Lysyl oxidases in mammalian development and certain pathological conditions

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Summary. Lysyl oxidase (LOX) catalyzes the oxidation of the side chain of a peptidyl lysine converting specific lysine and hydroxylysine residues of α-aminoadipic-δ-semialdehydes, which form covalent crosslinks in collagens and elastin. Five different but closely related lysyl oxidase isoenzymes have been identified to date, and they seem to have overlapping functions in many tissues. Modification of the extracellular matrix by lysyl oxidases has been shown to be a critical contributor to the development of various organs and certain pathological conditions.

Key words: LOX, Copper enzyme, Branching morphogenesis, Aneurysm

Introduction

Lysyl oxidase (LOX) has traditionally been described as an extracellular cuproenzyme that initiates lysine-derived crosslink formation in collagens and elastin. Its role in physiology, development and disease states has proved to be much more complicated, however, and cannot be entirely explained by its function in the extracellular matrix (ECM). LOX has also been identified inside cells, and even in the nucleus, and researchers have identified new functions and substrates for it in recent years. Current findings have shown that LOX is required for the hypoxia-mediated metastasis of certain cancer cells and for the development of major organs, such as the cardiovascular and respiratory systems, and have assigned it highly important roles in development and diseases in terms of migration, invasion, epithelial-mesenchymal transition (EMT) and even intracellular signalling (Fig. 1). In addition, other members of the lysyl oxidase gene family (LOXL1-4) seem to have a wide range of effects on the behaviour of different cell types.

LOX in mammalian development

The role of LOX in cardiovascular development and diseases has been studied mainly using animal models and tissues. Inactivation of the LOX gene in the mouse leads to perinatal death caused by major dysfunction and developmental defects in the cardiovascular and respiratory systems, and the mice have generalized elastolysis and abnormal collagen content in various tissues (Mäki et al., 2002, 2005; Hornstra et al., 2003). Mouse LOX mRNA is expressed prominently in the cardiovascular system during embryonic development, and its expression level is upregulated significantly between E11.5-E13.5 (Tsuda et al., 2003). Lysyl oxidation, reflecting the activity of LOX, can be detected in rat embryos starting from E9.5, which corresponds to a period of transition from the post-blastocystic stage to the pre-embryonic state and follows expression of the ATP7A gene, which provides a copper ion for the...
reaction catalyzed by LOX (Tchaparian et al., 2000). The temporal expression pattern of LOX overlaps with those of the main collagen types and elastin, and the roles of its substrates, especially those of collagen types I and IV, are crucial for normal tissue growth in the early development of mouse embryos, as observed in studies of corresponding mouse models (Löhler et al., 1984; Pöschl et al., 2004). It is therefore surprising that depletion in LOX activity causes major organ defects only at the later stages in embryonic development, suggesting that the other LOX isoenzymes probably have compensatory effects. Development of the vasculature in elastin-knockout mice is indistinguishable from that in the wild type until E17.5, clearly demonstrating the restricted role of elastin in the maturation of the cardiovascular system during embryonic development (Li et al., 1998), and also pointing to the fact that the defects observed in Lox−/− mice are not purely caused by elastinopathy. LOX seems to have a major role in all the cell types that contribute to the normal structure and function of vascular walls. Endothelial cells in the vascular system of Lox−/− E18.5 embryos showed degenerative changes, such as blebbing, vacuolization and detachment from the internal elastic lamina (Mäki et al., 2002). This observation supports the finding that LOX is needed for maintenance of the endothelial barrier. LOX thus seems to have a role in atherogenesis and endothelial dysfunction triggered by atherosclerotic risk factors and proinflammatory cytokines such as TNF-α (Alcudia et al., 2008). The most obvious affect of LOX during embryogenesis seems to be its ability to enhance the physical properties of the ECM of vascular walls and other tissues. Improperly crosslinked elastin and collagens not only affect the function of specific cell types, such as vascular smooth muscle cells, but have also wide systemic effects, as shown by ultrasonographic studies of Lox−/− embryos with increased pulsatility indices in the major arteries and veins (Mäki et al., 2002). LOX seems to be the major crosslinking enzyme before parturition, as it is responsible for over 80% of total lysyl oxidase activity in skin fibroblasts and aortic smooth muscle cells isolated from E18.5 mouse embryos (Mäki et al., 2003). Lack of its activity reduces the amount of desmosine crosslinks in embryonic lungs and aorta by approximately 60%, and immature collagen crosslinks in the whole embryonic body by 40% (Hornstra et al., 2003). These findings also reveal the partially compensatory mechanisms provided by the other lysyl oxidase family members. LOX activity seems to enhance the durability of elastic fibres and the ECM in the vascular system, since the prevalence of aneurysms and the destruction of elastic fibres are more prominent in the large arteries near the heart that have a higher blood pressure.

In addition to the cardiovascular system, LOX deficiency disturbs development of the respiratory system and some other tissues. Lung parenchymal and pleural urea-extractable lysyl oxidase activity is relatively high in rabbits in the first 3 weeks, but starts to decrease by approximately 50% during weeks 4-10. By contrast, lysyl oxidase activity in the airways remains high for the first 10 weeks and then decreases by 50%. Pneumonecetomy, and the subsequently decreased oxygen content of tissues (by 12-13%) resulted in a prompt and sustained increase in lung, but not pleural or airway enzyme activity (Kagan, 1986), thus suggesting differential roles and/or differential tissue distributions among the lysyl oxidases. Postnatal inhibition of LOX by β-aminopropionitrile (βapn) in rats during the first 4 weeks of life results in a 40-56% reduction in alveoli. Moreover, the alveoli are significantly enlarged, with fragmented elastic fibres and loosely arranged collagen fibres of widely varying diameter (Kida and Thurlbeck, 1980). Lysyl oxidation thus seems to play a role in the regulation of postnatal lung development. It nevertheless

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**Fig. 1.** Proposed functions of LOX in cell biology. Multifunctional LOX modifies ECM proteins and intracellular substrates, contributes to the motility and migration of cells and promotes cancer invasion and cellular transformation.
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has an even more crucial role in the development of the embryonic lungs and other respiratory organs, such as the diaphragmatic muscle (Mäki et al., 2005). The development and branching of the distal and proximal airways of Lox−/− mouse embryos are severely impaired, with characteristic abnormalities in elastic and collagen fibres. Lung morphogenesis in mice starts with the formation of two lung buds at E9.5, and thereafter branching is continued until the proper structures of the lung lobes are achieved (Ten Have-Opbroek, 1991). Branching morphogenesis itself is strictly regulated by various factors, such as the FGFs, Spry’s, BMP-4 and TGF-β1 (Cardoso, 2001). At the pseudoglandular stage of lung maturation in the rat (E9.5 to E14.2 in the mouse embryo), collagen fibres and fibronectin (FN) are predominantly concentrated at the sites of bifurcations in the primitive bronchial primordia, giving rise to further branching of the primitive bronchi and bronchioles at later stages of development (Wasowicz et al., 1996; Roman, 1997). FN has been found to bind LOX with high affinity, and to play a role in the regulation of its proteolytic activation (Fogelgren et al., 2005), so that it may enhance the LOX-mediated crosslinking of collagens at sites of bifurcation and subsequently promote branching. This may be one of the regulatory steps that control the ECM remodelling that is needed for proper branching of the lungs. Other known regulators of branching morphogenesis in lung tissue are hypoxia (Gebb and Jones, 2003) and various effectors, such as retinoic acid and TGF-β, all of which also regulate LOX transcription and activity (Smith-Mungo and Kagan, 1998; Cardoso, 2001; Csiszar, 2001). In addition to its contribution to inhibiting the proliferation and differentiation of epithelial cells in the lungs, TGF-β is known to promote the synthesis of the ECM, which, when deposited at the epithelial-mesenchymal interface, is thought to prevent local branching (Cardoso, 2001). TGF has also been found to upregulate the mRNA expression and activity of LOX (Csiszar, 2001) and may thus contribute to the remodelling of the ECM at branching sites via LOX itself.

The elastin and collagen defects caused by the decreased lysyl oxidation activity in Lox−/− animals is not limited to the respiratory and cardiovascular systems but is also manifested in other tissues. The total quantity of collagen crosslinks is reduced by 40% in the body of mutant embryos (Hornstra et al., 2003), and both elastic and collagen fibres have abnormal appearance in their skin (Mäki et al., 2005). LOX clearly makes a major contribution to ECM components, which not only regulate embryonic development but affect various pathological conditions.

LOX in pathological conditions

The role of LOX in cardiovascular diseases has been recently reviewed thoroughly by Alcudia and co-workers (2008), and will not be discussed further here. A great number of reports have suggested a strong association between organ fibrosis and increased lysyl oxidase activity in hepatic fibrosis in the rat, in models of lung, arterial and dermal fibrosis and in chronic human liver fibrosis, adriamycin-induced kidney fibrosis and other pathological conditions leading to fibrosis (see Kagan 1986, 1994; Smith-Mungo and Kagan, 1998). It has been suggested that chemotherapeutic inhibition of lysyl oxidase activity may prove to be a useful tool for the control of fibrosis, since it leads to the accumulation of insufficiently crosslinked and thus more soluble collagen molecules that are significantly more susceptible to proteolytic degradation.

Reduced lysyl oxidase activity has been detected in two X-linked recessively inherited disorders, Menkes disease and its milder variant occipital horn syndrome (OHS) (Horn and Tümer, 2002; OMIM 309400 and 304150). Both diseases are caused by mutations in the ATP7A gene that encodes a copper-transporting P-type ATPase, leading to inadequate availability of copper (Horn and Tümer, 2002). Due to the inefficient functioning of many cuproenzymes, including lysyl oxidase family members, the manifestations of these diseases are widespread and affect several tissues. Menkes disease patients have severe abnormalities in neural development and certain connective tissue abnormalities, and often die prematurely (Horn and Tümer, 2002), whereas OHS patients exhibit only mild neurological changes and mainly connective tissue defects, suggesting that the lysyl oxidases may be the most sensitive enzymes to copper availability in cells (Horn and Tümer, 2002). Mottled blotchy mice, an animal model for OHS, have a significantly higher incidence of aortic aneurysms, exhibit disrupted elastic fibres in the vascular walls and lungs, and have structurally and functionally abnormal lungs (Andrews et al., 1975; Fisk and Kuhn, 1976; Brophy et al., 1988), thus bearing a striking resemblance to lathyric, Lox−/− and Lox1+/− animal models (Mäki et al., 2002; Steinmann et al., 2002; Hornstra et al., 2003; Liu et al., 2004; Mäki et al., 2005) and animals exposed to copper deficiency (Kagan, 1986). Although the lysyl oxidases are implicated in the connective tissue problems associated with Menkes disease and OHS, no mutations in the LOX or LOXL genes are currently known that cause a predisposition to these diseases. Both upregulation and downregulation of LOX have been observed in Wilson disease, another condition which results from abnormal copper metabolism, attributed to mutations in the ATP7B gene (OMIM 277900), also encoding a copper-transporting P-type ATPase (Linz and Lutsenko, 2007). The fact that the change in LOX activity is clearly milder and more restricted to certain tissues in Wilson patients can be explained by differential expression of the ATP7A and ATP7B genes (Vadasz et al., 2005; Linz and Lutsenko, 2007).

The role of LOX in certain neurodegenerative diseases has also been studied. Upregulation and altered distribution of LOX has been observed in the central nervous system of a mouse model (mSOD1) for
amyotrophic lateral sclerosis (ALS) (Li et al., 2004). Interestingly, LOX is expressed not only in the fibrogenic cells, including the vascular walls of the central nervous system (CNS), but also in the brain matrix and neurons of normal mice and rats. In mSOD1 mice LOX expression and immunoreactivity is observed in sites where it is not normally observed, and the amount of LOX protein is increased in the neurons of the spinal cord, brain stem and cortex and the Purkinje cells of the cerebellum. In addition, the expression level is enhanced in the neurons of various parts of the CNS and the enzyme activity increases coincidentally with late-stage ALS (Li et al., 2004). LOX has also been characterized as an electroconvulsive shock-inducible gene of the CNS, and thus shows conditional and tissue-specific expression, which supports its potential function in the CNS (Sun et al., 2005). Lysyl oxidase activity is increased by approximately 30% in Alzheimer disease (AD) and in non-Alzheimer dementia, and is localized in the blood vessel walls and in plaque-like structures typical of these diseases (Gilad et al., 2005). Moreover, the number of LOX-positive plaque-like structures is more than two-fold higher in AD than in patients with non-AD dementia (Gilad et al., 2005). These findings suggest that LOX itself not only contributes to connective tissue manifestations of diseases, but may also affect the normal functioning and development of the CNS.

LOX is capable of suppressing cellular transformation, but paradoxically, also enhances invasion by certain cancer cells. The first evidence of the tumor suppressor activity of LOX was the observation that it is able to suppress the activation of c-H-ras in mouse NIH 3T3 cells, and thus subsequently inhibits transformation of these cells (Conte et al., 1990). Numerous studies have since supported this finding, demonstrating that the levels of expression and activity of LOX are reduced in many types of cancer cell (Csiszar, 2001; Payne et al., 2007). There is also a considerable amount of evidence that the tumor suppressor activity of LOX is caused by its intracellular actions, through its effects on intracellular signalling (Payne et al., 2007). The reduced expression of LOX mRNA in tumor cells may at least partly be the result of autocrine growth factor pathways, such as bFGF, or signalling cascades related to ras or other oncogenic processes (Palamakumbura et al., 2003). Ras itself mediates cellular transformation partly, and indirectly, by activation of the transcription factor NF-κB. Ectopic expression of LOX in ras-transformed NIH-3T3 cells resulted in decreased NF-κB transcriptional activity, inhibiting the nuclear intake of NF-κB. This effect was mediated via strong downregulation of both PI3K and Akt kinases and partial inhibition of MEK. Thus some of the anti-oncogenic effects of LOX on ras-mediated cellular transformation may result from a LOX-dependent inhibition of signalling pathways leading to the activation of NF-κB (Jeay et al., 2003). The exact role of the catalytic activity of LOX in transformation is currently unclear, but there is some evidence to suggest that the LOX propeptide (LOX-PP) may be mainly responsible for induction of the reversion of ras-transformed NIH-3T3 cells to the non-oncogenic phenotype (Palamakumbura et al., 2003, 2004; Min et al., 2007; Wu et al., 2007a). The LOX-PP was found to inhibit transformation of breast cancer cells driven by Her-2/neu, an upstream activator of ras, by suppressing activation of the extracellular signal-regulated kinase Akt and NF-κB. In addition, it was found to inhibit signalling cascades induced by Her-2/neu that promote a more invasive phenotype (Min et al., 2007). In addition, ectopic expression of LOX-PP in pancreatic cancer cells has been shown to reduce ERK and Akt activities, inhibit growth in soft agar and migration, and reduce levels of NF-κB and its target BCL2 (Wu et al., 2007a).

Both the catalytic activity of LOX and its mRNA expression are dramatically downregulated in most cancer cells. Recent studies have demonstrated, however, that the LOX mRNA level is elevated in highly invasive cancer cells, for example, and affects their invasive properties (Payne et al., 2007). LOX mRNA was found to be upregulated in invasive breast cancer cells, enhancing their invasive capability, and the mediator of this was shown to be the catalytically active form of LOX. The LOX-dependent chemotactic response of these cells was elicited by H2O2 (a by-product of the LOX-catalysed reaction) produced by LOX acting on one or more unidentified substrates (Payne et al., 2007). The role of H2O2 produced by the LOX-catalysed reaction in chemotaxis has also been pinpointed in other studies and cell lines (Lazarus et al., 1995; Li et al., 2000; Lucero et al., 2008), but the actual mechanism is still under investigation. Inhibition of LOX activity increases actin stress fibre formation and Rho activity in breast cancer cells through the p130(Cas)/Crk/DOCK180 signalling complex (Payne et al., 2006) and thus inhibition of the LOX catalytic activity could limit the invasiveness of cancer cells. Erler et al. (2006) observed that the elevation of LOX expression under hypoxic conditions appears to be essential for the hypoxia-induced metastatic response of breast cancer and head and neck squamous carcinoma cells (HNSCC), and that inhibition of LOX reduced the invasiveness of cancer cells. Mechanistically, the secreted and active form of LOX seemed to be responsible for the invasive properties of the hypoxic cancer cells through its effects on focal adhesion kinase (FAK) activity and cell-to-matrix adhesion. Postovit and co-workers (2008) confirmed that hypoxia greatly enhances LOX expression, but at the same time reduces its catalytic activity. The LOX-dependent activation of FAK/Src and migration of poorly invasive breast cancer cells is markedly increased during re-oxygenation following the hypoxia, but not in hypoxia alone. Furthermore, LOX expression seems to be only partially dependent on the hypoxia-inducible transcription factor 1 (HIF-1) and to be independent of a hypoxic environment at the later stages of tumor progression. An
association between LOX and EMT, the initial step in metastasis, has also been established (Higgins et al., 2007; Sahlgren et al., 2008). Recent findings have shown that LOX may be a good therapeutic target for preventing and treating tumor metastases.

**Lysyl oxidase-like proteins (LOXL1-4)**

The lysyl oxidase gene family consists of 5 members, coding for LOX itself and lysyl oxidase-like (LOXL) proteins 1-4. LOX and LOXL1 differ drastically from the other family members within their N-terminal regions, but all the members have high similarity in their catalytic domain (Fig. 2). The spectrum of the substrates and biological functions of LOXL1, 2, 3 and 4 remain to be established.

**Lysyl oxidase-like 1 (LOXL1)**

LOXL1 cDNA was originally characterized by Kenyon et al. (1993), and it has been identified as a secreted protein that is expressed in the ECM in active fibrotic diseases and in the early stromal reaction in breast cancer (Decitre et al., 1998). An inactive LOXL1 precursor has been isolated from the bovine aorta, and can be activated by BMP-1 *in vitro* on collagen and elastin (Borel et al., 2001). Liu and co-workers (2004) showed that LOXL1 is essential for elastic fibre homeostasis. Loxl1−/− mice do not deposit entirely normal elastic fibres in the uterine tract post partum and they develop pelvic organ prolapse, enlarged airspaces in the lungs, loose skin, intestinal diverticula and vascular abnormalities with concomitant tropoelastin accumulation. The LOXL1 protein was observed to colocalize with fibulin-5 at sites of elastogenesis. The study clearly showed the significance of elastic fibre renewal for tissues that are exposed to physical forces and therefore need the ability to deform repetitively and reversibly. In a continuation to this work, Liu and coworkers analysed the role of LOXL1 in elastic fibre formation and renewal in pelvic floor disorders using the Loxl1−/− mouse line as an animal model. LOXL1 was found to be highly expressed in the reproductive tract and downregulated during ageing. Loxl1−/− animals were unable to replenish their elastic fibres after parturition, which led to pelvic organ collapse, weakening of the vaginal wall, paraurethral pathology and bladder and lower urinary tract dysfunction (Liu et al., 2006, 2007). These findings are very similar to the phenotype of fibulin-5 knockout animals, thus strongly supporting an essential interaction between fibulin-5 and LOXL1 in elastic fibre homeostasis (Nakamura et al., 2002). LOXL1 is assumed to act via fibulin-5, a component of elastic fibres which has also been shown to bind tropoelastin, the uncrosslinked and soluble form of elastin (Liu et al., 2004). The role of fibulin-5 seems to be highly critical for elastogenesis, as it serves as an organizer molecule for some of the components of elastic fibres (Hirai et al., 2007). It deposits microfibrils, promotes aggregation of tropoelastin molecules through coacervation and also interacts not only with LOXL1, but also with LOXL2 and LOXL4, and may therefore tether these enzymes to microfibrils and subsequently facilitate aggregation and crosslinking of elastin itself to microfibrils (Hirai et al., 2007). The pro-region of LOXL1 is probably needed for its efficient secretion from cells and mediates its association in the ECM (Thomassin et al., 2005). This may be feasible, since the pro-regions of these enzymes do have a positive effect on their solubility and a negative effect on their activation, and therefore will change their physical properties, keeping the actual enzymatic activity latent until it is needed and then induced by the processing enzymes (Smith-Mungo and Kagan, 1998; Kagan and Li, 2003; Lucero and Kagan, 2006).

LOXL1 gene mutations (R141L, G153D) have recently been linked to the pseudoexfoliation syndrome (PXS) (Thorleifsson et al., 2007), which is described as
an age-related, systemic elastic microfibrillopathy (Ritch, 2008). PXS is also associated with an increased risk of cardiovascular and cerebrovascular diseases (Ritch and Schötzler-Schrehardt, 2001). Interestingly, the same G153D mutation of the LOXL1 gene was found to be associated with spontaneous cervical artery dissection (Kuhlenbäumer et al., 2007). Both detected mutations are located in the pro-region of LOXL1, which is thought to be involved on account of its association with the ECM. The effects of these mutations are not limited to the eye, however, since abnormal elastic fibres can also be found in the heart, lung, liver, kidney and other tissues of patients with PXS (Tarkkanen et al., 2008).

Overlapping manifestations can be seen in patients suffering from cutis laxa, a disease caused by mutations in the fibulin-5 and elastin genes (Zhang et al., 1999; Loeys et al., 2002), and in OHS (Horn and Tümer, 2002). The latter is also characterized by defects in collagen crosslinking and extractability (Horn and Tümer 2002), for which no direct association with LOXL1 defects has been found in humans or in animal models.

In cancer, LOXL1 has been localized to the stromal reaction in broncho-alveolar carcinomas and ductal breast tumors (Payne et al., 2007). LOXL1 expression can be detected in highly invasive/metastatic MDA-MB-231 and Hs578T breast cancer cell lines, but is absent from the poorly invasive cell lines MCF-7 and T47D (Kirschmann et al., 2002). The LOXL1 gene has been shown to be silenced by methylation in bladder cancer cells and primary tumors, and reintroduction of LOXL1 genes into bladder cancer cells leads to decreased colony formation ability (Wu et al., 2007b). In the same study, overexpression of LOXL1 was found to antagonize ras via the ERK signalling pathways. LOXL1 has also been shown to interact and cooperate with the Snail transcription factor, a regulator of EMT (Peinado et al., 2005).

**Lysyl oxidase-like proteins with scavenger receptor cysteine-rich repeats (LOXL2-4)**

In contrast to the C-terminal regions, the N-terminal regions of the LOX family members show only minor sequence homology, except that full-length LOXL2, LOXL3, and LOXL4 contain four repeated copies of scavenger receptor cysteine-rich (SRCR) domains in their N termini (Fig. 2). These domains, which are known to mediate protein-protein interactions in cell adhesion and cell signalling, are found on either cell surface proteins or secreted proteins (Csiszar, 2001). The actual function of the SRCR domains with respect to LOXL2-4 remains to be resolved.

Human LOXL2 was originally cloned and characterized by Saito et al. (1997), who named it WS9-14 due to its possible association with Werner syndrome, a disease characterized by premature ageing. LOXL2 is abundantly expressed in senescent fibroblasts and several adherent tumor cell lines, but is down-regulated in non-adherent cell lines, suggesting that LOXL2 may be involved in cell adhesion and may thus contribute to the metastasis of cancer cells (Saito et al., 1997). LOXL2 expression is increased in a variety of cancer cells, including colon adenocarcinomas, esophageal squamous cell carcinomas and invasive breast cancer cell lines (Kirschman et al., 2002; Fong et al., 2007). On the other hand, it is downregulated in some cancer cell lines such as ras-transformed rat fibroblasts, head and neck squamous cell carcinomas and ovarian tumors (Hough et al., 2000; Ono et al., 2000; Zuber et al., 2000; Rost et al., 2003), which suggests that it is regulated differentially in various cancer cell types. Akiri and co-workers (2003) found that LOXL2 expression in periductal carcinomas is significantly correlated with tumor malignancy. It is highly expressed in invasive/metastatic breast cancer cells, but not in the non-metastatic estrogen-dependent MCF-7 cells. Overexpression of recombinant LOXL2 in the MCF-7 cells of nude mice produces estrogen-dependent tumors which develop more rapidly than tumors originating from control cell lines, are surrounded by a high concentration of dense collagen fibres and contain many fibrotic foci. MCF-7 cells overexpressing LOXL2 invade the pseudocapsules that surround the tumors, and proceed to the adjacent blood vessels, nerves and muscles. In highly invasive cancer cell lines LOXL2, 3 and 4 expression was found to correlate with the production of Snail, one of the mediators of EMT. Further analysis using a two-hybrid yeast screen showed that Snail could interact with all the lysyl oxidases, the catalytic domains of LOXL2 and LOXL3 being the most potent interacting partners, and could collaboratively repress E-cadherin transcription (Peinado et al., 2005). Interaction in the Snail/LOXL2/LOXL3 protein complex is likely to be dependent on the Snail N-terminal (SNAG) domain and the lysine residues K98 and K137 within it, which seem to be important for Snail stability and function. Knockdown studies employing RNA interference in Snail-expressing metastatic carcinoma cells showed that the downregulation of LOXL2 leads to decreased tumor growth associated with an increase in apoptosis and reduced expression of mesenchymal and invasive/angiogenic markers.

LOXL2 (and LOX) have been found to be upregulated in the hepatocytes of patients with Wilson’s disease, primary biliary cirrhosis and some other conditions characterized by liver fibrosis, with an accompanying increase in collagen deposition (Vadasz et al., 2005). Moreover, recombinant LOXL2 seems to be able to oxidize type I collagen. This was inhibited by D-penicillamine, a copper-chelator, but not by D-pen at a concentration that completely abolishes the activity of LOX. In addition, LOXL2 inhibits the proliferation of HepG2 hepatoblastoma cells and catalyzes the oxidation of cell surface proteins (Vadasz et al., 2005). LOXL2 has also been identified as a gene conferring susceptibility to intracranial aneurysms (Akagawa et al., 2007). Lelièvre et al. (2008) suggested that LOXL2 is able to interact
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with VE-statin/egfll7, which co-localizes with elastic fibres in the vascular walls, thus suggesting a possible role for its regulation of elastogenesis. Moreover, VE-statin/egfl7 may also regulate the activity of other lysyl oxidase forms, since it binds directly to the catalytic domains of enzymes and its repression with siRNA enhances lysyl oxidation.

LOXL3 mRNA is expressed in a variety of tissues (Huang et al., 2001; Jourdan Le Saux et al., 2001; Mäki and Kivirikko, 2001), but its function is largely unknown. LOXL3 has a tissue-specific variant lacking exons 1-3 and 5, and thus lacking the SRCR domains 1-3, and it has also been suggested that this variant may be enzymatically active and oxidize collagens and elastin (Lee and Kim, 2006). LOXL3 is expressed in highly invasive/metastatic breast cancer cells but not in poorly invasive/metastatic ones (Kirschmann et al., 2002). Overexpression of LOXL3 in epithelial cells induces EMT, and is thought to co-operatively regulate Snail transcription factor in cancer cells (see previous chapter and Peinado et al., 2005). In mouse models of cardiac remodelling, LOXL3 expression and activity have also been associated with ventricular stiffness and incongruence with lymphocyte function (Yu et al., 2008).

LOXL4 was originally described as a cartilage-specific protein in mice with the ability to oxidize fibrillar collagens (Ito et al., 2001), but its mRNA expression pattern in human tissues was found to be much wider (Asuncion et al., 2001; Mäki et al., 2001). Like the other members of the lysyl oxidase gene family, it is expressed in highly invasive/metastatic breast cancer cells but not in poorly invasive/metastatic ones (Kirschmann et al., 2002). It is also upregulated in HNSCC cells relative to normal epithelial cells (Holtmeier et al., 2003), and in invasive HNSCC tumors and primary/metastatic HNSCC cell lines (Görögh et al., 2007; Weise et al., 2008). On the other hand, both LOXL4 and LOXL1 have been commonly observed to be silenced by methylation in human bladder cancer cells and to lose their expression in primary bladder tumors. Overexpression of the LOXL4 gene in bladder cancer cells leads to a decrease in colony formation ability and seems to antagonize ras via the ERK signalling pathways. Somatic mutations in LOXL4 have been found in bladder cancer, all of them in exon 8, which encodes for a SRCR domain (Wu et al., 2007b).

Concluding remarks and future prospects

Current results indicate that the lysyl oxidase family members have various roles, both intracellular and extracellular, in development and in pathological conditions, the most topical among these functions clearly being those connected with cancer biology. Regulation of these enzymes, especially LOX and LOXL2, clearly differs with the stage of cancer progression, being driven mainly by microenvironmental factors such as hypoxia. From a developmental biology viewpoint, the effects of the lysyl oxidases on cellular adhesion, migration and proliferation in particular may be a driving force during the development of various organs and should be investigated in more detail.

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