1 expression in a dose- and time-dependent fashion[117]. Co-treatment with aspirin and pravastatin synergistically reduced platelet LOX-1 expression, by favorably affecting ROS and NO's release from activated platelets[118].

Taken together, these studies provide evidence that LOX-1 expression can be modulated by various pharmacological treatments and could thus represent an attractive target for therapeutic intervention to limit vascular injury and its long-term effects.

Conclusion and Future Perspectives

The axis between LOX-1 and its ligands is an etiological factor that contributes to the atherosclerotic process. The experimental evidence gathered thus far demonstrates that ligand-LOX-1 interaction can alter cell phenotype in a pro-atherogenic sense, so that cells become dysfunctional and more prone to cellular damage and even death. Studies with transgenic and KO mouse models and LOX-1 association with the instability of plaques have in part confirmed the pathogenic role of LOX-1 in atherosclerosis. Therefore, although LOX-1 could represent a valid target for therapeutic intervention in cardiovascular disease and stroke, continued studies on transgenic and KO mice are needed to help us understand not only what this particular receptor does, but also what it does not do.

Indeed, clinical observations do not yet convincingly support the proposed central role of LOX-1 in experimental atherosclerosis and raise the question of whether findings made in animal models, particularly in KO mouse models, can easily be translated to human disease.

Truly, a limitation that applies to KO studies in general is worth considering. Since KO mice lack the targeted gene product not only in the tissue studied, but globally and throughout development, they have many chances to alter the expression of other functionally related genes. Thus, these studies may reveal more about the ability of mice to compensate developmentally for the absence of a specific protein than about the role of the protein in normal adult tissues. Until these issues are resolved, the potential utility of drugs that perturb LOX-1 activity to prevent vascular disease remains unclear.

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