

## COMMENTARIES

### Bone Marrow-Derived Cells – Fertilizer Necessary for the Seed and Soil

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**Commentary on:** Kaplan RN, Riba RD, Zacharoulis S, Bramley AH, Vincent L, Costa C, MacDonald DD, Jin DK, Shido K, Kerns SA, Zhu Z, Hicklin D, Wu Y, Port JL, Altorki N, Port ER, Ruggero D, Shmelkov SV, Jensen KK, Rafii S, Lyden D. VEGFR1-positive haematopoietic bone marrow progenitors initiate the pre-metastatic niche. *Nature*. 2005 Dec 8;438(7069):820-7.

Many cancers are known to have a predilection to an organ in which they establish and grow. This is particularly true for cancers of the head and neck, breast, prostate, kidney and lung, which favor metastases to bone (1). Many growth factors influence tumor growth in bone; well-documented examples include parathyroid hormone-related protein, gp130 cytokines, IL-8, endothelin, FGFs and prostaglandins, which either promote bone resorption or bone formation, thus allowing tumor establishment (1-4). However, the determinants that provide the selectivity of a tumor to a particular site are not known. This selectivity for tumors to home to an organ has been coined “the seed and soil” hypothesis, indicating that certain seeds will only germinate and sprout within a suitable environment. In such a concept, there is the requirement for a suitable seed as well as fertile soil for the seed type. While much is known about cancer cell lines and their ability to grow in bone in appropriate animal models (intracardiac or intratibial inoculation models), little is known about the host controlled factors that modulate cancers to grow in bone. The work by Kaplan and colleagues (5) identifies vascular endothelial growth factor receptor 1 (VEGFR1) as a key host factor, expressed by bone marrow-derived cells

(BMDCs), required for the successful colonization of B16 melanoma cells.

Using tagged ( $\beta$ -gal or GFP) BMDCs, the localization of these cells was tracked in mice that had received irradiation and subsequent implantation of tumor cells (DsRed-tagged to permit co-localization with BMDCs). BMDCs became established at metastatic sites prior to the establishment of tumor cells, yet tumor cells influenced BMDC cluster formation, with clusters being restricted to tissues where tumor metastasis occurred. Differential localization of BMDCs was apparent between mice injected with B16 melanoma cells or Lewis lung carcinoma cells. This finding implied that tumor cells were responsible to orchestrate the locality for establishment of BMDCs, but ultimately the presence of BMDCs was required for the subsequent establishment of tumor. The BMDCs consisted of hematopoietic progenitors that were VEGFR1<sup>+</sup>, CD133<sup>+</sup>, CD34<sup>+</sup> and CD117<sup>+</sup>, and were CD31 and VEGFR2 negative. Attention then focused upon the identification of the pre-metastatic BMDC signature and the potential role of VEGFR1. Through selective purification of VEGFR1<sup>+</sup> BMDC populations, it was revealed that these positive populations were able to support micrometastases, while VEGFR1 negative BMDCs could not produce pre-metastatic clusters. Furthermore, blockade of VEGFR1 function facilitated by use of a

neutralizing antibody to VEGFR1 diminished cluster formation and blocked metastasis while an anti-VEGFR2 antibody had no effect.

Since BMDCs could preferentially form clusters and required recruitment to potential metastatic sites, it was likely that VEGFR1 cells were endowed with enhanced invasive properties. These cells expressed integrin  $\alpha_4\beta_1$ , and at micrometastatic sites Kit-ligand, VEGF-A, MMP-9 and Id3, each of which has been implicated in cellular mobilization (6), were also expressed. The authors then performed similar studies in mice where integrin  $\alpha_4\beta_1$  expression was suppressed (facilitated by an anti-integrin  $\alpha_4\beta_1$  antibody), or in mice where MMP-9 or Id3 had been knocked out. In each model, reduced BMDC cluster formation and metastatic spread was noted.

Chemokine receptor 4 (CXCR4) and its ligand SDF-1 have been implicated in homing of breast cancers to bone (7;8) and retention of hematopoietic progenitor cells in the bone marrow – the expression of both SDF-1 and its receptor were elevated in pre-metastatic clusters.

The findings that BMDCs could establish at sites of micrometastasis and that this occurred prior to the detection of cancer cells suggested that secreted tumor-derived factors were responsible for the establishment of BMDC clusters. If so, a differential growth factor profile would be predicted to occur between cells capable of different metastatic potential. Analysis of conditioned media from melanoma and Lewis cell carcinoma revealed that placental growth factor (PlGF), a ligand that signals through VEGFR1, was elevated in B16 conditioned medium (CM). To address whether CM could dictate the site of metastasis, B16 CM was given intradermally prior to and following inoculation of Lewis cell carcinoma. CM from the melanoma cell line redirected the Lewis cell carcinomas from the lung to other sites frequently associated with melanoma, including kidney, spleen, intestine and oviduct.

This work provides new insights into the events governing metastasis formation, with particular emphasis on the role of bone marrow-derived cells and the secreted growth factors made by tumor cells that can influence BMDCs to take up residence at a site of metastasis. It also raises some questions. While the importance of VEGFR ligands has been explored, other growth factors will undoubtedly participate in the selection of BMDCs to respond to different tumor types permitting different sites of metastasis. Do the BMDCs contribute to site-specific metastases to bone, and are different subsets of BMDCs used in metastasis between organs? Are similar mechanisms involved? Do factors such as PTHrP, IL-8 and activators of gp130 signaling similarly invoke BMDC response or modulate their behavior to promote osteolytic outcome?

This work adds complexity to our growing knowledge of the events involved in site-specific metastasis. It is now apparent that the cell-surface signature of cells is not the initial selector for tumor establishment, but these are likely to act in concert with the secreted growth factors that the tumor produces, the BMDCs that are mobilized in response to growth factors and the cellular interactions between cancers, BMDCs, immune cells and stromal cells. Indeed, tumor-host interactions required for successful colonization and growth of tumor at distant sites require a tool box of factors expressed by the tumor and the host. The relative role of the respective factors as therapeutic targets for metastases needs to be defined, but the findings reported here provide rationale to use anti-VEGFR1 therapy in patients with high risk of metastasis. However, such patients would require treatment before clinical metastases were detected, a contrast to the standard of care in which drugs are first tested on patients with advanced disease. Thus, application of the novel findings reported by Kaplan *et al.* will require a paradigm shift in clinical trial design.

**Conflict of Interest:** The authors report that no conflict of interest exists.

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