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REVIEW ARTICLE

Mechanistic insight into lysyl oxidase in vascular remodeling and angiogenesis

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Abstract Vascular remodeling and angiogenesis are two key processes in the maintenance of vascular homeostasis and involved in a wide array of vascular pathologies. Following these processes, extracellular matrix (ECM) provides the mechanical foundation for vascular walls. Lysyl oxidase (LOX), the key matrix-modifying enzyme, has been demonstrated to significantly affect structural abnormality and dysfunction in the blood vessels. The role of LOX in vascular remodeling and angiogenesis has always been the subject in the current medical research. Therefore, we presently make a summarization of the biosynthesis of LOX and the mechanisms involved in vascular remodeling and angiogenesis, as well as the role of LOX in diseases associated with vascular abnormalities and the therapeutic potential via targeting LOX. In particular, we give a proposal that LOX likely reshapes matrisome-associated genes expressions in the regulation of vascular remodeling and angiogenesis, which serves as a mechanistic insight into the critical role of LOX in these two aspects. Additionally, LOX has also dual effects on the vascular dysfunction, namely, inhibition of LOX for improving hypertension, restenosis and malignant tumor while activation of LOX for curing arterial aneurysm and dissection. LOX-

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targeted therapy may provide a promising therapeutic strategy for the treatment of various vascular pathologies associated with vascular remodeling and angiogenesis.

Introduction

Blood vessels including arteries, capillaries and veins form one of the largest surfaces in the human body.[1] The establishment of an organized vascular system is key to tissue homeostasis via providing oxygen and some essential nutrients (such as vitamins and minerals) as well as removal of metabolic waste products. Extracellular matrix (ECM) is critical for structural support to the blood vessels.[2] A properly structured ECM is indispensable for maintenance of the mechanical integrity of the vessels. There is also evidence supporting that increased degradation of elastin, one of the most important ECM components in the aorta, leads to a loss of structural integrity and vessel wall dilation.[3] As the key matrix-modification enzymes, lysyl oxidase (LOX) and lysyl oxidase-like 1—4 (LOXL1—4) play a role in the remodeling of ECM. For instance, the mice lacking the protein Loxl1 which corresponds to LOXL1 in human exhibit the serious damage in lung, skin and blood vessel due to inability of depositing elastic fibers in the postpartum uterine tract.[4] Meanwhile, LOXL4 is also important for the development of elastic vessels and mechanical stress of the vessel wall.[5] Recently, LOX has grabbed great attention in remodeling ECM as it can initiate the covalent cross-linking of collagen and elastin, two well-known ECM proteins in the aortic wall, and thereby ensuring ECM stability.[6,7] What's more important, it is reported that the overall amount of LOX activity was reduced by 80% with deletion of Lox in cultured skin fibroblasts and aortic smooth muscle cells while Loxls proteins are merely responsible for the remaining 20% of the activity.[8,9] These data support that LOX encodes the majority of the LOX activity, at least in the aorta. Therefore, the present work focuses on the role of LOX in vascular biology. The direct evidence supporting a critical role of LOX in vascular biology arises from the study that the homozygous knockout of *Lox* gene in mice results in perinatal death due to aortic aneurysm (AA) and spontaneous dissection.[10] In addition, mutant *Lox* null mice also display highly abnormal aortic histology characterized by fragmented elastic fibers and aberrant smooth muscle cell (SMC) layers.[11] These results strongly support the functional significance of LOX in the maintenance of the integrity of blood vessels. Actually, in atherosclerosis, a chronic inflammatory disease of blood vessels,[12] it has been found that LOX modulates virtually all pathological stages, from endothelial dysfunction and plaque progression to vascular calcification, and aggravates vascular stiffness.

In vascular system, vascular remodeling and angiogenesis are key prerequisites in various conditions including embryonic development and vascular dysfunction (Fig. 1). For instance, vascular remodeling and angiogenesis are innate responses following ischemic cardiovascular events (such as peripheral artery disease and myocardial infarction), which is of vital importance for restoring blood supply and oxygenation.[13] With respect to the relationship of LOX and vascular remodeling or angiogenesis, LOX is the major isoform responsible for the proliferation and migration of the aortic vascular smooth muscle cells (VSMCs), which is a critical process in vascular remodeling, as the cells with LOX deficiency exhibits 80% reduction of LOX activity.[8] In the field of angiogenesis, LOX-mediated ECM stiffness binds to integrin β1 and subsequently results in activation of phosphatidylinositol-3 kinase (PI3K)/Akt signaling pathway and up-regulation of vascular endothelial growth factor (VEGF) expression.[14] In response to the increase in VEGF, elevated VEGF receptor 2 (VEGFR2) is observed in tip cells at the ends of angiogenic sprouts and thereby guide vessel outgrowth.[15] Taken together, these evidences support a critical role of LOX in vascular remodeling and angiogenesis. Until now, there is lack of systematic summarization of the relationship between LOX and vascular biology especially vascular remodeling and angiogenesis. Therefore, in this review, we aim to describe current knowledge of the role of LOX in vascular remodeling and angiogenesis and also further discuss the therapeutic potential against vascular dysfunction via targeting LOX.

Biosynthesis and localization of LOX

LOX was firstly identified in 1968 by the research group of Pinnell and Martin.[16] In addition to LOX, four other LOXLs members including LOXL1, LOXL2, LOXL3 and LOXL4 have been identified,[17] thus establishing the LOX family containing five isoforms. Structurally, LOXLs have significant similarity to LOX in C-terminal amine oxidase catalytic domain which can oxidize specific lysine and hydroxylysine residues for producing highly reactive aldehydes, finally resulting in the initiation of intra- and inter-molecular covalent cross-linkages. However, they are extremely variable in N-terminal signal peptide domain. The biosynthetic pathway of LOX is summarized in Figure 2. Briefly, it is translated as a 48-kDa preproenzyme in the ribosome. Following the removal of N-terminal signal peptide sequence in the endoplasmic reticulum (ER), N-glycosylation and copper incorporation and the cofactor lysyl tyrosine quinone (LTQ) formation occur in the golgi compartment to generate a proenzyme with the molecular weight of 50 kDa. Thereafter, the resulting 50 kDa proenzyme is secreted into the extracellular space and undergoes proteolytic cleavage by procollagen C-proteinases including bone morphogenic protein-1 (BMP-1) and to a less degree, by mammalian Tolloid-like 1 (mTLL-1) and mTLL-2 at the residues between Gly168 and Asp169,[18] yielding the mature form of LOX and LOX-PP, with the molecular weights at 32 kDa and 18 kDa, respectively. Intriguingly, the

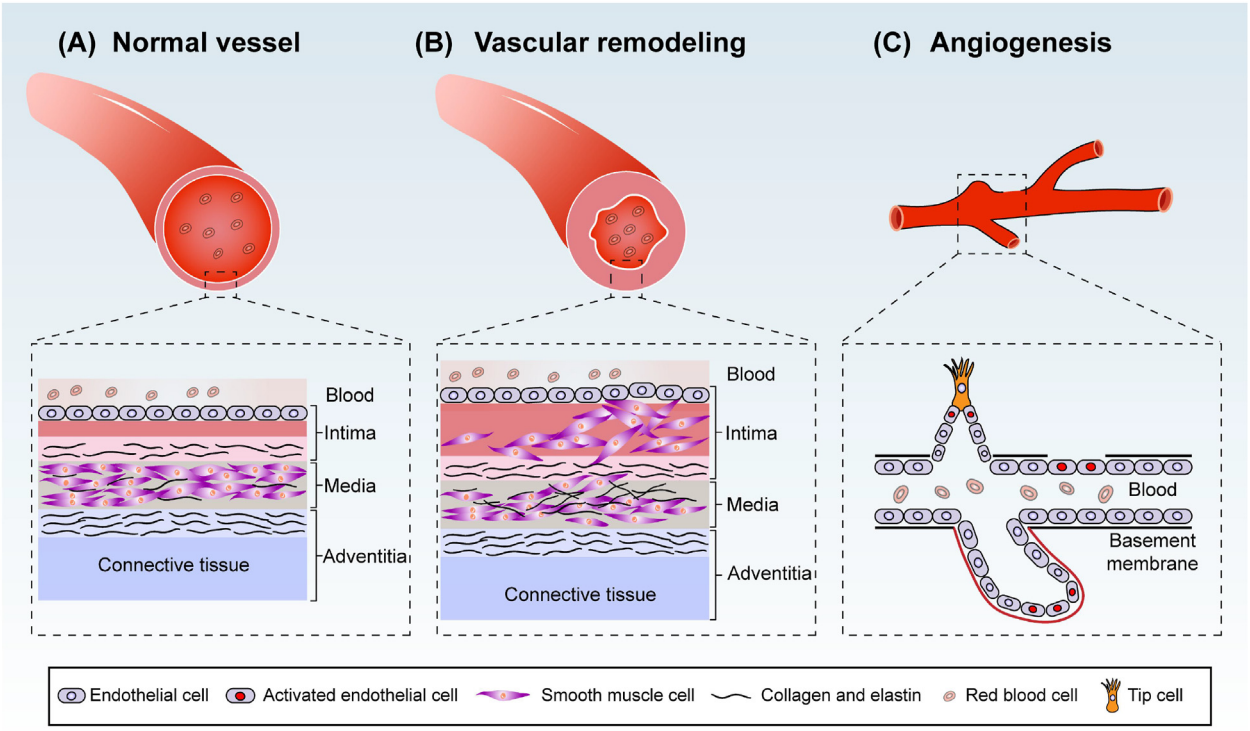

Figure 1 Vascular remodeling and angiogenesis. **(A)** Indicates the normal blood vessel, which generally has three layers: the intima, media, and adventitia. **(B)** Indicates vascular remodeling. Following this process, migration and proliferation of smooth muscle cells and extracellular matrix remodeling are observed, leading to adaptive change in vascular wall and neointimal hyperplasia. **(C)** Indicates angiogenesis. In the first place, endothelial cells are activated, releasing the enzymes such as proteases. Then, vascular basement membrane is degraded by these proteases. Finally, vascular sprouting is generated within interstitial matrix.

proteolytic process is also conducted between Asp218 and Tyr219 by a disintegrin and metalloproteinase with thrombospondin motifs 2/14 (ADAMTS2/14), generating a truncated form of LOX with the molecular weight at 25 kDa.[19] Although BMP-1 and ADAMTS2/14 share the similar substrate such as collagen, the capacity of substrate binding is different. In detail, cleavage by ADAMTS2/14 has been found to substantially decrease the capacity of LOX to bind telocollagen,[19] since ADAMTS2/14 get rid of sequence determinants in which the tyrosine residues are modified by sulfation.

LOX is differentially localized as shown in Table 1. The active LOX, the propeptide LOX-PP and proLOX all have the wide distribution within the cell and extracellular space.[6,19–38] In contrast, pre-proLOX with the molecular weight of 48 kDa is distributed in the cytoplasm.[39] However, the truncated form of LOX with the molecular weight of 25 kDa also exists although its location and biological are unclear.[40] Different forms of LOX exert distinct biological functions via binding to diverse types of substrates which usually include non-histone (Collagen I, II, III, V, IX and XI and elastin,[41–43] basic fibroblast growth factor (bFGF),[44] transforming growth factor β1 (TGF-β1),[45] platelet-derived growth factor receptor β (PDGFR-β) as well as Vgll3) and histone (H1 and H2) substrates (Table S1).[27,46,47] It is ubiquitous that the mature form of LOX has two possible destinations. On the one hand, extracellular localization of active form of LOX promotes covalent crosslinking of collagen and elastin in the ECM to maintain the tensile strength and structural integrity.[8,48] On the other hand, this form of LOX can also be transported back into the intracellular space. Cytosolic LOX is able to activate various cellular signaling such as Src/focal adhesion kinase (FAK) to control cell adhesion and motility while intranuclear LOX can oxidize histone H1 or histone H2, and therefore modulate expressions of genes including *collagen III* and *ELN*.[21,47,49–51] It is feasible that the nuclear LOX arises from secreted and processed enzyme in the extracellular space as there is evidence supporting that fluorescein-labeled LOX readily enters the cytosolic compartment and rapidly concentrates into the nucleus in VSMCs.[52] It is also noteworthy that the intracellular uptake and distribution are not affected by pharmacological inhibition of LOX activity with β-aminopropionitrile (BAPN), indicating that the mature form of LOX transports back into the cytosolic compartment and concentrates within nuclei independent of its catalytic activity. Another proteolytic form of LOX, namely, LOX-PP has been shown to possess intranuclear and extracellular functions. Nuclear LOX-PP is found to interfere with nuclear factor kappa B (NF-κB) RelA signaling and microtubule-associated protein regulation of microtubule stability, leading to underdevelopment of Purkinje cell dendrites.[29] In contrast, extracellular localization of LOX-PP has diverse biological functions including suppressions of tumor growth and migration,[34,53] angiogenesis and SMC proliferation.[36,54]

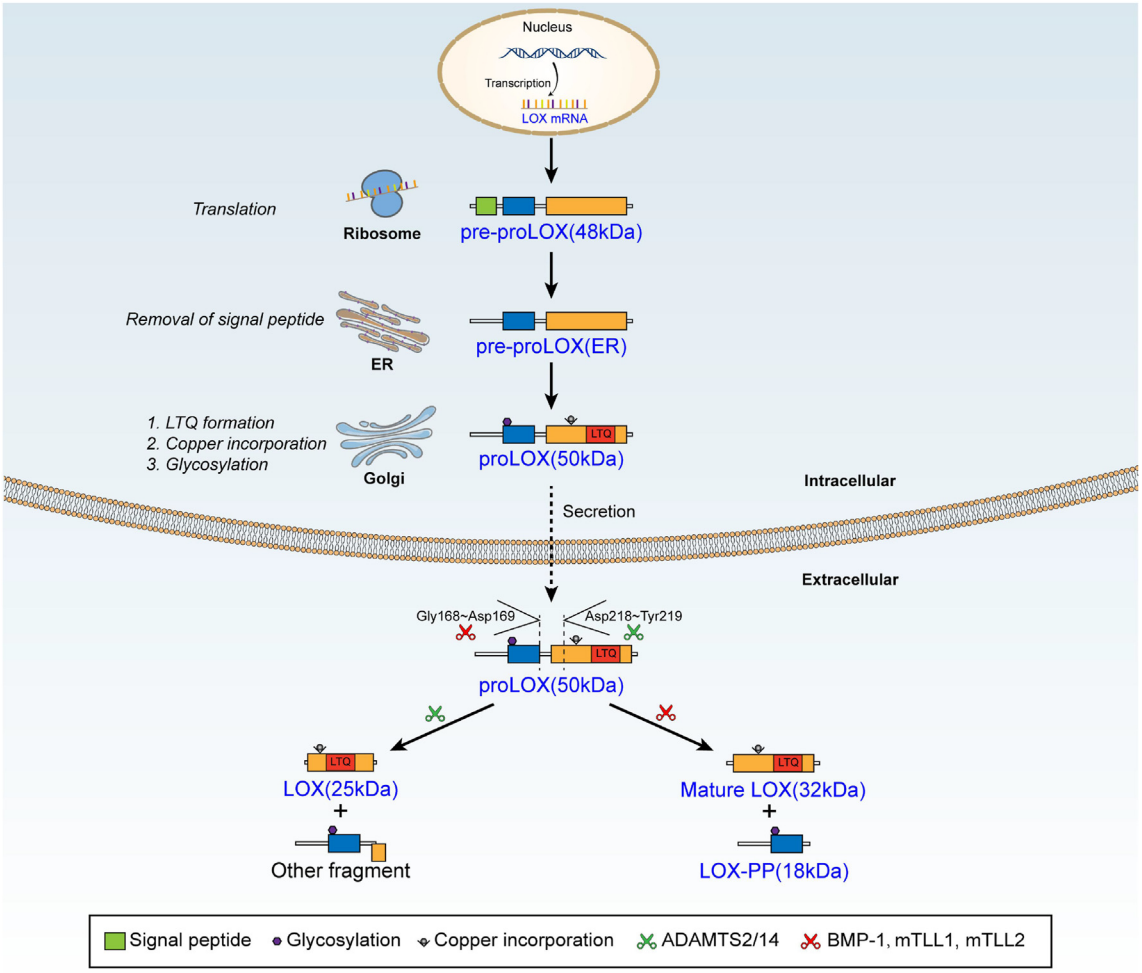

Figure 2 Biosynthesis of LOX. A 48-kDa preproenzyme for LOX (pre-proLOX) is translated in the ribosome and then transported into the endoplasmic reticulum for the removal of N-terminal signal peptide sequence, yielding a pre-proLOX fragment (pre-proLOX (ER)). After that, it enters the golgi apparatus with N-glycosylation, copper incorporation and the cofactor lysyl tyrosine quinone (LTQ) formation, releasing a 50-kDa proenzyme (proLOX). Following secretion into the extracellular space, proLOX is cleaved by the procollagen C-proteinases including BMP-1, mTLL1 or mTLL2 to form the mature LOX (32 kDa) and LOX-PP (18 kDa) at the residues between Gly168 and Asp169. The proteolytic cleavage is also carried out at the residues between Asp218 and Tyr219 by ADAMTS2/14, and subsequently forms of LOX with the molecular weight at 25 kDa and other fragments. ADAMTS2/14, a disintegrin-like and metalloproteinases with thrombospondin motifs 2/14; BMP-1, bone morphogenetic protein-1; ER, endoplasmic reticulum; LTQ, lysine tyrosylquinone; mTLL1, mammalian Tolloid-like 1; mTLL2, mammalian Tolloid-like 2; PP, propeptide.

LOX mutation and vascular pathology

There are at least ten reported genetic variants of *LOX*, which alter the activity of LOX in vascular pathologies. These variants are all located in the exons and they are marked in Figure 3A. The predicted three-dimension protein structure of LOX is shown in Figure 3B based on the link: https://alphafold.ebi.ac.uk/entry/P28300. Although there are no reports on the effect of majority of the genetic variants of *LOX* on protein conformation, the variant c.857T > G encoding p.M292R in Mus musculus which corresponds to p.M298R in Homo sapiens, localized in exon 4, is misfolded and trapped in the ER bound to calnexin, thus causing differential sensitivity to proteolytic degradation compared with the wild-type group.[55] The relationship between the ten variants of *LOX* gene and vascular diseases are summarized in Table 2. It is noted that expressions of the missense mutations in the *LOX* gene (c.839G > T encoding p.Ser280Arg and c.1044T > A encoding p.Ser348-Arg) significantly result in the decreased LOX activity in comparison with the wild-type group.[9] The patients succumbed to ascending aortic dissections (AADs) always carry these mutations and show fusiform enlargement of the root and ascending thoracic aorta.[9] It indicates that inhibition of LOX due to genetic variants in *LOX* increases the susceptibility to AAD. In addition, LOX activity is also found to be decreased by 50% by two nonsense mutations in the *LOX* gene (c.125G > A encoding p.Trp42* and c.604G > T encoding p.Gly202*), which can increase the risk of thoracic aortic aneurysm (TAA).[9] In two first cousins with TAA and dissection (TAAD), a missense mutation in the *LOX* gene (c.893T > G encoding p.Met298Arg) is identified which

Table 1 Location and biological function of LOX.

Category	MW	Location	Biological function	Refs
Mature LOX	32 kDa	Nucleus	Controlling gene transcription (*collagen III*, *ELN*)	20–22
		Cytoplasm	Controlling cell adhesion and motility Promoting tumor metastasis	23–25
		Extracellular space	Catalyzing ECM crosslinking Oxidizing cell membrane proteins and enhancing the chemotactic response of VSMCs Regulating VEGF expression to promote angiogenesis	6,26–28
LOX-PP	18 kDa	Nucleus	Inhibiting angiogenesis Leading to underdevelopment of Purkinje cell dendrites Interacting with DNA repair proteins and inhibiting tumor growth	29–31
		Cytoplasm	Promoting apoptosis and inhibit motility	32,33
		Extracellular space	Inhibiting angiogenesis Inhibiting proliferation of cancer cells to suppress tumor growth	34–36
ProLOX	50 kDa	Nucleus/Cytoplasm	Inhibiting signaling pathways that contribute to cell transformation	37
		Extracellular space	Unknown	38
Pre-proLOX	48 kDa	Cytoplasm	Unknown	39
Truncated LOX	25 kDa	Unknown	Unknown	19

cosegregates with disease in the family.[56,57] By introducing this human mutation into the homologous position in the mouse genome using CRISPR/Cas9 genome editing technology (c.857T > G encoding p.M292R in Mus musculus), it is confirmed that the amino acid substitution causes ruptured TAAD and perinatal death when both alleles are mutated ($Lox^{Mu/Mu}$).[55] The possible explanation may arise from the fact that the mutant Lox protein in the mice due to substitution from methionine-to-arginine leads to a secretion defect as this event causes the ER retention and does not transit to the Golgi, where copper incorporation occurs, finally causing the inactivated enzymatic activity of Lox. Similarly, another missense mutation for the *LOX* gene (c.871T > A encoding p.Cys291Ser) diminishes LOX activity and then increases the risk of TAAD.[58] In coronary artery diseases (CADs), patients carrying a missense mutation in the *LOX* gene (c.473G > A encoding p.Arg158Gln) is associated with increased susceptibility to this disease in a Chinese population.[59] Additionally, there are also three identified missense *LOX* variants including c.743C > T encoding p. Thr248Ile,[9] c.800A > C encoding p. Gln267Pro and c. 995A > G encoding p. Tyr332Cys,[9,60] although the roles of these mutations in vascular disease are not characterized. Taken together, it suggests that *LOX* variants may serve as an important risk factor of vascular pathologies.

Roles of LOX in vascular remodeling and angiogenesis

Vascular remodeling and angiogenesis are two critical events for both embryonic development and vascular pathologies. The former type often refers to the alteration in the size and/or composition of the blood vessel,[61] while the latter category indicates neovascularization. In the following description, we make an elaboration of the roles and regulatory mechanisms of LOX in these two vascular alterations as summarized in Fig. 4.

Role of LOX in vascular remodeling

Nowadays, at least four cellular processes are involved in vascular remodeling, which includes proliferative and migratory status of VSMCs, endothelial dysfunction, inflammatory processes and ECM reorganization.[62] All of these biological processes are dynamically regulated with each other following several stimulating factors such as growth factors and hemodynamic stimulus,[63] finally resulting in structural alteration and dysfunction of the vascular wall.[64] VSMCs and endothelial cells (ECs) are two major types of cells that are influenced in the process of vascular remodeling.[65] In detail, VSMCs in the medial layer exhibit phenotypic plasticity and switch from a contractile state to a synthetic state. These phenotypic modifications have been demonstrated to regulate cell proliferation and migration during arterial remodeling.[66] In contrast, ECs usually serve as vascular transducers and are responsible for producing vasoactive substances (either growth stimulators or inhibitors). To the best of our knowledge, the role of LOX in the vascular remodeling contains three aspects including the proliferation and migration of VSMCs, ECs dysfunction and ECM reorganization as shown below.

It has been demonstrated that the proliferation and migration of VSMCs are contributing factors for vascular remodeling. The direct role of LOX in the VSMCs stems from

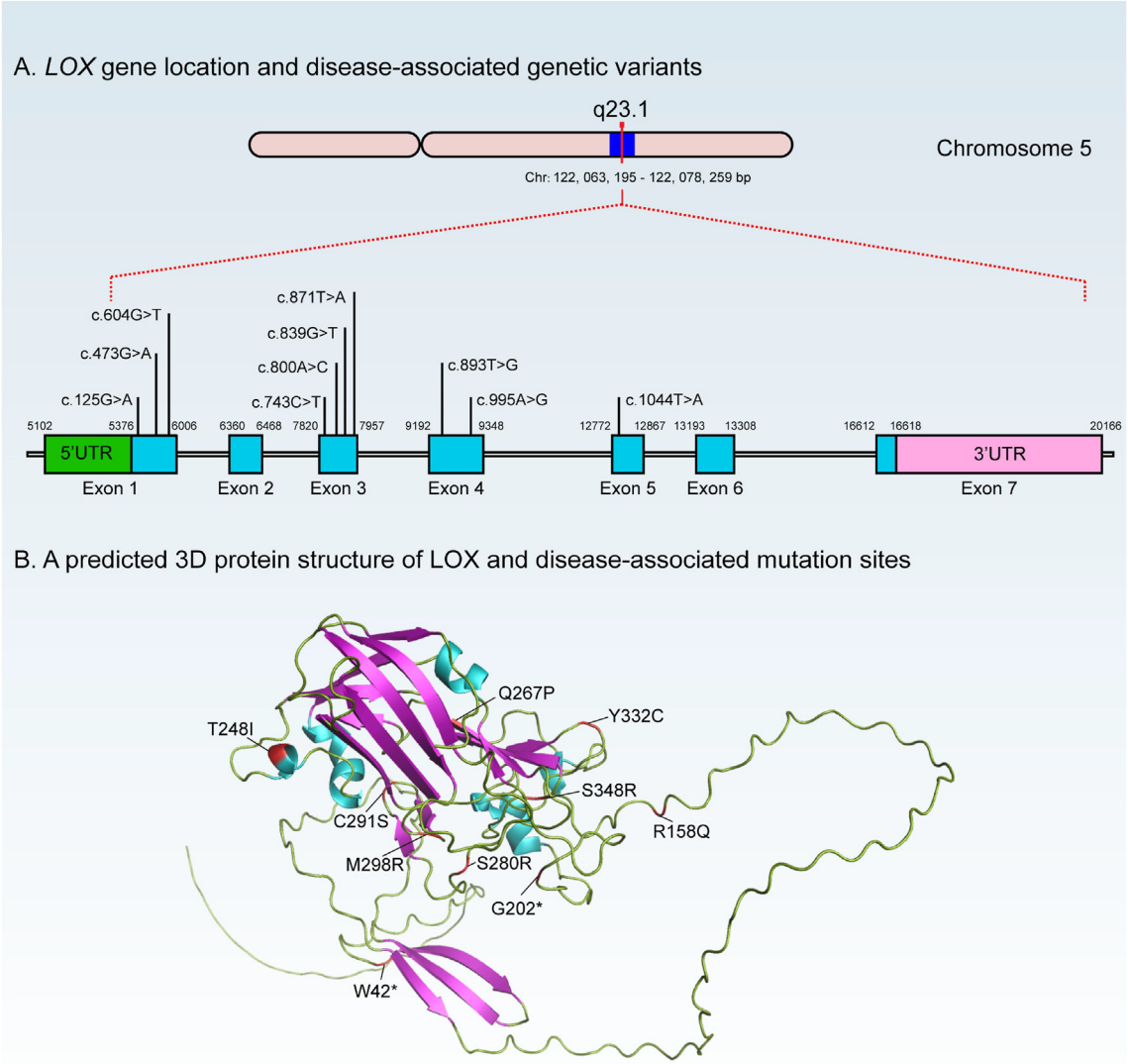

Figure 3 *LOX* gene location and disease-associated genetic variants. **(A)** Nucleotide numbering begins with the first nucleotide in the coding region (5376 nucleotide on the DNA). There are seven exons in *LOX* gene. The coding region in the exon is marked with blue and the non-coding regions including 5′UTR and 3′UTR are indicated with green and pink, respectively. The initiation and termination sites for each exon are marked in the diagram. **(B)** A predicted three-dimension protein structure of LOX according to the link: https://alphafold.ebi.ac.uk/entry/P28300 and disease-associated mutation sites. In the predicted structure, the peptide chain of LOX is color-coded according to structural components: alpha-helices, cyan; beta-pleated sheets, magenta; random coil and chartreuse. In addition, the disease-associated amino acid substitution is shown in red. UTR, untranslated region.

the result showing that the isoform responsible for the 80% of the LOX activity in aortic VSMCs is essential for ECM maturation.[8] *LOX*$^{-/-}$ aortas show location-specific differential gene expression in VSMCs.[67] Overproduction of LOX by constituent cells of the vascular wall may drive neointimal formation or medial hypertrophy by recruitment of VSMCs.[68] In the aspect of LOX in the VSMCs proliferation, there is conflicting data showing the expression of LOX is increased following the proliferative stimulus or proliferation deficiency.[69,70] However, it is definite that vascular LOX often contributes to the migration of VSMCs. For example, purified LOX is found to directly induce the migration of cultivated rat aortic SMCs.[71] Under this condition, the contribution of LOX to VSMCs migration is possibly due to the result of activation of chemotaxis by the elevated level of intracellular H_2O_2 following LOX overexpression via binding with hitherto uncharacterized substrate.[71] Besides, the chemotactic response of VSMCs is also enhanced by the LOX-dependent oxidization of cell membrane protein likePDGFR-β,[27] which subsequently leads to the high affinity and capacity for the binding of PDGF-BB. Pharmacological inhibition of LOX by BAPN reduces this

Table 2 Role of *LOX* variants in the vascular disease.

LOX variant	Amino acid substitution	rsID	Mutation type	Effects on LOX activity	Role in vascular disease	Refs
839G > T	Ser280Arg	rs886040965	Missense	Decrease	Causing AAD	[9]
1044T > A	Ser348Arg	rs1561417568	Missense	Decrease	Causing AAD	[9]
125G > A	Trp42*	—	Nonsense	Decrease	Causing TAA	[9]
604G > T	Gly202*	rs1473260982	Nonsense	Decrease	Causing TAA	[9]
893T > G	Met298Arg	rs876657852	Missense	Decrease	Causing TAAD	[56,57]
871T > A	Cys291Ser	—	Missense	Decrease	Causing TAAD	[58]
473G > A	Arg158Gln	rs1800449	Missense	Decrease	Causing CAD	[59]
743C > T	Thr248Ile	—	Missense	Decrease	Unknown	[9]
800A > C	Gln267Pro	rs886040967	Missense	Unknown	Unknown	[9]
995A > G	Tyr332Cys	—	Missense	Unknown	Unknown	[60]

Note. AAD, ascending aortic dissection; TAA, thoracic aortic aneurysm; TAAD, thoracic aortic aneurysm and dissection; CAD, coronary artery disease.

chemotactic response *in vitro*. These data suggest that LOX is essential to generate optimal chemotactic sensitivity of VSMCs via affecting cell proliferation.

LOX is abundantly expressed in the endothelium of healthy coronary arteries and recent studies indicate the relationship between LOX downregulation and endothelial dysfunction.[72] There is rare data showing the concrete role of LOX in the modulation of endothelial function. However, diverse evidences support the notion that LOX is critical for the maintenance of endothelial homeostasis. For instance, several risk factors of cardiovascular diseases which includes high levels of low-density lipoproteins (LDL),[73] hyperhomocysteinemia and pro-inflammatory cytokines,[74,75] are found to inhibit endothelial LOX expression and subsequently trigger endothelial dysfunction. Following LDL exposure, the exchange of macromolecules across an endothelial monolayer is increased, which is comparable with the irreversible LOX inhibitor BAPN.[73] It suggests that LOX downregulation by LDL impairs endothelial barrier function. Inhibitory effect of LOX is also reported to be observed by hyperhomocystinemia.[74] Additionally, the pro-inflammatory cytokine such as tumor necrosis factor-α (TNFα) is found to reduce endothelial LOX expression and activity in the vascular wall.[76] Treatment with protein kinase C inhibitors including Calphostin C and Go6976 can efficiently prevent TNFα-induced LOX downregulation *in vitro*.[76] Thus, these results support the hypothesis that LOX downregulation may contribute to the endothelial dysfunction, which remains a risk factor for cardiovascular diseases.

Vascular stiffness is always induced by ECM reorganization. It has been demonstrated that vascular stiffening is an important event in the process of vascular remodeling. Vascular stiffness is influenced by the structural elements of the arterial wall such as collagen and elastin. Given the central role of LOX in collagen and elastin cross-linking, it indicates that LOX has great contribution to vascular stiffness and vascular remodeling. In a transgenic LOX (TgLOX) mouse model, mesenteric resistance arteries in VSMCs are stiffer than control littermates and inhibition of LOX with BAPN significantly ameliorates stiffened vessel and abnormal elastin structure.[77] Mechanistically, LOX causes the elevations of H_2O_2 and the subtype of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, namely, NOX1 and mitochondrial dysfunction. Treatment with H_2O_2 scavenger and mitochondria-targeted antioxidant both diminish vascular stiffness and the elastin deposition in transgenic mice overexpressing human LOX, indicating the key role of LOX for vascular oxidative stress and stiffening property. Besides, the TgLOX mice are also characterized with the activation of p38 mitogen-activated protein kinase (MAPK) signaling in the vessel wall, especially following vascular oxidative stress. Abrogation of this signaling with the specific inhibitor SB203580 attenuates structural alteration and vascular stiffness. It indicates that LOX-mediated ECM reorganization is of vital importance for vascular stiffness, a basic characteristic of vascular remodeling.

Role of LOX in angiogenesis

There are three well-known types of angiogenesis, which include sprouting angiogenesis, splitting (intussusceptive) angiogenesis and vascular mimicry.[78] As the major type of angiogenesis,[79] the vessel sprouting refers to the process in which ECs become motile and are succumbed to be invasive, facilitating the formation of tip cells. In sprouting angiogenesis, the endothelial tip cells result in the outgrowth of blood-vessel sprouts and initiate the blood flow. There are extensive investigations supporting a critical role of LOX in sprouting angiogenesis. For instance, the results by the *ex vivo* aortic ring assay demonstrate that the sprouting events do not occur after the inhibition of LOX by BAPN.[80] Given that LOX possess the biological function via ECM remodeling or independent of ECM, the following description is performed to discuss the role and regulatory mechanism of LOX in vessel sprouting in the ECM-dependent and ECM-independent aspects.

LOX can modulate the vascular sprouting in an ECM-dependent or ECM-independent manner. With respect to the ECM-dependent role, LOX transmits signals into the cell via integrin β1 through matrix stiffness caused by ECM

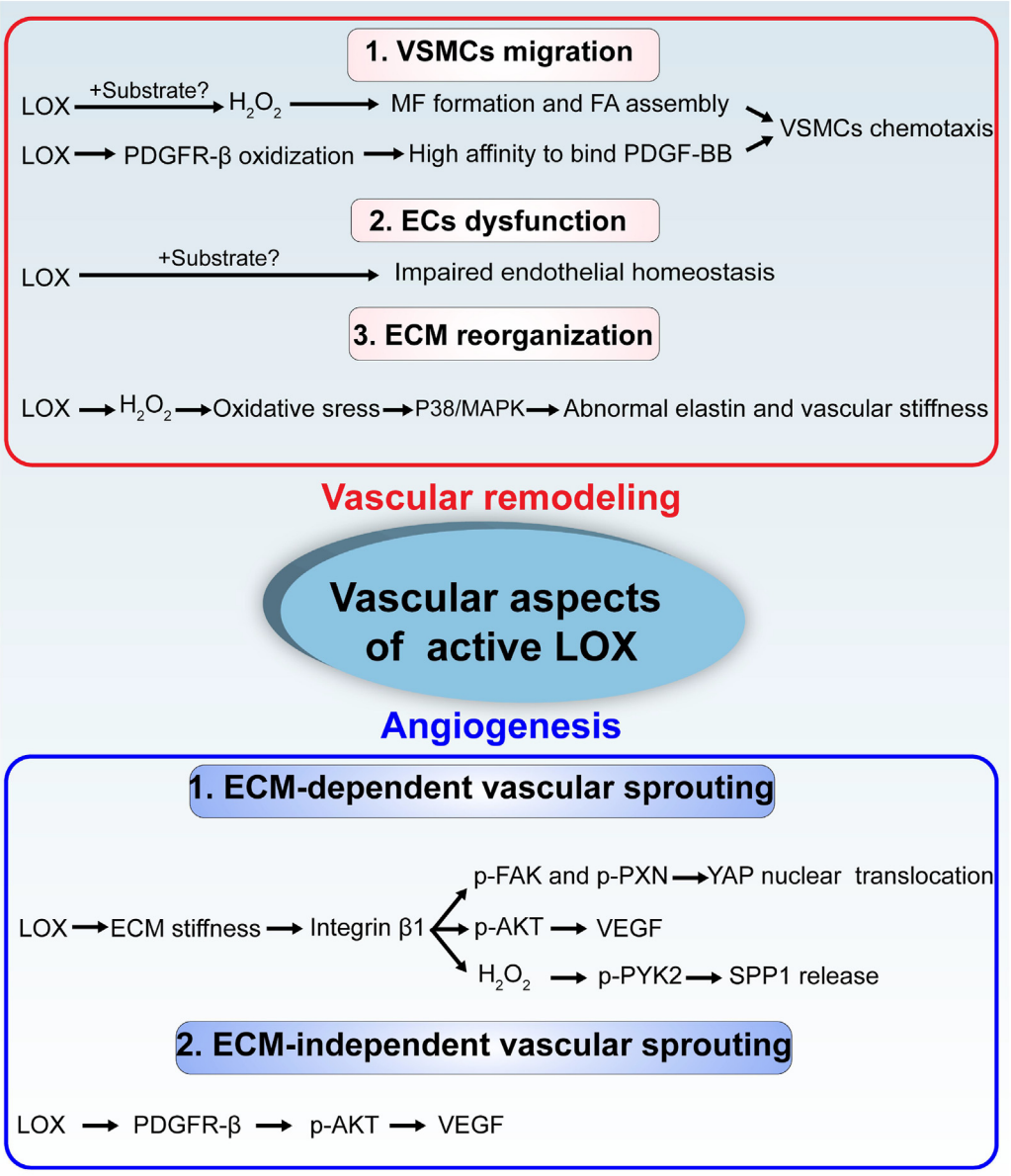

Figure 4 Role of LOX in vascular remodeling and angiogenesis. The left panel indicates the regulatory mechanism of LOX in vascular remodeling. Generally, LOX promotes vascular remodeling via VSMCs migration, ECs dysfunction and ECM reorganization. In the aspect of VSMCs migration, LOX binds to substrates and induces H_2O_2-dependent microfilament reorganization and focal adhesion assembly which is consistent with the induction of the chemotactic response. Furthermore, LOX activity is essential to generate optimal chemotactic sensitivity of cells to chemoattractants by oxidizing specific cell surface proteins, such as PDGFR-β. In addition, LOX is affected by various stimuli in vascular disorders and may bind to certain substrates or interfere with cellular signaling and thus impair endothelial homeostasis. As for the reorganization of ECM, H_2O_2 produced by LOX is associated with oxidative stress that promotes p38MAPK activation, elastin structural alterations and vascular stiffness. The right panel shows the regulatory role of LOX in angiogenesis. We propose that LOX promotes angiogenesis through ECM-dependent or ECM-independent manner. In the ECM-dependent way, LOX is able to transmit signals into the cell via integrin β1 through matrix stiffness caused by ECM crosslinking to activate and transfer YAP to the nucleus by increasing p-PXN of FAK, thus upregulating target gene expression such as CD34, VEGFR2, DLL4, CXCR4, EFNB2 and IGF2, and promoting tip cell formation. ECM crosslinking can also activate the PI3K/Akt pathway and up-regulate VEGF expression. Furthermore, LOX can activate the integrin β1-PYK2 pathway and then promote the release of SPP1 which can stimulate angiogenesis. On the other hand, LOX activates Akt through PDGFR-β stimulation, resulting in increased VEGF expression, which is ECM-independent. Akt, protein kinase B; ECs, endothelial cells; FAK, focal adhesion kinase; PDGFR-β, platelet-derived growth factor receptor β; PI3K, phosphoinositide-3-kinase; p-PXN, phosphorylation of paxillin; PYK2, proline-rich tyrosine kinase 2; SPP1, secreted phosphoprotein 1; VEGF, vascular endothelial growth factor; VSMCs, vascular smooth muscle cells; YAP, yes-associated protein.

crosslinking to activate the PI3K/AKT pathway and upregulate VEGF expression.[14] In turn, VEGF elevation binds to the integrin on the tip cells and subsequently promotes the vascular sprouting.[81] Meanwhile, LOX-mediated ECM crosslinking also activates integrin-related cellular signaling including angiogenic factors (PDGF and VEGF) signals and promote the endothelial cell proliferation and migration.[82,83] In detail, a recent study have shown that matrix stiffness caused by ECM crosslinking triggers the nuclear translocation of YAP by increasing phosphorylated levels of FAK (p-FAK) and paxillin (p-PXN), thus upregulating target genes like *CD34*, *VEGFR2*, *DLL4*, *CXCR4*, *EFNB2* and *IGF2* and ultimately promoting tip cell formation.[84] In addition, p-PXN can also reduce the intercellular connections to enhance this effect, which facilitates LOX-mediated ECM cross-linking and vascular sprouting.[84] Furthermore, the contribution of LOX to activation of integrin β1-PYK2 pathway makes the macrophages chemotactic and secretes SPP1 which stimulates angiogenesis.[14] Several ECM components such as collagens, vitronectin and fibronectin,[85] are found to act as the ligands of integrins and promoting VEGF-dependent angiogenesis via activating p-FAK and p-PXN.[86] Therefore, it is intriguing that LOX-mediated ECM cross-linking is conducted via possibly binding to the integrins and other ECM components, which underlies the ECM-dependent role of LOX in the process of angiogenesis, although the concrete regulatory mechanism by which LOX binds to integrins remains uncharacterized.

The effect of LOX on vessel sprouting is also conducted in an ECM-independent fashion. Although there is rare data for the validation of this notion, it has been demonstrated that tumor-derived LOX drives angiogenesis through activations of PDGFR-β and AKT, leading to the elevation of VEGF.[87] There is also evidence supporting that high level of LOX is able to promote tumor endothelial cell motility and tubulogenesis through activation of FAK signaling.[88] These data altogether indicate that LOX can directly regulate angiogenesis events without affecting ECM.

It is also interesting that LOX-PP, the propeptide of LOX, appears to play an opposite role in angiogenesis with the active form of LOX. Proteomic analysis reveals that LOX-PP can downregulate the levels of VEGF, MAPK, FGF,[89,90] FAK, fibronectin-mediated matrix formation and cytoskeleton regulation pathway which are the key mediators of angiogenesis. Overexpression of purified LOX-PP in human umbilical vein endothelial cells (HUVECs) is found to inhibit the migration of endothelial cell, cell adhesion and tube length and thereby exert anti-angiogenic effect.[36] In addition, a previous report shows that LOX-PP promotes the apoptosis of rat retinal endothelial cell and inhibits angiogenesis in response to high glucose.[91]

Matrisome-associated gene expressions are proposed to be involved in the regulation of LOX on vascular remodeling and angiogenesis

During vascular remodeling and angiogenesis, there are diverse molecular targets associated with structural ECM components and proteins that can interact with or remodel the ECM, namely, "matrisome". Nowadays, the matrisome is comprised of more than 300 core ECM proteins and associated ECM modifying molecules, ECM-affiliated proteins, ECM modulators and secreted factors in rodents.[92] There are a variety of ECM proteins like fibronectin,[93] type I/III/VIII collagen and fibrous macromolecules,[94–96] as well as ECM-associated regulators including LOXs,[67] and matrix metalloproteinases following the process of vascular remodeling, while vascular angiogenesis is associated with fibronectin,[97,98] type I/IV/VI collagen,[99–101] proteoglycans belonging to ECM molecules and LOX,[67,102] matrix metalloproteinases affiliated to the family of ECM-modifying enzymes.[103] It is especially intriguing that in a mouse model of TAAD induced by the deficiency of *Lox* gene, there are multiple alterations of genes associated with secreted factors, collagens, ECM-affiliated proteins and ECM glycoproteins,[67] which all belong to the matrisome proteins. Together with the knowledge mentioned above, we give a proposal that genetic manipulation of Lox in mice reshapes the expressions of matrisome-associated proteins in vascular biology such as vascular remodeling and angiogenesis. It is likely to serve as a mechanistic insight into the role of LOX in the process of vascular remodeling and angiogenesis.

Therapeutic potential via targeting LOX-mediated vascular remodeling or angiogenesis

The therapeutic potential via targeting LOX against disease pathology always either aggravates disease progression or cures disease is dependent upon specific context. In the following description, we will systematically discuss these two facets.

Inhibition of LOX-mediated vascular remodeling or angiogenesis is effective on disease pathology

Inhibition of LOX in the process of vascular remodeling has been reported to cure diverse pathological conditions such as hypertension-associated pathologies and restenosis. In hypertension research, increase of Lox expression is observed in the rat models of spontaneous hypertension and deoxycorticosterone acetate-salt (DOCA-salt) induction.[77,104] Administration of BAPN decreases hypertension-associated vascular remodeling and stiffness.[77] It indicates that LOX inhibition holds promise for treating hypertension. Similarly, in Angiotensin II (Ang II)-induced arterial hypertension with the pathological features including concentric mural hypertrophy smaller size in the intrarenal arteries,[105] enzymatic activity of vascular LOX is also enhanced after Ang II infusion.[106] Suppression of LOX-mediated ECM cross-linking has been shown to decrease vascular stiffness and ameliorate the detrimental end-organ effect in hypertension. Additionally, clinical and experimental evidences also support that LOX remains a causal factor in pulmonary arterial hypertension for which the pathological trait is the increase of muscularization and interruption of matrix structures in the wall of the pulmonary arteries.[107] In the aspect of hypertensive heart disease characterized by prominent fibrosis in the myocardium, collagen cross-linking induced by LOX has a positive relationship with fibrosis and left ventricular rigidity.[108] Treatment with BAPN in normal adult pigs is shown to significantly diminish collagen cross-linking and

myocardial stiffness in the left ventricle.[109] Collectively, it implies that enhanced LOX enzymatic function may be detrimental for hypertension and pharmacological inhibition of LOX is likely to have therapeutic potential against hypertension. With respect to restenosis, it is a maladaptive response to arterial injury during angioplasty, which is characterized with platelet aggregation, release of growth factors and inflammatory cytokines, and accumulation of SMCs and ECM production. Recent findings demonstrate that stabilization of ECM component such as collagen may facilitate restenosis following certain types of arterial injury.[110] As the major enzyme for the catalysis of ECM covalent crosslinking, LOX is positively correlated with type III collagen deposition in the restenotic plaques.[111] Similarly, in rabbits undergoing angioplasty, pharmacological inhibition of LOX by BAPN significantly reduces restenotic rates and constrictive remodeling.[112] These data indicate that LOX inhibition may act as a promising strategy for prevention and treatment of in-stent restenosis.

Angiogenesis is often one of the hallmarks of cancer and plays a critical role in the regulations of cancer cell growth, invasion, and metastasis.[113] There are diverse molecular players particularly VEGF and angiopoietin family, which are involved in the growth of vessel, finally causing tumor angiogenesis. The role of LOX in tumor angiogenesis has been extensively discussed. In hepatocellular carcinoma, LOX may promote angiogenesis by facilitating VEGF secretion.[28] LOX release by tumor endothelial cells (TECs) in renal cell carcinoma is found to contribute to the pro-angiogenic phenotype of TECs by promoting their tube formation and inhibition of LOX by BAPN can reduce tumor angiogenesis, the number of circulating tumor cells (CTCs) and pulmonary metastasis.[88] Under hypoxic conditions, it is also found that LOX stabilizes HIF-1α leading to a feed-forward mechanism that increases VEGF expression levels, ultimately promoting tumor-driven angiogenesis.[114,115] These data support the notion that LOX serves as a novel angiogenesis-associated target in malignancy.

Activation of LOX-mediated vascular remodeling or angiogenesis improves disease prognosis

Activation of LOX in the process of vascular remodeling and stiffness has been reported to cure diverse pathological conditions such as arterial aneurysm and artery dissection. Arterial aneurysm is defined as a 50% increase in the normal diameter of the vessel and characterized with the weakness and local dilation of the arterial wall. AA is the common type of arterial aneurysm with high morbidity and mortality which can lead to vascular rupture or dissection.[116] The potential role of LOX in AA was identified in the 1990s, which supports that LOX is of vital importance in the maintenance of the mechanical stability of the aorta using different experimental animal models.[117–119] In fact, it is reported that *Lox* deficient mice in aortas show TAA.[67] The irreversible inhibition of LOX by BAPN promotes aortic dilation.[120,121] Furthermore, administration of BAPN to Ang II-infused *ApoE* knockout mouse, a common model of aortic abdominal aneurysm (AAA), enhances the incidence of AAA.[122] Besides, in two other experimental models of AAA caused by the elastase and the $CaCl_2$, aneurysm development is accompanied by a reduction of LOX expression and enzymatic activity.[118,119] Local overexpression of LOX by aortic adenoviral delivery limits the development of $CaCl_2$-induced AAA, at least in part by inhibiting VSMC-mediated MCP-1 secretion and JNK activity.[119,123] Meanwhile, endogenous up-regulation of LOX represses AAA, indicating the production of LOX has a therapeutic potential against AAA.[124] A reduction of vascular LOX expression has also been described in rodent models and patients with cerebral aneurysm, a pathological condition characterized with the high vascular levels of interleukin-1 β and the activation of NF-κB signaling.[125] Altogether, these data indicate that aneurysmal diseases are associated with suppression of vascular LOX activity and therapeutic approaches aiming to normalize LOX activity could limit the progression of different forms of vascular aneurysms. In terms of artery dissection (AD), it is a longitudinal tear in the vessel wall, which can create a false lumen for blood flow and may propagate quickly.[126] Similar to aneurysms, AD is also a devastating cardiovascular diseases associated with disruption of elastin fibers which is an important feature of AD.[127] LOX serves as the enzyme that initiates the irreversible covalent cross-linking of collagen and elastin in vascular tissues and its deficiency plays an important role in the occurrence of AD. When murine *Lox* gene is disrupted, the aortic walls of the $Lox^{-/-}$ fetuses show highly fragmented elastic fibers and discontinuity in the SMCs. It urges us to believe that *Lox* gene can result in structural alterations in the arterial walls, leading to the cardiovascular dysfunction.[10] It is also consistent that the decrease of LOX is vulnerable to artery injury in a clinical case of spontaneous coronary AD via disorganisation of the collagen network.[128] In fact, there are diverse investigations showing that inhibition of LOX by BAPN aggregates AD.[129–132] These data indicate that activation of LOX has therapeutic potential against AD.

Activation of LOX-mediated angiogenesis has been reported to treat ischemic stroke, a prevalent neurological disease with high rate of mortality and disability.[133] Although there is no sufficient evidence to indicate the relationship between LOX and cerebral blood vessels, it should be mentioned that the brain ECM occupies ~20% of adult brain volume which is synthesized by neuronal, glial, and endothelial cells. ECM components are arranged into basement membranes that lie outside cerebral vessels and the external layer of ECM promotes adhesion of neural cells,[134] such as terminal nerve fiber and astrocyte end-feet, which contribute to numerous vasculature functions including the regulation of blood–brain barrier (BBB) permeability.[135] Thus, it is possible that LOX reshapes ECM, affects cerebrovascular function and affects BBB penetration. A previous work shows that the *LOX* 473AA genotype, A allele, and AC haplotype are associated with increased susceptibility to ischemic stroke in a Chinese population.[133] Several factors including VEGF, TGF-β and PDGF-BB seem to prompt both angiogenesis and axonal outgrowth after cerebral ischemia.[136] Given the effects of LOX on these mediators, the role of LOX in cerebral functional recovery after ischemia is of great interest.

Taken together, the role of LOX-mediated vascular remodeling or angiogenesis is different under different disease contexts. Whether the modulation is protective or detrimental for pathological conditions requires to be carefully evaluated.

Conclusion and perspective

The evidence discussed in the review supports that LOX modulates vascular remodeling and angiogenesis in the facets of physiology and pathophysiology. Although the concrete mechanism is still in its infancy, it is definite that regulations of VSMCs migration and ECM remodeling are involved in the LOX-mediated vascular remodeling while EC activation and vascular sprouting are important for LOX-mediated angiogenesis as described above. In fact, the biological function of LOX, possible affected by expression and genetic mutation, is extensively reported in diverse pathological conditions concerning vascular remodeling and/or angiogenesis. These data altogether conclude that targeting LOX may provide a promising therapeutic strategy for the treatment of various vascular diseases.

There are some issues required to be clarified. Firstly, in addition to the established role of LOX in the modulation of vascular function, there are also several studies supporting that other members of LOX family have an impact on vascular biology. For instance, germline deletion of *LOXL2* is found to cause embryonic and neonatal mortality due to severe cardiac and vascular defects,[137] suggesting the critical importance of LOXL2 for vascular development. The definite role of LOXL2 in angiogenesis is also widely reported. Following hypoxic stimulation, LOXL2 is induced and thereby initiates the proper deposition of collagen IV in the basement membrane by tip cells.[138] The direct role of LOXL2 in angiogenesis also arises from the result that treatment with the specific LOXL2 antibody AB0023 or simtuzumab can reduce bFGF-induced angiogenesis.[139] Additionally, this enzyme is also critical for vascular stiffness as the increase in aging-induced pulse wave velocity (PWV) is ameliorated in *Loxl2*$^{+/-}$ mice.[137] Further work demonstrates that LOXL2 enhances VSMCs stiffness and contractility and induces matrix deposition. It implicates that LOXL2 can serve as a good candidate gene for aging-associated vascular stiffness. In addition, other LOX family members including LOXL1,[140] LOXL3 and LOXL4 have also been demonstrated to play a role in the integrity of the vascular wall via regulating ECM deposition.[141,142] The detailed roles and regulatory mechanisms of various LOX isoforms in vascular biology deserve further research. Secondly, with respect to the certain vascular dysfunction such as atherosclerosis, inhibition of LOX can have beneficial effects on this pathology; however, it also results in plaque instability.[143] Therefore, ongoing research is essential to explore the underlying mechanism in order to ascertain the safety of this kind of therapeutic strategy. Besides, considering the important role of *LOX* gene mutation in vascular pathology as described above, it requires more in-depth clinical trials, and the implementation of multicenter clinical investigation with large sample size is also essential to ascertain the relationship between the reported genetic mutation and vascular dysfunction, which finally draws the conclusion that these variants may serve as good biomarker for the diagnosis and prognosis of patients with vascular pathology. On the other hand, high throughout sequencing is also employed to identify the novel variants in the *LOX* gene and explore their roles in vascular diseases. Despite some mysteries remain to be clarified in the future, LOX is undoubtedly a critical regulator of vascular remodeling and angiogenesis and LOX-targeted therapy may hold great promise in human disorders associated with vascular remodeling and angiogenesis.

Author contributions

X.-Y. Mao designed the manuscript. Z.-J. Wang wrote the manuscript. X.-Y. Mao, Q.-W. Guan, F.-H. Chen and H.-H. Zhou revised the manuscript.

Conflict of interests

All authors declare no potential conflict of interests.

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Appendix A. Supplementary data

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