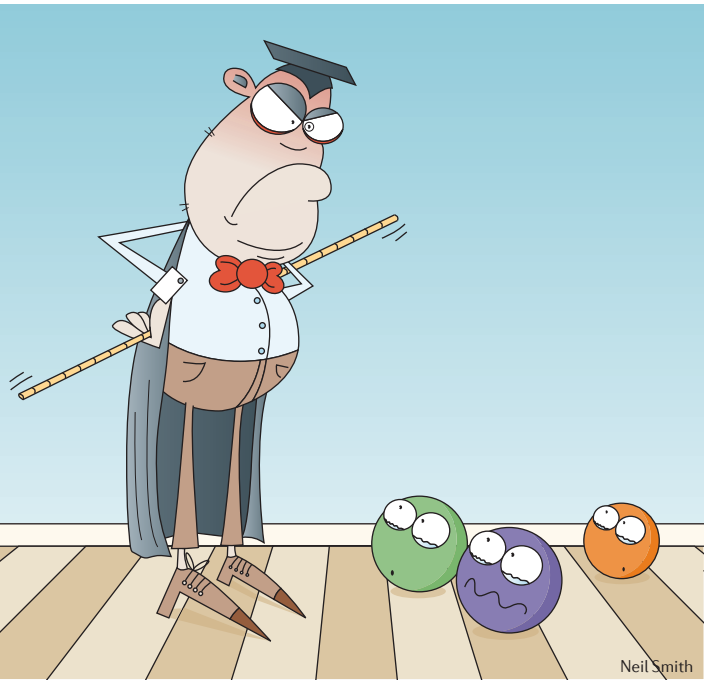
METASTASIS

SIX1 of the best

> SIX1 bound specifically to SIX1-binding sequences identified in the *VEGFC* promoter, and, along with its cofactor EYA2, it induced VEGFC expression.

Expression of the homeodomain-containing transcription factor SIX1 has previously been correlated with the presence of lymph node metastases in patients with breast cancer. Heide Ford and colleagues now show that SIX1 can promote lymphangiogenesis and metastasis through regulating the expression of the lymphangiogenic factor vascular endothelial growth factor C (VEGFC).



The authors had previously shown that exogenous expression of SIX1 in human MCF-7 non-metastatic breast cancer cells induced lymphatic metastases in immunocompromised mice. Further examination of these MCF-7–SIX1 tumours indicated increased numbers of lymphatic vessels in and surrounding the tumour compared with tumours formed by MCF-7 parental cells. Gene expression analyses and real-time PCR analyses indicated that *VEGFC* expression was increased in the MCF-7–SIX1 cell lines and in the tumours, whereas the expression of *VEGFA* and *VEGFD* was unaltered. Various techniques were used to verify that SIX1 bound specifically to SIX1-binding sequences identified in the *VEGFC* promoter, and, along with its cofactor EYA2, it induced VEGFC expression. Moreover, knockdown of *VEGFC* mRNA using short hairpin RNAs (shRNAs) in MCF-7–SIX1 cells reduced lymphangiogenesis in the primary tumours that formed in immunocompromised mice after orthotopic transfer and significantly reduced the number of lymph node and lung metastases.

To verify these findings the authors used a mouse metastatic breast cancer cell line that has high expression levels of endogenous SIX1. Similar results were found after orthotopic transfer into syngeneic mice, except that knockdown of either *Six1* or *Vegfc* in this cell line reduced primary tumour growth. However, comparison of tumours of an identical size verified the reduction in the number of metastases in the absence of SIX1. VEGFC re-expression was able to partially restore the lymphangiogenic and metastatic effect in this model in the absence of SIX1 expression, but distant lung metastases were not present, suggesting that SIX1 has other effects that are required for the promotion of distant metastases.

Analyses of a gene expression data set from human breast cancer cell lines and immunohistochemical analyses of 110 invasive ductal carcinoma samples indicated that SIX1 and VEGFC expression were significantly correlated. Thus, these findings indicate that SIX1 expression is important in the promotion of lymphangiogenesis and lymph node metastases. However, whether SIX1 will prove to be a therapeutic target for human breast cancer requires further analyses, as initial findings by these authors indicated that the effects of SIX1 knockdown could be overcome by upregulation of SIX2.

Nicola McCarthy

ORIGINAL RESEARCH PAPER Wang, C.-A. *et al.* SIX1 induces lymphangiogenesis and metastasis via upregulation of VEGF-C in mouse models of breast cancer. *J. Clin. Invest.* 2 Apr 2012 (doi:10.1172/JCI59858)