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Leukocytes as Paracrine Regulators of Metastasis and Determinants of Organ-Specific Colonization

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Abstract

It is now well recognized that tumor cell-host interactions regulate all aspects of cancer development. Amongst the various host response programs that facilitate primary cancer development, an emerging body of literature points to a critical role for leukocytes and their soluble mediators as regulating discrete events during primary tumor development and metastasis. This review focuses on the multiple aspects of leukocytes and their effector molecules as regulators of the metastatic process.

Keywords

leukocytes; inflammation; cancer; metastasis

Dissemination of malignant cancer cells to distant organs is a multistage process requiring detachment and escape from primary tumor sites, extravasation through multiple basement membranes and matrix, survival in peripheral blood or lymphatics, and ability to survive and proliferate in foreign tissue locales. Since, for many tumor types, there is a temporal lag (months to decades) between when malignant cells arrive in ectopic locations and when proliferative capabilities are acquired ^{1,2}, this implies that in addition to activation of survival programs at the metastatic site, disseminated malignant cells must acquire additional capabilities enabling their survival that likely rely on harnessing embedded regulatory programs at secondary sites. Thus, while cell-intrinsic programs are necessary for successful progression through each of these hurdles ^{2,3}, cell-extrinsic programs regulated by non-neoplastic cells of mesenchymal, vascular and immune origins are also critical determinants for successful metastatic progression ⁴.

Chronic infiltration of tissue by leukocytes, aka, chronic inflammation, is associated with predisposition to cancer ⁵. Chronic inflammation triggered by bacterial and viral infections or by autoimmune disease, is estimated to be linked with 20% of all deaths from cancer worldwide ⁶. Indeed, epidemiologic studies reporting that non-steroidal anti-inflammatory drugs (NSAIDs) reduce the risk of some cancers provide evidence for a causal link between inflammation and cancer ^{7–9}. Thus, chronic inflammation that precedes neoplasia provides a fertile microenvironment whereby secreted growth factors, reactive oxygen species and cytokines support epithelial proliferation and create a permissive microenvironment to foster ongoing genetic instability and accumulation of genetic alterations that predispose to malignancy ^{5,6}. Alternatively, inflammation can also be a physiological response to aberrant proliferation and tissue remodeling initiated by mutational activation of intrinsic programs that sustain proliferation and/or block cell death, and thus represent a secondary event

enabling *progression* of neoplastic cells¹⁰. In any event, hallmarks of inflammation, such as the infiltration of neoplastic tissue by innate and adaptive leukocytes, activated angiogenic vasculature, tissue remodeling, and high levels of chemokines and cytokines that regulate these processes, typify most solid tumors^{5, 6, 11}. This review discusses how key components and pathways of the immune microenvironment are associated with adult solid tumors and thereby promote the multistep cascade of tumor metastasis to distant organs.

Leukocytes implicated in mediating solid tumor metastasis

It has become generally accepted that the chronic presence of activated leukocytes in primary tumors is a “hallmark” of the tumorigenic process, and also represents a predictor of aggressive disease. Tumor-associated macrophages (TAMs) are one of the most abundant innate immune cells present in several types of human cancer^{12–16}, regulated in part by colony stimulating factor (CSF)1, a key cytokine involved in macrophage maturation, tissue recruitment and activation mediated by the CSF1 receptor (CSF1R/cFMS)¹⁷. A second CSF1R ligand, interleukin (IL)-34, possesses similar binding affinities and also regulates TAM recruitment to tissues, but exhibits distinct tissue distribution^{18–20}. TAM presence in several types of human cancer correlates with increased vascular density and worse clinical outcome^{21–26}. Studies in transgenic mouse models of mammary carcinogenesis revealed that TAMs promote tumor growth and enhance pulmonary metastasis by high-level expression of epidermal growth factor (EGF) and activation of EGF-regulated signaling in mammary epithelial cells (MECs) critical for invasive tumor growth and metastatic dissemination¹⁵. In mouse models of mammary carcinogenesis, TAMs activated by interleukin (IL)-4 and CSF1 have been identified as essential determinant of pulmonary metastasis due to the pro-metastatic mediators they secrete^{27–30}. The transcription factor *Ets2* was recently implicated in regulating some aspects of these activities as selective deletion of *Ets2* in TAMs decreased the frequency and size of pulmonary metastases in mouse models³¹.

T lymphocytes were classically studied in the context of their tumor surveillance and anti-tumor capabilities. However, recent investigations have revealed that CD4⁺ T cells and T regulatory cells instead promote pulmonary metastasis in part by regulating pro- versus anti-tumor bioactivity of innate leukocytes. We reported that interleukin (IL)-4-expressing CD4⁺ T cells promote invasion and metastasis of mammary adenocarcinomas by directly regulating TAM phenotype, bioeffector function and EGF expression, that in turn regulates invasion, presence of circulating tumor cells (CTCs) and pulmonary metastasis²⁹. Other mediators found to be significant with regards to T cell enhancement of pulmonary metastasis in mammary carcinomas are S100A4 and RANKL^{32, 33}. S100A4 protein mediates T cell attraction to developing neoplasms and premetastatic lungs of tumor-bearing mice, and in turn stimulates T cell production of cytokines, particularly granulocyte colony-stimulating factor and eotaxin-2³³. RANTES stimulates externalization of S100A4 via microparticle shedding from plasma membranes and induces upregulation of fibronectin (FN) from fibroblasts and a number of other cytokines, including RANTES in tumor cells that together enhance tumor cell motility³². During prostate carcinogenesis, T lymphocyte and macrophage-derived RANKL induces metastasis through activation and nuclear localization of IKK α leading to repression of *maspin*, a critical suppressor of metastasis^{34, 35}. Lung metastasis of mammary carcinomas is also regulated by CCR4⁺ T regulatory cells that can directly kill natural killer (NK) cells³⁶.

Other myeloid cell types implicated in regulating metastasis include neutrophils, mast cells and monocytes harboring T cell suppressive activity^{37–40} that are potent suppressors of anti-tumor adaptive immunity and directly facilitate metastasis by regulating angiogenic programs via enhanced metalloproteinase activity^{11, 41–43}. Cancer-Associated Fibroblasts

(CAFs) are implicated in regulating the activities of these myeloid cell types through their secretion of pro-inflammatory chemokines that recruit immune cells to sites of developing neoplasms^{33, 44, 45}.

Proinflammatory signals that impact exit from primary tumor sites

Regulators of the invasive phenotype

In order for malignant cells to detach from primary tumors and move through their substratum basement membrane, they must transiently acquire a motile and migratory phenotype, sometimes also referred to as *Epithelial to Mesenchymal Transition* (EMT)^{46, 47}. This motile state is characterized by loss of homotypic cellular adhesions and apical-basal polarity, and increased migratory capabilities. At the molecular level, this transition is largely driven by intrinsic alterations in gene expression, including suppression of *E-Cadherin*, mediated by activation of transcriptional repressors Snail, Slug, Twist and Zeb⁴⁸.

Extrinsic regulation is also important. Specifically, the chronic inflammatory microenvironment, provided by leukocytes and CAFs plays an important role in regulating the invasive and motile phenotype of would-be metastatic cells. Several leukocyte-regulated mediators have been identified as key to these processes. Notably, tumor necrosis factor (TNF) α secreted by TAMs activates the NF- κ B transcription factor in neoplastic (and other) cells, directly leading to expression of Snail1 and Zeb^{45, 49, 50}. Other leukocyte-derived cytokines (including IL-6 and IL-23) induce activation of intracellular STAT3 that in turn leads to induced expression of Twist^{51–53}.

Local hypoxia in neoplastic tissue also contributes to induction of motility programs^{54, 55} in part by activation of transcription factors hypoxia inducible factor (HIF)-1 α and NF- κ B, both implicated in EMT via transcription of Snail⁴⁹. Moreover, the chemokine receptor CXCR4 is upregulated in mammary carcinomas by hypoxia and is associated with invasive behavior in response to its ligand Stromal-Derived Factor-1 (SDF-1/CXCL12)^{56, 57}. Thus, hypoxic conditions select for a more metastatic phenotype partially through activation of pro-inflammatory signaling cascades.

Invasion

Movement of malignant cells through basement membrane and stromal matrix requires remodeling of matrix proteins. This process is coordinated by proteolytic enzymes spanning several catalytic classes and includes matrix metalloproteinases (MMPs), cysteine cathepsins and serine proteases⁴. Indeed, the increased expression and activity of various proteases has been observed in multiple human and murine tumor types, and can be used as a prognostic indicator of shorter survival rates in patients with breast, ovarian, colorectal and head and neck cancers^{58, 59}. While many proteolytic enzymes are produced by motile neoplastic cells, the majority of tumor-promoting proteases are produced by activated stromal cells in the local tumor microenvironment^{41, 60–63}, e.g., fibroblasts⁶⁴, or tumor-associated immune cells. Mast cells, neutrophils and macrophages secrete matrix remodeling proteases^{65–67} implicated in prometastatic activity, as well as serine proteases that are associated with higher tumor grades and lymph node metastasis in breast cancer³⁸.

In particular, murine macrophages are known to express elevated levels of the cysteine protease cathepsin B following exposure to IL-4⁶⁸. Macrophages at the invasive edge of pancreatic islet cancers express cathepsin B, and this is associated with loss of epithelial E-cadherin on neighboring malignant cells⁴. Secretion of proteases by cells within the tumor microenvironment may foster metastatic activity and motility of neoplastic cells through matrix and into vasculature, but also enhance and/or regulate presence and activity of leukocytes: over-expression of cathepsin B in a transgenic mouse model of mammary

carcinoma regulates pulmonary metastases, accompanied by increased numbers of B cells, Ig deposition and degranulation of mast cells in the primary tumor site⁶⁹

Protease secretion by TAMs is in part regulated by IL-6 emanating from neoplastic cells. Tumor cells-derived IL-6 induces secretion and activation of the cysteine protease cathepsin B, and secretion of matrix metalloproteinases (MMPs) by monocytes⁷⁰. Similarly, neoplastic cell and T cell-derived IL-4 induces cathepsin expression and activity in TAMs in several cancer types³⁰. TAMs in turn regulate neoplastic cell motility by secreting factors such as migration-stimulating factor (MSF)⁷¹. MSF is an oncofetal isoform of fibronectin and is induced in TAMs by macrophage-CSF, IL-4, and transforming growth factor beta (TGFβ). Notably, some immune cell-derived proteases also harbor tumor-suppressive activity: the aspartic proteinase cathepsin E, expressed predominantly by immune cells, including lymphocytes, macrophages, and dendritic cells, mediates neoplastic cell apoptosis by catalyzing the proteolytic release of soluble tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) from the cell surface. Tumor growth is subsequently enhanced by this cascade and survival in tumor-bearing mice impaired⁷².

Leukocytes and survival of CTCs

Before productive metastatic colonization is possible, circulating tumor cells (CTCs) must develop mechanisms enabling their survival within the circulation. Mechanical shear stress, detachment-induced cell death (anoikis) and cell-mediated cytotoxicity within the microcirculation effectively clear most CTCs⁷³. It has been estimated that only 0.01% of CTCs survive and eventually extravasate at distal locales⁴. Mechanisms that enhance the probability for CTC survival rely on physical interaction with leukocytes. Activated platelets aggregate around CTC and thereby protect them from NK cell-mediated lysis⁷³ by both thrombin-dependent and -independent mechanisms⁷⁴.

Adhesion to capillary walls is largely regulated by the availability of adhesion molecules on CTCs, the endothelium, and the composition of the underlying extracellular matrix (ECM). Platelets⁷⁴ and neutrophils facilitate these interactions however, via their production of matrix attachment molecules such as beta(2)-integrin/intracellular adhesion molecule-1 (ICAM-1)^{75, 76} and selectins⁷⁷. Once CTCs attach to capillary lumens, another obstacle to surmount is inhibition of detachment-induced cell death, or anoikis, which is thought to be a major impediment for productive metastatic spread. Chemokine receptors CXCR4 and CCR7, and their ligands reduce the sensitivity of CTCs to not only arrest on capillary lumens, but also CTC anoikis by selective regulation of pro-apoptotic Bmf and anti-apoptotic Bcl-xL proteins; thus, in the absence of appropriate cell-ECM interactions, selectins and chemokine receptors regulate CTC survival by mediating attachment and blocking cell death^{77, 78}.

Site-specific colonization

Organ-specific migration

Development of productive metastasis is a highly regulated process that is also subject to organ-specific mechanisms. Some solid tumors metastasize to preferred organ sites, for example, breast cancers metastasize to lung, liver, bone and brain; melanoma to liver, brain and skin; prostate cancer to bone; colorectal cancer to liver and lung, and lung adenocarcinoma metastasizes to bone, liver and brain^{2, 4}. Several studies have reported that tropism of CTCs to specific organ locales is regulated by the complexity of genetic alterations intrinsic to neoplastic cells², while also recognizing that altered expression of important genes can also regulate tropism. Cyclooxygenase (COX)2 (also known as PTGS2), the epidermal growth factor receptor (EGFR) ligand heparin bound (HB)EGF, and

the alpha2,6-sialyltransferase (ST6GALNAC5) all act as mediators of malignant cell passage through the blood-brain barrier when breast cancers metastasize to brain ^{79, 80}. In contrast, when breast cancer metastasizes to bone, IL-11 and connective tissue growth factor (CTGF) regulated by transforming growth factor (TGF) β are important ⁸¹.

A growing body of literature however also has identified cell-extrinsic mechanisms, in addition to intrinsic, that dictate organ-specificity of metastases, including differential expression of chemokines and their receptors. Chemokines expressed by specific organs promote tumor cell adhesion to microvessel walls, facilitate extravasation into target tissues and induce tumor cell migration. CXCL12-CXCR4, CCR7 and its corresponding chemokine ligands, CCL21 and CCL19, significantly regulate lymph node metastasis, whereas CCR10-CCL27 and CCR4-CCL22 regulate melanoma metastasis ^{82, 83}. Many malignant cells upregulate expression of chemokines receptors during premalignancy, partially as a result of autocrine and paracrine signaling mediated by TNF α , IL-1 and IL-6 at the primary tumor site and subsequent chemokines gradients then in part regulate migration towards specific organs ⁷¹. One such functional chemokines-signaling axis involves CXCR4 and its ligand CXCL12. Expression of CXCL12 by mesenchymal bone marrow-derived cells directs migration of metastatic breast cancer cells to bone. These cells constitutively secrete the chemokine stromal cell-derived factor-1 (SDF-1/CXCL12) and thereby attract CXCR4⁺ malignant cells. Activation of CXCR4 promotes tumor progression by enabling survival and growth programs in malignant cells in ectopic tissues, regulate survival and growth of neoplastic cells in a paracrine manner, and promote tumor angiogenesis by attracting endothelial cells ⁸⁴. This important axis has been implicated for metastasis of multiple solid tumors including pancreatic, hepatocellular, melanoma, lung and renal cancers ^{85–92}. Other functional chemokine receptors include CCR7, implicated in lymphatic metastasis, and CCR9 that is associated with metastasis to small intestine where its ligand is expressed ⁹³. Other chemokines receptors including CCR10, CXCR1, CXCR2, CXCR3, CXCR5 and CXCR7 expressed by malignant cells by a variety of solid tumors have also been implicated in organ-specific metastasis ¹¹.

Chemokine signaling is also an important feature of site-directed metastasis where neoplastic cell-secreted cytokines and chemokines signal to receptors expressed by various subtypes of myeloid cells, particularly significant with regards to colon carcinoma metastasis to liver ⁹⁴. Both murine and human colon cancer cells secrete the CC-chemokine ligands CCL9 and CCL15, and thereby induce recruitment of CD34⁺Gr1[−] immature myeloid cells that express the CCL9/15 receptor CCR1, activation of which directly induces MMP2 and MMP9 expression. Lack of the *Ccr1*, *Mmp2*, or *Mmp9* genes in myeloid cells suppresses disseminated tumor growth in the liver and significantly prolongs the survival of tumor-bearing mice ⁹⁴.

Autocrine signaling loops by malignant cells have also been implicated in organ-specific metastasis. Malignant cell-derived TGF β induces expression of the cytokine Angiopoietin-like 4 (ANGPTL4) in mammary carcinoma cells, which is critical for carcinoma dissemination and colonization in lungs. TGF β induces expression of Angptl4 through Smad signaling cascades in carcinoma cells just prior to their entry into circulation. This subsequently enhances their retention in lungs, but not in bone, by disruption of vascular endothelial cell-cell junctions which increases permeability of lung capillaries, and facilitates trans-endothelial passage of tumor cells ⁹⁵. These results indicate that a cytokine in the primary tumor microenvironment can induce expression of another cytokine in exiting tumor cells thus enabling those cells to disrupt lung capillary walls and seed pulmonary metastases.

Immune cell-support for colonization and organ-specific metastasis is mediated also by non-chemokine mechanisms: The two NF- κ B targets, S100A8 and S100A9 are inflammatory mediators with chemotactic activity expressed and secreted by neoplastic cells, as well as by tumor-associated myeloid cells, and are associated with metastasis and a poor outcome in a variety of human tumors ⁹⁶. Acting through the RAGE receptor (receptor for advanced glycation end-products), these induce migration of myeloid cells with T cell suppressive activity into mammary tumors in murine models of mammary and squamous carcinogenesis. Recruited suppressive myeloid cells facilitate tumor progression by inhibiting T and NK cell activation, and by polarizing immunity toward a tumor-promoting type 2 phenotype ^{97, 98}. Recently, S100A8/S100A9 were also implicated in site-specific colonization of melanoma to lungs: lack of the endogenous anti-inflammatory protein uteroglobin in mice leads to over expression of S100A8/S100A9. Over expression results in induction of MMP expression by neoplastic cells, and chemoattraction of melanoma cells according to the S100A8/S100A9 gradient, thus enhancing colonization of B16 melanoma in lungs ⁹⁹. Others studies implicate S100A8/S100A9 via secretion into pre-metastatic lungs by neoplastic cells, that in turn induce expression of serum amyloid A (SAA) 3, that acts through Toll-like receptor (TLR) 4 to recruit additional myeloid cells, thus creating an inflammatory environment that accelerates migration of primary tumor cells to lung parenchyma ¹⁰⁰.

Leukocyte support for tumor cell survival and colonization at the metastatic organ

The temporal gap between infiltration to distant organs and the ability to colonize and form macrometastases, a process sometimes requiring decades, depending on tumor type, suggests that in order to grow at the metastatic site, disseminated tumor cells must acquire an ability to “educate” their new microenvironment to support their own survival. Although changes in the metastatic microenvironment that enable growth of disseminated cells are poorly defined, emerging data indicate that immune-mediated signaling plays an important role. During the earliest stages of liver metastasis, microvascular arrest of neoplastic cells triggers a local inflammatory response: tumor-secreted vascular endothelial growth factor (VEGF) induces expression of pro-inflammatory cytokines by sinusoidal endothelial cells resulting in upregulation of adhesion molecules such as VCAM-1, allowing arrest of metastatic melanoma cells ¹⁰¹. Another prometastatic mechanism in liver supporting survival of CTCs is mediated by tumor-activated proinflammatory cytokine signaling by liver stellate cells, hepatocytes and myofibroblasts. These are recruited into sites of avascular micrometastases and create a microenvironment that supports metastatic growth through specific release of both proangiogenic factors and tumor cell invasion- and proliferation-stimulating factors provided by tumor-activated hepatocytes and myofibroblasts ¹⁰².

An intriguing mechanism by which tumor cells take advantage of immune pathways to increase their metastatic potential is the ectopic expression of Fc γ RIIB by metastatic melanoma cells. Fc γ RIIB is an inhibitory low-affinity receptor for IgG that terminates activation signals initiated by antigen cross-linking of the B cell receptor through its inhibitory ITIM motif ¹⁰³. 40% of human metastatic melanomas gain expression of Fc γ RIIB, in particular, in liver metastases (69%), suggesting that gain of expression supports their survival in liver by escaping humoral immunity ^{104, 105}. Experimental studies with B16 melanoma cells in immunocompetent mice indicate that tumor-expressed Fc γ RIIB operates as a decoy receptor inhibiting ADCC (Antibody-Dependent Cell Cytotoxicity) mediated by tissue macrophages, neutrophils and NK cells, that are abundant in liver: anti-tumor antibodies bind tumor cells via Fab domains while the Fc portion is “caught” by the tumor Fc γ RIIB and cannot be recognized by Fc γ R of the effector cells ¹⁰⁶. Thus, liver offers a prometastatic microenvironment that supports metastasis of cancer cells able to resist anti-

tumor hepatic defenses, and takes advantage of hepatic cell-derived factors that are key phenotypic properties of liver-metastasizing cancer cells.

The bone and bone marrow are also among the most frequent sites of cancer metastasis. During bone metastasis, breast carcinoma cells, through secretion of IL-6, IL-11, TNF α and parathyroid-hormone-related peptide (PTHrP) are able to activate osteoclasts through RANKL (receptor activator of nuclear factor- κ B ligand), critical for formation of osteolytic metastases¹⁰⁷. In other cancer types, such as neuroblastomas, IL-6 is secreted by bone marrow stromal cells and promotes osteolysis through the induction of RANKL in osteoblasts as well as in tumor cells¹⁰⁸. NF- κ B-regulated signaling in breast carcinoma cells promotes osteolytic bone metastasis by induction of osteoclastogenesis via GM-CSF¹⁰⁹. Late-stage breast cancers also metastasize to brain, where recruitment of glial cells and a brain inflammatory response correlates with tumor cell proliferation and growth in both experimental metastasis in mice and in human brain metastases¹¹⁰.

A pre-metastatic niche

Several studies suggest that primary tumors can “prepare” the distant target organ of metastasis by creating a pre-metastatic niche¹¹¹ whereas others studies indicate that a small number of metastatic cells activate their new microenvironment upon arrival¹¹². Regardless, neoplastic cells secrete factors that mobilize bone marrow-derived vascular endothelial growth factor receptor (VEGFR)1-expressing hematopoietic progenitor cells to sites of metastasis that induce expression of fibronectin by resident fibroblasts, thus creating favorable conditions for arrival of would-be metastatic cells¹¹³.

Gr1⁺CD11b⁺ myeloid cells have also been identified as playing a potential role in mediating changes that activate pre-metastatic lung into a permissive haven by diminishing immune protective programs¹¹⁴. Mammary tumor cells growing in mammary pads remotely activate expression of TARC/CCL17 and MDC/CCL22 in lungs. These chemokines acting through CCR4 attract both tumor and immune cells³⁶. Distant primary tumor-derived factors induce the expression of the inflammatory chemoattractants S100A8 and S100A9, which in turn attract Mac1⁺ myeloid cells to premetastatic lungs mediated by Toll-like receptor 4 (TLR-4) expressing cells that accelerate migration of primary tumor cells to lung tissues^{100, 115}. Lysyl oxidase (LOX) is a tumor-cell derived factor often induced in primary tumors in response to hypoxia¹¹⁶. However, systemic secretion of LOX leads to its accumulation in the lung, where it has been found to act on extracellular matrix proteins establishing a permissive niche for infiltrating cancer cells by crosslinking collagen IV in basement membranes, and by recruiting CD11b⁺ myeloid cells that adhere to crosslinked collagen IV, produce MMP2, and thereby enhance the invasion and recruitment of BMDCs and metastasizing tumor cells¹¹⁷. These data indicate that, through multiple mechanisms, creation of a pro-inflammatory microenvironment in metastatic organs, whether that be prior to or at the time of malignant cell arrival, enhances the survival and proliferative possibilities for metastatic cells.

Implications for therapy and perspectives

Elucidating the changes in metastatic microenvironments is a significant clinical goal for eradicating cancer-associated death. Immune-based signaling pathways have emerged as central players in facilitating growth of micrometastases into clinically relevant macrometastases. Anti-cancer therapies that target these programs are gaining attention and in a few cases are being evaluated in clinical trials¹¹⁸. Denosumab, an anti-RANKL antibody, originally developed for treatment of osteoporosis, and has been found effective for inhibiting bone metastasis in prostate cancer¹¹⁹. Disruption of tumor cell-adhesion to

protective stroma by targeting the CXCR4-CXCL12 axis using a small molecule CXCR4 antagonists, such as Plerixafor (AMD3100) is a novel, attractive therapeutic approach being explored in ongoing clinical trials for metastatic multiple myeloma, leukemia and other types of cancer^{120, 121}

Several therapeutic agents that limit IL-1 activity are approved for treating chronic inflammatory diseases, e.g., recombinant IL-1Ra (anakinra), neutralizing monoclonal antibodies to IL-1 β and a soluble receptor to IL-1, that have also been found to exert benefits in animal models of metastasis and tumor-associated angiogenesis. A goal for the future would be to evaluate this activity in clinical trials of IL-1 blockade¹²². Despite their critical involvement in invasion and metastasis, there has been conflicting results with anti-proteases, possibly due to of anti-tumorigenic activity of some enzymes¹²³.

As cancer research and clinical oncology progress increasingly towards a new era of integrative cancer therapy based on combinatorial drug regimens that act synergistically by targeting intrinsic pathways in neoplastic cells, as well as extrinsic pro-oncogenic pathways in the tumor microenvironment, the intensive research in deciphering the role of the metastatic microenvironment and of tumor-promoting inflammation will hopefully result in the coming years in innovative therapeutic strategies.

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Figure. Immune signaling in tumor microenvironments facilitates all stages of tumorigenesis Soluble mediators secreted by infiltrating and resident leukocytes and by carcinoma-associated fibroblasts (CAFs) within primary tumor sites support signaling programs within neoplastic cells that enable motile and invasive growth. Survival of circulating tumor cells (CTCs) in peripheral blood is facilitated by platelets, neutrophils and production of selectins and chemokine receptors. Organ-specific metastasis is directed by differential expression of chemokines and their receptors that together promote extravasation and retention of CTCs in distal organs. Colonization of distal organs is accomplished by mobilization of leukocytes and other stromal cells in ectopic organs, such as activation of osteoclasts in bone, and recruitment of glial cells in brain.