Trends in Cancer

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Review

It's all about the base: stromal cells are central orchestrators of metastasis

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The tumor microenvironment (TME) is an integral part of tumors and plays a central role in all stages of carcinogenesis and progression. Each organ has a unique and heterogeneous microenvironment, which affects the ability of disseminated cells to grow in the new and sometimes hostile metastatic niche. Resident stromal cells, such as fibroblasts, osteoblasts, and astrocytes, are essential culprits in the modulation of metastatic progression: they transition from being sentinels of tissue integrity to being dysfunctional perpetrators that support metastatic outgrowth. Therefore, better understanding of the complexity of their reciprocal interactions with cancer cells and with other components of the TME is essential to enable the design of novel therapeutic approaches to prevent metastatic relapse.

Highlights

Reprogramming and activation of stromal cells in metastatic organs precedes the formation of metastases.

Fibroblasts, osteoblasts, and astrocytes have similar roles in metastatic progression in their respective organs, as well as organ-specific roles.

During the metastatic cascade, stromal cells receive multiple activating signals, resulting in dysfunction: instead of resolving the tissue damage elicited by the disseminated cancer cells, they become culprits that support metastatic growth.

Stromal cells modulate the metastatic immune microenvironment by instigating inflammation and promoting the formation of an immunosuppressive metastatic microenvironment.

Activated stromal cells attract tumor cells, facilitate cancer cell dormancy and awakening, and promote tumor cell invasion, survival, and proliferation, ultimately supporting metastatic outgrowth.

Introduction

Metastatic spread of cancer cells to distant organs is the main cause of cancer-related death. It is now established that tumors are complex ecosystems comprising multiple cell types, including resident and recruited stromal and immune cells, blood vessels, and components of the extracellular matrix (ECM), collectively termed the TME [1]. The TME plays a central role in all stages of carcinogenesis. Most of our current knowledge on the TME is based on studies in primary tumors. However, when tumor cells disseminate to distant organs, they must adapt to distinct organ-specific microenvironments where the stromal cell composition and ECM differ from those at the primary site and employ unique molecular and cellular interactions to support or oppose the growth of disseminated cancer cells. To thrive at the metastatic site the cancer cells need to reshape their signaling pathways to effectively communicate with the new TME. These interactions are reciprocal, and stromal cells at the metastatic organs also undergo profound changes. Therefore, better understanding of organ-specific TMEs is an essential challenge to enable better therapeutic strategies that may prevent metastatic relapse.

Resident stromal cells (e.g., fibroblasts, osteoblasts, astrocytes) are essential components of all organs and play a central role as sentinels in sustaining tissue homeostasis (Figure 1). Stromal cells in primary tumors were shown to facilitate tumorigenesis in multiple cancer types [2–4]. While stromal cells at metastatic sites have similar functions in enabling metastatic growth, they have an additional key role in preparing the premetastatic niche. Secreted factors and extracellular vesicles (EVs) from cancer or stromal cells at the primary tumor reprogram stromal cells at the metastatic site to create a hospitable niche for disseminated cancer cells [5]. Like many other aspects of metastasis, this important aspect of the determination of metastatic growth was also shown to be organ-specific [6]. A large body of data led to our understanding of the role of fibroblasts (Box 1), osteoblasts (Box 2), and astrocytes (Box 3) in cancer initiation and progression.

The role of stromal cells in metastatic relapse is also emerging. Activated stromal cells in various organs prepare the 'soil' for metastatic seeding. A temporal outlook on the function of stromal

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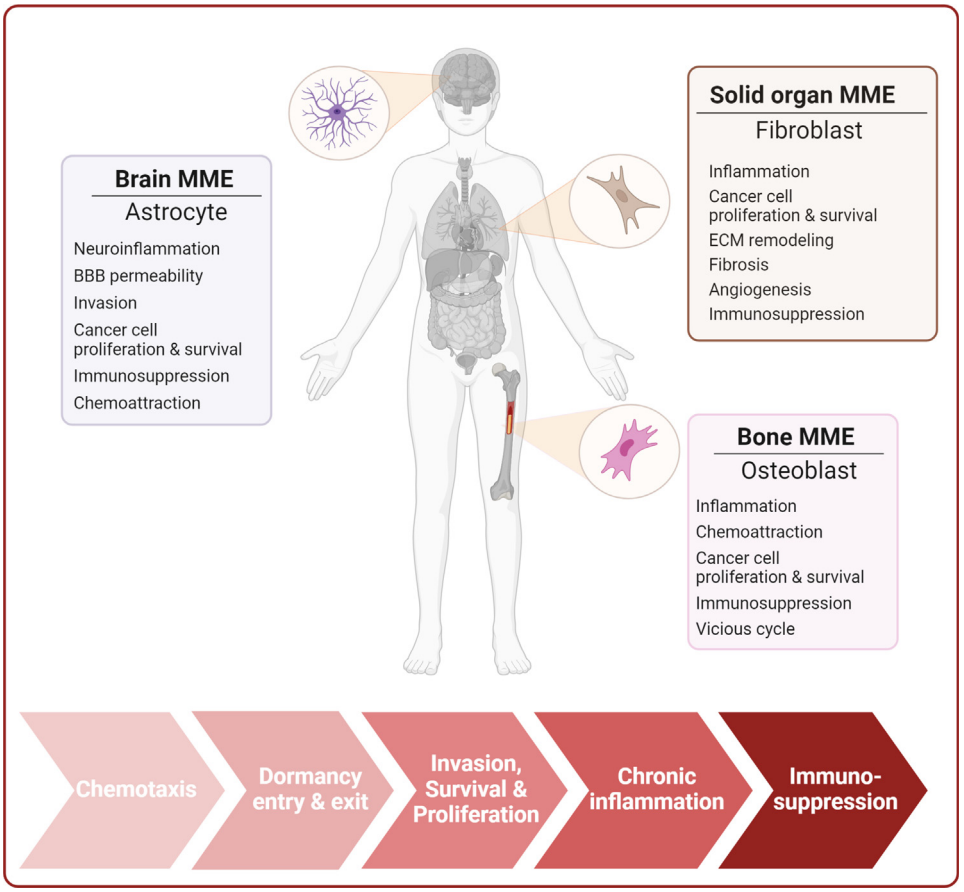

Figure 1. Stromal cells in the metastatic microenvironment (MME). Efficient metastasis requires preconditioning of the metastatic site prior to colonization. Primary tumors can remotely activate stromal cells in distinct organs. Systemic signaling from the primary tumor can activate astrocytes in the brain, osteoblasts in the bones, and fibroblasts in internal organs. Activated stromal cells prepare the soil for metastatic seeding, attract tumor cells to the metastatic site, sustain cancer cell dormancy, and mediate dormancy exit. Stromal cells can promote tumor cell invasion, survival and proliferation, supporting colonization of the metastatic site. Metastatic growth is accompanied by stroma-mediated inflammation, resulting in the formation of an immunosuppressive MME. Abbreviations: BBB, blood–brain barrier; ECM, extracellular matrix.

cells reveals that they facilitate the chemoattraction of disseminated cancer cells to the metastatic site, sustain cancer cell dormancy, and mediate dormancy exit. Stromal cells can also promote tumor cell invasion, survival, and proliferation and modulate the formation of a suppressive immune microenvironment at metastatic sites, thus supporting metastatic growth.

Here, we review recent findings on the metastasis-promoting roles of cancer-associated fibroblasts (CAFs), osteoblasts, and astrocytes in organ-specific metastasis, discuss the similarities and discrepancies between these stromal cells at different metastatic sites, and envisage possible avenues for future studies aimed at targeting stromal pathways to inhibit tumor metastasis.

Chemoattraction of tumor cell to metastatic sites

Metastasis is a multistage process. One of the early stages of the metastatic cascade includes the homing of disseminated cancer cells to metastatic organs. This can be facilitated by the secretion of chemoattractants by stromal cells in target organs, such as fibroblasts in lungs/liver/visceral

Box 1. Visceral organ stromal compartment: fibroblasts

Fibroblasts are a vastly heterogeneous cellular component of connective tissue in most organs. They provide structural scaffolding and growth regulatory elements in homeostatic tissues and are key players in tissue remodeling, wound healing, and scarring following tissue damage.

CAFs play a central role in the TME of various cancer types and metastatic sites. CAFs' functional heterogeneity and their roles in facilitating tumor progression as well as their ability to affect therapy response have been the topic of extensive research [7]. Reminiscent of their roles in wound healing, CAFs were shown to sense tissue damage, modulate ECM composition, affect matrix stiffness, and modify cancer cell trafficking. CAFs are also major modulators of immune responses in the TME: they were shown to promote inflammation, present antigens, induce type II immunity, and promote a permissive TME by recruiting immunosuppressive cells and by enabling T cell exclusion from tumors [1,8,9]. Targeting of subpopulations of CAFs was shown to potentiate immunotherapy [10,11].

Several studies have shown that CAFs at primary tumors can promote metastatic progression [12–18]. However, there remains much to learn on how fibroblasts are affected at the premetastatic niche and on their functional evolution during the early stages of metastatic progression.

Fibroblasts are prominent components of metastatic lesions even in organs in which there are normally few fibroblasts (like brain and bone). One of the origins of metastasis-associated fibroblasts is the BM: fibroblasts were shown to be specifically recruited from the BM into the lung metastatic niche in a transgenic mouse model of breast cancer [19].

organs, osteoblasts in the bones, and astrocytes in the brain (Table 1). Notably, the specific chemokine–chemokine receptor pathways engaged are physiologically employed by stromal cells to recruit immune cells to the relevant organ.

Fibroblasts

The role of fibroblasts in secreting cytokines and chemokines that promote metastasis begins at the primary tumor site: mammary fibroblasts secreted interleukin (IL)-1β that systemically affected lung endothelial cells to be more permissive for disseminated breast cancer cells [40]. CAF subsets can be distinct in primary tumors and their metastases. For example, $STEAP4^+$ $ADGRF5^+$, and $CXCR4^+$ $SRGN^+$ fibroblast subsets were significantly higher in lymphatic metastases compared with primary prostate lesions and were implicated in tumor progression, metabolism, and immunosuppression [41].

In breast cancer metastasis, signaling from lung fibroblasts enhanced metastatic colonization by cancer cells in multiple organs. Breast cancer cell-derived IL-1α/β upregulated lung fibroblast

Box 2. Bone metastasis stromal compartment: osteoblasts

Bone comprises a unique microenvironment that provides a favorable niche for metastatic cell seeding [20]. Breast, lung, prostate, and kidney cancers are the cancer types that most frequently spread to bones.

Osteoblasts are mesenchymal bone cells that arise from the differentiation of MSCs. Recent studies demonstrated that osteoblasts constitute a heterogeneous cell population, revealing a more diverse and complex ecosystem than previously believed [21–23]. In healthy adults, bone continuously undergoes remodeling. Bone homeostasis is a delicate balance between bone formation by osteoblasts and bone resorption by osteoclasts, reciprocally affecting each another with no net gain or loss in bone mass. This balance is drastically perturbed in cancer metastasis, leading to excessive bone destruction or production depending on the cancer type [20,24].

Since osteoblasts are a rare cell population, they are poorly represented in large-scale analyses of bone cells such as scRNA-seq [22] and there remains much to learn about their cancer-related tasks.

One of the changes in bone homeostasis during metastasis is the disruption in the composition and function of osteoblasts. Osteoblast abundance was found to be highly dynamic during the process of bone metastasis in multiple cancer types [25]. At early stages of metastatic colonization, when cancer cells homed to the bone, an initial increase in osteoblast numbers was observed, followed by a drastic decrease at later stages accompanied by elevated osteoclasts [26,27]. These findings suggest that osteoblasts may play distinctive roles during the early and late stages of bone metastasis and underline the need for deeper understanding of cancer-associated osteoblast plasticity.

Box 3. Brain metastasis stromal compartment: astrocytes

Brain metastases are up to ten times more frequent than primary brain tumors in adults and remain a significant challenge in clinical management, as surgery and standard therapies such as radiation and chemotherapy offer limited efficacy and very limited survival [28]. Lung, breast, and melanoma cancers are the most common malignancies associated with brain metastasis [29]. Reciprocal interactions between cancer cells and the brain microenvironment were shown to contribute to tumor progression and to organ-specific metastasis [30].

Astrocytes are star-shaped glial cells in the central nervous system (CNS) that perform many functions in maintaining homeostasis [31]. Under normal conditions, astrocyte end feet are part of the BBB. Astrocytes regulate extracellular ion and fluid composition and participate in the maintenance of synaptogenesis, synaptic plasticity, neurotransmitter clearance, and neurotrophin secretion. This functional diversity is performed by a highly heterogeneous cell population of astrocytes [32].

Astrocytes respond to injury by instigating a gene program that induces morphological and transcriptomic changes, which renders them reactive [33]. Reactive astrocytes are characterized by high levels of glial fibrillary acidic protein (GFAP); they perform neuroprotective functions that support neuronal recovery during the acute phase of tissue damage response, but may entail detrimental inflammatory responses that induce further tissue damage on chronicity [34]. Astrocyte subpopulations perform a range of tasks, some linked to neuroinflammation whereas others mediate tissue repair [35]. Thus, reactive astrocytes have been reported to improve or worsen the progression of various diseases, including brain metastasis [36]. While the initial response of reactive astrocytes to brain-infiltrating cancer cells was suggested to be antitumorigenic [37], multiple other studies indicated that reactive astrocytes surround and infiltrate brain metastases and support their subsequent growth [38,39].

CXCL9, which in turn attracted CXCR3-expressing cancer cells, thus fueling the growth of lung metastases in xenograft and syngeneic mouse models [42]. Similarly, CAFs derived from human colorectal liver metastasis secreted factors that enhanced the migration of colon cancer cells *in vitro*, which could be blocked by inhibiting nuclear factor kappa B (NF-κB) signaling [43]. These findings suggest that CAFs at the metastatic niche can facilitate the invasion of new metastatic sites via chemoattraction of cancer cells (Table 1).

Table 1. Chemoattraction and dormancy

Effect on cancer cells	Stromal cells	Cancer type	Metastatic site	Upstream pathway/activator	Molecule produced by stromal cells	Molecule targeted in cancer cells	Targeting therapy	Refs
Chemoattraction	Fibroblasts	Breast	Lung	IL-1a/b	CXCL9	CXCR3		[42]
	Fibroblasts	CRC	Liver	TNF-a	NF-κB related; IL-8		NF-κB inhibitor (parthenolide)	[43]
	Osteoblasts	Prostate	Bone		SDF-1 (CXCL12)	CXCR4		[45,54]
	Astrocytes	Melanoma	Brain		CXCL10	CXCR3	Genetic inhibition of CXCR3	[47]
Dormancy	Aged fibroblasts	Melanoma	Lung		sFRP1	WNT5A		[51]
	Aged fibroblasts	Breast	Lung		PDGFc			[52]
	Osteoblasts	Prostate	Bone		TGFb2 and GDF10	TGFβRIII to activate phospho-p38MAPK		[56]
	Osteoblasts	Prostate	Bone		Annexin II, GAS6	Annexin II receptor; AXL, Sky, and Mer		[55]
	Osteoblasts	Prostate	Bone		TGFb2 and GDF10	TGFβRIII to activate phospho-p38MAPK		[56]
	Osteoblasts	Prostate and AML	Bone		CXCL12	CXCR4	Combination of chemotherapy with CXCR4 antagonist	[54]
	Astrocytes	Breast	Brain		Laminin-211	YAP sequestration		[58]

Osteoblasts

Multiple studies have shown that cancer cells home to bones by hijacking the SDF-1 (CXCL12)–CXCR4 signaling axis in various cancer types such as breast, prostate, myeloma, and leukemia [44,45]. Osteoblasts express the chemokine CXCL12 while metastatic cancer cells express its receptor, CXCR4. By hijacking this signaling axis, which is physiologically relevant to the mobilization of hematopoietic stem cells (HSCs), cancer cells home to the HSC niche in bones, competing with local HSCs. Mobilizing HSCs to the circulation prior to tumor cell inoculation resulted in increased numbers of tumor cells colonized in the trabecular region of the bone [46].

Astrocytes

Astrocytes were shown to mediate brain tropism of melanoma cells via hijacking of the CXCL10–CXCR3 signaling axis, which is physiologically important for T cell recruitment. A subset of melanoma cells in a mouse model of melanoma expressed CXCR3, which facilitated their chemoattraction to activated proinflammatory astrocytes that secreted CXCL10 [47]. Genetic inhibition of this astrocyte–melanoma crosstalk reduced brain metastasis formation [47].

Dormancy

Once disseminated tumor cells (DTCs) enter metastatic organs, they may remain in a state of quiescence for extended time periods, resulting in dormant, clinically undetectable microlesions [48]. This emerging research field has potential clinical implications as discussed elsewhere [49]. Importantly, metastatic dormancy is largely dependent on cues from the microenvironment, and specifically from the stromal environment in the niche where the DTCs reside [50] (Table 1 and Figure 2), as discussed later.

Fibroblasts

The fate of DTCs regarding dormancy and awakening was shown to depend on the aging of the host. Aged lung fibroblasts in mouse models of melanoma were shown to support the awakening of dormant cancer cells in the lung premetastatic niche, thus enabling melanoma metastatic outgrowth. Aged CAFs showed increased secretion of the WNT antagonist sFRP1, which inhibited WNT5A in melanoma cells, forcing them to exit their dormant state [51]. Similarly, aged mice were reported to be more prone to develop breast cancer lung metastasis than young mice. PDGF-C was identified as a key mediator of both dormancy and dormancy exit, depending on aging in the microenvironment: high PDGF-C in aged and fibrotic lungs was associated with an accumulation of activated fibroblasts and enhanced metastatic outgrowth [52]. Another role of fibroblasts in sustaining tumor cell dormancy is via their production of ECM components, particularly collagens. Collagens were implicated in sustaining tumor cell quiescence and in the formation of the dormancy-promoting niche [53]. Thus, fibroblasts affect cancer cell dormancy, and an aged stromal compartment fosters a permissive niche (Table 1 and Figure 2).

Osteoblasts

The HSC niche in bones comprises mesenchymal stem cells (MSCs) and osteoblasts. Under physiological conditions, the niche environment maintains HSCs in a dormant state. This function of maintaining the dormancy state may be hijacked by bone-metastasizing cells: human prostate cancer cells were shown to compete with HSCs on occupation of the osteoblastic niche [54], implicating a determining role for osteoblasts in bone colonization. Interestingly, animals with a greater number of HSC niches also presented with enhanced cancer cell dissemination [54]. Osteoblasts were shown to secrete factors such as GAS6 [55] or GDF10 and transforming growth factor (TGF)b2 [56] that inhibited the growth of metastasizing prostate cancer cells, leading to tumor cell dormancy. This growth-arresting effect of osteoblasts on cancer cells is not limited to prostate cancer: myeloma cells were also shown to enter a state of dormancy when interacting with

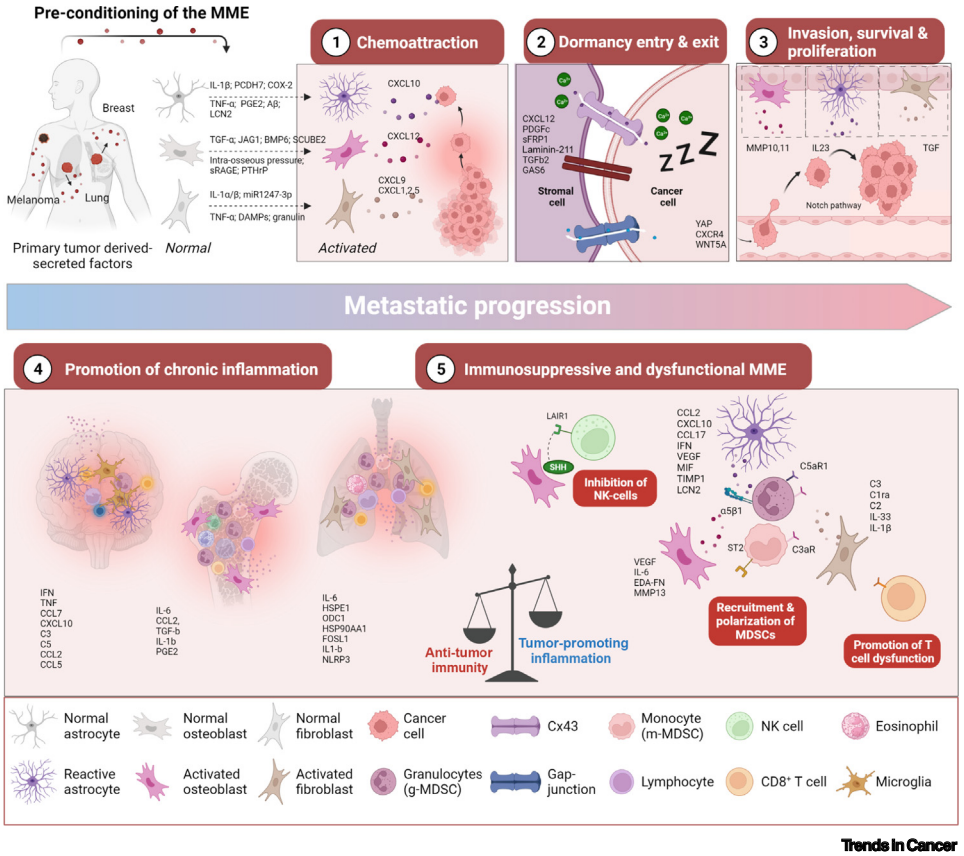

Figure 2. Astrocytes, osteoblasts, and fibroblasts play important roles in all stages of the metastatic process. The contribution of stromal cells in metastasis differs according to the metastatic stage. Systemic signaling from the primary tumor activates stromal cells in distant organs, leading to their activation. In response, stromal cells secrete factors that eventually chemoattract cancer cells to a specific metastatic site (1). Arriving in the new organ, cancer cells may enter dormancy, a process facilitated by stromal cells (2). When exiting dormancy, stromal cells sustain the invasion, survival, and proliferation of cancer cells, leading to metastatic outgrowth (3). This is accompanied by chronic tissue inflammation (4), resulting in the formation of an immunosuppressed and dysfunctional metastatic microenvironment (MME) (5). Abbreviations: COX-2, cyclooxygenase 2; DAMP, damage-associated molecular pattern; EDA-FN, extradomain A-fibronectin; IFN, interferon; IL, interleukin; LCN, lipocalin-2; MDSC, myeloid-derived suppressor cell; MMP, matrix metalloprotease; NK, natural killer; PGE2, prostaglandin E2; PTHrP, parathyroid hormone-related protein; TGF, transforming growth factor; TNF-α, tumor necrosis factor alpha; VEGF, vascular endothelial growth factor.

osteoblasts in the bone niche [57]. Moreover, prostate cancer cells enter dormancy when injected directly into the femur of mice but not when injected subcutaneously [56], further demonstrating the critical role of the organ microenvironment in determining the fate of metastatic cancer cells.

Astrocytes

Astrocytes were implicated in maintaining dormancy and actively inhibiting the growth of brain metastatic cells during the early stages of brain colonization. Astrocytes promoted the dormancy of brain-disseminated cancer cells. Intravital imaging in mouse models of triple-negative breast cancer (TNBC) demonstrated that astrocyte end feet in the brain formed a vascular niche that limited the proliferation of disseminated cancer cells occupying this niche. Dormancy was maintained via deposition of laminin-211 by astrocytes, which promoted quiescence by sequestering cancer cell YAP in the cell membrane, via the dystroglycan receptor [58]. In addition to maintaining dormancy, astrocytes promoted active killing of cancer cells. Studies in mouse models of lung

and breast cancer brain metastasis revealed that astrocytes respond to brain invasion by cancer cells by secreting plasminogen activator (PA), which generates plasmin. Plasmin released membrane-bound FasL from astrocytes, which actively killed cancer cells. In addition, plasmin inhibited the migration of cancer cells along brain capillaries by inactivating L1CAM [37]. Brain-metastatic cells secreted elevated levels of inhibitory serpins, which blocked these antimetastatic effects of astrocytes and enabled cancer cell survival and migration [37]. Astrocytes may also inhibit the growth of brain-metastasizing cells by exosome-mediated signaling: astrocyte-derived exosomal miRNA (miRNA-142-3p) was shown to deplete TRPA1 in lung cancer cells, thus inhibiting FGFR2 signaling and reducing the proliferation and invasion of lung cancer cells [59].

Taken together, these studies suggest that better understanding of the molecular mechanisms in various stromal niches may provide the basis for therapeutic intervention to sustain cancer cell dormancy.

Survival and outgrowth of metastatic cancer cells

In addition to supporting the attraction of cancer cells to metastatic sites and affecting their state of dormancy, stromal cells can support the outgrowth of metastasizing cancer cells by affecting the colonization, invasion, survival, and growth of DTCs (Table 2).

Fibroblasts

Fibroblasts in lymph nodes play a role in facilitating metastatic spread. Flow cytometry analysis of metastatic lymph nodes from breast cancer patients revealed two specific subsets of CAFs that accumulate in metastatic lymph nodes and can promote distant metastatic progression [60]. FAP^{High} $CD29^{Med\text{-}High}$ αSMA^{High} $PDPN^{High}$ $PDGFR\beta^{High}$ CAFs (CAF-S1) were found to stimulate cancer cell migration and to initiate an epithelial-to-mesenchymal transition in cancer cells through the CXCL12 and TGFβ pathways. The second CAF subset, $FAP^{Low\text{-}Med}$ $CD29^{High}$ αSMA^{High} $PDPN^{Low}$ $PDGFR\beta^{Med}$ (CAF-S4), were highly contractile, promoted cancer cell motility and invasiveness via NOTCH signaling, and were strongly associated with metastasis [60]. Importantly, the proportions of each CAF subset could be used as a prognostic factor for metastatic disease. In high-grade serous ovarian cancer (HGSOC), fibroblasts were found to play a critical role in early peritoneal dissemination of aggressive cells: HGSOC cancer cells and fibroblasts isolated from HGSOC patients formed protective spheroids that enabled cancer cells to circulate in ascites fluid and to invade new metastatic sites in the peritoneal cavity [61]. Comparison of omental fibroblasts derived from HGSOC patients with normal omental fibroblasts revealed that cancer cell-derived TGFβ1 induced upregulation of HGF and matrix metalloprotease (MMP)2 expression in omental fibroblasts, resulting in increased adhesion and invasion of ovarian cancer cells in a 3D co-culture model [62]. CAF-derived MMP2 was further implicated in HGSOC peritoneal metastasis: human CAF-derived EVs downregulated miR-29c-3p, which normally targets MMP2. This CAF-induced MMP2 increase resulted in enhanced metastatic potential of ovarian cancer cells in mouse peritoneal xenografts [63].

Osteoblasts

Multiple physiological functions of osteoblasts can be hijacked by bone-metastasizing cells. Secreted factors from immature osteoblasts enhanced the expression of adhesion molecules on bone vascular endothelial cells (BVECs) *in vitro*, thus facilitating the extravasation of cancer cells into the bone marrow (BM) parenchyma [64]. Following extravasation, breast cancer cells predominantly resided in the osteogenic niche, supported by E-cadherin expression on cancer cells and osteogenic N-cadherin expression. These interactions with osteoblasts activated the mammalian target of rapamycin (mTOR) pathway in breast cancer cells, consequently driving the progression to micro/macrometastasis [65]. Another cell-contact-mediated pathway by which osteoblasts

Table 2. Invasion, colonization, proliferation, and survival of cancer cells

Effect on cancer cells	Stromal cell	Cancer type	Metastatic site	Upstream pathway/activator	Molecule produced by stromal cells	Molecule targeted in cancer cells	Targeting therapy	Refs
Invasion	Fibroblasts	Breast	Lymph node		CXCL12/TGFb	NOTCH pathway		[60]
	Fibroblasts	Ovarian	Omentum	TGFβ1	HGF, MMP2		Inhibition of HGF or MMP2	[62,63]
	Fibroblasts	Ovarian	Peritoneum		EGF	$ITGA5^{high}$		[61]
	Osteoblasts	Breast, lung, and prostate	Bone	Pressure	CCL5 and MMPs			[72]
	Osteoblasts	Breast	Bone		MMP10, MMP11, TGFb, and CXCL12		Anabolic PTH	[67]
	Astrocytes	Breast	Brain		S1P3, IL-6, and CCL2		S1P3 inhibition	[75]
	Astrocytes	Melanoma	Brain		IL-23	MMP2		[39]
Colonization	Osteoblasts	Breast	Bone		HIF-1			[68]
	Astrocytes	Melanoma	Brain		CXCL10	CXCR3	Genetic inhibition of CXCR3	[47]
	Astrocytes	Breast	Brain	IL-1b and TNF-a	TGFb2	ANGPTL4 through TGFbR and SMAD signaling		[73]
	Astrocytes	Breast	Brain	IL-1b	JAG1	Notch signaling		[38]
	Astrocytes	Breast and melanoma	Brain		EVs containing miRs	PTEN loss		[76]
	Astrocytes	Lung	Brain		HA			[91]
Proliferation and survival	Osteoblasts	Breast	Bone		N-cadherin	E-cadherin, activating mTOR signaling		[65]
	Osteoblasts	Breast and prostate	Bone		Gap junctions (Cx43)	Calcium influx and NFAT		[66]
	Astrocytes	Breast, melanoma, and lung	Brain		STAT3		STAT3 inhibition	[33]
	Astrocytes	Breast	Brain		IGFBP2 and CHI3LI		Inhibition of either IGFBP2 or CHI3LI	[77]
	Astrocytes	Melanoma and breast	Brain		Polyunsaturated fatty acids	PPARγ pathway	PPARγ antagonists	[78]

were shown to actively support the growth of bone-metastasizing cancer cells is via the increase of NFAT activity through connexin 43 (cx43) gap junctions: breast and prostate cancer cells used osteoblasts in the osteogenic niche as a cx43-mediated calcium reservoir to increase their intracellular calcium concentration, thus promoting bone metastatic progression [66]. Elevated expression of Cx43 was validated in human breast and prostate bone metastasis samples.

Additionally, osteoblasts enhanced cancer cell migration and tumor cell growth *in vitro* and *in vivo* by expressing MMPs (*Mmp10*, *Mmp11*, and *Mmp13*) and CXCL12. These growth-promoting effects could be reversed by treating osteoblasts or mice with parathyroid hormone (PTH) [67]. CXCL12 expression, induced by local hypoxia in the bone osteogenic niche, affected not only breast cancer bone metastasis but also colonization of other distant sites via the activation of hypoxia-inducible factor (HIF)-1α in osteoblasts. Osteoprogenitor cells (OPCs) are physiologically

located in hypoxic niches in the BM. Activation of HIF signaling in these cells resulted in increased breast cancer metastasis to bone but also induced elevated CXCL12 in the blood, which led to a systemic increase of breast cancer cell proliferation and dissemination through direct activation of the CXCR4 receptor [68].

Notably, enhanced bone metastasis was described in mice with active bone turnover, further suggesting a key role for osteoblasts in metastasis progression [69,70]. A study using multiple mouse models of bone metastasis showed that healing of induced bone fractures, mediated by $NG2^+$ OPGs, promoted spontaneous metastasis in the injured bone, further demonstrating the importance of active bone turnover in determining metastatic fate [71].

Mechanical factors also play a role in stroma-supported bone metastasis. Tumor-induced pressure, mimicking the increased intraosseous pressure associated with bone metastasis, enhanced osteocyte activation and secretion of growth factors that in turn increased the viability and aggressiveness of breast, lung, and prostate cancer cells. This was mediated by CCL5, as neutralizing CCL5 in the conditioned media of pressure-activated osteocytes reversed the enhanced migration and invasion of cancer cells [72].

Thus, osteoblasts are key players in the orchestration of all stages of bone metastasis (Table 2).

Astrocytes

The growth-promoting functions of astrocytes can be induced by cancer cell-derived proinflammatory factors including IL-1β, tumor necrosis factor alpha (TNF-α) and cyclooxygenase 2 (COX2) [38,73,74]. Activated astrocytes can support the growth of DTCs in the brain by multiple mechanisms.

Astrocytes promote the invasion of cancer cells into the brain by modulating the blood–brain barrier (BBB). The BBB, which normally protects the brain by limiting the access of molecules and cells, is modulated into the more permeable blood–tumor barrier (BTB) in cancer [34,75]. Astrocytes modulated BTB permeability in mouse models of breast cancer brain metastasis by overexpressing sphingosine 1-phosphate receptor 3 (S1P3), leading to increased secretion of IL-6 and CCL2, reducing endothelial cell adhesion and promoting increased BTB permeability. Inhibition of S1P3 resulted in restored BTB tightening both *in vitro* and *in vivo* [75].

Once DTCs enter the brain, astrocytes facilitate their survival and growth. In mouse models of breast and melanoma brain metastasis, astrocytes epigenetically regulated PTEN expression in cancer cells by secreting exosomes containing PTEN-targeting miRNAs. Uptake of these miRNAs by tumor cells led to loss of their PTEN expression, thus enhancing their survival in the brain at the early stages of brain metastasis [76]. Proteomic analysis of human cerebrospinal fluid (CSF) identified astrocyte-derived IGFBP2 and CHI3LI, which supported the proliferation of $HER2^+$ breast cancer cells and enhanced the formation of metastatic lesions [77]. Tumor-derived IL-1β and TNF-α reprogrammed astrocytes leading to the upregulation of TGFβ2, which in turn induced the expression of ANGPTL4 in breast cancer cells, enhancing their seeding and growth in the brain [73]. In addition, direct contact between astrocytes and cancer cells promoted the growth of metastasized breast cancer cells in the brain [38]. IL-1β secreted from breast cancer cells activated surrounding astrocytes, which upregulated Notch signaling in cancer cells, depending on the direct interaction of cancer cells and astrocytes [38]. Similarly, brain-metastasizing breast cancer cells secreted COX2 and its downstream product prostaglandin, which directly activated astrocytes leading to the secretion of CCL7, which promoted the self-renewal of tumor-initiating cells in the brain [74].

Another mechanism by which astrocytes support brain metastasis is by affecting the metabolism of brain-metastasizing cancer cells. Under physiological conditions, astrocytes are central regulators of neuron metabolism and are a major cellular source of fatty acids. Astrocyte-derived fatty acids enhanced breast cancer and melanoma cell proliferation and metastatic outgrowth in the brain by activating the proliferator-activated receptor gamma (PPARγ) pathway in the surrounding cancer cells. Systemic blockage of the PPARγ pathway decreased brain metastases in mouse models [78]. Thus, astrocytes facilitated metastatic outgrowth by supporting the adaptation of cancer cells to the unique metabolic microenvironment of the brain.

In summary, stromal cells are reprogrammed by reciprocal interactions with DTCs to support the survival and outgrowth of cancer cells in metastatic sites (Table 2).

ECM remodeling and angiogenesis

The ECM in tumors is drastically changed, and its altered composition and stiffness were shown to affect a wide range of properties during tumorigenesis and cancer progression, as reviewed in [79]. Organ-specific changes in the ECM at different metastatic niches is emerging as a key feature of premetastatic niches and an integral component of metastatic tumors [79,80]. Many of the ECM components and much remodeling are produced and mediated by stromal cells (Table 3).

Fibroblasts

Enhanced collagen deposition and stiffness were previously shown to be associated with metastasis [81]. Recent findings suggest that systemic factors remotely activate stromal cells in future metastatic organs to foster ECM modulations that create a permissive niche. Breast cancer cells secrete high levels of Activin A (ActA), a TGFβ superfamily member, inducing profibrotic rewiring of resident lung fibroblasts, which facilitated the formation of a fibrotic premetastatic niche, and enhanced lung metastasis [82]. Genetic targeting of ActA in a spontaneous model of breast cancer lung metastasis significantly attenuated lung metastasis and improved survival in mice [82]. Reprogramming of fibroblasts in metastatic sites may also be mediated by signaling from fibroblasts at the primary tumor: EVs from fibroblasts in the primary tumor of a salivary adenoid cystic carcinoma (SACC) murine model were found to remotely activate lung fibroblasts. Integrin α2β1-mediated uptake of EVs by fibroblasts in the liver induced matrix remodeling and the formation of a premetastatic niche, thus enhancing lung metastasis [83]. These studies identified systemic factors that remotely activate stromal cells in future metastatic organs and therefore represent potential targets to inhibit the metastatic cascade.

Fibroblast-mediated remodeling of the ECM can have an important role in enabling melanoma metastasis, in association with aging. While young skin fibroblasts produced abundant ECM components, aged fibroblasts lost expression of the hyaluronic and proteoglycan link protein (HAPLN1), resulting in enhanced alignment of ECM matrices that promoted metastasis in a mouse model of transplantable melanoma [84]. Interestingly, age-related changes in HAPLN1 increased the lymphatic permeability, which affected melanoma lymph node metastasis by enabling the escape of melanoma cells from the lymphatic system to distant metastatic sites [85]. Stiffness of the ECM modulated by CAFs also affects metastatic growth: liver metastasis-associated fibroblasts were highly activated compared with fibroblasts from colorectal cancer (CRC) primary tumor due to the renin-angiotensin system (RAS), which triggered fibrosis thus enhancing tissue stiffness. Liver metastases were shown to be stiffer than their respective primary tumors in human samples of CRC [86].

Fibroblast activation and ECM stiffness were also linked to increased angiogenesis and with resistance to antiangiogenic therapy in CRC. Hepatic fibroblasts supported pancreatic ductal adenocarcinoma (PDAC) metastasis by promoting angiogenesis in a mouse model of pancreatic

Table 3. CAF-specific effects on the TME: ECM and angiogenesis

Effect on MME	Stromal cell	Cancer type	Metastatic site	Upstream pathway/activator	Molecule produced by stromal cells	Molecule targeted in cancer cells	Targeting therapy	Refs
ECM production and remodeling	Fibroblasts	Melanoma	Lung		Downregulated fibulin, agrin, HAPLN1			[84]
	Fibroblasts	CRC	Liver		RAS pathway			[86]
	Fibroblasts	Pancreatic	Liver	Macrophage-derived granulin	Periostin		Granulin-secreting macrophages	[88]
	Fibroblasts	SACC	Lung	CAF-derived EVs via α2β1	TGFβ pathway; periostin		α2β1 inhibitor (TC I-15)	[83]
	Fibroblasts	Breast	Lung		Myc and Hsf1 signaling; *Hspe1*, *Hsp90aa1*, *Odc1*, *Fosl1*			[95]
	Fibroblasts	Breast	Lung	ActA	Collagen		Act-A genetic ablation	[82]
	Astrocytes	Lung	Brain		HA			[91]
Endothelial permeability and angiogenesis	Fibroblasts	Melanoma	Lymph node		HAPLN1			[85]
	Fibroblasts	Pancreatic	Liver		CXCL8 and CCL2			[87]
	Fibroblasts	Breast	Lung		VEGF-A and Tenascin-C			[14]

cancer metastasis to liver [87]. This is of particular interest, as patients receiving both antiangiogenic therapy and RAS inhibitors for their hypertension exhibited reduced liver stiffness and metastasis, thus consolidating the central role of liver fibroblasts and fibrosis in metastatic progression [86].

Profibrotic activation of fibroblasts can also be mediated by immune cells. In PDAC, granulin-secreting macrophages accumulated in premetastatic livers and activated hepatic fibroblasts, thus forming a fibrotic microenvironment that supported PDAC metastatic growth [88]. ECM remodeling was also implicated in omental metastasis from ovarian cancer in mouse and human tissues [89]. Single-cell RNA-seq (scRNA-seq) of patient-derived samples from either primary ovarian cancer or metastatic tissue revealed that a subpopulation of fibroblasts, which was prominent in metastasis, was implicated in angiogenesis and high collagen production, further promoting cancer propagation. This subpopulation of CAFs was characterized by enriched activity of the transcription factors SOX4 and SRF [90].

Astrocytes

Changes in the unique ECM of the brain during brain metastasis formation remains largely uncharted territory. However, some evidence suggests that astrocytes may play a role in these processes. Increased hyaluronic acid (HA) deposition correlated with the presence of reactive astrocytes around disseminated lung cancer cells in mice, and co-culture of tumor cells with HA-producing astrocytes increased the invasive outgrowth of lung cancer cells [91]. These findings suggest that better understanding of the brain ECM may provide novel nodes for therapeutic interventions (Table 3).

Promotion of a proinflammatory and immunosuppressive microenvironment

Tumor-promoting inflammation and avoidance of immune destruction are hallmarks of cancer and metastasis [92]. Stromal cells are central modulators of the tumor immune microenvironment in multiple cancer types [1]. Stromal cells have also emerged as key players in the modulation of the immune microenvironment in tumor metastasis (Table 4). Stroma-mediated promotion of an

Table 4. Promotion of a proinflammatory and immunosuppressive MME

Effect on cancer cells	Stromal cell	Cancer type	Metastatic site	Upstream pathway/activator	Molecule produced by stromal cells	Molecule targeted in cancer cells	Targeting therapy	Refs
Promotion of a proinflammatory MME	Fibroblasts	HCC	Lung	Tumor-derived miR-1247-3p	Downregulation of *B4GALT3*; activate β1-integrin–NF-κB			[93]
	Fibroblasts	CRC	Liver and lung	Tumor-released ITGBL1-rich EVs	TNFAIP3-mediated NF-κB signaling; *IL-6* and *IL-8*			[94]
	Fibroblasts	Breast	Lung		Myc, NF-κB, and STAT3 signaling; *Hspe1*, *Hsp90aa1*, *Odc1*, *Fosl1*			[95]
	Fibroblasts	Breast	Lung	DAMPs, ATP, necrotic fluid	Il-1b, NLRP3			[40]
	Osteoblasts	Breast	Bone		IL-6, IL-8, CCL2			[103]
	Osteoblasts	Breast	Bone		TGFb signaling, PI3Kinase and Rac			[104]
	Osteoblasts	Breast	Bone		IL-1b	NF-κB, CREB signaling, and Wnt ligand production		[124]
	Osteoblasts	Breast	Bone		IL-6, IL-8, CCL2			[103]
	Osteoblasts	Breast	Bone		TGFb signaling, PI3Kinase and Rac			[104]
	Osteoblasts	Breast	Bone		IL-1b	NF-κB, CREB signaling, and Wnt ligand production		[124]
	Osteoblasts	Breast	Bone	Senescence	IL-6		IL-6 neutralization	[102]
	Osteoblasts	Prostate	Bone	Cancer-derived TGFa	PGE-2			[105]
	Astrocytes	Breast and lung	Brain	PCDH7	Gap-junction (Cx43)-induced cGAMP; STING pathway; IFNα and TNF	STAT1 and NF-κB signaling	Gap junction inhibitors meclofenamate and tonabersat	[36]
	Astrocytes	Breast	Brain	COX2 and PGE2	CCL7			[74]
	Astrocytes	Breast, melanoma and lung	Brain	Granulocyte-derived LCN2	CXCL10, CCL5, CCL2, C3, C5, and TNF-a			[114]

Vicious cycle	Osteoblasts	Breast	Bone	Cancer-derived Jagged-1	Notch signaling, IL-6			[101]
	Osteoblasts	Prostate	Bone	Cancer-derived BMP6	SMAD			[125]
Recruitment of immunosuppressive cells	Fibroblasts	Breast	Lung		*Il-33*	ST2 on immune cells	Neutralizing αIL-33	[96]
	Fibroblasts	Breast	Lung	DAMPs, ATP, necrotic fluid	Il-1b, NLRP3			[40]
	Fibroblasts	Breast	Lung		Complement signaling, C1ra, C1s2, C2, C3		Combination of chemotherapy and complement receptor antagonist	[97]
	Osteoblasts	Lung	No metastasis	sRAGE			Deletion of OCN^+ osteoblasts	[110]
	Osteoblasts	Breast	Bone	PTHrP	VEGF-A, IL-6 via α4β1 (VLA-4)	VCAM-1 on m-MDSCs	Src inhibitors	[106]
	Astrocytes	Melanoma	Brain		CCL2, CXCL10, CCL17			[112]
	Astrocytes	Breast and melanoma	Brain		Type I IFN and CCL2		CCR2 inhibition	[115]
Exclusion of T cells	Fibroblasts	Urothelial	Multi		TGFb signaling		Combination of TGFβ and PD-L1 blockade	[116]
Promotion of an immunosuppressive MME	Fibroblasts	Breast	Lung		Il-33	ST2 on immune cells	Neutralizing αIL-33	[96]
	Fibroblasts	Ovarian	Ascites		Complement, CXCL1/2/10/12, IL-6, IL-10			[98]
	Osteoblasts	Breast	Bone		EDA-FN	α5β1 on MDSCs	Integrin α5β1 receptor or arginase inhibition	[107]
	Osteoblasts	Breast and lung	Bone	Tumor-derived EVs (HTRA1)	MMP13		MMP13 inhibition or osteoprogenitor deletion	[25]
	Osteoblasts	Breast	Bone	SCUBE2	SHH, Hedgehog pathway	LAIR1 signaling on NK cells	Targeting the Hedgehog pathway	[23]
	Astrocytes	Breast, melanoma, and lung	Brain		VEGF, MIF, TIMP-1, and LCN2		STAT3 inhibition	[33]
	Astrocytes	Breast, melanoma, and lung	Brain	Granulocyte-derived LCN2	CXCL10, CCL5, CCL2, C3, C5, and TNF-a			[114]

inflammatory microenvironment directly affects tumor cell survival and growth and is associated with the establishment of an immunosuppressive metastatic niche. An immunosuppressive microenvironment is rich in cells that inhibit T cell killing functions [macrophages, myeloid-derived suppressor cells (MDSCs), T regulatory (T_{reg}) cells, etc.] and is partially deprived of functional killer cells [cytotoxic T cells, natural killer (NK) cells, etc.]. Stromal cells are central to the recruitment of immune cells to the metastatic niche and interact both directly and indirectly with innate and adaptive immune cells to hinder their antitumor immune responses (Table 4).

Fibroblasts, osteoblasts, and astrocytes can be rewired towards a proinflammatory phenotype in cancer and metastasis by systemic or paracrine signaling from cancer cells or immune cells. However, the specific signaling pathways that these activated stromal cells employ can be organ specific.

Fibroblasts

Much as in the TME of primary tumors, one of the main prometastatic functions of fibroblasts is the promotion of an inflammatory microenvironment (Table 4). Enhanced secretion of proinflammatory mediators by fibroblasts can either affect cancer cells directly or modulate immune responses. For example, activated fibroblasts in a mouse model of hepatocellular carcinoma (HCC) lung metastasis facilitated inflammation and metastasis via activation of the β1-integrin–NF-κB signaling pathway in the lung premetastatic niche. The activation of fibroblasts in the lung premetastatic niche was instigated by tumor-derived exosomal miR-1247-3p. High expression of miR-1247-3p in serum exosomes was correlated with lung metastasis in HCC patients [93]. Similarly, integrin beta-like 1 (ITGBL1)-rich EVs released into the circulation by CRC primary tumors activated fibroblasts in premetastatic niches in a NF-κB-dependent manner resulting in fibroblast secretion of proinflammatory cytokines, which promoted liver and lung metastasis [94].

Metastasis-associated fibroblasts are highly dynamic and coevolve with metastatic progression. Transcriptome profiling of lung fibroblasts at distinct stages of lung metastasis in a transgenic breast cancer mouse model (MMTV-PyMT) revealed their transcriptional rewiring towards a protumorigenic state. Lung fibroblasts from an early metastatic stage showed enhanced cellular response to stress, while lung fibroblasts isolated from macrometastases upregulated proinflammatory pathways and ECM remodeling pathways [95]. Specifically, CAF-derived IL-33 instigated type 2 immunity in the lung metastatic microenvironment (MME), which mediated the recruitment of eosinophils, neutrophils, and inflammatory monocytes to lung metastases. Targeting of IL-33 *in vivo* resulted in attenuation of immune cell recruitment and type 2 immunity, and inhibition of lung metastasis. Fibroblast-derived IL-33 was also evident in lung metastatic tissue of breast cancer patients [96]. Proinflammatory signaling by activated fibroblasts was shown to be associated with the recruitment of MDSCs and the fostering of an immunosuppressive niche [8]. CAFs from primary tumors can instigate proinflammatory signaling at the metastatic site. Mammary fibroblasts that were activated by the sensing of damage-associated molecular patterns (DAMPs) secreted IL-1β, which upregulated the expression of adhesion molecules by lung endothelial cells, thus enhancing MDSC infiltration and facilitating both primary tumor growth and lung metastasis [40]. In addition, fibroblast-derived complement signaling induced an immunosuppressive microenvironment rich in MDSCs and dysfunctional T cells in lungs in early and late metastatic breast cancer [97]. Similarly, in metastatic ovarian cancer, single-cell analysis of ascites samples from HGSOC patients revealed the presence of a subset of immunomodulatory fibroblasts characterized by their expression of complement components CXCL1/2/10/12 and cytokines IL-6 and IL-10 [98]. Thus, fibroblasts at metastatic sites are reprogrammed to become proinflammatory, and their secretion of cytokines and chemokines shapes a growth-permissive, immunosuppressive metastatic niche.

Osteoblasts

In bone metastasis, increased inflammatory signaling leads to the instigation of the 'vicious cycle' [99]. Osteolytic bone metastases (from breast, kidney, lung, and colon cancers) are characterized by excessive bone resorption. Activated osteoclasts degrade the surrounding mineralized bone matrix resulting in the release of growth factors, which in turn fuel cancer cell colonization, survival, and invasiveness. One example of the multiple signaling pathways that are hijacked by bone metastasis is TGFβ signaling. Cancer cells initiate RANKL secretion by osteoblasts, which in turn induces TGFβ release from the bone matrix. TGFβ induces the accumulation of suppressive myeloid and T_{reg} cells that further produce RANKL, thus enhancing bone destruction by osteoclasts [100]. Osteoblasts also promote bone degradation by enhancing osteoclast differentiation: breast cancer-derived Jagged1 engaged Notch signaling in osteoblasts, enhancing IL-6 production and triggering osteoclast activation in a mouse model of breast cancer bone metastasis [101]. Moreover, induction of osteoblast senescence *in vivo*, causing enhanced IL-6 production, increased osteoclastogenesis and promoted metastatic seeding in a mouse model of breast cancer [102]. Like fibroblasts, proinflammatory activation of osteoblasts can be induced by cancer cells. Osteoblasts reacted to tumor-secreted factors by undergoing an inflammatory stress response (enhanced expression of cytokines, including IL-6, IL-8, and MCP-1/CCL2) and by impairing mineralization of the bone matrix [103]. These modifications were accompanied by morphological changes, reminiscent of CAF activation [104].

Unlike osteolytic bone metastasis, prostate cancer bone metastases are mostly osteoblastic, characterized by excessive bone production, which enhances skeletal pressure and causes severe bone pain. This type of bone remodeling is associated with proinflammatory signaling in osteoblasts. Prostate cancer-derived TGFα enhanced prostaglandin E2 (PGE2) production in osteoblasts, resulting in increased formation of calcified bone nodules [105]. Thus, osteoblasts are central culprits in changes in bone homeostasis, which accompany and enable bone metastasis (Table 4).

Osteoblasts also facilitate the formation of immunosuppressive niches in the bone and in the periphery, conducive to metastatic progression. Breast cancer-derived PTH-related protein (PTHrP) activated osteoblasts, which in turn expressed vascular endothelial growth factor A (VEGF-A) and IL-6, leading to phosphorylation of SFK in monocytic MDSCs. These MDSCs subsequently expressed the ADAM17 and MMP7 proteases, resulting in their detachment from the osteogenic niche and mobilization into the circulation [106]. In addition to promoting MDSC differentiation, osteoblasts also directly interact with MDSCs in mouse models of breast cancer and melanoma [106,107]. Extradomain A-fibronectin (EDA-FN) originating from osteoblasts can bind α5β1 on MDSCs thus increasing arginase activity, resulting in increased cancer growth [107]. Moreover, osteoprogenitors activated by tumor-secreted HTRA1-containing EVs, can interact with granulocyte-monocyte progenitors, resulting in aberrant myelopoiesis and systemic immunosuppression [25]. Osteoblasts can directly affect lymphoid cells in the bone metastatic niche by suppressing NK cell antitumor immunity, thus promoting bone colonization of luminal breast cancer [23]. This was driven by tumor cell-derived SCUBE2, which facilitated the release of SHH to activate Hedgehog signaling in MSCs, which eventually lead to NK suppression via LAIR1 signaling. Targeting of Hedgehog signaling reduced bone metastasis in mice [23]. This activation of the Hedgehog pathway is reminiscent of the crosstalk between cancer cells and fibroblasts in PDAC and breast tumors [108,109], suggesting convergence and similarities between the roles of distinct stromal cells in cancer and metastasis. The findings that osteoblasts can directly affect NK cells also raise the question of whether osteoblasts interact with or directly affect the functions of T cells in bone metastasis.

In addition to their local functions facilitating bone metastasis, osteoblasts can affect the immune microenvironment in remote tumors. Osteoblasts can remotely supply lung tumors with SiglecF$^+$ neutrophils that promote tumor progression. This was operative in lung cancer patients even in the absence of bone metastatic lesions [110]. Ablation of osteocalcin-expressing osteoblasts reduced not only tumor growth but also the infiltration of tumor-promoting neutrophils [110]. Similarly, osteoblast-derived Dkk1 was suggested to promote primary tumor growth and the formation of an immunosuppressive microenvironment in mouse models of melanoma and lung cancer [111]. In summary, osteoblasts are reprogrammed by cancer-secreted factors to support tumor growth. Further characterization of cancer-associated osteoblasts will better clarify the underlying mechanisms by which tumor cells activate osteoblasts to facilitate bone colonization and modulate the immune microenvironment in bone metastasis.

Astrocytes

In the brain, astrocytes support the growth of brain-metastasizing cells by proinflammatory signaling, affecting both innate and acquired immunity in the brain. Activation of astrogliosis and neuroinflammation at the early stages of melanoma brain metastasis upregulated multiple cytokines and chemokines in astrocytes and enhanced the growth of melanoma cells [112]. Proinflammatory activation of astrocytes via bidirectional signaling with melanoma cells led to the formation of a proinflammatory milieu in the brain. Brain-metastasizing melanoma cells upregulated the expression of proinflammatory genes in astrocytes, including IL-23, which induced the secretion of MMP2 and enhanced the invasiveness of melanoma cells [39].

The reciprocal interactions of tumor cells and astrocytes included the formation of functional gap junctions, thereby establishing a physical communication channel between cancer and stromal cells. Breast and lung cancer cells reprogrammed astrocytes by providing cGAMP via gap junctions, which upregulated a variety of proinflammatory cytokines [e.g., interferon (IFN)α, TNFα] in astrocytes, which supported the outgrowth of brain metastatic cells [36]. Pharmacological targeting of gap junction formation blocked this proinflammatory signaling loop and attenuated brain metastasis in a mouse model of breast cancer [36].

Neuroinflammation may also have antimetastatic roles that are mediated by astrocytes. Brain-metastasizing melanoma cells suppressed astrocyte-mediated neuroinflammation, which inhibited the phagocytosis of melanoma cells by microglia [113]. This inhibition was mediated by melanoma-derived amyloid beta (Aβ), previously implicated in Alzheimer's disease, which suppressed the complement pathway in astrocytes [113]. These findings create a possible mechanistic link between brain metastasis and Alzheimer's disease, which may be exploited therapeutically.

Proinflammatory activation of astrocytes was shown to be initially activated by systemic signaling from primary melanoma tumors, mediated by lipocalin-2 (LCN2) [114]. Activated astrocytes recruited immunosuppressive granulocytes from the BM to the brain MME, which in turn became a main source of LCN2 in the brain. Genetic ablation of LCN2 in the host significantly inhibited neuroinflammation and attenuated brain metastasis [114]. Reactive astrocytes promoted the formation of an immunosuppressive microenvironment by expressing type I IFN in brain metastatic lesions from breast cancer and melanoma [115]. Type I IFN activated CCL2 expression in reactive astrocytes, which facilitated the infiltration of monocytic MDSCs into the brain MME [115].

Immune modulation by astrocytes in the brain is not limited to myeloid cells. A subpopulation of reactive astrocytes surrounding brain-metastatic lesions from multiple cancer types in mouse models and in patients express the immune-modulatory transcription factor STAT3 and promote brain metastasis by facilitating macrophage/microglial infiltration in the brain and inhibiting CD8$^+$ T

cell activation [33]. Pharmacological targeting of STAT3 in patients with advanced systemic disease that included brain metastasis resulted in significant improvement in their survival [33].

Taken together, these studies demonstrate that astrocytes are activated by cancer cell-secreted factors to modulate neuroinflammation and immune suppression in the brain MME, which supports the growth of brain-metastasizing cancer cells.

Resistance to therapy

Among the plethora of stromal cell functions during all stages of metastasis formation, they were implicated in the host response to therapy, thus affecting the efficacy of treatments and therapy resistance (Table 5). While most cancer therapies target cancer cells, and more recently immune

Table 5. Therapy resistance

Effect on cancer cells	Stromal cell	Cancer type	Metastatic site	Upstream pathway/activator	Molecule produced by stromal cells	Molecule targeted in cancer cells	Targeting therapy	Refs
Chemoresistance	Fibroblasts	Breast	Lung		Complement signaling, C1ra, C1s2, C2, C3		Combination of chemotherapy and complement receptor antagonist	[97]
	Osteoblasts	Prostate	Bone		Annexin II, GAS6	Annexin II receptor; AXL, Sky, and Mer		[55]
	Osteoblasts	Prostate	Bone		Annexin II, GAS6	Annexin II receptor; AXL, Sky, and Mer		[55]
	Osteoblasts	Breast	Bone		VWF and VCAM1		Integrin-mediated interactions	[50]
	Osteoblasts	Prostate and AML	Bone		CXCL12	CXCR4	Combination of chemotherapy with CXCR4 antagonist	[54]
	Osteoblasts	Breast	Bone		Jagged-1	Notch signaling	Neutralizing Jagged1 (clone 15D11)	[117]
	Astrocytes	Melanoma, breast, and lung	Brain		Gap junctions (Cx43)			[118,120]
	Astrocytes	Breast	Brain		Gap junctions, ET-1	IL-6, IL-8, and ETR; AKT/MAPK signaling		[119]
	Astrocytes	Breast and lung	Brain	PCDH7	Gap junction (Cx43)-induced cGAMP; STING pathway; IFNα and TNF	STAT1 and NF-κB signaling	Gap-junction inhibitors meclofenamate and tonabersat	[36]
Antiangiogenic resistance	Fibroblasts	CRC	Liver		RAS signaling		Combination of antiangiogenic + RAS inhibitor	[86]
Immunotherapy resistance	Fibroblasts	Urothelial	Multi		TGFb signaling		Combination of TGFβ and PD-L1 blockade	[116]

cells, better understanding of stromal responses to therapy and their intricate interactions with immune cells in different organs may provide the basis for stroma-targeted therapeutics that will potentiate immunotherapy.

Fibroblasts

CAFs from the primary tumor have been suggested to be important in affecting the response to chemotherapy and immunotherapy in multiple cancer types, thus enabling tumor progression and metastatic relapse [10,11]. For example, TGFβ signaling in fibroblasts in metastatic urothelial cancer was shown to be associated with the exclusion of $CD8^+$ T cells from the tumor, which were instead found in the fibroblasts and collagen-rich peritumoral stroma, and with poor response to anti-PD-L1 treatment [116]. Therapeutic co-administration of TGFβ-blocking reagents and anti-PD-L1 antibodies reduced TGFβ signaling in stromal cells, facilitated T cell tumor penetration, and promoted tumor regression [116].

Fibroblasts also support chemoresistance and lung metastasis via the activation of complement signaling [97]. Following resection of the primary tumor in two models of spontaneous TNBC lung metastasis, adjuvant chemotherapy upregulated complement signaling in lung fibroblasts resulting in enhanced recruitment and functional reprogramming of MDSCs, thus promoting T cell dysfunction. Pharmacological targeting of complement signaling in combination with chemotherapy alleviated immune dysregulation and attenuated lung metastasis [97]. These studies highlight the therapeutical importance of understanding the operative pathways of CAFs at the metastatic site and harnessing this knowledge to design more effective therapeutic strategies.

Osteoblasts

In bone metastasis, dormant cells can be resistant to chemotherapy as they enter a non-dividing state [57]. In prostate cancer bone metastasis as well as in acute myeloid leukemia (AML), chemotherapy had no effect on dormant DTCs in the BM. Resistance to chemotherapy was partially reversed by combining cytotoxic treatment with a CXCR4 antagonist, thus releasing cancer cells from the protective osteogenic niche [54]. Osteoblasts in the osteogenic niche were also reported to support chemoresistance in a model of breast cancer bone metastasis: following chemotherapy, osteoblasts expressed elevated levels of Jagged-1, inducing inflammation and providing a survival niche for cancer cells, thus sustaining bone metastasis [117]. Osteoblasts and bone vascular endothelium can synergistically trigger chemoresistance of prostate cancer DTCs [50]. These studies demonstrate that osteoblasts play a central role in providing a protective niche for disseminated cancer cells in the bone (Table 5 and Figure 3).

Astrocytes

Brain tumors can harness the neuroprotective effects of astrocytes for their own survival: reactive astrocytes were shown to protect cancer cells from chemotherapy by activating signaling pathways related to cell survival (Table 5). Astrocytes and cancer cell interactions are important in inducing these chemoprotective functions in astrocytes. In co-culture experiments, astrocytes reduced the apoptosis of melanoma cells when treated with chemotherapeutic drugs. This chemoprotective effect was dependent on physical contact and gap junction communication between astrocytes and lung or breast cancer cells [118]. Chemoprotection was further shown to be mediated by activation of the endothelin axis in a breast cancer model [119]. Gap junction signaling between breast cancer cells and astrocytes induced endotaxin signaling in astrocytes, which affected survival proteins that protected cancer cells from chemotherapy [119]. These cancer cell–astrocyte gap junctions comprised connexin 43 (Cx43) in mouse models of lung and breast cancer brain metastasis. Cx43 gap junctions induced the secretion of proinflammatory cytokines in astrocytes, which in turn activated the STAT1 and NF-κB pathways in brain metastatic

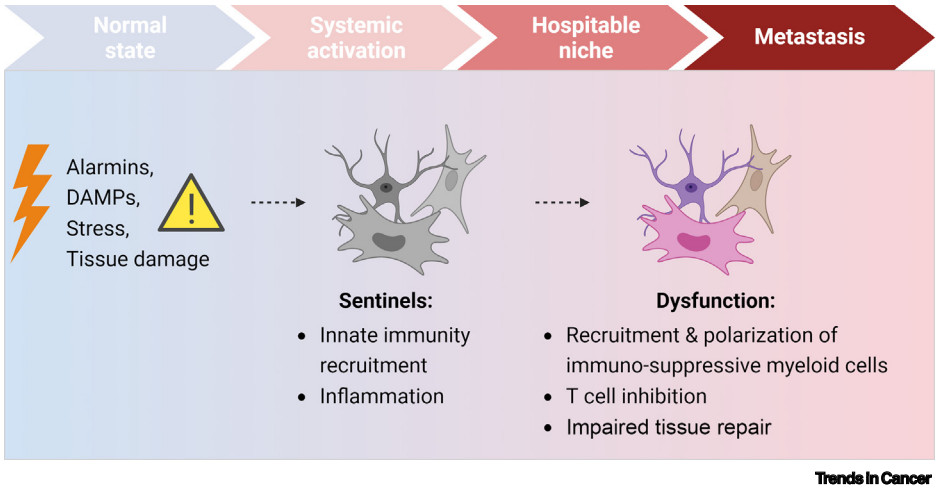

Figure 3. Stromal cells are activated during metastatic progression. Under physiological conditions, stromal cells function as sentinels of tissue homeostasis and integrity. They alert the immune system in case of danger or tissue damage and are at the center of wound-healing processes. In cancer, primary tumors systemically activate stromal cells in distant organs, favoring the preparation of an inflammatory and protumorigenic hospitable niche. During the metastatic cascade, stromal cells receive a variety of signals, which lead to their reprogramming. Instead of resolving the tissue damage elicited by the disseminated cancer cells, they become culprits that support metastatic growth.

cells, thus increasing their survival and chemoresistance [36]. Cx43 gap junctions between cancer cells and stromal cells played a central role in the interactions of cancer cells with multiple stromal cells in various organs, including not only astrocytes but also fibroblasts and osteoblasts, further emphasizing shared stromal mechanisms [36,66,120,121].

Concluding remarks

The fast evolution of the TME field has revealed the vast heterogeneity of stromal cells and the complexity of their reciprocal interactions with cancer cells, as well as with other components of the TME. The studies reviewed herein reveal that fibroblasts, osteoblasts, and astrocytes have similar tasks in metastatic progression in the respective organs in which they reside, as well as organ-specific roles (Figures 1 and 2). Many of these tasks result from the hijacking and exacerbation of the physiological roles of resident stromal cells that normally function as sentinels of tissue integrity. When tissue damage occurs, stromal cells alert the immune system and are at the center of the tissue repair and wound-healing processes. During the metastatic cascade, stromal cells receive a large variety of activating signals, which lead to their dysfunction: instead of resolving the tissue damage caused by metastasis, they aggravate the never-healing-wound process of cancer (Figure 3).

A temporal outlook on the shared prometastatic mechanisms of fibroblasts, osteoblasts, and astrocytes indicates that reprogramming and activation of stromal cells in metastatic organs precedes the formation of metastases (Figure 1). Primary tumors can remotely activate each type of stromal cell in distinct organs by systemic signaling and EVs [25,122]. These activated stromal cells prepare the soil for further metastatic seeding, attract tumor cells to the metastatic site (Table 1), sustain cancer cell dormancy, or mediate dormancy exit (Table 1). Stromal cells can promote tumor cell invasion to metastatic organs and support their survival and proliferation, thus enabling successful colonization of metastatic sites (Table 2). This is accompanied by modulation of the immune microenvironment by stromal cells, the instigation of tumor-promoting inflammation, and the fostering of an immunosuppressive and dysfunctional microenvironment (Table 4), which may eventually result in therapy resistance (Table 5 and Figures 2 and 3).

Outstanding questions

Are the functional mechanisms activated in stromal cells cancer-type specific or organ specific? For example, are lung CAFs in primary lung tumors similar to metastasis-associated fibroblasts in lung metastases?

Can we translate existing knowledge on one stromal cell type (e.g., fibroblasts) to stromal cells in other organs (osteoblasts and astrocytes)?

How do systemic perturbations such as aging, sex, and metabolic status affect the prometastatic functions of organ-specific stromal cells?

How can we overcome the plasticity and heterogeneity of stromal cells when designing co-targeting therapeutic strategies?

Can we integrate understanding of the similarities and differences between stromal cells to improve and potentiate existing therapeutics?

Collectively, these insights underscore the opportunities and the challenges of harnessing knowledge on stromal cells for novel therapeutic strategies. We have emphasized herein the importance of understanding the unique organ-specific pathways that affect metastasis in various organs. However, systemic unifying mechanisms that affect stromal cell function should also be taken into account when studying the MME, such as aging, sex, and metabolic status [123]. In the coming years, we anticipate additional studies that will shed light on the functional heterogeneity of stromal cell populations, providing context-specific understanding of their organ-distinct functions as well as unifying mechanisms that are common to their tasks across organs and cancer types (see Outstanding questions).

An ongoing challenge in the investigation and targeting of stromal cells in organ-specific metastasis is the immense plasticity and robust adaptive mechanisms of stromal cells that allow them to evade standard-of-care therapeutics and protect cancer cells, thus enabling metastatic growth. Better understanding of their vulnerabilities will allow the design of co-targeting strategies, to potentiate existing therapeutics.

Hopefully, we will see in the future integration of the preclinical findings described in this review into a comprehensive, integrative understanding of MMEs in both local and systemic outlooks. Integration of this knowledge will propel the design of novel therapeutic combination strategies aimed at impairing the tumor-supportive and immunosuppressive responses of stromal cells, to prevent metastatic relapse.

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Declaration of interests

The authors declare no conflicts of interest.

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