The Biology Behind

CCR9 Homes Metastatic Melanoma Cells to the Small Bowel

Commentary on Ameresi et al., p. 638

Ann Richmond

The question of why certain types of tumors often metastasize to the same organs has intrigued scientists for over a century. In 1889, Paget put forth the “seed and soil” concept that cancer cells (seed) spread in a nonrandom fashion to distant target organs where they are drawn based on a conducive microenvironmental milieu (soil; ref. 1). A second concept that has been around for sometime is that tumor cells express “homing signals” to direct their movement to appropriate microenvironments for growth of metastatic lesions. A homing signal acts like a lighthouse to allow the ship to move toward the land. We have recently come to understand that chemokine receptors displayed on tumor cells allow the tumor cell (seed) to follow a gradient of chemokine to its target organ milieu (soil) that expresses the ligand for this chemokine receptor. The chemokine receptors are seven-transmembrane, G protein–coupled receptors that respond to ligand binding to activate a series of downstream signals that result in a polarized distribution of the actin cytoskeleton to form a leading pseudopod, followed by organization of the uropod cytoskeleton to allow a myosin-mediated contraction. This process repeats as the cell senses higher and higher concentrations of the ligand gradient and, as a result, the cell moves up the chemokine gradient and stops when the concentration of the ligand saturates receptors.

Chemokine receptors are divided into four classes, based on whether they bind CXC, C, CC, or CX3C chemokines. The CXC chemokine binding receptors (CXCR1-7) bind largely CXC chemokines (CXCL1-16); the C chemokine binding receptor (CR) binds CL1; the CC chemokine receptors (CCR1-10) bind CC chemokines CCL1-27, and the CX3C chemokine receptor binds CX3CL1 (2). Most of these chemokine/chemokine receptor interactions are quite promiscuous such that receptors bind multiple chemokine ligands and, likewise, the ligands bind a number of receptors. These receptors are abundantly expressed by leukocytes, which, in turn, usually display a number of chemokine receptors, establishing the necessary redundancy to ensure appropriate recruitment of leukocytes to sites of wounding, repair, infection, or inflammation. The expression of chemokines and their receptors is not limited to leukocytes. In fact, most cell and tissue types express chemokines or chemokine receptors at specific stages of differentiation.

Chemokine/chemokine receptor engagement results in activation of downstream signals, including phosphatidylinositol 3-kinase; ras/raf/mitogen-activated protein kinase; small GTPases such as Rac, Rho, Cdc42, and others; pAKT; src kinases; DOCK/ELMO; p130Cas; FAK; machinery involved in the organization of the actin cytoskeleton; and PKC-mediated signals that lead to mobilization of intracellular free calcium (3). These events occur in a cyclic fashion that involves local excitation followed by global inhibition, allowing for modulation of the actin cytoskeleton to facilitate extension of pseudopods and retraction of uropods in a continuously cycling manner such that the end result is directed migration up the chemokine gradient. Activation of integrins is an important component of the events required for directed migration. This directed migration is highly important during development, in the immune response, in the inflammatory response, in angiogenesis, and in tumor cell extravasation and intravasation as is essential for metastasis.

In addition, a number of transcriptional enhancers are activated by chemokine receptors that allow transcription of factors that modulate apoptosis and cell growth (4). Some of the CXC chemokines stimulate angiogenesis (CXCR1, CXCR2, and several CC chemokine receptors), whereas CXCR3 regulates angiostasis. CXCR4 has been described as angiogenic in some studies (5, for review), but not in others (6). CXCR7 is involved in tumor cell proliferation (7, 8). CXCL12 binds both CXCR4 and CXCR7 (7, 8).

Our knowledge of the role of chemokine/chemokine receptor interactions has been developing for >27 years. However, only in the last 6 to 7 years have we come to understand that chemokine receptor display on tumor cells can have a major effect on directed metastasis of tumor cells to organs that strongly express the chemokine ligand for the specific chemokine receptor the tumor cell expresses. Initial documentation of chemokine receptors serving as “homing signals” came from the landmark paper of Muller et al. (9), where CXCR4 chemokine receptor expression in breast cancer tumor cell lines and human ductal breast carcinoma biopsy tissue samples was enhanced, compared with cell lines established from normal mammary gland or the normal mammary gland tissue itself. Muller et al. also correlated expression of CXCR4 with metastasis to distant organs expressing CXCL12, the chemokine ligand for CXCR4 (bone, liver, lung, and lymph node). These early studies have been verified by a host of other studies showing CXCR4 expression in melanoma and a number of other metastatic tumor types (5, 10). Several antagonist for CXCR4/CXCL12 interactions (CXCR4 blocking antibody, CXCR4 small-molecule antagonists, peptide antagonist for CXCR4, small interfering RNA for CXCR4) have been tested in animal models and were shown to inhibit the number or size of tumor metastasis in breast cancer, non–small cell lung cancer, melanoma, renal cancer, osteosarcoma, colorectal cancer,
esophageal cancer, gastric cancer, and ovarian cancer (11–17). These studies indicate that antagonism of appropriate chemokine receptors displayed on homing tumor cells may offer new tools for therapy.

CCR7 is expressed in metastatic breast cancer cell lines, metastatic melanoma cell lines, as well as metastatic breast and melanoma tumor tissues. Lymph nodes, a common site of melanoma and breast cancer metastasis, strongly express CCL21, a ligand for CCR7 (18, 19). In contrast, lymph nodes also express CXCL9 to 11, all of which activate CXCR3 expressed on some melanoma cells as well as T cells and endothelial cells (20, 21).

CCR10 expression on melanoma tumor cell lines was shown to be associated with enhanced tumor growth for these cell lines and xenografts expressing CCR10 migrate to CCL27-expressing skin. However, CCR10 expression was not observed by Siedl et al. in primary or metastatic melanoma lesions (18, 19). Other studies have shown that CCR1 expression is up-regulated in melanoma and that CCR5 is expressed in primary melanoma lesions and some cutaneous metastases (19). Low levels of expression of CXCR1 have been detected in congenital nevi and in some, but not all, primary melanoma lesions, whereas CXCR2 mRNA expression was detected in congenital nevi, ~40% of primary melanoma lesions, and 30% of cutaneous metastases (19).

CCR9 is expressed on human melanoma cells and participates in the enhanced motility of melanoma cells and is likely a “homing receptor” for melanoma to the small bowel (19, 22–24). The ligand for CCR9, CCL25, is strongly expressed in the small bowel and the thymus (24–26). The work of Lentz et al. showed that 8 of 20 melanoma cell lines expressed functional CCR9 and CXCR4 receptors and of those eight lines positive for CCR9 and CXCR4, three were established from small-bowel metastases. Another of the eight CCR9-positive lines was taken from a patient that later developed metastasis to the small bowel. This group went on to study melanoma metastases to the small bowel and verify that at the time the tissue was taken, CCR9 was expressed in small-bowel metastasis, suggesting a possible link between CCR9 expression and the migration of these cells to organs expressing CCL25. For 11 of melanoma cells that did not express CCR9, none metastasized to the small bowel. There was one CCR9-negative cell line that developed small-bowel metastases 2 years after resection of the skin metastases, but by then there were multiple sites of visceral metastasis (23). For the three melanoma tumor lines expressing CCR9 and metastasizing to the small bowel, treatment with CCL25 down-regulated CCR9 and induced actin polymerization (23).

In this issue of Clinical Cancer Research, Amerisi et al. (27) have done an in-depth analysis of CCR9 expression in 198 melanoma lesions. Using reverse transcription-PCR, fluorescence-activated cell sorting analysis, and immunohistochemistry, they show that 102 of 198 metastatic melanomas metastasize to the small bowel. Of the 102 that metastasize to the small bowel, 88 express CCR9. The remaining 96 metastases were CCR9 negative and metastasized to the liver (n = 19), kidney (n = 5), lung (n = 14), gallbladder (n = 9), pancreas (n = 8), adrenal (n = 7), stomach (n = 18), and colon (n = 16). Of the 14 CCR9-negative patients with small-bowel metastases, these tumors also metastasized to colon, spleen, kidney, or adrenal glands. Moreover, examination of 23 paraffin-embedded archival primary melanomas revealed that 11 of 23 were CCR9 positive and of those 11 positives, 7 (64%) later developed small-bowel metastases. CCL25 expression in the small bowel was higher in those melanoma lesions that were metastatic to the small bowel than for those patients where CCR9-positive melanoma did not metastasize to the small bowel. Melanoma lesions that undergo metastasis to the small bowel also showed expression of α4 integrin and β1 integrin, both of which have been associated with lymphoid tissue in the gut and T cells in the small bowel. It is of interest that T cells expressing CCR9 or CCR10 also home to the organs that express the ligands for these chemokines, enabling the potential for immune surveillance, which would in turn impair establishment of metastatic lesions (24–26, 28–33).

For those CCR9-positive metastatic melanoma lesions that metastasized to the lymph nodes, there was no evidence of expression of CCL25 in the nodes. Because lymph nodes express CCL21 and CXCL12, these chemokines likely mediate the homing of melanoma lesions also expressing CCR7 and/or CXCR4 to lymph node (34, 35). Altogether, the data of Amerisi et al. confirm that CCR9 expression on primary tumors suggest a >60% likelihood that there will be metastasis to the small bowel. Based on this information, physicians can carefully follow their patients after surgical excision with a clear indication that there is a strong chance that if metastases occur, they will be to the small bowel. Both blocking antibodies to CCR9 as well as small interfering RNA for CCR9 blocked melanoma cell migration and invasion in response to CCL25 in vitro. These data offer promise that targeting CCR9 might offer rational postsurgical treatment for CCR9-positive melanoma patients.

Acknowledgments

We thank Albert Zlotnik and Fran Balkwill for their critical reading of the manuscript.

References

11. Yoon Y, Liang Z, Zhang X, et al. CXC chemokine receptor-4 antagonist blocks both growth of primary
CCR9 Homes Metastatic Melanoma Cells to the Small Bowel
Ann Richmond


Updated version Access the most recent version of this article at: http://clincancerres.aacrjournals.org/content/14/3/621

Cited articles This article cites 34 articles, 15 of which you can access for free at: http://clincancerres.aacrjournals.org/content/14/3/621.full.html#ref-list-1

Citing articles This article has been cited by 2 HighWire-hosted articles. Access the articles at: /content/14/3/621.full.html#related-urls

E-mail alerts Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.