Determinants of Organotropic Metastasis

Heath A. Smith and Yibin Kang

Department of Molecular Biology, Princeton University, Princeton, New Jersey 08544; email: ykang@princeton.edu

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Abstract

The spread of cancer from a primary tumor to distant organ sites is the most devastating aspect of malignancy. Dissemination to specific organs depends upon blood flow patterns and characteristics of the distant organ environment, such as the vascular architecture, stromal cell content, and the biochemical milieu of growth factors, signaling molecules, and metabolic substrates, which can be permissive or antagonistic to metastatic colonization. Metastatic tumor cells possess intrinsic cellular properties selected for adaptation to specific organ environments, where they co-opt growth and survival signals, undergo metabolic reprogramming, and subvert resident stromal cell activities to promote extravasation, immune evasion, angiogenesis, and overt metastatic growth. Recent work and new experimental models of metastatic organotropism are uncovering crucial details of how malignant cells metastasize to specific tissues, revealing key mediators that prepare metastatic niches in specific organs and identifying new targets that offer attractive options for therapeutic intervention.

INTRODUCTION

The clinical detection of metastasis signals a dire prospect for the long-term survival of cancer patients and often alters the focus of cancer treatment, from achieving a cure to providing better palliative care (Massague & Obenauf 2016, Wan et al. 2013). Although the development of metastasis appears to be the final step of cancer progression, it is, in fact, the culmination of a series of events that can begin early in the tumorigenesis process. Cellular changes favorable to metastatic spread and survival in harsh new environments are enriched in clonal populations of tumor cells, both prior to and after dissemination (Celià-Terrassa & Kang 2016). Nevertheless, even the most aggressively growing primary tumor cells have a very low rate of success (estimated to be less than 0.01%) in seeding metastasis in distant organs (Fidler 1970). Anoikis, immune attack, metabolic stress, and the lack of prosurvival and growth signals in new organ environments constitute major bottlenecks between dissemination and overt metastatic outgrowth.

A predictable pattern of organ-specific metastasis, which often depends upon the histological origin of the cancer, is consistently observed in both the clinic and animal models, as distinct cancer types are predisposed to metastasize to specific secondary organs (Obenauf & Massague 2015, Valastyan & Weinberg 2011, Wan et al. 2013). For example, prostate cancer almost always develops bone metastasis first, and uveal melanoma and colorectal cancer often generate liver metastasis. In fact, the peculiar organ distribution pattern of metastases from different cancer types was the inspiration for Stephen Paget's [1989 (1889)] visionary "seed and soil" hypothesis of metastasis. Contributing significantly to organotropic metastasis are the blood flow patterns and features of the vasculature that dictate specific organ accessibility, genetic and epigenetic traits intrinsic to the tumor cells that define metastatic propensity, as well as secreted factors produced by the primary tumor and the stromal cells in specific organ environments that establish supportive metastatic niches for disseminated tumor cells (DTCs). In this review, we focus on these complicated factors governing the unique patterns of metastatic organotropism and the implications for therapeutic intervention.

BLOOD FLOW PATTERNS AND VASCULAR ARCHITECTURE

Circulating tumor cells (CTCs) typically follow distinct blood flow patterns to disseminate from the primary organ to distant tissues, where the tumor cells become spatially entrapped in capillary vessels or attach to adhesion molecules on the endothelium. The mechanical or biochemical entrapment of tumor cells may be enhanced by the dissemination of clusters of tumor cells (Aceto et al. 2014). The venous blood that circulates through most organs drains back to the lungs, which may partly explain why the lungs are such a common site of metastasis. Likewise, blood from the colon and gastrointestinal tract drains first into the liver, which is frequently the initial site of colorectal cancer metastasis (Deneve et al. 2013). However, blood flow pattern and capillary entrapment alone cannot sufficiently explain all of the clinical observations of metastatic organotropism. For example, the liver, kidneys, and brain each receive approximately an equal volume of blood flow, yet the liver has a much higher metastatic seeding potential, the brain has intermediate potential, and the spleen is a relatively rare site of metastasis (Budczies et al. 2015).

The architecture of blood vessel walls and endothelial cell morphology also differ among organs and can influence the ease and likelihood of extravasation and colonization (**Figure 1**). Sinusoid vessels in the liver and the bone marrow are fenestrated, or lined, with a discontinuous layer of endothelial cells that provides greater permeability than blood vessels in the lung, which have more tight junctions between endothelial cells and a basement membrane, making transendothelial migration (TEM) difficult (Aird 2007). Breast cancer and melanoma cells can enhance lung extravasation by breaking these endothelial junctions through the activity of SPARC, ANGPTL4,

and cANGPTL4, which can increase the leakiness of the vessel walls (Huang et al. 2011, Padua et al. 2008, Tichet et al. 2015). Other factors overexpressed by metastatic cells to mediate lung extravasation include epiregulin, COX2 (cyclooxygenase-2), MMP1 (matrix metalloprotease-1), and MMP2, which promote degradation and invasion through the extracellular matrix (ECM) and vascular network (Gupta et al. 2007).

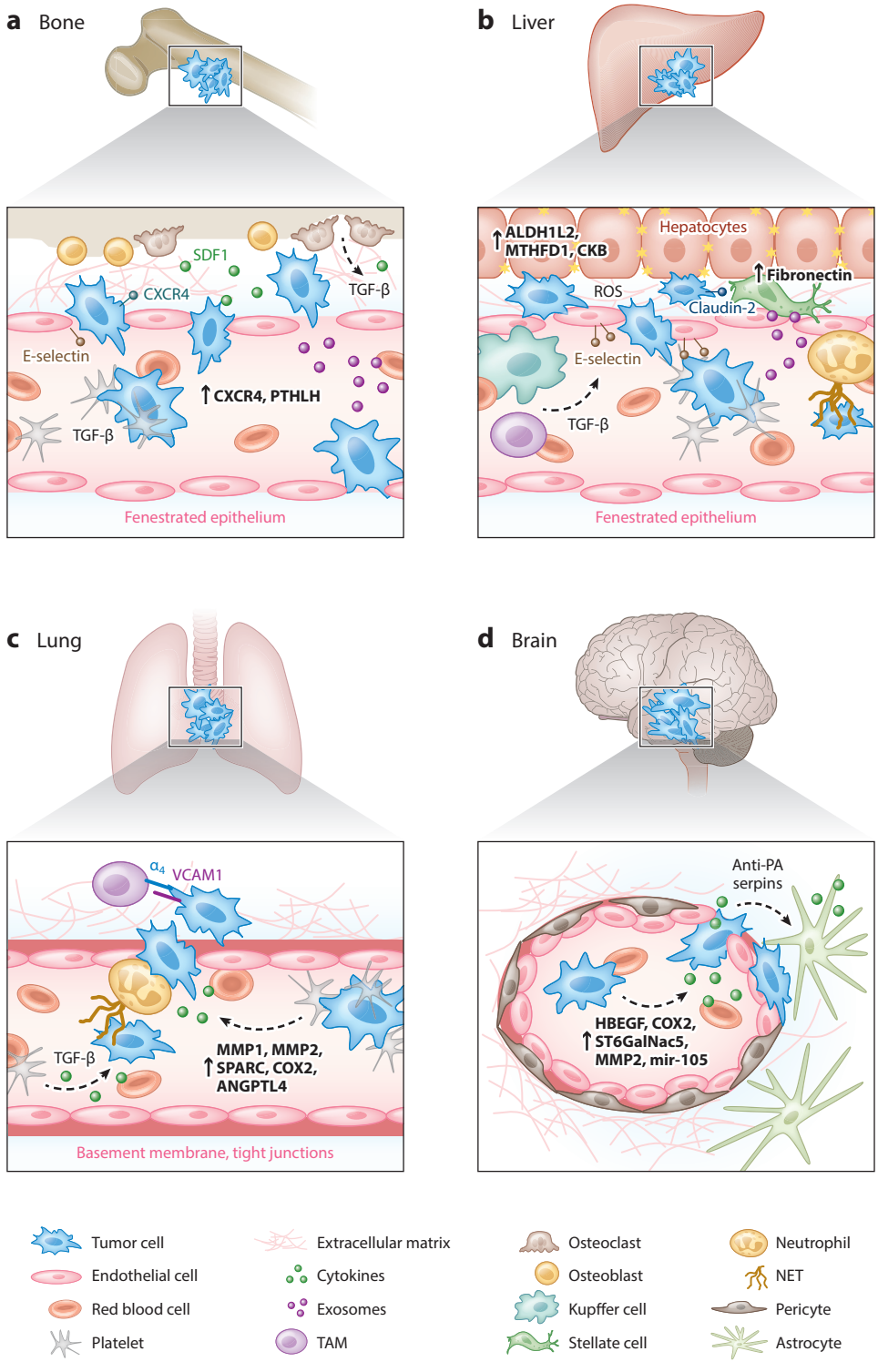
The blood–brain barrier (BBB) represents the toughest organ-specific deterrent to vascular extravasation and a unique challenge for metastatic colonization. Astrocytes and pericytes form a protective network around capillaries to reinforce vessel walls (Aird 2007). However, tumor cells can cross this barrier by upregulating the expression level of COX2, HBEGF, MMP2, miR-105, and ST6GalNac5, which increase vascular permeability in the brain (Bos et al. 2009, Sevenich & Joyce 2014, Zhou et al. 2014). Recently, Heregulin binding of HER3–HER2 dimers on breast cancer cells was shown to promote metalloproteinase-dependent TEM in vitro, and thus suggested another possible mechanism for traversing the BBB in vivo in which Heregulin expression in the brain stroma is prevalent (Momeny et al. 2015).

The ECM content and the presence of adhesion molecules on endothelial cells also differ by organ environment and may contribute to metastatic tropism. For example, E-selectin expression on endothelial cells in the bone marrow has been shown to contribute to CTC adhesion and metastasis to the bone (Barthel et al. 2013), and induced E-selectin expression in the liver by inflammatory cytokines, such as TNF-α (tumor necrosis factor-α) secretion by tumor-recruited macrophages and Kupffer cells, enhances adhesion and liver metastasis (Auguste et al. 2007, Eichbaum et al. 2011). Different repertoires of adhesion molecules may be expressed in distinct organs to promote metastatic seeding, such as N-cadherin (Qi et al. 2005) and ICAM1 (intracellular adhesion molecule 1) (Rahn et al. 2005), which enhance TEM, although the contribution of various adhesion molecules to colonization in specific tissues is still unclear, as are the receptors to these ligands on CTCs, which may differ between cancer types.

GENETIC ADAPTION OF TUMOR CELLS DURING ORGAN-SPECIFIC COLONIZATION

Once embedded in a capillary, CTCs may undergo apoptosis, extravasate into the organ parenchyma, or proliferate to form an embolus that can rupture the vessel wall (Al-Mehdi et al. 2000). However, in animal models, the majority of DTCs that reach distant organs die within days (Fidler 1970, Luzzi et al. 1998, Minn et al. 2005b). These findings suggest that only a small portion of malignant cells have attributes conferring metastatic colonization potential. However, no mutations unique to metastatic cells have been identified in recent genomic sequencing studies (Bozic et al. 2010, Campbell et al. 2010). Instead, clonal enrichment for preexisting mutations present in primary tumors, such as RAS^{G13D} and $BRAF^{G464V}$, was found to be upregulated in metastasis models and may explain the increased fitness and survival of metastatic cells in specific organs (Jacob et al. 2015).

Using the MDA-MB-231 model of breast cancer metastasis, specific gene signatures have been identified in isogenic subpopulations of tumor cells with an enhanced ability to colonize different organs, such as bone, lung, and brain (Bos et al. 2009, Kang et al. 2003, Minn et al. 2005a, Nguyen & Massague 2007). Similar approaches were used to identify other organotropic metastatic gene signatures (Loo et al. 2015, Piskounova et al. 2015, Tabaries et al. 2012). Genes with these signatures promote DTC survival in the vasculature, capillary adhesion, extravasation, migration, angiogenesis, and the mobilization of stromal components that can promote organ-specific colonization. The different gene signatures enriched in metastatic cells correlate with the colonization challenges inherent to specific environments. For example, successful metastatic colonization


a Bone
SDF1
CXCR4
TGF-β
E-selectin
↑CXCR4, PTHLH
TGF-β
Fenestrated epithelium
b Liver
↑ALDH1L2, MTHFD1, CKB
Hepatocytes
↑Fibronectin
ROS
Claudin-2
E-selectin
TGF-β
Fenestrated epithelium
c Lung
α4 VCAM1
TGF-β
MMP1, MMP2, ↑SPARC, COX2, ANGPTL4
Basement membrane, tight junctions
d Brain
Anti-PA serpins
HBEGF, COX2, ↑ST6GalNac5, MMP2, mir-105
Tumor cell
Endothelial cell
Red blood cell
Platelet
Extracellular matrix
Cytokines
Exosomes
TAM
Osteoclast
Osteoblast
Kupffer cell
Stellate cell
Neutrophil
NET
Pericyte
Astrocyte

of the bone is mediated by proteins [such as IL11 (interleukin-11), OPN, CTGF, and FGF5] that facilitate the cross talk with stromal components of the bone microenvironment to promote osteolytic bone colonization (Kang et al. 2003), and genes enriched in signatures for lung and brain metastasis promote extravasation or crossing of the BBB (Bos et al. 2009, Minn et al. 2005a). In the liver, where metabolic stress is a dominant factor, the enrichment of genes involved in oxidative stress and metabolism, such as *ALDH1L2*, *MTHFD1*, and *CKB*, is observed (Loo et al. 2015, Piskounova et al. 2015). Interestingly, some of these organotropic gene expression signatures are shared among tumors of different histological origin. For example, similar to lung-tropic breast cancer cells, the upregulation of *SPARC*, *VCAM1*, and *ANGPTL4* expression in metastatic hepatocellular carcinoma cells was also observed in lung metastasis (Wan et al. 2015), suggesting a similar genetic program directing metastatic seeding to specific organs regardless of tumor origin.

METABOLIC ADAPTATION IN NEW ORGAN ENVIRONMENTS

Tumor cells arriving in new environments frequently encounter stressful metabolic conditions, such as elevated levels of reactive oxygen species (Pani et al. 2010). Upregulation of redox signaling in some cancer cells promotes metastatic survival in specific organ environments by attenuating the cytoxic effects of stressful metabolites. The increased expression of detoxifying enzymes, such as

Figure 1

Vascular architecture conferring organ-specific barriers to extravasation and metastatic seeding. Major obstacles to metastatic colonization in the (*a*) bones, (*b*) liver, (*c*) lung, and (*d*) brain are outlined, with the relative difficulty for metastatic seeding in each organ increasing from panels *a* to *d*. Shown are clinically relevant organs that are frequent sites of metastases, with inset images detailing major morphological and physiological features of the vasculature in each organ. Blood vessels in the bone and liver are composed of a fenestrated epithelium lacking tight junctions between endothelial cells, making colonization relatively easy; however, the lung and brain have tight endothelial junctions and a basement membrane, making these tissues more impervious to extravasation. In addition, astrocytes and pericytes extend podia that wrap around blood vessels in the brain, contributing to the formation of the BBB, which confers an additional obstacle to metastasis. Immune surveillance significantly inhibits efficient metastatic colonization in each organ, and in the liver, metastatic cells must adapt molecular mechanisms to tolerate higher levels of toxic metabolites. Genes and tumor-secreted factors responsible for organ-specific extravasation and survival are shown. In the bone, the SDF1–CXCR4 chemokine axis has a significant role in metastatic homing to the bone, and exosomes and inflammatory cytokines released by tumor cells have been shown to prepare a metastatic niche. In the liver, high levels of ROS and other toxic metabolites inhibit metastatic colonization, and primary tumor–derived exosomes induce stellate cell upregulation of fibronectin, which enhances the adhesion of CTCs. Likewise, Claudin-2 expression on tumor cells acts as an adhesion molecule for attachment to stellate cells in the liver. E-selectin expression in the liver by inflammatory cytokines, such as TNF-α secretion by tumor-recruited macrophages and Kupffer cells, enhances adhesion and liver metastasis. In the liver and lungs, neutrophils extend biomolecular nets [neutrophil extracellular traps (NETs)] that ensnare CTCs to promote seeding. Moreover, tumor cell secretion of MMPs and other factors promotes ECM degradation and extravasation into the parenchyma. Interactions with TAMs and platelets also protect CTCs in the circulation and at the sites of extravasation in the lungs. In the brain, a number of factors are upregulated to cross the BBB, including proteases that degrade endothelial junctions and the ECM, as well as factors that inhibit astrocyte-derived plasminogen activator. The key below the panels identifies the various cells and cell products depicted in the diagram. Abbreviations: BBB, blood–brain barrier; COX2, cyclooxygenase-2; CTC, circulating tumor cell; CXCR4, C-X-C chemokine receptor type-4; ECM, extracellular matrix; MMP, matrix metalloprotease; NET, neutrophil extracellular trap; PTHLH, parathyroid hormone-like hormone; RBC, red blood cell; ROS, reactive oxygen species; SDF1, stromal cell–derived factor-1; TAM, tumor-associated macrophage; TGF-β, transforming growth factor-β; TNF-α, tumor necrosis factor-α; VCAM, vascular adhesion molecule.

ALDH1L2 and MTHFD1 in the folate pathway, has been shown to increase melanoma metastasis to the liver, pancreas, and lung, favoring the survival of melanoma cells in these tissues (Piskounova et al. 2015). Many other types of cancer cells may upregulate or express certain isoforms of ALDH to adapt to toxic environments (Rodriguez-Torres & Allan 2016).

In addition to avoiding death in the new environments of a distant organ, metastatic cells must procure new sources of energy to meet the metabolic demands for survival and growth. The unique cocktail of nutrients and growth factors present, or sometimes absent, in different tissues will strongly dictate the likelihood of metastasis to specific organs. Most primary tumors depend on aerobic glycolysis for energy production, and DTCs have to adopt new means of energy acquisition when they colonize foreign organs, such as mitochondrial oxidative phosphorylation, peroxide signaling, autophagy, and fatty acid metabolism (LeBleu et al. 2014, Liang et al. 2007, Nieman et al. 2011, Weber 2016). Normal glucose uptake is disrupted when tumor cells detach from the ECM and enter circulation. This is compensated for by the activation of metabolic pathways involved in oxidative phosphorylation and peroxide signaling (Weber 2016). For example, invasive cancer cells increase PGC1-α transcriptional activation to drive mitochondrial oxygen consumption to colonize the lung (LeBleu et al. 2014). Tumor cells may also enter a dormant state in foreign environments in which autophagy pathways are activated to meet the metabolic requirements for quiescence (Liang et al. 2007). Metastatic breast cancer cells can exit dormancy and reinitiate their growth potential in the brain by adapting mechanisms to scavenge energy from various substrates and pathways, including glycolysis, the tricarboxylic acid cycle, and oxidative phosphorylation (Chen et al. 2007, Sansone et al. 2016).

In general, the brain and lungs provide high levels of glucose and oxygen (DeBerardinis et al. 2008), but the liver has low oxygen and glucose levels. Thus, tumor cells coming from primary tumors adapted to aerobic glycolysis will find it easier to colonize the brain or lungs, and tumor cells homing to the liver must engage in glycolysis for their survival. The activation of PDK1-mediated glycolysis through HIF1-α activation is a mechanism by which breast cancer cells efficiently colonize the liver (Dupuy et al. 2015). Likewise, colon cancer cells that metastasize to the liver have been shown to scavenge energy by downregulating miR-483 and miR-551, which increases CKB (creatine kinase) expression and leads to extracellular production of phosphocreatine from creatine and ATP (adenosine triphosphate) (Loo et al. 2015). Phosphocreatine is then taken up by the tumor cells for metabolic energy requirements. Alternatively, ovarian cancer cells can metabolize fatty acids secreted from adipocytes by uptake of FABP4 as they metastasize into abdominal fat tissues (Nieman et al. 2011). Thus, metastatic adaptation to specific metabolic environments has a significant role in organotropism.

SECRETED FACTORS, INFLAMMATION, AND ORGAN-SPECIFIC NICHES

The development of metastasis in various organs also depends upon systemic changes to normal physiological states that make certain tissues more conducive to metastatic seeding. These changes are often mediated by secreted factors, such as cytokines, growth factors, and extracellular vesicles (Peinado et al. 2011, Quail & Joyce 2013). One of the first studies to demonstrate the systemic organotropic effects of tumor-secreted factors revealed that preconditioned media from B16 melanoma cells could redirect lung cancer cells to metastatic sites typical of melanoma, such as the spleen, intestine, kidney, and oviduct, where lung cancer cells rarely metastasize (Kaplan et al. 2005). This finding demonstrated that primary tumor factors contribute to metastatic tropism, perhaps by preparing a premetastatic niche in specific organs. Indeed, tumor-secreted VEGFA and PlGF have been shown to directly mobilize cells from the bone marrow (BM), including

hematopoietic progenitor cells and immature myeloid cells (Deng et al. 2012; Hiratsuka et al. 2006, 2008; Kaplan et al. 2005) that home to specific organ sites to establish metastatic niches. By reorganizing the vasculature and ECM components in the tumor microenvironment (TME), these immature myeloid cells prepare a receptive soil for metastatic colonization. Secreted cytokines and exosomes from primary tumors have often been found to alter the expression of adhesion molecules, such as fibronectin, in distant organs to promote metastatic tropism; fibronectin can help recruit VLA-4-expressing BM-derived cells (Kaplan et al. 2005, Webber et al. 2010).

Exosomes, which are small (0.1–1 μm) vesicles containing proteins and nucleic acids, are released from both cancer and normal host cells, and also significantly alter the biological activity of recipient cells and promote metastatic colonization of specific organs, particularly the lung, bone, liver, and brain (Hoshino et al. 2015, Lakkaraju & Rodriguez-Boulan 2008, van Niel et al. 2006). These vesicles can directly contribute to tumor niche formation, and profoundly regulate immune responses, hematopoietic mobilization, and TME remodeling (Janowska-Wieczorek et al. 2001, 2005; Liu et al. 2010; Xiang et al. 2009). Recent work has shown that exosomes released by tumor cells with specific metastatic organotropism preferentially fuse with stromal cells at the destination of metastasis, including fibroblasts, Kupffer cells, and endothelial cells (Hoshino et al. 2015). Protein expression analyses of these exosomes revealed that specific integrin cargo was responsible for distinct organotropic effects. Specifically, $\alpha_6\beta_4$ and $\alpha_6\beta_1$ expression in exosomes was associated with lung metastasis, and $\alpha_v\beta_5$ was shown to promote liver metastasis.

Exosomes from primary tumors can also deliver c-MET and other factors to the BM, activating and mobilizing stromal cells that alter metastatic niches (Peinado et al. 2012). Exosomes can alter the premetastatic environment by delivering TGF-β (transforming growth factor-β) and other biomolecules to fibroblasts and turning them into metastasis-promoting myofibroblasts (Valenti et al. 2006, Webber et al. 2010). During lung metastasis, exosomes derived from primary renal carcinoma stimulate angiogenesis in the lungs (Grange et al. 2011). Likewise, in the liver, exosomes released from pancreatic carcinoma can stimulate TGF-β secretion and fibronectin expression on hepatic stellate cells, and macrophage recruitment, to enhance liver metastasis (Costa-Silva et al. 2015).

An important component of metastatic niche conversion is primary tumor hypoxia and systemic inflammation (Mantovani et al. 2008). Chronic inflammation has been shown to be a key factor promoting tumor metastasis (Balkwill & Mantovani 2001, de Visser et al. 2006, Grivennikov et al. 2010), and hypoxic tumors release many inflammatory factors that establish metastatic niches in the lung and bone. Hypoxic breast tumors mobilize $CD11b^+$ myeloid cells from the BM that accumulate in the lung and enhance metastasis by directly inhibiting antitumor natural killer (NK) cell activity (Sceneay et al. 2012). Hypoxia also triggers the release of lysyl oxidase (LOX), which accumulates in lung tissues to form a premetastatic niche (Erler et al. 2006) by cross-linking collagen IV proteins in the basement membranes of the epithelium (Finger & Giaccia 2010). $CD11b/Gr1^+$ myeloid cells bind to these cross-linked fibers and recruit other BM cells that reorganize the local niche. LOX also promotes bone metastasis by enhancing osteoclast differentiation (Cox et al. 2015).

The lung is a frequent site of metastasis in many cancer types, which could be due, in part, to the fact that the lungs are prone to inflammation in which cytokines and stromal cells prepare a metastatic niche. In fact, acute inflammation has been shown to enhance lung metastasis in an allergy-induction model (Taranova et al. 2008). Similarly, bacterial infection in the lungs can create an acute inflammatory state that can promote the seeding of melanoma, lung, prostate, and colorectal cancer cells through a ubiquitin–CXCR4 (C-X-C chemokine receptor type-4) axis (Yan et al. 2013). In mouse models of arthritis, systemic inflammation contributes to breast cancer metastasis to both the bones and lungs (Roy et al. 2011).

Bone harbors several particularly receptive niches for DTCs (Esposito & Kang 2014, Ren et al. 2015). Specialized subniches within the BM provide an abundant assortment of growth factors and cytokines, such as CXCL12 (C-X-C motif ligand-12) and IGF1 (insulin-like growth factor-1), that foster the seeding and survival of DTCs (Vivanco & Sawyers 2002). The hematopoietic stem cell niche, in particular, is favored by metastatic prostate cells, which outcompete healthy hematopoietic stem cells for niche occupation (Shiozawa et al. 2011), whereas the osteogenic niche can be readily subverted by breast cancer cells for efficient outgrowth (Wang et al. 2015). Moreover, inflammatory TGF-β signaling has been shown to induce the expression of bone metastasis genes, such as *PTHLH*, *Jagged1*, and *SPHK1*, in breast cancer to promote bone metastasis (Sethi et al. 2011, Stayrook et al. 2015, Yin et al. 1999), and elevated expression of the TGF-β signaling pathway inhibitor PMEPA1 or DLC1 reduces bone metastasis in, respectively, prostate and breast cancers (Fournier et al. 2015, Wang et al. 2014). Importantly, bone destruction during the development of metastatic lesions in bone releases large amounts of TGF-β that are embedded in the bone matrix, which can feed back to tumor cells to enhance their bone metastatic ability (Korpal & Kang 2008).

These findings clearly suggest that inflammatory cytokines can promote metastatic dissemination and survival in specific organs. In addition to lung and bone, tumor-derived factors have also been shown to prepare metastatic niches in the liver, lymph nodes, and adrenal glands as well (Kim et al. 2009, Schelter et al. 2011), demonstrating the importance of systemic factors in establishing a variety of specific organ niches for metastasis.

STROMAL INFLUENCE ON METASTATIC COLONIZATION IN DIFFERENT ORGANS

The physiological function of different organs often depends on specialized resident stromal cells. Therefore, it is not surprising that stromal cells have crucial roles in the development of organotropic metastasis (Hanahan & Coussens 2012, Joyce & Pollard 2009, Quail & Joyce 2013). In particular, the importance of immune cells in cancer metastasis is gaining attention in light of recent breakthroughs in cancer immunotherapy and the growing recognition of the importance of various immune cell types in cancer progression. Immunosuppression is clearly a prerequisite for metastasis in most organs, and an impaired or absent immune system will not only affect how many cells escape from the primary tumor but will also alter the survival and dormancy of DTCs and change the characteristics of the premetastatic niche. $CD8^+$ T cells and NK cells, in particular, restrict the metastatic outgrowth of DTCs in many tissues (Eyles et al. 2010, Paolino et al. 2014). The liver parenchyma, in particular, has a high frequency of innate immune cells and NK cells, requiring metastatic cells to circumvent detection (Takeda et al. 2001). In multiple organs, SOX2 and SOX9 expression was recently shown to be critical for DTCs to avoid immune-mediated eradication (Malladi et al. 2016). This SOX-dependent phenotype relies on DKK1 inhibition of Wnt signaling for a slow cell cycling state and downregulation of ULBP ligands that can be recognized by NK cells. Different organs have unique repertoires and frequencies of specific immune populations that can inhibit metastatic growth, and play a key roles in metastatic tropism (**Figure 2**).

Bone

Bone is a particularly frequent site of metastasis, in part due to the activity of a particularly rich stromal environment, including hematopoietic cells, mesenchymal cells, and resident tissue-specific cells, such as osteoclasts and osteoblasts, responsible for bone maintenance (Ren et al. 2015, Weilbaecher et al. 2011). Secreted factors, such as OPN, RANKL (receptor activator of nuclear

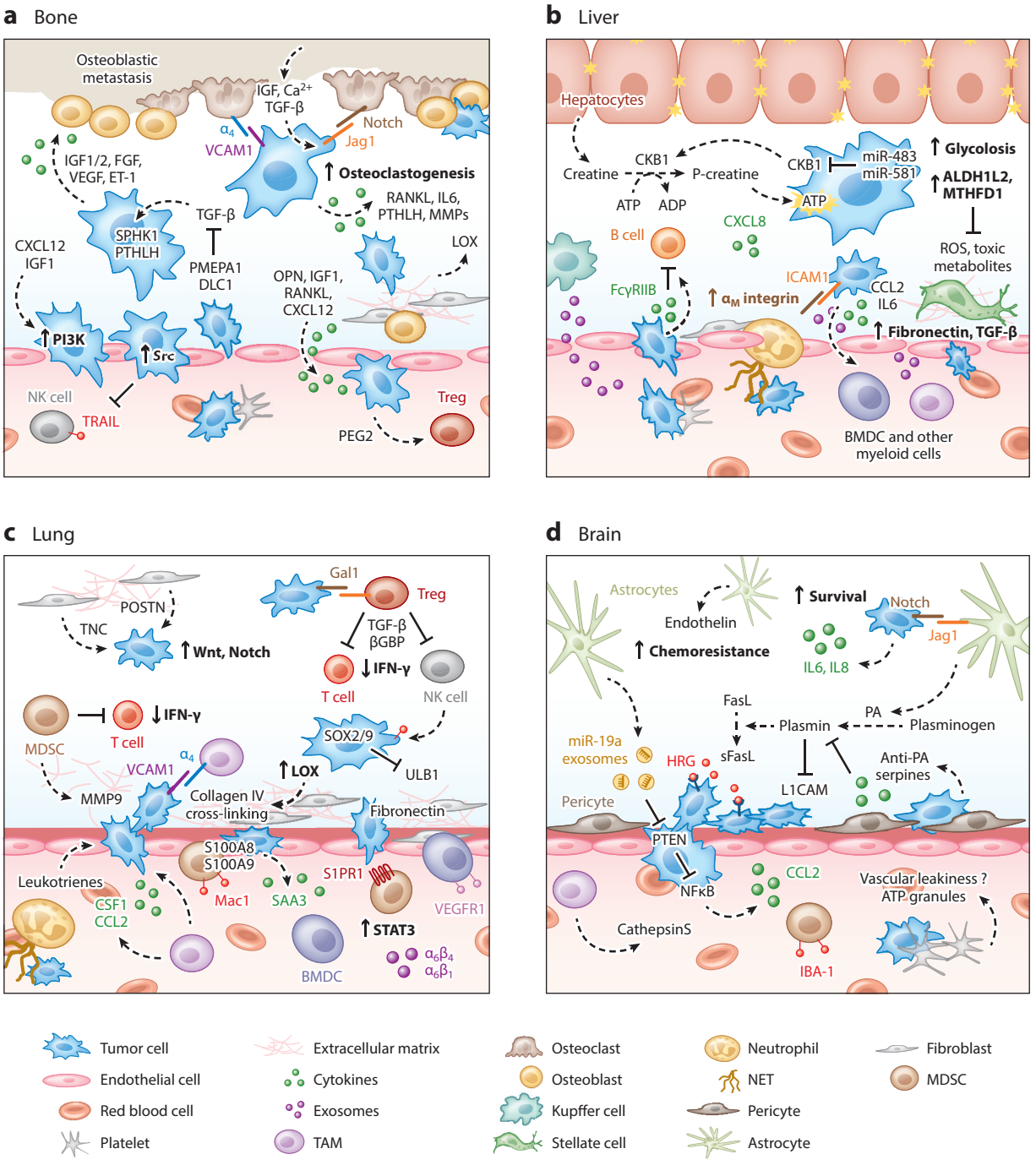
factor κb ligand), IGF1 (insulin-like growth factor-1), and CXCL12 from the bone marrow, serve as a chemotactic source to recruit metastatic cells from primary tumors, which subsequently engage in cross talk with several stromal populations to promote colonization, survival, and outgrowth (Weilbaecher et al. 2011). Most notably, interactions between metastatic cells and osteoclasts and osteoblasts induce a pattern of bone resorption and destruction that releases cytokines, feeding back to the metastatic cells to promote growth in a feedback loop termed the vicious cycle (Ell & Kang 2012). Depending on pathological features reflecting the imbalance of bone homeostasis, bone metastasis may be considered osteoblastic or osteolytic, or contain features of both (Weilbaecher et al. 2011). The activation of osteoblasts by tumor cell–secreted FGF, IGF, VEGF, endothelin-1, and other cytokines promotes osteoblastic metastasis, which is often associated with prostate cancer (Logothetis & Lin 2005); the osteolytic lesions frequently observed in breast and lung cancers display hyperactive osteoclast-mediated bone degradation (Weilbaecher et al. 2011). RANKL secreted directly from metastatic cells, or indirectly from osteoclasts activated by tumor-secreted PTHLH (parathyroid hormone-like hormone) and IL6, induces osteoclast formation and bone destruction (Guise et al. 2006, Weilbaecher et al. 2011). Alternatively, VCAM1 (vascular adhesion molecule-1) expression in DTCs can induce osteoclastogenesis by binding to $\alpha_4\beta_1$ integrin on osteoclast progenitor cells, which facilitates their recruitment and maturation (Lu et al. 2011). Furthermore, elevated expression of Jagged-1 in tumor cells engages bone stromal cells through Notch signaling, directly promoting osteoclast differentiation and the production of tumor-promoting growth factors, such as CTGF and IL6 from osteoblasts (Sethi et al. 2011). DTCs in the BM also avoid TRAIL-mediated (tumor necrosis factor–related apoptosis-inducing ligand-mediated) cell death by innate immune cells through increased Src–kinase activation of AKT (Zhang et al. 2009).

Other cells promoting bone metastasis include $CD4^+$ T cells, myeloid-derived suppressor cells (MDSCs), T regulatory cells (Tregs), and dendritic cells. Surprisingly, instead of exhibiting antitumor immune activity, tumor-specific $CD4^+$ T cells promote a premetastatic niche in the bone by inducing aberrant bone remodeling through RANKL secretion (Monteiro et al. 2013). The secretion of prostaglandin E2 from breast tumor cells recruits Tregs that promote bone metastasis, perhaps systemically establishing a bone metastatic niche (Karavitis et al. 2012). Likewise, plasmacytoid dendritic cells have been shown to recruit MDSCs and Tregs to breast tumors, which suppresses antitumor immune responses and promotes breast cancer metastasis to the bone by inhibiting the cytotoxicity of $CD8^+$ T cells (Sawant et al. 2012), again highlighting the importance of immune evasion for metastatic tropism.

Liver

The abundance of innate immune cells in the liver, particularly NK cells, makes immune–stromal interactions and immune evasion particularly important features for liver metastasis. TRAIL is constitutively expressed on NK cells in the liver and has a substantial role in suppressing tumor metastasis by inducing apoptosis in TRAIL-sensitive tumor cells (Takeda et al. 2001). Similarly, melanoma cells have been shown to avoid immune attack in the liver by secreting FcγRIIb, which functionally blocks B cell recognition (Cohen-Solal et al. 2010).

Inflammatory pathways also promote important metastatic effects in the liver. TGF-β secretion from colorectal carcinoma cells activates IL11 production from resident fibroblasts, which signals through STAT3 to promote tumor cell survival (Calon et al. 2012). Likewise, exosomes released from pancreatic carcinoma can stimulate TGF-β secretion from the liver stroma, fibronectin expression on hepatic stellate cells, and macrophage recruitment to enhance liver metastasis (Costa-Silva et al. 2015). Indeed, tumor-associated macrophages (TAMs) play an important part in liver


a Bone
Osteoblastic metastasis
IGF, Ca2+
TGF-β
α4
VCAM1
Notch
Jag1
IGF1/2, FGF, VEGF, ET-1
↑ Osteoclastogenesis
RANKL, IL6, PTHLH, MMPs
TGF-β
SPHK1
PTHLH
CXCL12
IGF1
PMEPA1
DLC1
LOX
OPN, IGF1, RANKL, CXCL12
↑ PI3K
↑ Src
NK cell
TRAIL
Treg
PEG2
b Liver
Hepatocytes
Creatine
CKB1
P-creatine
ATP
ADP
CKB1
miR-483
miR-581
↑ Glycolysis
↑ ALDH1L2, MTHFD1
ROS, toxic metabolites
CXCL8
B cell
FcγRIIB
ICAM1
↑ αM integrin
CCL2
IL6
↑ Fibronectin, TGF-β
BMDC and other myeloid cells
c Lung
POSTN
TNC
↑ Wnt, Notch
Gal1
Treg
TGF-β
βGBP
↓ IFN-γ
T cell
NK cell
MDSC
↓ IFN-γ
T cell
SOX2/9
ULB1
↑ LOX
α4
VCAM1
MMP9
Collagen IV cross-linking
Fibronectin
S100A8
S100A9
Mac1
SAA3
S1PR1
↑ STAT3
VEGFR1
α6β4
α6β1
Leukotrienes
CSF1
CCL2
BMDC
d Brain
Astrocytes
Endothelin
↑ Chemoresistance
↑ Survival
Notch
Jag1
IL6, IL8
FasL
PA
Plasmin
Plasminogen
miR-19a exosomes
HRG
sFasL
L1CAM
Anti-PA serpines
Pericyte
PTEN
NFκB
CCL2
Vascular leakiness ? ATP granules
CathepsinS
IBA-1
Tumor cell
Endothelial cell
Red blood cell
Platelet
Extracellular matrix
Cytokines
Exosomes
TAM
Osteoclast
Osteoblast
Kupffer cell
Stellate cell
Neutrophil
NET
Pericyte
Astrocyte
Fibroblast
MDSC

tropism by protecting CTCs from immune attack in the circulation and at sites of organ colonization in sinusoids. Recent studies also identified a myeloid cell subset (CD11b/Gr1^{mid}) that is recruited by CCL2 (chemokine C-C motif ligand-2) to the liver to enhance the metastasis of colorectal cancer cells (Zhao et al. 2013).

Neutrophils also promote metastatic seeding in the liver by entrapping or retaining CTCs. This entrapment depends upon CXCL8 secretion from tumor cells to upregulate αM integrin expression in neutrophils, which in turn engages ICAM1 on the cancer cells. The αM–ICAM1 interaction between neutrophils with tumor cells has been shown to be necessary for lung cancer cells to form metastases by increasing CTC adhesion and colonization in liver sinusoids (Spicer

Figure 2

Stromal interactions promoting organotropic metastasis. (*a*) Some of the stromal interactions in bone-specific metastasis are depicted, including alteration of bone homeostasis leading to osteoblastic or osteolytic lesions, creation of an osteoblastic niche for DTCs in bone, immune evasion, metastatic chemotaxis, and prosurvival signaling. Hypoxia and inflammatory pathways also contribute significantly to bone metastasis: HIF1-α induction of LOX promotes bone metastasis by increasing osteoclast activity; TGF-β-induced Jag1, PTHLH, and SPHK1 expression in tumor cells significantly increases bone metastasis of breast cancer, and the TGF-β inhibitors PMEPA1 and DLC1 reduce bone metastasis. Hyperactive Src signaling activity in DTCs protects tumor cells from TRAIL-induced cell death in the bone microenvironment and increases response to prosurvival signals (CXCL12, IGF1) from the bone stroma. Tumor cells produce PEG2 to recruit immunosuppressive Treg cells to suppress antitumor immune responses in bone metastasis. (*b*) In the liver, hepatocyte production of creatine provides an energy substrate that can be utilized by DTCs, and tumor cells upregulate enzymes, such as ALDH1L2 and MTFHFD1, to neutralize toxic metabolic by-products in the TME. Metastatic cells also secrete cytokines (e.g., CCL2, IL6) and exosomes to recruit BMDCs and a number of immune myeloid cells to establish a premetastatic niche in the liver and to avoid immune detection and destruction by cells of the innate and adaptive immune systems. Tumor cells also avoid immune attack in the liver by secreting FcγRIIb, which blocks B cell recognition. Exosomes in the liver are taken up by a variety of stromal cells, including Kupffer cells, to promote metastasis. Exosomes further induce local TGF-β production, stellate cell upregulation of fibronectin for CTC attachment, and macrophage recruitment. Exosomes also deliver $\alpha_v\beta_5$ integrins to endothelial cells, fibroblasts, and Kupffer cells in the liver, which could increase the vascular adhesion of CTCs. Tumor cells secrete CXCL8 to promote αM integrin expression in neutrophils, which interacts with ICAM1 in tumor cells to promote adhesion. (*c*) In the lung, BMDC recruitment is important for establishing a premetastatic niche and remodeling the ECM and vasculature through protease and cytokine secretion. Upregulation of adhesion molecules by recruited BM cells and exosomes containing $\alpha_6\beta_4$ and $\beta_6\beta_1$ integrins promotes CTC adhesion and extravasation into the lung parenchyma. Similarly, α_4 integrin binding on TAMs with VCAM1 on DTCs promotes their survival in the lungs. Tumor cells also produce Gal1 to drive Treg immune suppression and upregulate SOX2 and SOX9 for dormancy and to avoid NK cell destruction. Similarly, tumor cell–induced S1PR1/STAT3 activation in MDSCs is important for recruiting and activating these myeloid cells, which degrade ECM via MMP9 production to enhance CTC extravasation and suppress antitumor immune cell activity to enhance DTC survival and outgrowth. Fibroblast secretion of POSTN and TNC activates, respectively, Wnt and Notch signaling in metastatic cells, enhancing DTC survival. (*d*) In the brain, astrocyte interactions with DTCs are critical for BBB extravasation and the survival of metastatic cells. miR-19a-containing exosomes produced by astrocytes inhibit PTEN expression, which upregulates NF-κB signaling and CCL2 production. CCL2 recruits IBA1$^+$ MDSCs that promote BBB extravasation of CTCs. Similarly, HRG expressed in the brain stroma can interact with receptors on CTCs to promote extravasation, and cathepsin S production by TAMs and tumor cells in the brain microenvironment promotes the extravasation of CTCs across the BBB. Anti-PA serpin expression by metastatic cells blocks plasmin-mediated L1CAM cleavage on endothelial cells to promote adhesion and spreading on vascular basement membranes. Anti-PA serpins also inhibit the production of proapoptotic sFasL from FasL and promote the survival of DTCs. Tumor cells can also co-opt signaling interactions with astrocytes to promote their survival (by Jag1–Notch interaction) as well as chemoresistance (by endothelin production). The key below the panels identifies key stromal components. Abbreviations: ADP, adenosine diphosphate; ATP, adenosine triphosphate; BBB, blood–brain barrier; BMDC, bone marrow–derived dendritic cell; BMP, bone morphogenetic protein; CCL2, chemokine C-C motif ligand-2; CTC, circulating tumor cell; CXCL12, chemokine C-X-C motif ligand-12 (SDF1); DTC, disseminated tumor cell; ECM, extracellular matrix; Gal1, galectin-1; HRG, Heregulin; ICAM, intracellular adhesion molecule; IFN, interferon; IGF1, insulin-like growth factor-1; IL, interleukin; Jag1, Jagged-1; LOX, lysyl oxidase; MDSC, myeloid-derived suppressor cells; MMP, matrix metalloprotease; NK, natural killer; PEG2, prostaglandin E2; POSTN, periostin; PTHLH, parathyroid hormone-like hormone; RANKL, receptor activator of nuclear factor κb ligand; ROS, reactive oxygen species; sFasl, soluble Fas ligand; SPHK1, sphingosine kinase-1; TAM, tumor-associated macrophage; TGF-β, transforming growth factor-β; TME, tumor microenvironment; TNC, tenascin C; TRAIL, tumor necrosis factor–related apoptosis-inducing ligand; Treg, T regulatory cell; VCAM, vascular adhesion molecule.

et al. 2012). Interestingly, it has also recently been shown that neutrophils can secrete a network of DNA and antimicrobial proteins to form a biomolecular mesh, also called a neutrophil extracellular trap (NET), that promotes liver and lung colonization of cancer cells, supposedly by entrapping CTCs (Cools-Lartigue et al. 2013). The exact role of neutrophils in metastasis is still somewhat controversial as neutrophil depletion can actually promote spontaneous breast cancer metastasis (Granot et al. 2011). This suggests that neutrophils have antitumor functionality in some contexts, and it stands to reason that the prometastatic phenotype of neutrophils is driven by specific factors in the tumor microenvironment that change their polarity from N1 to N2 neutrophils. Further research is warranted to address the timing and context for therapeutic targeting of neutrophils to prevent liver metastasis.

In addition to the metastatic regulatory roles of immune cells in metastasis, signaling between metastatic cancer cells and hepatocytes and other tissue-specific stromal cells also significantly impacts liver colonization. For example, Claudin-2 expression on breast cancer cells acts directly as an adhesion molecule with stellate hepatocytes to promote cell–cell interactions and metastasis (Tabaries et al. 2012).

Lung

The lung has been intensively studied for the roles of several immune populations in establishing premetastatic niches. For example, $CD11b^+/Gr1^+$ MDSCs promote metastasis by suppressing antitumor effector T cells and NK cells and by inhibiting IFN-γ (interferon-γ) production (Talmadge & Gabrilovich 2013). MDSCs also prepare a favorable niche for DTCs by altering adhesion molecule expression on resident stromal cells through inflammatory cytokine production and by remodeling the ECM and vasculature through MMP9 expression (Yan et al. 2010). Similarly, VEGFR1-positive bone marrow–derived dendritic cells accumulate in the lungs through fibronectin binding to form premetastatic niches (Kaplan et al. 2005). These bone marrow–derived dendritic cells are mobilized by serum amyloid A3 (SAA3) in $Mac1^+$ myeloid cells and lung-resident endothelial cells in response to tumor-secreted factors, such as S100A8 and S100A9 (Hiratsuka et al. 2008). Similarly, melanoma cell activation of S1PR1/STAT3 signaling in MDSCs is important for recruiting and activating myeloid cells in the premetastatic lung (Deng et al. 2012).

TAMs and monocytes also contribute significantly to lung tropism by promoting CTC extravasation and survival. The recruitment of macrophages and monocytes by tissue factor–mediated coagulation in the lungs is an important step in premetastatic niche formation (Gil-Bernabe et al. 2012). $CD11b^+/LY6C^+$ monocytes recruited to the lung in response to CCL2 promote melanoma metastasis (van Deventer et al. 2013), and their depletion by a Ly6G-specific antibody reduces dissemination and seeding (Toh et al. 2011). Macrophage α_4-integrin binding to VCAM1 on breast tumor cells triggers ezrin activation and enhances PI3K/AKT signaling to promote cancer cell survival in the lungs (Chen et al. 2011). Consistently, genetic ablation of macrophages by CSF1 knockout significantly inhibits lung metastasis (Lin et al. 2001), and COX2 inhibitors inhibit breast cancer lung metastasis by suppressing VEGFA and MMP9 expression in TAMs (Na et al. 2013).

Tregs have also been found to promote lung tropism by suppressing NK and T cell frequency and antitumor activity. CCL22 expression in the lung stroma of mice injected with conditioned media from 4T1 breast tumor cells has been shown to directly recruit immunosuppressive Tregs to the premetastatic lung and to increase metastasis (Olkhanud et al. 2009). Galectin-1 expression by tumor cells in the lung further drives Treg polarization and metastasis (Dalotto-Moreno et al. 2013). Reducing $CD4^+/CD25^+$ Treg numbers in the primary tumor (Kitamura et al. 2015), inhibiting their expansion in the lungs by blocking TNF signaling (Chopra et al. 2013), and neutralizing immunosuppressive Treg-secreted factors, such as β-GBP and TGF-β (Olkhanud

et al. 2009, Smyth et al. 2006), have all been shown to inhibit lung metastasis, suggesting options for therapeutic intervention.

Several other stromal populations have been found to promote lung tropism, including neutrophils, platelets, and fibroblasts. Neutrophils promote metastatic seeding in the lungs by secreting leukotrienes, which increase vascular permeability and extravasation (Wculek & Malanchi 2015), and platelets protect CTCs in circulation, induce epithelial to mesenchymal transition, and recruit macrophages and granulocytes to sites of extravasation and the premetastatic niche to promote seeding (Camerer et al. 2004; Gil-Bernabe et al. 2012; Labelle et al. 2011, 2014; Palumbo et al. 2005). Fibroblasts stimulate lung metastasis by secreting periostin, which activates Wnt signaling in cancer stem cells (Malanchi et al. 2012), as well as tenascin C, which amplifies Notch signaling to promote tumor cell survival in the lungs (O'Connell et al. 2011, Oskarsson et al. 2011).

Brain

Metastasis in the brain is perhaps the most clinically devastating outcome for most cancer patients. This is due to the many areas of the brain that are not amenable to surgical resection and because of the impervious nature of the BBB to many chemotherapies and targeted drugs.

The interactions between metastatic cells and stromal cells in the brain also have very important roles in metastatic seeding. Astrocytes, in particular, display key properties that specifically block metastasis by preventing extravasation, survival, and outgrowth. In the brain, astrocytes secrete plasminogen activator, which cleaves L1CAM and prevents metastatic adhesion and spreading on the basement membrane of brain capillaries, thus inhibiting extravasation and colonization (Valiente et al. 2014). In addition, plasminogen activator production by astrocytes results in cleavage of the proapoptotic Fas ligand (FasL) into an activated form to kill infiltrating metastatic cells (Valiente et al. 2014). To circumvent astrocyte-mediated apoptosis, metastatic cells produce anti-PA serpins that block production of the apoptotic form of FasL, protecting the metastatic cells from direct killing. Interestingly, astrocytes have also been shown to promote metastasis through alterations in stromal signaling. Tumor cells lose PTEN expression after dissemination specifically to the brain but not other organs (Zhang et al. 2015). This PTEN loss is mediated by PTEN-targeting exosomal microRNAs secreted from resident brain astrocytes. PTEN loss leads to increased CCL2 expression by metastatic cells, which recruit IBA1^{+}- myeloid cells that further promote metastatic progression.

Other stromal interactions promote the extravasation, survival, and protection of metastatic cells from chemotherapy in the brain. Cathepsin S expression, by both breast cancer cells and macrophages in the brain microenvironment, has been shown to cooperatively mediate BBB transmigration and metastatic brain tropism (Sevenich et al. 2014). Moreover, metastatic cells can co-opt stromal Notch signaling from astrocytes, which can be subverted by metastatic cells for survival in the brain (Xing et al. 2013). Resistance to chemotherapy is a hallmark of metastasis in many organ sites. In the brain, breast cancer and astrocyte interaction induces endothelin production from astrocytes and endothelin receptor expression on tumor cells, and it has been shown to protect metastatic cells from chemotherapy-induced cell killing (Kim et al. 2014). This endothelin production depended upon IL6 and IL8 signaling from metastatic cells, and the protective effect also depended upon endothelial cell signaling in the brain.

THERAPEUTIC APPLICATIONS AND FUTURE DIRECTIONS

The various systemic factors that favor premetastatic niche formation and metastatic organotropism highlight a variety of potential therapeutic options for targeting metastasis.

Tumor-derived cytokines, exosomes, and mobilized stromal cells are all attractive targets amenable to antibody therapies or small-molecule targeting. Moreover, new drugs and technologies to deliver chemotherapeutics to specific organs and metastatic sites are rapidly developing in preclinical trials, and these include viral, stem cell, and nanoparticle-based systems.

The metastatic microenvironment is composed of corrupted and reactive stroma, matrix, and immune cells that support malignant growth. Targeting the stroma instead of the tumor may provide a means for controlling tumor growth without battling the Darwinian selection mechanisms of acquired therapy resistance seen in cancer cells. Blocking the recruitment and activation of inflammatory stromal cells by neutralizing secreted factors—such as VEGF, TNF, TGF-β, S100A8, S100A9, LOX, and CCL2—or signaling pathways—such as S1PR1/STAT3, NF-κB, and SAA3—may provide a means for clinically preventing or treating metastasis in at-risk patients.

Inhibiting the development of the premetastatic niche might be a particularly beneficial therapy for preventing metastasis to the lung and other organs in the adjuvant setting. CXCL1- and CXCL5-neutralizing antibodies may block MDSC recruitment and premetastatic niche formation. Similarly, CCL2 blockade could prevent monocyte recruitment, and CCL5 and CCL22 inhibition could block Treg recruitment to premetastatic sites. Likewise, using CSF1-neutralizing antibodies to target macrophage recruitment has been effective in combination with angiogenic and cytotoxic therapies for treating metastasis (De Palma & Lewis 2013). In fact, the pharmacological inhibition of platelet clot formation likely reduces metastases in the lungs (Gil-Bernabe et al. 2012), in part by blocking macrophage recruitment.

Several drugs are available that specifically treat bone metastasis, including bisphosphonates, which inhibit osteoclast bone adhesion and induce osteoclast apoptosis (Wong & Pavlakis 2011). These drugs, along with zoledronic acid and denosumab, a monoclonal antibody antagonist of RANKL, are the standard of care for patients with bone metastasis (Li et al. 2014); however, these drugs are mainly palliative, treating the skeletal-related effects of bone metastasis. Targeting chemotaxis mechanisms that drive the seeding of metastatic cells to bone niches could also be therapeutically evaluated to prevent bone metastasis. For instance, breast tumor cells have been shown to utilize CX3CR1 to exit circulation and enter the bone to form metastases (Fatatis et al. 2016). Blocking this receptor activity with neutralizing antibodies and a small-molecule antagonist reduced bone-tropic metastasis of breast cancer. Additionally, many tumor types metastasize to the bone and other sites via the CXCL12–CXCR4 axis (Muller et al. 2001). Several clinical therapies to block this signaling axis are being evaluated, and CXCR4-blocking antibodies and peptides have shown efficacy in metastatic inhibition (Houshmand & Zlotnik 2003).

Targeting liver metastasis should focus on bolstering the already rich immune cell populations present in this tissue, particularly NK cells and B cells, potentially utilizing immunotherapies to activate these cells or block antibodies to prevent immunosuppression. Neutrophils are particularly important for liver tropism, and preventing their polarization and recruitment to liver sinusoids by targeting the CXCL2, CXCL5, and CCL9 axes might be beneficial for treating or preventing metastases in this tissue. Indeed, $Ly6G^+$ neutrophil depletion has been shown to decrease breast cancer liver metastasis in preclinical studies (Tabaries et al. 2015). Moreover, blocking the hepatocyte–CTC claudin-2-mediated cell–cell interaction in breast cancer metastasis could be an attractive means for directly inhibiting liver colonization (Tabaries et al. 2011).

The delivery of chemotherapeutics or other targeted therapies to specific organ environments, particularly where the bioavailability of a drug is limited, could enhance the efficacy of antimetastasis therapies, particularly in the brain where the BBB poses a significant obstacle to drug delivery. Recently, a neural stem cell line engineered to secrete a therapeutic HER2 antibody has been found to home to brain metastases in mice and prolong survival by inhibiting PI3K/Akt signaling in tumor cells (Kanojia et al. 2015). Likewise, mesenchymal stem cells (MSCs) have been heavily

investigated for their tumor-tropic potential to multiple organ sites. A HER2-retargeted oncolytic herpes simplex virus infected into tumor-tropic MSCs has recently been found to have significant effects on ovarian cancer metastases in the lungs and breast cancer metastases in the brain (Leoni et al. 2015). Indeed, MSCs loaded with chemotherapeutics have shown preclinical efficacy in treating metastasis in a number of studies (Pacioni et al. 2015).

Similarly, viruses that display inherent organ, tissue, or cellular tropism could be used as delivery agents for antimetastasis therapeutics. For instance, a neurotropic vesicular stomatitis virus, engineered with reduced neurotoxicity but with oncolytic potential, has recently been shown to home to the brain, where it destroyed gliomas and metastatic melanoma cells, prolonging survival in mice without adverse effects on normal brain tissue (Wollmann et al. 2015).

Nanoparticles are also being evaluated for their potential in the tropic delivery of antimetastasis therapies. Interestingly, cellulose nanocrystals localize to the bone, likely through interactions between Ca^{2+} in the bone matrix and the negative charged surface of the cellulose nanocrystal (Colombo et al. 2015). These nanoparticles have proven safety in vivo, and they easily penetrate the cytoplasm of tumor cells, which makes them attractive carriers of tumor-reactive payloads for killing metastatic cells, specifically in the bone. Similarly, injectable nanoparticle generators offer another potential route for chemotherapeutic delivery to organs not easily permeated by antibodies or biomolecules, and they have been found to cure metastatic breast cancer in 40–50% of mice (Xu et al. 2016). Optimizing nanoparticle delivery systems offer an attractive therapeutic option for metastases in specific organs.

The rich stromal–tumor–cytokine interactions described above provide many potential targets for therapeutic intervention. With this information at hand, it should be possible to tailor personalized therapies that block metastases or reduce their progression in different organs.

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