METASTASIS

New recruits

> the recruitment of … inflammatory monocytes to metastatic sites following tumour cell injection was blocked by antibodies against CCL2

Macrophages in the tumour microenvironment have diverse roles in tumour progression. Pollard and colleagues previously identified a distinct macrophage subpopulation that is involved specifically in promoting metastasis; they have now examined the origin and function of these metastasis-associated macrophages (MAMs).

Mouse monocytes expressing green fluorescent protein (GFP) were sorted into inflammatory and resident monocytes. These two subpopulations differ in that resident monocytes are present in tissues under steady-state conditions, whereas inflammatory monocytes are specifically activated by inflammation. Equal numbers of each monocyte population were adoptively transferred into syngeneic mice carrying advanced mammary tumours and pulmonary metastases that were induced by polyoma middle T antigen (*PyMT*) under the control of the mouse mammary tumour virus (*MMTV*) promoter. Despite equivalent availability of both cell types, threefold more GFP-positive inflammatory monocytes than resident monocytes were present in metastases. This enrichment was not seen in mice with pre-metastatic *MMTV–PyMT* tumours, but was observed in pulmonary foci that arose following tail vein injection of a *PyMT*-induced mouse mammary tumour cell line (Met-1 cells) and in the lungs shortly after injection of Met-1 cells. Furthermore, a substantial proportion of inflammatory monocytes at metastatic sites differentiated into MAMs by 2 days following adoptive transfer.

Similar results were observed using human monocytes obtained from healthy donors. Adoptive transfer of inflammatory and resident human monocytes into nude mice intravenously injected with the human metastatic breast cancer cell line 4173 (derived from MDA-MB-231 cells) led to preferential recruitment of inflammatory monocytes to the lungs.

Inflammatory monocytes are known to respond to the chemokine CCL2, and the authors found that the recruitment of both mouse and human inflammatory monocytes to metastatic sites following tumour cell injection was blocked by antibodies against CCL2. CCL2-specific antibodies also reduced the number of experimental metastases that developed in mice following tail vein injection of Met-1 cells and reduced the number of spontaneous metastases following orthotopic injection of MDA-MB-231 cells. Interestingly, metastasis resulting from tail vein injection of 4173 cells was partially blocked by either mouse or human CCL2-specific antibodies, indicating that both host and tumour cell CCL2 contribute to metastasis formation.

How do inflammatory monocytes enhance metastasis? Imaging of intact lungs showed that CCL2-specific antibodies reduced the number of macrophage–Met-1 cell interactions and tumour cell extravasation *in vivo*, as well as inflammatory monocyte-promoted transendothelial migration of Met-1 cells or 4173 cells *in vitro*. Transcriptome analysis revealed that the expression of vascular endothelial growth factor A (*Vegfa*) was enhanced in inflammatory monocytes compared with resident monocytes. Inducible knockout of *Vegfa* in mouse monocytes reduced transendothelial migration of tumour cells *in vitro* and experimental metastasis of Met-1 cells *in vivo*. Furthermore, co-injection of wild-type inflammatory monocytes with Met-1 cells in monocyte-*Vegfa*-null mice restored metastasis.

CCL2 expression and macrophage infiltration are both associated with metastatic disease and poor prognosis in breast cancer. If this pathway is confirmed in humans, it may be advantageous to target it therapeutically to prevent metastasis.

Sarah Seton-Rogers

ORIGINAL RESEARCH PAPER Qian, B. *et al.* CCL2 recruits inflammatory monocytes to facilitate breast-tumour metastasis. *Nature* 8 Jun 2011 (doi:10.1038/nature10138)