Simon Bradbrook/NPG

METASTASIS

Polarizing metastasis

The planar cell polarity (PCP) pathway controls the distribution of cells within a plane and involves factors including WNTs, frizzled (FZD) receptors and prickle-like proteins (PKs). Although various components of this WNT–PCP pathway are overexpressed in different cancers, and are associated with metastasis, its role in cancer is unclear and was the focus of a paper by Jeffrey Wrana and colleagues.

Luga *et al.* found that conditioned media from the mouse L fibroblast cell line induced protrusive structures and motility of human breast adenocarcinoma MDA-MB-231 cells and other carcinoma cell lines. Furthermore, co-injection of L fibroblasts with MDA-MB-231 cells in the mammary fat pads of SCID mice significantly increased the number and size of lung metastases produced by these normally poorly metastatic cells. As SMURF1 and SMURF2 regulate breast cancer cell protrusive activity and motility, the authors knocked down the expression of these ubiquitin ligases in MDA-MB-231 cells. This prevented the effects of the L fibroblast-conditioned media on protrusive activity and motility, and so the authors investigated whether the role of the SMURFs in the PCP pathway might explain these results. Indeed, knockdown of members of the WNT–PCP pathway (DVL1, FZD6, VANGL1 and PK1) prevented L fibroblast-conditioned-media-induced breast cancer cell motility and protrusion. Moreover, PK1 knockdown in MDA-MB-231 cells that were co-injected with L fibroblasts into mammary fat pads suppressed the metastatic propensity of the breast tumour cells, indicating that the PCP pathway is activated in breast cancer cells by a soluble factor derived from fibroblasts and that this promotes metastasis.

> “the PCP pathway is activated in breast cancer cells by a soluble factor derived from fibroblasts”

The PCP pathway is regulated by the WNT ligands WNT5A and WNT11, among others. Surprisingly, the authors found that L fibroblasts did not express either of these ligands, and suppression of WNT secretion had no effect on the response of MDA-MB-231 cells to L fibroblast-conditioned media, indicating that the soluble factor from L fibroblasts induced autocrine WNT–PCP signalling in breast tumour cells. Consistently, suppression of WNT secretion in MDA-MB-231 cells inhibited the protrusion and motility induced by L fibroblast-conditioned media, as did knockdown of WNT11 (but not of WNT5A). Immunofluorescence analyses showed that WNT11 localized in the cytoplasm of MDA-MB-231 cells, and, on treatment with L fibroblast-conditioned media, became associated with FZD6 at the tips of cell protrusions, indicating that a fibroblast-derived soluble factor induced autocrine WNT11–FZD6 PCP signalling in MDA-MB-231 cells.

So, what do L fibroblasts secrete? Analyses of the L fibroblast-conditioned media revealed that proteins contained in exosomes were the likely culprit. Exosomes isolated from the conditioned media induced motility and protrusive activity of MDA-MB-231 cells, which were prevented when exosomes were depleted from conditioned media. Proteomic analysis of L fibroblast-derived exosomes and subsequent knockdown studies revealed that reduced expression of the tetraspanin CD81 in L fibroblasts prevented the induction of MDA-MB-231 cell motility and inhibited the increased metastasis *in vivo*. Importantly, exosomes isolated from patient-derived cancer-associated fibroblasts also expressed CD81 and induced MDA-MB-231 cell motility and protrusive activity (that was PK1-dependent). Finally, analyses of MDA-MB-231 cells treated with conditioned media from L fibroblasts that expressed fluorescently labelled CD81 revealed that CD81 colocalized in vesicular structures with WNT11. Further investigation led the authors to suggest that CD81-positive exosomes derived from cancer-associated fibroblasts are endocytosed by recipient breast tumour cells, where they are loaded with WNT11, which in turn activates the WNT–PCP signalling pathway in an autocrine manner and drives tumour cell motility and protrusion, which promotes metastasis.

Gemma K. Alderton

ORIGINAL RESEARCH PAPER Luga, V. *et al.* Exosomes mediate stromal mobilization of autocrine Wnt-PCP signaling in breast cancer cell migration. *Cell* **151**, 1542–1556 (2012)