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Lysyl Oxidase Isoforms and Potential Therapeutic Opportunities for Fibrosis and Cancer

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Abstract

Introduction—The lysyl oxidase family of enzymes is classically known as being required for connective tissue maturation by oxidizing lysine residues in elastin and lysine and hydroxylysine residues in collagen precursors. The resulting aldehydes then participate in cross-link formation, which is required for normal connective tissue integrity. These enzymes have biological functions that extend beyond this fundamental biosynthetic role, with contributions to angiogenesis, cell proliferation, and cell differentiation. Dysregulation of lysyl oxidases occurs in multiple pathologies including fibrosis, primary and metastatic cancers, and complications of diabetes in a variety of tissues.

Areas covered—This review summarizes the major findings of novel roles for lysyl oxidases in pathologies, and highlights some of the potential therapeutic approaches that are in development and which stem from these new findings.

Expert opinion—Fundamental questions remain regarding the mechanisms of novel biological functions of this family of proteins, and regarding functions that are independent of their catalytic enzyme activity. However, progress is underway in the development of isoform-specific pharmacologic inhibitors, potential therapeutic antibodies and gaining an increased understanding of both tumor suppressor and metastasis promotion activities. Ultimately, this is likely to lead to novel therapeutic agents.

Keywords

cancer; copper amine oxidases; fibrosis; lysyl oxidase propeptide; lysyl oxidases; lysyl tyrosylquinone

1. Introduction

The well-established functions of lysyl oxidases are to catalyze the final enzyme reaction required for biosynthetic cross-linking of collagens and elastin [1]. Studies over the last 15

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years, however, have identified a variety of additional biological activities including keratinocyte [2, 3] and chondrocyte differentiation [4], tumor growth inhibition [5, 6], promotion of metastasis in cancer [7], stimulation of cell proliferation [8], cell migration [9], angiogenesis [10], and extracellular matrix maturation [11]. Understanding the biology and mechanisms of all functions of each lysyl oxidase isoform is likely to provide significant therapeutic opportunities in addressing both cancer and fibrosis. This review is focused on fibrosis and cancer, and particularly highlights novel mechanistic relationships that extend beyond the known functions of lysyl oxidases in collagen biosynthesis.

2. Body

The lysyl oxidase family consists of five members in mammals designated lysyl oxidase (LOX) and lysyl oxidase like-1 through lysyl oxidase like-4 (LOXL1 - LOXL4). All five members have a conserved C-terminal domain that contains the active enzyme region, and a more variable N-terminal pro-region. All members have a signal peptide and are secreted into the extracellular environment. LOX and LOXL1 both undergo extracellular proteolytic processing by procollagen C-proteinases [12, 13], while it is unclear whether or not LOXL2 – LOXL4 require processing for activation [14]. LOXL2 – LOXL4 is a sub-group within the family based on the common feature of their propeptide domains which all contain four scavenger receptor cysteine rich domains which are not present in LOX or LOXL1 [15, 16]. The functions of the pro-regions of LOXL2 – LOXL4 are currently unknown. The propeptide domain of LOX, known as LOX-PP, has tumor growth inhibitory properties that will be reviewed in detail below. The pro-region of LOXL1 appears to help with its own trafficking and activation by procollagen C-proteinase [17].

All lysyl oxidase isoforms incorporate copper intracellularly, likely dependent on the copper chaperone ATP7a [18]. Copper binding is dependent on conserved amino acid residues in the active site in LOX [19, 20], and copper incorporation is required for optimal lysyl oxidase enzyme activity [21]. All five isoforms have a conserved tyrosine and lysine residue which in LOX becomes the active site carbonyl cofactor lysyl tyrosyl quinone (LTQ). This cofactor is required for lysyl oxidase oxidation of its primary amine substrates: the epsilon amino group of lysine or hydroxylysine residues in collagens and lysine residues in elastin [22, 23]. By analogy to serum amine oxidases, copper is required for biogenesis of the LTQ cofactor, and this conversion does not appear to require a different enzyme protein for LTQ generation [23-25]. In support, copper-binding residues were experimentally identified by site-directed mutagenesis loss of function studies of recombinant mouse LOX in which histidine residues 292, 294, and 296 were found to be required for LTQ generation in mouse LOX. Thus, a major function of copper bound in LOX is LTQ cofactor generation, which is a spontaneous auto-catalyzed post-translational modification required for ultimate acquisition of enzyme activity. In addition, it has been suggested that copper may have a role in stabilizing the structure of LOX [21], though this has not been directly evaluated.

The catalytic mechanism of the LOX reaction has been reviewed previously [26] and is presented in Figure 1. Noteworthy is the requirement of Schiff base formation with the LTQ cofactor in the first step, which then facilitates the required redox reaction [26]. This reaction mechanism is the same as that which is known for other copper-dependent quinone

containing amine oxidases which catalyze oxidative deamination of primary amines and for which considerable experimental evidence in support also exists [27-29]. It is notable that secondary and teritiary amines have been shown not to function as substrates for lysyl oxidase and are not oxidized [30] likely because Schiff base formation cannot occur.

2.1 Activities of each lysyl oxidase isoform and potential therapeutic opportunities

2.1.1 Lysyl oxidase (LOX)—Lysyl oxidase (LOX) may be the best studied isoform to date and was the first one cloned [31, 32]. It is synthesized as a 50 kDa pre-proenzyme and like all five isoforms undergoes signal peptide removal and intracellular N-glycosylation in the endoplasmic reticulum. The 50 kDa LOX proenzyme is secreted into the extracellular environment as an inactive precursor [33]. Pro-LOX then undergoes proteolytic processing by procollagen C-proteinases which are products of the *BMP1*, *TLL1* and *TLL2* genes [12, 13]. This processing releases the active enzyme and the lysyl oxidase propeptide LOX-PP. Active LOX and LOX-PP each have independent biological activities.

2.1.1.1 Active LOX biological activities: Lysyl oxidase (LOX) is the most abundantly expressed isoform in most, but not all tissues [34-36]. As noted, LOX is required for the biosynthesis of normal collagens and elastin, and therefore LOX is critical for vascular, mineralized and non-mineralized connective tissues. LOX knockout mice exhibit perinatal death due to vascular and cardiovascular defects [37, 38]. In fibrotic disease, elevated lysyl oxidase activity is consistently observed and contributes to resistance of the extracellular matrix to proteolytic degradation, thereby contributing to connective tissue accumulation and fibrosis (reviewed in [39]). Some recent studies of fibrosis have included a comprehensive analysis of LOX isoforms expression, while other studies have focused on LOX activity without assessing for the genetic source, or assessed for LOX expression only, or only by inhibition by β-aminopropionitrile (BAPN) which inhibits all five isoforms [40-48]. Although some reports may suggest that BAPN is inactive against some LOX isoforms in vivo, it should be understood that BAPN can serve as a substrate for some serum amine oxidases which do not belong to the LOX family. Thus, if BAPN appears to not inhibit a LOX isoform in vivo, then it is likely that (1) either BAPN has been metabolized, or (2) that the activity in question occurs as a result of a non-enzymatic function of LOX or LOX isoform. Data consistently show LOX up-regulated in a variety of fibrotic conditions, sometimes accompanied by additional LOX isoforms, including lung- [49-53], liver- [43, 54-56], and heart- [57, 58], and skin- [59-61] fibrosis, and hypertension [47, 58]. One notable exception is phenytoin-induced gingival overgrowth which appears to be accompanied by LOXL2 elevations and not LOX [62, 63]. Thus, tissue-specific regulation of different LOX isoforms in fibrosis and other related pathologies can occur and should be carefully monitored which may ultimately aid in developing therapeutic strategies.

Recent studies have identified an important and novel role for LOX enzyme activity in the development of myelofibrosis that extends beyond its role in collagen cross-linking. LOX expression is high early in megakaryocyte differentiation and its expression and enzyme activity is required for optimal PDGF signaling and cell proliferation. Decreased LOX expression with time permits appears to slow proliferation to permit differentiation of megakaryocytes to platelets. The GATA-1 low mouse is a model of myelofibrosis, and a

high level of LOX expression was found which results in extracellular matrix accumulation and bone marrow fibrosis. Inhibition of LOX activity with BAPN resulted in attenuation of myelofibrosis [64]. Thus, excess LOX activity in the bone marrow is a major contributor to myelofibrosis. Myeloproliferative neoplasms often result in bone marrow fibrosis, and assessments of expression of LOX isoforms in human myeloproliferative neoplasms was recently reported. Primary myelofibrosis is accompanied by clearly elevated expressions of 4 of the 5 LOX isoforms studies (LOXL4 was not studied), while some variation in elevated isoforms expression was observed in other myeloproliferative neoplasms [65]. Taken together, these studies point to inhibitors of LOX isoforms as a potential therapeutic strategy to treat myelofibrosis emanating from a variety of myeloproliferative neoplasms.

LOX-PP may also be involved in regulating the differentiation of megakaryocytes into platelets. rLOX-PP inhibits endomitosis and polyploidy of megakaryocytes by inhibiting TPO-stimulated ERK1/2 signaling which is required for differentiation into platelets [66]. Thus, high early LOX expression and enzyme activity promotes megakaryocyte accumulation via enhancing PDGF signaling and cell proliferation while correspondingly expressed LOX-PP inhibits polyploidy. In summary, data taken together suggest that as megakaryocytes differentiate into platelets, LOX down-regulation drives lower cell proliferation (via lower enzyme activity) and permits polyploidy (via lower LOX-PP levels).

Diabetic retinopathy is a major cause of blindness in aging subjects and in diabetic individuals. This condition is accompanied by perivascular cell death [67] and basement membrane thickening and an acellular fibrotic lesion. Increased basement membrane thickening is associated with increased vascular permeability leading to bleeding and blindness [68, 69]. Increased expression of LOX has been associated with increased basement membrane thickening and permeabilization [70]. This is thought to be caused by uneven cross-linking of the basement membrane and distortion of its structure, leading to increased permeability of retinal capillaries. Elevated LOX and LOXL2 levels and inhibition of choroid neovascularization in vivo with neutralizing anti-LOX and – LOXL2 antibodies further supports functional contributions of LOX and LOXL2 to diabetic retinopathy [71].

There is now great interest in observations that elevated LOX expression accompanies and may drive metastatic cancer [7, 9, 72-78]. At least three ideas have emerged which are related to contributing mechanisms. Displastic tissues are characterized by fibrosis and increased tissue stiffness that can enhance cell migration. LOX-dependent collagen cross-linking increases stiffness, enhances fibrosis and integrin signaling, and thereby can create a permissive environment for tumor cell migration and intravasation [73, 79]. Some studies support the notion that, in addition, LOX promotes the formation of a metastatic niche at distant sites. In this model LOX is seen to localize at a distant site and then serves to attract tumor cells to extravasate. LOX has also been linked to promoting angiogenesis in tumors [80]. Although there is now little doubt that LOX activity contributes to metastasis, some aspects of some of studies related to LOX and metastatic niche identify the molecular size of LOX as 70 – 80 kDa [72, 81, 82], while pro-lysyl oxidase is well-established to be a 48 – 50 kDa protein. This and other biochemical aspects of the study of LOX biology and chemistry should be carefully re-examined. If indeed LOX is somehow structurally modified

to result in a higher apparent molecular weight in some cancers, these modifications could be of significant biological consequence.

2.1.1.2. Lysyl oxidase propeptide (LOX-PP): The studies on active LOX and cancer summarized above are in contrast to early studies which indicated that LOX expression is reduced in tumor cell lines, and that LOX re-expression is required for stable phenotypic reversion of wildtype or mutant c-H-*ras*-expressing mouse and human cells [83-87]. This complexity is related to findings which demonstrate that stimulation of metastatic progression is dependent on active LOX enzyme, while LOX-PP inhibits Ras-signaling in vitro, and primary tumor growth in vivo [5, 6, 88-90]. Thus, excess LOX activity emanating from all LOX isoforms has the potential to contribute to metastasis. Evidence that LOX-PP is a tumor suppressor as well as a tumor growth inhibitor comes from a study in which a polymorphism in the LOX-PP sequence resulted in a higher incidence of cancer in a subpopulation of triple-negative breast cancer patients [91]. As far as is known now, only LOX-PP which is unique in sequence, is a tumor growth inhibitor/tumor suppressor. No evidence exists that propeptides from the other four isoforms are tumor growth inhibitors. It is, therefore, of interest that LOXL2 and LOXL4 have been observed as highly up-regulated in metastatic cancers, and may be more effective promoters of metastatic disease than LOX possiblydue to the absence of a tumor inhibiting domain [92-105].

Several molecular targets of LOX-PP which mediate its inhibitory effects on tumor promoting signaling pathways have been identified in breast cancer and prostate cancer cell lines. Targets in prostate cancer cell lines include FGF-2 signaling mediated by FGFR1 and AKT activation [106, 107], and inhibition of DNA repair pathways mediated by binding to MRE11-containing DNA repair foci after nuclear localization [108]. In breast cancer cells, targets are fibronectin-stimulated FAK signaling and ERK1/2 activation [109], inhibition of β-catenin activation by targeting the receptor-type protein tyrosine phosphatase kappa [110], inhibition of Ras-signaling by direct targeting of Hsp70 and Raf leading to reduced ERK1/2 activation [111], inhibition of CIN-85-mediated invasion [112], and inhibition of proliferation and stimulation of apoptosis in vivo [88]. An interesting study investigated the phenotype of a spontaneous mutation in the mouse *NNA1* transcription factor gene revealed ataxia caused by overexpression of LOX in Purkinje cells in the brain [113]. Purkinje cells are critical for development of normal neural networks in the brain. Effects of elevated LOX expression resulted in reduced Purkinje cell growth. This effect was shown not to be related to LOX enzyme activity, but instead directly depends on LOX-PP. LOX-PP was shown to inhibit the RelA subunit of NF-κB from entering the nucleus, resulting in deficient mRNA and protein levels of MAP1B and MAP2 which are microtubule binding proteins required for Purkinje cell growth. Thus, in normal mice, normal NNA1 represses production of LOX. If LOX is overexpressed, LOX-PP then inhibits RelA nuclear localization leading to deficient MAP1B and MAP2 expression, deficient Purkinje cell production, contributing to ataxia. As noted above, LOX-PP targets many aspects of RAS signaling [111], and RAS can drive NF-κB activation [114]. Thus, one possibility is that the primary molecular target of LOX-PP in this context could be RAF, though other RAS effectors are also possible.

LOX-PP is generated extracellularly by proteolytic processing by procollagen C-proteinases [13, 33]. Molecular binding partners and targets of LOX-PP identified so far are

intracellular, except possibly cell surface FGFR1. Therefore, the pathways of LOX-PP cell uptake was investigated in a variety of cell lines to assess for possible avenues to enhance uptake and therefore potentially block cancer cell growth and metastatic potential. Data demonstrated that macropinocytosis is employed by most cell lines, while clathrin-dependent pathways serve as secondary uptake pathways in some cell lines [89]. Ongoing work is focusing on modifying the structure of LOX-PP to enhance its uptake by cancer cells and increase its effectiveness, and to establish which targets are the most important in mediating the tumor inhibitor properties of LOX-PP.

2.1.2. Lysyl oxidase like-1 (LOXL1)—LOXL1 has been linked primarily to elastin maturation. LOXL1 null mice experience skin, uterine, and lung abnormalities and females exhibit uterine prolapse [115-117]. LOXL1 has been shown to be closely associated with elastic fibers in vivo [118]. However, a recent report identifies a bone abnormality in *LOXL1* null mice which occurs only in females. Mutant mice have a deficiency in trabecular bone in both long bones and vertebrae [119]. Immunohistochemistry demonstrated strong expression of LOXL1 in growth plate chondrocytes, suggesting that LOXL1 is important not only for elastin maturation, but also possibly for type II collagen. Moreover, data implicate sex hormone regulation of LOXL1 may be an important aspect of its biological control.

A variety of polymorphisms in LOXL1 have been genetically linked to ocular exfoliation syndrome and glaucoma which may be caused by aberrant regulation of expression of LOXL1 and/or missense mutations combined with environmental stressors [118, 120-123]. LOXL1 null mice do not exhibit glaucoma, but do exhibit intracellular subcapsular vesicles [118]. Intracellular vesicle accumulations in subcapsular cells precedes development of cataracts in some animal models of cataract development [124]. Abnormalities in LOXL1 expression or structure seem likely to contribute to some ocular abnormalities, but may not be primary determinants.

Only a few papers and conflicting data exist regarding roles for LOXL1 in cancer biology. One report indicates that LOXL1 and LOXL4 expression are anti-tumorigenic and inhibit RAS and ERK1/2 signaling in bladder cancer [125], while LOXL1 was suggested to enhance lung metastasis in low pH hypoxic tumor microenvironments [126]. A role for caffeine-stimulated LOXL1 expression in inhibiting tumor metastasis has also been suggested [127]. Additional studies seem to be required to better understand these findings.

2.1.3. Lysyl oxidase-like 2—There is a great deal of interest in LOXL2, particularly in cancer biology. It is widely over-expressed in cancers, including metastatic cancers. For example, high levels of LOXL2 occur in colon cancer associated fibroblasts [128], skin cancer [129], oral cancer [130], skin and head and neck squamous cell carcinoma [101], liver metastasis [103], cholangiocarcinoma [131], breast cancer [76, 92, 132, 133], and gastric cancer metastasis [102]. Attenuation of LOXL2 expression was shown to inhibit metastasis or the invasive cell phenotypes in most of these studies. One study documented LOXL2 down regulation in non-small cell lung cancers [134].

A variety of mechanisms for LOXL2 promotion of metastasis are being investigated and a rather complex picture has emerged. MicroRNA down-regulation of LOXL2 has recently

been shown to be diminished in head and neck, renal and lung cancers [97, 135, 136] suggesting that regulatory pathways which control expression of LOXL2 may be significant drivers of metastasis. Evidence for both tumor secreted and stromal cell secreted LOXL2 in promoting metastasis has been found which may contribute to matrix stiffness, FAK activation and integrin signaling and control of cancer cell migration [94, 128]. LOXL2 expression in cancer cells is stimulated under hypoxic conditions, and evidence for HIF-1 transcriptional regulation of LOXL2 has been presented [137]. Extracellular interaction of LOXL2 with HSP90 to stimulate migration and metastasis has recently been highlighted [138]. Several papers investigate roles and mechanisms of intracellular LOXL2 in promoting cancer metastasis. Direct nuclear interactions of LOXL2 with Snail and down-regulation of E-cadherin to stimulate epithelial to mesenchymal transition and migration of cancer cells has been reported, but no evidence for LOXL2 oxidation of Snail has so far been reported [139]. Interestingly, enzymatically inactive forms of LOXL2 were also able to interact with Snail and down-regulate E-cadherin [133, 140]. Moreover, expression of full length LOXL2 or of an enzymatically inactive truncated form of LOXL2 were both found to down-regulate the promoter activity of claudin 1 and *Lgl2* promoters independent of Snail in MDA-MB-231 breast cancer cells. Claudin 1 and Lgl2, like E-cadherin are epithelial cell adhesion proteins critical for normal epithelial polarity [100]. LOXL2 in which essential copper-binding histidine residues were mutated to glutamine, thus eliminating enzyme activity, still bound to Snail and down-regulated E-cadherin expression [140]. Similarly, binding to the *E-cadherin* and *claudin 1* promoters remained unaffected by these mutations in MDA-MB-231 human breast cancer cells and in MDCK cells, an epithelial cell line. Finally, FAK activation and cell migration in these cell lines was equally stimulated by active an inactive forms of LOXL2. The authors conclude that stimulation of epithelial to mesenchymal transition (EMT) mediated by intracellular LOXL2 occurs independent of its enzyme activity [140]. As noted, however, LOXL2, like all other isoforms, is a secreted protein, and intracellular trafficking that leads to nuclear localization of LOXL2 and details regarding its interactions with transcription factors remain to be determined, and are of importance regarding under what conditions LOXL2 modulation of EMT is regulated. Reports of splice variants, and intracellular protein proteolytic processing are of interest and may be related to these questions of intracellular trafficking [99, 141].

Several reports suggest that LOXL2 can directly oxidize tri-methylated lysine residues in histones and TAFIID, another nuclear protein, and thereby promote an invasive phenotype [142-145]. Although there is no doubt that excess LOXL2 expression can promote EMT and an invasive phenotype, the notion of direct oxidation of nuclear trimethyl lysine residues by LOXL2 in our opinion requires further investigation. As noted above, secondary and tertiary amines do not serve as substrates of LOX [30]. The LTQ cofactor cannot form a Schiff base with tertiary amines; and the LTQ cofactor which functions in LOX is presumed to function in all other lysyl oxidase isoforms due to sequence conservation and the fact that they all oxidize primary amines. Thus, basic biochemical questions remain regarding the mechanism of action of LOXL2 and its apparent ability to oxidize tri-methylated lysine residues. In light of the proposed mechanism for tri-methyl lysine oxidation by LOXL2 [145], one approach might be to directly assess the substrate potential of a variety of primary,

secondary and tertiary amines and corresponding primary alcohols for aldehyde generation by purified LOXL2.

LOXL2 is upregulated under hypoxic conditions and has been shown to participate in normal angiogenesis which was dependent on both non-enzymatic and enzymatic activities. Initial organization of endothelial cells into tubes was dependent on LOXL2 expression but not enzyme activity, while stabilization of the basement membrane structures and stabilization of vessels required active LOXL2 [146]. Tumor growth requires a blood supply and LOXL2 has been shown to participate in tumor angiogenesis angiogenesis [147].

In formation of mineralized tissues, LOX is the predominant isoform elaborated by osteoblasts [35, 148]. Interestingly, LOXL2 appears to have negative consequences for mineralization by osteoblasts and odontoblasts. In odontoblast differentiation, LOXL2 expression was lower than all other isoforms [149], while in BMP-2-stimulated osteoblast differentiation, LOXL2 addition resulted in highly cross-linked collagen and poor mineralization [150]. However, LOXL2 is critically required for chondrocyte differentiation. Knock-down of LOXL2 profoundly inhibits development of chondrocytes in vitro, and expression of LOXL2 in healing long bones correlates positively with the chondrogenic phase in vivo [4].

LOXL2 null and overexpressing mice have recently been generated [129]. LOXL2 deletion was accomplished by deletion of exon 2 and resulted in perinatal lethality in 50% of the mice. Heart defects characterized by disrupted ventricular septa were noted in 40% of the surviving homozygous null mice. Homozygous mice overexpressing LOXL2 were almost all sterile with poor testicle formation and low sperm production. Fibrosis in the epididymis and inflammation was observed histologically, accompanied by low expression of E-cadherin and claudin of basal epithelial cells of the epididymis, consistent with the EMT activity of LOXL2 previously identified [100, 139, 140]. LOXL2 null and over-expressing mice were subjected to a chemically-induced model of squamous cell carcinoma [129]. Interestingly, LOXL2 over-expressing mice developed cancer earlier and with greater severity than the LOXL2 null mice. Lesions in LOXL2 null mice were smaller than the corresponding wildtype littermates. LOXL2 was observed to inhibit epidermal cell differentiation mediated by its inhibition of the Notch1 pathway apparently mediated by LOXL2 binding to the Notch1 promoter, implicating a mechanism of action of LOXL2 which occurs in the nucleus of cells without oxidation of trimethylated histone H3K4 [129].

2.1.4. Lysyl oxidase-like 3 (LOXL3)—LOXL3 null mice have recently been characterized and are perinatal lethal, exhibit cleft palate and vertebral defects. Collagen density was abnormally low in both the palate and vertebral primordia in knockout embryos. Both cleft palate and ocular abnormalities occur in a human family which has missense mutation in the *Loxl3* gene, but the ocular phenotype was not observed in mice [151, 152]. Two splice variants of LOXL3 have been identified [153, 154]. One variant is initiated from an alternative promoter and has been shown to have catalytic activity and distinct tissue-specific expression patterns compared to full-length LOXL3 [154]. The biological relevance of these findings remain to be determined. As noted, LOXL3 is often up-regulated in concert with the

other four isoforms in fibrosis, but no specific therapeutic opportunities for LOXL3 have become apparent so far to our knowledge.

2.1.5. Lysyl oxidase-like 4 (LOXL4)—LOXL4 is expressed in vascular tissues and has normal functions in connective tissue remodeling. Excess LOXL4, like LOX, has been linked to vascular fibrotic pathologies [155]. Splice variants of LOXL4 have been identified, and it has been proposed that shorter isoforms could stimulate metastasis while full length LOXL4 may have tumor inhibitory properties [156, 157]. LOXL4 expression appears to be a reliable marker for head and neck cancer development [158-160]. LOXL4 is overexpressed in gastric cancer and contributes to FAK activation and integrin signaling [104].

3. Therapeutic opportunities

Lysyl oxidase enzyme activity has long been considered to be a viable target to treat fibrosis [39]. BAPN is a highly effective and specific inhibitor of all lysyl oxidases in vitro, but has the disadvantage that it is oxidized by other amine oxidases that can lead to the production of toxic products [161, 162]. The copper chelator tetrathiomolybdate is an effective lysyl oxidase activity inhibitor as well [163-165], but it has the potential to cause significant toxicity or side effects due to the importance of copper in mitochondrial respiration and energy production to normal physiology [166]. Interestingly, novel LOX active site-directed inhibitor development is underway, some of which are selective for specific lysyl oxidase isoforms [167]. These inhibitors have enormous potential for treating LOXL2 driven metastatic cancers which have been summarized above. Preliminary data indicate that LOXL2 inhibitors can effectively inhibit bleomycin-induced lung fibrosis without toxicity [168]. Clearly much work needs to be done before human trials are undertaken, but there is now reason to be optimistic that the development of effective small molecule lysyl oxidase inhibitors to address fibrosis and cancer can be accomplished.

Therapeutic antibodies targeting LOX and LOXL2 have been described and employed in preclinical studies [40, 94, 169], and some human clinical trials have apparently been initiated by at least one pharmaceutical company. One limitation to these reagents may be that the negative effects of some LOX isoforms have been reported to be mediated not in the extracellular environment, but by intracellular targets which may not be accessible to antibody-based reagents.

LOX-PP has tumor growth inhibitor properties, is a natural product expressed by mammalian cells, and can enter cells from the extracellular environment. LOX-PP has a variety of molecular targets all of which appear to promote cancer development or metastasis, except possibly when injected into bones [170]. Thus, LOX-PP may have an advantage over reagents that are designed to target only one molecule, due to the plasticity of cancer and the seemingly inevitable development of resistance to chemotherapeutics. A slow release formulation has been shown to be effective at inhibiting the growth of a pre-existing breast cancer xenograft, suggesting that formulations of LOX-PP or a derivative to extend its half-life so that it is effective in vivo are possible [88].

4. Conclusion

At least three therapeutic approaches, therefore, exist based on what is known so far regarding the biology of the lysyl oxidases: novel small molecule inhibitors, therapeutic antibodies, and molecules and formulations based on LOX-PP structure and activity. Additional opportunities seem likely to be developed after the biological functions and binding partners of the propeptide regions of LOXL1 and LOXL2 – LOXL4 and splice variants have been determined, and the novel substrates of all five active lysyl oxidase enzymes implied in many recent studies are actually identified.

5. Expert Opinion

Key findings are that lysyl oxidase enzyme activity from all 5 isoforms drives both fibrosis and cancer metastasis. Very significant progress in developing small molecule pan-lysyl oxidase isoform inhibitors and isoform-specific lysyl oxidase inhibitors is a promising current avenue of ongoing research that seems likely to lead to new therapies for fibrosis and possibly metastatic cancer [167, 168]. Therapeutic antibodies that block the enzyme activity of lysyl oxidase isoforms have been developed in pre-clinical studies [171]. LOXL2 has emerged as an isoform that is of particular importance in the development of cancer and metastasis. LOX-PP derived from pro-LOX, however, is a tumor growth inhibitor and tumor suppressor [6, 91]. As a natural product, an increased understanding of its mechanisms of action and intracellular trafficking and identification of its most important targets will inform the degree to which a LOX-PP-derived therapeutic can be developed, possibly with little or no associated toxicity.

Although the evidence for the existence of novel substrates of lysyl oxidases that lead to some of these enzymes' pathologic effects is convincing, identification of these substrates and which lysine residue(s) are oxidized is lacking. This structural information is critically important for the development of, for example, strategies to block substrate activity perhaps with substrate-specific therapeutic antibodies that would prevent access by lysyl oxidases. It is also important to pay close attention to the structure of each lysyl oxidase isoform and to the enzymology of this family. The biosynthetic pathway of LOXL2 – LOXL4 is unclear at this time with respect to whether or not biosynthetic proteolytic processing occurs, and whether this is needed for enzyme activation. The origins and fates of intracellular lysyl oxidases are largely unknown regarding how these secreted proteins become intracellular, and how they traffic through the cell, sometimes localizing in the nucleus. No substrate specificity studies or classical enzymology has been reported on any isoform except LOX, and only one paper has been published along these lines on LOXL2 [14]. Confirmation of copper and LTQ cofactor content is important to establish in all isoforms. There is a great need to crystalize lysyl oxidase isoforms to gain detailed structural information, and recombinant expression of active lysyl oxidase isoforms has been very challenging. Lysyl oxidases from some commercial sources are inactive. LOXL2 from R&DSystems, however, is clearly an active enzyme and is an important contribution to the field.

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Reference annotations

* Of interest

** Of considerable interest

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Article highlights box

- Dysregulation of lysyl oxidases is linked to fibrosis and cancer in a variety of tissues and organs.
- The lysyl oxidase family of proteins consists of five members and is required for normal biosynthesis of the extracellular matrix.
- Functions of the lysyl oxidase family of proteins extend beyond the classically known contributions to collagen and elastin cross-linking and include regulation of cell proliferation and differentiation.
- Respective unique domains of lysyl oxidase family members mediate novel functions and some of these are independent of active lysyl oxidase enzyme activity.
- The current understanding of contributions of both enzyme-dependent and non-enzyme dependent activities of lysyl oxidases to fibrosis and cancer points to the high likelihood of future development of potential therapeutic opportunities. Such opportunities will grow out of an increased mechanistic understanding of the variety of functions of the versatile lysyl oxidase family.

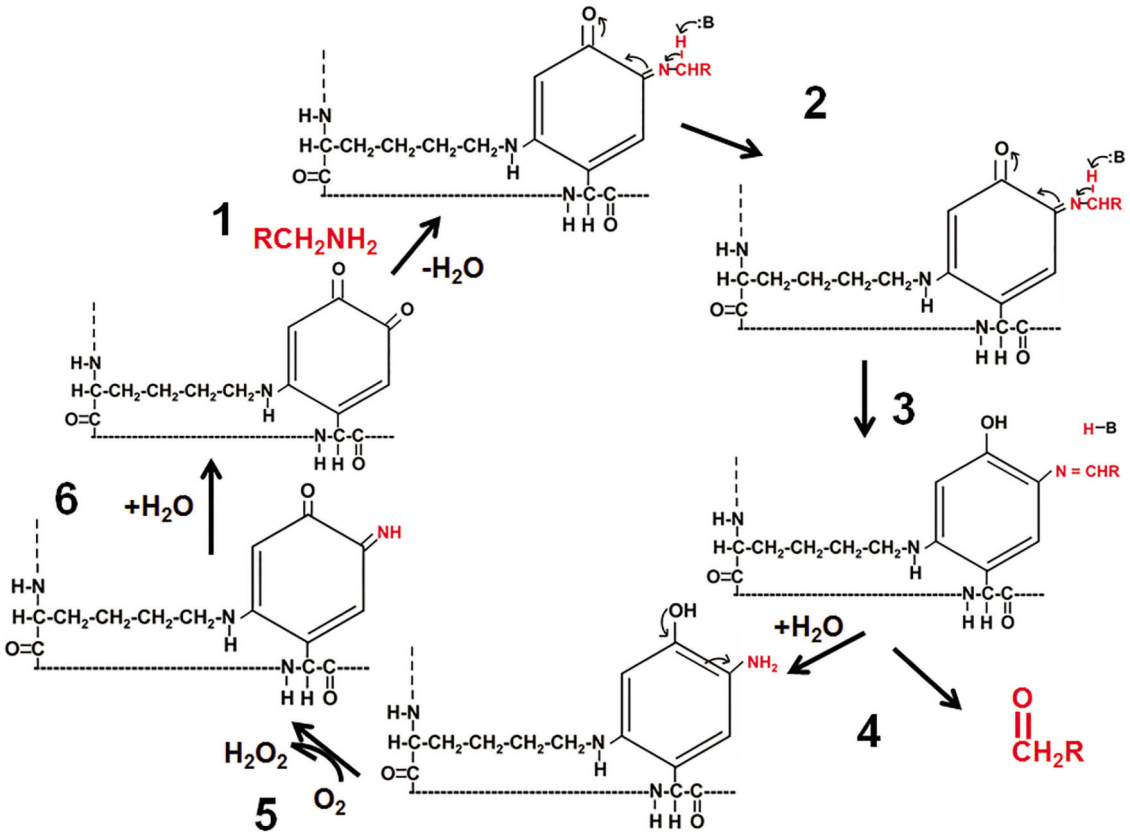

Figure 1.

The mechanism of action of the lysyl oxidase-catalyzed reaction [26]. The red molecule represents the primary amine substrate or lysine side chain substrate. The numbers identify the sequence of the reaction steps.