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Enzymatic and non-enzymatic functions of the lysyl oxidase family in bone

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Abstract

Advances in the understanding of the biological roles of the lysyl oxidase family of enzyme proteins in bone structure and function are reviewed. This family of proteins is well-known as catalyzing the final reaction required for cross-linking of collagens and elastin. Novel emerging roles for these proteins in the phenotypic development of progenitor cells and in angiogenesis are highlighted and which point to enzymatic and non-enzymatic roles for this family in bone development and homeostasis and in disease. The explosion of interest in the lysyl oxidase family in the cancer field highlights the need to have a better understanding of the functions of this protein family in normal and abnormal connective tissue homeostasis at fundamental molecular and cellular levels including in mineralized tissues.

Keywords

lysyl oxidase; collagen; bone; progenitor cell; angiogenesis; cell proliferation; cell differentiation

Introduction

Determinants of bone strength

Bone strength depends not only on bone mineral density, but also on determinants of bone quality. The structure of the organic components of bone, of which about 90% consists of type I collagen synthesized by osteoblasts, is now understood to determine the quality and material properties of bone. Strength measurements of bones in which the bone mineral density is minimally altered and the organic phase is significantly altered support this understanding [1-6]. Major determinants of the functional integrity of the organic phase are the degree and nature of enzymatic collagen cross-links, and the negative effects of non-enzymatic collagen glycation which can be considered in a competitive relationship with enzyme-derived cross-linking. The current review will focus on aspects of enzyme-dependent biosynthetic cross-linking in bone with emphasis on the lysyl oxidases.

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Lysyl hydroxylase and lysyl oxidase

Enzymes and reactions responsible for collagen cross-linking

Enzyme-dependent collagen cross-linking depends on a combination of intracellular modifications of procollagen alpha chains by lysyl hydroxylases, and on extracellular modifications by lysyl oxidases. Lysyl hydroxylases are endoplasmic reticulum-associated oxidases which require α -ketoglutarate and ascorbate. These cofactors which are also required for collagen prolyl hydroxylases. Lysyl hydroxylases catalyze hydroxylation of the penultimate carbon atom on some lysine residues of procollagen alpha chains. Lysyl hydroxylase 2b catalyzes the hydroxylation of some lysine residues in the telopeptide region of fibrillar collagens, while lysyl hydroxylase 1 performs this reaction on some lysine residues in the triple helical regions [7–9]. Only a subset of lysine residues are hydroxylated, and the degree of hydroxylation is tissue-specific, as recently reviewed [4]. These hydroxyl groups further serve as sites of attachment of the carbohydrate moieties of collagen, and only a subset of these hydroxyl groups become glycosylated. Glycosylation of collagens is different from the well-known N- and O-glycosylation pathways which occur on asparagine and serine/threonine residues of other proteins. Collagen glycosylations are catalyzed by hydroxylysyl galactosyltransferase, followed by galactosylhydroxylysyl glucosyltransferase to produce glucosylgalactosyl hydroxylysine. Interestingly, galactosylhydroxylysyl glucosyltransferase activity has been shown to be carried out by lysyl hydroxylase 3 [10, 11].

The final enzyme reaction required for cross-link formation is catalyzed by the lysyl oxidase family which is made up of five members: lysyl oxidase (LOX) and the four lysyl oxidase like enzymes, lysyl oxidase like 1 – 4 (LOXL1 – LOXL4). These secreted extracellular enzymes can all catalyze the oxidation of the lysine and hydroxylysine side chains of collagens in the telopeptide regions of fibrillar procollagens to form peptidyl α -aminoadipic- δ -semialdehyde residues or peptidyl- δ -hydroxy- α -aminoadipic- δ -semialdehyde residues. These residues are also known as allysine and hydroxyallysine, respectively [12, 13]. Substrate preferences for LOXL1 for elastin [14] and LOXL2 for type IV collagen [15] are apparent. However, direct comparative analyses of substrate specificities among the 5 purified LOX isoforms have not been performed. Substrate sequence requirements for purified bovine aorta LOX using synthetic peptides mimicking relevant collagen sequences have been determined [16]. For example, G₄EKG₆ was a more efficient substrate than G₅KEG₄, while DEK sequences within peptides which therefore have two negatively charged residues on the N-terminal side of lysine were unfavorable. Consistent with this understanding is the fact that EK sequences are oxidized in the C-terminal telopeptide region of fibrillar collagens, while KE sequences are not, determined by structure analysis of actual collagen cross-links [16]. It is clear, however, that collagen fibrils are better LOX substrates than denatured collagen and collagen peptides. The physical chemical basis for this is only partially understood [17]. This observation does not exclude the fact that LOX can oxidize soluble substrates, but the enzyme may be less efficient than with insoluble or semi-soluble substrates. The concept was put forth that LOX may bind to one collagen chain in the triple helical domain to oxidize a lysine residue in the neighboring juxtaposed telopeptide. This notion has not been further investigated to our knowledge, but would provide an explanation

for identification of cross-links throughout collagen fibrils, rather than only on the surface of fibrils, while also being consistent with the observation that insoluble or semi-soluble collagen fibrils more readily undergo LOX oxidation [16]. In this model, LOX would bind to a procollagen molecule in the triple helix domain before fibril formation, and then act on a neighboring collagen molecule telopeptide lysine residue as fibril formation commenced and progressed.

The importance of an insoluble or semi-soluble substrate for optimal LOX activity is also true for elastin [18]. Synthetic peptide elastin-like copolymers with the same sequences around lysine residues but which form coacervates are superior substrates to those copolymer peptides that do not coacervate [19]. Thus, semi-soluble structures consistently provide an optimum environment for LOX activity [19]. The physical state of protein structures is a major determinant of the optimal potential for lysyl oxidase catalyzed oxidative deamination, while the primary sequence surrounding the lysine residues to be oxidized appears to be of secondary importance. Electrostatic charge is, in addition, an important determinant of substrate potential. Soluble cationic proteins generally are good substrates for lysyl oxidase in vitro [20]. This finding may be of considerable biological importance because many regulatory proteins such as growth factors are cationic, and some of these have been shown to serve as substrates for lysyl oxidase in vitro, thereby attenuating their ability to initiate signaling [21, 22].

As noted, lysyl oxidases act on lysine and hydroxylysine residues exclusively in the telopeptide regions of fibrillar collagens. These aldehydes are highly reactive and result in the formation of the normal immature and mature biosynthetic collagen cross-links over time without apparent involvement of LOX or other enzymes [23–25]. Cross-links form within collagen trimers at each telopeptide end, and between some modified lysine residues in the telopeptide region and non-modified lysine residues located in neighboring juxtaposed triple helical regions. These cross-links stabilize collagen structure and confer tensile strength of connective tissues. The microenvironment and favorable juxtapositions of modified and non-modified lysine- and hydroxylysine residues determine the actual cross-links that can form. Thus, lysyl oxidases catalyze the final enzyme-mediated reactions required for biosynthetic collagen cross-linking, but lysyl oxidases do not directly cross-link collagen.

Hydroxylysine reactivity

The structures of collagen cross-links have been reviewed elsewhere and will not be repeated in detail here [4, 13, 26, 27]. However, an important point is that the chemical reactivity of respective lysine residues is modulated by whether or not lysyl hydroxylation has occurred. For example the pKa of hydroxylysine residues is 8.5 while that of lysine residues is more typically about 9.5 [28]. Thus, hydroxylysine residues are more nucleophilic and chemically reactive than non-modified lysine residues at physiologic pH values, and therefore are more likely to form immature and mature cross-links. Glycosylation of the hydroxyl group either with galactose only, or with the successive addition of glucose to form the galactosyl-glucose modification, would both be expected to raise the pKa back to non-modified lysine, reducing, but not eliminating, the tendency to cross-link. These considerations are consistent

with the up-regulation of lysyl hydroxylase 2b by vitamin D in vitro, and with an in vivo study showing an increased proportion of hydroxylysine containing cross-links in bones from ovariectomized monkeys treated with a vitamin D pro-drug [29, 30]. Moreover, a high percent of non-glycosylated pyridinoline cross-links compared to the proportion of non-glycosylated immature cross-links in bovine bone suggests an increased reactivity of non-glycosylated hydroxylysine leading to efficient cross-linking [31]. Increased chemical reactivity of hydroxylysine relative to lysine could contribute to increased rates and degree of maturation of immature cross-links, because mature collagen hydroxypyridinium cross-links (also called pyridinoline) contain one hydroxylysine residue not oxidized by LOX family members (Figure 1). Steric effects of di-glycosylation of hydroxylysine residues may further inhibit cross-linking beyond reducing the chemical reactivity of lysine. For example, glycosylated mature pyridinoline cross-links contained in type I and type II collagen contain primarily only mono-glycosylated cross-links, and only rarely di-glycosylated cross-links [27, 31]. Confirmation of the role of hydroxylysine glycosylation in regulating subsequent cross-linking may require the study of peptides which contain hydroxyallysine residues derivatized with a variety of carbohydrate-like structures, and then assess for chemical reactivity and the potential to form intermolecular cross-links in vitro.

Due to the increased nucleophilic character of hydroxylysine, these residues theoretically could form hydroxylysylpyridinoline cross-links more efficiently compared to lysylpyridinoline formation from non-modified lysine residues (see Figure 1 for structures). Based on this idea, one prediction could be that hydroxylysylpyridinoline collagen cross-links would be more abundant than lysylpyridinoline cross-links which contain one non-hydroxylated lysine residue. This is the case in mature human bone and dentin [13]. However, these cross-links are present at about the same level in some cultured differentiating osteoblast cultures [7, 32]. Interestingly, due to their more nucleophilic character, hydroxylysine-containing residues could in principle be better substrates for lysyl oxidase. Thus, if hydroxylysyl residues were more efficiently oxidized by lysyl oxidase and converted to aldehydes, non-modified hydroxylysine residues would be present at lower than expected levels. This could contribute to the equivalent levels of pyridinoline and hydroxypyridinoline levels observed in some bones [7, 32]. There is, however, no direct experimental evidence available to our knowledge regarding whether or not hydroxylysine residues or lysine-residues are more efficient substrates for lysyl oxidases in collagens.

Elastin

Lysyl oxidases are critically important for elastin cross-linking [33]. Elastin is abundant in non-mineralized tissues such as arteries and skin and in hyaline cartilage (cartilage found in the ear and nose) but is scarce in bone. Mature elastin cross-links are known as desmosine and isodesmosine, and consist of four lysine residues instead of three contained in the pyridinolines of collagens (Figure 1). Collagens contain additional tri- and tetravalent cross-links which contain histidine in addition to lysine and lysine-derivatives [13] (Figure 1). In bone tissues, elastin is a component of the periosteum, in which a population of stem cells also occurs [34–38]. The periosteum has been shown to participate in lateral growth of cortical bone, possibly driven by periosteum-derived stem- or progenitor cells. Interestingly, expression of LOXL4, elastin (ELN), decorin, natriuretic peptide receptor C (NPR3) and C-

type lectin domain family 3, member B (CLEC3B) by some bone marrow-derived progenitor cell lines has been correlated with the ability to differentiate into bone forming osteoblasts [39]. The mechanistic basis of elastin expression in this context has not been determined.

Lysyl oxidase family

Properties of lysyl oxidases

LOX was originally discovered as a result of people and grazing animals consuming naturally occurring LOX inhibitors in *Lathyrus oderatus* peas resulting in severe connective tissue abnormalities which were especially apparent in bone [40]. Identification of the active compound was accomplished leading finally to the identification of the chemistry of the collagen modification and the crucial enzyme activity that is now known as lysyl oxidase [41–43]. The inhibitor, contained in these *Lathyrus oderatus* pea plants consumed so long ago, β -aminopropionitrile (BAPN), is still employed and its use remains the criteria for defining LOX enzyme activity. The inhibition of LOX by BAPN is unique to this family of enzymes, while an abundance of other tissue-associated and serum amine oxidase enzymes exist that are either unaffected by BAPN, or oxidize BAPN as a substrate [44]. Thus, an amine oxidase that is inhibited by low concentrations of BAPN is, by definition, a lysyl oxidase, and an amine oxidase resistant to BAPN is not. BAPN is an active-site directed irreversible inhibitor of LOX [45, 46].

In mammals the lysyl oxidase family of enzymes consists of five members. Each protein is encoded by a different gene: *lysyl oxidase (LOX)* and *lysyl oxidase-like 1 - 4 (LOXL1 – LOXL4)*. All share a common C-terminal domain that encompasses the catalytic enzyme domain (Figure 2). The enzyme domain includes a copper binding site and an unusual modified tyrosine residue known as lysyltyrosyl quinone, or LTQ, that serves as the carbonyl cofactor required for the catalytic reaction (Figure 3). Evidence has been presented that copper incorporation depends on a copper transporter ATP7a that occurs in the endoplasmic reticulum, before pro-LOX is secreted into the extracellular environment [47]. The *ATP7a* X-linked gene is mutated in Menkes disease and its negative effects on copper transport has been proposed as the basis for reduced LOX activity in this disease [48]. The structure of the active site carbonyl cofactor in lysyl oxidase was established as LTQ based on a thorough mass spectrometry and site-directed mutagenesis study resulting from a collaboration of the laboratories of Judith Klinman and Herbert Kagan [49]. The structure shown in Figure 3 was established in bovine and rat LOX proteins, and consists of a quinone derived by post-translational modification of a LOX tyrosine residue cross-linked to a LOX lysine residue in the same polypeptide chain which correspond to Lys 320 and Tyr 355, of human LOX, respectively. Biogenesis of the quinone cofactor is thought to be copper-dependent, by analogy to other quinone-cofactor containing amine oxidase enzymes [50, 51]. Interestingly, all five LOX isoforms have conserved copper binding amino acid residues and conserved tyrosine and lysine residues which suggest that all LOX isoforms having the same essential active site components of tightly bound copper, and the LTQ cofactor Figure 4. The rigorous identification of the active site carbonyl of LOX as LTQ argues against a direct role of pyridoxal phosphate or another vitamin B6 derivative as the active site carbonyl cofactor

required for LOX catalytic activity. Studies show, however, that vitamin B6 deficient animals have poor collagen cross-linking in bone [52]. It can be concluded that vitamin B6 has an indirect mechanism that regulates the level of LOX isoforms in bone which remains to be fully understood.

The LOX family can be divided into two subgroups consisting of LOX and LOXL1, and LOXL2 – LOXL4, respectively. LOX and LOXL1 are structurally more similar to each other than they are to LOXL2 – LOXL4. LOX and LOXL1 each have unique propeptide regions with limited similarity to each other, while LOXL2 – LOXL4 each have four conserved scavenger receptor cysteine-rich (SRCR) domains in their longer propeptide regions. LOX biosynthesis includes secretion of the inactive 50 kDa glycoprotein precursor thought to already contain copper and quinone cofactors. Pro-LOX then undergoes extracellular proteolytic processing by procollagen C-proteinases encoded by the *BMP1* and the related *TLL1* and *TLL2* genes, resulting in generation of active ~30 kDa lysyl oxidase and the ~18 kDa N- and O-glycosylated lysyl oxidase propeptide (LOX-PP) [53–55]. Processing is optimal in the presence of fibronectin [56], or periostin [57] which may provide a scaffold for assembly of processing complexes that may be context-dependent. LOXL1 appears to similarly processed by BMP1, resulting in an active LOX that is slightly larger than LOX resulting in a ~33 kDa active enzyme [58]. LOXL2 – LOXL4 are larger proteins, and it is currently unclear whether removal of any part of the propeptide regions is required for enzyme activity to develop. Data so far appear to suggest that proteolytic processing of LOXL2 may not be required for its activity [59].

LOX expression and enzyme activity in differentiating MC3T3-E1 murine osteoblasts has been analyzed in relationship to collagen expression, collagen accumulation and fibril diameter, BMP-1 expression, and effects of BAPN [60]. Data show that total LOX activity increases just before collagen accumulation accelerates, as expected. BMP1 and TLL1 were not strongly regulated as a function of differentiation. In the presence of BAPN, collagen became more extractable and less cross-linked and exhibited a less uniform and wider fibril diameter. Effects on collagen of BAPN are similar to that observed in chick chondrocyte cultures, in which collagen extractability, fibril diameter, and accumulation were increased [32]. Data support the notion that lysyl oxidase family regulation supports the observed temporal accumulation of collagen into mature fibrils in osteoblast cultures. These early studies did not measure the role of each LOX isoform in osteoblast differentiation or collagen accumulation, and gene expression studies were done only on the LOX isoform. A recent study has indicated that *LOX* is by far the principal lysyl oxidase isoform expressed in developing osteoblasts in vitro [61], with kinetics of expression similar to an earlier publication focused only on LOX [60]. Calvaria osteoblasts from *LOX*^{-/-} perinatal mice exhibited deficient osteoblast differentiation and abnormal collagen fibril diameters [62]. In vivo studies which determine the expression of all five isoforms in bone as a function of age have not yet been published, to our knowledge.

Studies of *LOX*^{+/-} and of *LOXL1*^{-/-} adult mice each show evidence of deficient bone formation. Data regarding the bone phenotype of *LOXL1*^{-/-} mice have now been published, and reveal a trabecular deficiency in females by micro-computed tomography, but hardly any abnormalities in males [63]. LOXL1 was expressed primarily by growth plate chondrocytes

in wildtype littermates. Mutant mice at 13 weeks of age are all normal in size, but growth plates in long bones in females exhibit chondrocyte disorganization. Collagen structure in bone was impaired, with evidence of increased resorption and decreased synthesis supported by Sirius red and TRAP staining of bones. High levels of bone resorption markers RANKL and C-terminal cross-linked type 1 collagen telopeptide (CTX1) and low levels of the bone biosynthetic marker type 1 collagen propeptide (PINP) in the serum of mutant mice further supported active bone resorption occurring in female mutant mice. The basis for the sex-difference and the mechanism of development of this bone phenotype in *LOXL1*^{-/-} mice is under investigation.

Studies of fracture healing implicated lysyl oxidase isoforms as differentially expressed during long bone healing in mice [64]. The temporal expression pattern of LOXL2 suggested possible association with chondrogenesis. This was further investigated in differentiating murine ATDC5 cells, which mimic the normal chondrocyte differentiation pathway, in which a specific differentiation-dependent up-regulation of LOXL2 on day 7 occurred [65]. The functional importance of LOXL2 in ATDC5 chondrocytes in maturing cells was established by shRNA knockdown of LOXL2 which resulted in deficient expression of early and late chondrocyte differentiation markers, including SOX9, type II collagen, type X collagen, and aggrecan, and deficient deposition of mineral and proteoglycans [65]. Interestingly, LOXL2 appears not to be expressed by the widely used normal osteoblast MC3T3-E1 cell line [66], suggesting that LOXL2 has a tissue-specific distribution and function in chondrocytes. Since the early differentiation marker SOX9 was somehow regulated by LOXL2 in ATDC5 cells, the question is raised whether LOXL2 promotes chondrocyte differentiation independent of its effects on collagen maturation.

Diabetic osteopenia

LOX-dependent cross-linking is deficient in bone, while non-enzymatic glycation of lysine and arginine residues is increased in bones in diabetic subjects [4, 67–69]. Non-enzymatic glycation occurs normally as a function of age, but is accelerated in diabetes due to increased glucose concentrations, and increased oxidative stress, resulting in non-enzymatic modifications of proteins. These heterogeneous modifications are known as advanced glycation end products (AGE's) and alter biology by activating receptors for advanced glycation end products [70, 71]. Alternatively, glycation of long-lived proteins directly alters their respective functions, including collagens. For example, in vitro studies have shown that type 1 collagen chemically modified so that some lysine residues are converted to carboxymethyl lysine, a known AGE, results in attenuated binding and activation of the osteoblast non-integrin collagen receptor: discoidin receptor-2 (DDR2) [72]. Thus, the diabetic condition can effectively lower LOX production by osteoblasts due to attenuated maintenance of LOX expression normally maintained by normal collagen/DDR2 interaction and signaling.

In a related study, LOX expression was measured in healing of calvaria defects in diabetic mice. Defects exhibited an abundance of hematomas in diabetic mice which appeared to stimulate a fibrotic soft tissue response accompanied by increased expression of lysyl oxidase and lysyl oxidase enzyme activity [73]. It was suggested that the robust fibroblast

activity associated with the fibrotic response effectively inhibited osteoblast development. It was further suggested that the model of calvaria defect healing, although relevant to bone healing, is not an appropriate model to understand diabetic bone homeostasis in the absence of an injury in which osteoblast-derived lysyl oxidases are envisioned to be down-regulated, consistent with the in vitro study summarized above [72]. Clearly, the role of lysyl oxidase isoforms in the context of diabetic osteopenia on one hand, and diabetic bone healing on the other, requires further investigation.

Novel functions of LOX isoforms

Extracellular matrix-independent activities of lysyl oxidase family members which are important for bone biology have been identified in studies of LOX regulation in primary bone marrow cells, and in the C3H10T1/2 cell line model of progenitor cells which can differentiate into osteoblasts, adipocytes or chondrocytes. For example, LOX was found to be up-regulated by Wnt3a at the transcriptional level in C3H10T1/2 progenitor cells and in primary bone-marrow derived cells, but not in MC3T3-E1 cells which are already committed to the osteoblast lineage. Because C3H10T1/2 cells produce little or no extracellular matrix under these conditions, the question was raised regarding LOX function. LOX shRNA studies showed that LOX was required for normal proliferation of C3H10T1/2 cells, and that differentiation into osteoblasts was blocked in cells expressing diminished levels of LOX [74]. Similarly, LOX expression was found to be required for differentiation of C3H10T1/2 cells to adipocytes [75, 76]. Finally, LOX activity is required for the optimal response of PDGF receptor to PDGF-BB ligand in smooth muscle cells and megakaryocytes [77, 78]. By contrast, additional in vitro studies show that LOX can oxidize FGF-2 and TGF- β and thereby inhibit their ability to signal [21, 22]. Taken together, data support the notion that effects of lysyl oxidase on cell biology and physiology extend beyond its role in collagen cross-linking, and that LOX and LOX isoforms appear to have physiologic substrates in addition to collagens and elastin.

LOXL2 was shown to be important in sprouting angiogenesis [15], a process important in endochondral bone formation and bone healing [79]. Hypoxia induces a variety of gene expressions in endothelial cells to induce angiogenesis. LOXL2 was shown to be the primary lysyl oxidase isoform expressed in HUVEC cells under hypoxic conditions. In a mouse model of hindlimb ischemia-induced revascularization, LOXL2 expression was increased dramatically and was localized to endothelial cells. Knockdown of LOXL2a in zebrafish in the same study showed defects in intrasegmental vessel (ISV) growth. ISV development in zebra fish is a model of the study of angiogenesis. BAPN treatment of wild type fish did not affect ISV formation, suggesting that a non-enzymatic function of LOXL2 could be responsible for poor ISV formation. Similarly, in vitro co-culture experiments of HUVECs with fibroblasts in which LOXL2 expression in HUVECs was either knocked down or overexpressed revealed that HUVEC LOXL2 expression was required for initial vessel formation. Interestingly, vessel formation was minimally inhibited by BAPN, indicating that LOXL2 effects on initial tube formation were independent of its enzyme activity. By contrast, BAPN reduced the stability of previously formed vessels, suggesting that LOXL2 enzyme activity is important in the stabilization of vessels. Finally, LOXL2 activity was shown to be required for deposition of a collagen IV matrix by HUVECs. These studies [15]

taken together suggest that LOXL2 promotes collagen IV assembly in the initial formation of micro vessels independent of its enzyme activity, while stable micro vessels depended on LOXL2 enzyme activity to result in collagen IV cross-linking and stabilization. Although lysyl oxidase family-dependent cross-linking is known to occur in the 7S region of type IV collagen [80], only indirect evidence has been published so far that the LOXL2 isoform can oxidize this protein [15]. It will be interesting to learn more about both the non-enzymatic and enzymatic interactions which occur between type IV collagen and LOXL2 in angiogenesis. For example, it will be important to discover the structural determinants which define LOXL2 to collagen IV interactions, and what if any role the pro-region of LOXL2 might have in the initial and later stages of angiogenesis. Global LOXL2 knockout and overexpressing mice were recently generated [81]. Knockout mice exhibit perinatal lethality with heart and liver vascular defects in affected mice, while a subset of null mice survive. Global overexpression of LOXL2 resulted in male sterility, and an increased susceptibility to cancer development in a carcinogen-induced skin cancer model. No bone phenotypes of these mice have been reported so far.

The phenotype of the LOXL3 null mouse has been recently reported. Homozygous embryos exhibit perinatal lethality. The LOXL3 null mice have cleft palate, mandibular deformities, and spinal deformities [82]. Unlike a Stickler Syndrome in a human family, in which a LOXL3 missense mutation has been reported and which exhibits ocular defects, no eye defects were observed in the homozygous LOXL3 null mice [83]. However, the cleft palate manifestations are common between the human missense mutant individuals and the LOXL3 null mice. LOXL3 was expressed primarily by mesenchymal cells in the palate, tongue and in cartilage in wild type embryos. Stickler Syndrome is more often caused by mutations in cartilage collagens including *COL2A1*, *COL11A1* or *COL11A2*. It was, therefore, suggested that likely substrates for LOXL3 may include these collagens.

LOXL4 was first cloned from a cDNA library made from the chondrocyte cell line ATDC5 [84], and was later shown to be expressed in a variety of other tissues [85]. LOXL4 is catalytically active with collagen substrates [84]. LOXL4 expression in bone marrow progenitor cells is one of several markers that may be predictive of in vivo bone formation, however this remains to be confirmed [39, 86].

Lysyl oxidases and bone metastasis

The lysyl oxidase family is currently the subject of intense research in the context of cancer. LOX has been shown to suppress tumor growth mediated by its propeptide region [87, 88], while active lysyl oxidase enzymes are most often increased in the context of metastasis [89]. Metastasis to bone is frequent in prostate, renal, and breast cancers, and is painful and crippling and can lead to premature death [90]. In the context of breast cancer metastasis to bone, a working model has been recently proposed in which circulating active LOX enzyme emanating from breast tumors creates a permissive environment, or metastatic niche, in bone that promotes the subsequent initiation and development of bone metastatic tumors [91]. Data suggested that LOX enzyme activity in some way directly promotes osteoclast differentiation. Much of the data was based on full length pro-LOX knockdown, or introduction or overexpression of an atypical 70 kDa form of pro-LOX contained in

conditioned media [91]. However, other work shows that purified recombinant N-terminal LOX propeptide (LOX-PP), which contains no LOX enzyme activity, inhibits proliferation and promotes differentiation of both primary bone-marrow derived osteoclasts and osteoblasts, suggesting that LOX-PP modulates coupling between osteoblasts and osteoclasts [92]. These findings are consistent with LOX-PP inhibition of normal osteoblast proliferation reported earlier [93]. New data demonstrated that LOX-PP inhibits OPG production by bone marrow stromal cell cultures in the presence of M-CSF and RANKL. LOX-PP increased CCN2 levels in whole bone marrow-derived primary mouse cells [92]. Decreased OPG and increased CCN2 can each contribute to osteoclast development and fusion [94, 95] which was observed to be increased in the presence of rLOX-PP [92]. The relative contributions and mechanisms of abnormal high levels of active LOX enzyme on the one hand [60, 74, 93], and LOX-PP on the other, to decouple osteoblast and osteoclast interactions in the presence or absence of cancer cells [92] remain to be adequately understood, and are likely to influence bone metastasis. For example, direct injection of the osteolytic prostate cancer PC3 cell line over expressing rLOX-PP into the tibia of mice increased bone destruction compared to PC3 control cells determined by μ CT analyses, while injection of DU145 osteogenic cancer cells overexpressing rLOX-PP created osteoblastic lesions that were similar compared to its DU145 control [92].

LOX enzyme activity has an important role in stimulating EMT of breast cancer cell lines augmented by mesenchymal stem cells in microenvironments of primary tumors. This activity was shown to have relevance to metastasis to bone [96]. Xenografts of MCF7 cells over-expressing both RAS and full length pro-LOX leading to increased levels of active LOX were better able to metastasize to bone than MCF7 cells expressing only Ras. In vitro, mesenchymal stem cells strongly stimulated LOX production and activity in breast cancer cell lines highlighting the importance of stromal interactions with cancer cells in tumors to regulate gene expression and exacerbated tumor growth and metastasis. This elevated LOX activity was then shown to be required in cancer cells for nuclear localization and activation of TWIST and increased production of vimentin, fibronectin, N-cadherin, and smooth muscle actin, which are all markers of the mesenchymal phenotype. LOX activity promoted breast cancer cell migration in vitro. Interestingly, the authors proposed and provided evidence that extracellular high molecular weight hyaluronic acid elaborated by mesenchymal stem cells stimulates LOX production in breast cancer cell lines through the CD44 receptor on the cancer cells. This conclusion was based on studies in which cancer cell lines were plated on hyaluronan of different molecular weights, showing that high molecular weight hyaluronan stimulated CD44 activation and TWIST and LOX expression. In response, CD44 was proposed to migrate to the nucleus of cancer cells and directly promote transcription of LOX. Evidence included identification of a 50 kDa nuclear form of CD44, and successful CD44 ChIP assay of the LOX promoter in mesenchymal stem cell-stimulated LOX producing cancer cells [96].

Metastasis is a complex multistage process and involves tumor initiation and tumor growth in which EMT is important, intravasation of cells into the circulation, travel to the distant site, extravasation, and growth at the distant site [97]. Understanding each stage of metastasis requires a thorough analysis of tumor-stromal cell and extracellular matrix interactions at the cellular and molecular levels. The lysyl oxidase family plays key roles in

several of these processes, with multiple isoforms and multiple domains which can have opposing functions which are only beginning to be understood. Moreover, the processing and functions of the pro-domains of many of the lysyl oxidase isoforms are largely yet to be discovered.

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References

1. Paschalis EP, Tatakis DN, Robins S, Fratzl P, Manjubala I, Zoehrer R, Gamsjaeger S, Buchinger B, Roschger A, Phipps R, Boskey AL, Dall'Ara E, Varga P, Zysset P, Klaushofer K, Roschger P. Lathyrism-induced alterations in collagen cross-links influence the mechanical properties of bone material without affecting the mineral. *Bone*. 2011; 49:1232–1241. [PubMed: 21920485]
2. Oxlund H, Barckman M, Ortoft G, Andreassen TT. Reduced concentrations of collagen cross-links are associated with reduced strength of bone. *Bone*. 1995; 17:365s–371s. [PubMed: 8579939]
3. McNerny EM, Gong B, Morris MD, Kohn DH. Bone fracture toughness and strength correlate with collagen cross-link maturity in a dose-controlled lathyrism mouse model. *Journal of bone and mineral research: the official journal of the American Society for Bone and Mineral Research*. 2015; 30:455–464.
4. Saito M, Marumo K. Effects of Collagen Crosslinking on Bone Material Properties in Health and Disease. *Calcif Tissue Int*. 2015; 97:242–261. [PubMed: 25791570]
5. Alford AI, Kozloff KM, Hankenson KD. Extracellular matrix networks in bone remodeling. *The international journal of biochemistry & cell biology*. 2015; 65:20–31. [PubMed: 25997875]
6. Garnero P. The Role of Collagen Organization on the Properties of Bone. *Calcif Tissue Int*. 2015; 97:229–240. [PubMed: 25894071]
7. Uzawa K, Grzesik WJ, Nishiura T, Kuznetsov SA, Robey PG, Brenner DA, Yamauchi M. Differential expression of human lysyl hydroxylase genes, lysine hydroxylation, and cross-linking of type I collagen during osteoblastic differentiation in vitro. *Journal of bone and mineral research: the official journal of the American Society for Bone and Mineral Research*. 1999; 14:1272–1280.
8. Pornprasertsuk S, Duarte WR, Mochida Y, Yamauchi M. Lysyl hydroxylase-2b directs collagen cross-linking pathways in MC3T3-E1 cells. *Journal of bone and mineral research: the official journal of the American Society for Bone and Mineral Research*. 2004; 19:1349–1355.
9. van der Slot AJ, Zuurmond AM, Bardoe AF, Wijmenga C, Pruijs HE, Sillence DO, Brinckmann J, Abraham DJ, Black CM, Verzijl N, DeGroot J, Hanemaaijer R, TeKoppele JM, Huizinga TW, Bank RA. Identification of PLOD2 as telopeptide lysyl hydroxylase, an important enzyme in fibrosis. *The Journal of biological chemistry*. 2003; 278:40967–40972. [PubMed: 12881513]
10. Heikkinen J, Risteli M, Wang C, Latvala J, Rossi M, Valtavaara M, Myllylä R. Lysyl hydroxylase 3 is a multifunctional protein possessing collagen glucosyltransferase activity. *The Journal of biological chemistry*. 2000; 275:36158–36163. [PubMed: 10934207]
11. Sricholpech M, Perdivara I, Nagaoka H, Yokoyama M, Tomer KB, Yamauchi M. Lysyl hydroxylase 3 glucosylates galactosylhydroxylysine residues in type I collagen in osteoblast culture. *The Journal of biological chemistry*. 2011; 286:8846–8856. [PubMed: 21220425]
12. Lucero HA, Kagan HM. Lysyl oxidase: an oxidative enzyme and effector of cell function. *Cellular and molecular life sciences: CMLS*. 2006; 63:2304–2316. [PubMed: 16909208]
13. Eyre DR, Paz MA, Gallop PM. Cross-linking in collagen and elastin. *Annu Rev Biochem*. 1984; 53:717–748. [PubMed: 6148038]
14. Liu X, Zhao Y, Gao J, Pawlyk B, Starcher B, Spencer JA, Yanagisawa H, Zuo J, Li T. Elastic fiber homeostasis requires lysyl oxidase-like 1 protein. *Nature genetics*. 2004; 36:178–182. [PubMed: 14745449]
15. Bignon M, Pichol-Thievent C, Hardouin J, Malbouyres M, Brechot N, Nasciutti L, Barret A, Teillon J, Guillon E, Etienne E, Caron M, Joubert-Caron R, Monnot C, Ruggiero F, Muller L,

- Germain S. Lysyl oxidase-like protein-2 regulates sprouting angiogenesis and type IV collagen assembly in the endothelial basement membrane. *Blood*. 2011; 118:3979–3989. [PubMed: 21835952]
16. Nagan N, Kagan HM. Modulation of lysyl oxidase activity toward peptidyl lysine by vicinal dicarboxylic amino acid residues. Implications for collagen cross-linking. *The Journal of biological chemistry*. 1994; 269:22366–22371. [PubMed: 7915281]
17. Siegel RC. Biosynthesis of collagen crosslinks: increased activity of purified lysyl oxidase with reconstituted collagen fibrils. *Proceedings of the National Academy of Sciences of the United States of America*. 1974; 71:4826–4830. [PubMed: 4531019]
18. Narayanan AS, Page RC, Kuzan F, Cooper CG. Elastin cross-linking in vitro. Studies on factors influencing the formation of desmosines by lysyl oxidase action on tropoelastin. *Biochem J*. 1978; 173:857–862. [PubMed: 30449]
19. Kagan HM, Tseng L, Trackman PC, Okamoto K, Rapaka RS, Urry DW. Repeat polypeptide models of elastin as substrates for lysyl oxidase. *The Journal of biological chemistry*. 1980; 255:3656–3659. [PubMed: 6102568]
20. Kagan HM, Williams MA, Williamson PR, Anderson JM. Influence of sequence and charge on the specificity of lysyl oxidase toward protein and synthetic peptide substrates. *The Journal of biological chemistry*. 1984; 259:11203–11207. [PubMed: 6147351]
21. Li W, Nugent MA, Zhao Y, Chau AN, Li SJ, Chou IN, Liu G, Kagan HM. Lysyl oxidase oxidizes basic fibroblast growth factor and inactivates its mitogenic potential. *Journal of cellular biochemistry*. 2003; 88:152–164. [PubMed: 12461785]
22. Atsawasuwan P, Mochida Y, Katafuchi M, Kaku M, Fong KS, Csiszar K, Yamauchi M. Lysyl oxidase binds transforming growth factor-beta and regulates its signaling via amine oxidase activity. *The Journal of biological chemistry*. 2008; 283:34229–34240. [PubMed: 18835815]
23. Kang AH. Studies on the location of intermolecular cross-links in collagen. Isolation of a CNBr peptide containing -hydroxylysine norleucine. *Biochemistry*. 1972; 11:1828–1835. [PubMed: 5025627]
24. Wu JJ, Eyre DR. Identification of hydroxypyridinium cross-linking sites in type II collagen of bovine articular cartilage. *Biochemistry*. 1984; 23:1850–1857. [PubMed: 6426508]
25. Nicholls AC, Bailey AJ. Identification of cyanogen bromide peptides involved in intermolecular cross-linking of bovine type III collagen. *Biochem J*. 1980; 185:195–201. [PubMed: 6769428]
26. Knott L, Bailey AJ. Collagen cross-links in mineralizing tissues: a review of their chemistry, function, and clinical relevance. *Bone*. 1998; 22:181–187. [PubMed: 9514209]
27. Yamauchi M, Sricholpech M. Lysine post-translational modifications of collagen. *Essays in biochemistry*. 2012; 52:113–133. [PubMed: 22708567]
28. Gonzalez E, Hamabata A, Rojkind M. Some properties of the reactive hydroxylysyl residues in collagen: their possible role in nucleation during fibril formation. *Collagen and related research*. 1984; 4:339–350. [PubMed: 6509890]
29. Nagaoka H, Mochida Y, Atsawasuwan P, Kaku M, Kondoh T, Yamauchi M. 1,25(OH)2D3 regulates collagen quality in an osteoblastic cell culture system. *Biochemical and biophysical research communications*. 2008; 377:674–678. [PubMed: 18930711]
30. Saito M, Grynpas MD, Burr DB, Allen MR, Smith SY, Doyle N, Amizuka N, Hasegawa T, Kida Y, Marumo K, Saito H. Treatment with eldecacitol positively affects mineralization, microdamage, and collagen crosslinks in primate bone. *Bone*. 2015; 73:8–15. [PubMed: 25482210]
31. Terajima M, Perdivara I, Sricholpech M, Deguchi Y, Pleshko N, Tomer KB, Yamauchi M. Glycosylation and cross-linking in bone type I collagen. *The Journal of biological chemistry*. 2014; 289:22636–22647. [PubMed: 24958722]
32. Gerstenfeld LC, Riva A, Hodgins K, Eyre DR, Landis WJ. Post-translational control of collagen fibrillogenesis in mineralizing cultures of chick osteoblasts. *Journal of bone and mineral research: the official journal of the American Society for Bone and Mineral Research*. 1993; 8:1031–1043.
33. Wagenseil JE, Mecham RP. New insights into elastic fiber assembly. *Birth defects research. Part C, Embryo today: reviews*. 2007; 81:229–240.

34. Rooker GD, Steins GN, Tate LJ, Keeley FW. Biochemical identification of elastin in periosteum and pericranium. *Clinical orthopaedics and related research*. 1980; 152:292–295. [PubMed: 7438619]
35. Frankenhuys-van den Heuvel TH, Kuijpers-Jagtman AM, Maltha JC. Microscopic study of the rabbit mandibular periosteum and attached structures. *Acta anatomica*. 1991; 142:33–40. [PubMed: 1781237]
36. Chillemi C, Bevilacqua C, Gigante A. In vitro effects of elastase on periosteum of long bones: an histochemical, immunohistochemical and morphometric study. *European journal of histochemistry: EJH*. 1999; 43:227–233. [PubMed: 10563255]
37. Gigante A, Chillemi C, Quaglini D, Miselli M, Pasquali-Ronchetti I. DL-penicillamine induced alteration of elastic fibers of periosteum-perichondrium and associated growth inhibition: an experimental study. *Journal of orthopaedic research: official publication of the Orthopaedic Research Society*. 2001; 19:398–404. [PubMed: 11398852]
38. Cha HM, Kim SM, Choi YS, Kim DI. Scaffold-free three-dimensional culture systems for mass production of periosteum-derived progenitor cells. *J Biosci Bioeng*. 2015; 120:218–222. [PubMed: 25641580]
39. Larsen KH, Frederiksen CM, Burns JS, Abdallah BM, Kassem M. Identifying a molecular phenotype for bone marrow stromal cells with in vivo bone-forming capacity. *Journal of bone and mineral research: the official journal of the American Society for Bone and Mineral Research*. 2010; 25:796–808.
40. Selye H. Lathyrism. *Revue canadienne de biologie/editee par l'Universite de Montreal*. 1957; 16:1–82.
41. Bornstein P, Piez KA. The nature of the intramolecular cross-links in collagen. The separation and characterization of peptides from the cross-link region of rat skin collagen. *Biochemistry*. 1966; 5:3460–3473. [PubMed: 5972327]
42. Pinnell SR, Martin GR. The cross-linking of collagen and elastin: enzymatic conversion of lysine in peptide linkage to alpha-amino adipic-delta-semialdehyde (allysine) by an extract from bone. *Proceedings of the National Academy of Sciences of the United States of America*. 1968; 61:708–716. [PubMed: 5246001]
43. Mc KG, Lalich JJ, Schilling ED, Strong FM. A crystalline “lathyrus factor” from *Lathyrus odoratus*. *Archives of biochemistry and biophysics*. 1954; 52:313–322. [PubMed: 13208255]
44. Tang SS, Chichester CO, Kagan HM. Comparative sensitivities of purified preparations of lysyl oxidase and other amine oxidases to active site-directed enzyme inhibitors. *Connective tissue research*. 1989; 19:93–103. [PubMed: 2571459]
45. Tang SS, Trackman PC, Kagan HM. Reaction of aortic lysyl oxidase with beta-aminopropionitrile. *The Journal of biological chemistry*. 1983; 258:4331–4338. [PubMed: 6131892]
46. Trackman PC, Kagan HM. Nonpeptidyl amine inhibitors are substrates of lysyl oxidase. *The Journal of biological chemistry*. 1979; 254:7831–7836. [PubMed: 38246]
47. Tchapanian EH, Uriu-Adams JY, Keen CL, Mitchell AE, Rucker RB. Lysyl oxidase and P-ATPase-7A expression during embryonic development in the rat. *Archives of biochemistry and biophysics*. 2000; 379:71–77. [PubMed: 10864443]
48. Rucker RB, Kosonen T, Clegg MS, Mitchell AE, Rucker BR, Uriu-Hare JY, Keen CL. Copper, lysyl oxidase, and extracellular matrix protein cross-linking. *The American journal of clinical nutrition*. 1998; 67:996s–1002s. [PubMed: 9587142]
49. Wang SX, Mure M, Medzihradsky KF, Burlingame AL, Brown DE, Dooley DM, Smith AJ, Kagan HM, Klinman JP. A crosslinked cofactor in lysyl oxidase: redox function for amino acid side chains. *Science*. 1996; 273:1078–1084. [PubMed: 8688089]
50. Dubois JL, Klinman JP. Mechanism of post-translational quinone formation in copper amine oxidases and its relationship to the catalytic turnover. *Archives of biochemistry and biophysics*. 2005; 433:255–265. [PubMed: 15581581]
51. Mure M, Wang SX, Klinman JP. Synthesis and characterization of model compounds of the lysine tyrosyl quinone cofactor of lysyl oxidase. *Journal of the American Chemical Society*. 2003; 125:6113–6125. [PubMed: 12785842]

52. Fujii K, Kajiwaru T, Kurosu H. Effect of vitamin B6 deficiency on the crosslink formation of collagen. *FEBS letters*. 1979; 97:193–195. [PubMed: 33064]
53. Panchenko MV, Stetler-Stevenson WG, Trubetskoy OV, Gacheru SN, Kagan HM. Metalloproteinase activity secreted by fibrogenic cells in the processing of prolysin oxidase. Potential role of procollagen C-proteinase. *The Journal of biological chemistry*. 1996; 271:7113–7119. [PubMed: 8636146]
54. Uzel MI, Scott IC, Babakhanlou-Chase H, Palamakumbura AH, Pappano WN, Hong HH, Greenspan DS, Trackman PC. Multiple bone morphogenetic protein 1-related mammalian metalloproteinases process pro-lysin oxidase at the correct physiological site and control lysin oxidase activation in mouse embryo fibroblast cultures. *The Journal of biological chemistry*. 2001; 276:22537–22543. [PubMed: 11313359]
55. Cronshaw AD, Fothergill-Gilmore LA, Hulmes DJ. The proteolytic processing site of the precursor of lysin oxidase. *Biochem J*. 1995; 306(Pt 1):279–284. [PubMed: 7864821]
56. Fogelgren B, Polgar N, Szauter KM, Ujfaludi Z, Laczko R, Fong KS, Csiszar K. Cellular fibronectin binds to lysin oxidase with high affinity and is critical for its proteolytic activation. *The Journal of biological chemistry*. 2005; 280:24690–24697. [PubMed: 15843371]
57. Maruhashi T, Kii I, Saito M, Kudo A. Interaction between periostin and BMP-1 promotes proteolytic activation of lysin oxidase. *The Journal of biological chemistry*. 2010; 285:13294–13303. [PubMed: 20181949]
58. Borel A, Eichenberger D, Farjanel J, Kessler E, Gleyzal C, Hulmes DJ, Sommer P, Font B. Lysin oxidase-like protein from bovine aorta. Isolation and maturation to an active form by bone morphogenetic protein-1. *The Journal of biological chemistry*. 2001; 276:48944–48949. [PubMed: 11684696]
59. Lugassy J, Zaffryar-Eilat S, Soueid S, Mordoviz A, Smith V, Kessler O, Neufeld G. The enzymatic activity of lysin oxidase-like-2 (LOXL2) is not required for LOXL2-induced inhibition of keratinocyte differentiation. *The Journal of biological chemistry*. 2012; 287:3541–3549. [PubMed: 22157764]
60. Hong HH, Pischon N, Santana RB, Palamakumbura AH, Chase HB, Gantz D, Guo Y, Uzel MI, Ma D, Trackman PC. A role for lysin oxidase regulation in the control of normal collagen deposition in differentiating osteoblast cultures. *Journal of cellular physiology*. 2004; 200:53–62. [PubMed: 15137057]
61. Sharma-Bhandari A, Park SH, Kim JY, Oh J, Kim Y. Lysin oxidase modulates the osteoblast differentiation of primary mouse calvaria cells. *International journal of molecular medicine*. 2015; 36:1664–1670. [PubMed: 26497171]
62. Pischon N, Maki JM, Weissaupt P, Heng N, Palamakumbura AH, N'Guessan P, Ding A, Radlanski R, Renz H, Bronckers TA, Myllyharju J, Kielbassa AM, Kleber BM, Bernimoulin JP, Trackman PC. Lysin oxidase (lox) gene deficiency affects osteoblastic phenotype. *Calcif Tissue Int*. 2009; 85:119–126. [PubMed: 19458888]
63. Alsofi L, Daley E, Hornstra I, Morgan EF, Mason ZD, Acevedo JF, Word RA, Gerstenfeld LC, Trackman PC. Sex-Linked Skeletal Phenotype of Lysin Oxidase Like-1 Mutant Mice. *Calcif Tissue Int*. 2015; doi: 10.1007/s00223-015-0076-4
64. Bais M, McLean J, Sebastiani P, Young M, Wigner N, Smith T, Kotton DN, Einhorn TA, Gerstenfeld LC. Transcriptional analysis of fracture healing and the induction of embryonic stem cell-related genes. *PloS one*. 2009; 4:e5393. [PubMed: 19415118]
65. Iftikhar M, Hurtado P, Bais MV, Wigner N, Stephens DN, Gerstenfeld LC, Trackman PC. Lysin oxidase-like-2 (LOXL2) is a major isoform in chondrocytes and is critically required for differentiation. *The Journal of biological chemistry*. 2011; 286:909–918. [PubMed: 21071451]
66. Atsawasuwan P, Mochida Y, Parisuthiman D, Yamauchi M. Expression of lysin oxidase isoforms in MC3T3-E1 osteoblastic cells. *Biochemical and biophysical research communications*. 2005; 327:1042–1046. [PubMed: 15652501]
67. Saito M, Fujii K, Mori Y, Marumo K. Role of collagen enzymatic and glycation induced cross-links as a determinant of bone quality in spontaneously diabetic WBN/Kob rats. *Osteoporosis international: a journal established as result of cooperation between the European Foundation for Osteoporosis and the National Osteoporosis Foundation of the USA*. 2006; 17:1514–1523.

68. Saito M, Marumo K. Collagen cross-links as a determinant of bone quality: a possible explanation for bone fragility in aging, osteoporosis, and diabetes mellitus. *Osteoporosis international: a journal established as result of cooperation between the European Foundation for Osteoporosis and the National Osteoporosis Foundation of the USA*. 2010; 21:195–214.
69. Saito M, Marumo K. Bone quality in diabetes. *Frontiers in endocrinology*. 2013; 4:72. [PubMed: 23785354]
70. Vlassara H. The AGE-receptor in the pathogenesis of diabetic complications. *Diabetes/metabolism research and reviews*. 2001; 17:436–443. [PubMed: 11757079]
71. Litwinoff E, Hurtado Del Pozo C, Ramasamy R, Schmidt AM. Emerging Targets for Therapeutic Development in Diabetes and Its Complications: The RAGE Signaling Pathway. *Clinical pharmacology and therapeutics*. 2015; 98:135–144. [PubMed: 25974754]
72. Khosravi R, Sodek KL, Faibish M, Trackman PC. Collagen advanced glycation inhibits its Discoidin Domain Receptor 2 (DDR2)-mediated induction of lysyl oxidase in osteoblasts. *Bone*. 2014; 58:33–41. [PubMed: 24120383]
73. Khosravi R, Trackman PC. Diabetes-induced fibrotic matrix inhibits intramembranous bone healing. *Journal of cell communication and signaling*. 2014; doi: 10.1007/s12079-014-0242-x
74. Khosravi R, Sodek KL, Xu WP, Bais MV, Saxena D, Faibish M, Trackman PC. A novel function for lysyl oxidase in pluripotent mesenchymal cell proliferation and relevance to inflammation-associated osteopenia. *PloS one*. 2014; 9:e100669. [PubMed: 24971753]
75. Huang H, Song TJ, Li X, Hu L, He Q, Liu M, Lane MD, Tang QQ. BMP signaling pathway is required for commitment of C3H10T1/2 pluripotent stem cells to the adipocyte lineage. *Proceedings of the National Academy of Sciences of the United States of America*. 2009; 106:12670–12675. [PubMed: 19620713]
76. Halberg N, Khan T, Trujillo ME, Wernstedt-Asterholm I, Attie AD, Sherwani S, Wang ZV, Landskroner-Eiger S, Dineen S, Magalang UJ, Brekken RA, Scherer PE. Hypoxia-inducible factor 1alpha induces fibrosis and insulin resistance in white adipose tissue. *Molecular and cellular biology*. 2009; 29:4467–4483. [PubMed: 19546236]
77. Eliades A, Papadantonakis N, Bhupatiraju A, Burrridge KA, Johnston-Cox HA, Migliaccio AR, Crispino JD, Lucero HA, Trackman PC, Ravid K. Control of megakaryocyte expansion and bone marrow fibrosis by lysyl oxidase. *The Journal of biological chemistry*. 2011; 286:27630–27638. [PubMed: 21665949]
78. Lucero HA, Ravid K, Grimsby JL, Rich CB, DiCamillo SJ, Maki JM, Myllyharju J, Kagan HM. Lysyl oxidase oxidizes cell membrane proteins and enhances the chemotactic response of vascular smooth muscle cells. *The Journal of biological chemistry*. 2008; 283:24103–24117. [PubMed: 18586678]
79. Wan L, Zhang F, He Q, Tsang WP, Lu L, Li Q, Wu Z, Qiu G, Zhou G, Wan C. EPO promotes bone repair through enhanced cartilaginous callus formation and angiogenesis. *PloS one*. 2014; 9:e102010. [PubMed: 25003898]
80. Bailey AJ, Sims TJ, Light N. Cross-linking in type IV collagen. *Biochem J*. 1984; 218:713–723. [PubMed: 6426460]
81. Martin A, Salvador F, Moreno-Bueno G, Floristan A, Ruiz-Herguido C, Cuevas EP, Morales S, Santos V, Csiszar K, Dubus P, Haigh JJ, Bigas A, Portillo F, Cano A. Lysyl oxidase-like 2 represses Notch1 expression in the skin to promote squamous cell carcinoma progression. *The EMBO journal*. 2015; 34:1090–1109. [PubMed: 25759215]
82. Zhang J, Yang R, Liu Z, Hou C, Zong W, Zhang A, Sun X, Gao J. Loss of lysyl oxidase-like 3 causes cleft palate and spinal deformity in mice. *Human molecular genetics*. 2015; 24:6174–6185. [PubMed: 26307084]
83. Alzahrani F, Al Hazzaa SA, Tayeb H, Alkuraya FS. LOXL3, encoding lysyl oxidase-like 3, is mutated in a family with autosomal recessive Stickler syndrome. *Human genetics*. 2015; 134:451–453. [PubMed: 25663169]
84. Ito H, Akiyama H, Iguchi H, Iyama K, Miyamoto M, Ohsawa K, Nakamura T. Molecular cloning and biological activity of a novel lysyl oxidase-related gene expressed in cartilage. *The Journal of biological chemistry*. 2001; 276:24023–24029. [PubMed: 11292829]

85. Mäki JM, Tikkanen H, Kivirikko KI. Cloning and characterization of a fifth human lysyl oxidase isoenzyme: the third member of the lysyl oxidase-related subfamily with four scavenger receptor cysteine-rich domains. *Matrix Biology*. 2001; 20:493–496. [PubMed: 11691589]
86. Gothard D, Greenhough J, Ralph E, Oreffo RO. Prospective isolation of human bone marrow stromal cell subsets: A comparative study between Stro-1-, CD146- and CD105-enriched populations. *Journal of tissue engineering*. 2014; 5:1–17.
87. Min C, Kirsch KH, Zhao Y, Jeay S, Palamakumbura AH, Trackman PC, Sonenshein GE. The tumor suppressor activity of the lysyl oxidase propeptide reverses the invasive phenotype of Her-2/neu-driven breast cancer. *Cancer research*. 2007; 67:1105–1112. [PubMed: 17283144]
88. Palamakumbura AH, Jeay S, Guo Y, Pischon N, Sommer P, Sonenshein GE, Trackman PC. The propeptide domain of lysyl oxidase induces phenotypic reversion of ras-transformed cells. *The Journal of biological chemistry*. 2004; 279:40593–40600. [PubMed: 15277520]
89. Barker HE, Cox TR, Erler JT. The rationale for targeting the LOX family in cancer. *Nature reviews. Cancer*. 2012; 12:540–552. [PubMed: 22810810]
90. Ulmert D, Solnes L, Thorek D. Contemporary approaches for imaging skeletal metastasis. *Bone research*. 2015; 3:15024. [PubMed: 26273541]
91. Cox TR, Rumney RM, Schoof EM, Perryman L, Hoyer AM, Agrawal A, Bird D, Latif NA, Forrest H, Evans HR, Huggins ID, Lang G, Linding R, Gartland A, Erler JT. The hypoxic cancer secretome induces pre-metastatic bone lesions through lysyl oxidase. *Nature*. 2015; 522:106–110. [PubMed: 26017313]
92. Alsulaiman M, Bais MV, Trackman PC. Lysyl oxidase propeptide stimulates osteoblast and osteoclast differentiation and enhances PC3 and DU145 prostate cancer cell effects on bone in vivo. *Journal of cell communication and signaling*. 2015; doi: 10.1007/s12079-015-0311-9
93. Vora SR, Palamakumbura AH, Mitsi M, Guo Y, Pischon N, Nugent MA, Trackman PC. Lysyl oxidase propeptide inhibits FGF-2-induced signaling and proliferation of osteoblasts. *The Journal of biological chemistry*. 2010; 285:7384–7393. [PubMed: 20048148]
94. Nishida T, Emura K, Kubota S, Lyons KM, Takigawa M. CCN family 2/connective tissue growth factor (CCN2/CTGF) promotes osteoclastogenesis via induction of and interaction with dendritic cell-specific transmembrane protein (DC-STAMP). *Journal of bone and mineral research: the official journal of the American Society for Bone and Mineral Research*. 2011; 26:351–363.
95. Shalhoub V, Faust J, Boyle WJ, Dunstan CR, Kelley M, Kaufman S, Scully S, Van G, Lacey DL. Osteoprotegerin and osteoprotegerin ligand effects on osteoclast formation from human peripheral blood mononuclear cell precursors. *Journal of cellular biochemistry*. 1999; 72:251–261. [PubMed: 10022507]
96. El-Haibi CP, Bell GW, Zhang J, Collmann AY, Wood D, Scherber CM, Csizmadia E, Mariani O, Zhu C, Campagne A, Toner M, Bhatia SN, Irimia D, Vincent-Salomon A, Karnoub AE. Critical role for lysyl oxidase in mesenchymal stem cell-driven breast cancer malignancy. *Proceedings of the National Academy of Sciences of the United States of America*. 2012; 109:17460–17465. [PubMed: 23033492]
97. Valastyan S, Weinberg RA. Tumor metastasis: molecular insights and evolving paradigms. *Cell*. 2011; 147:275–292. [PubMed: 22000009]

Highlights

- The lysyl oxidase family of proteins is well-known to be critical for collagen and elastin cross-linking required for the normal function of connective tissues.
- This family of proteins has more recently been found to have multiple functions in biology.
- This review is focused on summarizing evolving information regarding functions of lysyl oxidases in bone biology.
- Topics covered include effects on cell proliferation, differentiation and function of a variety of cell types, and emerging roles in diabetic osteopenia and metastatic cancers relevant to bone.

Figure 1. Major biosynthetic collagen and elastin cross-links

Red residues indicate the lysine or hydroxylysine residues not modified by lysyl oxidases.

Dehydroaldohistidine can react further with an unmodified hydroxylysine residue to form an aldimine resulting in the tetravalent cross-link dehydrohydroxymerodesmsine (not shown).

Figure 2. Domain structures of lysyl oxidase family members

Signal peptide, propeptide and enzyme domains are shown schematically with numbers of amino acids per domain indicated for human family members. The enzyme domains (red) contain copper binding sequences and the LTQ cofactor produced after translation. The lysyl oxidase family can be thought of as having two subfamilies consisting of LOX and LOXL1 as one subfamily, and LOXL2 – LOXL4 as the second, based on the degrees of sequence identity, particularly in the propeptide regions.

Figure 3. The structure of the LTQ cofactor

This structure is generated by posttranslational modification in the enzyme domain of all lysyl oxidase isoforms, determined by analogy with rat and bovine LOX [49]. The amino acid numbering shown are the relevant human LOX lysine and tyrosine residues.


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SDFLPSRPRYSWEVHSCQHYHSMDEFSHYDLDANTQRRVAEGHKASFCLEDTSCDYGYHRRFACIAH-TQGLSPGCDYTGADIDCQWIDI LOX (275-366)
ADFLPNRPRTWEVHSCQHYHSMDEFSHYDLDAAATGKVAEGHKASFCLEDTSCDPGNLKRYACTSH-TQGLSPGCDYTGADIDCQWIDI LOXL1
SDFRPKNGRHAWIWHDCRRHYHSMDEFTHYDLDLNLNGT-KVAEGHKASFCLEDTCEGDQKRYECANFGDQGITMGCDWITRHDIDCQWIDI LOXL2
ADFRPKAGRHSWVWHECHGHYSMDIFTHYDILTFNGT-KVAEGHKASFCLEDTCEQEDVSKRYECANFGDQGITMGCDWITRHDIDCQWIDI LOXL3
TDFRPKTGDRDSWVWHECHGHYSMDIFTHYDILTLNGS-KVAEGHKASFCLEDTINCPDGLQRRYACANFGDQGITMGCDWITRHDIDCQWIDI LOXL4

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Figure 4. Conserved copper-binding cysteine and histidine-rich region (yellow) and LTQ lysine and tyrosine residues (blue)

These amino acid sequences contain key features of lysyl oxidase family enzyme active sites. Numbers to the right indicate residue numbers shown for human LOX.