Metastatic Cells Will Take Any Help They Can Get

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Two recent papers in *Cancer Cell* (Lu et al., this issue of *Cancer Cell*, and Kang et al.) illustrate means whereby acquisition of VCAM-1 by tumor cells can promote metastasis. First, monocyte/macrophages expressing α4 integrin bind VCAM-1 and provide survival signals enhancing establishment of metastases. Second, VCAM-1 allows dormant tumor cells to interact with osteoclasts, yielding paracrine signals and enhancing osteolytic metastatic growth.

Cancer is a leading cause of death, and most patients die from metastases rather than from primary tumors. Therefore, metastasis is subject to intense research investigations seeking to understand this interesting biological process and to provide better means of treatment. Much has been learned about the changes giving rise to primary tumors, and novel targeted therapies are based on that knowledge. However, it is clear that tumor cells have often already left the primary tumor site and dispersed throughout the body when primary tumors are diagnosed. Circulating tumor cells (CTCs) are found in the blood, and disseminated tumor cells (DTCs) can be found in ectopic sites such as lymph nodes and bone marrow, where they can persist for years and only later develop into clinically relevant metastases. The incidences of both types are indicators of poor prognosis for cancer progression.

The probability for both CTCs and DTCs to become clinically relevant metastases is low; most CTCs fail to seed productively in a distant site, and many DTCs remain dormant for years. Arrest and establishment of a potentially metastatic cell at a secondary site requires several steps: (1) survival in the circulation, (2) initial arrest, (3) extravasation, (4) initial seeding and establishment of a supportive niche allowing survival of the displaced cancer cells, (5) eventual growth into a colony or micrometastasis, and (6) subsequent growth into a secondary tumor/macrometastasis. While only the last presents an actual clinical problem, the earlier steps are essential and, based on results of various analyses, frequently fail. Tumor dormancy, which can last a long time, intervenes at steps 5 and 6. The challenges are to deduce what mechanisms allow some CTCs to home and seed effectively at distant sites and establish a proliferating secondary tumor and what changes allow dormant DTCs to resume growth.

Two recent papers in *Cancer Cell* provide new information bearing on these questions. In the first, Chen et al. (2011) describe investigations of a gene whose expression they found upregulated in derivatives of MDA-MB-231, a human mammary carcinoma line commonly used in studies of metastasis. It is possible to introduce such cells into the circulation and select for variants that show high propensity for metastasis to one or another secondary site. In this case, selection was for metastasis to the lung, and a variety of variants were selected and analyzed. These variants shared a gene expression signature, lung metastasis signature, differing from that of the parental MDA-MB-231 line and from those of variants selected for metastasis to bone or brain (Minn et al., 2005). Among the genes in the lung metastasis signature were several subsequently shown to enhance extravasation of the introduced CTCs in the lung. Another upregulated gene encodes VCAM-1, a cell-cell adhesion protein best known for its involvement in recruiting leukocytes to inflamed endothelium (which expresses VCAM-1) or to the bone marrow. Many hematopoietic cells express α4 integrins, α4β1 or α4β7, which bind to either VCAM-1 on other cells or fibronectin in the extracellular matrix. The α4-integrin-mediated adhesion plays important roles in recruiting and maintaining hematopoietic stem and progenitor cells and in the homing of lymphocytes and myeloid cells to sites of inflammation.

The occurrence of VCAM-1 on metastatic tumor cells naturally suggested that it might play some analogous role in recruitment, arrest, or extravasation of the tumor cells. However, Chen et al. (2011) showed that the presence of VCAM-1 on these cells did not enhance transendothelial migration and had little if any effect on the early recruitment of metastatic cells to the lung. Thus, it appeared that VCAM-1 interactions were not important for arrest, extravasation, or initial seeding (steps 2–4 above). However, VCAM-1 did enhance longer-term tumor cell survival and growth of lung metastases and Chen et al. (2011) showed that this was because VCAM-1 on the tumor cells binds monocytes through α4-integrin-mediated adhesion. Furthermore, this cell-cell adhesion provides survival signals to the tumor cells and prevents their apoptosis. This requires clustering of the surface VCAM-1 and recruitment of phosphorylated ezrin by the cytoplasmic domain of VCAM-1; ezrin in turn recruits PI3-kinase and activates Akt, providing the antiapoptotic signal. Chen et al. (2011) further show that VCAM-1 levels are elevated in breast cancer metastases to lung and bone, but not in those to brain, and that high levels of VCAM-1 correlate with elevated leukocyte infiltration. This suggested that leukocytes promote growth and survival of metastases if the tumor cells express VCAM-1 and thus select for VCAM-positive cells. It is somewhat surprising that overexpression of VCAM-1 did not enhance metastasis of MDA-MB-231 cells to bone even though bone marrow is replete with α4-integrin-positive cells, both hematopoietic cells and others—presumably, additional factors are needed for establishment of metastases

in the bone marrow. The results of Chen et al. (2011) establish a role for VCAM-1/α4 integrin interactions in steps 5 and 6: survival and growth of a macrometastasis.

The second paper, published in this issue of *Cancer Cell*, reveals additional roles for VCAM-1 in emergence from dormancy of DTCs (Lu et al., 2011). These researchers were studying clones of MDA-MB-231 that failed to give rise to bone metastases for several months after introduction into mice. However, after 4 months, tumors appeared in various bones in a small proportion of the injected mice. Further investigation showed that some of the initially injected cells had lodged in the bones but failed to grow into metastases—that is, they were dormant. However, with low frequency, some could emerge from dormancy and develop into frank metastases. Cells isolated from such metastases were vigorously metastatic to bone when retested. Comparison of gene expression signature of these new variants with that of others originally isolated for their ability to establish rapid bone metastases showed that the new DTC-derived cells did not recapitulate the original bone metastasis signature, which includes a set of genes that allow the cells to set up a paracrine signaling network between tumor cells and bone cells (Kang et al., 2003) and instead expressed a different set of genes. Testing some of these genes for functional involvement by knockdowns implicated VCAM-1 and overexpression of VCAM-1 in appropriate cell lines produced actively metastatic cells. Examination of other lines confirmed the generally prometastatic role of VCAM-1 and antibodies against VCAM-1 or α4 integrin-reduced growth of the metastases.

So, how does VCAM-1 confer metastatic capacity in this case? It turned out that expression of VCAM-1 increased the numbers of differentiated osteoclasts and established a paracrine support circuit between osteoclasts and the tumor cells. VCAM-1 contributed to the increase in osteoclast numbers in two ways: soluble VCAM-1 released from the tumor cells acts as a chemoattractant for preosteoclasts (which are α4-integrin-positive), and α4 integrin/VCAM-1-mediated adhesion of preosteoclasts to the tumor cells enhances their differentiation and establishes a paracrine cycle between tumor cells, osteoclasts and osteoblasts that enhances tumor growth.

So, acquisition of VCAM-1 by potentially metastatic mammary carcinoma cells can enhance their metastatic capacity through at least two different mechanisms: by monocyte/macrophage lineage cells clustering VCAM-1 on tumor cells and providing survival signals or by paracrine interactions between dormant tumor cells and osteoclasts to establish a vicious cycle of intercellular stimulation to enhance osteolytic metastatic growth. Since α4 integrins are expressed by a variety of cells present in the tumor microenvironment (lymphocytes, lymphatics, cells of the blood vessel wall, as well as myeloid cells), it seems plausible that additional advantages may accrue to tumor cells that express VCAM-1, in the form of additional VCAM-1/ α4 integrin interactions. On the brighter side, drugs (e.g., nataluzimab) are already in clinical use to inhibit such interactions in the context of autoimmune diseases and, with further research, it may be possible to deploy them also against metastasis. Since both examples discussed in these two papers concern long-term maintenance of metastases rather than their initial seeding, it is realistic to consider such approaches targeting preexisting metastases.

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