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Leukocytes as paracrine regulators of metastasis and determinants of organ-specific colonization

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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.

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. As, 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, although 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, *i.e.*, 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, epidemiological studies reporting that nonsteroidal anti-inflammatory drugs 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 cells.[10] 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.

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Leukocytes Implicated in Mediating Solid Tumor Metastasis

It has been 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 (DeNardo *et al.*, manuscript submitted),[12–15] regulated in part by colony-stimulating factor (CSF)-1, a key cytokine involved in macrophage maturation, tissue recruitment and activation mediated by the CSF-1 receptor (CSF-1R/cFMS).[16] A second CSF-1R ligand, interleukin (IL)-34, possesses similar binding affinities and also regulates TAM recruitment to tissues, but exhibits distinct tissue

distribution.[17–19] TAM presence in several types of human cancer correlates with increased vascular density and worse clinical outcomes.[20–25] 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 by activation of EGF-regulated signaling in mammary epithelial cells critical for invasive tumor growth and metastatic dissemination.[14] In mouse models of mammary carcinogenesis, TAMs activated by IL-4 and CSF-1 have been identified as essential determinants of pulmonary metastasis because of the prometastatic mediators they secrete.[26–29] 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 models.[30]

T lymphocytes were classically studied in the context of their tumor surveillance and antitumor capabilities. However, recent investigations have revealed that $CD4^+$ T cells and T-regulatory cells instead promote pulmonary metastasis in part by regulating protumor *versus* antitumor bioactivity of innate leukocytes. We reported that IL-4-expressing $CD4^+$ T cells promote invasion and metastasis of mammary adenocarcinomas by directly regulating TAM phenotype, bioeffector function and EGF expression, which in turn regulates invasion, presence of circulating tumor cells (CTCs) and pulmonary metastasis.[28] Other mediators found to be significant with regards to T-cell enhancement of pulmonary metastasis mammary carcinomas are S100A4 and receptor activator of nuclear factor-B ligand (RANKL).[31,32] S100A4 protein mediates T cell attraction to developing neoplasms and premetastatic lungs of tumor-bearing mice, and in turn, it stimulates T-cell production of cytokines, particularly granulocyte CSF and eotaxin-2.[32] 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 motility.[31] During prostate carcinogenesis, T lymphocyte and macrophage-derived RANKL induces metastasis through activation and nuclear localization of inhibitor of nuclear factor kappa-B kinase subunit alpha leading to repression of *maspin*, a critical suppressor of metastasis.[33,34] Lung metastasis of mammary carcinomas is also regulated by $CCR4^+$ T regulatory cells that can directly kill natural killer (NK) cells.[35]

Other myeloid cell types implicated in regulating metastasis include neutrophils, mast cells and monocytes harboring T-cell suppressive activity[36–39] that are potent suppressors of antitumor adaptive immunity and directly facilitate metastasis by regulating angiogenic programs via enhanced metalloproteinase (MMP) activity.[11,40–42] Cancer-associated fibroblasts (CAFs) are implicated in regulating the activities of these myeloid cell types through their secretion of proinflammatory chemokines that recruit immune cells to sites of developing neoplasms.[32,43,44]

Proinflammatory Signals that Impact Exit from Primary Tumor Sites

Regulators of the invasive phenotype

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).[45,46] 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 Zeb.[47]

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 potential 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-κB transcription factor in neoplastic (and other) cells, directly leading to expression of Snail1 and Zeb.[44,48,49] Other leukocyte-derived cytokines (including IL-6 and IL-23) induce activation of intracellular STAT3 that in turn leads to induced expression of Twist.[50–52]

Local hypoxia in neoplastic tissue also contributes to induction of motility programs[53,54] in part by activation of transcription factors hypoxia inducible factor-1α and NF-κB, both implicated in EMT via transcription of Snail.[48] 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).[55,56] Thus, hypoxic conditions select for a more metastatic phenotype partially through activation of proinflammatory 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 MMPs, cysteine cathepsins and serine proteases.[4] 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.[57,58] Although 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,[40,59–62] *e.g.*, fibroblasts[63] or tumor-associated immune cells. Mast cells, neutrophils and macrophages secrete matrix remodeling proteases[64–66] implicated in prometastatic activity, as well as serine proteases that are associated with higher tumor grades and lymph node metastasis in breast cancer.[37]

In particular, murine macrophages are known to express elevated levels of the cysteine protease cathepsin B following exposure to IL-4.[67] 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 cells.[4] Secretion of proteases by cells within the tumor microenvironment may not only foster metastatic activity and motility of neoplastic cells through matrix and into vasculature but also enhance and/or regulate the presence and activity of leukocytes: overexpression 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 site.[68]

Protease secretion by TAMs is in part regulated by IL-6 emanating from neoplastic cells. Tumor cell-derived IL-6 induces secretion and activation of the cysteine protease cathepsin B and secretion of matrix MMPs by monocytes.[69] Similarly, neoplastic cell and T-cell-derived IL-4 induces cathepsin expression and activity in TAMs in several cancer types.[29] TAMs in turn regulate neoplastic cell motility by secreting factors such as migration-stimulating factor (MSF).[70] MSF is an oncofetal isoform of FN and is induced in TAMs by macrophage-CSF, IL-4 and transforming growth factor beta (TGF-β). 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 TNF-related apoptosis-inducing ligand from the cell surface. Tumor growth is subsequently enhanced by this cascade, and survival in tumor-bearing mice is impaired.[71]

Leukocytes and Survival of CTCs

Before productive metastatic colonization is possible, 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 CTCs.[72] It has been estimated that only 0.01% of CTCs survive and eventually extravasate at distal locales.[4] 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 lysis[72] by both thrombin-dependent and -independent mechanisms.[73]

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). Platelets[73] and neutrophils facilitate these interactions via their production of matrix attachment molecules such as beta(2)-integrin/intracellular adhesion molecule-1 (Refs. 74 and 75) and selectins.[76] 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 on CTC anoikis by selective regulation of proapoptotic Bmf and antiapoptotic 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.[76,77]

Site-specific colonization

Organ-specific migration. The 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 cells,[2] while also recognizing that altered expression of important genes can also regulate tropism. Cyclooxygenase-2 (also known as PTGS2), the EGF receptor (EGFR) ligand heparin-bound EGF and the α-2,6-sialyltransferase (ST6GALNAC5) all act as mediators of malignant cell passage through the blood–brain barrier when breast cancers metastasize to brain.[78,79] In contrast, when breast cancer metastasizes to bone, IL-11 and connective tissue growth factor regulated by TGF-β are important.[80]

However, a growing body of literature has also 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.[81,82] Many malignant cells upregulate expression of chemokine 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 chemokine gradients and then in part regulate migration toward specific organs.[70] One such functional chemokine-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 SDF-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, regulates survival and growth of neoplastic cells in a paracrine manner and promotes tumor angiogenesis by attracting endothelial cells.[83] This important axis has been implicated for metastasis of multiple solid tumors including

pancreatic, hepatocellular, melanoma, lung and renal cancers.[84–91] 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.[92] Other chemokine 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.[93] 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 MMP-2 and MMP-9 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.[93]

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 transendothelial passage of tumor cells.[94] 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 also mediated by nonchemokine mechanisms: The two NF-κB 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.[95] S100A8 and S100A9 act through the RAGE receptor (receptor for advanced glycation end products). They induce migration of myeloid cells with T-cell suppressive activity into tumors, in murine models of mammary and squamous carcinogenesis. Recruited suppressive myeloid cells facilitate tumor progression by inhibiting T cell and NK cell activation and by polarizing immunity toward a tumor-promoting type 2 phenotype.[96,97] 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 overexpression of S100A8/S100A9. Overexpression 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.[98] Another mechanism by which tumor cell-secreted S100A8/S100A9 facilitate metastasis is through serum amyloid A-3 (SAA). Secretion of S100A8/S100A9 into premetastatic lungs induces local expression of SAA, 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.[99]

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 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 proinflammatory cytokines by sinusoidal endothelial cells resulting in upregulation of adhesion molecules such as VCAM-1, allowing arrest of metastatic melanoma cells.[100] 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.[101]

An intriguing mechanism by which tumor cells take advantage of immune pathways to increase their metastatic potential is the ectopic expression of FcγRIIB by metastatic melanoma cells. FcγRIIB is an inhibitory low-affinity receptor for IgG that terminates activation signals initiated by antigen crosslinking of the B-cell receptor through its inhibitory immunoreceptor tyrosine-based inhibiting motif.[102] Forty percent of human metastatic melanomas gain expression of FcγRIIB, in particular, in liver metastases (69%), suggesting that gain of expression supports their survival in liver by escaping humoral immunity.[103,104] Experimental studies with B16 melanoma cells in immunocompetent mice indicate that tumor-expressed FcγRIIB operates as a decoy receptor inhibiting antibody-dependent cell cytotoxicity mediated by tissue macrophages, neutrophils and NK cells, which are abundant in liver: antitumor antibodies bind tumor cells via Fab domains, whereas the Fc portion is "caught" by the tumor FcγRIIB and cannot be recognized by FcγR of the effector

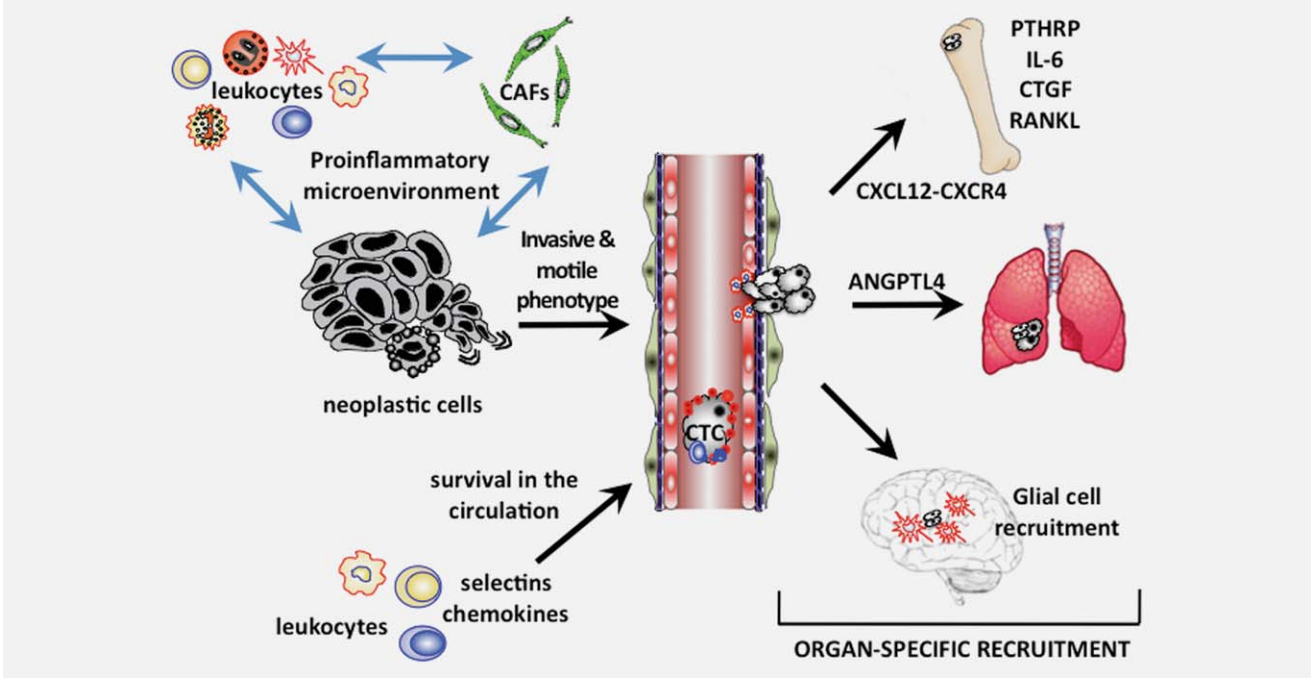

Figure 1. Immune signaling in tumor microenvironments facilitates all stages of tumorigenesis. Soluble mediators secreted by infiltrating and resident leukocytes and by carcinoma-associated fibroblasts (CAFs) within primary tumor sites support signaling programs within neoplastic cells that enable motile 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. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

cells.[105] Thus, liver offers a prometastatic microenvironment that supports metastasis of cancer cells able to resist antitumor 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, are able to activate osteoclasts through RANKL, which are critical for the formation of osteolytic metastases.[106] 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 cells.[107] NF-κB-regulated signaling in breast carcinoma cells promotes osteolytic bone metastasis by induction of osteoclastogenesis via granulocyte macrophage colony-stimulating factor.[108] 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 metastases (Figure 1).[109]

A Premetastatic Niche

Several studies suggest that primary tumors can "prepare" the distant target organ of metastasis by creating a premetastatic niche,[110] whereas other studies indicate that a small number of metastatic cells activate their new microenvironment on arrival.[111] Regardless, neoplastic cells secrete factors that mobilize bone marrow-derived VEGF receptor (VEGFR)-1-expressing hematopoietic progenitor cells to sites of metastasis that induce expression of FN by resident fibroblasts, thus creating favorable conditions for arrival of would-be metastatic cells.[112]

$Gr1^+CD11b^+$ myeloid cells have also been identified to play a potential role in mediating changes that activate premetastatic lung into a permissive haven by diminishing immune-protective programs.[113] 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 cells.[35] 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 TLR-4-expressing cells that accelerate migration of primary tumor cells to lung tissues.[99,114] Lysyl oxidase (*LOX*) is a tumor cell-derived factor often induced in primary tumors in response to hypoxia.[115] However, systemic secretion of LOX leads to its accumulation in the lung, where it has been found to act on ECM 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 MMP-2 and thereby enhance the invasion and recruitment of BMDCs and metastasizing tumor cells.[116] These data indicate that, through multiple mechanisms, creation of a proinflammatory 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

Elucidation of 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. Anticancer therapies that target these programs are gaining attention and in a few cases are being evaluated in clinical trials.[117] Denosumab, an anti-RANKL antibody, originally developed for treatment of osteoporosis, has been found effective for inhibiting bone metastasis in prostate cancer.[118] Disruption of tumor cell adhesion to protective stroma by targeting the CXCR4-CXCL12 axis using a small molecule of CXCR4 antagonist, 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.[119,120]

Several therapeutic agents that limit IL-1 activity are approved for treating chronic inflammatory diseases, *e.g.*, recombinant IL-1Rα (anakinra), and neutralizing monoclonal antibodies to IL-1β and a soluble receptor to IL-1, which 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 blockade.[121] Despite their critical involvement in invasion and metastasis, there has been conflicting results with antiproteases, possibly due to antitumorigenic activity of some enzymes.[122]

As cancer research and clinical oncology progress increasingly toward 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 innovative therapeutic strategies in the future.

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