REVIEW ARTICLE

THE ROLE OF LYSYL OXIDASE (LOX) IN PROMOTING CANCER METASTASIS

N. Ab Latif[1], H.R. Evans[2], N. Wang[2], J.T. Erler[3], A. Gartland[2].
[1]Faculty of Medicine, Universiti Kuala Lumpur, Royal College of Medicine Perak, Malaysia
[2] Human Metabolism, The University of Sheffield, Sheffield, UK
[3] Section of Cell and Molecular Biology, The Institute of Cancer Research, London, UK

Abstract

The mechanism of cancer metastasis has been well conceptualized which involves the crosstalk between the primary tumor cells, the premetastatic niche permissive for subsequent tumor cell colonization, cells from the myeloid lineage and even the noncancerous stromal cells within the premetastatic niche. Hypoxia and LOX have been indicated in various studies to have close relationship with numerous malignant cancer cell types and are associated with poor prognosis and low patient survival rate. There is also a distinct connection of hypoxia with increased expression of LOX protein by the primary tumor cells via hypoxia-inducible transcription factor (HIF-1α). Together with other proteins like matrix metalloproteinase-2 and -9 (MMP-2 and MMP-9) and fibronectin (FN), this LOX protein poses a synergistic effect that will promote cancer metastasis. LOX from the primary tumor has been found to migrate to a distant site and initiates matrix remodeling which will result in extracellular matrix (ECM) with high tensile strength conducive for the bone marrow derived cells (BMDCs) to invade. At this new site so called premetastatic niche, there will be a crosstalk between the LOX, FN, MMPs, BMDCs and the stromal fibroblast cells within the surrounding microenvironment. Such crosstalk will cause further invasion by the BMDCs to take place and causes increased matrix remodeling and subsequent matrix degradation, creating a tempting environment for the tumor cells to invade. The BMDCs and stromal fibroblasts will secrete various growth factors which among them is the vascular endothelial growth factor (VEGF), a crucial promoter of angiogenesis. Apart from that, increasing evidence has shown that LOX also conveys its metastatic effect by promoting epithelial-mesenchymal transition (EMT) activities, by intervening the signaling pathways controlling stroma-induced EMT. Angiogenesis and EMT are the two important hallmarks of cancer metastasis which allow the tumor cells to become motile and invasive. Hence, all these factors contribute to the progression of premetastatic niche to become an actual metastases and subsequently acquiring invasive capability and malignancy that allow them to metastasize to other sites.

Keywords: *Hypoxia, Lysyl Oxidase, LOX, FN, MMPs, Extracellular Matrix (ECM), angiogenesis, matrix remodeling, bone marrow-derived cells (BMDCs), Epithelial-to-mesenchymal transition (EMT), stromal fibroblast, metastasis*

Introduction

In most cancer cases, poor survival rate is not caused by the primary tumor itself, but rather from the secondary metastatic tumor that renders the patients to traumatic long endurance cancer symptoms. It is also well-known that metastasis contributes to cancer recurrence and resistance to therapeutic interventions. In the case of breast cancer for example, the main metastatic site in many cases is in the bones. There are many studies being carried out to study the mechanism of such preference (Chambers *et al.*, 2002). Nevertheless, the biology of metastasis itself has long been studied and identified to involve a series of cascade events which includes cell intravasation, transport and immune evasion of the cells within the circulatory system, intrude at the secondary site, extravasation, and finally colonization and growth at the new site (Kaplan *et al.*, 2006). The factors that initiate primary cancer cells to migrate to a new metastatic site are continuously being studied, and one increasing popular "culprit" for this event is the Lysyl Oxidase (LOX) protein (Chang *et al.*, 2014). Numbers of research have been carried out looking at different points of view on the role of LOX in promoting cancer metastasis. Therefore, this review will bring those research findings together and draw an overall conclusion on the malignant role of LOX protein.

The LOX protein

Contrary to the grim connection associated with this protein, LOX itself is an important protein especially in early embryonic development, as well as for functional connective tissue and effective wound healing. Defective LOX expression or activity may cause various disorders involving connective tissue such as Ehler-Danlos syndrome, Menkes' syndrome, arteriosclerosis and scleroderma (Kirschmann *et al.*, 2002). The *LOX* gene which is located on chromosome 5 in human expresses LOX protein, a copper-dependent amine oxidase enzyme which mediates the covalent crosslinking of collagen fibril by oxydizing peptidyl lysine to α-aminoadipic-δ-semialdehyde in elastin and collagen (Rucker *et al.*, 1998; Sion & Figg, 2006). Therefore this protein functions as a crucial enzyme which promotes collagen and elastin crosslinking in the extracellular matrix (ECM) to produce mature ECM with high tensile strength (Lucero & Kagan, 2006; Finger & Giaccia, 2010). This mature and stable ECM acts as a physical frame where cells are organized into functional tissues and organs. Apart from that, four family members of LOX coined as LOX-like proteins (LOXL1, LOXL2, LOXL3 and LOXL4) with varying degrees of similarity with LOX have also been explained and shown to involve in various degree of cancer progression (Cano *et al.*, 2012; Lucero & Kagan, 2006). Therefore, the mechanism on how such physiologically important enzyme contributes to most metastatic event in cancer patient has gained tremendous attention worldwide.

LOX and cancer

LOX protein has become the centre of attention of many cancer research groups since the finding of Erler *et al.* (2006). This protein has been associated with poor prognosis and low survival rate among cancer patients. In estrogen receptor (ER)-negative breast tumor bearing patients as well as in head and neck cancers, LOX has been associated with highly-invasive hypoxic tumor cells particularly the breast tumor cells, and is significantly correlated with lower distant metastasis-free survival as well as patients overall survival (Erler *et al.*, 2006). In addition, Kirshmann *et al.* (2002) found that LOX mRNA is highly expressed in highly metastatic MDA-MB-231 and Hs578T cells as compared to the non-metastatic MCF-7 tumor cells as evidenced from a reverse transcription polymerase chain reaction (RT-PCR) analysis. Transfection of murine *LOX* gene fused with a

FLAG marker protein into non-metastatic MCF-7 tumor cells showed localization of this fusion protein in the cytoplasm. This provides the idea that MCF-7 non-metastatic phenotype can be reversed by introducing LOX mRNA into the cells and interestingly, such invasive ability could then be reversed by the administration of β-aminopropionitrile (BAPN), an irreversible LOX inhibitor (Kirschmann *et al.*, 2002, Chen *et al.*, 2012).

Bondareva *et al.* (2009) studied the LOX-mediated metastatic colonization in breast cancer cells. They found metastases and tumor-induced bone lesions in mouse model injected with MDA-MB-231 cells flagged with luciferase, and the administration of BAPN was able to reduce the frequency of metastasis. Furthermore, in surgically dissected esophageal squamous cell carcinome (ESCC), Sakai *et al.* (2009) found positive correlation of the level of LOX expression alone with the presence and number of lymph node metastases; as well as the patients' overall and cancer-specific survival rate. Using nonsmall cell lung cancer (NSCLC) cell line with knocked down LOX expression, Wei *et al.* (2012) also revealed the role of LOX in promoting invasion and migration under hypoxic condition, which is in accordance with the increased level of LOX mRNA and protein. LOXL2, a family related LOX protein had also been associated with highly invasive basal-like breast cancer cells and lower survival of the patients (Ahn *et al.*, 2013); and lymph node metastasis and poor prognosis of esophageal squamous cell carcinoma (Li *et al.*, 2012).

Hypoxia induces LOX expression through hypoxia-inducible transcription factor (HIF-1α)

Hypoxia is one of the determining factors for the expression of LOX protein. As the primary tumor expands, a hypoxic condition will develop within the cancer cell mass as a result of blood vessels fail to reach the inner cells. As compared to hypoxia-inducible transcription factor-β (HIF-1β) which is constitutively expressed, hypoxia will eventually activate the HIF-1α and angiopoietin-2 (Sion *et al.*, 2006; Zhang *et al.*, 2013). A study by Ji *et al.* (2013) using ovarian cancer cells found that HIF-1α and LOX protein was highly expressed in epithelial ovarian cancer tissues and that the level of expression is positively correlated with the cancer stage, diameter and lymph node metastasis. The expression of LOX itself correlated positively with the expression of HIF-1α. Interestingly they found that the expression of these proteins were increased in hypoxic condition and decreased after reoxygenation. In addition, both the shRNA of LOX and administration of BAPN decreased the HIF-1α expression, LOX catalytic activity and inhibit the ovarian cancer cell line HO-8910 ability to migrate and invade even under hypoxia. Wong *et al.* (2012) earlier studied the inhibition of HIF using two drug blockers, digoxin and acriflavine which inhibit HIF in orthotopic breast cancer model. The drugs were found to block LOX and LOXL expression, collagen crosslinking and lung metastasis. Therefore, overexpression of HIF-1α is a characteristic feature of malignant cancer cells and associated with poor prognosis. Interestingly, it is believed that the way HIF-1α exerts such effect is by the role of LOX protein.

The expression of HIF-1α also will turn on the signaling pathway involved in cancer metastasis. One such pathway is the Focal Adhesion Kinase (FAK) pathway (Erler *et al.*, 2006). Chen *et al.* (2012) in their study found increased LOX protein expression and activity in the invasive MDA-MB-231 breast cancer cell line and this increased level of active LOX was positively correlated with the phosphorylation of FAK at Tyr-576. This finding is also supported by Ji *et al.* (2013) which found LOX-dependent FAK/AKT activation during the hypoxic condition; and by Osawa *et al.* (2013) which revealed the positive correlation between inhibited cell migration and reduced tube formation in LOX inhibited-mouse tumor endothelial cells (TECs) with

decreased phosphorylation of FAK at tyrosine 397. In their study, Baker *et al.* (2013) also showed direct correlation between LOX-mediated tissue stiffness and the activation of FAK signaling pathway via phosphorylation at tyrosine 397 which renders the colorectal cancer cells to become more proliferative and invasive. Erler group (Erler *et al.*, 2009) in earlier study showed that the ECM of cancer mass was relatively stiffer when compared to the normal counterpart. LOX protein had been implicated in this scenario through its catalytic activity. They also found close correlation between matrix stiffness and activation of the FAK pathway. Therefore, there is a clear connection between hypoxia and LOX via HIF1-α expression and this connection causes the activation of pathways involved in cancer progression.

LOX interacts with other proteins to pose synergistic effect

It has been postulated that metastasis occurs when expression of specific proteins from the primary tumor promotes the tumor cells to migrate from the primary site into the blood vessels, and intrude a new metastatic site by attaching to the endothelial cells and invading the extracellular matrix (ECM) forming micrometastases (Hiratsuka *et al.*, 2006). Hypoxic condition has been known to increase the expression of metastatic proteins like LOX, fibronectin (FN, protein important for matrix assemble; Singh *et al.*, 2010), MMP-2 and MMP-9 (Psaila & Lyden, 2009). In a study by Ma *et al.* (2011) using gastric cancer with and without lymph node metastasis, the LOX and MMP-2 mRNA and protein were significantly higher in the former than the latter; and that the expression of LOX protein was positively correlated with MMP-2 in gastric cancer with lymph node metastases. Apart from that, the expression of LOX and MMP-2 proteins were significantly higher in gastric cancer than that in pericancerous tissues.

Liu *et al.* (2012) used RNA interference to knock down *Lox* gene expression. They transfected human breast cancer cell line MDA-MB-231 with LOX-RNAi and determined the mRNA and protein expression of LOX, MMP-2 and MMP-9. They subsequently found decreased expression of both mRNA and protein of these proteins as compared to the control group (MDA-MB-231 cells without transfection). Furthermore the migrative and invasive abilities of the cells in RNAi group were also significantly lower than in the control group. Using immunohistochemistry study on breast cancer tissue, cancer-adjacent breast tissue and benign lesion tissues, they found high expression of LOX protein in breast cancer tissue when compared with the other two. Hence, they also proposed synergistic effect of these proteins in promoting invasion and hence cancer metastasis. Cox & Erler (2013) in their review supported the notion that there is a synergistic effect of LOX with fibronectin and protein from the metxincin superfammily particularly the MMPs. They postulated the idea that colocalization of FN and LOX is important at tumor initiation site in which FN enhances LOX's catalytic activity. They also suggested that LOX and MMPs work synergistically in promoting ECM remodeling and subsequent metastatic progression. To a certain extent, LOX has also been implicated to regulate the activation of the MMP proteins.

LOX colocalizes with bone marrow-derived cells at the premetastatic niche

Apart from inducing the expression of LOX and other related proteins under hypoxic condition, the HIF-1α transcription factor also activates the expression of various growth factors that involve in cancer metastasis which include placental growth factor, platelet-derived growth factor B, epithelial growth factor and vascular endothelial growth factor. These growth factors are claimed to be responsible to direct the interaction of the primary tumor with cells from myeloid lineage

such as the bone marrow-derived cells (BMDCs), bone marrow-derived angiogenic cells (BMDACs) and blood vessel endothelial cells (BECs); as well as the lymphatic vessel endothelial cells (LECs). These cells involve in promoting metastasis by inducing the formation of premetastatic niche through angiogenesis and lymphangiogenesis (Semenza, 2013). BMDCs such as the macrophages, monocytes, neutrophils and mast cells, all contribute to tumor angiogenesis by secreting growth factors (GF), cytokines, vascular endothelial growth factor A (VEGF-A), epithelial growth factor (EGF), fibroblast growth factor-2 (FGF2), protein kinase 2 and matrix metalloproteinase enzymes (MMPs). Macrophages are especially, capable of altering the tumor cell characteristics and promote tumor angiogenesis and metastasis. The myeloid cells which are responsible in the formation of pre-metastatic niche are characterized as CD11b+ CD34+ F4/80- immature myeloid cells where these cells will promote the invasion of tumor cells by expressing MMP-2 and MMP-9 at the premetastatic niche (Joyce & Pollard, 2009; Psaila & Lyden, 2009; Spano & Zollo, 2012).

In conjunction with this, Erler *et al.* (2009) found out that mice bearing wildtype MDA-MB-231 tumor cells had higher population of BMDCs particularly the CD11b+ myeloid cells and c-Kit myeloid progenitor cells, where those cells colocalized with LOX in the lung, as compared to mice bearing *LOX* shRNA tumors. Furthermore they reported FN staining 3 days after tumor injection followed by colocalization of LOX after 7 days and such colocalization increased over the time. After 14 days, CD11b+ myeloid cells were found to accumulate at site of the FN and LOX colocalization. The MMP-2 activity, a protein that cleaves collagen IV into peptides, from BMDCs had also been discovered. The activation of MMP-2 at the FN and LOX colocalization site increased the subsequent invasion of BMDCs. Kaplan *et al.* (2005) demonstrated that the MMP expression was increased at the premetastatic niche by Integrin ($\alpha4\beta1$) signalling produced after FN binding. Finally, tumor cells were observed at the premetastatic niche 3-5 weeks after tumor injection. Interestingly, LOX-containing conditioned medium (CM) harvested from hypoxic tumor cells *in vitro* and injected into mice daily for three weeks could also cause increased accumulation of CD11b+ myeloid cells and c-Kit+ myeloid progenitor cells in the lung, proposing that the LOX-containing CM itself is capable of inducing the accumulation of BMDCs at the favourable premetastatic niche without the presence of metastatic tumor cells (Erler *et al.*, 2009). Noteworthy, Spano & Zollo (2012) also pointed out that the recruitment of certain types of myeloid cells does not only contribute to the progression of cancer metastasis via the establishment of an inflammatory milieu, but also acts as a means of escaping the host's immune response.

LOX promotes matrix remodeling and hence initiates angiogenesis

Extracellular matrix is defined as the interconnected scaffold constituted of various macromolecules mainly proteins and sugars which surround and interact with cells in a particular solid tissue. Mature and stable ECM serves as biochemical and biomechanical supports for normal cellular and tissue functions. The interaction of cell with its surrounding ECM has been associated with crucial functions like cell adhesion, proliferation, differentiation, polarity, survival and apoptosis. Such interaction is also implicated to be a symbiotic phenomenon where ECM supports the cell function and integrity, while cells help to create a strong and mature ECM. One important characteristic feature of ECM is that it is continuously being remodeled in a tightly-controlled mechanism. Continuous remodeling of ECM is important for its normal function. Therefore, disruption to this controlled process will drive to a severe pathologic event as normally found in the case

of fibrosis and malignant cancers. Hence, in cancer progression and metastatic process, ECM loses its integrity caused by increased deposition, repeated cleavage and repatterning, and increased breakdown of the ECM. These aberrant processes will eventually promote cell transformation, proliferation, angiogenesis and invasiveness (Cox & Erler, 2013; Maller *et al.*, 2010).

Since LOX involves in collagen and elastin crosslinking, this protein which is produced in abundance by hypoxic tumor cells causes increased ECM remodeling at the new metastatic site, so called the premetastatic niche. Matrix remodeling will consequently promote the recruitment and invasion of the BMDCs to this new metastatic site and promotes cancer progression (Semenza, 2013). Osawa *et al.* (2013) found high LOX mRNA and protein expressions as well as its catalytic activity in mouse tumor endothelial cells (TECs) than in normal endothelial cells (NECs), and that mouse TECs with knocked down LOX expression demonstrated inhibited cell migration and reduced tube formation. Interestingly they also found high LOX protein concentration in the tumor vessels of mouse TECs and that BAPN inhibited angiogenesis and micrometastases formation in a mouse *in vivo* model. A study by Dong *et al.* (2014) further supported this finding when they found gradual increase of VEGF in hepatocellular carcinoma (HCC) with increasing LOX expression, whereby high expression of VEGF was always associated with HCC cells on high stiffness gel. Therefore, there is a close relationship between the role of LOX in promoting matrix remodeling which will produce high matrix stiffness with subsequent expression of growth factor which is known to initiate angiogenesis, a hallmark of tumor malignancy.

Perhaps, the most interesting finding pertaining to matrix remodeling and angiogenesis was made by Fang *et al.* (2013) whom used quantum dot-based multiplexed imaging system in order to prove the coexistence of collagen IV, LOX and angiogenesis in the metastatic process of hepatocellular carcinoma. They found that the presence of LOX protein was high at the area of small cancer mass but the expression was lower in large cancer mass. This probably gives an insight that LOX highly involves in metastatic progression. The group also found that LOX expression was significantly correlated with the presence of collagen IV and tumor angiogenesis; and that LOX promoted ECM remodeling by means of collagen IV cleavage and repatterning which resulted in harder and stiff ECM, able to promote cancer cell invasion. The stiff ECM however was less resistant towards invading cancer cells and this was proven by their finding that the ECM was hydrolyzed at the invasion front. In addition, the neovessel formation was found mainly close to the broken linear collagen IV fibers indicating that angiogenesis is initiated by degrading the ECM.

LOX promotes epithelial-to-mesenchymal transition and hence invasive ability of the tumor cells

Epithelial to mesenchymal transition (EMT) is defined as a transition of cells from non-motile and polarized epithelial phenotype to motile and non-polarized mesenchymal phenotype that allows the cells to travel to a distant site through blood stream. Hypoxia and LOX protein have also been implicated in the repression of E-cadherin expression, which subsequently causes EMT, a phenomenon known to promote cancer progression. The loss of E-cadherin from the adheren junction renders tumor cell to become motile and hence becomes invasive since the adhesive factor that binds cancer cells together has lost (Finger & Giaccia, 2010). This is shown in the study by Peinado *et al.* (2005) which found the association of overexpression of LOXL2 and LOXL3 proteins in cancer epithelial cells with EMT process. The LOXL2 protein regulates EMT via the transcription factor Snail and down-regulate E-cadherin expression.

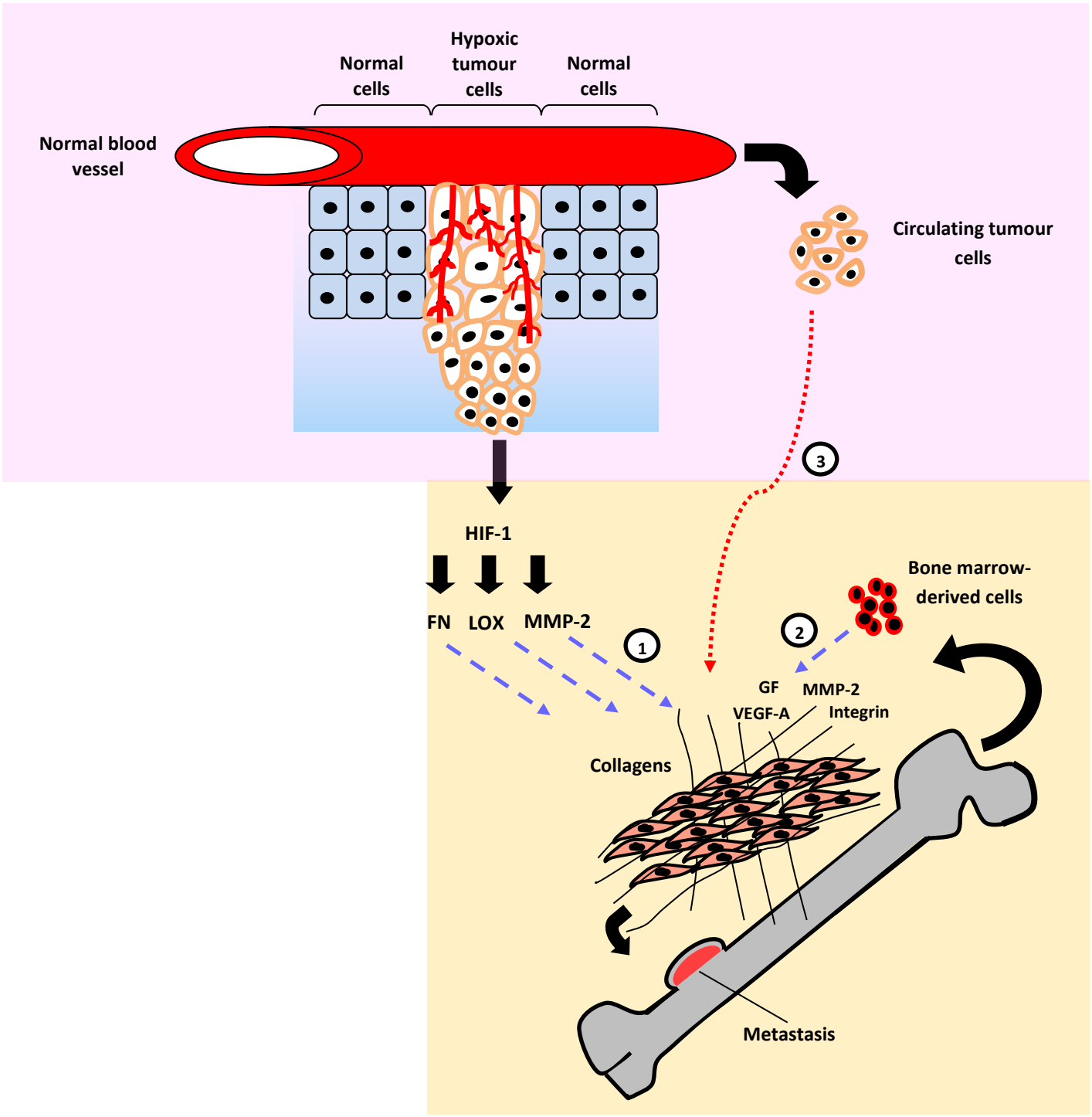

Figure 1. The postulated mechanism of cancer metastasis in bone – the synergistic effect of LOX and other proteins (FN and MMPs) that migrate from the hypoxic primary tumor to the premetastatic niche and crosslink collagen producing extracellular matrix (ECM) with high tensile strength. This is a permissive environment for the bone marrow-derived cells (BMDCs) particularly the CD11b+ CD34+ F4/80- immature myeloid cells to invade. In return, BMDCs produce a plethora of growth factors, cytokines, VEGF-A, integrin and MMPs which promote further invasion of the BMDCs and is subsequently followed by the invasion of the primary tumor cells. LOX also mediates the post-translational modification of the ECM components (cleavage by MMPs, crosslinking and glycosylation), angiogenesis and EMT in the premetastatic niche which render the tumor cells to become invasive. There is also communication between the recently migrated tumor and its neighboring cells (stromal fibroblasts) that further promote cancer metastasis via LOX-dependent mechanism.

Schietke *et al*. (2010) also found up-regulation of HIF-1α followed by elevated expression of LOX and LOXL2 proteins coexist with the repression of E-cadherin and the induction of EMT. Thus, the pathogenesis of LOXL2 has now become remarkably important as this protein not only involved in ECM remodeling, but there is also strong evidence showing that this protein involves in promoting EMT process via transcriptional mechanism. This probably explains why LOXL2 is associated with cancers of poor prognosis and distant metastases (Ahn *et al.*, 2013; Cano *et al.*, 2012; Taylor *et al.*, 2010).

These findings are further supported by Barker *et al.* (2012) whom highlighted the role of LOX and its family members (LOXL2 and LOXL3) in facilitating EMT process. Overexpression of LOX is able to convert a non-invasive tumor cells from an epithelial form to a fibroblastic feature by altering the expression of vimentin and suppressing E-cadherin expression. They also highlighted that EMT activity could be induced following changes in the ECM stiffness. This gives the idea that LOX promotes EMT not only directly by regulating vimentin and E-cadherin expression, but also indirectly by altering ECM microenvironment. Fang *et al.* (2013) also pointed out that EMT could happen due to the imbalance mechanical force within the tumor-stroma microenvironment produced by ECM stiffening that compromises the cell-cell junction integrity and therefore promoting cancer cell invasion. Apart from that, the MMP-2 and MMP-9 proteins are also commonly linked to EMT by their role in degrading type IV collagen in the ECM and hence causing the cells to become invasive (Finger & Giaccia, 2010). Xu *et al.*, explains the transcriptional regulations of EMT in details which starts by the induction of the growth factor TGF-β and activated Smad proteins and their interactions on transcription factors like Snail, ZEB and twist. This results in the upregulation of the target genes fibronectin, vimentin, N-cadherin, MMPs, and collagen; and downregulation of target genes like E-cadherin and Claudins.

In addition, immunohistochemistry study by Zhang *et al.* (2013) on surgical resection of HCC samples found increased HIF-1α expression, decreased E-cadherin expression and overexpression of the Snail1, N-cadherin and vimentin. They also cultured HCC cells under hypoxic condition and found EMT within the cells and an increase in the cells' migration and invasiveness; and such features are reversible when normoxic condition is reintroduced. Study using shRNA of HIF-1α also revealed the inhibition of EMT process. In fact, the role of HIF-1α in promoting EMT was also seen in an earlier study by Higgins *et al.* (2007) who knocked down HIF-1α expression in primary renal epithelial cells. They found that HIF-1α promotes EMT *in vitro* and that this transcription factor induced the migration of renal epithelial cells by upregulating LOX and LOXL2 genes expression.

Tumor cells communicate with stromal fibroblasts to promote metastasis through LOX-dependent mechanism

In many cancer cases, fibroblasts are the main constituent of the tumor-stroma apart from the localized epithelial cell, endothelial cell and inflammatory cells. The role of these cells in cancer and metastatic progression has now become more apparent. Fibroblasts have high proliferation rate and communicate with the cancer and surrounding cells by secreting various growth factors and chemokines. These cells also contribute to the high deposition of ECM components like collagen type I and III, produce MMPs which promote cell invasion via ECM degradation and also produce VEGF which is an important proangiogenic factor. In addition, fibroblast also secretes the growth factor TGF-β, which is known to promote cancer invasion by inducing EMT process and mediates inflammatory response via

chemokines. Together with other stromal cells, fibroblasts regulate cell survival and tumor cell migration by upregulating MMPs production and downregulating the protein inhibitors for MMPs (Spano & Zollo, 2012). Earlier, Taylor *et al.* (2010) also supported this idea when they mentioned that cancer metastatic progresses from the dynamic interplay between cancer cells and their accompanying stroma.

To further evaluate the invasive ability of particularly LOX protein, Cox *et al.* (2013) studied the role of LOX in promoting metastasis in fibrosing tissues. They found that LOX-dependent collagen crosslinking created a permissive environment for fibrosis formation capable of developing metastasis by supporting tumor cell proliferation, colonization and growth. However knocking down LOX expression prevented the fibrosis from promoting metastatic colonization. In much earlier study, Kirschmann *et al.* (2002) found that LOX mRNA was highly expressed when MCF-7 cancer cells were cultured in a conditioned medium from fibroblast. This suggested the role of stromal fibroblast in regulating LOX in the presence of breast cancer cells. Barker *et al.* (2012) also highlighted that stromal fibroblast works synergistically with cancer cells to perturb the expression and organization of ECM components mediated by remodeling enzymes particularly LOX protein. Cox & Erler (2013) support this finding when they highlighted that the stromal cells surrounding tumor milieu also secrete LOX protein which is only able to promote metastatic progression when it coexists with other tumorigenic factors such as LOX protein from the hypoxic tumor cells and activated fibroblasts.

Conclusion

Consequently, cancer metastasis is a complex process involving a constructive interplay between the primary tumor cells, the microenvironment of the premetastatic niche, the myeloid cells, stromal fibroblast, the proteins and various growth factors produced by both the primary tumor cells and cells at the premetastatic niche. The idea presented in this article is that LOX which is overexpressed by the primary tumor cells when hypoxic condition is encountered becomes the key player that regulates the important cellular and molecular changes within a premetastatic niche which could be progressively transformed to cancer metastasis. Even though this idea is well-accepted and adopted in the current setting of cancer research, there are still loop holes and gaps that need to be addressed within this notion. Nevertheless, there are many on-going researches being carried out studying this "idea" in depth and looking seriously at a specific research area. Continuous findings by these research groups are of great importance in order to merge the ideas so that the mechanism of cancer metastasis could be drawn in greater details and in a multi-angle perspective.

This complete understanding is thus remarkably important to facilitate the effort of tackling cancer progression at the earliest stage. The clinical implications can be seen both at the diagnosis and the prognostic levels, as well as in developing therapeutic interventions that can cease or even prevent cancer metastasis. Understanding the overall mechanism of metastatic progression would enable specific biomarkers to be targeted which would indicate the cancer patient's disease progression. Perhaps the presence of CD11b+ myeloid cells in the blood circulation would serve as an indicator for metastatic progression in cancer patients. Moreover, measuring growth factors and specific pro-metastatic proteins in the blood has become one of the approaches used nowadays to screen cancer patients with high probability of having metastases. Apart from that, therapeutic interventions could also be designed by addressing specific target proteins, growth factors or cytokines that involve in every critical step of metastatic progressions.

One such example is the anti-VEGF drug bevacizumab which currently being used to inhibit angiogenesis (Harbeck, 2008). Inhibition of proteins or growth factors involved in ECM remodeling, fibrotic progression and EMT process within the tumor milieu are among other attractive approaches. Besides, targeting the inflammatory cells particularly the CD11b+ myeloid cells and interfering the infiltration of these cells at site of premetastatic niche could also serve as a promising approach.

Despite all these interventions, resolving the gaps exist within the concept of metastatic progression itself is the main priority to all cancer researchers. There are still questions that need to be addressed in order for us to understand the whole metastatic process. As been pointed out by Sleeman (2012), it is worth to study the fate of the recently migrated tumor cells upon arrival at the premetastatic niche, whether they survive and are progressively transformed to the invasive state, or perhaps they survive but remain in a dormant state at the new site. How long will they be in the dormant state is another issue worth to be pondered upon. Numbers of literatures support targeting LOX as a promising drug that could block metastatic progression. However, since LOX also involves in normal cellular and tissue function, targeting LOX could be somewhat tricky. Besides, if this drug is to be used, what is the right effective dose that is safe for the patients is another point to be addressed. The time frame at which the premetastatic niche is transformed to a malignant cancer capable of metastasizing is another major issue. Such information is needed if we are to design a therapeutic intervention that targets one crucial step in the metastatic progression before an overt metastasis is formed. Perhaps the best method to address all these questions is by producing an animal model that could mimic the metastatic progression of cancer in human. By this way, the interconnections of various metastatic factors as well as the main role players involved in the whole "system" could be studied in greater details and with better confidence. However, the biggest challenge is to produce different animal models for different types and subtypes of cancers. Nevertheless, this could be achieved by collaborative study by different research groups. The outcomes of this study will help to unravel the nature and the actual mechanism involved in cancer progression that are important for medical benefits.

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