Microenvironment in metastasis: roadblocks and supportive niches

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Pein M, Oskarsson T. Microenvironment in metastasis: roadblocks and supportive niches. Am J Physiol Cell Physiol 309: C627–C638, 2015; doi:10.1152/ajpcell.00145.2015.—In many cancers, malignant cells can spread from the primary tumor through blood circulation and initiate metastasis in secondary organs. Metastatic colonization may depend not only on inherent properties of cancer cells, but also on suitable microenvironments in distant sites. Increasing evidence suggests that the nature of the microenvironment may determine the fate of disseminated cancer cells, providing either hindrance or support for cancer cell propagation. This can result in strong selective pressure where the vast majority of cancer cells, invading a secondary organ, are either eliminated or maintained in a dormant state. The ability of cancer cells to fend off or circumvent anti-metastatic signals from the stroma and the capacity to manipulate the local microenvironment towards a supporting environment, a metastatic niche, may be essential for metastatic growth. The molecular interactions between cancer cells and the stroma are still enigmatic, but recent studies are beginning to reveal their nature. Here, we discuss the interactive relationship between metastatic cancer cells and host stroma, involving selection and adaptation of metastasis-initiating cells and host tissue remodeling. Understanding the dynamic and continuously evolving cross talk between metastatic cancer cells and the stroma may be crucial when developing cancer treatments.

cancer; microenvironment; metastasis; niche; stem cells

METASTASIS IS THE PRIMARY cause of mortality in many cancers, yet today, metastatic progression is still poorly understood. The metastatic process consists of several sequential steps that are needed for cancer cell dissemination from the primary site and growth of secondary tumors in distant organs. Early steps include cancer cell invasion into local stroma and entry into vascular circulation (intravasation), where the cells must survive without adhesion and evade host immune recognition (137). Disseminated cancer cells that reach a secondary organ need to exit the circulation (extravasate) and invade a newly found parenchyma. In secondary sites, cancer cells face an unfamiliar microenvironment and must survive and grow under potentially suboptimal conditions to develop micrometastasis that can bring forth organ colonization and establishment of macrometastasis (102) (Fig. 1).

Despite its clinical importance and global prevalence, metastasis is an exceedingly improbable and inefficient process at the cellular level. Evidence from mouse models suggests that only a fraction of disseminated cancer cells are able to grow out and form secondary tumors in distant sites. Xenograft studies on melanoma metastasis to liver and lungs showed that while extravasation was efficient in these models, tumor growth in secondary sites was rate-limiting (26, 85). Moreover, models of brain metastasis of numerous cancers demonstrated that colonization of the brain is highly inefficient (67, 138). Based on these animal studies, initiation of micro- and macrometastatic growth may represent a prime threshold for disseminated cancer cells of several malignancies. This is in line with clinical observations of metastatic relapses in different cancers long after successful removal of the primary tumor. For example, metastasis in breast cancer and prostate cancer can occur years to decades after resection of the primary lesion (60, 66), suggesting that the survival and outgrowth of cancer cells at the distant site present a rate-limiting step in the metastatic progression of these malignancies.

Conditions that favor metastatic growth depend on inherent properties of cancer cells as well as on the microenvironment in secondary organs. The notion of fitting interactions between cancer cells and stroma was already recognized in the late nineteenth century, when the English surgeon Stephen Paget proposed the so-called “seed and soil” hypothesis to describe organ preference in metastasis (105). He built on the work of Ernst Fuchs as well as his own study of over 700 lethal breast cancer cases and noticed that metastatic spread appeared to be nonrandom. Paget suggested that cancer cells, which he termed the “seed,” require a specific and appropriate microenvironment, a “soil,” for propagation and that metastases will only form if seed and soil are compatible (105).

Given the high genetic and epigenetic heterogeneity of cancer cells within primary tumors, disseminated cancer cells may exhibit immense diversity with varying metastatic poten-
SCs may overlap with important properties of normal stem cells. Signaling pathways such as Wnt, Notch, and transforming growth factor-β (TGF-β), which are associated with stem/progenitor cell function, are essential for metastatic progression in model systems (104, 121, 129). Moreover, expression of stem cell signatures in human tumors is associated with increased aggressiveness and poor clinical outcome in different cancers (11, 88, 109). Recent evidence suggests that the ability of cancer cells to acquire stem cell- and tumor-initiating properties may be more plastic than previously thought (27, 54, 87, 141). Consequently, non-cell autonomous mechanisms such as stromal signals may influence stem cell properties and the metastatic potential of cancer cells (112).

Today, the importance of the microenvironment in cancer progression and metastasis is widely recognized and further evidence accumulates in support of Paget’s hypothesis (15, 42, 61, 112). The significance of microenvironmental cues in the development of metastasis involves most steps in the metastatic process (61). Depending on its composition, the microenvironment can have a negative effect on cancer cell viability, serving as a source of selective pressure, or it can promote metastatic progression by protecting cancer cells from harmful signals and by supporting their fitness under stressful conditions (15, 42, 102). In light of this, it is likely that metastatic disease is driven by the coevolution of cancer cells and the stromal microenvironment. In this review, we focus on the dynamic interactions between cancer cells and the microenvironment that may initially select Paget’s “seeds” of metastasis, the MetSCs, and subsequently fuel metastatic growth.

Selection Within the Primary Lesion

Selection of potential MetSCs may already occur in the primary tumor. This notion was instigated by evidence such as gene signatures expressed in human primary tumors that can predict metastatic risk, even in an organ-specific manner (33, 139). The site of selection has not been determined, but invasive tumor fronts are likely locations. The invasive front is the interface between the growing primary tumor and the tissue stroma and is exposed to significant selective pressure. The front is abundant with blood vessels and stromal cells such as fibroblasts, myofibroblasts, and immune cells like tumor-associated macrophages (TAMs) and myeloid progenitor cells (61). Signals from these cells facilitate tumor cell invasion and may select for increased metastatic ability. Indeed, a study on cancer cells isolated from the invasive front of human pancreatic tumors showed that a subpopulation expressing CD133 and CXCR4 exhibits high migratory capacity and ability to form metastasis when injected into mice (56). This suggests that invasive fronts of primary tumors may host MetSCs.

To disseminate from the primary tumor, cancer cells may acquire an increased migratory and invasive capacity. In carcinomas, this can occur via a mechanism known as epithelial-mesenchymal transition (EMT), during which cells lose polarity and cell-cell adhesion typical for epithelium and acquire a highly motile mesenchymal phenotype (133). In several cancers, EMT has been shown to occur at the invasive front (28). EMT can be induced by the microenvironment via the secretion of several factors (133). Recruited myeloid-derived suppressor cells were found to promote EMT via the TGF-β-, epidermal growth factor (EGF)-, and hepatocyte growth factor...
(HGF)-signaling pathways in a spontaneous mouse melanoma model (135). Moreover, TAMs at the invasive front have been shown to induce EMT in cancer cells by producing TGF-β, which positively correlates with tumor grade in non-small cell lung cancer (16). In prostate cancer, cancer-associated fibroblasts (CAFs) can induce EMT via the secretion of matrix metalloproteinases (MMPs) (51). Importantly, studies showed that circulating tumor cells (CTCs) in the blood of cancer patients with lung, breast, or hepatocellular carcinoma present molecular features of EMT, implying relevance for EMT in metastatic spread in humans (59, 89, 149).

EMT may not only allow cancer cells to leave the primary tumor via loss of cell-cell contacts and increased motility, but may also facilitate the acquisition of stem cell properties (87). In breast and pancreatic cancer cells, the expression of EMT transcription factors such as Twist and Snail generated cells with stem-like features (87, 121). Furthermore, coexpression of EMT and stem cell markers in CTCs from patients with metastatic breast cancer further underscores this link (5, 7). In hepatocellular carcinoma, TAMs at the invasive front can promote invasion and cancer stem cell (CSC)-like properties via TGF-β1-induced EMT (41). In breast cancer cells, EMT results in an increased expression of the cell adhesion molecule CD90/Thy1 and the receptor tyrosine kinase ephrin type-A receptor 4 (EphA4), which mediate physical interactions with TAMs (81). These interactions induce NF-κB signaling and promote the secretion of cytokines including interleukin-6 (IL-6), IL-8, and granulocyte-macrophage colony-stimulating factor (GM-CSF) that sustain stem cell properties of breast cancer cells (81). Thus, stromal-induced EMT may be linked to the acquisition of stem cell properties in some cancers and thereby select for MetSCs.

While EMT is crucial for metastasis of certain tumors, other malignancies may metastasize in the absence of this mechanism. EMT is not the only means by which cancer cells can migrate and invade. Collective cell migration is a frequently observed migratory pattern in tumors where a group of cells migrates together without losing cell-cell adhesion or exhibiting other apparent indicators of EMT (45). Furthermore, in carcinoma models that undergo EMT, only partial EMT is often observed (14). In line with this, epithelial markers such as EpCAM and cytokeratins are regularly used to identify CTCs in the blood and disseminated tumor cells (DTCs) lodged in the bone marrow (106). Evidence suggests that CSCs may have the ability to transition between epithelial and mesenchymal states (80), a property that may be essential for overt metastatic colonization, which will be discussed later in this review.

MetSC evolution within primary tumors is driven by complex cellular interactions that are still poorly understood. However, recent evidence is beginning to reveal how these cells can be selected within the primary tumor lesion. In the primary tumor, stromal signals that resemble the milieu of a distant organ may select cancer cell clones that are primed for metastasis in that particular organ. Indeed, a role for CAFs in organ-specific pre-seed selection within the primary tumor has been demonstrated in mouse models for breast cancer metastasis to the bone. CAFs in the stroma of triple-negative breast cancer were found to secrete the chemokine CXCL12 and insulin-like growth factor I (IGF-I), which are normally high in bone microenvironment, and thereby select cancer cell clones that are primed for bone metastasis (150). CXCL12 and IGF-I select for cancer cells with high Src activity, which exhibit an increased responsiveness to these survival signals. Src enhances the response of the phosphatidylinositol-3-kinase (PI3K)-Akt survival pathway to CXCL12 and IGF-I, thereby promoting survival of breast cancer cells in the bone marrow microenvironment (150, 151). Taken together, molecular signals provided by the primary tumor stroma may already preselect for properties that subsequently benefit cancer cells on the path to metastasis.

Underscoring the complexity of MetSC evolution, evidence suggests that cancer dissemination may not be unidirectional and that CTCs can return back to the tumor of origin, a process termed self-seeding (69, 94). In mouse models, self-seeding was shown to select cancer cells with increased aggressiveness and a molecular profile of seeder cells revealed an upregulation of metastasis-associated genes (69). Indeed, functional analysis demonstrated that seeder cells exhibit superior ability to metastasize compared with cancer cells within the bulk tumor (69). The self-seeding process may contribute to the cellular heterogeneity within the primary tumor and influence its development and cancer cell evolution.

Selection at the Distant Site

Whether metastasis manifests in a particular organ can be determined by numerous factors involving blood circulatory routes, cancer cell’s ability to traverse divergent vasculature, and metastatic fitness within the secondary microenvironment. Circulation patterns may increase the probability of metastasis to a particular organ by a direct and effective blood flow, delivering great numbers of cancer cells to the organ. This could, for example, partially explain the incidence of liver metastasis in colon cancer and lung metastasis in breast cancer, as these sites receive fairly direct blood flow from the organ harboring the primary tumor (29). Importantly, vascular anatomy and physiological properties can vary significantly in different organs and this may generate another selective barrier for metastasis (93). Vascular permeability differs greatly between organs and is reflected by the genes and cellular functions required for extravasation (19, 53). Furthermore, stromal components such as platelets and macrophages have been shown to significantly promote extravasation at the metastatic site (75, 116). However, lodging at the distant site is only an early step towards manifest metastasis and most disseminated cancer cells will die at the secondary site despite successful infiltration. The probability of metastatic outgrowth is likely to be shaped by microenvironmental factors in secondary organs (Fig. 2A).

Responses to inhospitable microenvironments. Propagation of cancer cells is highly context-dependent and thus their growth within distant organs may be determined by the tissue microenvironment. This is underscored by different studies showing that normal microenvironments that support tissue homeostasis can effectively suppress malignant growth (24, 36, 131). A pioneering study published four decades ago showed that an embryonic mouse blastocyst microenvironment can suppress and reprogram teratocarcinoma cells, resulting in mosaic mice in which cancer cells contributed to apparently normal differentiation of several tissues (91). Other studies showed that normal stromal fibroblasts isolated from different tissues can prevent cancer cell growth (6, 44, 97). Conse-
Fig. 2. Suppressive and supportive roles of secondary microenvironments in metastasis. A: tissue microenvironment can inhibit or promote metastatic cancer growth depending on cellular and molecular composition. Upon initial arrival at distant sites, cancer cells may be suppressed and restrained by inhibitory stromal factors. For example, resident fibroblasts inhibit cancer cell proliferation and apoptotic signals derived from the stroma, such as Fas-L and Trail, contribute to cancer cell elimination. Cytotoxic T cells, natural killer cells, and other immune cells of the adaptive and innate immune system pose a significant barrier to cancer cell survival and proliferation. Tissue-specific architecture as well as extracellular matrix (ECM) composition and cytokine profiles may differ significantly from the stromal composition of the primary organ, thus hindering cell-ECM interactions that are required for metastatic outgrowth. Significant stromal alterations can be induced by cancer cells as well as by chemical, physical, and inflammatory stimuli and disturbed microenvironments may be supportive for cancer growth and promote metastasis. Cancer-associated fibroblasts are analogous to activated myofibroblasts in wound healing and similarly secrete a variety of proinflammatory cytokines and growth factors including CXCL12, HGF, PDGF, and VEGF. ECM protein composition encompasses fibrillary collagens, fibronectin, and matricellular proteins. The modified ECM and growth factor milieu promote survival and stemness of metastatic cells, may reactivate dormant metastatic cancer cells, and initiate angiogenesis. Recruited cells such as macrophages, myeloid progenitors, and mesenchymal cells further promote metastatic growth by the establishment of an inflammatory milieu and paracrine signaling loops. Therapeutic treatment that can counteract and revert these stromal changes may be a promising therapeutic strategy against metastatic cancer. B: most cancer cells that invade a secondary organ and avoid immediate elimination maintain a dormant state. Microenvironmental factors from the resting physiological stroma may play a major role in this process. For example, stromal cells of the bone marrow secrete bone morphogenetic protein 7 (BMP7), forcing invading cancer cells into a dormant state via p38 pathway activation. Growth arrest-specific protein 6 (GAS6) is produced by osteoblasts and mediates dormancy in the bone marrow. Stromal-derived exosomes from the bone marrow containing microRNAs such as miR-23b can also contribute to cancer cell dormancy. In contrast to resting stroma, a reactive microenvironment harbors factors that can induce awakening of dormant DTCs. β1-integrin enables cancer cell adhesion to the ECM that results in FAK signaling and triggers reactivation of dormant DTCs. Increased ECM stiffness is common to desmoplasia and supports proliferation of DTCs, as opposed to soft ECM associated with dormancy. Transforming growth factor-β1 (TGF-β1) is another factor that can induce a switch of dormant DTCs towards reactivation. TGF-β1 can promote fibrosis within the secondary tissue, leading to stiff ECM and escape from dormancy.

consequently, the microenvironment in secondary organs can constitute a major challenge for invading cancer cells and exert a strong selective pressure. In addition to eradication by natural killer (NK) cells and cytotoxic T-cells, pro-apoptotic stimuli such as Fas ligand (Fas-L) and Trail derived from a secondary microenvironment can pose a high risk to cancer cells, resulting in their elimination (138, 151). However, successful metastatic cancer cells are able to counteract these defensive mechanisms imposed by the stroma. In brain metastasis, lung and breast cancer cells prevent plasmin-dependent conversion of membrane-bound Fas-L on astrocytes to a soluble form induced by the brain stroma (138). The cancer cells protect themselves from Fas-L-induced death by expressing serpins that inhibit plasmin generation (138). Moreover, PI3K-Akt signaling is an important survival pathway for DTCs and amplification of this signaling pathway may provide a significant advantage under unfavorable conditions. In the lungs, infiltrating breast cancer cells expressing high levels of vascu-
lar cell adhesion protein 1 (VCAM-1) may be selected (90). VCAM-1-producing breast cancer cells were found to interact with pulmonary macrophages via α4-integrins (32). This engagement triggers PI3K-Akt activation and protects cancer cells from the pro-apoptotic cytokine Trail present in the leukocyte-rich environment of the lungs (32).

In addition to cancer cell properties that promote resistance to antitumor signals, a selection of cancer cells that bring their own niche components may be favored. Breast cancer cells infiltrating the lungs have been shown to produce the extracellular glycoprotein tenasin-C (TNC), resulting in a superior ability to form lung metastases in an orthotopic mouse model (101). Autocrine TNC was found to increase MetSCs’ responsiveness to stem/progenitor cell signaling pathways, supporting their metastatic fitness (101). Consequently, as the lung stroma may not provide infiltrating cancer cells with all necessary niche components upon arrival, the selected cancer cells that bring their own stromal constituents may have a survival advantage.

**Specialized endogenous niches.** In secondary organs, invading cancer cells may benefit from specialized tissue structures such as stem cell niches. In a mouse model of prostate cancer metastasis, cancer cells were shown to compete with hematopoietic stem cells (HSCs) for occupancy in the bone marrow niche, which may provide a supporting environment in favor of metastasis (126). In line with this, a recent study on breast cancer metastasis to the bone revealed interactions between breast cancer cells and the osteogenic niche (143). Osteogenic niche cells induce mTOR signaling in breast cancer cells via heterotypic adherens junctions and promote the formation of micrometastasis (143).

Vascular niches are receiving increased attention as promoters of cancer cell fitness. Vascular affinity is well-studied in brain malignancies such as gliomas. In glioblastoma, the most aggressive form of glioma, signals from the perivascular niche induce Hedgehog-, Notch-, Wnt-, and PI3K-signaling in CSCs, thereby supporting tumor propagation (31). In metastasis, vascular structures have also been demonstrated to be an important site for disseminated cancer cells. Invading cancer cells from breast, lung, and melanoma lesions have been shown to adhere to and stretch around blood capillaries in the brain and significantly benefit from the perivascular niche (67, 138). Real-time imaging revealed that cancer cells in the brain stay at a perivascular niche in close contact to endothelial cells after extravasation through the vascular wall (67). Over 90% of cancer cells invading the brain die, but the surviving cells stretch around capillaries and grow out on coopted vessels (138). In breast and lung cancer models, adhesion to brain capillaries was found to be dependent on the expression of the cell adhesion molecule LICAM by metastatic cancer cells, mediating vascular cooption and metastatic outgrowth (138). Very little is known about the specific supportive signals that the perivascular niche produces in favor of cancer cell viability. However, the perivascular location may provide oxygen and nutrients as well as attachment to support MetSCs and to further the development of brain metastasis (67, 112). Moreover, evidence suggests that Notch signaling may be promoted by endothelial niches. In a mouse model of colorectal cancer, tumor growth was promoted by the production of the Notch ligand Jagged-1 by endothelial cells that induced Notch signaling and promoted stemness (82). While specific tissue structures can support metastatic cancer cells, remodeling of the microenvironment may be required additionally to produce a fully competent metastatic niche.

**Pre-metastatic niche.** The stroma of secondary organs can be modified by systemic signals from the primary tumor. This effect may even occur before the arrival of cancer cells at the secondary site and the modified microenvironment has therefore been termed a pre-metastatic niche (115). Tumor-derived secreted factors such as VEGF-A and placental growth factor (PIGF) promote mobilization of bone marrow-derived cells (BMDCs) and their accumulation in the lung (64, 115). This is associated with significant changes in extracellular matrix (ECM) composition and matrix remodeling. Fibronectin is upregulated within the niche and the matrix remodeling enzyme MMP9 is induced (57, 64). Moreover, hypoxic tumor cells within the primary lesion secrete the enzyme lysyl oxidase (LOX) that cross-links collagen, promoting adhesion of BMDCs that express MMP2 and enhancing cancer cell invasion in the lungs (39). In a recent study, systemically released LOX was also identified as a promoter of the pre-metastatic niche in the bone, where it regulates osteoclast differentiation, supporting osteolytic bone metastases (35). S100 calcium-binding proteins A8/9 (S100A8/9) are upregulated in the pre-metastatic lung niche and have been shown to promote the expression of serum amyloid A3 (SAA3), which in turn recruits CD11b-expressing myeloid cells and induces ECM remodeling enzymes (58, 128). Interestingly, recent studies suggest that exosomes, which are secreted membrane-bound vehicles carrying protein, DNA and RNA, can play a role in the development of pre-metastatic niches (8). Exosomes from primary melanoma lesions can enhance lung endothelial permeability and promote tumor growth and metastatic progression through education of bone marrow progenitor cells by delivering the receptor tyrosine kinase Met to the cells (110). These results underscore the importance of variable systemic signals induced by the primary tumor, that lead to changes in secondary microenvironments and promotion of metastatic colonization.

**Dormancy**

Most infiltrating cancer cells fail to reinitiate growth at distant sites when they are confronted with a foreign cellular composition and a distinct ECM and cytokine milieu in secondary organs. Some cancer cells can enter dormancy in distant sites and remain as residual disease without causing the patient adverse symptoms (2). Evidence from human cancer patients indicates that most DTCs are nonproliferative and arrested in the G0 phase of the cell cycle (2, 108). The understanding of mechanisms involved in dormancy is still exceedingly rudimentary. However, recent studies have provided some insights. The mitogen-activated protein kinases (MAPKs) p38 and extracellular signal-regulated kinase (ERK) have been suggested to regulate dormancy. A balance between the activity of p38 and ERK was shown to determine whether cancer cells enter dormancy, in which dormant cells exhibit high p38 activity, but low ERK activity (3, 4). Since chemotherapy drugs primarily target rapidly dividing cells, dormant cancer cells exhibit chemotherapy resistance (49, 73).

While inherent properties of cancer cells may significantly affect the prospects of dormancy (70), the microenvironment
also plays a crucial role. Signals from the foreign environment may force DTCs into dormancy until a receptive microenvironment is established (130) (Fig. 2B). Alternatively, microenvironmental factors may select cells that enter dormancy and thereby avoid elimination. A nonpermissive microenvironment may lack nutrients, oxygen, and necessary growth factors and induce the activation of stress signaling pathways in cancer cells, ultimately leading to a state of dormancy (2). Moreover, factors like bone morphogenetic protein 7 (BMP7) secreted by stromal cells residing in the bone marrow can result in dormancy of prostate cancer cells injected into the bone (74). Interestingly, growth of these cells could be reinitiated upon withdrawal of BMP7 (74). Other identified stromal factors that promote dormancy include BMP4 and growth arrest-specific protein 6 (GAS6) (130). BMP4 is abundantly present in the lung and was shown to mediate breast cancer cell dormancy (48). GAS6 produced by osteoblasts induced prostate cancer cell dormancy in the bone marrow (127). In addition, stromal-derived exosomes from the bone marrow carrying microRNAs such as miR-23b were shown to induce dormancy in metastatic breast cancer cells (77, 98).

Dormancy may shelter DTCs from environmental stress. However, to resume metastatic growth, dormant cancer cells must reenter a proliferative state. Several observations support the notion that reactivated DTCs are the source of metastasis. For example, the correlation between DTCs in the bone marrow of breast, colorectal, esophageal, and lung cancer patients and a reduced metastasis-free survival suggests a plausible link (22, 79, 107, 134). Indeed, experiments show that DTCs isolated from distant organs can effectively grow when placed in a different context. In a mouse model for metastasis, dormant breast cancer cells isolated from seemingly metastasis-free organs of tumor-bearing mice were shown to resume proliferation when cultured in vitro and subsequently to produce tumors and metastases in vivo after reimplantation into the mouse mammary gland (132). Of note, these reimplanted cells remained dormant in the bone marrow, which underlines the importance of cellular context in DTC fate. Consequently, some microenvironments may induce tumor cell dormancy and suppress cancer cell reactivation, while other tissues may be conducive to metastatic outgrowth by the same cancer cells (20, 49).

Reactivation

How cancer cells are reactivated from dormancy is still enigmatic and although recent studies are beginning to break ground, immense work still lies ahead. To overcome dormancy and to initiate metastatic growth, disseminated cancer cells likely need to adapt to the particular tissue microenvironment of the distant organ. In a mouse model for breast cancer metastasis, cancer cells were shown to express the secreted protein Coco, a BMP antagonist, to counteract BMP-mediated dormancy in lungs (48). It is unknown how Coco is induced in dormant cancer cells, but signals from the microenvironment may be involved. Several microenvironmental factors have been shown to trigger reactivation of dormant DTCs (130) (Fig. 2B). Barkan et al. (9) demonstrated that breast cancer cells can induce the switch from dormancy to metastatic growth by engaging the ECM. The switch was found to be dependent on fibronectin production and β1-integrin signaling that resulted in cytoskeletal reorganization and formation of actin stress fibers (10). Moreover, induction of fibrosis at the metastatic site has been suggested to trigger the transition from dormancy to cancer growth (9). While mammary cancer cells remained dormant in normal lungs, TGF-β1-induced pulmonary fibrosis with extensive type I collagen deposition stimulated mammary cancer cells to exit dormancy and to form proliferative metastatic lesions in vivo (9). The inflammatory state of the secondary microenvironment and ECM stiffness induced by TGF-β1 may thus regulate DTC escape from dormancy (21). This is further supported by an in vitro study on hepatocellular carcinoma showing that an increased ECM stiffness, which is characteristic of inflammation, promotes proliferation regulated by β1-integrin and FAK, whereas a soft ECM induces dormancy in hepatocellular carcinoma cells (122). In a mouse model of breast cancer, metastatic cancer cells invading the lungs were found to activate FAK signaling that was dependent on cell-matrix adhesions via β1-integrins and enabled cell proliferation and growth of micrometastases (125). Finally, evidence from three-dimensional culture models suggests that sprouting neovascularization may also trigger the outgrowth of dormant DTCs (50).

To initiate tumor cell proliferation in distant sites, MetSCs that have undergone EMT may need to reacquire an epithelial phenotype after extravasation. The reversion towards an epithelial phenotype, termed mesenchymal-epithelial transition (MET), can be induced by microenvironmental factors. Myeloid cells recruited to pre-metastatic lungs were shown to induce MET in disseminated breast cancer cells by expressing the ECM component versican (46). Myeloid cell-derived versican reverts TGF-β pathway activity, resulting in MET and accelerated metastasis formation (46). Moreover, metastatic colonization of breast cancer cells was found to require the loss of the homeobox factor paired related homeobox 1 (Prrx1), which reverted EMT and enabled the acquisition of stem cell properties (96). In a spontaneous squamous cell carcinoma mouse model, repression of the transcription factor and EMT regulator Twist1 was required in cancer cells at distant sites to enable metastasis formation (136). MET facilitates cancer cell adhesion to the parenchyma, establishing interactions with cells of the foreign microenvironment, and thus promotes metastatic outgrowth (147).

Reconstructing and Educating the Microenvironment

While the reacquisition of an epithelial phenotype may help invasive cancer cells to adapt to ectopic stroma, a subsequent modification of the environment could be needed to grow further and develop macrometastases. MetSCs may modify the microenvironment and build a metastatic niche that supports a continued metastatic growth. Aberrant (pathological) tissue microenvironments can promote cancer and metastasis formation. Ageing, chronic inflammation, and responses to chemicals or radiation have been associated with cancer and are all characterized by a disturbed and remodeled microenvironment (8). Increasing evidence suggests that metastatic cancer cells are also able to remodel the stroma of the secondary organ. Thus, while the stroma may initially suppress metastasis, a reactive stroma can be more permissive to metastatic outgrowth (15).
Inflammatory environment. Disseminated cancer cells can recruit several distinct inflammatory cell types to their local milieu, such as myeloid precursor cells and TAMs (61, 112). In lung cancer metastasis models, cancer cells activate myeloid cells by secreting the matricellular component versican, which binds to Toll-like receptors (TLRs) 2 and 6 and thereby stimulates the production of proinflammatory cytokines IL-6 and TNF-α by myeloid cells (71). Recruited stromal cells establish paracrine loops with cancer cells, promoting metastatic growth. For example, disseminated breast cancer cells overexpressing CXCL1/2 attract myeloid progenitor cells to the lungs, which in turn secrete S100A8/9, supporting cancer cell survival in lung metastasis (1). Moreover, recruited cells can further modify the stroma, facilitating metastatic growth. TAMs recruited to the metastatic site secrete VEGF and proteases that promote matrix remodeling and angiogenesis (114). In mouse models of breast cancer metastasis to the lung, CCR2-expressing inflammatory monocytes and TAMs are recruited to growing lung metastases via CCL2 (116, 117). Recruited TAMs in turn promote extravasation, seeding, and metastatic outgrowth of breast cancer cells in the lung (117). Moreover, breast cancer cells expressing the adhesion molecule VCAM-1 bind to α4-integrins on metastasis-associated macrophages in the lungs, promoting cancer cell survival and metastatic growth (32).

Reactive fibroblasts: myofibroblasts. Resting fibroblasts can inhibit cancer cell growth, whereas activated fibroblasts, termed myofibroblasts or cancer-associated fibroblasts (CAFs), provide a favorable environment for metastatic propagation (37, 62). Activation of fibroblastic stroma is firmly linked to wound healing and tissue regeneration. Fibroblast activation is accompanied by increased proliferation, an enhanced secretion of ECM proteins, the expression of α-smooth-muscle actin (αSMA), and a spindle-shaped morphology (62). Importantly, CAFs may be highly heterogeneous based on their origin and inducing signals. Normal fibroblasts have for example been shown to exhibit already significant heterogeneity between distinct tissues and CAFs may originate from different cell types, such as resident stromal fibroblasts, mesenchymal stem cells, pericytes, adipocytes, and endothelial cells (30, 62). The fibroblast transition from a resting state that represses cancer growth to an activated state that promotes cancer progression appears to be crucial to macrometastatic growth. The identification of several myofibroblast-inducing cytokines suggests that cancer cells transform resting fibroblasts into myofibroblasts by secreting factors that overlap with those of normal wound healing processes, during which fibroblasts are physiologically activated.

Although the molecular interactions between cancer cells and myofibroblasts in metastasis are poorly known, evidence from primary tumors provides insights that may apply to metastatic disease. TGF-β is one of the primary factors driving fibroblast activation in wound healing and fibrosis (18, 40) and has been implicated in cancer in the conversion of mesenchymal stem cells into CAFs (8, 13, 118). Colorectal cancer cells stimulate a pro-metastatic program in stromal cells via TGF-β, which promotes IL-11 secretion by activated fibroblasts (25). Fibroblast-derived IL-11 feeds back to cancer cells and triggers glycoprotein 130 (gp130)/signal transducer and activator of transcription 3 (STAT3) signaling, resulting in enhanced survival of metastatic colorectal cancer cells (25). In addition, cancer cells have been shown to stimulate fibroblast proliferation and differentiation into myofibroblasts by secreting basic fibroblast growth factor (bFGF), EGF, IL-6 and platelet-derived growth factor (PDGF) (51, 62). Whereas activated fibroblasts return to a resting state after tissue repair in wound healing, CAFs stay in a continuously activated state in tumors (38, 62). The sustained activation of fibroblasts may be attributed to the perpetual secretion of activating cytokines by the tumor (65). Activated fibroblasts were shown to play an important role in metastasis, producing ECM proteins that are essential for metastatic colonization (86, 95, 101, 112). Moreover, activated fibroblasts secrete cytokines such as CXCL12, VEGF, PDGF, and HGF (112). CAFs isolated from primary human breast cancer samples express high levels of CXCL12, promoting recruitment of endothelial cells and growth of CXCR4-expressing breast cancer cells in model systems (99). During wound healing, myofibroblasts recruit endothelial precursor cells to the wound site by CXCL12 and induce their differentiation into mature endothelial cells by the secretion of VEGF (12, 145). Similarly, VEGF-A secreted by S100A4+ fibroblasts was found to induce an angiogenic microenvironment in a mouse model for breast cancer metastasis to the lung, fostering metastatic colonization (95). The evidence indicates that activation of stromal fibroblasts may be an important factor in the establishment of a metastatic niche.

Matrix alterations. Stromal fibroblast activation in metastasis coincides with the production of specific ECM proteins. The matrix composition in metastatic lesions is also concordant with ECM produced during wound healing and under pathological conditions such as fibrosis (120). The ECM exhibits high density, and proteins include fibrillar collagens, fibronec tin, and matricellular proteins (9, 17, 64, 100). CAFs are likely to be a major source of these matrix proteins (62). Matricellular proteins are an intriguing group of heterogeneous matrix proteins that serve an important regulatory role in the ECM. Interestingly, matricellular proteins such as TNC and periostin (POSTN) are expressed in normal stem cell niches, suggesting that they may regulate stem cell properties and behavior (100). Breast cancer cells that express TNC have a survival advantage upon extravasation into the lungs (101). However, following initial colonization CAFs become a significant source of TNC, fueling continued metastatic growth (95, 102). TNC supports MetSC survival and fitness by increasing responsiveness to Wnt and Notch signaling pathways (101). The Wnt pathway is also regulated by POSTN, that supports stem cell maintenance by presenting Wnt ligands to cancer cells within the metastatic niche (86). Metastatic breast cancer cells were found to induce POSTN in lung stromal fibroblasts by the secretion of TGF-β3 (86). Of note, POSTN has been shown to directly bind TNC and to facilitate TNC incorporation into the ECM (68). Considering this, it is tempting to hypothesize that TNC and POSTN may be close collaborators within the metastatic niche (103).

ECM remodeling is important for the generation of metastatic niches, and changes in levels of ECM remodeling enzymes such as MMPs, cathepsins, and LOX family members are frequently observed in metastasis (17, 61). Cancer cells have been shown to cause downregulation of Fibulin-5 in stromal fibroblasts, thereby increasing MMP9 production and enhancing metastatic colonization (92). In breast cancer models, cathepsin B promotes degradation of collagen, leading to
increased invasion and metastasis (140, 142). Biomechanical attributes of the matrix change significantly during cancer progression and metastasis, where tissue tension is induced via LOX-mediated collagen cross-linking (76). Matrix stiffness can modulate Hippo signaling, leading to activation of the transcriptional coactivator with PDZ-binding motif (TAZ) and increasing self-renewal and growth initiation capacities of breast cancer cells (34).

Remodeling vasculature. Sufficient blood supply must be ensured for metastatic outgrowth, and the lack of vascularization may prevent the development of macrometastasis (28, 47). Since the normal vasculature is usually quiescent in the adult, tumor cells must activate endothelial cells to stimulate the growth of new blood vessels (55). Metastatic breast cancer cells have been shown to recruit endothelial cells by secreting insulin-like growth factor-binding proteins 2 (IGFBP2) and soluble c-Mer tyrosine kinase (MERTK) receptor, and endothelial cell recruitment was inhibited by miR-126-mediated repression of these factors, resulting in significantly reduced metastasis (113). Other cancer cell-derived miRNAs were shown to promote endothelial recruitment and metastatic outgrowth. miR-1908, miR199a-5P, and miR-199a-3P were found to target Apolipoprotein E (ApoE), an inhibitor of endothelial recruitment, and thereby supported angiogenesis and melanoma metastasis (111). Induction of proangiogenic factors such as VEGF and bFGF has been observed in advanced cancer as well as perturbation of antiangiogenic factors like angiotatin and endostatin (55). Cancer cells can invoke neovascularization by the induction of the transcription factor inhibitor of DNA binding 1 (Id1) in bone marrow-derived endothelial precursors (47). In contrast, Id1 blocking was found to impair angiogenesis and macrometastatic outgrowth in the lungs (47). Moreover, in a mouse model of breast cancer, Wnt7b expression in myeloid cells was found to be required for neovascularization by inducing VEGF-A in endothelial cells (148).

Tissue-specific stromal reconstruction. Depending on the site of metastasis, cancer cells may face a unique stromal composition. To establish a supportive environment and grow out successfully, interactions with particular stromal components may be required. Osteolytic bone metastasis is a prime example of this. Invading cancer cells in the bone interact with osteoclasts, resulting in a feed-forward loop that can lead to significant bone loss (119, 144). Osteoclast mobilization and activation are induced by metastatic cancer cells via numerous factors, including parathyroid hormone-related protein (PTHrP), the cytokines IL-11 and TNF-α, the proteolytic enzymes A disintegrin and metalloproteinase with thrombospondin motifs 1 (ADAMTS1) and MMP1, VCAM-1 and the notch ligand Jagged-1 (52, 63, 83, 84, 124, 144). Metastatic cancer cells stimulate osteoclast-mediated bone resorption, releasing matrix-bound growth factors such as TGF-β, IGFs, FGF, PDGF, and BMPs (144). These factors in turn feed back to the cancer cells, inducing a vicious growth cycle and stimulating metastatic progression (119, 144).

The brain also represents a unique microenvironment within the human body, which stands out with a significantly different ECM composition and brain-specific cell types. The brain microenvironment frequently responds to insults such as infection, trauma, stroke, or neurodegeneration with reactive gliosis, a process that is also observed in and around human and experimental brain metastases (19, 23, 43, 123). The main mediators of this response are reactive glial cells including astrocytes, microglia, and oligodendrocytes. Evidence suggests that metastatic cancer cells interact with reactive glial cells to establish a permissive metastatic niche. For example, metastatic cells may hijack the neuroprotective effect of reactive glia for their own survival. Brain metastatic melanoma cells were found to be protected from chemotherapy in vitro by reactive astrocytes via direct cell-cell contact (78). Similarly, direct interaction with astrocytes induces upregulation of the survival factors gluthathione S-transferase A5 (GSTA5), BCL2-like 1 (BCL2L1), and Twist1 in breast and lung cancer cell lines and protects them from chemotherapy (72). Furthermore, brain-metastatic lung cancer cells were shown to activate astrocytes in vitro by secreting IL-8, macrophage migration inhibitory factor (MIF), and plasminogen activator inhibitor-1 (PAI-1) (123). Activated astrocytes in turn secrete the proinflammatory cytokines IL-6, TNF-α, and IL-1β and thereby promote lung cancer cell proliferation (123). Reactive astrocytes may also sustain stem cell properties of metastatic cancer cells. Metastatic breast cancer cells were found to produce high levels of IL-1β, which upregulates the Notch ligand Jagged-1 in reactive astrocytes, thereby promoting self-renewal of breast MetSCs through activation of the Notch pathway and induction of the transcription factor hairy and enhancer of split 5 (HES-5) (146). However, not all signals from reactive astrocytes are beneficial for cancer cells, underscoring the complexity of reactive gliosis. For example, plasminogen activator (PA) proteins are secreted by astrocytes and convert plasminogen to the active protein plasmin, which in turn inhibits cancer cell adhesion to brain capillaries (138). The ability of cancer cells to interact with organ-specific stromal cells may lead to stromal reconstruction and development of metastasis at these sites.

Perspectives

Growing evidence suggests that during metastatic progression, cancer cells and the host microenvironment coevolve. We have discussed how dynamic interactions between metastatic cancer cells and the distant stroma influence the metastatic process. A healthy stroma may initially eliminate or suppress the growth of most cancer cells that invade a secondary organ. In several malignancies, such as cancers of the breast and prostate, the resting microenvironment may induce cancer cell dormancy, clinically presenting as disease latency that can last for several years. The few cancer cells that survive this may initiate metastatic growth and reconstruct the surrounding microenvironment, leading to further support of expanding metastasis. However, it is important to note that a significant difference is observed in disease progression between different cancer entities. For example, pancreatic cancer and lung cancer do not exhibit long latency and instead metastasize quickly (93). This suggests that the dependency of cancer cells on the secondary microenvironment and the need for adaptation to the foreign stroma may vary between cancers.

Currently, therapeutic treatments against advanced cancer metastasis are extremely challenging. Emerging studies demonstrating a pivotal role of the microenvironment in metastasis stress the importance of considering both cancer cell properties and the reactive stroma when developing cancer treatments.
Disrupting the cross talk between cancer cells and reactive stroma could be an effective strategy. However, since interactions between metastatic cancer cells and the stroma may continuously evolve during disease progression, an understanding of the dynamic interplay and evolution may be necessary to identify the appropriate treatment for a particular stage in the development of metastasis.

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