Lessons Learned From Lymphocytes: CC Chemokine Receptor-7 Involved in Lymphogenic Metastasis of Melanoma

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The metastatic potential of primary tumors is the chief prognostic determinant of malignant disease *(1)*. Although distinct patterns of metastasis have long been recognized by clinicians, the underlying molecular mechanisms along with the alterations in gene expression of tumor cells required for these processes have begun to emerge only recently. Metastasis and invasion are regulated by extensive interactions and signaling events between the tumor and the local host tissue [reviewed in *(2)*]. Two principal pathways of metastasis can be distinguished: lymphogenic metastasis to regional lymph nodes and hematogenic metastasis to distant organs. Lately, it has become clear that the two major routes of metastasis, through the lymphatic system and the bloodstream, are not paths by which disseminated tumor cells are distributed in a haphazard fashion. Rather, the process of metastasis is intimately related to the pathways and mechanisms used in physiologic leukocyte migration. The potential of tumor cells to metastasize is determined by their individual expression profile of genes involved in cell migration.

Lymphocytes and monocytes can enter lymph nodes either through afferent lymphatic vessels or through specialized blood vessels, the high endothelial venules (HEVs). Although naive B and T cells enter lymph nodes through HEVs, dendritic cells (DCs) and some memory T cells use afferent lymphatics. Recent evidence suggests that cell migration along these paths is regulated not only by adhesion molecules but also by chemokines and chemokine receptors. Chemokines are a family of small *chemo*attractant cyto*kines* that bind to G protein-coupled receptors expressed on target cells, thus allowing these cells to follow chemokine concentration gradients into selected tissues [reviewed in *(3)*]. Apparently, not only lymphocyte traffic but also the migration of metastatic tumor cells but also the migration of metastatic tumor cells is regulated by chemokines.

Homing of lymphocytes through HEVs has been the subject of intense research in recent years, and the key events in this process have been characterized. It is thought to proceed in a sequential, multistep fashion [reviewed in *(4)*]. Initially, there is a loose, selectin-mediated rolling and "tethering" of the lymphocyte to the endothelium, followed by the interaction of surface-bound chemokines on HEVs and chemokine receptors expressed on lymphocytes. This interaction in turn mediates a signal that rapidly activates integrins on the lymphocyte membrane. The binding of activated integrins to their ligands, the intracellular adhesion molecules, on the endothelium allows a firm adhesion of the lymphocyte to the endothelial wall and subsequent extravasation into the surrounding lymphoid tissue. In this process, the CC chemokine receptor-7 (CCR7) and its ligands, the chemokines Epstein-Barr virus-induced molecule-1 ligand chemokine (ELC/CCL19) and secondary lymphoid tissue chemokine (SLC/CCL21), play a key role: CCL19 and CCL21 are expressed on the luminal walls of HEV, where they are recognized by CCR7, thus initiating the migration of lymphocytes through the HEV into the lymph node *(5,6)*. After entering the lymph node, most of the T cells take advantage of, again, CCR7 to follow a CCL19 gradient built up by DCs to screen these cells for presented antigens. B cells and follicular T-helper cells, however, exploit the receptor CXCR5 to follow a B-lymphocyte chemoattractant (BLC/CXCL13) gradient that guides them into the B-cell-rich follicles *(7,8)*.

The mechanisms governing entry into lymph nodes via afferent lymphatic vessels are not yet as well understood. Recently, research concerning DCs in the skin, such as Langerhans cells, has begun to shed new light on the regulatory mechanisms involved in the maturation and migration of DCs to regional lymph nodes [reviewed in *(9)*]. Langerhans cells reside in the epidermis and, after receiving proinflammatory cues, such as signaling by interleukin 1β and tumor necrosis factor-α (TNF-α), migrate to regional lymph nodes via afferent lymphatic vessels. For the mobilization of skin DCs toward the draining lymph nodes, CCR7 and its ligands CCL19 and CCL21 are required. CCL21 is expressed on lymphatic ducts in the dermis, and expression of CCR7 on Langerhans cells is increased after exposure to TNF-α or other inflammatory stimuli. Furthermore, neutralization of CCL19 and CCL21 inhibits chemotactic activity of DCs *(10,11)*. Additional regulatory mechanisms are at work in this system, however. DCs express different integrins during their migration. α6 Integrin is required for the migration from the epidermis but gets decreased once DCs reside in the lymph nodes *(12)*. Furthermore, the lipid transporter multidrug resistance protein-1 (MDR1) and the leukotriene transporter multidrug resistance-associated protein-1 (MRP1) are required for DCs to emigrate from the epidermis *(11,13)*. This finding is of special importance for metastasis, because many cancer cells are known to stimulate the expression of MDR1, and the expression of MDR1 in some cases is associated with the metastatic potential of cancer cells *(14)*.

In this issue of the Journal, Wiley et al. *(15)* examine the role of CCR7 in regional lymph node metastasis in a murine melanoma model. They transduced a murine melanoma cell line, B16, with CCR7 and, surprisingly, transduction with this single gene was sufficient to substantially increase the metastasis to draining lymph nodes of B16 cells, which otherwise have a low propensity to spread. In the model used by Wiley et al., transduced cells were injected subcutaneously, thus mimicking lymphogenic metastasis of melanoma cells from the skin. Of interest, intravenous application of CCR7-transduced B16 melanoma

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cells (imitating a blood-borne route of metastasis) did not show increased spread of the tumor cells to lung or peripheral lymph nodes. Earlier findings had already pointed to the involvement of chemokine receptors in metastasis. Müller et al. *(16)* have analyzed the expression of chemokine receptors in breast cancer and melanoma cells and found a substantial increase in the expression of CXCR4, CCR7, and, in the case of melanoma cells, also CCR10. On treatment with the corresponding chemokines, CXCL12 and CCL21, breast cancer cells responded with formation of pseudopodia and increased chemotactic migration. Furthermore, treatment with anti-CXCR4 antibody resulted in a considerable reduction of tumor spread in an animal model for breast cancer metastasis *(16)*. What is indeed remarkable about the study by Wiley et al. is the fact that expression of a single gene, CCR7, enabled melanoma cells to acquire new metastatic properties. A closer look at the changes induced in these cells and how they are brought about is warranted: Is the expression pattern of integrins, such as $\alpha 4$ or $\alpha 6$ integrin, subsequently changed? Are other molecules relevant to this new chemotactic response, for instance selectins, differently expressed? If so, are these changes induced by chemokine receptor signaling or what other mechanisms might be accountable?

Recent advances in the knowledge of cell migration are changing our understanding of tumor metastasis. Undoubtedly, ongoing research will reveal further metastatic processes to be regulated, nonrandom, and highly analogous to physiologic cellular migration. These advances clearly present opportunities for the development of prognostic markers and therapeutic intervention.

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