Emerging Role of Lysyl Oxidases in Promoting Tumour Metastasis

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The spread of tumour cells to distant organs, a process known as metastasis, is responsible for >90% of cancer-related deaths. Metastasis is a complex, multi-step process [1] (Figure 1), and the development of metastases is not random, as indicated in the 'seed and soil hypothesis' [2], which states that primary tumour cells ('seeds') survive preferentially in certain metastatic microenvironments ('soils'). Metastasis is therefore dependent on complex interactions between the tumour cells and the metastatic site. As a result the metastatic process is highly inefficient, with only an estimated 0.01% of circulating tumour cells being capable of initiating metastatic tumour growth [3]. There are few effective treatments for advanced metastatic cancer [4], hence a better understanding of the process at a molecular level is urgently needed.

Metastasis is largely driven by the properties of the environment surrounding tumour cells [5]. Factors that are involved in modifying the 'tumour microenvironment' are therefore of great clinical relevance and have provided a number of novel pharmaceutical targets. One such target is the lysyl oxidase (LOX) family of proteins, consisting of 5 members (LOX, LOX-like 1 [LOXL1], LOX-like 2 [LOXL2], LOX-like 3 [LOXL3] and LOX-like 4 [LOXL4]), all containing a conserved catalytic region and being implicated in cancer progression (Figure 2) [6]. The first member of this family to be characterised was LOX, a secreted copper-dependent enzyme that catalyses the oxidative deamination of lysine residues, a process resulting in covalent cross-linking of collagens and elastin in the extracellular matrix [7]. LOX is synthesised within the cell as an inactive 'proenzyme' that is cleaved into the active mature enzyme and a propeptide fragment after secretion into the extracellular environment (Figure 3) [8].

LOX expression is induced by factors such as transforming growth factor-β (TGF-β), tumour necrosis factor-α (TNF-α) and interferon-γ (IFN-γ) [8], all of which play a role in tumour development. The LOX gene is also a notable target of hypoxia inducible factor-1 (HIF-1) [9], a transcription factor that promotes target gene expression in response to low oxygen conditions ('hypoxia'), a common feature of solid tumours.

Initial studies identified LOX as a gene capable of reversing malignant transformation in vitro [10]. It has been proposed that the tumour suppressor function of LOX is due to the activity of the propeptide fragment, cleaved from the proenzyme with release of active LOX enzyme [11-13]. In contrast, evidence is accumulating in support of a role for the mature LOX enzyme in promoting tumour progression. LOX influences invasion, the first step of metastasis, of breast cancer cells [14, 15], an effect reported to be due to the increased stiffness that occurs as a result of collagen cross-linking catalysed by LOX [16, 17]. In support of this, we have recently shown that a catalytically inactive mutant form of LOX does not promote tumour progression in a colorectal cancer model [18], suggesting that LOX catalytic activity mediates metastasis. Importantly, we have shown that LOX levels in cancer patients are correlated with metastasis and decreased survival, and provided preclinical evidence that inhibition of LOX can suppress metastasis [14, 18]. Indeed, the dramatic suppression of tumour metastasis observed upon inhibition of LOX in preclinical models suggests that LOX influences a number of steps in the metastatic process.

Metastasis is strongly influenced by the formation of 'pre-metastatic niches' [19], in which clusters of immune cells (bone marrow-derived cells; BMDCs) and mobile factors secreted by tumour cells reach a distant site of future metastasis and facilitate the invasion and growth of tumour cells in that environment [20]. The presence of these BMDCs at pre-metastatic niches enhances metastasis; therefore preventing their accumulation at pre-metastatic sites may be a target for therapeutic intervention. We have recently shown that BMDCs and LOX co-localise in human metastatic tissue, and inhibition of LOX can prevent BMDC recruitment and metastasis in models of breast cancer metastasis [21].

Although many early studies focused on a role for LOX in metastatic breast cancer, more recent studies have revealed that LOX has value as a prognostic or metastatic marker for head and neck [22, 23], lung [24] and colorectal cancers [18]. Using a preclinical colorectal cancer model, we confirmed in vitro reports [25] that activation of Src protein kinase is

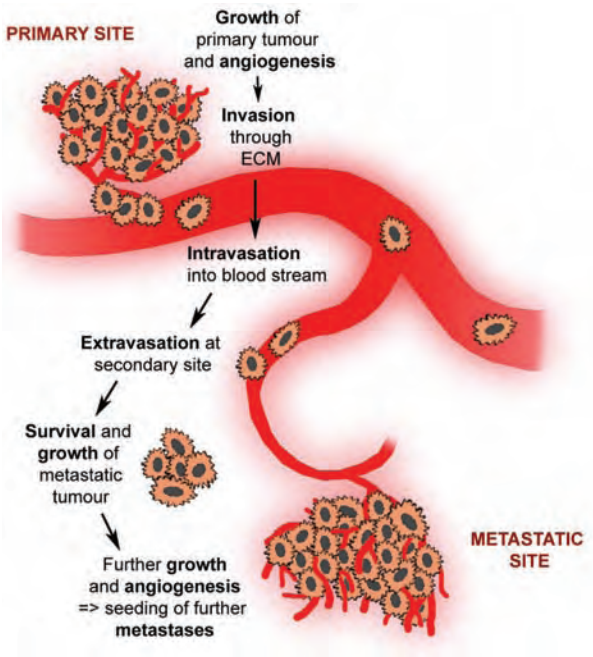

Figure 1: The multistep process of metastasis.
The metastatic process consists of a series of distinct, sequential steps, each of which must be achieved for metastasis to be successful (adapted from McGee et al. *Mammary gland biology and breast cancer.* Conference on Common Molecular Mechanisms of Mammary Gland Development and Breast Cancer Progression. EMBO Rep 2006;7(11):1084-8).

required for LOX to promote metastasis in vivo [18]. This is noteworthy as Src is a target of several drugs already in clinical use (e.g. dasatinib), and our work suggests that LOX expression may identify patients who will respond to these treatments. Furthermore, this model has provided further preclinical evidence that inhibition of LOX is effective in suppressing metastasis, supporting the work carried out in the breast cancer model [14].

Recent work has also suggested that LOX may promote primary tumour growth as well as metastasis in colorectal cancer [18, 26]. Further studies are needed to confirm whether LOX plays a similar role in other types of cancers, and if inhibition of LOX might be effective and viable in patients. The success of LOX as a therapeutic target in preclinical models has led to detailed investigation of the other members of the LOX protein family, in particular LOXL2.

LOXL2, like LOX, is an extracellular matrix copper-dependent enzyme thought to be involved in the cross-linking of stromal collagens and elastin [27, 28]. In addition to conserved C-terminal region the LOXL2 protein has scavenger receptor cysteine-rich regions that are commonly found in cell surface receptors and adhesion molecules, as well as a cytokine receptor-like domain (Figure 2).

The expression of LOXL2 is upregulated in breast, prostate [15], colon, esophageal [29], head and neck [30], and pancreatic carcinomas [31]. High LOXL2 expression has been associated with poor prognosis in patients with squamous cell carcinoma [30], increased liver metastases in colon cancer [32], as well as drug resistance in pancreatic cancer cells [31]. We have also established a correlation between LOXL2 expression, metastasis and poor survival in breast cancer patients [33]. Additionally, Akiri et al. [34] have shown that LOXL2 up-regulation increases the invasiveness of otherwise non-invasive breast cancer cells.

Recent work has shown LOXL2 is a direct target of HIF-1 at a transcriptional level, and upregulation of LOXL2 in hypoxia caused downregulation of E-cadherin, a classical hallmark of epithelial to mesenchymal transition (EMT; Figure 3) [35]. This is in accordance with previous findings where LOXL2 was shown to be involved in both EMT and tumour progression in murine squamous and spindle cell carcinomas [30, 36, 37]. It has since been suggested that LOXL2 contributes to tumour progression via Snai1 [36, 37]. Research from our laboratory has identified a role for LOXL2 in invasion and metastasis of mammary carcinoma cells, and has recently shown that LOXL2 promotes invasion of breast cancer cells by regulating the expression and activity of two extracellular proteins, tissue inhibitor of metalloproteinase-1 (TIMP-1) and matrix metalloproteinase-9 (MMP-9) [33]. This supports a previous report which positively associates LOXL2 with TIMP-1 expression in colorectal cancer [38]. LOXL2 has also been linked to Src kinase/focal adhesion kinase (Src/FAK) pathway activation, and this appears to be the major pathway where secreted LOXL2 induces gastric tumour cell invasion and metastasis [39].

Perhaps the most important aspect of LOXL2 is its potential as a therapeutic target. We have recently provided preclinical evidence that LOXL2 inhibition is highly effective against spontaneous lung, liver and bone metastases of mammary carcinoma cells [33]. This supports a study carried out by Barry-Hamilton et al. [40], which reported that LOXL2 antibody treatment significantly reduces bone metastases from intracardiac injection of breast carcinoma cells.

Consistent and encouraging results have established both LOX and LOXL2 as excellent therapeutic targets against both primary and metastatic tumours. LOX is required for both early and late stages of metastatic disease progression [14, 18, 21], and Bondareva et al. have shown that the use of β-aminoproprionitrile (BAPN), a known LOX inhibitor that may also inhibit

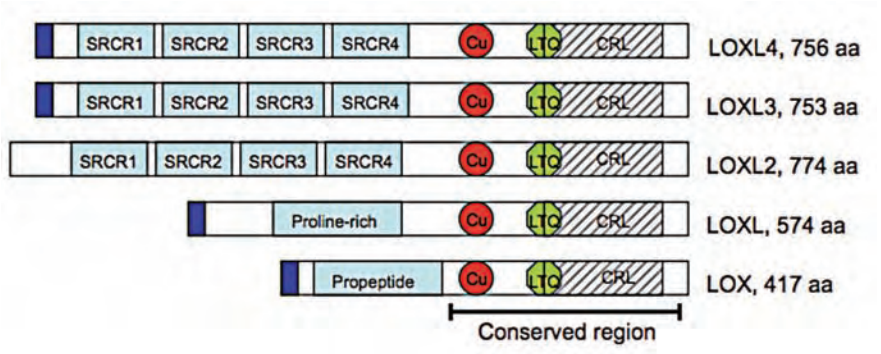

Figure 2: Structure of the LOX family members. LOXL2 has 4 scavenger receptor cysteine-rich regions (SRCR), which are replaced by a propeptide domain in LOX, and a proline-rich domain in LOXL. Red circles indicate the sites of histidine-containing putative copper-binding ('Cu') domains, and LTQ denotes lysine tyrosylquinone cofactor formation. Grey shaded boxes represent the cytokine receptor-like domains (CRL). Purple boxes indicate signal peptides. (aa = amino acids).

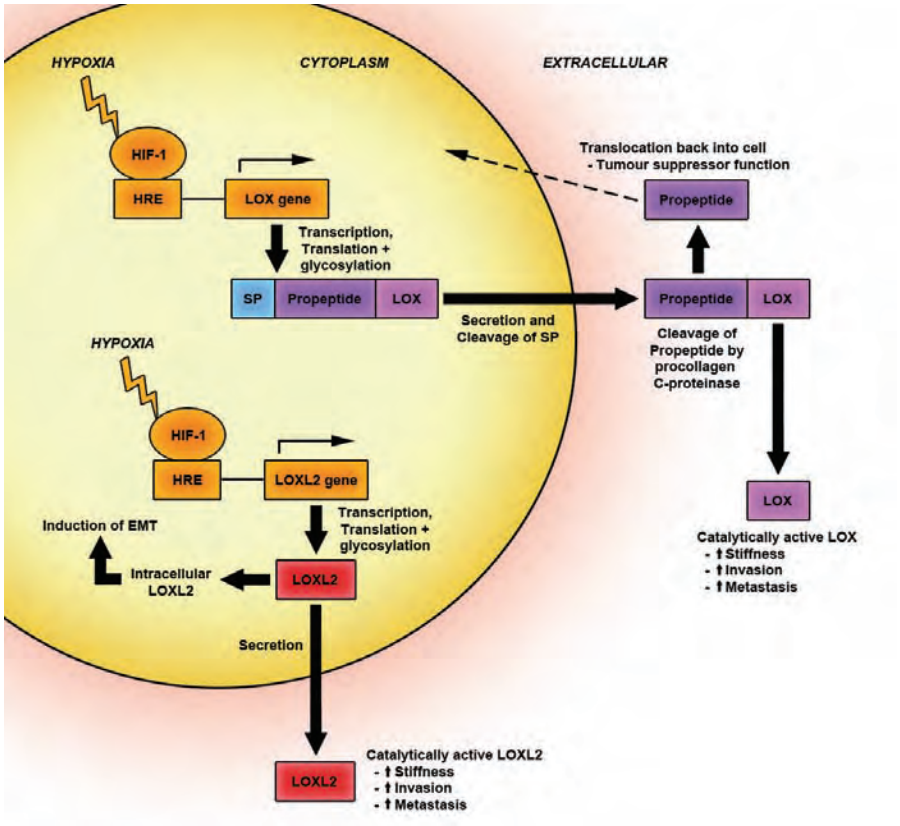

Figure 3: The roles of LOX and LOXL2 in tumorigenesis. The expression of the LOX and LOXL2 enzymes is induced by low oxygen ('hypoxia') . After secretion from the cell, they catalyse the cross-linking of collagens and elastin, resulting in increased tissue stiffness and invasive capacity. Additionally, both LOX and LOXL2 are thought to carry out intracellular roles, which may also influence tumour progression as indicated. (HIF-1 = hypoxia inducible factor, HRE = hypoxia response element, EMT = epithelial mesenchymal transition, SP = signal peptide).

LOXL2 [40], inhibits the metastatic colonisation potential of breast cancer cells [41]. Interestingly, our results suggested that LOXL2 is required only for early stages of metastatic breast cancer progression, as late treatments of MMTV-PyMT transgenic spontaneous tumourigenic mouse models with the non-specific LOXL2 inhibitor D-penicillamine [27] did not reduce metastatic burden [33]. However, the use of a more specific antibody against LOXL2 was effective against late-stage metastatic bone colonisation according to Barry-Hamilton et al. [40]. We have also reported no effects of LOXL2 genetic, chemical or antibody inhibition on primary tumour growth, whereas others have shown that treatment with different LOXL2-targeting antibodies resulted in decreased primary tumour growth [39, 40]. This may be due to differences in inhibitor efficiencies and methodologies, and indicates that further research is required to fully understand the mechanisms of LOXL2-mediated tumour progression.

Interestingly, our data showed that LOX and LOXL2 do not compensate one another, as the induced expression of one molecule is unable to compensate for the loss of the other (unpublished), which is supported by the embryonic lethality of LOX knockout mice [42, 43]. Furthermore, manipulation of LOX expression did not affect LOXL2 levels in our preclinical colorectal cancer model [18]. This suggests that while LOX and LOXL2 might be involved in similar processes, they have distinct pivotal roles.

Antibodies are effective therapeutic agents against secreted proteins such as LOX and LOXL2 since they do not cross the cell membrane and hence specifically target the extracellular functions of the molecules. However, this kind of treatment is expensive and not always supported by public health services. The alternative use of small molecule inhibitors is less costly and easier to administer. In addition, these compounds can cross the blood-brain barrier. So far, no deleterious side-effects have been reported in the use of LOX antagonists in preclinical studies.

The future of studies involving LOX and LOXL2 is exciting. The outcome of the LOXL2 antibody AB0024 from Arresto Biosciences (bought by Gilead Sciences) in human patient clinical trials is eagerly anticipated. Production of LOX monoclonal antibodies is expected to yield highly favourable results in cancer treatments, both in breast cancer and colorectal cancer, and LOX small molecule inhibitors are currently under development. Downstream effectors of LOX and LOXL2, such as Src kinase and TIMP-1, can be further validated and developed as biomarkers in the clinic to monitor the efficacy of treatments. The next years ahead will be a particularly interesting time for validation of these promising anti-metastatic targets. ■

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