

 METASTASIS

Epithelial to mesenchymal and back again

Epithelial–mesenchymal transition (EMT) is associated with metastasis, and it is thought that EMT confers invasive and stem cell-like features that allow cells to disseminate;

whereas, the reverse process —MET— is thought to allow the out-growth of

these cells at distant sites. Two papers now provide evidence for this idea of plasticity in epithelial and mesenchymal transitions *in vivo*.

EMT is a developmental process; so, to identify additional regulators of EMT, Ocaña and colleagues screened chick embryos for transcription factors that are involved in early developmental stages. The authors identified the transcription factor paired related homeobox 1 (PRRX1). PRRX1 expression correlated with invasive phenotypes and TWIST1 (an EMT transcription factor) expression in zebrafish embryos and a panel of human cancer cell lines. Furthermore, knockdown of PRRX1 or TWIST1 decreased the ability of BT-549 human breast carcinoma cells to degrade collagen, whereas expression of PRRX1 induced the invasion of HBL-100 breast epithelial cells (which are non-invasive and which express TWIST1 but not PRRX1).

Is PRRX1 involved in metastasis? Cells expressing PRRX1 that were injected into the tail veins of nude mice did not develop lung metastatic foci, whereas stable knockdown of PRRX1 in these cells induced lung colonization. Orthotopic implantation in nude mice of BT-549 cells in which

either PRRX1 alone or both PRRX1 and TWIST1 were knocked down developed primary tumours, whereas implantation of BT-549 cells expressing PRRX1 did not. The tumours that formed did not exhibit an invasive margin and did not form overt metastases. Further experiments revealed that PRRX1 loss induces MET (in cells that have undergone EMT), stem cell-like properties and proliferation. Additionally, analyses of gene expression data sets revealed that high levels of *PRRX1* mRNA correlated with relapse-free survival in breast adenocarcinoma and lung squamous cell carcinoma. Together, these data show that PRRX1 expression is associated with EMT and invasion, but its expression must be lost at later stages for metastatic colonization.

To investigate the plasticity of epithelial and mesenchymal transitions, Tsai and colleagues analysed the expression of TWIST1 in primary human breast carcinoma and matched lymph node metastases. Of the primary tumours with high TWIST1 expression, most of the matched lymph node metastases no longer expressed TWIST1. The authors generated transgenic mice in which *Twist1* expression could be induced in the skin by doxycycline and used the DMBA–TPA carcinogenesis protocol to induce papillomas. Doxycycline was then applied orally (to induce *Twist1* in all skin tumour cells even if they had disseminated) or topically (to induce *Twist1* at primary sites), and both methods increased the conversion rate to invasive squamous cell carcinoma compared with controls. However, topical induction of *Twist1* significantly increased the

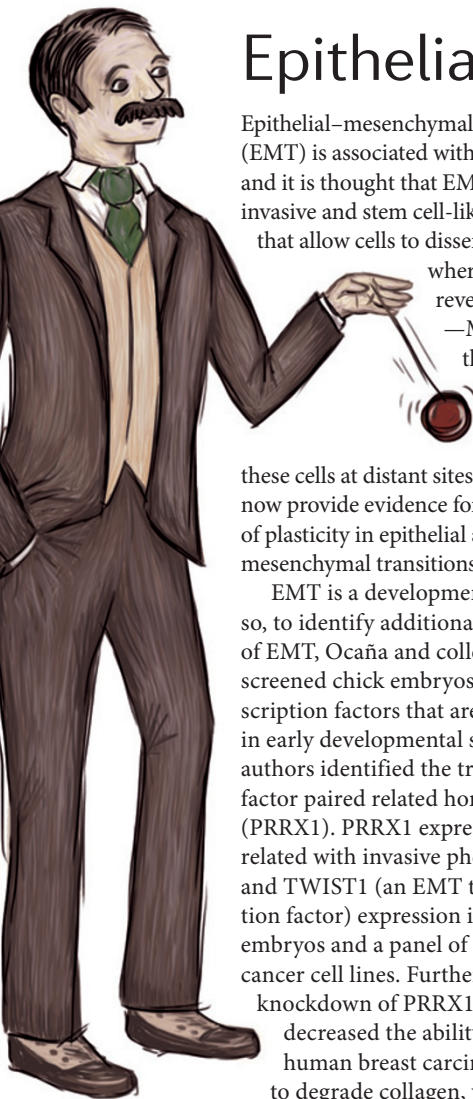
metastasis frequency compared with mice receiving oral doxycycline. This indicates that turning off *Twist1* expression (which occurs as cells disseminate from the primary sites) is required for metastasis formation. Consistently, the metastases that formed in mice treated with oral or topical doxycycline no longer expressed TWIST1 and had epithelial characteristics, indicating EMT reversion.

At what stage is EMT reversed in metastasis? Analyses of peripheral blood from these mice revealed that *Twist1* induction doubled the number of circulating tumour cells, and these cells expressed TWIST1. Tail-vein injection of primary tumour cells expressing TWIST1 also extravasated from the lung vasculature. Importantly, turning off *Twist1* expression in these cells allowed their proliferation *in vivo*. This indicates that EMT reversion is crucial for metastatic outgrowth.

These two papers provide *in vivo* evidence for the plasticity of epithelial and mesenchymal transitions, indicating that EMT (induced by PRRX1 and/or TWIST1) is required for invasion, intravasation and extravasation, and that the suppression of EMT regulators (and consequently EMT reversion) is required for metastatic outgrowth.

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ORIGINAL RESEARCH PAPERS Ocaña, O. H. *et al.* Metastatic colonization requires the repression of the epithelial–mesenchymal transition inducer *Prrx1*. *Cancer Cell* 11 Dec 2012 (doi:10.1016/j.ccr.2012.10.012) | Tsai, J. H. *et al.* Spatiotemporal regulation of epithelial–mesenchymal transition is essential for squamous cell carcinoma metastasis. *Cancer Cell* 11 Dec 2012 (doi:10.1016/j.ccr.2012.09.022)



Lara Crow/NPG

“ EMT reversion is crucial for metastatic outgrowth ”