METASTASIS

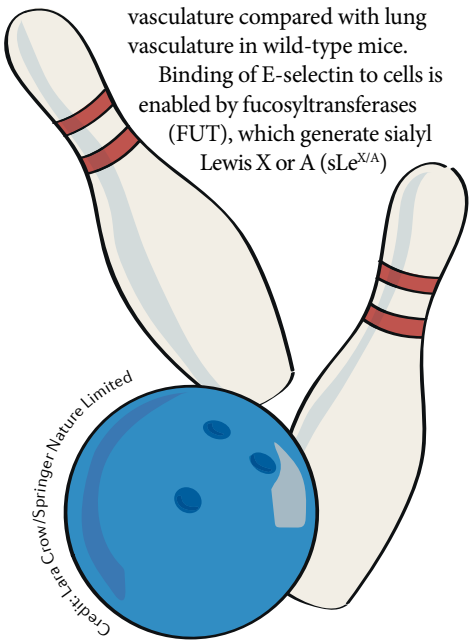
E-selectin fills two needs for metastasis

> GLG1 was a significant prognostic marker for bone metastasis

Metastasis initiation is enabled by cellular plasticity, including mesenchymal-to-epithelial transition (MET) and the gain of stem cell-like properties by cells in the metastatic organ site. However, it is unclear how these seemingly paradoxical programmes are induced. Esposito et al. showed that endothelial selectin (E-selectin) in the bone vascular niche interacts with disseminated tumour cells and controls both programmes.

To study the necessity of E-selectin in bone and lung metastasis of breast cancer in xenograft models, the authors used immunodeficient E-selectin-knockout mice (*Sele*$^{-/-}$). Intracardiac injection of the bone-tropic BM2 cells, a subline of MDA-MB-231 breast cancer cells, led to a reduced bone metastatic tumour burden in *Sele*$^{-/-}$ mice compared with wild-type mice. By contrast, the lung metastatic burden of the lung-tropic LM2 subline of MDA-MB-231 was similar to that in wild-type mice. This finding correlated with a higher expression of E-selectin in bone vasculature compared with lung vasculature in wild-type mice.

Binding of E-selectin to cells is enabled by fucosyltransferases (FUT), which generate sialyl Lewis X or A (sLe$^{X/A}$) tetrasaccharides on cell-surface proteins. Of the six isoforms of FUT, overexpression of FUT3, FUT5 and FUT6 significantly increased bone metastasis of bone-tropic M1a cells, a subline of SUM150 breast cancer cells — although only endogenous expression of FUT3 and FUT6 positively correlated with E-selectin binding. When M1a cells overexpressing these isoforms were subjected to a multi-organ metastasis model, FUT3 and FUT6 promoted bone metastasis, whereas primary tumour growth or lung metastasis were not affected.



To identify substrates of FUT3 and FUT6, the authors performed N-glycoprotein capture mass spectrometry in M1a and BM2 cells. Following further analysis, Golgi apparatus protein 1 (GLG1) was identified as one of the top candidates. The researchers then performed fluorescence microscopy and western blotting under denaturing conditions to analyse binding of GLG1 to E-selectin, and found that even though GLG1 and E-selectin colocalized, direct binding could not be detected. Yet, CRISPR-mediated *Glg1* knockout in BM2 cells or M1a cells reduced E-selectin binding in vitro, and reduced their ability to metastasize in bone upon intracardiac injection. Thus, binding of E-selectin to tumour cells required FUT3 or FUT6 and GLG1 expression and contributed to bone metastasis in mice.

These findings were corroborated in patients with oestrogen receptor-negative (ER–) breast cancer, in whom multi-organ metastasis is common. Patients from two cohorts who had high expression of FUT3 and GLG1 or FUT6 and GLG1 showed worse prognosis for distant metastasis-free relapse, and GLG1 was a significant prognostic marker for bone metastasis. Could therapeutic inhibition of E-selectin in stratified patients prevent bone metastasis? To test this in vivo, mice were given the sLe$^{X/A}$ mimetic GMI-1271 following intracardiac injection with BM2 cells. Indeed, GMI-1271 conferred increased survival and preserved bone tissue compared with PBS.

E-selectin is thought to act as an adhesive trap for tumour cells in the vasculature, but in bone vasculature, blood flow is usually slow. Could E-selectin induce changes in tumour cell behaviour instead? Indeed, whereas BM2 or M1a cells seeded on IgG-coated plates formed monolayers, cells seeded on E-selectin-coated plates formed 3D multi-layered cell clusters. This was accompanied by increased expression of N-cadherin and tight junction proteins, and reduced expression of epithelial-to-mesenchymal transition (EMT)-related gene signatures in cells growing on E-selectin, overall indicating that E-selectin induced MET. This MET programme was non-canonical because the expression of master regulators of EMT such as SNAIL or TWIST was unaffected. In addition, the three most prominent downregulated mesenchymal genes were WNT repressors, which translated into increased canonical WNT signalling in BM2 cells seeded on E-selectin, and led to an increase in stem cell-like features including SOX2 and OCT4 expression.

This study offers a functional mechanism for the simultaneous induction of MET and stem cell traits in disseminating breast cancer cells in the bone, and provides therapeutic opportunities to prevent initiation of bone metastasis.

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