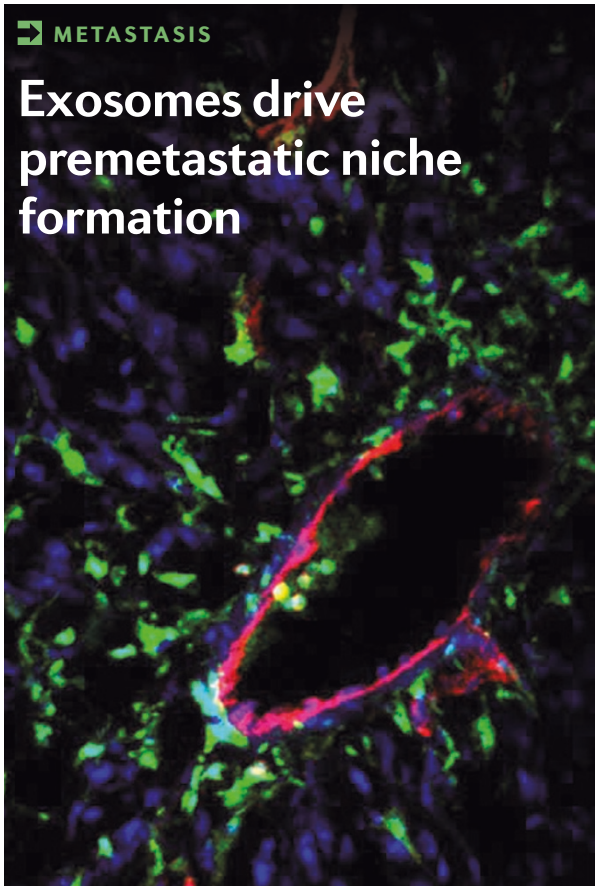
METASTASIS

Exosomes drive premetastatic niche formation



▲ Confocal microscopic analysis of BMDCs (GFP^+) and vasculature (lectin, red) in primary tumours from mice with exosome-exposed bone marrow. Image by Hector Peinado and David Lyden (Weill Cornell Medical College).

> “tumour-derived exosomes promote metastasis”

The premetastatic niche is a specialized microenvironment that forms at the sites of future metastases and promotes the survival and outgrowth of disseminated tumour cells. Evidence suggests that systemic factors from the primary tumour are involved in premetastatic niche formation, so David Lyden, Jacqueline Bromberg and colleagues investigated the possibility that exosomes, which can disseminate systemically, are one such factor.

Exosomes are small, membrane-bound vesicles that transfer RNAs and proteins to the cells they fuse with. To establish whether exosomes are relevant to metastasis, Peinado *et al.* isolated exosomes from the plasma of patients with melanoma and found that the protein concentration was higher in the exosomes from patients with stage 4 disease (metastatic melanoma) than from patients with earlier stages of melanoma. Moreover, increased levels of tyrosinase-related protein 2 (TYRP2; also known as DCT) in exosomes from patients with stage 3 melanoma (disease involving lymph nodes) correlated with the future development of metastasis. To investigate how melanoma-derived exosomes promote metastasis, the authors turned to model systems and melanoma cell lines (which also produce exosomes). They intravenously injected fluorescently labelled exosomes from B16-F10 metastatic melanoma cells into mice and found that exosomes exited the circulation to localize in common sites of melanoma metastasis: lung, bone marrow, liver and spleen. B16-F10 exosomes also caused endothelial permeability in the lung, which is an early event in premetastatic niche formation. Analyses of lung tissue after treatment with B16-F10 exosomes revealed that the expression of many genes associated with premetastatic niche formation was increased compared with controls. Furthermore, intravenous injection of B16-F10 exosomes prior to orthotopic implantation of B16-F10 cells significantly increased the metastatic tumour burden compared with control-treated mice.

To investigate how exosomes might mediate metastasis, the authors focused on bone marrow-derived cells (BMDCs), a crucial component of the premetastatic niche. They used bone marrow from B16-F10 exosome-exposed mice to reconstitute the bone marrow of lethally irradiated mice, which were then subcutaneously injected with B16-F10 cells. Primary tumour growth and the number and size of metastases were significantly increased in these mice. Moreover, the number of BMDCs (particularly vasculogenic BMDCs) was substantially increased in the metastases that formed, indicating that exosomes can cause the mobilization of BMDCs to premetastatic sites and mediate premetastatic niche formation.

As exosomes from B16-F10 cells promote metastasis, whereas exosomes from non-metastatic B16-F1 cells do not, the authors compared exosome protein content and found that MET, among others, was highly expressed in B16-F10 exosomes. MET expression and downstream signalling was increased in bone marrow progenitor cells (BMPCs) from mice that were treated with B16-F10 exosomes, and B16-F10 exosomes from cells in which MET was knocked down reversed these effects. When they were injected into mice implanted with B16-F10 cells, MET-depleted B16-F10 exosomes also reduced the number of lung and bone metastases and circulating vasculogenic and haematopoietic BMDCs, indicating that horizontal transfer of MET to BMPCs promotes BMDC mobilization. Total levels of MET and MET phosphorylation were both higher in exosomes derived from patients with stage 3 and 4 melanoma, and MET expression was increased in vasculogenic BMPCs from patients with stage 4 melanoma.

Therefore, tumour-derived exosomes promote metastasis by conditioning both the bone marrow and the premetastatic niche. Exosomes could potentially be used to identify patients who are likely to develop metastatic disease, and the process of exosome production could yield new targets for antimetastatic therapy.

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ORIGINAL RESEARCH PAPER Peinado, H. *et al.* Melanoma exosomes educate bone marrow progenitor cells toward a pro-metastatic phenotype through MET. *Nature Med.* 27 May 2012 (doi:10.1038/nm.2753)