Keeping Out the Bad Guys: Gateway to Cellular Target Therapy

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Abstract

Tumor-stromal interaction is implicated in many stages of tumor development, although it remains unclear how genetic lesions in tumor cells affect stromal cells. We have recently shown that inactivation of transforming growth factor-β family signaling within colon cancer epithelium increases chemokine CC chemokine ligand 9 (CCL9) and promotes recruitment of the matrix metalloproteinase (MMP)-expressing stromal cells that carry CC chemokine receptor 1 (CCR1), the cognate receptor for CCL9. We have further shown that lack of CCR1 prevents the accumulation of MMP-expressing cells at the invasion front and suppresses tumor invasion. These results provide the possibility of a novel therapeutic strategy for advanced cancer—prevention of the recruitment of MMP-expressing cells by chemokine receptor antagonist.

Introduction

Adenocarcinomas develop progressively from epithelial cells that accumulate genetic lesions. A representative example in the multistep tumor progression is seen in colon cancer. Namely, most colorectal adenomas are initiated by inactivation of the APC gene in the epithelial cells and progress into malignant adenocarcinomas through additional genetic alterations in the K-ras, p53, SMAD4, and transforming growth factor-β (TGF-β) receptor type II (TGFBR2) genes (1). These genetic lesions can contribute to tumor progression through increased proliferation and decreased apoptosis in the tumor cells themselves. In addition to these cellular changes, it has been recognized that the stromal cells and extracellular matrix (ECM) surrounding the tumor epithelium play key roles in multiple steps of tumor development (2–4). For example, tumor-infiltrating leukocytes contribute to skin carcinogenesis (5), and cancer-associated fibroblasts (CAF) stimulate prostate tumor formation (6), not to mention the essential roles of angiogenesis in tumor growth (7, 8). CAFs also promote the growth of breast cancer xenografts (9). Furthermore, depletion of tumor-associated macrophages reduces metastasis in a breast cancer mouse model (10), suggesting that the stromal cells affect not only tumor initiation and growth but also its metastasis. However, precise roles of the stromal cells in tumor invasion, apparently the first step in metastasis, have not been understood. It also remains unclear how genetic lesions in the tumor epithelial cells, such as inactivation of the SMAD4 or TGFBR2 genes, induce stromal cell accumulation and activation.

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Tumor-Stromal Interaction in Tumor Invasion

As a model of colorectal tumor progression, we earlier constructed compound mutant mouse strain "cis-Apc/Smad4" that carries homozygous inactivation of both Apc and Smad4 genes specifically in the tumor epithelium (11). The cis-Apc/Smad4 mice develop intestinal adenocarcinomas that show marked invasion, in contrast to the simple Apc mutants that develop only benign adenomas. Like most epithelial cancers, the cis-Apc/Smad4 adenocarcinomas invade the intestinal wall as protruding glands or sheets, a typical pattern of "collective migration" (12). Such type of invasion is assumed as an early stage of epithelial cancer metastasis because tumor collectives or clusters can be detected in the circulation of patients with adenocarcinomas (12).

In a recent study, we found that the invading tumor glands of the cis-Apc/Smad4 adenocarcinomas were associated with clusters of "immature myeloid cells" that expressed myeloid progenitor cell marker CD34, pan-leukocyte marker CD45, and myeloid cell marker CD11b (13). These cells did not express monocyte marker CD14, granulocyte marker Gr-1, macrophage marker F4/80, dendritic cell marker CD11c, T-cell marker CD3ε, B-cell marker CD20, endothelial marker CD31, vascular endothelial growth factor receptor 1 (VEGFR1) or von Willebrand factor, or myofibroblast marker αSMA (13). Furthermore, these cells did not express hematopoietic stem cell marker c-kit or CD133.1

To examine the mechanism underlying the accumulation of these cells, we looked for chemokines that were increased specifically in the cis-Apc/Smad4 polyps because chemokines stimulate directed cell movements (see below). Among the chemokines tested, only CC chemokine ligand 9 (CCL9) was increased significantly in the cis-Apc/Smad4 tumor epithelium. Because the Smad4 genes are lost only in the tumor epithelial cells, by the genetic design of the cis-Apc/Smad4 mice, these results indicate that loss of the TGF-β family signaling in the tumor epithelium can promote the production of chemokine CCL9. Consistently, we found that expression of CCL9 in mouse colon cancer cell line CT26 was suppressed by TGF-β family ligands, such as TGF-β, activin, bone morphogenetic protein (BMP) 4, and BMP7. We further examined expression of CC chemokine receptor 1 (CCR1), the cognate receptor for CCL9, and found that the CD34+ immature myeloid cells at the invasion front express CCR1 and migrate toward its ligand CCL9.

We then assessed the role of CCR1 in recruiting CD34+ immature myeloid cells toward the polyps. To this end, we introduced a homozygous Ccr1 knockout mutation into the cis-Apc/Smad4 mice and found that lack of CCR1 prevents the accumulation of immature myeloid cells at the invasion front. Importantly, the loss of CCR1 dramatically suppressed tumor invasions into the smooth muscle layer and serosa in the cis-Apc/Smad4 mice. Because immature myeloid cells at the invasion front showed gelatinolytic...
activities and expressed matrix metalloproteinase (MMP) 2 and MMP9, these results indicate that the immature myeloid cells help tumor invasion, at least in part through the production of MMPs.

Taken together, our recent findings indicate that loss of TGF-β family signaling within tumor epithelium stimulates chemokine CCL9 secretion, which results in the accumulation of CCR1+ immature myeloid cells that help tumor invasion (Fig. 1).

Because the invasion mechanism of the primary colon cancers and that of the metastatic lesions are likely to be similar (14), micrometastatic and colonizing cancer cells may also depend on the immature myeloid cells for invasion into the parenchyma of the target tissues. It should be investigated whether CCR1+ immature myeloid cells are also recruited to the metastatic lesions and help metastatic invasion in human colon cancer.

Bone Marrow–Derived Myeloid Cells in Cancer

Whereas CCR1+ immature myeloid cells assist tumor invasion, other types of myeloid cells can also contribute to the tumor growth and metastasis. For example, CD11b+ monocytes that express Tie-2 angiopoietin receptor promote angiogenesis and subsequent tumor growth (15). Hematopoietic cells expressing CD11b and a granulocyte marker Gr-1 also promote tumor growth by increasing angiogenesis and vasculogenesis (16). Interestingly, a subset of perivascular progenitor cells that express CD11b differentiate into pericytes and stabilizes tumor vessels (17). Furthermore, hematopoietic progenitor cells that express CD11b and VEGFR1 cluster at the premetastatic sites and support tumor metastasis (18).

In the intestinal polyps of the cis-Apc/Smad4 mice, however, the CCR1+ immature myeloid cells at the invasion front seem different from these cells because they do not express VEGFR1 or Gr-1. One of the important mechanisms that recruit CCR1+ immature myeloid cells to the invasion front is increased secretion of its ligand CCL9 from the cancer epithelium caused by the block in the Smad4 signaling. The mechanisms that mediate recruitment of other bone marrow–derived myeloid cells described above are unclear and remain to be investigated. Once the ligand receptor systems are identified for such cells, these cells may also become novel therapeutic targets to inhibit their recruitment (see below).

Chemokines and Chemokine Receptors in Cancer

As mentioned above, we have revealed that immature myeloid cells in the intestinal polyps are recruited by chemokine CCL9. Although it has been reported that breast cancer cells release colony-stimulating factor-1 (CSF-1) and recruit macrophages to the tumor (10, 19), we found no expression of CSF-1 in the cis-Apc/

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Figure 1. SMAD4-deficient tumor cells produce chemokine CCL9 and recruit the receptor-expressing cells that promote tumor invasion. i, the inactivation of the TGF-β family signaling within the tumor epithelium causes increased production of chemokine CCL9 because it is suppressed by TGF-β, activin-A, and BMPs. ii, increased levels of CCL9 recruit immature myeloid cells that carry the CCL9 receptor CCR1 from the blood to the tumor invasion front. These immature myeloid cells produce MMP9 and MMP2 (iii) and help the tumor epithelium to migrate and invade into the stroma en masse (iv).
Smad4 polyps (13), excluding its contribution to the recruitment of the CCR1+ immature myeloid cells.

Chemokines are chemotactic cytokines that direct the migration of chemokine receptor-expressing cells. Although originally identified for leukocyte trafficking in infections, the chemokine-chemokine receptor system plays important roles in organ-specific tumor metastasis. For example, tumor cells from various types of cancer carry chemokine receptor CXCR4 and metastasize to the bone and lung, where its ligand CXCL12 is expressed (20). Furthermore, we have shown that CXCR3 expressed on melanoma and colon cancer cells promotes their metastasis to the lymph nodes but not to the lung (21, 22). These results collectively suggest that distinct chemokine receptors control metastasis of tumor cells to specific organs (e.g., CXCR3 to the lymph nodes and CXCR4 mainly to the lung and bone). In addition to metastasis, tumor promotion and growth are also aided by chemokines. Namely, CXCL14 helps recruitment of macrophages that promote hyperplastic tumors in the stomach (23). In a breast cancer xenograft model, CXCL12 promotes angiogenesis by recruiting endothelial progenitor cells and stimulates tumor growth (9). Our recent study has revealed an additional role of chemokines in tumor development (i.e., promotion of tumor invasion by CCL9). These reports clearly indicate that chemokine-chemokine receptor interactions play important roles at various stages in tumor development.

In cancer tissues, the major source of chemokines, including CXCL12, seems to be the stromal cells, such as fibroblasts and leukocytes, although it remains unclear how genetic alterations in tumor cells cause the stromal cells to secrete chemokines. Our study has shown that the tumor epithelial cells can secrete chemokine CCL9 through inactivation of the TGF-β family signaling. To our knowledge, this is the first demonstration that a genetic lesion (i.e., loss of Smad4 gene in the tumor epithelial cells) can promote chemokine production that contributes to tumor invasion. Interestingly, another CC chemokine, CCL2, is produced by the tumor epithelium of human ovarian cancer and correlates with the extent of leukocyte infiltration and tumor progression (20). These results suggest that recruitment of leukocytes by tumor cells themselves can promote tumor progression in various types of cancer and that chemokines and their receptors may provide novel therapeutic targets.

**CCR1 Antagonists**

Although most primary tumors can be resected surgically, their metastatic spreads are often lethal. If the tumor invasion at the metastatic sites is also helped by CCR1+ immature myeloid cells, it should be possible to treat or prevent it with CCR1 antagonists.

It has been reported that CCR1 binds several chemokines, including CCL3, CCL5, CCL7, as well as CCL9, and is associated with various autoimmune diseases, such as multiple sclerosis, rheumatoid arthritis, and organ transplant rejection (24). Therefore, some pharmaceutical companies have developed CCR1 antagonists and are testing some compounds in phase II clinical trials for multiple sclerosis and rheumatoid arthritis (24).

Among the reported CCR1 antagonists, one of well-characterized compounds seems to be BX471 developed by Berlex. In competitive binding assays, BX471 inhibits in a dose-dependent manner the binding of CCL3, CCL5, and CCL7 to human CCR1 with Ki value of 2.8, 1.0, and 5.5 nmol/L, respectively (24). Furthermore, BX471 suppresses human leukocyte migration stimulated by the CCR1 ligands, but not those by ligands of CCR2, CCR5, or CXCR4, suggesting that it is a functional antagonist with selective specificity to CCR1. Using animal models of autoimmune diseases, many reports suggest the efficacy of this compound as therapeutics. Namely, BX471 reduces a clinical score in a rat experimental allergic encephalomyelitis model of multiple sclerosis (25) and blocks rat heart transplant rejection in combination with cyclosporine (26). Because some CCR1 antagonists, including BX471, have already been tested for their safety, we can advocate these compounds as potential drug candidates for colon cancer progression.

**From “Molecular Target” Therapy to “Cellular Target” Therapy**

As described above, lack of CCR1 prevented the accumulation of MMP-expressing cells at the invasion front and suppressed tumor invasion in the cis-Apc/Smad4 mice. Similar to the cis-Apc/Smad4 polyps, the CCR1+ cells that expressed MMP9 infiltrated at the invasion front of some human colon cancers with TGFBR2 mutations (13), suggesting the key roles of the stromal cells that express MMPs in invasion. Accordingly, these results provide a novel strategy for cancer therapy that targets the “assisting stromal cells” using chemokine receptor antagonists to prevent accumulation of the MMP-expressing cells.

Because MMPs degrade the ECM that separates tumor epithelium from the initially normal stroma, they contribute to the tumor invasion and subsequent metastasis. Consistent with this notion, many clinical studies of colon cancer have shown that expression levels of MMP2 and MMP9 correlate with tumor progression (27). However, the attempts to block MMPs directly to prevent tumor progression have failed in the extensive phase III clinical trials. Almost all MMP inhibitors from multiple companies could not show clinical efficiency. Even worse, some compounds had severe side effects, such as inflammation, musculoskeletal pain, and joint strictures (28–30). In retrospect, the attempts to inhibit MMPs failed because they cleave not only ECM components but also many other proteins, including growth factor binding proteins, cytokine precursors, and chemokines (30). The proteolysis of these MMP substrates results in the release and/or activation of secreted factors that stimulate stromal cell infiltration and proliferation. Therefore, it is not surprising that systemic and prolonged inhibition of MMP activities causes aberrant immune responses and other stromal reactions. The ineffectiveness of MMP inhibitors may be explained partly by the recent findings that cancer stromal cells have non-MMP proteinases that degrade ECM, such as cathepsins (31) and uPARAP/Endo 180 (32).

Compared with the systemic “molecular target” therapy using MMP inhibitors, the “cellular target” therapy using CCR1 antagonists may cause much less side effects because chemokines affect only tissue-specific and therefore localized cell migration. Consistently, a mouse study indicates that the effect of CCR1 deficiency is rather benign unless the mice are infected (33). Instead of direct and systemic inhibition of MMPs, the blockade of MMP-producing cell accumulation by CCR1 antagonists might help improve management of some advanced tumors.

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