13. EFFECTS OF CHEMOKINES ON TUMOR METASTASIS

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INTRODUCTION

One of the puzzling questions in the study of cancer metastasis has been: "Why do tumor cells metastasize preferably to specific organs and not to others?" The mechanism of cancer metastasis has been debated for several decades after the "seed and soil" theory, which was described by Paget (1). Paget theorized that cancer metastasis preferentially occur to organs or sites that support the growth of cancer cells. On the other hand, Ewing (2) described "anatomical mechanical theory" to account for cancer metastasis. He theorized that the patterns of blood flow from the primary tumor can predict the first metastasized organs. Recent progress in cancer metastasis biology has introduced development of a new concept, "homing theory," which incorporates the previous two theories to describe the mechanics associated with cancer metastasis. It is *hypothesized* that cancer cells are drawn to specific organ sites as a result of complex signaling between the tumor cells and the cells of the organ (3).

One of the highly important key factor recently identified in the homing theory is the chemokine receptor–ligand axis. Chemokines are small molecular weight chemotactic cytokines that are involved in a myriad of cell trafficking events. The chemokines are currently grouped into four major subfamilies (CC, CXC, CX3C,

and C) based on the arrangement of the two NH_2-terminal cysteine residues (4, 5). There are more than 50 chemokine family members to date, with at least 18 chemokine receptors defined containing seven transmembrane-spanning G-protein domains (6). Chemokines, which are induced by inflammation or pathogenic stimulation, activate the migration of leukocytes, dendritic cells (DCs), and other hematopoietic cells to specific sites (7, 8). Chemokine signaling is known to activate cell motility, invasion, interaction with the extracellular matrix, and modify overall disease outcome (6, 9). Chemokine receptors can bind and activate heterotrimeric G proteins. The activated G-protein subunits stimulate multiple signal transduction pathways, such as phosphoinositide-3 kinases (PI3K), phospholipase Cβ (PLCβ), Src family kinases, and cytoskeleton-related elements. Tumor cells of different origins bear different chemokine receptors. Similarly, many embryonic and fetal cells of different origins are known to express specific chemokine receptors. In this chapter, we will focus on CCR7 and CXCR4, since they are the most predominantly studied chemokine receptors studied in human cancers.

CCL21/SLC

Of particular interest in tumor progression to lymph nodes is chemokine CCL21/SLC, also referred to as 6Ckine or exodus, which is involved in recruiting naïve T-cells, memory T-cells, natural killer cells, and DCs (7–12). CCL21/SLC is constitutively expressed in the high endothelial venules (HEV) of lymph nodes, Peyer's patches, and thymus, spleen, and mucosal tissue (11, 13). It has a high affinity for its receptor, CCR7, a member of the chemokine receptor family (14–17). CCR7 is prevalent in various subsets of T-cells of different differentiation sites and DCs. The release of CCL21/SLC by HEV cells recruits CCR7(+) cells to draining lymph nodes. Abnormal expression of CCL21/SLC affects lymphocyte circulation and recruitment to lymph nodes. Lymphocytes and DCs of the DDD/1-plt/plt (paucity of lymph node T-cells) mouse do not migrate into peripheral lymph nodes because these nodes express no detectable SLC (18). Antigen-stimulated lymph nodes, when activated, express CCL21/SLC, which can attract of CCR7(+) immune cells such as DCs, T-cells, specific subsets of activated T-cells, and naïve T-cells (11, 12, 17). This is a very significant mechanism whereby tumor-draining lymph nodes can orchestrate and accentuate tumor immunity. Without this mechanism of immune cell recruitment, lymph nodes would likely have limited capacity to control tumor progression.

Studies have demonstrated that tumor cells of different embryonic organs can express functional chemokine receptors and respond to specific chemokine ligands (19). Chemokine physiological effects on tumor cells have been shown to be similar to effects on hemopoietic-derived cells. The chemokine receptor–ligand axis is a highly complicated but efficient physiological mechanism whereby immune cells can be induced to rapidly migrate to distant organ sites where injury or infection has occurred. Tumor cells appear to have taken advantage of this same physiological mechanism to migrate to specific organ sites. This may be a mechanism that is inherently programmed in cells recapitulating events during embryonic and fetal development. The focus of our laboratory has been on studying how human tumor

cells may utilize these chemokine signals to facilitate tumor cell metastasis from the primary tumor to the sentinel lymph nodes (SLNs) or distal sites in the body. The SLN is the first tumor-draining lymph node and site if metastatic disease was to establish in the regional draining node basin. We have found that metastatic tumor cells, which express functional chemokine receptors, respond to specific chemokine ligands in regional and distant organ sites. Understanding these events that promote tumor metastasis is highly important in that the tumor involvement status of the draining lymph node basin is one of the most important prognostic factors in many tumor types, such as breast, colorectal, gastric, esophageal, and melanoma.

MELANOMA

We previously reported that human melanoma cells express functional chemokine receptors CCR7 and CXCR4 (20–23). At first, we hypothesized that CCL21/SLC regulates the migration of CCR7-bearing melanoma cells from a primary lesion to the SLN. We demonstrated that melanoma cell lines and microdissected tumor tissues have heterogeneous expression of CCR7 mRNA as assessed by quantitative RT-PCR assay (20). Some melanoma cells did not express CCR7. This indicated that CCR7 receptor expression level may determine the fate of melanoma cells. There was strong functional correlation between CCR7 mRNA expression and cell migration induced by CCL21/SLC. CCL21/SLC did not induce migration of CCR7(–) cells. However, cell lines with higher CCR7 mRNA expression had a greater response to CCL21/SLC. These studies indicated the functional CCL21–CCR7 axis in human melanoma cells. Immunohistochemical (IHC) staining, a flow cytometry analysis, verified CCR7 protein expression in melanoma cells. CCR7 expression levels in primary melanomas significantly correlated with increasing Breslow thickness, which is one of the most important factors in determining prognosis of primary melanomas in patients.

CXCR4

Melanoma cell lines and metastatic melanoma in liver were shown to express various levels of CXCR4 as assessed by quantitative RT-PCR assays and IHC (21, 22). There was a significant correlation between CXCR4 mRNA expression and melanoma cell migration induced by its ligand, CXCL12. CXCR4 mRNA expression was observed in 24 of 27 (89%) melanoma liver metastases, and metastatic melanoma cells were demonstrated by IHC for immunostaining of CXCR4 (22). The ligands for CXCR4, CXCL12/SDF, are highly expressed in liver, and results suggest that CXCL12 may specifically attract melanoma CXCR4(+) cells and promote tumor progression. CXCL12/SDF are expressed by other organs, including lymph nodes as well.

EPIGENETIC REGULATION OF CHEMOKINE RECEPTORS

Variable expression of CXCR4 and CCR7 was observed in melanoma cell lines. We hypothesized that epigenetic events may regulate these receptors. One type of epigenetic event is the methylation of the promoter region of CpG islands, which can cause gene silencing (24). Another epigenetic event is the deacetylation of

chromatin regions in the promoter area of the gene. To investigate this, we assessed the effect of 5-aza-2-deoxycytidine (5-Aza) and trichostatin A (a histone deacetylase inhibitor; TSA) treatment of melanoma cells in culture, and then assessed receptor expression by flow cytometric analysis and quantitative real-time RT-PCR. From these studies, we observed that the two reagents could reactivate expression and enhance function CXCR4 and CCR7 on melanoma cells (21). However, this was variable with individual cell lines. For several cell lines, enhancement of chemokine receptors was quite significant. The results strongly suggested that an epigenetic mechanism may endogenously regulate chemokine receptor expression on melanoma cells and that melanoma cells are capable of expressing very high levels of the receptors. The internal or external cell mechanisms of turning these receptors on and off are still not fully understood. However, this suggests that microenvironment factors around tumor cells may promote chemokine receptor activation.

TUMOR-DRAINING LYMPH NODES

Lymph nodes are known to produce the chemokines CCL21/SLC and CXCL12. These chemokines are elevated during inflammation and immune responses of tissue site draining lymph nodes. We assessed the expression of these chemokines in the SLN of melanoma patients with and without micrometastasis. In our results, CCL21/SLC and CXCL12 production in the SLN correlated with level of metastasis involvement. Most interestingly, chemokines were more suppressed as metastatic tumor burden increased in the SLN. There is likely to be a feedback mechanism by which CCL21/SLC production is inhibited in the lymph nodes to prevent further recruitment of T-cells and DCs/LCs. However, metastatic cells in the SLN may also directly downregulate CCL21/SLC expression via immune suppressive factors to inhibit recruitment of DCs and naïve T-cells, thus providing a mechanism for escaping destruction. Future investigations are needed to determine the mechanism of suppression of these chemokines in lymph nodes with melanoma metastasis. Chemokine suppression in lymph nodes may be a very critical event in regulating immune responses and preventing overexpression of these secondary lymphoid organs. Suppression of key regulatory immune cells such as DCs to the lymph node would significantly dampen any major attack to metastatic tumor cells. Mechanisms involved in chemokine regulation may be useful in developing more effective immunotherapeutic strategies for augmenting regional tumor immunity.

COLORECTAL CANCER

Liver metastasis is the predominant factor affecting colorectal cancer (CRC)-related high mortality. Previous studies have shown that cellular extracts from liver parenchyma have high concentrations of CXCL12, the ligand specific to CXCR4. Our *hypothesis* is that the high levels of CXCL12 in the liver could provide a specific

homing target for CXCR4-bearing CRC cells, and the CXCR4 expressed by CRC is a prognostic factor for poor disease outcome. We assessed the CXCR4 expression in CRC primary and metastatic tumor specimens in the liver (22, 25). High CXCR4 expression in primary tumor specimens ($n = 57$) from AJCC stage I/II CRC patients was significantly correlated with increased risk for local recurrence and/or distant metastasis (risk ratio 1.35; 95% CI 1.09–1.68; $p = 0.0065$). High CXCR4 expression in primary tumors ($n = 35$) from AJCC stage IV patients significantly correlated with worse overall median survival (9 vs. 23 months; risk ratio 2.53; 95% CI 1.19–5.40; $p = 0.016$). CXCR4 expression was significantly higher in liver metastases ($n = 39$) compared to primary CRC tumors. Moreover, low vs. high CXCR4 expression in CRC liver metastases correlated with a significant difference in overall survival ($p = 0.036$). This suggested that the CXCL12–CXCR4 axis signaling mechanism may be clinically relevant for patients with CRC. Primary and metastatic CRC tumor cells demonstrated focal immunoactivity for CXCR4 protein in both the cytoplasm and cell membrane. Other studies using IHC have also demonstrated the prognostic significance of CXCR4 in CRC (26, 27).

CHEMOKINE RECEPTORS ON CARCINOMAS

CXCR4 has been demonstrated to be expressed by many types of carcinomas, and is associated with tumor progression and poor prognosis (4, 28). CCR7 expression has been reported for breast cancer, nonsmall-cell lung cancer, gastric cancer, and esophageal cancer (29–32). CCR7 expression in various carcinomas, in general, appears to correlate with lymph node metastasis and worse prognosis. Melanoma cells have been reported to express not only CXCR4 and CCR7, but also CCR10 and CXCR2 (19, 33). CCR10 may be related to skin metastasis of melanoma cells. CCR3 and CCR4, which are expressed in T-cell lymphomas, have been reported to correlate with tumor progression and poor prognosis of disease (34, 35).

Interestingly, sites of metastasis can be controlled by transfection of the cancer cells with different chemokine receptors in mouse B16 melanoma models (36–38). Transfection with CCR7 resulted in lymph node metastasis, transfection with CXCR4 caused lung metastasis, and transfection with CCR10 resulted in skin metastasis. Chemokine receptors on cancer cells and chemokines expressed in target organs may strongly support the "seed and soil" theory for organ-specific metastasis.

MICROENVIRONMENT

Tumor cells expressing specific chemokine receptors are likely to be more progressive in an organ microenvironment that produces specific chemokine ligands. Tumor cell adaptation to the microenvironment would promote metastasis. Tissue insult and inflammation would activate chemokine production, whereby tumor cells circulating or invading would take advantage of this favorable type of microenvironment. This

chemokine receptor-axis physiological mechanism is highly effective in rapid orchestration of hematopoietic cells to specific sites. We often associate chemokine-induced migration of tumor cells to distant sites. However, migration within an organ via chemokines also is likely to occur as the microenvironment may facilitate this intraorgan dissemination. This is observable particularly in lymph nodes and in the liver. Metastasis colonization within a tissue organ is poorly understood. Chemokines as well as blood/lymphatic drainage are likely to play a significant role in this process. Once a metastatic cell reaches a distant organ site its success depends on many factors such as migration and invasion both of which are likely to be regulated by chemokine gradients status in the organ at that time.

TARGETS FOR CANCER THERAPY

Chemokines and their respective receptors are now becoming attractive molecular targets for cancer therapy. Blocking agents for chemokine receptors or the chemokines themselves may contribute significantly to suppressing tumor invasion and metastasis. For instance, anti-CXCR4 monoclonal antibody significantly inhibits the metastasis of human breast cancer cells to the lymph nodes, as well as the growth of non-Hodgkin lymphoma cells in various animal models (19, 39). A small molecule CXCR4 antagonist, AMD 3100, developed for the treatment of AIDS, has been indicated in new cancer therapy. AMD 3100 inhibited the growth of intracranial glioblastoma and medulloblastoma in a xenograft model (40). However, clinical applications of these CXCR4 inhibitors must be closely monitored because CXCL12–CXCR4 signaling plays significant roles in the development and migration of hematopoietic stem cells and immune cells. Further studies on the cancer chemokine receptor network will reveal new therapeutic approaches for the treatment of malignant tumors. Similarly, identification of new chemokine receptor–ligand axis mechanisms defined in tumor cells will likely move this field further.

CONCLUSION

There is now sufficient observation reported in multiple human tumor systems to indicate that the chemokine receptor–ligand axis is a critical component of the metastatic process. Although the chemokine receptor–ligand axis is often associated with migration, other physiological functions are turned on. Observation to date strongly suggests the chemokine receptor–ligand response of tumors appears to be related to the aggressive tumor cell phenotype. The physiological properties of metastatic solid tumor cells are very similar to hematopoietic cells during metastasis. This also suggests that regulating tumor cell metastasis may be a significant problem at various stages of progression. As we understand the chemokine receptor–ligand axis as it applies to tumor metastasis, it will open new potential approaches of targeted therapy.

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