Abstract: Cu-dependent lysyl oxidase (LOX) plays a catalytic activity-related, primary role in the assembly of the extracellular matrix (ECM), a dynamic structural and regulatory framework which is essential for cell fate, differentiation and communication during development, tissue maintenance and repair. LOX, additionally, plays both activity-dependent and independent extracellular, intracellular and nuclear roles that fulfill significant functions in normal tissues, and contribute to vascular, cardiac, pulmonary, dermal, placenta, diaphragm, kidney and pelvic floor disorders. LOX activities have also been recognized in glioblastoma, diabetic neovascularization, osteogenic differentiation, bone matrix formation, ligament remodeling, polycystic ovary syndrome, fetal membrane rupture and tumor progression and metastasis. In an inflammatory context, LOX plays a role in diminishing pluripotent mesenchymal cell pools which are relevant to the pathology of diabetes, osteoporosis and rheumatoid arthritis. Most of these conditions involve mechanisms with complex cell and tissue type-specific interactions of LOX with signaling pathways, not only as a regulatory target, but also as an active player, including LOX-mediated alterations of cell surface receptor functions and mutual regulatory activities within signaling loops. In this review, we aim to provide insight into the diverse ways in which LOX participates in signaling events, and explore the mechanistic details and functional significance of the regulatory and cross-regulatory interactions of LOX with the EGFR, PDGF, VEGF, TGF-β, integrins, inflammation and steroid signaling pathways.

Keywords: lysyl oxidase (LOX); epidermal growth factor receptor (EGFR); platelet derived growth factor (PDGF); vascular endothelial growth factor (VEGF); transforming growth factor β (TGF-β); integrins; inflammation; steroid signaling

1. Introduction

Cu-dependent lysyl oxidase (LOX), one of five members of the LOX family, contributes to functions of the extracellular matrix (ECM) by promoting the formation of intra- and inter- molecular cross-linkages of ECM components and the development of tissue-specific insoluble matrices [1,2]. The ECM provides a local structural and signaling environment that controls cell fate, adhesion, proliferation, differentiation, migration and communication during development, tissue maintenance and repair, and various pathological processes. In addition to its activity in the ECM of skin, lung, cardiovascular, epithelial and other tissues, LOX has also been recognized as playing a significant role in cellular and nuclear functions.

The LOX gene is tightly regulated during development and aging and has been noted for its aberrant expression patterns in a range of disorders. The activation of the LOX protein is critically dependent on Cu concentrations and redistribution involving the Cu-uptake transporter-1 (CTR1) and the Cu-efflux pump ATP7A. In the ECM, the pro-LOX is proteolytically activated by procollagen C-proteinases [3] into a 30 kDa enzyme and an 18 kDa N-terminal pro-peptide fragment (LOX-PP).
The LOX-PP has a range of well-characterized functions which are independent of LOX. These activities, primarily associated with carcinogenesis, are beyond the focus area of the present manuscript, but were the subject of a recent, comprehensive review [4].

Extracellular matrix processed LOX can reenter the cytoplasm to associate with the cytoskeleton, and has been localized in the nuclei in different cell types, wherein, it was proposed to modify the chromatin structure in interactions with histones as part of epigenetic regulatory mechanisms [5,6]. The internalization and translocation of LOX into the nucleus appears to involve nuclear localization sequences, but the mechanistic details of the nuclear import of LOX remain only partly characterized [7].

A recent three dimensional (3-D) model of LOX generated by homology modeling recaptured the essential domains, the coordination of the Cu ion, the lysyl tyrosylquinone LTQ cofactor, disulfide bridges and the catalytic site within a groove surrounded by two fluctuating loops with variable openings which are suitable for the accommodation of LOX substances of different sizes [8]. LOX also participates in various activity-independent functions, the mechanistic details of which remain to be fully characterized.

Altered LOX expression and functions have been associated with vascular, cardiac, pulmonary, dermal, placenta, diaphragm, kidney and pelvic floor disorders, glioblastoma, diabetic neovascularization, osteogenic differentiation and bone matrix formation, ligament remodeling, polycystic ovary syndrome, fetal membrane rupture and stages of tumor progression and metastasis in various cancer types. Many of these processes involve interactions of LOX with signaling pathways including the epidermal growth factor receptor (EGFR), platelet derived growth factor (PDGF), vascular endothelial growth factor (VEGF), transforming growth factor β (TGF-β), ERK, NF-κB, PI3K/AKT, SMAD, MAPK, FAK/AKT, inflammatory and steroid regulatory pathways.

In this review, we aim to explore the involvement of LOX in major signaling events, including the relationships and/or mechanistic elements and functional significance of regulatory and cross-regulatory LOX interactions. Secondary signaling mechanisms due to peroxide generated during the catalytic reaction of LOX, including HIF-1α, and the involvement of LOX in various tumor types, have been reviewed extensively and are not discussed here. Tumor cell-related functions of LOX have emerged, however, within signaling frameworks, and brief summaries are provided in the relevant contexts.

2. Caveolae Compartmentalized LOX: Angiotensin II and Epidermal Growth Factor Receptor Cross-Regulation

In a murine model of angiotensin II (Ang II)-induced abdominal aortic aneurysm (AAA), genome-wide transcriptional profiling studies identified LOX, together with ADAM17 and epidermal growth factor receptor (EGFR), as highly ranked gene subnetworks [9], and localized LOX within vascular smooth muscle cell (VSCM) caveolae [10]. The membrane microdomain caveolae facilitate the temporal and spatial coordination of signaling events, including those promoted by Ang II characterized in both VSMC and endothelial cells. Caveolae have been linked to cardiovascular diseases including atherosclerosis, dyslipidemia, cardiac fibrosis, insulin resistance, inflammation, oxidative stress and AAA, many of which also involve LOX. While many of the specific details of LOX functioning in these contexts remain to be determined, those relevant to EGFR provide some degree of insight, as detailed below.

Caveolae-compartmentalized and Ang II-induced signals involve metalloprotease ADAM17/tissue necrosis factor-α (TNF-α), converting the enzyme responsible for epidermal growth factor receptor (EGFR) transactivation, as analyzed in VSMC/ADAM17-deficient mice [11]. The activated EGFR is known to regulate LOX expression via the PI3K/AKT, MEK/ERK and SAPK/JNK pathways, as observed in human non-small cell lung carcinoma cell lines and confirmed in vivo in an orthotopic metastasis mouse model and in human tumor tissue microarray analysis [12]. In turn, LOX regulation of EGFR also occurs, and was found, in primary and metastatic breast cancer cells, to be linked to the suppression of TGFβ1 signaling with the involvement of HTRA1, leading to increased expression of the EGF-like domain-containing MATN2 that traps EGFR at the cell surface, as part of its activation by EGF [13].
Ang II-induced and catalytically active (responsive to β-aminopropionitrile (BAPN) inhibition) LOX was also reported to contribute to vascular stiffening and arterial hypertension in vivo in mice [14], and to accelerate cardiac remodeling and hypertrophy in a mouse model with elevated expression of LOX in cardiomyocytes and cardiofibroblasts [15]. The Ang II-stimulated hypertensive mice overexpressing active LOX accompanied by increased H₂O₂ (the byproduct of the catalytic reaction of LOX with its substrates) showed both increased vascular stiffness and oxidative stress that promoted p38MAPK activation as part of the signaling pathway involved in hypertension-associated vascular remodeling [16].

The contribution of Ang II-associated signals to EGFR transactivation that target LOX is supported by several mouse models, human carcinoma cell lines and human tumor tissue relevant to the pathomechanisms of AAA, vascular remodeling and hypertension, and to primary and metastatic lung carcinoma. Reverse LOX-mediated regulation of EGFR cell surface availability appears to be specific to primary and metastatic breast cancer cell lines.

3. LOX in Platelet Derived Growth Factor Signaling

Among other functions, a role of catalytically-active LOX has been identified in platelet-derived growth factor (PDGF) signaling through the modification/oxidation of lysyl residues of the cell surface PDGF receptor-β (PDGFRβ) with modified (faster) turnover of the phosphorylated elements of the PDGFR-dependent regulatory pathway, including SHP2, AKT1 and ERK1/2, a process characterized during in vitro chemotactic response in rat aortic smooth muscle cells and mouse embryonic fibroblasts [17,18]. In a network analysis of low shear stress-induced vascular remodeling of rat aorta focused on endothelial and vascular smooth muscle cell interactions, increased LOX was found in parallel to increases of PDGF, TGF-β1 and phosphorylated ERK1/2 [19].

Increased LOX was found to contribute to myelofibrosis in bone marrow biopsies of patients with myeloproliferative neoplasms [20], and the link between LOX and PDGF was further explored. During megakaryocyte proliferation and differentiation, enzymatically-active LOX is highly expressed in the early stage of expansion and differentiation, and is involved in optimal PDGF signaling and cell proliferation. In later differentiation stages, LOX activity subsides, slowing proliferation and allowing differentiation of megakaryocytes into platelets, as confirmed and experimentally recaptured under BAPN inhibitory conditions in GATA-1(low) mice, where inhibition of LOX activity, via the reduction of PDGFRβ binding to cells, also reduced PDGF signaling [21].

LOX was also shown to be essential in endothelial cells in vitro and in angiogenesis in vivo by activating AKT through PDGFRβ stimulation, resulting in increased VEGF expression, a mechanism characterized in models of both colorectal cancer and breast cancer, and supported by clinical correlations between LOX, VEGF levels and blood vessel formation in colorectal tumors [22].

The role of LOX in optimizing PDGF signaling is further supported by studies of a LOX paralogue, the enzymatically active LOXL2, that, in an orthotopic oral cancer mouse model and human gingival fibroblasts, provided direct evidence for the oxidation of PDGFRβ promoting oral fibrosis and oral cancer [23,24].

Platelet derived growth factor signaling is not only modulated by active LOX, but has been reported to also control LOX. In human periodontal, ligament-derived, mesenchymal stem cells, PDGF treatment stimulated cell proliferation and induced a significant increase in LOX activity [25]. The details of the role of PDGF in controlling LOX activity, and whether this interaction is more general or limited to these stem cells, remain unclear.

Consistent in vitro and in vivo evidence demonstrated in various cell types and animal models, and supported by data with LOXL2, collectively confirm the role of LOX in modifying PDGF signaling by oxidizing the lysyl residues of the cell surface receptor PDGFRβ, thereby contributing both to the regulation of megakaryocyte expansion and differentiation, and to vascular remodeling and angiogenesis.
4. LOX and Vascular Endothelial Growth Factor

Mutual regulatory activities exist between LOX and vascular endothelial growth factor (VEGF). During angiogenesis in stimulated endothelial cells, LOX was shown to activate AKT via PDGFRβ, resulting in enhanced VEGF expression. Furthermore, LOX-promoted angiogenesis was diminished through inhibition of PDGFRβ, AKT and VEGF signaling [22]. Positive correlation of LOX and VEGF expression levels, dose-dependent upregulation of both by TGF-β, and under siRNA LOX conditions, decreased VEGF and p38 MAP signaling were noted in hepatocellular carcinoma cells [26]. In tumor tissues of hepatocellular carcinoma, upregulated LOX similarly correlated with increased VEGF and reduced overall survival related to increased tumor cell proliferation, migration and invasion [24]. In proliferative diabetic retinopathy, neovascularization and basement membrane changes are enhanced with increased glucose concentration and time exposure. The diabetic neovascular membrane was found to have increased LOX immunostaining, in spite of reduced LOX protein and activity, both of which were effectively restored by VEGF in ARPE cells [27].

Further evidence was reported for LOX in mediating VEGF-induced in vitro angiogenesis and osteogenic differentiation of human dental pulp cells. Exogenic VEGF significantly upregulated LOX mRNA and activity, together with an increase of angiogenic mRNAs and capillary tube formation. In contrast, LOX gene silencing and BAPN inhibition of LOX activity diminished the angiogenic effects of VEGF. Under these conditions, VEGF-induced phosphorylation of AKT, ERK, JNK and p38, and activation of NF-κB were inhibited by LOX silencing and BAPN [28]. Beta-aminopropionitrile (BAPN) inhibition of LOX activity, resulting in decreased phosphorylation of AKT and MAPK, was also noted during umbilical vein endothelial cell angiogenesis [29]. In a highly tumorigenic subpopulation of hepatocellular carcinoma containing tumor-initiating, sphere-forming cells that generate tumors with vascular enrichment, the upregulation of LOX correlated with an increase in secreted VEGF that promoted tube formation of endothelial cells, an effect that was blocked by BAPN, demonstrating the pivotal role of LOX in these cells [30].

The VEGF axis and LOX in HIF-1α-driven solid tumor angiogenesis showed branching [31] of the activated pathways that also induced each other [22,27]. There is a direct positive regulatory loop linking LOX and HIF-1α. LOX activated by HIF-1α can further induce HIF-1α through PI3K/AKT signaling, a process that involves LOX-generated H2O2 promoting colorectal carcinoma cell proliferation and in vivo tumor growth [32].

Other mechanisms by which LOX and VEGF are coregulated are Cu-related and involve HIF-1α and HDAC2. The chaperone function of the Cu-transport protein Antioxidant-1 (Atox-1) is an important contributor to VEGF-induced angiogenesis that involves LOX activation, as demonstrated in Atox1-deficient mice [33]. VEGF and LOX are also coregulated by the Cu-dependent activation of HIF-1α, the absence of which contributes to emphysema (shown in rat lungs) and involves histone deacetylase HDAC2-mediated expression of HIF-1α, VEGF [34].

In conclusion, there is positive coregulation of LOX and VEGF expression controlled by TGF-β in hepatocellular carcinoma cells and tumors that correlates with reduced overall survival. The coordinated upregulation of both LOX and VEGF also occurs in in vitro angiogenesis and in vivo in neovascularization in diabetic retinopathy. The Cu-dependent coregulation of VEGF and LOX plays roles in angiogenesis and in emphysema. VEGF and LOX can, furthermore, regulate each other, although this finding has only been observed in in vitro angiogenesis.

5. Cross-Regulation of Transforming Growth Factor β and LOX

Active LOX has strong associations with lung, arterial, cardiac, dermal and kidney fibrosis, and with cellular fibrotic mechanisms including platelet-induced epithelial-mesenchymal transition and fibroblast to myofibroblast transdifferentiation, specifically in wounds characterized by injury and repair cycles. In the fibrotic process, transforming growth factor-β1 (TGF-β1) signaling drives significant upregulation of the LOX mRNA, protein levels, and activity in fibroblasts, osteoblasts, epithelial and aortic smooth muscle cells. In an in vivo TGF-β1-induced synovial fibrosis model, and in end-stage
human osteoarthritis, TGF-β upregulated LOX expression was confirmed [35]. Increased TGF-β and TGF-βRII contributed to the maintenance of symptoms; however, TGF-β1 and TGF-βRII induction of LOX was limited to the initial stages of capsule deformation noted in shoulder instability [36].

LOX and the type I A1 and A2 collagen genes (COL1A1/A2) have TGF-β response promoter elements that ensure their coregulated expression [37]. Consequently, TGF-β/p38 mitogen-activated protein kinase (p38MAPK) signaling promotes both LOX levels and collagen synthesis characterized in obesity-associated, fetal muscle fibrogenesis. In adult rat cardiac fibroblasts, TGF-β1 upregulation of LOX mRNA, protein and activity (accompanied by increases in collagen type I, III and BMP-1 expression) involved PI3K, Smad3, p38-MAPK, JNK and ERK1/2, and indicated the merging of the PI3K/Akt and Smad pathways [38]. LOX upregulation by TGF-β1Smad2/3 signaling was shown to promote myocardial fibrosis and chronic heart failure. In this rat model, TGF-β1Smad2/3 promoted the expression of C-jun, an AP-1 transcription factor subunit that induced LOX gene expression. Furthermore, LOX inhibition by BAPN was shown to attenuate TGF-β-mediated downstream effects, including cardiac dysfunction, ventricular remodeling and myocardial fibrosis [39].

The involvement of the TGF-β/Smad3 pathway in regulating LOX expression was also confirmed in preeclampsia, a pregnancy-specific condition, in which decreased LOX was shown to be in close association with impaired trophoblast invasion in preeclamptic placenta. In testing trophoblast cells, knockdown of LOX suppressed cell migration and invasion with activation of the TGF-β/Smad3 pathway. This effect could be rescued by inhibiting the TGF-β/Smad3 pathways, known to be active not only in cells, but also in clinical samples [40].

In skeletal growth, SMAD4, a central element of the TGF-β/BMP pathway, controls LOX. Mice lacking Smad4 present a combination of phenotypic features which are typical in osteogenesis imperfecta, cleidocranial dysplasia and Wnt deficiency syndromes. In this model, osteoblasts fully differentiated, but the lack of Smad4- and Runx2-regulated Lox resulted in a disorganized and hypomineralized collagen matrix [41].

TGF-β is a central determinant of the cellular response to hypoxia. Coordinated upregulation of LOX and TGF-β, both at the protein and mRNA levels, have been shown to be induced in vitro and in vivo by hypoxia, and downregulated by hypercapnia in a rat model of hypoxic pulmonary hypertension [42].

A LOX/TGF-β feedback loop, characterized in skeletal muscle development, was shown to play a role in maintaining balance between muscle components. In a mouse model, myofiber-derived, secreted LOX was able to control the balance between myofibers and muscle connective tissue by modulating TGF-β1 signaling, thereby inhibiting myofiber differentiation and enhanced muscle connective tissue development [43]. LOX modulation of TGF-β signaling also occurs in idiopathic pulmonary fibrosis. In the inflammatory stage, the inhibition of LOX manifests in diminished inflammatory cell infiltration, TGF-β signaling and myofibroblast accumulation, mechanisms that are not active in the fibrogenic phase. Conversely, ectopic LOX sensitizes fibrosis-resistant mice to inflammation and bleomycin-induced lung fibrosis and is critical for the progression of lung fibrosis by enhancing the inflammatory response [44].

A notable direct interaction occurs between active LOX and TGF-β1, through which LOX can reduce TGF-β1-stimulated SMAD3 phosphorylation. While this effect depends on the amine oxidase activity of LOX, it does not involve peroxide-related mechanisms. In this direct interaction, LOX was proposed, but not proven experimentally, to be responsible for oxidative deamination of lysine residues in TGF-β1, either by changing charges or by covalently stabilizing TGF-β1 conformation. Both of these changes may reduce the efficiency of receptor binding of TGF-β1 and result in reduced signaling via SMAD3 in a cross-talk with phosphoinositide 3-kinase (PI3K) and AKT serine-threonine protein kinase [45].

There is consistent data to support TGF-β1/Smad3 signaling-driven LOX induction resulting in cellular fibrotic mechanism, multiple tissue fibrosis, ventricular remodeling and impaired trophoblast invasion in preeclampsia. LOX can also modulate TGF-β via a feedback loop which is active in skeletal muscle development and idiopathic pulmonary fibrosis. Additionally, LOX in direct interaction with TGF-β1 may reduce TGF-β1-stimulated SMAD3 activation.
6. LOX in Integrin-Mediated Mechano-Transduction

Cell-ECM interactions involve reciprocal forces that are prominently mediated and translated into intracellular signals by integrins [46], and contribute to the mechano-regulation of LOX with distinctly different control of LOX expression by low- and high-level mechanical forces. High-level stretching does not affect LOX activity, while low-level stretching enhances LOX gene expression and activity, that, together with increased expression of COL1A1 and COL3A1 genes, promote ECM stabilization, as demonstrated in human periodontal ligament cells [47]. The physical properties of the cellular microenvironment influence LOX expression via interactions of integrin α2β1 with collagen type I, as seen in cardiac fibroblasts [48]. Type I collagen (COL1) is an abundant scaffolding protein stabilized by LOX cross-linking that promotes the formation of integrin complexes, leading to the activation of the TGF-β pathway and further increase in LOX expression [49]. These pathways have also been recognized as playing significant roles within the tumor microenvironment [50]. A tissue microarray analysis of genes associated with increased stromal stiffness confirmed a relationship between elevated levels of LOX and COL1 expression involving integrin β1/GSK-3β/β-catenin signaling associated with hepatocellular carcinoma development and progression [51].

ECM stiffness promotes inflammatory activation in lung endothelial cells, a process that involves LOX activation and enhanced expression of the microtubule-associated Rho A activator GEF-H1, and that can be reversed by LOX suppression, resulting in diminished GEF-H1 and inflammation control [52]. LOX is also involved in the matrix-mediated mechanotransduction observed in posttraumatic osteoarthritis. This process involves age- and mechanical stress-induced increases in matrix stiffness, and destabilized chondrocyte catabolism and anabolism via the Rho-Rho kinase myosin light chain axis [53]. In an organotypic skin culture model of recessive dystrophic epidermolysis bullosa characterized by high risk for early cutaneous squamous cell carcinoma, injury-driven modifications of the microenvironment, including increased TFG-β, ECM cross-linking and tissue stiffening, the prominent integrin β1/pFAK/pAKT mechano-signaling proved to be both TGF-β- and LOX-dependent [54].

Elements of regulatory interactions involving LOX-mediated matrix stiffness were also revealed in hepatocellular carcinoma. The gradual upregulation of LOX and VEGF resulted in increased matrix rigidity that promoted angiogenesis with integrin β1 as the major mediator activating the PI3K/AKT pathway [55].

LOX was also demonstrated to function as a macrophage chemoattractant involving the integrin β1/PYK2 pathway to promote macrophage infiltration and, in heterotypic cell interactions, glioma cell survival and angiogenesis. The results from this study also revealed YAP1 regulation of LOX expression via SRC/AKT/YAP1. The transcriptional coactivator YAP1 functions together with TEAD transcription factors through distal enhancers involving H3K27 acetylation (H3K27ac). Specific binding of YAP1 and H3K27ac to the LOX promoter was confirmed in glioblastoma cell models. Furthermore, SRC/AKT/YAP1 and YAP1/LOX signaling were shown to positively correlate with macrophage density and reduced overall survival in glioblastoma multiforme patients [56]. The involvement of LOX, not only as a target, but as a modulator of YAP1, was reported in unilateral ureteral obstruction, where upregulation of the ERK/YAP1/Kifl5/cyclin D axis was suppressed by LOX inhibition [57].

Related to both mechano-transduction and inflammation, the mechano growth factor-E (MGF-E) in osteoarthritic fibroblast-like synoviocytes was reported to upregulate LOX mRNA, concomitant with downregulated TNF-α and interleukin-1 beta (IL-1β), resulting in mitigated inflammation [58]. Table 1 provides a summary of LOX interactions with, and contribution to, signaling mechanisms, including the regulatory elements involved and the cell, tissue types, model systems and/or pathological condition in which these interactions were identified or characterized.

In conclusion, LOX/integrin-associated interactions include a LOX/integrin/TGF-β/LOX feedback loop, integrin β1/pFAK/pAKT mechano-signaling (that is both TGF-β and LOX dependent), the integrin β1/PYK2 pathway, LOX regulation though SRC/AKT/YAP1 and a proposed LOX-mediated modulation of YAP1. These processes contribute to matrix stiffness-driven epithelial cell activation, angiogenesis, macrophage infiltration and glioma cell survival.
**Table 1.** Lysyl oxidase (LOX) regulatory and cross-regulatory interactions with signaling pathways.

| Signaling Mediators | Interaction/Activity | Signaling Pathways Involved                                                                 | Refs. |
|---------------------|----------------------|-----------------------------------------------------------------------------------------------|-------|
| Ang II             | Ang II upregulated LOX via EGFR transactivation | EGFR transactivator ADAM17, EGFR/P38/AKT, MEK/ERK and SAPK/JNK (lung carcinoma); Oxidative stress-activated p38MAPK (vascular remodeling) | [11,12,16] |
| EGFR               | LOX-controlled modulation of EGFR cell surface availability and EGF activation | Suppressed TGF-β signaling leading to HTRA1/increased MATN2 that traps EGFR at cell surface (tumor progression) | [13] |
| PDGF               | LOX-induced modification/oxidation of cell surface PDGFRβ | Faster turnover of PDGFRβ-induced VEGF (endothelial cells, hepatocellular carcinoma, diabetic neovascularization); VEGF-promoted LOX activity via Akt/ERK/JNK/p38/NF-κB (endothelial angiogenesis) | [17–20,22] |
| VEGF               | Mutual positive regulation | LOX-activated AKT via PDGFRβ/increased VEGF (endothelial cells, hepatocellular carcinoma, diabetic neovascularization); VEGF-promoted LOX activity via Akt/ERK/JNK/p38/NF-κB (endothelial angiogenesis) | [22,26–30] |
| Cu-related coregulation | VEGF/LOX upregulation by Cu-dependent activation of HIF-1α (angiogenesis); VEGF/LOX expression coordinated with HIF-1α by DAC2 | | [34] |
| Coregulation of LOX with ECM substrates | TGF-β/p38MAPK via TGF-β response promoter elements in the LOX and the COL1A1/A2 genes | | [37] |
| TGF-β              | Induction of LOX gene expression | TGF-β and TGF-β1; PI3K, Smad3, p38-MAPK, JNK, ERK1/2 (fibrosis); Smad2/3 promoted C-JUN/AP-1 (myocardial fibrosis); TGF-β/SMAD3 (preeclampsia); SMAD4 (osteogenesis) | [35,36,38–41] |
|                    | LOX/TGF-β feedback loop | LOX-modulated TGF-β1 regulating myofiber and muscle ECM balance and in inflammatory fibrotic stage (pulmonary fibrosis) | [43,44] |
|                    | Direct interaction: LOX-induced oxidative changes altered TGF-β receptor binding | Diminished TGF-β1 induced SMAD3 activation in a cross-talk with PI3K and AKT | [45] |
|                    | LOX-stabilized ECM-mediated regulation | TGF-β pathway activation and a positive feedback for LOX expression | [49,50] |
|                    | Stromal stiffness promoted LOX | Activation of integrin β1/GSK-3β-catenin (hepatocellular carcinoma) or Rho-Rho kinase myosin light chain axis (osteoarthritis) | [51,53] |
| Integrins          | ECM stiffness-driven inflammation, elevated LOX | Involving Rho activator GEF-H1 (lung endothelia) | [52] |
|                    | Injury-driven stromal alterations | TGF-β and LOX-dependent activation of integrin β1/pFAK/pAKT (epidermolysis bullosa subtype) or PI3K/AKT (angiogenesis) | [54,55] |
|                    | LOX promoted macrophage infiltration | Integrin β1/PYK2 activation via SRC/AKT/YAP1 (macrophages, glioblastoma) | [58] |

Ang II: type 2 angiotensin; EGFR: epidermal growth factor receptor LOX: lysyl oxidase; PDGF: platelet derived growth factor; VEGF: vascular endothelial growth factor; TGF-β: transforming growth factor beta; ADAM17: ADAM metallopeptidase domain 17; PI3K: phosphoinositide 3-kinase; AKT: protein kinase B; ERK: extracellular signal-regulated kinase; MEK: mitogen-activated protein kinase kinase/extracellular signal regulated kinase; SAPK: stress activated protein kinase; JNK: Jun N-terminal kinase; p38MAPK: P38 mitogen-activated protein kinase; HTRA1; M: HtrA serine peptidase 1; ATN2: atrophin 2; SHP2: SH2 domain protein tyrosine phosphatase, NF-κB: nuclear factor kappa B; HIF: hypoxia-inducible factor; ECM: extracellular matrix; COL1: type I collagen; AP-1: activator protein-1; GSK3: glycogen synthase kinase 3; GEF: guanine nucleotide exchange factor; FAK: focal adhesion kinase; PYK2: proline-rich tyrosine kinase 2; YAP: yes-associated protein.
7. LOX in Inflammatory Pathways

Chronic TNF-α-induced inflammation is a feature of lung diseases with an inflammatory component, including asthma, emphysema, fibrosis and chronic obstructive pulmonary disease. The inflammatory process is characterized by TNF-α-dependent Cu deficiency, leading to the downregulation of LOX, and is accompanied by decreased expression of Vegf and Fak, as reported in a mouse model [59]. Additionally, TNF-α regulates LOX through diverse cell- and tissue-specific transcriptional and posttranscriptional mechanisms.

In pluripotent mesenchymal cells, TNF-α was shown to downregulate LOX, independent of the Wnt3a signaling that is required for osteoblast development. In LOX-overexpressing C3H10T1/2 cells, TNF-α-mediated LOX downregulation occurred via a posttranscriptional mechanism involving miR203. Silencing of LOX in these cells resulted in suppressed growth and osteoblast differentiation, supporting the conclusion that interference with LOX expression in inflammatory conditions such as diabetes, osteoporosis and rheumatoid arthritis may result in a diminishing pluripotent cell pool, a condition known to also contribute to osteopenia [60]. Furthermore, TNF-α modulates/decreases vascular LOX expression and activity that, in endothelial cells, occurs through a transcriptional mechanism involving TNF-α receptor and protein kinase C activation [61].

In TNF-α-dependent myocardial fibrosis, however, LOX upregulation was noted and found to be due to TNF-α activation of TGF and PI3K signaling [38]. In eosinophilic esophagitis patients, fibroblast-derived TNF-α also induced epithelial LOX expression by activating nuclear factor NF-κB and TGF-β-mediated signaling. LOX upregulation in these patients was indicative of disease complications and fibrostenotic conditions [62].

The pro-inflammatory Interleukin 1β (IL-1β) contributes to LOX overexpression in polycystic ovary syndrome (PCOS), with increased LOX and IL-1β levels being observed in granulosa cells and follicular fluid of PCOS patients. In a rat model of PCOS, IL-1β was shown to increase LOX expression via ERK1/2 and JNK, and via the subsequent activation of the transcription factor c-JUN, while inhibition of LOX activity by BAPN improved some of the symptoms [63]. A contrasting IL-1β-mediated downregulation of LOX was reported in several tissues. In human amniotic tissue explants and amnion fibroblasts, IL-1β inhibited LOX via activating p38 and ERK1/2 mitogen-activated protein kinase pathways, resulting in NF-κB phosphorylation as well as GATA3. Subsequently, the activated NF-κB interacted with GATA3 at the NF-κB binding site within LOX promoter inhibiting gene expression [64].

In cerebral aneurysms, prominent degradation of the extracellular matrix and the vascular wall involves reduced LOX expression and elevated IL-1β that, in aortic smooth muscle cells, was confirmed to activate NF-κB, the inhibition of which restored LOX levels [65]. The IL-1β is a known mediator in the acute inflammatory phase of ligament injury with reduced LOX expression in ligaments [66]. In synovial fibroblasts, the combined effect of elevated TNF-α and IL-1β resulted in reduced LOX expression that, together with increased MMPs, was proposed to impede remodeling and contribute to the poor healing ability of ligaments [67].

Both, IL-1α and IL-4 are involved in ovarian surface epithelial postovulation injury and repair cycles in both pro- and anti-inflammatory mechanisms via different signaling pathways. The anti-inflammatory actions of IL-4 involving SATA6, PI3K and the p38MAPK pathways in these cells were shown to upregulate LOX mRNA levels [68]. In contrast, IL-6 stimulation in osteoblasts was reported to result, via JAK2, Fli1 and Dnmt1, in the downregulation of LOX expression by epigenetic CpG methylation, a mechanism that negatively affects bone matrix formation [69]. In a cohort of keratoconus patients with corneal thinning and structural abnormalities, reduced LOX mRNA and activity were noted in the corneal epithelia that correlated with disease severity. However, in this study, no causality was established between LOX and the parallel increase in IL-6 levels [70].

Similarly, the pro-inflammatory Interferon gamma (IFN-γ), active in aortic aneurysm, and arteriosclerotic plaque rupture, were demonstrated to reduce LOX mRNA and activity in rat aortic smooth muscle cells, partly via transcriptional downregulation, and also by reducing the LOX
mRNA half-life [71]. Additionally, IFN was shown to also reduce TGF-β-induced LOX levels in cardiac fibroblasts [72]. The mechanistic details of LOX regulation by inflammatory mediators are summarized in Table 2.

The involvement of LOX in disorders with inflammatory components has been well-established. The role of TNF-α is pleiotropic; it inhibits the functions of LOX in diabetes, osteoporosis and rheumatic arthritis, but also upregulates LOX in myocardial fibrosis and in esophagitis patients. The overall regulatory roles, mechanisms and in vivo significance of IL-1β, IL-1α, IL-4, IL-6 and IFN-γ, while recognized to affect LOX, are less clear at this stage.

Table 2. LOX regulation in inflammatory pathways.

| Inflammatory Mediators | Regulatory Activity | Signaling Pathways Involved | Refs. |
|------------------------|---------------------|-----------------------------|-------|
| TNF-α                  | LOX inhibition in chronic inflammation | TNF-α downregulation via Vegf and Fak (mouse model); miR203-mediated silencing (mesenchymal cells); TNF-α receptor and protein kinase C activation-mediated (endothelial cells) | [59–61] |
|                        | LOX upregulation    | TGF-β/PI3K signaling (myocardial fibrosis); NF-κB/TGF-β-mediated signaling (fibroblast-epithelial interactions, esophagitis) | [38,62] |
| IL-1β                  | Induced/inhibited LOX expression | Overexpression via ERK1/2/JNK and c-JUN activation (rat granulosa cells); inhibition by p38 and ERK1/2, NF-κB activation and interaction with GATA3 at the NF-κB binding LOX promoter site (amnion); via IL-1β-activated NF-κβ (aortic smooth muscle cells); IL-1β-mediated inhibition (ligaments) | [63–66] |
| IL-4                   | Pro-/anti-inflammatory activity-related upregulation of LOX | SATA6, PI3K, p38MAPK (ovarian epithelium) | [68] |
| IL-6                   | Epigenetic control of LOX expression | Downregulation through JAK2, Fli1 and Dnmt1 (osteoblasts) | [69] |
| IFN-γ                  | Pro-inflammatory control of LOX | Downregulation by transcription and mRNA half-life control (aortic smooth muscle cells, cardiac fibroblast) | [71,72] |

TNF: tumor necrosis factor; IL: interleukin; IFN: interferon; VEGF: vascular endothelial growth factor; TGF-β: transforming growth factor beta; PI3K: phosphoinositide 3-kinase; ERK: extracellular signal-regulated kinase; JNK: c-Jun N-terminal kinase; p38MAPK: P38 mitogen-activated protein kinase; NF-κB: Nuclear Factor kappa B; PI3K: phosphatidylinositol-3-kinase FAK: focal adhesion kinase; JAK: Janus kinase; Fli1: Flagellar protein; Dnmt1: DNA (cytosine-5)-methyltransferase 1; LOX: lysyl oxidase.

8. LOX in Steroid Signaling Regulatory Loops

An earlier report identified LOX as part of endocrine-, paracrine- and autocrine-level coordinated regulatory loops in rat ovaries during follicular development. Follicle stimulating hormone (FSH) was shown to be a determinant of activation or inhibition of both LOX mRNA and catalytic activity by local dihydrotestosterone, growth differentiation factor-9 (GDF-9), activin A and TGF-β1 [73]. In primary human ovarian surface epithelial cells, following IL-1α stimulation, expression profiling identified the genes involved in the synthesis of steroid hormones and their receptors, steroids and retinoids, upregulation of 11beta-hydroxysteroid dehydrogenase type 1 (11β-HSD1), suppression of steroidalogenic gene 3betaHSD1 and a subset of genes including IL-6, IL-8, NF-κB inhibitor alpha and, notably, LOX as inflammation-associated genes responsive to glucocorticoid regulation [74].

Estradiol (E2) is a positive regulator of LOX gene expression, as confirmed both in the urogenital tissues of mice with accelerated ovarian aging, and in human Ishikawa cells. In these models, as a result of inhibitory tests, it was proposed that TGF-β1 signaling mediates the E2 upregulation of LOX [75].
Studies aimed at the mechanisms responsible for fetal membrane rupture and preterm birth (including cortisol regeneration in the amnion and its role in modulating LOX regulation) identified, in human primary amniotic fibroblasts, the induction of components of prostaglandin E2 (PGE2) synthesis, cortisol stimulated 11-hydroxysteroid dehydrogenase 1 (11β-HSD1) and reciprocal inhibition of LOX. The same mechanism was confirmed in human amnion tissue explants. In contrast, LOX inhibition was effectively reversed by glucocorticoid receptor inhibition and a mutation of a negative glucocorticoid LOX promoter element. These results highlighted the role of local cortisol in the amnion in downregulating LOX gene expression through a negative response element in the LOX promoter with deficient LOX functions contributing to fetal membrane rupture [76]. Further details for prostaglandin E2 (PGE2) modulating EP2/EP4 receptor-coupled cAMP/PKA signaling have also emerged, including a feed-forward loop diminishing LOX levels in human amnion fibroblasts during end stage gestation, a mechanism that may similarly contribute to fetal membrane rupture [77]. Table 3 summarizes the LOX regulatory interactions of FHS, estradiol, cortisol and prostaglandin.

LOX has been known to be subject to glucocorticoid regulation via a negative LOX promoter element. More recent studies emphasized the significance of steroid regulation of LOX and its contribution to fetal membrane rupture.

| Steroid Hormones | Regulatory Activity | Signaling Pathways Involved | Refs. |
|------------------|---------------------|-----------------------------|-------|
| Follicle stimulating hormone | FHS activation/inhibition of LOX mRNA/activity | Local dihydrotestosterone, GDF-9, activin A, and TGF-β1 (rat ovaries) | [73] |
| Estradiol (E2) | Intersection with TGF-β1/LOX | TGF-β1-mediated E2 upregulation of LOX gene expression (mouse urogenital tissue, Ishikawa cells) | [75] |
| Cortisol | LOX inhibition by cortisol induced PGE2 and 11β-HSD1 | Regulation via the negative steroid LOX promoter element (amniotic fibroblasts and tissue, fetal membrane rupture) | [76] |
| Prostaglandin E2 (PGE2) | PGE2-induced feed-forward loop targeting LOX | EP2/EP4 receptor-coupled cAMP/PKA pathway (amniotic fibroblasts, fetal membrane rupture) | [77] |

**Table 3.** Steroid regulation of LOX in ovarian and urogenital cells and tissues and in fetal membrane rupture.

9. Conclusions and Future Directions

The involvement of LOX in regulatory pathways has long been recognized. More recent studies have revealed LOX-associated mechanisms, in which LOX is not only a target of regulatory pathways, but also an active player in signaling events that include LOX-mediated modifications of cell surface receptor functions and mutual regulatory activities in interactions with signaling mediators in processes that contribute to a range of pathological conditions.

There is, however, some degree of limitation in our current understanding of the more general roles of LOX in regulatory contexts, as the downstream consequences have not yet been fully characterized, and data were obtained in specific cell lines and/or in cell and animal disease models with only a subset of results derived from or confirmed in human in vivo studies.

It is particularly important to fill these knowledge gaps, as LOX has been shown to be a promising target for therapeutic intervention in cancer [3,78,79] and in vascular [80], myocardial [81,82], peritoneal [83] liver [84] and urological disorders [85], in adipose tissue dysfunction with an
Inflammatory component [86] and in inflammation in Crohn's disease [87]. In some of these conditions, fibrosis is the pathological endpoint linking aberrant LOX to its ECM cross-linking activity as an attractive target for LOX-inhibitory therapies. In other disorders, including those with inflammatory components, multiple upstream regulatory mechanisms are involved in controlling LOX and, importantly, LOX also contributes to signaling interactions with wide-ranging in vivo downstream effects. In order to realize the full potential of LOX as a therapeutic target with mitigated negative consequences, a more comprehensive understanding of the full spectrum of LOX functions is needed, including the mechanistic details of its involvement in regulatory pathways.

**Funding:** No external funding support was received.

**Conflicts of Interest:** The authors declare no conflict of interest.

**References**

1. Csiszar, K. Lysyl oxidases: A novel multifunctional amine oxidase family. *Prog. Nucleic Acid Res. Mol. Biol.* 2001, 70, 1–32. [CrossRef] [PubMed]

2. Umana-Diaz, C.; Pichol-Thievend, C.; Marchand, M.F.; Atlas, Y.; Salza, R.; Malbouyres, M.; Barret, A.; Teillon, J.; Ardidie-Robouant, C.; Reggiero, F.; et al. Scavenger Receptor Cysteine-Rich domains of Lysyl Oxidase-Like2 regulate endothelial ECM and angiogenesis through non-catalytic scaffolding mechanisms. *Matrix Biol.* 2020, 88, 33–52. [CrossRef] [PubMed]

3. Trackman, P.C. Lysyl Oxidase Isoforms and Potential Therapeutic Opportunities for Fibrosis and Cancer. *Expert Opin. Ther. Targets* 2016, 20, 935–945. [CrossRef] [PubMed]

4. Trackman, P.C. Functional importance of lysyl oxidase family propeptide regions. *J. Cell Commun. Signal.* 2018, 12, 45–53. [CrossRef] [PubMed]

5. Oleggini, R.; di Donato, A. Lysyl oxidase regulates MMTV promoter: Indirect evidence of histone H1 involvement. *Biochem. Cell Biol.* 2011, 89, 522–532. [CrossRef]

6. Iturbide, A.; de Herreros, A.G.; Peiró, S. A new role for LOX and LOXL2 proteins in transcription regulation. *FEBS J.* 2015, 282, 1768–1773. [CrossRef]

7. Ricard-Blum, S.; Gondelaud, F. Shuttling from the extracellular matrix to the nucleus. *Biol. Aujourd'hui* 2016, 210, 37–44. [CrossRef]

8. Vallet, S.D.; Guéroult, M.; Belloy, N.; Dauchez, M.; Ricard-Blum, S.A. Three-Dimensional Model of Human Lysyl Oxidase, a Cross-Linking Enzyme. *ACS Omega* 2019, 4, 8495–8505. [CrossRef]

9. Spin, J.M.; Hsu, M.; Azuma, J.; Tedesco, M.M.; Deng, A.; Dyer, J.S.; Maegdfessel, L.; Dalman, R.; Tsao, P.S. Transcriptional profiling and network analysis of the murine angiotensin II-induced abdominal aortic aneurysm. *Physiol. Genom.* 2011, 43, 993–1003. [CrossRef]

10. Takayanagi, T.; Crawford, K.J.; Kobayashi, T.; Obama, T.; Tsuji, T.; Elliott, K.J.; Takayanagi, T.; Crawford, K.J.; Kobayashi, T.; Obama, T.; et al. Caveolin 1 is critical for abdominal aortic aneurysm formation induced by angiotensin II and inhibition of lysyl oxidase. *Clin. Sci.* 2014, 126, 785–794. [CrossRef]

11. Kawai, T.; Takayanagi, T.; Forrester, S.J.; Preston, K.J.; Obama, T.; Tsuji, T.; Kobayashi, T.; Boyer, M.J.; Cooper, H.A.; Kwok, H.F.; et al. Vascular ADAM17 (a Disintegrin and Metalloproteinase Domain 17) Is Required for Angiotensin II/β-Aminopropionitrile-Induced Abdominal Aortic Aneurysm. *Hypertension* 2017, 70, 959–963. [CrossRef]

12. Hou, X.; Du, H.; Quan, X.; Shi, L.; Zhang, Q.; Wu, Y.; Liu, Y.; Xiao, J.; Li, Y.; Lu, L.; et al. Silibinin Inhibits NSCLC Metastasis by Targeting the EGFR/LOX Pathway. *Front. Pharmacol.* 2018, 9, 21. [CrossRef] [PubMed]

13. Tang, H.; Tang, H.; Leung, I.; Saturno, G.; Viros, A.; Smith, D.; Di Leva, G.; Morrison, E.; Niculescu-Duvaz, D.; Lopes, F.; et al. Lysyl oxidase drives tumour progression by trapping EGF receptors at the cell surface. *Nat. Commun.* 2017, 8, 14099. [CrossRef] [PubMed]

14. Ebersohn, L.S.; Sanchez, P.A.; Majeed, B.A.; Tawinwung, S.; Secomb, T.W.; Larson, D.F. Effect of lysyl oxidase inhibition on angiotensin II-induced arterial hypertension, remodeling, and stiffness. *PloS ONE* 2015, 10, e0124013. [CrossRef]

15. Galán, M.; Varona, S.; Guadall, A.; Orriols, M.; Navas, M.; Aguiló, S.; de Diego, A.; Navarro, M.A.; García-Dorado, D.; Rodriguez-Sinovas, A.; et al. Lysyl oxidase overexpression accelerates cardiac remodeling and aggravates angiotensin II-induced hypertrophy. *FASEB J.* 2017, 31, 3787–3799. [CrossRef] [PubMed]
16. Martínez-Revelles, S.; García-Redondo, A.B.; Avendaño, M.S.; Varona, S.; Palao, T.; Orriols, M.; Roque, F.R.; Fortuño, A.; Touyz, R.M.; Martínez-González, J.; et al. Lysyl Oxidase Induces Vascular Oxidative Stress and Contributes to Arterial Stiffness and Abnormal Elastin Structure in Hypertension: Role of p38MAPK. *Antioxid. Redox. Signal.* 2017, 27, 379–397. [CrossRef]

17. Lucero, H.A.; Ravid, K.; Grimsby, J.L.; Rich, C.B.; DiCamillo, S.J.; Mäki, J.M.; Myllyharju, J.; Kagan, H.M. Lysyl oxidase oxidizes cell membrane proteins and enhances the chemotactic response of vascular smooth muscle cells. *J. Biol. Chem.* 2008, 283, 24103–24117. [CrossRef]

18. Lucero, H.A.; Mäki, J.M.; Kagan, H.M. Activation of cellular chemotactic responses to chemokines coupled with oxidation of plasma membrane proteins by lysyl oxidase. *J. Neural Transm.* 2011, 118, 1091–1099. [CrossRef]

19. Qi, Y.X.; Jiang, J.; Jiang, X.H.; Wang, X.D.; Ji, S.Y.; Han, Y.; Long, D.K.; Shen, B.R.; Yan, Z.Q.; Chien, S.; et al. PDGF-BB and TGF-β1 on cross-talk between endothelial and smooth muscle cells in vascular remodeling induced by low shear stress. *Proc. Natl. Acad. Sci. USA* 2011, 108, 1908–1913. [CrossRef]

20. Tadmor, T.; Bejar, J.; Attias, D.; Mischenko, E.; Sabo, E.; Neufeld, G.; Vadasz, Z. The expression of lysyl-oxidase gene family members in myeloproliferative neoplasms. *Am. J. Hematol.* 2013, 88, 355–358. [CrossRef]

21. Eliades, A.; Papadantonakis, N.; Bhupatiraju, A.; Burridge, K.A.; Johnston-Cox, H.A.; Migliaccio, A.R.; Crispino, J.D.; Lucero, H.A.; Trackman, P.C.; Ravid, K.; et al. Control of megakaryocyte expansion and bone marrow fibrosis by lysyl oxidase. *J. Biol. Chem.* 2011, 286, 27630–27638. [CrossRef]

22. Baker, A.M.; Bird, D.; Welti, J.C.; Gourlaouen, M.; Murray, G.I.; Reynolds, A.R.; Cox, T.R.; Erler, J.T. Lysyl oxidase plays a critical role in endothelial cell stimulation to drive tumor angiogenesis. *Cancer Res.* 2013, 73, 583–594. [CrossRef] [PubMed]

23. Saxena, D.; Mahjour, F.; Findlay, A.D.; Mously, E.A.; Kantarci, A.; Trackman, P.C. Multiple Functions of Lysyl Oxidase Like-2 in Oral Fibroproliferative Processes. *J. Dent. Res.* 2018, 97, 1277–1284. [CrossRef] [PubMed]

24. Mahjour, F.; Dambal, V.; Shrestha, N.; Singh, V.; Noonan, V.; Kantarci, A.; Trackman, P.C. Mechanism for Oral Tumor Cell Lysyl Oxidase like-2 in Cancer Development: Synergy With PDGF-AB. *Oncogenesis* 2019, 8, 34. [CrossRef] [PubMed]

25. Mihaylova, Z.; Tsikandelova, R.; Sanimirov, P.; Gateva, N.; Mitev, V.; Ishkittiev, N. Role of PDGF-BB in proliferation, differentiation and maintaining stem cell properties of PDL cells in vitro. *Arch. Oral Biol.* 2018, 85, 1–9. [CrossRef]

26. Zhu, J.; Huang, S.; Wu, G.; Huang, C.; Li, X.; Chen, Z.; Zhao, L.; Zhao, Y. Lysyl Oxidase Is Predictive of Unfavorable Outcomes and Essential for Regulation of Vascular Endothelial Growth Factor in Hepatocellular Carcinoma. *Dig. Dis. Sci.* 2015, 60, 3019–3031. [CrossRef]

27. Coral, K.; Madhavan, J.; Pukhraj, R.; Angayarkanni, N. High glucose induced differential expression of lysyl oxidase and its isoform in ARPE-19 cells. *Curr. Eye Res.* 2013, 38, 194–203. [CrossRef]

28. Bae, W.J.; Yi, J.K.; Park, J.; Kang, S.K.; Jang, J.H.; Kim, E.C. Lysyl oxidase-mediated VEGF-induced differentiation and angiogenesis in human dental pulp cells. *Int. Endod. J.* 2018, 51, 335–346. [CrossRef]

29. Shi, L.; Zhang, N.; Liu, H.; Zhao, L.; Liu, J.; Wan, J.; Wu, W.; Lei, H.; Liu, R.; Han, M. Lysyl oxidase inhibition via β-aminopropionitrile hampers human umbilical vein endothelial cell angiogenesis and migration in vitro. *Mol. Med. Rep.* 2018, 17, 5029–5036. [CrossRef]

30. Yang, M.; Liu, J.; Wang, F.; Tian, Z.; Ma, B.; Li, Z.; Wang, B.; Zhao, W. Lysyl oxidase assists tumor-initiating cells to enhance angiogenesis in hepatocellular carcinoma. *Int. J. Oncol.* 2019, 54, 1398–1408. [CrossRef]

31. Fraga, A.; Ribeiro, R.; Principe, P.; Lopes, C.; Medeiros, R. Hypoxia and Prostate Cancer Aggressiveness: A Tale with Many Endings. *Clin. Genitourin. Cancer* 2015, 13, 295–301. [CrossRef] [PubMed]

32. Pez, F.; Dayan, F.; Durivault, J.; Kaniekswi, B.; Aimond, G.; Le Provost, G.S.; Deux, B.; Clézardin, P.; Sommer, P.; Pouysségur, J.; et al. The HIF-1-inducible lysyl oxidase activates HIF-1 via the Akt pathway in a positive regulation loop and synergizes with HIF-1 in promoting tumor cell growth. *Cancer Res.* 2011, 71, 1647–1657. [CrossRef]

33. Chen, G.; Sudhabhar, Y.; Youn, S.W.; Das, A.; Cho, J.; Kiamiya, T.; Urao, N.; McKinney, R.D.; Surenkhuu, B.; Hamakubo, T.; et al. Copper Transport Protein Antioxidant-1 Promotes Inflammatory Neovascularization via Chaperone and Transcription Factor Function. *Sci. Rep.* 2015, 5, 14780. [CrossRef]

34. Mizuno, S.; Yasuo, M.; Bogaard, H.J.; Kraskauskas, D.; Alhussaini, A.; Gomez-Arroyo, J.; Farkas, D.; Farkas, L.; Voelkel, N.F. Copper deficiency induced emphysema is associated with focal adhesion kinase inactivation. *PLoS ONE* 2012, 7, e30678. [CrossRef] [PubMed]
35. Remst, D.F.; Blom, A.B.; Vitters, E.L.; Bank, R.A.; van den Berg, W.B.; Blaney Davidson, E.N.; van der Kraan, P.M. Gene expression analysis of murine and human osteoarthritis synovium reveals elevation of transforming growth factor β-responsive genes in osteoarthritis-related fibrosis. *Arthritis Rheumatol.* 2014, 66, 647–656. [CrossRef]

36. Belangero, P.S.; Leal, M.F.; Cohen, C.; Figueiredo, E.A.; Smith, M.C.; Andreoli, C.V.; de Castro Pochini, A.; Ejnisman, B.; Cohen, M. Expression analysis of genes involved in collagen cross-linking and its regulation in traumatic anterior shoulder instability. *J. Orthop. Res.* 2016, 34, 510–517. [CrossRef] [PubMed]

37. Hong, H.H.; Trackman, P.C. Cytokine regulation of gingival fibroblast lysyl oxidase, collagen, and elastin. *J. Periodontol.* 2002, 73, 145–152. [CrossRef]

38. Voloshenyuk, T.G.; Hart, A.D.; Khutorova, E.; Gardner, J.D. TNF-α increases cardiac fibroblast lysyl oxidase expression through TGF-β and PI3Kinase signaling pathway. *Biochem. Biophys. Res. Commun.* 2011, 413, 370–375. [CrossRef]

39. Lu, M.; Qin, Q.; Yao, J.; Sun, L.; Qin, X. Induction of LOX by TGF-β1/Smad/AP-1 signaling aggravates rat myocardial fibrosis and heart failure. *IUBMB Life* 2019, 71, 1729–1739. [CrossRef]

40. Xu, X.H.; Jia, Y.; Zhou, X.; Xie, D.; Huang, X.; Jia, L.; Zhou, Q.; Zheng, Q.; Wang, K.; Jin, L.P. Downregulation of lysyl oxidase and lysyl oxidase-like protein 2 suppressed the migration and invasion of trophoblasts by activating the TGF-β/collagen pathway in preeclampsia. *Exp. Mol. Med.* 2019, 51, 20. [CrossRef]

41. Salazar, V.S.; Zarkadis, N.; Huang, L.; Norris, J.; Grimston, S.K.; Mbalaviele, G.; Civitelli, R. Embryonic ablation of osteoblast Smad4 interrupts matrix synthesis in response to canonical Wnt signaling and causes an osteogenesis-imperfecta-like phenotype. *J. Cell Sci.* 2013, 126, 4974–4984. [CrossRef] [PubMed]

42. Xia, X.D.; Peng, Y.P.; Lei, D.; Chen, W.Q. Hypercapnia downregulates hypoxia-induced lysyl oxidase expression in pulmonary artery smooth muscle cells via inhibiting transforming growth factor β1 signaling. *Cell Biochem. Funct.* 2019, 37, 193–202. [CrossRef] [PubMed]

43. Kutchuk, L.; Laitala, A.; Soueid-Bomgarten, S.; Shentzer, P.; Rosendahl, A.H.; Eilot, S.; Grossman, M.; Sagi, I.; Sormunen, R.; Myllyharju, J.; et al. Muscle composition is regulated by a Lox-TGFβ feedback loop. *Development* 2015, 142, 983–993. [CrossRef] [PubMed]

44. Cheng, T.; Liu, Q.; Zhang, R.; Zhang, Y.; Chen, J.; Yu, R.; Ge, G. Lysyl oxidase promotes bleomycin-induced lung fibrosis through modulating inflammation. *J. Mol. Cell Biol.* 2014, 6, 506–515. [CrossRef] [PubMed]

45. Atsawasuwun, P.; Mochida, Y.; Katafuchi, M.; Kaku, M.; Fong, K.S.; Csizsar, K.; Yamauchi, M. Lysyl oxidase binds transforming growth factor-beta and regulates its signaling via amine oxidase activity. *J. Biol. Chem.* 2008, 283, 34229–34240. [CrossRef]

46. Handorf, A.M.; Zhou, Y.; Halanski, M.A.; Li, W.J. Tissue stiffness dictates development, homeostasis, and disease progression. *Organo genesis* 2015, 11, 1–15. [CrossRef]

47. Chen, Y.J.; Jeng, J.H.; Chang, H.H.; Huang, M.Y.; Tsai, F.F.; Yao, C.C. Differential regulation of collagen, lysyl oxidase and MMP-2 in human periodontal ligament cells by low- and high-level mechanical stretching. *J. Periodontal Res.* 2013, 48, 466–474. [CrossRef]

48. Gao, A.E.; Sullivan, K.E.; Black, L.D. Lysyl oxidase expression in cardiac fibroblasts is regulated by α2β1 integrin interactions with the cellular microenvironment. *Biochem. Biophys. Res. Commun.* 2016, 475, 70–75. [CrossRef]

49. Voloshenyuk, T.G.; Landesman, E.S.; Khutorova, E.; Hart, A.D.; Gardner, J.D. Induction of cardiac fibroblast lysyl oxidase by TGF-β1 requires PI3K/Akt, Smad3, and MAPK signaling. *Cytokine* 2011, 55, 190–197. [CrossRef]

50. Amendola, P.G.; Reuten, R.; Erler, J.T. Interplay Between LOX Enzymes and Integrins in the Tumor Microenvironment. *Cancers* 2019, 11, 729. [CrossRef]

51. You, Y.; Zheng, Q.; Dong, Y.; Wang, Y.; Zhang, L.; Xue, T.; Xie, X.; Hu, C.; Wang, Z.; Chen, R.; et al. Higher Matrix Stiffness Upregulates Osteopontin Expression in Hepatocellular Carcinoma Cells Mediated by Integrin β1/GSK3β/β-Catenin Signaling Pathway. *PLoS ONE* 2015, 10, e0134243. [CrossRef] [PubMed]

52. Mambetsariev, I.; Tian, Y.; Wu, T.; Lavoie, T.; Solway, J.; Birukov, K.G.; Birukova, A.A. Stiffness-activated GEF-H1 expression exacerbates LPS-induced lung inflammation. *PLoS ONE* 2014, 9, e92670. [CrossRef] [PubMed]

53. Kim, J.H.; Lee, G.; Won, Y.; Lee, M.; Kwak, J.S.; Chun, C.H.; Chun, J.S. Matrix cross-linking-mediated mechanotransduction promotes posttraumatic osteoarthritis. *Proc. Natl Acad. Sci. USA* 2015, 112, 9424–9429. [CrossRef] [PubMed]
54. Mittapalli, V.R.; Madl, J.; Löffek, S.; Kiritsi, D.; Kern, J.S.; Römer, W.; Nyström, A.; Bruckner-Tuderman, L. Injury-Driven Stiffening of the Dermis Expedites Skin Carcinoma Progression. *Cancer Res.* 2016, 76, 940–951. [CrossRef]

55. Dong, Y.; Xie, X.; Wang, Z.; Hu, C.; Zheng, Q.; Wang, Y.; Chen, R.; Xue, T.; Chen, J.; Gao, D.; et al. Increasing matrix stiffness upregulates vascular endothelial growth factor expression in hepatocellular carcinoma cells mediated by integrin β1. *Biochem. Biophys. Res. Commun.* 2014, 444, 427–432. [CrossRef]

56. Chen, P.; Zhao, D.; Li, J.; Liang, X.; Chang, A.; Henry, V.K.; Lan, Z.; Spring, D.J.; Rao, G.; Wang, Y.A. Symbiotic Macrophage-Glioma Cell Interactions Reveal Synthetic Lethality in PTEN-Null Glioma. *Cancer Cell* 2019, 35, 868–884.e6. [CrossRef]

57. Chen, W.C.; Lin, H.H.; Tang, M. Matrix-Stiffness-Regulated Inverse Expression of Krüppel-Like Factor 5 and Krüppel-Like Factor 4 in the Pathogenesis of Renal Fibrosis. *Am. J. Pathol.* 2015, 185, 2468–2481. [CrossRef]

58. Li, H.; Lei, M.; Yu, C.; Lv, Y.; Song, Y.; Yang, L. Mechano growth factor-E regulates apoptosis and inflammatory responses in fibroblast-like synoviocytes of knee osteoarthritis. *Int. Orthop.* 2015, 39, 2503–2509. [CrossRef]

59. Liu, L.; Geng, X.; McDermott, J.; Shen, J.; Corbin, C.; Xuan, S.; Kim, J.; Zuo, L.; Liu, Z. Copper Deficiency in the Lungs of TNF-α Transgenic Mice. *Front. Physiol.* 2016, 7, 234. [CrossRef]

60. Khosravi, R.; Sodek, K.L.; Xu, W.P.; Bais, M.V.; Saxena, D.; Faibish, M.; Trackman, P.C. A novel function of lysyl oxidase in pluripotent mesenchymal cell proliferation and relevance to inflammation-associated osteopenia. *PLoS ONE* 2014, 9, e100669. [CrossRef]

61. Alcudia, J.F.; Martínez-Gonzalez, J.; Guadall, A.; Gonzalez-Diez, M.; Badimon, L.; Rodriguez, C. Lysyl oxidase and endothelial dysfunction: Mechanisms of lysyl oxidase down-regulation by pro-inflammatory cytokines. *Front. Biosci.* 2008, 13, 2721–2727. [CrossRef] [PubMed]

62. Kasagi, Y.; Dods, K.; Wang, J.X.; Chandramouleeswaran, P.M.; Benitez, A.J.; Gambanga, F.; Kluger, J.; Ashorobi, T.; Gross, J.; Tobias, J.W.; et al. Fibrostenotic eosinophilic esophagitis might reflect epithelial lysyl oxidase induction by fibroblast-derived TNF-α. *J. Allergy Clin. Immunol.* 2019, 144, 171–182. [CrossRef] [PubMed]

63. Zhang, C.; Wang, C.; Yin, L.; Xu, C.; Zhang, Y.; Sung, K.L. Interleukin-1 beta influences on lysyl oxidase expression in pluripotent embryonal stem cells. *Int. Orthop.* 2015, 39, 1080–1086. [CrossRef] [PubMed]

64. Zhang, C.; Wang, W.; Liu, C.; Lu, J.; Sun, K. Role of NF-κB/GATA3 in the inhibition of lysyl oxidase by IL-1β in human amnion fibroblasts. *Immunol. Cell Biol.* 2017, 95, 943–952. [CrossRef] [PubMed]

65. Aoki, T.; Kataoka, H.; Ishibashi, R.; Nozaki, K.; Morishita, R.; Hashimoto, N. Reduced collagen biosynthesis is the hallmark of cerebral aneurysm: Contribution of interleukin-1beta and nuclear factor-κB. *Arterioscler. Thromb. Vasc. Biol.* 2009, 29, 1080–1086. [CrossRef]

66. Xie, J.; Wang, C.; Yin, L.; Xu, C.; Zhang, Y.; Sung, K.L. Interleukin-1 beta influences on lysyl oxidases and matrix metalloproteinases profile of injured anterior cruciate ligament and medial collateral ligament fibroblasts. *Int. Orthop.* 2013, 37, 495–505. [CrossRef] [PubMed]

67. Zhang, Y.; Jiang, J.; Xie, J.; Xu, C.; Wang, C.; Yin, L.; Yang, L.; Sung, K.P. Combined effects of tumor necrosis factor-α and interleukin-1β on lysyl oxidase and matrix metalloproteinase expression in human knee synovial fibroblasts. *Exp. Ther. Med.* 2017, 14, 5258–5266. [CrossRef]

68. Papacleovoulou, G.; Crichley, H.O.; Hillier, S.G.; Mason, J.I. IL1α and IL4 signalling in human ovarian surface epithelial cells. *J. Endocrinol.* 2011, 211, 273–283. [CrossRef]

69. Thaler, R.; Agsten, M.; Spitzer, S.; Paschalis, E.P.; Karlic, H.; Klaushefer, K.; Varqa, F. Homocysteine suppresses the expression of the collagen cross-linker lysyl oxidase involving IL-6, Fli1, and epigenetic DNA methylation. *J. Biol. Chem.* 2011, 286, 5578–5588. [CrossRef]

70. Shetty, R.; Sathyarayananmooorthy, A.; Ramachandra, R.A.; Arora, V.; Ghosh, A.; Srivatsa, P.R.; Pahuja, N.; Nuijs, R.M.; Sinha-Roy, A.; Mohan, R.R. Attenuation of lysyl oxidase and collagen gene expression in keratoconus patient corneal epithelium corresponds to disease severity. *Mol. Vis.* 2015, 21, 12–25.

71. Song, Y.L.; Ford, J.W.; Gordon, D.; Shanley, C.J. Regulation of lysyl oxidase by interferon-gamma in rat aortic smooth muscle cells. *Arterioscler. Thromb. Vasc. Biol.* 2000, 20, 982–988. [CrossRef]

72. Pappritz, K.; Savvatis, K.; Koschel, A.; Miteva, K.; Tschöpe, C.; van Linthtout, S. Cardiac (myo)fibroblasts modulate the migration of monocyte subsets. *Sci. Rep.* 2018, 8, 5575. [CrossRef] [PubMed]

73. Harlow, C.R.; Rae, M.; Davidson, L.; Trackman, P.C.; Hillier, S.G. Lysyl oxidase gene expression and enzyme activity in the rat ovary: Regulation by follicle-stimulating hormone, androgen, and transforming growth factor-beta superfamily members in vitro. *Endocrinology* 2003, 144, 154–162. [CrossRef] [PubMed]
74. Rae, M.T.; Niven, D.; Ross, A.; Forster, T.; Lathe, R.; Critchley, H.O.; Ghazal, P.; Hillier, S.G. Steroid signalling in human ovarian surface epithelial cells: The response to interleukin-1alpha determined by microarray analysis. *J. Endocrinol.* 2004, 183, 19–28. [CrossRef] [PubMed]
75. Zong, W.; Jiang, Y.; Zhao, J.; Zhang, J.; Gao, J.G. Estradiol plays a role in regulating the expression of lysyl oxidase family genes in mouse urogenital tissues and human Ishikawa cells. *J. Zhejiang Univ. Sci. B* 2015, 16, 857–864. [CrossRef] [PubMed]
76. Liu, C.; Guo, C.; Wang, W.; Zhu, P.; Li, W.; Mi, Y.; Myatt, L.; Sun, K. Inhibition of Lysyl Oxidase by Cortisol Regeneration in Human Amnion: Implications for Rupture of Fetal Membranes. *Endocrinology* 2016, 157, 4055–4065. [CrossRef]
77. Liu, C.; Zhu, P.; Wang, W.; Li, W.; Shu, Q.; Chen, Z.J.; Myatt, L.; Sun, K. Inhibition of lysyl oxidase by prostaglandin E2 via EP2/EP4 receptors in human amnion fibroblasts: Implications for parturition. *Mol. Cell Endocrinol.* 2016, 424, 118–127. [CrossRef]
78. Cox, T.R.; Gartland, A.; Erler, J.T. Lysyl Oxidase, a Targetable Secreted Molecule Involved in Cancer Metastasis. *Cancer Res.* 2016, 76, 188–192. [CrossRef]
79. Leung, L.; Niculescu-Duvaz, D.; Smithen, D.; Lopes, F.; Callens, C.; McLeary, R.; Saturno, G.; Davies, L.; Aljarah, M.; Brown, M.; et al. Anti-metastatic Inhibitors of Lysyl Oxidase (LOX): Design and Structure-Activity Relationships. *J. Med. Chem.* 2019, 62, 5863–5884. [CrossRef]
80. Páramo, J.A. New mechanisms of vascular fibrosis: Role of lysyl oxidase. *Clin. Investig. Arterioscler.* 2017, 29, 166–167. [CrossRef]
81. López, B.; González, A.; Hermida, N.; Valencia, F.; de Teresa, E.; Diez, J. Role of lysyl oxidase in myocardial fibrosis: From basic science to clinical aspects. *Am. J. Physiol. Heart Circ. Physiol.* 2010, 299, H1–H9. [CrossRef] [PubMed]
82. Rodriguez, C.; Martinez-González, J. The Role of Lysyl Oxidase Enzymes in Cardiac Function and Remodeling. *Cells* 2019, 8, 1483. [CrossRef] [PubMed]
83. Harlow, C.R.; Wu, X.; van Deemter, M.; Gardiner, F.; Poland, C.; Green, R.; Sarvi, S.; Brown, P.; Kadler, K.E.; Lu, Y.; et al. Targeting lysyl oxidase reduces peritoneal fibrosis. *PLoS ONE* 2017, 12, e0183013. [CrossRef] [PubMed]
84. Chen, W.; Yang, A.; Jia, J.; Popov, Y.V.; Schuppan, D.; You, H. Lysyl oxidase (LOX) family members: Rationale and their potential as therapeutic targets for liver fibrosis. *Hepatology* 2020. accepted for publication. [CrossRef]
85. Li, T.; Wu, C.; Gao, L.; Qin, F.; Wei, Q.; Yuan, J. Lysyl oxidase family members in urological tumorigenesis and fibrosis. *Oncotarget* 2018, 9, 20156–20164. [CrossRef]
86. Pastel, E.; Price, E.; Sjöholm, K.; McCulloch, L.J.; Rittig, N.; Liversedge, N.; Knight, B.; Möller, N.; Svensson, P.A.; Kos, K. Lysyl oxidase and adipose tissue dysfunction. *Metabolism* 2018, 78, 118–127. [CrossRef]
87. De Bruyn, J.R.; van den Brink, G.R.; Steenkamer, J.; Buskens, C.J.; Bemelman, W.A.; Meisner, S.; Muncan, V.; Velde, A.A.T.; D’Haens, G.R.; Wildenberg, M.E. Fibrostenotic Phenotype of Myofibroblasts in Crohn’s Disease is Dependent on Tissue Stiffness and Reversed by LOX Inhibition. *J. Crohns Colitis* 2018, 12, 849–859. [CrossRef]