

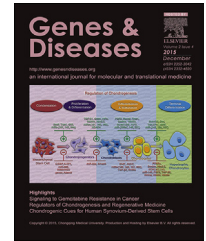
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COMMENTARY

Opening the LOX to bone metastasis: The role of secreted lysyl oxidase in skeletal recurrence of breast cancers



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Breast cancer is the second-most prevalent cancer in the United States, with 234,190 new cases projected to be diagnosed in 2015. The initial diagnosis is commonly made early in its disease course due to the use of routine screening mammography.¹ Combined with advances in adjuvant chemotherapy, this has led to promising overall outcomes, including a 5-year survival rate of 89.4% and a 10%–12% rate of locoregional recurrence at 20 years for stage I/II disease.^{1–3} By comparison, prognosis remains poor for patients with metastatic disease at diagnosis or for those who present after initial cure of disease with distant recurrence.⁴ Among cases of distant recurrence, skeletal recurrence occurs frequently and can be accompanied by hypercalcemia, pain, and functional compromise often requiring a protracted course of palliative therapy.^{5–7}

The mechanisms underlying the predilection for skeletal metastases seen with breast cancer have heretofore been poorly understood. Hypotheses include expression of osteolysis-stimulating factors such as parathyroid hormone-related protein (PTHrP), interleukin-11 (IL-11), macrophage colony stimulating factor (M-CSF), and vascular endothelial growth factor (VEGF) by cancer cells.⁸ Expression of these factors leads to osteoclast-mediated bone resorption and chemotaxis of tumor cells to the osteolytic lesion upon release of growth factors stored within the bone microenvironment, creating a self-perpetuating cycle.⁸

In June 2015, Cox et al published their work establishing a link between expression of a copper-dependent amine oxidase, lysyl oxidase (LOX), and induction of pre-metastatic osteolytic lesions in patients with estrogen receptor-negative (ER-negative) breast cancer. It has previously been established that hypoxia in a primary breast adenocarcinoma is associated with an increased risk for metastasis secondary to expression of hypoxia-inducible factors (HIFs).⁹ In the study by Cox et al, expression of a previously-established primary hypoxic tumor signature was found to be correlated to metastasis in samples from a cohort of lymph-node negative breast cancer patients who did not receive adjuvant therapy. However, the relationship held true only with samples from ER-negative breast cancers. The authors investigated this correlation further by performing mass-spectrometry-based analysis of the hypoxic secretome of the ER-negative MDA-MB-231 breast cancer parent cell line and an osteotropic clone line (MDA-BT). Compared to expression in the parental MDA-MB-231 line, expression of LOX was upregulated more than 1.5-fold in MDA-BT cells. Expression of LOX, known to be mediated by HIF-1 α , appeared to be induced by hypoxia more than other osteotropic genes in the hypoxic secretome.¹⁰ Retrospective analysis of LOX expression in both the authors' patient cohort and a supporting patient cohort showed it to be correlated with frequency of metastasis in ER-negative breast cancers.

In vivo studies were performed using 4T1-BALB/c mice, a syngeneic model of spontaneously-metastasizing ER-negative breast cancer with high levels of LOX expression. Mice were injected with 4T1Luc (control), 4T1shLOX cells which have low LOX expression compared to controls, or 4T1

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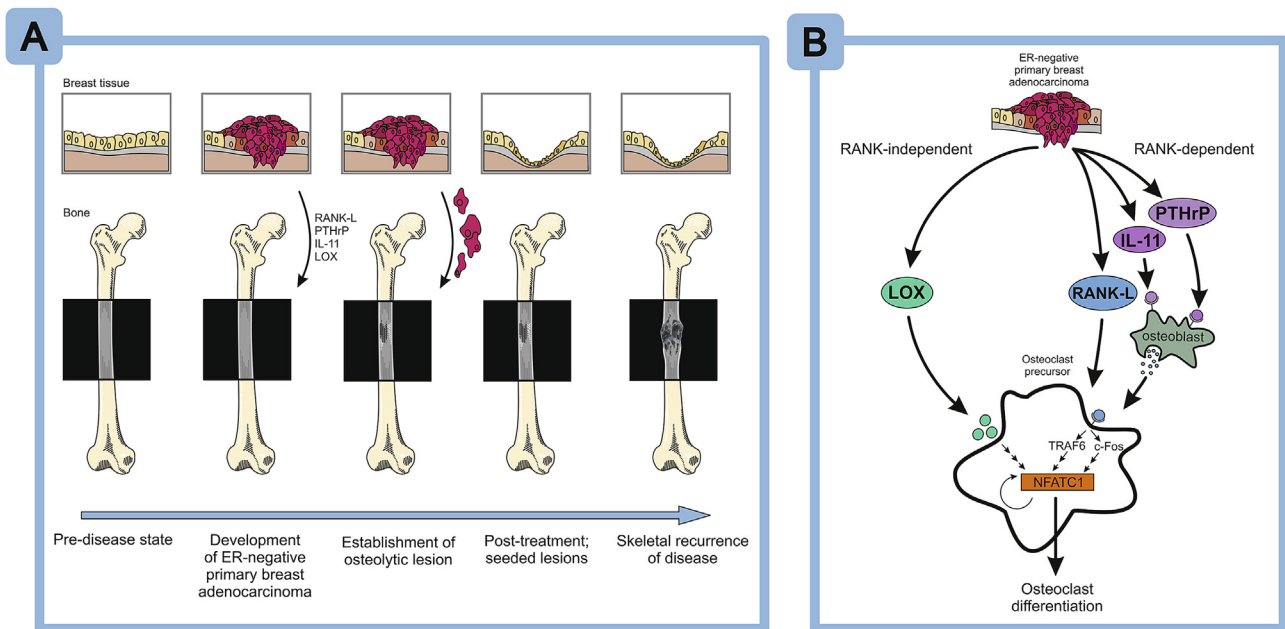


Figure 1 Tumor overproduction of LOX in ER-negative breast adenocarcinoma induces formation of pre-metastatic osteolytic lesions. A) Overproduction of cytokines, including PTHrP, IL-11, RANKL, and newly described LOX, induces formation of osteolytic lesions which may be seeded by cells from the primary tumor. Skeletal recurrence of disease occurs at a distant time point. B) The mechanism for formation of pre-metastatic osteolytic lesions by cytokines has previously been described to be RANK-dependent, where osteoblasts are activated to produce RANKL, which binds RANK on the surface of osteoclasts. Downstream effects include osteoclastogenesis. Cox et al demonstrate that activation of osteoclasts by LOX occurs, but via a RANK-independent pathway.

scrambled control (4T1scr). Relative to the control, 4T1scr tumors were associated with increased osteolytic lesions with time while 4T1shLOX tumors led to comparatively fewer lesions. The role of tumor-secreted, hypoxia-induced factors in the development of osteolytic lesions is supported by the fact that the authors obtained similar results upon injecting tumor-free mice with tumor-conditioned media (CM) from 4T1shLOX or 4T1scr cell lines. The role of LOX in the induction of pre-metastatic osteolytic lesions has been explored across multiple cancer types and appears to be independent of tumor presence. This was demonstrated by increased frequency and size of osteolytic lesions in non-tumor-bearing mice injected with CM from originally LOX-deficient SW480 colorectal cancer cells transformed to overexpress LOX. By comparison, CM from SW480 cells overexpressing a catalytically-inactive mutant did not have a similar effect.

The formation of pre-metastatic osteolytic lesion and subsequent skeletal metastases is due to disruption of bone homeostasis, which normally consists of a balance between osteoid deposition and resorption (Fig. 1A).⁸ The mechanism by which LOX causes or perpetuates this imbalance was studied further. When added to pre-osteoclast cultures, recombinant LOX (rLOX) stimulated osteoclastogenesis more potently than receptor activator of nuclear factor kappa-B ligand (RANKL). Absence of autocrine production of RANKL was confirmed by ELISA, allowing the effect to be attributed wholly to LOX. Greater nuclear localization of nuclear factor of activated T-cells 1 (NFATc1), a regulator of osteoclastogenesis, was demonstrated with rLOX treatment compared to treatment with RANKL. This localization was disrupted in a dose-dependent

manner when osteoclast cultures were treated with anti-LOX antibody. LOX was additionally shown to increase terminal differentiation and decrease proliferation of calvarial mouse osteoblasts. This effect was also reversed by treatment with anti-LOX antibodies. Similar induction of differentiation was observed when human osteoblasts of the SaOS-2 cell line were treated with 4T1scr CM and when osteoblast and osteoclast numbers were quantified on tibial endosteal surfaces from tumor-bearing mice.

In a final set of experiments, the authors established the pre-metastatic nature of osteolytic lesions induced by LOX. Mice pre-conditioned with 4T1scr CM and LOX antibody developed a reduced tumor burden than their counterparts conditioned with 4T1scr CM without the LOX antibody. Addition of bisphosphonate therapy to CM pre-conditioning reduced the number of bony metastases at 5 weeks after injection with tumor cells. Although the priming of sites before establishment of metastases has been demonstrated across multiple cancer types, this study is the first to characterize the molecular mediators of osteolytic lesion formation in ER-negative breast cancer. LOX and other proteins of the lysyl oxidase family, which primarily function to covalently cross-link collagen and/or elastin in the extracellular matrix (ECM), have previously been shown to play a role in initiation and progression of multiple tumor types. Notably, increased expression in both primary tumor cells and surrounding stroma has been linked to metastasis of several cancers, including breast cancer.¹¹ However, this is the first study to establish the specific mechanisms underlying this association with ER-negative breast cancer. It is also the first to characterize a RANKL-independent mechanism for osteoclastogenesis by LOX during the

development of skeletal metastases (Fig. 1B). These findings suggest a role for development of novel, LOX-specific inhibitors for prophylaxis against skeletal recurrence of ER-negative breast cancers. Finally, further work is needed to resolve why the correlation between LOX expression and skeletal metastasis of breast cancer is limited to the ER-negative subtype.

Conflicts of interest

The authors declare no conflict of interest.

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