

## Review

Surviving at a Distance:  
Organ-Specific MetastasisAnna C. Obenauf<sup>1</sup> and Joan Massagué<sup>1,\*</sup>

**The clinical manifestation of metastasis in a vital organ is the final stage of cancer progression and the main culprit of cancer-related mortality. Once established, metastasis is devastating, but only a small proportion of the cancer cells that leave a tumor succeed at infiltrating, surviving, and ultimately overtaking a distant organ. The bottlenecks that challenge cancer cells in newly invaded microenvironments are organ-specific and consequently demand distinct mechanisms for metastatic colonization. We review the metastatic traits that allow cancer cells to colonize distinct organ sites.**

## Organ Tropism of Metastatic Cells

Metastasis results from disseminated cancer cells that initiate new tumors at distant organ sites. The metastatic cascade involves multiple steps, including invasion, entry into the circulation from the primary tumor, systemic dissemination, arrest and extravasation in secondary organs, settlement into latency, reactivation, outgrowth, and potential seeding of tertiary metastasis (Box 1 summarizes the early steps of the metastatic cascade) [1–3]. The pattern of affected organs is remarkably variable depending on the cancer type [1,2,4,5] (Figure 1, Key Figure). Some cancer types predominantly spread to one organ (e.g., prostate cancer to bone, pancreatic cancer and uveal melanoma to liver), or show sequential organ-specific colonization (e.g., colorectal cancer, CRC, frequently metastasizes first to the liver, and later to the lungs). Other cancer types, such as breast cancer, lung cancer, and melanoma, are able to colonize many different organ sites, either sequentially or synchronously [1,5,6]. Although defined organ tropisms are not rigid phenomena, the organ-specific patterns of metastasis are clear (Figure 1). Beyond lymph node spread, the liver, lung, bone, and brain are frequently colonized by a variety of cancer types. The skin, ovaries, and spleen are less common sites of metastasis. Skin metastases generally occur in melanoma and breast cancer, ovarian metastases in breast and gastric cancers, and spleen metastases almost exclusively in melanoma [5].

What determines the organ tropism of metastases? Each organ varies in its physical accessibility, vascular and nutrient supply, and stromal composition, thus placing different demands on infiltrating cancer cells [1]. The organ-specific circulation pattern and the anatomy of vessels certainly influence metastatic spread. However, this does not fully explain the organ-specific pattern of metastasis clinically observed in most cancers. For example, kidneys, liver, and brain equally receive approximately 10–20% of blood volume, but each shows a very different pattern of metastasis [5]. This discrepancy between anatomy and metastasis in different organs has long been observed and forms the basis for the ‘seed and soil’ hypothesis, according to which cancer cell seeds have intrinsic compatibilities with particular welcoming organ microenvironment soils [7,8]. This view is supported by recent observations that distinct cancer subtypes display significant variations in their organ specificity. For instance, adenocarcinoma of the lung spreads more frequently to the brain and adrenal gland than does squamous carcinoma of the lung [5]. Among different breast cancer subtypes, luminal breast tumors have a higher propensity to form bone metastasis, and HER2<sup>+</sup> (human epidermal growth factor receptor 2) breast cancer is

## Trends

The pattern of affected organs in metastasis is variable depending on the tumor of origin, indicating that intrinsic cancer cell traits, the physical accessibility of target organs, and the composition of host-organ microenvironments are important determinants of distant metastasis.

Metastasis is an inefficient process in which few cells succeed at reestablishing a tumor at a distant organ.

Organ-specific metastasis involves cancer cell interactions with the host microenvironment, including activation of paracrine cytokine loops, modification of the host cellular composition, and alteration of extracellular matrix structures.

Immune cell evasion, association with a supportive niche, and the ability to amplify survival pathways, often achieved through interaction with the stroma, are essential for successful metastatic colonization.

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### Box 1. The Metastatic Cascade

Overt metastasis is the final manifestation of a series of stochastic events collectively known as the ‘metastatic cascade’. The cascade can be parsed into distinct steps: (1) local invasion, (2) intravasation, (3, 4) dissemination in the circulation and arrest at the distant site, (5) extravasation, and (6, 7) colonization of target organs (Figure 1). These steps have been extensively reviewed in [1,2,122].

*Step (1).* To invade from the confined primary tumor to the adjacent parenchyma, tumor cells utilize the action of variety of extracellular proteases, including matrix metalloproteinases (MMPs) and cathepsins, which break down the extracellular matrix (ECM) and trigger the release of growth factors that influence tumor growth and invasion [79,123]. The invasive front of a tumor is an important interface at which cancer and stromal cells interact closely [124]. Myeloid cells accumulate at the invasive front, generating an immunosuppressive environment. Tumor-associated macrophages and fibroblasts promote the invasion of cancer cells by producing pro-migratory factors or by depositing fibrillar collagen [125–128].

*Step (2).* Departure from a primary tumor is favored by the epithelial-to-mesenchymal transition (EMT) of cancer cells. EMT involves loss of intercellular adhesion, epithelial polarization, and the gain of mesenchymal traits [122]. In cancer cells, EMT supports self-renewal, motility, and invasiveness, traits that favor metastatic dissemination [122,129,130]. A leaky neovasculature generated by the primary tumor contributes to easier access to the circulation.

*Step (3, 4).* Cancer cells may invade and intravasate as single cells or as multicellular clusters, and associate with non-neoplastic cells which may enhance their survival during dissemination [120,125,131]. At distant organ sites, circulating tumor cells arrest in narrow capillary beds and extravasate. Rapid physical trapping due to the size of the vasculature likely plays a major role in this process [132]. The capacity to arrest at distant organs may also be determined by specific functions of the cancer cells, for example by forming adhesive connections in specific organs as has been described for breast cancer in the lung vasculature [133].

*Step (5).* Cancer cells lodged in the microvasculature may initiate intraluminal growth and form an embolus that eventually ruptures the blood vessel or, more frequently, cancer cells may extravasate directly into the tissue parenchyma by penetrating the microvascular wall. In the bone marrow or the liver, the vasculature is fenestrated and poses a lower physical barrier than in other organs such as the lungs or the brain [1,2]. There, the vasculature is surrounded by a tight basement membrane that is additionally reinforced by pericytes and astrocytes, and the cancer cells therefore require additional specialized functions to extravasate into the parenchyma [14,64].

*Step (6, 7).* The vast majority of cancer cells that extravasate into the parenchyma will die, but a minority of these cells may enter a period of dormancy and survive for months to decades. From such disseminated tumor cell populations a few cancer cells may reinitiate growth and establish a full-fledged tumor at the distant site.

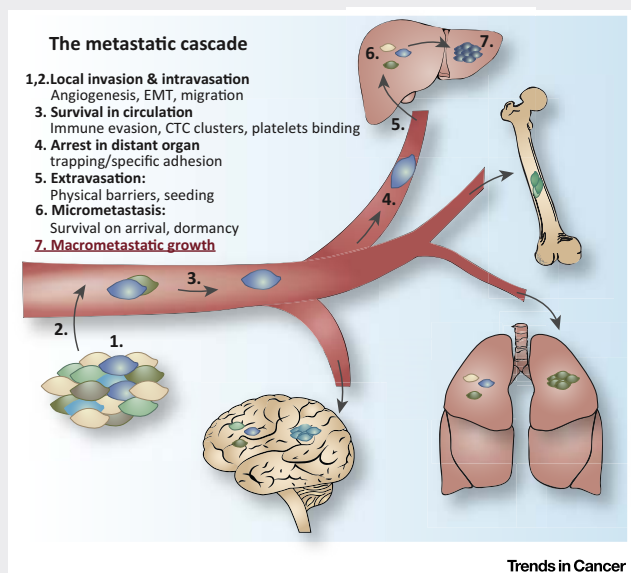
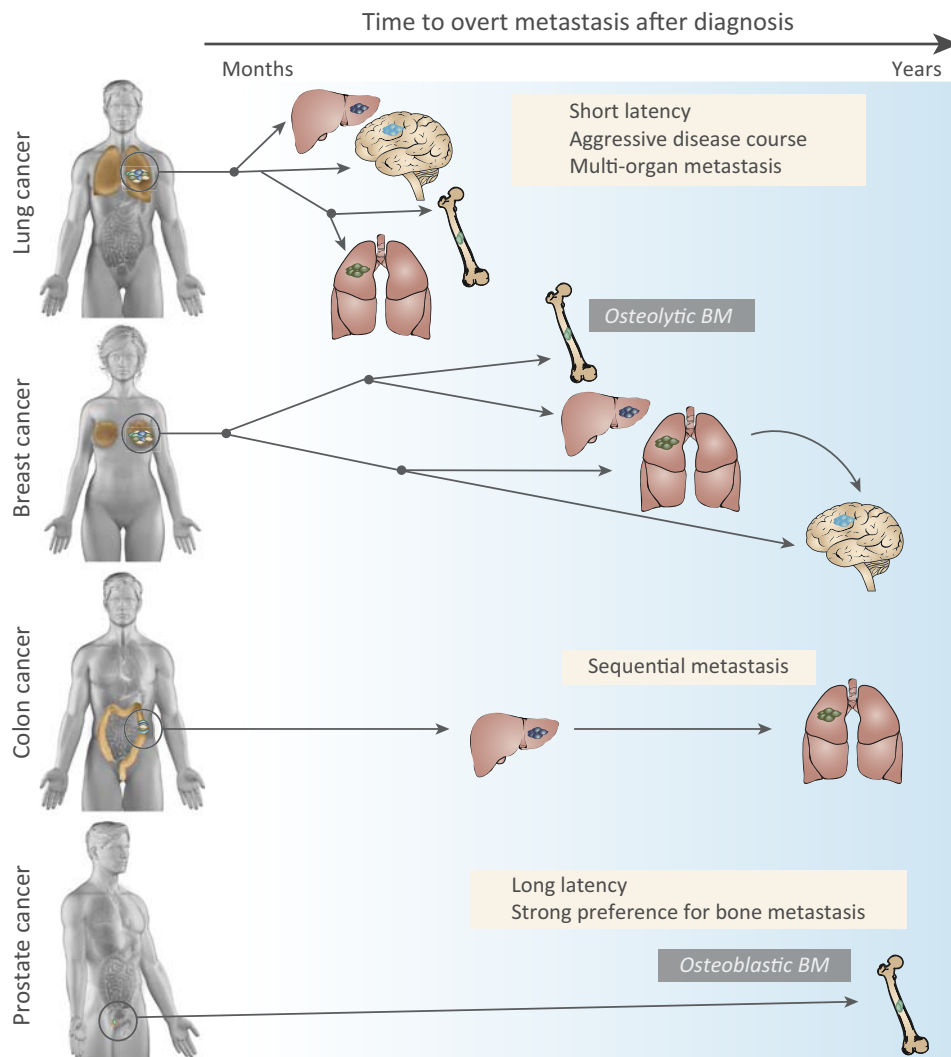


Figure 1. The Metastatic Cascade.

## Key Figure

## Patterns of Metastatic Spread of Solid Tumors



Trends in Cancer

**Figure 1.** Different cancer types exhibit remarkable variability in their metastatic course, reflected in the length of the latency period (months to years), the organs affected (most commonly the liver, lung, bone, and brain) and the type of metastasis (e.g., osteolytic or osteoblastic bone metastasis). Latency period (denoted by the arrow on top of the figure – left: months, right: years after diagnosis): lung cancer metastasis typically occurs within months after initial diagnosis, whereas prostate cancer and some subtypes of breast cancer can produce distant relapse up to decades after initial diagnosis. Lung cancer is the main contributor to brain metastasis, whereas it is a late occurrence in breast cancer. Organ pattern (the most-frequently affected organ is located on the top of each cancer type): lung and breast cancers metastasize to different organs (with a different propensity), whereas colon cancer most frequently metastasizes to liver, and from established liver metastasis secondarily to lung. Prostate cancer typically although not exclusively metastasizes to bone. Different cancer types also vary in the type of metastatic lesions they induce, well illustrated by the development of osteolytic bone metastasis in breast and lung cancer, and osteoblastic bone metastasis in prostate cancer. Abbreviation: BM, bone metastasis.

associated with a higher frequency of liver metastases [9–11]. Nonetheless, the proportion of disseminated cancer cells that survive to achieve metastatic colonization is vanishingly low [2,12,13], meaning that most seeds are poorly endowed and no soil is really very welcoming.

These clinical observations are complemented by a wealth of data from experimental mouse models. These models have revealed tumor-intrinsic and -extrinsic mechanisms dictating organ-specific metastatic progression against a background of massive attrition of the disseminated cancer cells [13–31]. These studies support the notion that organ-specific metastasis depends not only on extrinsic factors enabling cancer cell access to organs, such as circulation patterns and vascular wall accessibility, but also on the intrinsic abilities of the metastatic cancer cells themselves. For example, intrinsic abilities to interact with the host microenvironment allow cancer cells to cross physical barriers, survive in distant sites, engage with a distinct organ-specific cell types, and eventually overtake the host tissue (Box 1).

Metastasis is above all a Darwinian selection process in which cancer cells with distinct metastatic traits that enable them to overcome metastatic bottlenecks are selected from a genetically- and epigenetically-heterogeneous tumor cell population [32,33]. The bottlenecks that exert selective pressures are distinct at each step of the metastatic cascade. Cancer cell clones expand as a function of their ability to surpass the specific demands of each step of the metastatic cascade, and continue to evolve thereafter [33–36].

General mediators of metastasis, such as those supporting invasion, the ability to amplify survival pathways, or immune evasion, increase the probability of cancer cells to adapt and, consequently, survive through multiple specific challenges in multiple organs. By contrast, specific genes and pathways enable passage through crucial organ-specific barriers, such as crossing the blood–brain barrier, or mediate beneficial interactions with organ-specific cell types, such as the osteoclasts in the bone marrow. In addition to tumor cell-autonomous traits that increase the probability of disseminated cancer cells to establish overt metastasis, the paracrine interaction with stromal cells and tumor-driven systemic processes can have a profound impact on metastasis, for instance by stimulating the growth of distant tumor cells or by generating a pre-metastatic niche at a distant site [37]. During all stages of the metastatic cascade tumor cells enlist the help of non-neoplastic cells (extensively reviewed in [38–40]). Individually, all these traits promote survival of individual cancer cells when facing a harsh encounter with a new organ microenvironment and, with that, these traits increase the probability of achieving clinically-overt metastasis. In this review we focus on the mechanisms that enable cancer cells to grow out and take over the distant organ.

### The Final Stage of the Metastatic Cascade: Organ Colonization

Fine-tuned crosstalk between cancer cells and their microenvironment is required for successful colonization of a distant organ. This may be achieved by distinct mechanisms including, but not limited to, (i) evasion of the immune system or other detrimental signals that may threaten cancer cell survival, (ii) interaction with stem cell niches and resident cell populations to promote survival signals in the local microenvironment, and (iii) recruitment of cell populations that modify the new host microenvironment to better match the growth requirements of the cancer cells. Mechanistic dissection of organ-specific metastatic colonization in experimental mouse models over the past decade has shed light on these organ-specific metastatic traits, the composition of permissive metastatic niches, and how complex interactions between cancer cells and their niche result in overt metastasis.

#### Bone Metastasis

Approximately 60–85% of patients with metastatic breast and prostate cancer harbor bone metastases, often resulting in pathological fractures, chronic pain, and neurological

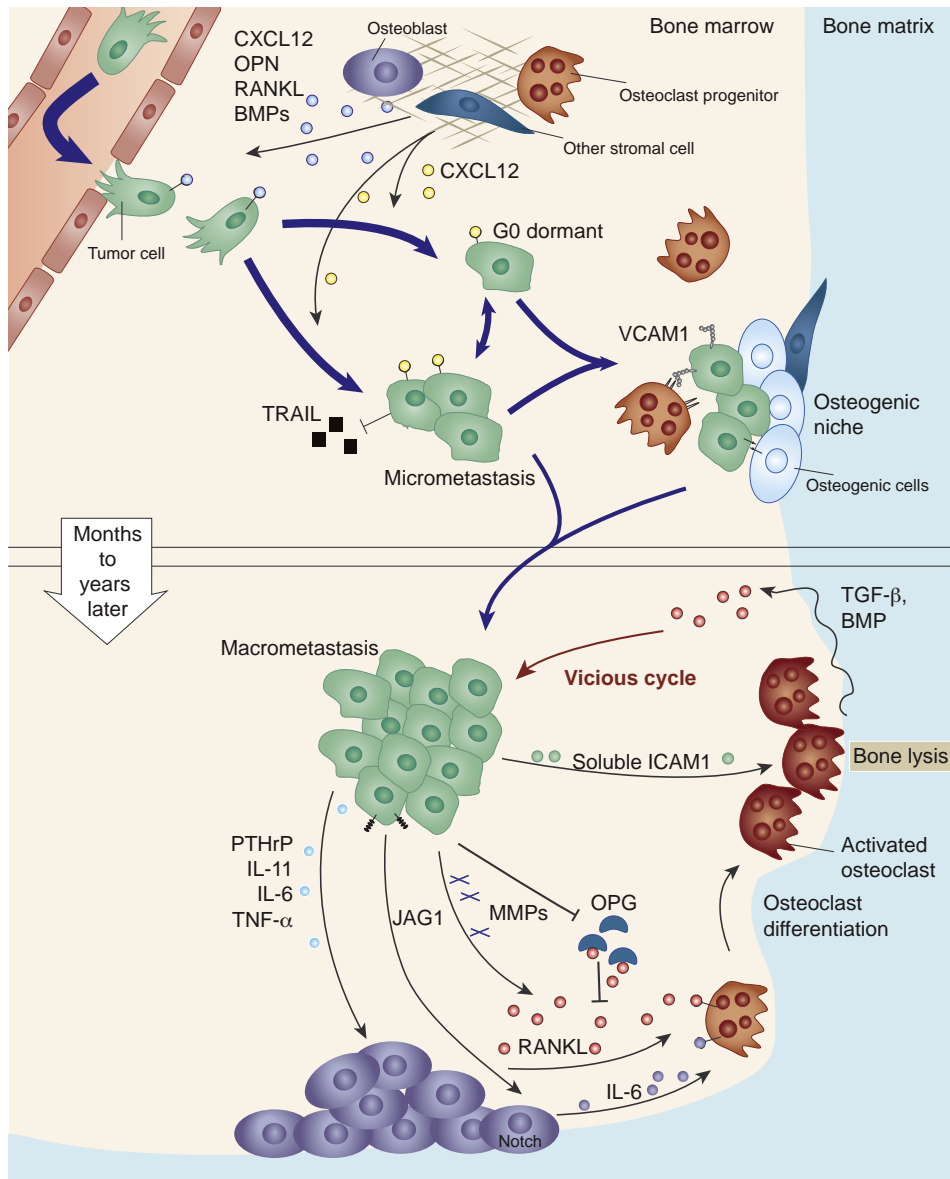
compression syndromes [41]. The small blood vessels in the bone marrow, the sinusoids, are lined with fenestrated endothelia to allow the traffic of hematopoietic cells. The bone marrow sinusoids are likely more permissive to circulating tumor cells (CTCs) than are other types of capillaries. In addition, bone matrix cells like osteoblasts secrete a variety of chemo-attracting factors (e.g., CXCL12, RANKL, OPN, or BMPs) that recruit cancer cells to the bone marrow [41,42] (Figure 2, top).

After extravasation into the bone marrow, cancer cells may benefit from abundantly expressed soluble factors, such as CXCL12 (C-X-C motif chemokine 12) and IGF1 (insulin-like growth factor 1), that stimulate PI3K (phosphoinositide 3-kinase)–AKT (protein kinase B) signaling – a pathway well known to enhance cancer cell survival in challenging environments [43] (Figure 2, top). Cancer cells with elevated SRC (SRC proto-oncogene tyrosine kinase) signaling and high expression levels of CXCR4 (the receptor for CXCL12) are especially primed to utilize the physiological survival signals in the bone marrow, thereby increasing the probability of establishing overt metastasis later on [21,26]. In addition, high SRC activity has been shown to counteract proapoptotic signaling of TRAIL [tumor necrosis factor (TNF)-related apoptosis-inducing ligand/TNFSF10], a cytokine also present in bone metastatic lesions [21,44]. These findings from animal models are also reflected in clinical datasets in which CXCR4 expression and expression of the SRC signature in tumor cells is associated with breast cancer bone relapse [21].

The need to find supportive niches within an organ is of importance for the survival of disseminated metastatic stem cells [45]. Cancer cells may take up residence in stem cell niches of the bone marrow. Prostate cancer cells compete with hematopoietic stem cells (HSCs) for occupancy of stem cell niches [46] and breast cancer cells can occupy osteogenic niches [47] (Figure 2, top). In these niches, cancer cells may benefit from heterotypic adherens junctions between E-cadherin on cancer cells and N-cadherin on osteogenic cells. E-cadherin expression correlates with bone metastasis in patient samples, and early disruption of the adherens junctions reduces bone metastasis in mouse models [47]. Similarly, the expression of  $\alpha 4 \beta 1$  integrin and its ligand, vascular cell adhesion molecule-1 (VCAM1), facilitates microenvironmental crosstalk in the bone marrow to promote the expansion of micrometastases in preclinical models [28,48,49].

During the final phase of overt colonization metastatic cancer cells can also actively modify the bone microenvironment in their favor by disturbing the complex and tightly regulated network of signals that control bone homeostasis by regulating osteoblasts and osteoclasts (Figure 2, bottom). Depending on the signals released from cancer cells, bone metastases manifest as osteoblastic lesions, osteolytic lesions, or a combination thereof [41,50]. In osteoblastic lesions, which are typically of prostate cancer metastasis, tumor cells stimulate bone matrix deposition by osteoblasts, resulting in increased bone density and eventual displacement of the bone marrow [51]. Factors secreted by prostate cancer cells that promote osteoblastic bone metastasis include fibroblast (FGF), insulin-like (IGF), vascular endothelial (VEGF), and platelet-derived (PDGF) growth factors, as well as endothelin 1 (ET1), WNT (wingless/int) family members, and bone morphogenetic proteins (BMPs) [41,51–55].

In osteolytic lesions, which are caused most commonly by breast cancer and lung cancer, the metastatic cells activate bone-resorbing osteoclasts, which produce collagenases and other proteases that degrade extracellular matrix (ECM) proteins and demineralize the bone matrix [41,56]. Taking center stage in the formation of osteolytic bone metastasis is NF- $\kappa$ B ligand [RANKL, receptor activator of nuclear factor  $\kappa$ B (RANK) ligand/TNFRSF11A] signaling [41]. Tumor cell-derived parathyroid hormone-like hormone (PTHrP/PTHrH), interleukin (IL)-11, IL-6, and TNF- $\alpha$  cue osteoblasts to release RANKL, which induces osteoclast formation and the subsequent resorption of the bone [41,50]. Bone metastatic cancer cells also secrete matrix



## Trends in Cancer

**Figure 2. Osteolytic Metastatic Colonization of the Bone.** The capillaries in the bone, known as sinusoids, are lined with fenestrated endothelia that facilitate the traffic of hematopoietic cells. Thus, the bone marrow sinusoids are likely permissive to cancer cell passage. (Upper panel) Upon infiltrating the bone marrow, cancer cells are exposed to a variety of growth- and death-promoting signals which are thought to force cancer cells into a latent state until they acquire the necessary traits for overt metastasis. In this state cancer cells benefit from secreted survival signals (CXCL12) from bone-resident cells and by direct interaction with osteogenic cells and pre-osteoclasts. (Lower panel) A crucial step in the formation of overt osteolytic bone metastasis is the activation of osteoclasts. This process is locally facilitated by cancer cell-derived mediators including PTHrP, IL-11 and others that stimulate the secretion of RANKL by osteoblasts. Cleavage and release of membrane bound RANKL, or inactivation of the antagonist OPG can also contribute to increasing RANKL activity. Alternatively, cancer cells trigger the secretion of IL-6 by osteoblasts, which in turn induces osteoclast differentiation. Activated osteoclasts execute bone resorption, which releases TGF- $\beta$  and other growth factors that are embedded in the mineralized bone matrix. TGF- $\beta$  then further stimulates the expression of osteolytic factors in the cancer cells, resulting in a vicious cycle of bone metastasis. Abbreviations: BMP, bone morphogenetic protein; CXCL12, chemokine C-X-C motif ligand 12, also known as SDF1; ICAM1, intracellular adhesion molecule 1; IL, interleukin; JAG1, Jagged 1; MMP, matrix metalloproteinase; OPG, osteoprotegerin; PTHrP, parathyroid hormone-like hormone; RANKL; receptor activator of nuclear factor  $\kappa$ B ligand; TGF- $\beta$ , transforming growth factor  $\beta$ , TRAIL, tumor necrosis factor (TNF)-related apoptosis-inducing ligand; VCAM, vascular cell adhesion molecule.

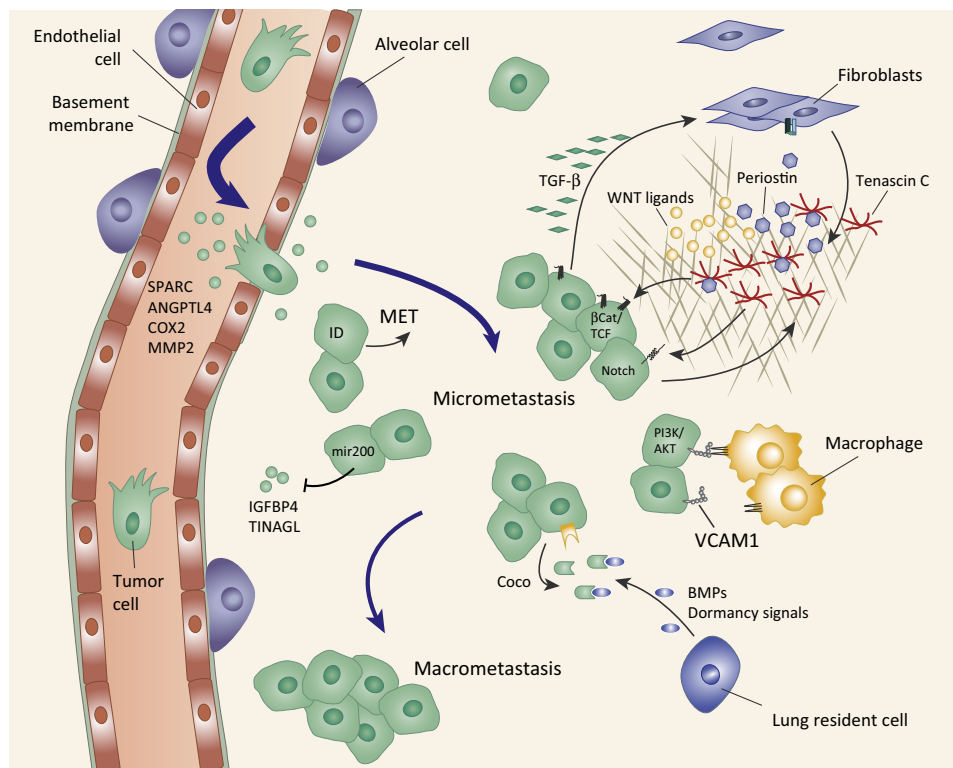
metalloproteases (MMPs), which increase local RANKL activity, either directly by cleaving and releasing membrane-bound RANKL [57], or indirectly, by reducing the level of the RANKL-antagonist osteoprotegerin [58].

One consequence of bone resorption in osteolytic metastasis is the release of growth factors that are normally embedded in the mineralized bone matrix (Figure 2, bottom). The released growth factors then stimulate tumor growth, leading to the production of additional osteolytic and osteoblastic factors, and resulting in the ‘vicious cycle’ of bone metastasis [41,50,56]. Transforming growth factor  $\beta$  (TGF- $\beta$ ) is abundant in the bone matrix and is released during osteoclastic bone resorption [41]. In breast and melanoma models, TGF- $\beta$  signaling plays an essential role in the establishment of bone metastasis. TGF- $\beta$  signaling is activated in bone metastasis of breast cancer patients, and inhibition of the TGF- $\beta$  pathway reduces bone metastasis formation in preclinical models [24,59–61]. It has recently been shown that bone tropic prostate cancer cells also benefit from TGF- $\beta$  signaling, which is further amplified by reduced levels of PMEPA1 (prostate transmembrane protein, androgen-induced 1), a negative regulator of TGF- $\beta$  signaling. In patients, PMEPA1 levels decreased in metastatic lesions compared with the primary tumor, and low PMEPA1 levels correlated with worse prognosis [62].

Additional mechanisms that promote osteolytic bone metastasis involve the Notch ligand Jagged 1 (JAG1), the expression of which is also regulated by TGF- $\beta$ . JAG1 overexpression mediates bone metastasis in a human hormone receptor-negative (‘triple negative’) breast cancer cell line [24] and is associated with bone metastatic relapse in different patient cohorts [30]. In xenograft models, JAG1 promotes osteolytic bone metastasis by activating Notch signaling in osteoblasts, which induces the secretion of IL-6 and directly activates osteoclast differentiation [30] (Figure 2, bottom). Osteoclast differentiation is also influenced by tumor-derived factors (e.g., soluble intracellular adhesion molecule 1, sICAM-1), which induce widespread changes in microRNA abundance [31]. In *in vitro* experiments osteoclast differentiation could be blocked by the ectopic expression of several microRNAs which target osteoclast genes. In a xenograft model, the delivery of these microRNAs inhibited osteoclast activity and reduced osteolytic bone metastasis from breast cancer cells. Clinically, serum levels of sICAM-1 and two microRNAs that were elevated during osteoclast differentiation, mir-16 and mir-378, are associated with bone metastasis burden [31]. These examples show that factors secreted by cancer cells can modulate the bone metastatic microenvironment and determine the type of bone metastases formed.

### Lung Metastasis

Lung metastasis is frequent in different types of cancer, including breast cancer, gastrointestinal tumors, renal carcinomas, melanoma, different types of sarcoma, and lung cancer itself [5]. Lung capillaries are lined with endothelial cells that are surrounded by a basement membrane and adjacent alveolar cells [1]. To cross these structural obstacles, breast cancer and melanoma cells express specific mediators such as SPARC (secreted protein, acidic, cysteine-rich/osteonectin), the TGF- $\beta$ -inducible factor angiopoietin-like 4 (ANGPTL4), and the secreted C-terminal fibrinogen-like domain of angiopoietin-like 4 (cANGPTL4) [63–65]. The expression of these mediators enhances the extravasation of tumor cells in the lung by dissociating the cell–cell junctions between endothelial cells (Figure 3). Other factors expressed by cancer cells are the EGF (epidermal growth factor) receptor ligand epiregulin, the prostaglandin synthase COX2 (cytochrome c oxidase polypeptide II), and the metalloproteinases MMP1 and MMP2, which foster the breaching of lung capillaries to seed metastasis [66]. All these mediators are upregulated in breast tumors and their expression predicts relapse to the lungs [16,63,66], reinforcing the concept that metastatic traits required in early steps of the metastatic cascade are already selected for in the primary tumor, where they may play a different role in processes such as tumor angiogenesis.



Trends in Cancer

**Figure 3. Metastatic Colonization of the Lung.** The lung capillaries are lined with a basement membrane and mediators of cancer cell extravasation in the lung have been identified (e.g., SPARC, ANGPTL4, COX2, MMP2). The expression of ID proteins in breast cancer cells induces mesenchymal–epithelial transition (MET) at the metastatic site and supports breast cancer cells in bypassing senescence. The cancer cell- and myofibroblast-derived extracellular matrix proteins POSTN and TNC stimulate cancer cell survival by enhancing Wnt access to their receptors (periostin) or by amplifying WNT and Notch signaling (TNC). Cancer cells express high levels of VCAM1, which tethers macrophages to cancer cells and triggers activation of the cell survival AKT pathway in cancer cells. Cancer cells overcome dormancy signals (BMPs) from lung resident cells by overexpressing Coco, which increases formation of macrometastasis. Abbreviations: ANGPTL4, angiopoietin-like 4; AKT, protein kinase B;  $\beta$ Cat,  $\beta$ -catenin; BMP, bone morphogenetic protein; PI3K, phosphoinositide 3-kinase; POSTN, periostin; SPARC, secreted protein, acidic, cysteine-rich/osteonectin; TCF, T cell factor; TINAGL, tubulointerstitial nephritis antigen-like; TNC, tenascin C; VCAM1, vascular cell adhesion molecule 1; WNT, wingless/int.

After extravasation in the lung parenchyma, tumor–stroma interactions play a crucial role in amplifying the output of survival and stemness pathways in cancer cells, consequently increasing their chances of surviving (Figure 3). In an MMTV (mouse mammary tumor virus)-driven polyomavirus middle T (PyMT) mouse breast cancer model, lung metastatic cancer stem cells stimulate the expression of the ECM protein periostin in lung fibroblasts via secretion of TGF- $\beta$ 3 [67]. Increased periostin levels recruit WNT ligands and stimulate WNT signaling preferentially in cancer stem cells, ultimately promoting lung colonization [67]. In the ECM, periostin interacts with the hexameric glycoprotein tenascin C (TNC) [68]. TNC is expressed at the invasive front of tumors where it also binds to fibronectin, integrins, and syndecan membrane proteoglycan, and is associated with poor prognosis and lung relapse in breast cancer patients [69]. TNC expression by fibroblasts or the tumor cells amplifies the Notch signaling output, and promotes the survival of the tumor cells and their colonization of the lung [69,70]. As the metastatic lesion grows and recruits cancer-associated fibroblasts, tumor cell derived TNC is joined by TNC from the stroma in this supportive role [69]. Lung-tropic human breast cancer cells express high levels of VCAM1, which is engaged by  $\alpha$ 4 $\beta$ 1-integrins on tumor-associated macrophages. In



xenograft models this interaction triggers VCAM1 activation of ezrin, which subsequently enhances PI3K–AKT signaling in the cancer cells and increases their survival [71].

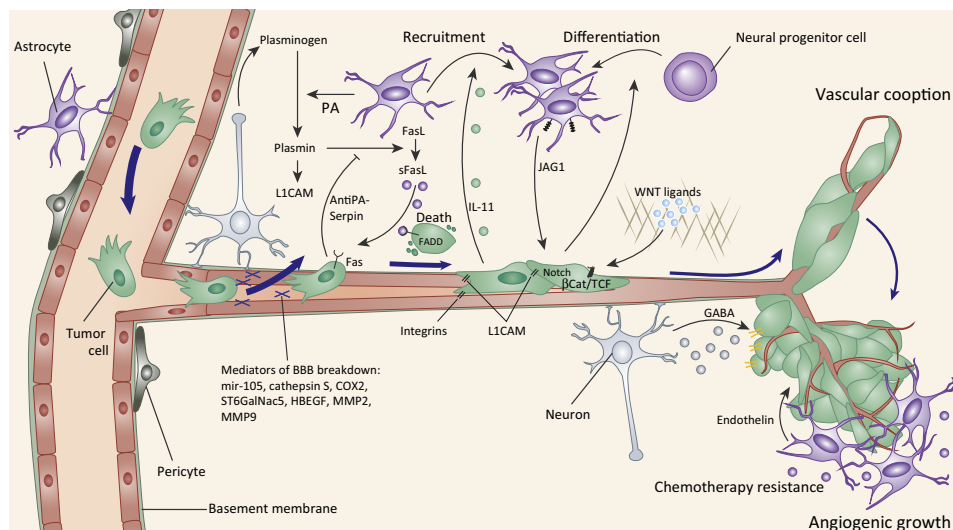
In addition to these interactions with the metastatic niche, cell-intrinsic mechanisms are also essential for the outgrowth of disseminated tumor cells. For example, the expression of inhibitor of differentiation 1 (ID1) and ID3 in breast cancer cells supports metastasis initiation after infiltration of the target parenchyma [72]. ID1 is under the control of TGF- $\beta$  signaling and induces mesenchymal–epithelial transition (MET) at the metastatic site by antagonizing the transcription factor Twist1. Loss of ID1 dramatically reduced lung colonization in a xenograft model [73]. The microRNA mir-200 is intricately linked to the epithelial–mesenchymal transition (EMT)–MET program, and its overexpression promotes metastatic colonization of the lung. In addition to regulating E-cadherin, expression of mir-200 promotes metastatic colonization by targeting SEC23A (Sec23 homolog A), which regulates the secretion of metastasis suppressive proteins IGFBP4 (IGF binding protein 4) and TINAGL1 (tubulointerstitial nephritis antigen-like 1) [74]. Metastatic cells in the lungs may also have to overcome antagonistic signals from the stroma [75]. For example, BMP signals can promote differentiation of allograft breast cancer cells in the lungs. In this model, the BMP-sequestering protein Coco promotes metastatic outgrowth [76] (Figure 3).

#### Brain Metastasis

Metastasis in the central nervous system (CNS) principally involves the brain parenchyma and the leptomeninges, and it has a particularly poor prognosis with high morbidity and mortality. The median survival of patients with brain metastasis is in the order of months, and few effective treatments are currently available [77]. More than half of brain metastases derive from lung adenocarcinoma, followed by breast cancer and melanoma [5]. To enter the brain parenchyma, cancer cells must traverse microcapillary walls that constitute the blood–brain barrier, which consists of tightly adjoined endothelial cells that are lined by a basement membrane, pericytes, and astrocyte foot processes [78]. To cross this barrier and access the brain parenchyma, cancer cells require specialized mechanisms. Some of the molecular mediators of this process have been identified, including the acetylgalactosaminide sialyltransferase ST6GalNac5, COX2, HBEGF (heparin-binding EGF-like growth factor), MMP2, mir-105, and the protease cathepsin S [14,79,80] (Figure 4).

Metastatic colonization of the brain proceeds with close apposition of cancer cells at the abluminal side of the microcapillaries [81,82]. Small lesions often develop without establishing new vasculature [83]. Recently, lung cancer and breast cancer metastatic cells were shown to express the cell adhesion molecule L1CAM for spreading on the basement membrane of brain capillaries after extravasation into the brain parenchyma (Figure 4). Brain metastatic cells also produce specific serpin protease inhibitors to prevent L1CAM cleavage by astrocyte-derived plasminogen activator (PA) [84]. High expression of serpin B2 and neuroserpin correlates with lower brain metastasis-free survival in patients with lung adenocarcinoma [84]. Integrins also play a crucial role in mediating brain metastatic cell spreading and angiogenesis [85,86]. Human lung adenocarcinoma and murine myeloma cells that reached mouse brain tissue and failed to establish required  $\beta$ 1 integrin mediated adhesion to the vascular basement membrane were less efficient at forming overt brain metastasis [87,88].

Metastatic cancer cells encounter a variety of resident cell types in the brain parenchyma. Astrocytes can provide a growth-permissive microenvironment for infiltrated cancer cells, first, however, cancer cells must evade astrocyte-induced cell death. In xenograft models of brain metastasis, activated astrocytes overexpress the proapoptotic cytokine Fas ligand (FasL/FASLG) and release it from a membrane-anchored form by the action of PA to kill infiltrated metastatic cells. Brain metastatic cells express anti-PA serpins that shield cancer cells from



## Trends in Cancer

**Figure 4. Metastatic Colonization of the Brain.** To establish parenchymal brain metastasis cancer cells have to cross the vascular walls that constitute the blood–brain barrier (BBB), which consists of tightly connected endothelial cells lined with a basement membrane and contacting astrocyte and pericytes. Several classes of mediators of cancer cell passage through the BBB have been identified (mir-105, cathepsin S, COX2, ST6GalNac5, HBEGF, MMP2, MMP9). Cancer cells express high levels of anti-PA serpins which prevent the release of cytotoxic soluble FAS ligand (sFasL) from reactive astrocytes and the inactivation of the L1CAM adhesion molecule that mediates vascular cooption by the cancer cells. Once cancer cells evade astrocyte-mediated killing they can take advantage of astrocyte-derived survival and chemo-protective functions of largely unknown nature. Cancer cells may stimulate the accumulation of astrocytes in metastatic lesions. Cancer cells may also utilize neuron-secreted GABA as support for metastasis. Abbreviations:  $\beta$ -cat;  $\beta$ -catenin; COX 2, cytochrome c oxidase polypeptide II; FADD, FAS-associated via death domain; FasL, FAS ligand; GABA,  $\gamma$ -amino butyric acid; HBEGF, heparin-binding EGF-like growth factor; JAG1, Jagged 1; L1CAM, cell adhesion molecule L1; mir-105, microRNA 105; PA, plasminogen activator; MMP, matrix metalloproteinase; ST6GalNac5, acetylgalactosaminide sialyltransferase; TCF, T cell factor.

PA-released FasL [84]. The surviving cancer cells can induce astrocytes to establish Notch signaling [25] and endothelin production, which favor metastasis in experimental systems [89,90]. Conversely, cancer cells can increase the density of astrocytes by promoting the differentiation of neural progenitor cells towards the astrocyte lineage [91] (Figure 4).

The contribution of other brain cells, including oligodendrocytes, pericytes, microglia, and neurons, is less well defined. Although brain metastatic cells need to neutralize cytotoxic microglia signals, microglia infiltration correlates with metastatic progression [81,92]. Brain metastatic cells may upregulate GABA ( $\gamma$ -amino butyric acid) transporters and utilize neuron-released GABA neurotransmitter as a metabolite, supporting outgrowth in the brain [93] (Figure 4).

The WNT pathway was identified to support colonization of brain and bone by KRAS (Kirsten rat sarcoma viral oncogene homolog)-mutant and EGFR-mutant human lung adenocarcinoma cells [20]. Clinically, a specific WNT-responsive gene signature is associated with metastatic relapse in lung adenocarcinoma patients [20]. Two WNT-regulated genes, LEF1 (lymphoid enhancer-binding factor 1) and HOXB9 (homeobox B9), were specifically implicated in metastatic cell invasiveness and colony formation [20]. Brain metastases in patients show upregulation of WNT target genes [94].

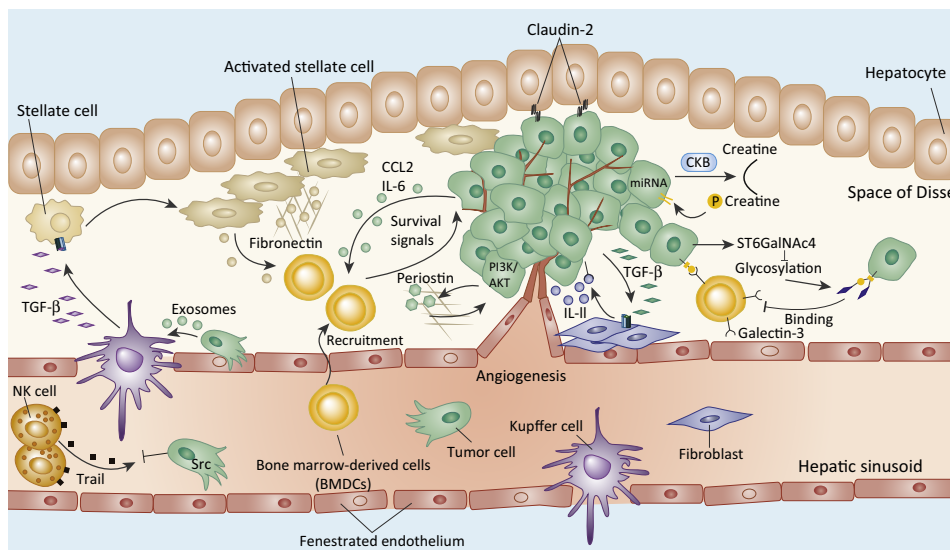
### Liver Metastasis

The liver is the most common site of distant metastasis in solid tumors. Gastrointestinal cancers such as CRC, pancreatic cancer, and tumors of the gallbladder, which are drained by the

enterohepatic circulation, reach the liver first. As such, the liver provides a large number of cancer cells with ample opportunity to arrest, extravasate, and colonize the hepatic parenchyma [22]. Indeed, a recent study found a higher number of circulating CRC cells in the portal venous blood than in the peripheral blood, suggesting that a significant percentage of tumor cells are trapped in the liver [95]. Other primary tumors that metastasize to the liver include lung and breast cancers [4,5]. Uveal (ocular) melanoma almost exclusively relapses in the liver, providing a clear indication that, beyond circulation patterns, particular compatibilities of metastatic cells with the host stroma also count in organ-specific metastasis [96].

In contrast to the vessels in the brain or the lungs, the hepatic vasculature is fenestrated (sinusoidal endothelium) and lacks an organized subendothelial basement membrane. Therefore, cancer cell extravasation is less restricted in the liver than it is in the brain or the lungs, as shown in quantitative cell tracking studies in mice [12]. However, the liver parenchyma is rich in cells of the innate immune system, potentially posing an obstacle to cancer cells. Indeed, the neutralization of proapoptotic TRAIL on resident natural killer cells in the liver increases experimental metastasis [97] (Figure 5).

Particular liver parenchyma cell types favor metastatic outgrowth (Figure 5). In experimental models, claudin 2-mediated cell–cell interactions between breast cancer cells and hepatocytes led to induction of c-Met (MET proto-oncogene, receptor tyrosine kinase) and stimulate metastasis to the liver [98]. In an allograft model, exosome vesicles released by murine pancreatic ductal adenocarcinoma cells caused TGF- $\beta$  secretion, stimulated fibronectin production by hepatic stellate cells, and triggered recruitment of bone marrow-derived



## Trends in Cancer

**Figure 5. Metastatic Colonization of the Liver.** The extravasation into the liver is facilitated by the hepatic vascular endothelium, which is fenestrated and lacks an organized basement membrane. High SRC signaling protects cancer cells from TRAIL-mediated apoptosis. Cancer cells release MIF containing exosomes that trigger TGF- $\beta$  production for the activation of stellate cells, leading to the recruitment of bone marrow-derived cells (BMDCs). BMDCs can also be attracted by secretion of CCL2 and IL-6. Other survival signals may be provided by galectin-3. In the liver, colon cancer cells secrete periostin, which induces PI3K/AKT signaling. Cancer cells also interact with hepatocytes via claudin-2, stimulating overt metastasis. The secretion of CKB by cancer cells contributes to metastatic outgrowth by generating phosphocreatine as a metabolite to regenerate ATP in cancer cells. Abbreviations: AKT, protein kinase B; CCL2, C-C motif chemokine ligand 2; CKB, creatine kinase B; IL, interleukin; NK cell, natural killer cell; MIF, macrophage migration inhibitory factor; PI3K, phosphoinositide 3-kinase; SRC, SRC proto-oncogene tyrosine kinase; TGF- $\beta$ , transforming growth factor  $\beta$ ; TRAIL, tumor necrosis factor (TNF)-related apoptosis-inducing ligand.

macrophages [99]. The macrophage migration inhibitory factor (MIF) was highly enriched in murine and human pancreatic cancer exosomes, and its blockade inhibited metastasis in the mice [99] (Figure 5). CRC and lung cancer cells mobilize myeloid cell populations through soluble factors, such as CCL2 (chemokine C-C motif ligand 2) or IL-6, that promote liver metastasis [100,101] (Figure 5).

Some cancer cells express the glycosyltransferases St6GalnAc4 and C2GnT2, which alter the glycosylation of a galectin 3 ligand on tumor cells and thereby increase interaction with galectin 3 expressed on myeloid cells [101] (Figure 5). Clinically, aberrant glycosylation and high galectin 3 levels are associated with metastatic progression [101,102]. Another significant case of metastatic interaction with the hepatic microenvironment is provided by CRC stem cells. The cells are often unresponsive to TGF- $\beta$  owing to mutations that disable the TGF- $\beta$  receptors or the SMAD (SMA/mothers against decapentaplegic) signal-transducer proteins. However, these cells abundantly secrete TGF- $\beta$ , which enhances metastasis formation in the liver by activating a paracrine loop with production of IL-11 from stromal fibroblasts. IL-11 then activates STAT3 (signal transducer and activator of transcription 3) signaling in CRC stem cells to support their survival in the liver [27] (Figure 5).

Proliferating cancer cells in the liver have high biosynthetic demands and compete with hepatocytes for glycolytic substrates. *In vivo* screens identified two microRNAs (miR-551a and miR-483) that were downregulated in liver-tropic CRC cells, leading to increased expression of the brain-type creatine kinase CKB [103]. The cancer cells benefit from high levels of creatine in the liver, which CKB converts into phosphocreatine that the CRC cells import for their bioenergetic needs [103] (Figure 5).

### Concluding Remarks

Genomics and other systems-level approaches, combined with extensive work in experimental models, have started to shed light on the traits of cancer cells, the composition of stromal niches, and the interaction between cancer cells and these niches that increase the probability of overt colonization of a specific organ by cancer cells from different tumors of origin. However, many questions remain open (see Outstanding Questions).

The manifestation of organ-specific metastasis can take months to decades and is the result of multiple different traits that each provide a small advantage to individual cancer cells to survive and thrive. Despite physical barriers that need to be overcome, the arrival in a distant organ does not seem to be the most-limiting factor. Tumor cells can be found in the blood in early-stage cancer [104], in some cases even before tumors are overtly invasive [105–107]. Notably, even non-transformed epithelial stem cells are able to infiltrate and survive in the lung when injected in large numbers into the circulation [108]. It remains unknown whether cancer cells that leave the primary site very early during tumor progression are able to initiate clinically-manifest metastasis or whether cancer cells that leave the primary tumor later during tumor progression stand a better chance.

Disseminated tumor cells can survive for decades after surgical resection of a tumor. Latent cancer cell populations may reside in specialized protective niches in the bone marrow or in other organs, as suggested by cases in which recipients of liver, kidney, or heart transplant developed metastasis from dormant cancer cells carried by the donor organ [109–111]. The identification of mediators of cancer cell survival during metastatic dormancy is of interest because targeting such mediators with adjuvant therapy could prevent overt metastasis. However, one of the most limiting steps of the metastatic cascade appears to be the transition from infiltration of an organ to overt colonization, which involves the evasion of organ-derived detrimental signals and the exploitation of organ-derived survival signals.

### Outstanding Questions

When and where do the traits for organ-specific metastasis arise – in the primary tumor or at the distant organ site?

What is the origin of these metastatic traits – genetic or epigenetic?

Do metastatic cells utilize different niches for their initial survival on arrival, for dormancy, and for aggressive outgrowth?

What gives cancer cells the ability to enter a dormant state for up to several years while retaining tumor-initiating capacity?

What are the signals that allow cancer cells to exit dormancy and reactivate their proliferative programs?

How do cancer cells acquire metastatic traits for organ colonization during the dormant state?

Are organs that serve as sanctuary sites for dormant metastatic cells the same organs as those in which overt metastasis eventually emerges?

Are the mechanisms that support the survival of cancer cells after extravasation related to those that support the survival of residual cancer cells under anticancer therapy?

What is the basis for the notorious drug-resistance of metastatic cells in distant organ microenvironments, such as the brain?

Would therapeutic targeting of the mechanisms that specifically support the survival of dormant metastatic cells prove an efficient strategy to prevent metastasis?

Several of the mediators of organ-specific colonization, such as those involved in cancer cell interactions with osteoblasts and osteoclasts, are only relevant to metastasis in that particular organ site. However, many mediators of metastasis that were identified in studies on one or another organ are not necessarily restricted to that particular organ. For example, periostin was originally implicated in lung metastasis by breast cancer cells [67] but is also utilized by CRC cells for liver metastasis [112]. Similarly, VCAM1 gives tumor cells in the lung a distinct survival advantage by fostering interactions with macrophages [71], whereas in the bone marrow VCAM1 mediates the interaction of tumor cells with myeloid osteoclast progenitors, promoting their osteolytic expansion [28]. Other examples are COX2 and MMP1, which were initially shown to be mediators of breast cancer cell extravasation in the lungs but also play a role in extravasation through the blood–brain barrier [14,16,22,113,114].

Tumor heterogeneity, cancer cell plasticity, and complex cooperations between different cancer clones provide additional challenges in the modeling, interpretation, and therapeutic intervention of metastatic cancer [115,116]. Already at the primary site different cancer cell clones may cooperate to sustain the growth of the tumor [117,118], and crosstalk between tumor cells stimulates metastasis [119]. Collective invasion of multicellular clusters increases the survival and metastatic efficiency of disseminated tumor cells in preclinical models [120], and multiclonal seeding has been detected in prostate cancer patients [35,36].

Each of the steps of the metastatic cascade poses natural vulnerabilities of the cancer cells that could be targeted to prevent overt metastasis and to improve the outcome of patients with metastatic cancer. In a therapeutic setting, signals released by cancer cells under the stress of targeted kinase therapy stimulate the proliferation and dissemination of drug-resistant cancer cell minorities [121]. It is possible that the mechanisms that provide a survival benefit during the crucial steps of metastasis may also increase the survival of cancer cells during drug treatment, thus contributing to therapy resistance and disease progression. Future research must be directed to identifying the most crucial mediators of metastatic colonization as therapeutic targets. The most valuable of these targets might well be those that mediate not organ-specific metastasis, but multi-organ metastasis.

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