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Lysyl oxidase (LOX) – a future new ally on the stage of the fight against cancer

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ABSTRACT

The local extracellular matrix (EM) remodelling is considered one of the critical events in metastasis, both at primary and the secondary sites. One of the main actors on the EM remodelling is lysyl oxidase (LOX). In this connection, the aim of this paper is to bring to light a possible future systemic (and why not, salivary) biomarker of the metastatic process.
LOX best-characterized role is in the EM remodelling by oxidative deamination of collagens and elastin peptidyl lysine residues, in order to facilitate covalent cross-linking.
The LOX family members seem to play extremely important roles in these extracellular matrix interactions.
Future research is needed to confirm whether simple LOX blocking or its downstream actions control could be regarded as target of preventive treatment in patients at a higher risk of metastasis. This is how the portrait of the future biomarker of LOX is becoming more and more outlined.

Keywords: extracellular matrix, lysyl oxidase, biomarker, metastatic process

INTRODUCTION

Primary tumors spread to nonadjacent unrelated tissues has been proven to be a complex and very difficult challenge for researchers. This is because metastasis represents a complex systemic process involving the primary tumor cells, but also non-malignant cells at different secondary sites.

It has been shown that cancer cells secrete factors able to modulate the molecular behavior of host cells resident in secondary tissues, in the absence of the originating tumor cells (1). The local extracellular matrix (EM) remodelling is considered one of the critical events in metastasis, both at primary and the secondary sites (1). One of the main actors on the EM remodelling is Lysyl oxidase (LOX) (1). In this connection, the aim of this paper is to bring to light a possible future systemic (and why not, salivary) biomarker of the metastatic process (1-3).

So, let us find out who actually LOX is.

LOX is a member of a five secreted copper-dependent enzymes family, whose main biochemical function is to oxidize primary amines to reactive aldehydes (4,5).

Proteins with LOX activity have been identified not only in animals, but also in other eukaryotes, bacteria and archaea many, highlighting the pre-metazoan origin for this gene family (6).

LOX was first discovered as a result of analysing the effects of *Lathyrus oderatus* peas consumption. *Lathyrus oderatus* peas contain a natural LOX inhibitor – β-aminopripionitrile. *Lathyrus oderatus*

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peas consumption resulted in men and animals in severe connective tissue abnormalities which were especially apparent in bone (7). β-Aminopripionitrile is actually a active-site directed irreversible inhibitor of LOX (8). β-aminopripionitrile is still used in order to define LOX enzymatic activity. LOX inhibition by β-aminopripionitrile is unique to this enzymes family, while all other tissue-associated and serum amine oxidases remain unaffected or oxidize β-aminopripionitrile as a substrate (9). So, it can be sustained that an amine oxidase inhibited by low concentrations of β-aminopripionitrile is, by definition, a LOX, and an amine oxidase resistant to β-aminopripionitrile is not.

The LOX family includes 2 subgroups (10):

- LOXL1
- LOXL2 – LOXL4; the members of this subgroup have four conserved scavenger receptor cysteine-rich (SRCR) domains in the longer propeptidic region (10).

LOX are expressed in various cell types: basal and suprabasal keratinocytes, fibroblasts, adipocytes, osteoblasts, smooth muscle cells, and endothelial cells (4). Moreover, during fibrosis, LOX mRNA molecules are overexpressed in the oral submucosa, bone marrow, liver, lung, kidney, and skin (11).

LOX BIOSYNTHESIS

Biosynthesis of LOX enzymes includes the following steps (10):

- secretion of an inactive 50 kDa glycoprotein precursor (Pro-LOX). This precursor contains the copper and quinone cofactors (10);
- Pro-LOX undergoes extracellular proteolytic processing by the procollagen C-proteinases, resulting an active ~30 kDa lysyl oxidase and a~18 kDa N- and O-glycosylated lysyl oxidase propeptide (LOX-PP) (10).

LOX FUNCTIONS

The essential role played by mammalian LOX was demonstrated using $LOX^{-/-}$ mice, which died just before or soon after birth because of severe cardiovascular malformations, due to defective elastogenesis (12).

LOX best-characterized role is in the EM remodelling by oxidative deamination of collagens and elastin peptidyl lysine residues, in order to facilitate covalent cross-linking (13,14).

Enzyme-dependent collagen cross-linking is based on a constellation of intracellular procollagen alpha chains modifications of by lysyl hydroxylases and the extracellular modifications catalysed by LOX. The lysyl hydroxylases are endoplasmic reticulum-associated enzymes depending on α-ketoglutarate and ascorbate, cofactors also required by collagen prolyl hydroxylases (15). The lysyl hydroxylases catalyze the hydroxylation of the penultimate carbon atom of specific of procollagen alpha chains lysine residues (15). It is worth noting that only some specific lysine residues are hydroxylated. The hydroxylation degree is tissue-specific (16). Some of these new hydroxyl groups will further become sites of attachment for the collagen carbohydrate moieties of collagen (16). Collagen molecules glycosylation is quite different from the well-known N- and O-glycosylation pathways, occuring in the case of asparagine and serine/threonine residues of other proteins (16). Collagen glycosylation reactions are catalyzed by hydroxylysyl galactosyltransferase, followed by galactosylhydroxylysyl glucosyltransferase activity in order to produce glucosylgalacosyl hydroxylysine (17).

LOX activity is very important in ensuring the EM structural integrity and stability (13). In this way LOX contributes to the tensile strength of many connective tissues (18). Moreover, LOX can also activate the collagen III and elastin genes promoters, but is able indirectly to represses the cyclin D1gene promoter (18,19). So, it can be sustained that LOX has a main role in the EM biology, by intra- and extra-cellularly mechanisms.

Also, interesting *in vitro* studies revealed that LOX is able to oxidize FGF-2 and TGF-β, inhibiting in this way their ability to generate signals (20).

It has been shown that LOXL2 played important roles on the angiogenesis stage, in the context of endochondral bone formation and bone healing (11).

In the bone marrow progenitor cells LOXL4 expression may be considered a future, predictive marker of vivo bone formation (21).

Taken all together, the experimental data highlights the fact that LOX biological functions extend beyond collagen cross-linking. Nagan's model regarding LOX activity sustained that this enzyme would bind to the triple helical domain of the procollagen molecule before fibril formation, and then would act on a neighboring collagen molecule telopeptide lysine residue, during fibril formation (22).

The LOX enzyme family is more and more subject of intense research in the cancer context. Very interesting to note is the fact that LOX expression is typically induced under hypoxia conditions, as a result of the hypoxia response element (HRE) embedded within its promoter sequence (23).

Hypoxia represents a typical feature of almost all solid tumors. It is well known that solid tumors are characterized by increased growth and decreased cell death, causing significant oxygen deficiency (hypoxia) (23). In this regard, the expression and the enzymatic activity of LOX in tumors should be considered an important indicator of the transition from oxygen sufficiency to deficiency (23).

Active LOX molecules were most often associated with metastasis molecular mechanisms (24). In the context of metastasis EMT is a very important player, being involved in cells intravasation into the circulation, cells travel to the distant site, extravasation, and growth at this distant site (25). In order to understand the complex mechanism of each step of metastasis it is necessary a deep and thorough analysis of the extracellular matrix interactions at the cellular and molecular levels site (25). The LOX family members seem to play extremely important roles in these extracellular matrix interactions. These enzymes have multiple isoforms and different domains with opposing functions, which are incompletely understood yet site (25).

CONCLUSIONS

Elucidating the deep molecular mechanisms of the early events associated with solid tumor metastasis, including the discovery and characterization of the key effectors of this process, represent essential tasks for the development of efficient targeted cancer therapies. Further studies are required in order to elucidate the intimate mechanisms by which LOX molecules play their roles on the metastasis stage. Moreover, future research is needed to confirm whether simple LOX blocking or its downstream actions control could be regarded as target of preventive treatment in patients at a higher risk of metastasis. This is how the portrait of the future biomarker of LOX is becoming more and more outlined.

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