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Review

A glitch in the matrix: organ-specific matrisomes in metastatic niches

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Modification of the extracellular matrix (ECM) is a critical aspect of developing a metastasis-supportive organ niche. Recent work investigating ECM changes that facilitate metastasis has revealed ways in which different metastatic organ niches are similar as well as the distinct characteristics that make them unique. In this review, we present recent findings regarding how ECM modifications support metastasis in four frequent metastatic sites: the lung, liver, bone, and brain. We discuss ways in which these modifications are shared between metastatic organs as well as features specific to each location. We also discuss areas of technical innovation that could be advantageous to future research and areas of inquiry that merit further investigation.

Highlights

The extracellular matrix (ECM) of different organs are composed of distinct combinations of matrisomal proteins; metastasis-supportive modifications of these ECMs can be similarly specific to each organ niche while sharing commonalities.

Tumor-derived extracellular vesicles (EVs) home to lung fibroblasts, activate them to cancer-associated fibroblasts (CAFs), and induce a metastasis-supportive microenvironment through collagen and fibronectin deposition and ECM modification.

Immune cells play a significant role in profibrotic changes in the liver and lung metastatic niches.

The bone and brain have unique ECM compositions that produce singular types of modifications.

Increased understanding of metastasis-supportive ECM changes has enabled development of more accurate models and revealed important clinical considerations.

Novel therapeutic approaches for targeting of ECM changes in the premetastatic niche may be beneficial in preventing metastatic relapse.

The extracellular matrix plays an integral role in establishing metastatic niches

Metastasis is responsible for ~90% of cancer-related deaths. Extensive research has focused on what causes cancer cells to spread and how these mechanisms can be targeted or prevented. Since the introduction of Paget's now renowned 'seed and soil hypothesis' in 1889 [1], it has been recognized that the environment of the secondary site plays a crucial role in determining the success of metastatic colonization. More recently, the discovery that the primary tumor can influence the formation of a **premetastatic niche** (PMN; see Glossary) in metastatic organs [2] revealed a novel aspect of the tumorigenic process that added complexity to our understanding of tumor biology and provided new opportunities for therapeutic intervention. One of the elements of the PMN that has garnered increased attention in the past few years is the **ECM**. The composition and functions of the ECM in cancer were comprehensively reviewed recently in [3]. Functioning as both a support and signaling regulator, the ECM and its modifications are known to affect tumorigenesis through various mechanisms [4]. Moreover, the ECM and the diverse components of the **matrisome** that comprise it (Table 1) are now known to support metastatic outgrowth in multiple secondary organs. However, the ECM of the PMN is immensely complex, and its establishment and maintenance require crosstalk between a variety of cell types (Figure 1) with unique functions and players in different tissues. These intricacies have given rise to a burgeoning field of study that holds great potential for novel antimetastatic strategies.

In this review, we present recent findings regarding ECM changes that support metastasis in four frequent metastatic sites, the lung, liver, bone, and brain, and highlight both commonalities and unique features in each location. While these changes can directly modify the function of tumor cells, this review will primarily discuss recent findings regarding ECM alterations that facilitate the formation of a hospitable metastatic niche. We also discuss areas that hold potential for future research, including technical innovations that could help facilitate studies and clinical considerations that merit further investigation.

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Normal ECM composition in common metastatic organs

The tasks of the ECM in all organs are largely consistent: provide structural support for tissues through the ECM architecture and act as a modulator of cell function and phenotype. The latter is achieved by (i) regulating biochemical signals through the sequestration and release of signaling molecules and acting as ligands that drive cellular signaling and (ii) regulating mechanical signals by altering the stiffness of the microenvironment. Thus, remodeling of the ECM is a normal physiological occurrence. Although major similarities are present in ECM of the lung, liver, bone, and brain, there are also organ-specific characteristics and functions mediated by the prevalence, state, and location of ECM components. For example, collagens are abundant in the lung, liver, and bone but are much less prevalent in the brain where hyaluronan (HA) comprises the ECM backbone [5]. Similarly, elastins are uniquely prevalent in lung where they facilitate tissue recoil [6]. The state of collagen also differs; while many organs contain similarly organized fibrillar collagen matrices, the unique strength of bone is provided by mineralization of type 1 collagen fibers with carbonated hydroxyapatite [7]. Finally, the location of ECM components within organs can dictate their organ-specific function. Fibronectin, for example, is distributed in the interstitial space of the lung and liver, but in the brain it is more concentrated in or around the endothelial **basement membrane** (BM) [5]. These characteristics can produce distinct functional microenvironments within an organ, such as strong cortical bone from densely packed ECM and flexible trabecular bone from more porous matrix [7]. The specialized functions of these organs also require organ-specific cell types that contribute to ECM formation. The core matrisomal proteins, especially collagen and fibronectin, are primarily produced by activated tissue-resident fibroblasts in normal lung [6] and by **hepatic stellate cells** (HStCs) in the liver [8]. In addition to fibroblasts, osteoblasts also contribute ECM components in the bone [7]. By contrast, the more unique matrisomal proteins in the brain are produced by a diverse set of both neurons and **glial** cells [5].

Thus, there are similarities in ECM composition, structure, and function between the lung, liver, bone, and brain, but their distinct physiological roles necessitate organ-specific differences. These physiological conditions provide a useful comparison to the diseased state when examining the changes that occur to support metastases.

ECM modifications that support metastasis

Lung

The lung is a common site of metastasis for various human cancers, including breast, melanoma, colorectal, bladder, and kidney cancer. Many mouse models of metastasis have a propensity for lung tropism, making it a frequent site of study for PMN characterization. Lung-resident fibroblasts have been identified by numerous studies as key players in metastatic niche formation [9–17]. Reprogramming resident fibroblasts and other recruited mesenchymal cells to **cancer-associated fibroblasts** (CAFs) [18–21] induces the expression of profibrotic and proinflammatory genes, which modulate the immune microenvironment [11,15,22–27]. Fibroblasts are the main producers of ECM components and, as such, are central to matrisome remodeling, which promotes lung metastasis [23]. Recent advances in single-cell technologies have facilitated in-depth analysis of CAF heterogeneity, revealing specific roles for different CAF subpopulations, including matrix-producing and immunomodulatory phenotypes that may have distinct roles in PMN establishment [21,25,28,29].

Education of lung fibroblasts is mediated by primary tumor-derived secreted factors and **extracellular vesicles** (EVs). S100A4^{+} lung fibroblasts in mice were the target of EVs from multiple human lung-tropic cancer cell lines and a human colorectal cancer (CRC) cell line through EV expression of specific integrins [9,11], highlighting the general importance of fibroblasts for

Glossary

Basement membrane (BM): sheets of specialized ECM deposited between epithelial tissue and the supporting connective tissue. The BM is typically composed of laminins, type IV collagens, and fibronectin. It functions to provide support, aid tissue compartmentalization, and regulate cell behavior.

Bleomycin: a chemotherapeutic that when instilled into the lungs of mice induces a fibrotic response.

Bone marrow-derived cells (BMDCs): a heterogeneous population of pluripotent stem and progenitor cells, such as mesenchymal stem cells and hematopoietic stem cells, originating from the bone marrow that are recruited to distant sites by chemokines and contribute to the formation of a premetastatic niche.

Cancer-associated fibroblasts (CAFs): a heterogeneous cell population found in and around the tumor that have been activated by the signaling milieu of the microenvironment. CAFs are characterized by a mesenchymal phenotype with a lack of tumor-specific mutations. Their functions include production and remodeling of collagen and other ECM molecules, crosstalk with immune cells, and secretion of signaling factors.

Extracellular matrix (ECM): a composition of secreted protein and glycosaminoglycan molecules that provide structural and functional support to the cellular components of tissue.

Extracellular vesicle (EV): lipid-delimited vesicles of various sizes that are released from all cells and contain molecules, including proteins and nucleic acids. Tumor cells use EVs to establish supportive niches at local and distant metastatic sites by systemic EV dissemination.

Fibrosis: a pathological state of overproduced ECM components, particularly collagen and fibronectin, from activated fibroblasts. This state can also include immune activation.

Glial: non-neuronal cells of the brain.

Hepatic stellate cells (HStCs): liver-specific mesenchymal cells found in the space of Disse that are responsible for ECM production and wound healing in the liver. Like fibroblasts, HStCs can be activated to a myofibroblast-like state.

Matrisome: the full profile of core and matrix-associated proteins expressed by the genome.

lung PMN establishment. These fibroblasts were typically found in laminin-rich microenvironments [9], suggesting that specific ECM molecules may help establish hospitable microniches. Education of fibroblasts by EVs affects the composition and organization of the ECM; in an autochthonous pancreatic ductal adenocarcinoma (PDAC) model, collagen 1 filaments were shorter and less organized following EV education of fibroblasts by tumors carrying mutations for Tp53 [30]. Activated CAFs can, in turn, produce and release their own EVs to influence the ECM of the metastatic site. A study in a mouse model of salivary adenoid cystic carcinoma (SACC) metastasis suggested that CAF-derived EVs have superior lung ECM remodeling and metastasis-promoting ability compared with EVs from tumor cells [16]. Further investigation is needed to determine if this finding is widely applicable.

ECM components expressed on EVs can also enhance their capacity to induce metastasis-supportive lung changes. EVs from a human breast cancer cell line were shown to have fibrillar fibronectin on their surface, which was dependent on ECM regulators tissue transglutaminase-2 and tensin-1 [12]. Disrupting this fibronectin coating reduced the ability of EVs to activate fibroblasts and resulted in fewer lung metastases from either tail vein injection into NOD *scid* gamma (NSG) mice or orthotopic injection into immunocompetent mice.

In addition to influencing the premetastatic matrisome through education of fibroblasts, cancer cells have direct effects on the matrix once they arrive in the lung. Breast cancer cell lines are able to directly produce and secrete fibronectin [31]. However, they are incapable of assembling fibronectin into a robust matrix, an ability reserved by fibroblasts, emphasizing their centrality in ECM modulation [13]. Breast cancer cells can also promote collagen 1 and 3 stability by utilizing pyruvate, a nutrient that is particularly abundant in the lung, to modify the collagen by hydroxylation [32]. Lung ECM production and remodeling can also be accomplished by other **stromal** cells beyond fibroblasts. In response to tumor-derived factors, mouse perivascular cells, including pericytes, can undergo phenotypic switching to an activated state, characterized by increased migration, proliferation, and production of ECM components to establish the lung PMN and promote metastatic progression in immunocompetent mice [33].

Myeloid-derived suppressor cells: a heterogenous population of cells derived from the myeloid lineage typically composed of monocytes and immature neutrophils that have acquired an immunosuppressive phenotype in response to a pathological state.
Neutrophil extracellular traps (NETs): trapping structures composed of DNA, histones, and other proteins such as proteases that are extruded from neutrophils in response to pathogens or other threats.
Oncomaterial: as proposed and defined by Aguado and colleagues [83], 'biomaterials that enable the detection and treatment of cancer metastasis.'
Premetastatic niche (PMN): a metastasis-supportive microenvironment induced by tumor-derived factors that is initiated in a secondary organ before the arrival of metastatic cells. This specific microenvironment can include characteristics such as infiltration by immunosuppressive cells and ECM modification such as fibrosis.
Stroma: components of the connective tissue in the tumor microenvironment, including the ECM, BM, vasculature, immune cell populations and fibroblasts.

Table 1. Components of the matrisome[a]

Category	Subcategory	Description	Examples	Refs
Core	Collagens	The most abundant ECM proteins, 28 different structural proteins divided among functional subgroups	Fibrillar: types I, II, III, V, XI Network-forming: types IV, XV, XVIII	[88]
	Glycoproteins	Proteins with glycosylation post-translational modifications Function as connectors between cells and the ECM and provide structural support Comprise the majority of core matrisomal genes	Fibronectins, laminins, tenascins, osteopontin (SPP1), periostin	[3,88–90]
	Proteoglycans	A subgroup of glycoproteins characterized by their glycosaminoglycan side chains Help regulate ECM assembly and facilitate signaling by sequestering secreted factors	Versican, perlecan, heparan sulfates, hyaluronan	[3,88]
Matrix-associated	ECM-affiliated	A heterogeneous group of proteins defined by their close association with the ECM	Galectins, syndicans, collagen-related proteins	[89,90]
	ECM regulators	Capable of modifying and remodeling ECM components to release sequestered molecules and impact ECM stiffness and turnover, which affect cancer-promoting mechanotransduction signaling and cell behavior	Crosslinkers: LOX and LOX-like family members, TGs Proteases: MMPs, ADAMs Inhibitors: TIMPs	[4,57,89,90]
	Secreted factors	Modulate ECM production, structure, and function	TGF-β proteins, Wnts, IL-6, IL-8, IL-1β	[89,91]

[a]Abbreviations: ADAMs, a disintegrin and metalloproteinases; ECM, extracellular matrix; IL-1β, interlekin-1β; IL-6, interleukin-6; IL-8, interleukin-8; LOX, lysyl oxidase; MMPs, matrix metalloproteinases; TGs, transglutaminases; TGF-β, transforming growth factor-β; TIMPs, tissue inhibitors of metalloproteinases.

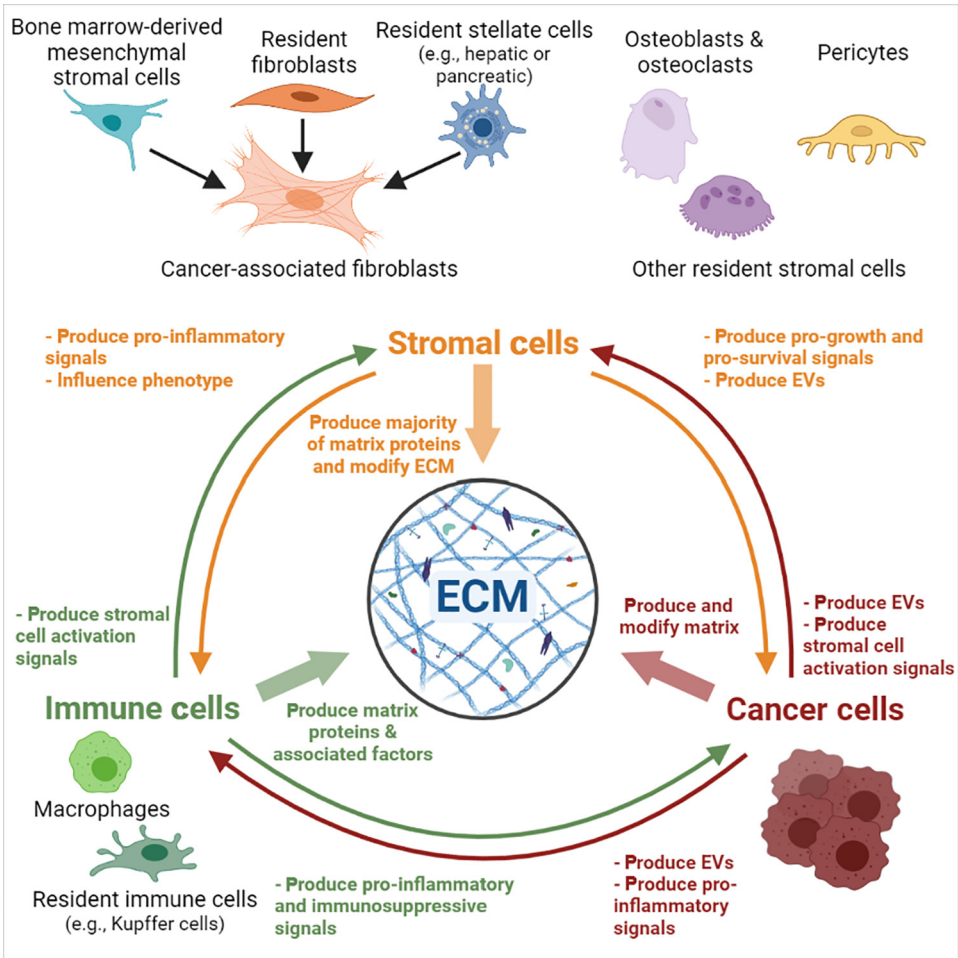

Figure 1. Cells that modify the matrisome in cancer microenvironments. The cells of the metastatic niche, including stromal cells, immune cells, and cancer cells, exhibit dynamic and reciprocal crosstalk that modifies the extracellular matrix (ECM). Stromal cells: activation of stromal cell populations results in overproduction of ECM components and dysregulated remodeling of the ECM. These stromal populations include resident fibroblasts, recruited bone marrow-derived mesenchymal stromal cells and resident pancreas and liver stellate cells, which are activated to cancer-associated fibroblasts (CAFs) as well as other organ-specific resident stromal cells, including osteoblasts and osetoclasts in the bone and pericytes in the lung. These activated stromal cells also produce signaling factors that support cancer cells and recruit immune cells and affect their functional phenotype. Immune cells: in addition to their established role in creating metastasis-supporting proinflammatory and immunosuppressive primary tumor and metastatic microenvironments, resident and recruited immune cells also promote stromal cell activation. Moreover, immune cells in the tumor microenvironment can directly modulate the ECM by producing matrix proteins and matrix-associated factors. Cancer cells: cancer cells produce a plethora of signaling molecules, cytokines, chemokines, and ECM components to modulate their local tumor microenvironment. These factors, whether secreted directly or packaged into extracellular vesicles (EVs), can also reach the metastatic organ and instigate ECM changes to establish a hospitable premetastatic niche (PMN). Furthermore, disseminated tumor cells can also establish a metastatic niche in the environment of their target organs using these mechanisms.

As discussed earlier, fibronectin and collagen are critical components of the lung metastasis-supporting matrix. These proteins are also the predominant ECM core components that contribute to **fibrosis**, a critical aspect of metastasis [34,35]. Although there is debate about whether fibrosis is primarily cancer preventing, promoting, or context dependent [36–38], multiple studies show it

plays a role in producing a supportive metastatic environment [10,39,40]. A proteomic analysis using Raman spectroscopy of premetastatic lungs in a xenograft model of breast cancer metastasis identified consistent differences in fibrotic ECM components, especially collagen matrix density and proteoglycan content, that correlated with metastatic potential [41], suggesting that profibrotic changes are supportive of metastatic outgrowth. In agreement with these findings, an aggressive human triple-negative breast cancer cell line implanted orthotopically induced more ECM changes in the premetastatic lung and lung fibroblasts of nude mice than a less metastatic luminal-A cell line [42]. The supportive functions of a fibrotic ECM and fibroblast expression of fibronectin and osteopontin (SPP1) are mediated by direct chemoattractive and antiapoptotic effects on tumor cells [10]. Another characteristic of fibrosis, increased matrix stiffness generated by crosslinking of collagen fibers by lysyl oxidase (LOX) (Box 1), also facilitated the survival and outgrowth of tail vein-injected breast cancer cells arriving at the lung of immunocompetent mice [39]. Thus, the induction of fibrosis is an impactful prometastatic change in the lung microenvironment. Further understanding of its role in metastasis may be informed by studies of idiopathic pulmonary fibrosis, which have revealed previously underappreciated roles for other cell types, including macrophages [43] and lung epithelial cells [44], in the development of this pathology. These findings may contribute to understanding the cell types, stromal influences, and matrisomal protein functions that operate in metastasis-associated fibrosis.

The metastasis-supportive roles of another ECM molecule, periostin, has also gained interest. Periostin was the top upregulated gene in mouse lung fibroblasts activated by human SACC CAF-derived EVs, and its deposition could be seen early in the formation of the lung PMN [16]. Furthermore, its expression coincided with activation of lung fibroblasts in mice [40]. Targeting periostin diminished **bleomycin**-induced lung fibrosis and subsequently reduced lung colonization after tail vein injection of the B16 model of melanoma [40]. In the mouse mammary tumor virus-polyoma middle tumor antigen (MMTV-PyMT) breast cancer model, targeting periostin revealed its additional roles in the lung PMN, including promoting the activation of LOX and

Box 1. The varied roles of lysyl oxidase (LOX) family members in producing metastasis-supporting ECM modifications

The LOX family of proteins has attracted increased attention in recent years that has revealed its varied roles for supporting metastasis of multiple cancer types. LOX is a collagen crosslinker, which puts it in the category of ECM regulator. This type of ECM modification increases tissue stiffness, as seen in the bone where increased crosslinking by LOX changes the tissue's biomechanical properties [7]. LOX expression was increased following induction of lung fibrosis in immunocompetent mice by bleomycin or irradiation and was accompanied by increased metastasis from tail vein-injected mouse breast cancer cells. Inhibiting LOX reduced both fibrosis and metastatic burden [39]. The same effect was observed in the liver, where blocking HStC-derived LOX reduced dimethylnitrosamine-induced liver fibrosis and metastatic burden [39]. In addition to this known profibrotic function, investigation of the LOX family of proteins has uncovered a variety of ways by which these molecules influence the ECM of the metastatic site. In the lung, LOX expression and activity were upregulated in immunocompetent mice following a peritoneal incision, an effect accompanied by increased tumor cell seeding following tail vein injection [92]. Furthermore, the LOX identified in these lungs was derived from the wound site and was not found in the liver or spleen, suggesting specific trafficking and indicating a possible source of metastasis-promoting signals besides the primary tumor. In addition to LOX, the related LOX-like proteins LOXL1, LOXL2, and LOXL4 were upregulated when hepatocellular carcinoma cells were grown under high stiffness conditions *in vitro* [93]. LOXL2 specifically upregulated fibronectin, tenascin-c, S100A8 and A9 chemokines, and MMP9 expression by lung fibroblasts and promoted **bone marrow-derived cell (BMDC)** invasion and motility [93,94], further indicating prometastatic functions of LOX proteins other than collagen crosslinking, including modulating other ECM components and affecting the immune milieu toward immunosuppression. In addition to tumor cells and fibroblasts as known sources of LOX proteins, M2-like polarization of macrophages due to a high stiffness tumor microenvironment promoted macrophage LOXL2 expression, revealing yet another source of the protein [95]. Additionally, circulating orthotopic breast cancer-derived LOX was shown to be involved in mouse bone PMN formation via a collagen-independent mechanism by facilitating osteolytic lesions that were subsequently colonized by cancer cells [56]. These studies bolster the important role of the LOX protein family in ECM modulation and indicate multiple sources that would need to be considered if targeting LOX is pursued as an antimetastatic strategy.

recruiting **myeloid-derived suppressor cells**, thereby mediating an immunosuppressive environment [45].

While most studies focus on the interstitial matrix, a recent study discovered a previously unknown role for the BM in promoting metastatic cell seeding and outgrowth in the lungs. Loss of Net4, a BM component, promoted lung metastasis of mouse breast cancer and melanoma cell lines by increasing BM stiffness, enabling cancer cell transmigration. Notably, increased metastasis occurred despite stiffer BM having smaller pores, suggesting that matrix stiffness is more important to metastasis than pore size [46]. Neutrophils were also shown to modify the BM in the lung PMN. Following an inflammatory signal, recruited neutrophils used **NETs** (neutrophil extracellular traps) to target neutrophil elastase (NE) and matrix metalloproteinase 9 (MMP9) to the BM. These proteases acted on BM laminins to reveal epitopes that signal via $\alpha_3\beta_1$ integrins on cancer cells, resulting in the awakening of dormant breast cancer cells in mouse lungs [47].

The lung metastatic niche, induced by education of lung fibroblasts and direct modifications of matrisomal molecules, is a complex and dynamic environment built on extensive ECM modification. Further understanding of the niche will benefit from the findings of related pathologies to elucidate other potential mechanisms at play.

Liver

The liver is a more common site of metastasis than of primary liver cancer. Many solid tumors metastasize to the liver, including colorectal, pancreatic, lung, breast, melanoma, and prostate cancer. The cells that initiate the establishment of the PMN in liver include immune cells, especially macrophages, in addition to resident fibroblastic cells. EVs from PDAC xenografts are 'postmarked' for the resident liver macrophage population (Kupffer cells) through specific integrin expression, preferring Kupffer cells located in fibronectin-rich microenvironments [9]. Kupffer cells further promote the production of fibronectin by producing transforming growth factor-β (TGF-β), which activates the fibroblast-like HStCs [48]. Macrophage recruitment and secretion of the glycoprotein granulin was also critical to liver myofibroblast activation and expression of metastasis-supportive periostin [49], suggesting potentially dual activation mechanisms involving both recruited and resident macrophages. In a related mechanism in CRC, human CRC cell line-derived EVs expressing a different integrin molecule homed to $F4/80^+$ macrophages in nude mice in addition to HStCs [11]. Thus, in the liver, activation of resident or recruited immune cells can precede or coincide with activation of fibroblastic cells in facilitating metastasis-promoting matrix changes. Like in the lung, activated HStCs express myofibroblast markers and deposit fibronectin, which contributes to liver PMN formation [11]. In addition, HStCs upregulate the expression of pro-inflammatory genes, consequently mediating the recruitment of macrophages and neutrophils from the bone marrow, which further activate liver fibroblast-like cells.

As in the lung, liver fibrosis has become increasingly recognized as supportive of metastatic colonization and outgrowth across various tumor types. Immunocompetent mice with induced liver fibrosis showed increased frequency of breast cancer metastases, and attenuating liver fibrosis decreased metastatic burden [39]. Like in the lung, inflammation is associated with fibrosis and the formation of a metastatic niche in the liver. In a transgenic mouse model of PDAC, livers of mice with autochthonous early stage tumors had increased collagen 1 and fibronectin expression and deposition, resulting in higher metastatic burden [50]. Matrix deposition was mediated by hepatocyte interleukin-6–signal transducer and activator of transcription 3 (IL-6–Stat3) signaling and enhanced production of the acute-phase reactants serum amyloid A proteins, emphasizing the connection between fibrosis and inflammation in establishing the PMN. It was also shown that a fibrotic microenvironment induced by metastatic cancer cell activation of HStCs could support

the survival of a nonmetastatic clone following its arrival in the fibrotic niche, revealing a mechanism by which cells that could not otherwise complete the metastatic cascade are supported by a fibrotic metastatic site [51]. The importance of fibrosis is also evident in the clinic. CRC patients with high liver fibrosis exhibited increased metastatic incidence and worse survival than those with low fibrosis scores, regardless of age, gender, body mass index, or diabetes status [52]. Additionally, liver fibrosis was determined to be an independent prognostic factor for metastasis and relapse for CRC patients, emphasizing its significant role in establishing a metastasis-supporting environment.

As discussed in the lung, modifications of ECM directly affect tumor cell characteristics. The ECM modification citrullination was abundant in a matrisome analysis of human CRC liver metastases where it increased tumor cell adhesion and promoted an epithelial phenotype necessary for metastatic colonization, demonstrating a connection between ECM and tumor cell plasticity [53]. Citrullinated matrix was uniquely increased in liver metastases and was not found in primary CRC tumors or normal liver [53].

In summary, the lung and liver exhibit overlap in metastatic niche mechanisms yet also display organ-specific nuances. While the mechanisms of fibroblast cell activation, fibrosis, and ECM modification seen in the lung also apply to HStCs and the liver, further investigation of the intricacies of the interaction with immune cell populations, especially macrophages, and distinct collagen modifications may be interesting avenues to identify liver-specific metastasis-supportive matrisome modifications.

Bone and brain: underexplored matrisomes

The bone and brain present intriguing tissues to study metastasis-supportive ECM changes due to their relative abundance (bone) and paucity (brain) of matrix components. Extensive bone remodeling by osteoblasts and osteoclasts occurs physiologically but can also become pathologically dysregulated. Two pronounced changes in the bone metastatic matrix are unorganized collagen fibrils and disrupted collagen and calcium apatite structure, which reduces bone toughness and impairs bone mechanics [7,54,55]. These changes in collagen structure increase tissue stiffness, while increased MMP and osteoclast activity leads to excess bone matrix remodeling [7,56,57]. Characteristic imbalance of bone resorption and deposition occurs in distinct tumor types, producing osteolytic (predominant resorption) or sclerotic (predominant deposition) metastases [7]. In osteolytic metastasis, enhanced bone degradation supports metastasizing cells by releasing ECM regulators and growth factors sequestered in the matrix while simultaneously supporting further bone resorption by osteoclasts, resulting in the 'vicious cycle' of osteolytic metastases [7]. Lesions in the bone PMN that support metastases formation can also be induced systemically by tumor-secreted factors. Osteolytic lesions and cortical bone loss have been observed in immunocompetent mice before arrival of orthotopically injected breast cancer cells in the bone and following injection with breast cancer cell-conditioned medium [56].

A search for miRNAs differentially expressed in breast cancer patients with and without bone metastasis revealed that human breast cancer cells inhibit osteoblast collagen production by secreting EV-packaged miR-218, which was elevated in the blood of those patients with bone metastases [58]. Bone colonization can also be supported by direct action of cancer cell-derived proteases. MMP2 produced by prostate cancer cells and regulated by the glycoprotein thrombospondin-2 promoted bone colonization of intratibially injected cells in nude mice [59]. Other proteases, including MMP1 and ADAMTS1, support osteoclastogenesis, providing a hospitable niche that enhanced bone metastasis of a human breast cancer cell line in mice [57].

Thus, the establishment of a bone PMN, particularly regarding modifications to matrisomal proteins, is a developing area of research with potentially significant clinical impact. Clinical studies have demonstrated that bone-targeted treatments that prevent bone destruction, such as bisphosphonates and the monoclonal antibody denosumab, are efficient adjuvants for preventing bone metastasis in breast cancer patients, indicating that targeting ECM changes is a viable therapeutic approach [60–62].

In brain, PMN establishment is largely a 'black box'. Being a highly unique organ in cell composition and function, it is unsurprising that our knowledge about the brain metastatic niche suggests it entails a singular matrix environment. A mass spectrometry (MS) study of ECM in various metastatic niches of breast cancer xenografts in mice demonstrated that the brain is characterized by relatively fewer but more diverse and unique matrisomal proteins than other niches [63]. Further investigation of these unique proteins may reveal opportunities for targeted therapeutic approaches.

While the brain matrisome is unique, tissue stiffness still plays a role in producing a hospitable niche. A study of the primary brain cancer glioblastoma revealed that a hypoxic microenvironment elevated tenascin-C expression, driving stiffer matrix that facilitated tumor growth [64]. Supporting this, an *in vitro* biomimetic study using HA, a common brain ECM glycoprotein, showed that stiff hydrogels facilitated increased proliferation of a human brain-tropic breast cancer cell line [65]. Similarly, HA-mediated motility receptor (HMMR), the receptor for HA, was upregulated in a metastatic human lung adenocarcinoma cell line, and its knockdown reduced brain metastasis resulting from intra-arterial injection into nude mice [66]. Moreover, the survival of brain micrometastases was found to be supported by HA deposits produced by nearby astrocytes, the most common glial cell type [66]. Astrocytes are known to be activated by metastasizing cells and can produce many prometastatic factors including proinflammatory signals [22], ECM regulators such as MMPs, and matrisomal secreted factors including TGF-β [67]. These studies indicate that modifications in the brain matrisome are functionally important for metastatic growth and offer numerous inroads into investigating the brain metastatic and premetastatic niches and the role of ECM modification.

ECM changes exhibit organ specificity

As illustrated earlier, ECM changes supporting distinct metastatic niches in different organs share numerous characteristics, including the functions of activated cells, upregulated molecules, and resulting effects. Studies that directly compare metastatic niches of the same tumor type in different organs showed that some ECM-related mechanisms of niche formation are robust across metastatic sites [11]. However, these types of studies also uncovered organ-specific mechanistic nuances. For example, the broadly applicable mechanism of tumor EV homing through integrin expression subsequently demonstrated organ-specific nuances in the unique integrin combinations, predominant ECM components, and distinct target cell types for the lung, liver, and brain [9]. These specificities are unsurprising as each organ has unique ECM environments that metastasizing cells must adapt to and, therefore, require unique ECM alterations to facilitate those adaptations. In a study of claudin-low breast cancer metastasis, integrin subunit α5 (ITGA5) expression was determined to be crucial for metastasis to the bone but dispensable for the lung, consistent with the organ-specific localization of the ITGA5 binding partner fibronectin in each of these organs [68]. Proteomic profiling of ECM in metastases from a triple-negative breast cancer xenograft in immunocompromised mice, which can occur in the lung, liver, bone, and brain, showed induction of distinct ECM changes in each organ [63]. Bioinformatic comparison of ECM components from both the normal and metastatic tissues showed that while ECM compositions generally grouped by organ, some metastatic ECM samples exhibited vastly different changes despite being

produced by the same cancer cells. For example, metastatic liver showed a particular increase in the abundance of glycoproteins compared with normal, which was not observed in other metastatic sites [63]. Moreover, while matrisomal proteins were mainly produced by the metastasis-associated stroma in all sites, the compositions of those proteins were different [63]. Thus, the changes induced in the metastatic stroma by the same primary tumor were organ specific. This was also seen in a study comparing gene expression changes of proteases and their inhibitors (ECM regulators) in the stroma of lung, bone, and brain early versus late metastases of breast cancer xenografts in immunocompromised mice [69]. The expression changes were found to be organ specific, with more robust differences in the stroma of brain and bone.

When examining the ECM contributions from the metastatic cells, bioinformatic analysis revealed that these contributions from the same parental cancer cell line were also specific to each metastatic site [63]. Similarly, the changes in expression of proteases in organotropic cell line variants growing in the same metastatic microenvironment were more alike than the expression changes of the same variant outgrowing in different organs [69], providing further evidence that ECM changes are dictated by the metastatic tissue. These observations highlight unique organ-specific ECM changes and reveal the distinct contributions made by each cellular compartment to developing a metastasis-supporting ECM environment.

Matrisome research perspective

Investigating the metastatic ECM is tricky due to its dynamic nature of subtle and cumulative changes involving a complex array of players. One approach to overcome this has been to integrate multiple analyses to maximize the information that can be gleaned from samples, particularly limited patient tissues. In an MS-based proteomic analysis, the matrisomes of normal colon, CRC, and liver metastases from three patients were compared to identify tissue-specific changes that could act as novel biomarkers or therapeutic targets. Integration of these results with larger patient gene expression studies allowed for validation of the resulting signatures while also revealing opportunities for personalized targets [70]. In another study, integrating analyses of six microenvironmental parameters from metastatic ovarian cancer patient samples representing a range of disease states allowed for evaluation of ECM remodeling throughout metastasis development [71]. These analyses revealed direct correlations between disease severity and matrisome complexity, collagen alignment, and tissue stiffness. Moreover, transcriptomic and proteomic analysis produced a matrix index that correlated with disease severity and outcome and was shown to be an indicator of prognosis and survival in 13 of 15 cancers examined, suggesting a common matrix response with prognostic value [71].

Other innovative technical approaches to study the ECM unveiled new opportunities for exploring ECM structure and content. Proteomic analysis using Raman spectroscopy has allowed for investigation of subtle changes in multiple proteins simultaneously without requiring protein labeling [41]. Label-free analysis of lung PMNs was also accomplished using Fourier transform infrared imaging in conjunction with polarization contrast [72]. Advancements in MS-based proteomic analysis based on protein solubility have been used to analyze direct interactions of ECM-associated factors with the matrix in the lungs of mice at different states following bleomycin-induced injury [73]. MS approaches were also used to analyze the abundance of LOX- and lysyl hydroxylase 2-mediated type 1 collagen crosslinking in human breast cancer tissues [74]. Moreover, MS was applied to simplify annotation and absolute quantification of ECM components, which could improve the accuracy of *in vitro* models of the PMN and diseased tissue [75,76]. Modeling and analysis have further benefited from advances in methods to produce physiologically relevant *ex vivo* materials. ECM produced by primary fibroblasts can be grown in culture for study or use in coculturing experiments [77,78]. Similarly, tissue decellularization,

Key figure

Organ-specific extracellular matrix (ECM) alterations produce metastasis-supportive microenvironments

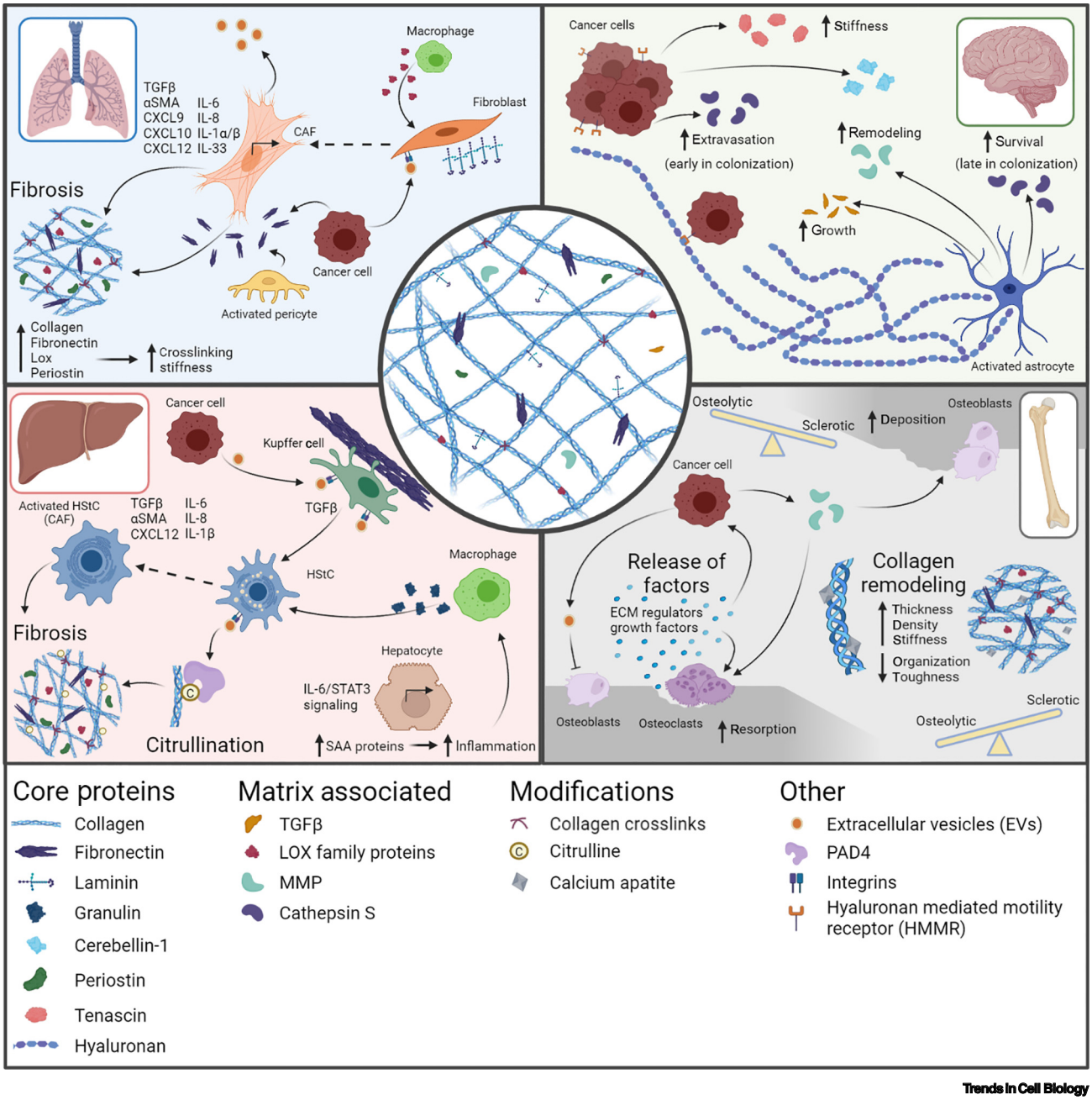

Figure 2. Changes to the ECM of the metastatic organ are induced by distant or disseminated tumor cells to produce a metastasis-supportive microenvironment. Some changes are shared between two or more metastatic organs, whereas other changes are organ-specific. Lung (upper left): tumor-produced extracellular vesicles (EVs) and

(Figure legend continued at the bottom of the next page.)

Box 2. Clinical implications of the effect of treatment strategies on ECM modifications

Recent work is beginning to uncover how clinical interventions designed to improve patient outcome could have unanticipated impacts on the ECM that promote metastasis. Mouse breast cancer cells treated with the chemotherapy paclitaxel produced EVs with enhanced capability for lung PMN formation in mice, inducing increased expression of fibronectin, collagen, and MMP9 compared with EVs from control-treated cells [96]. A similar phenomenon was observed in the liver of immunocompetent mice where pretreatment with cisplatin increased expression of MMP2 and periostin by liver cells and induced fibrosis, thereby increasing experimental melanoma metastasis to the liver [97]. Chemotherapy treatment of human breast cancer cells also upregulated osteopontin and tenascin-c mRNA via JNK signaling, which promoted lung metastasis in a xenograft model, whereas targeting these glycoproteins sensitized both primary tumor and metastatic cells to chemotherapy and significantly reduced metastasis [98]. Even surgical intervention to remove primary tumors and metastatic lymph nodes for treatment or staging can have detrimental effects, as evidenced by the increase in fibrillar collagen production and metastatic seeding of tail vein-injected mouse breast cancer cells observed in response to a 1-cm peritoneal incision and suturing, resulting in increased mortality [92]. In a breast cancer model of lymph node metastasis, removal of tumor-bearing lymph nodes accelerated metastasis to the lung along with significantly increased LOX and MMP2 expression, infiltration by $CD11b^+$ myeloid cells, and ECM remodeling, indicating that surgical interventions might enhance ECM alterations that support metastasis [99]. These studies highlight the potential benefit of therapeutic combinations that include targeting the ECM. ECM-targeting and antifibrotic therapies considered for use against cancer generally utilize three strategies: targeting the ECM, targeting the ECM-producing CAFs, and targeting profibrotic signaling (reviewed in [100]). Further investigation of these therapeutic strategies and the benefit of combining them with other treatment modalities will likely lead to improvements in anticancer treatment.

Outstanding questions

How do the ECM changes of distinct metastatic organs differ? How are they similar? What do these changes mean for developing antimetastatic therapies?

What can be learned from related lung pathologies, such as idiopathic pulmonary fibrosis, to inform our understanding of ECM changes that support metastasis?

What is the temporal sequence of macrophage and fibroblast activation in the liver?

How are the ECMs of bone and brain PMNs modified? What could the contribution of unique matrisomal proteins, particularly in the brain, reveal about organ-specific or shared mechanisms in metastasis-supportive metastatic niches?

How do clinical interventions affect premetastatic/metastatic niche ECM modifications? Do these need to be considered when devising therapeutic strategies?

such as *in situ* decellularization of tissues (ISDoT) technology, which leaves native ECM structure intact but removes the cellular components, allows for ECM-specific imaging or proteomic analysis [79,80]. The decellularized matrices can also be used to coat scaffold biomimics, allowing for a more accessible comparison of ECM effects in different disease states [81]. Scaffolds were also used to study bone metastasis *in vitro* and *in vivo* to determine how changes in pore size and rigidity affect myeloid cell infiltration, gene expression, and drug response [82]. These advancements in analysis approaches have inspired new uses for **oncomaterials** in monitoring disease progression and treating patients [83]. One innovative example is the concept of a 'biomaterial sink', an implantable material that could mimic the PMN to attract and capture metastasizing cells, preventing them from reaching patient organs. This approach produced survival benefit in both immunodeficient and immunocompetent mouse models harboring human ovarian and mouse breast cancer cells, respectively [84,85]. These feats of bioengineering have opened possibilities for leveraging the ECM to advance both research and therapeutic intervention.

Concluding remarks

Recent work investigating the ECM has revealed its important role in contributing to the formation of metastasis-supportive environments. The variety of lung metastasizing models provided an abundance of data illuminating the complexity of interactions and modifications that contribute to the PMN. From this point, both robust mechanisms shared between common metastatic organs and unique changes identified through comparative analysis revealed new avenues for research and presented opportunities for global and targeted treatment strategies (Figure 2, Key figure). However, there is still much to be learned, and some tissues remain underexplored (see Outstanding questions). Specifically, the bone and brain are two metastatic sites with

infiltrating macrophages activate quiescent fibroblasts to cancer-associated fibroblasts (CAFs), which produce proinflammatory molecules and promote fibrosis. Liver (lower left): tumor-produced EVs activate Kupffer cells to produce transforming growth factor- β (TGF-β), which activates hepatic stellate cells (HStCs) to CAFs. These cells produce proinflammatory molecules and promote fibrosis and collagen citrullination. Hepatocytes are also influenced to promote inflammation and infiltration of recruited macrophages. Bone (lower right): disruption in the balance of bone resorption and deposition results in osteolytic or sclerotic metastases by different tumor types. Release of sequestered factors from bone resorption by osteoclasts supports disseminated cancer cells and further promotes osteoclast activity. Collagen remodeling increases stiffness while reducing bone integrity. Brain (upper right): secretion of factors from both the tumor and stromal compartments contribute to a metastasis-supportive microenvironment. Organ-specific characteristics of the microenvironment, such as production of unique proteins by the tumor cells and increased production of hyaluronan by activated astrocytes, are more prevalent here compared with other metastatic organs. Abbreviations: αSMA, α-smooth muscle actin; CXCL9, C-X-C motif chemokine ligand 9; CXCL10, C-X-C motif chemokine ligand 10; CXCL12, C-X-C motif chemokine ligand 12; IL-6, interleukin-6; IL-8, interleukin-8; IL-1α/β, interleukin-1α/β; IL-33, interleukin-33; SAA, serum amyloid A; STAT3, signal transducer and activator of transcription 3.

immense clinical impact where current understanding of ECM changes that support PMNs is lacking. Access to human tissue samples and the limited number of mouse models have hindered the progress in studying these sites, but recent innovations in *in vitro* ECM acquisition [77–80], analysis [42,72,76], and *in vivo* modeling may help [86,87]. Technical innovations have also presented novel therapeutic strategies that are 'outside the box' but may hold potential. Finally, it is imperative to better understand the impact of chemotherapy and surgery on ECM alterations, as recent work suggests these treatments could simultaneously be targeting the tumor and also promoting PMN-forming ECM changes (Box 2). Learning how to address these effects may be instrumental in improving patient outcome.

In summary, studies in recent years have revealed 'glitches' in metastatic niche matrices, suggesting that understanding the shared and organ-specific modifications that characterize these sites will be valuable for understanding the complex intricacies mediating metastasis.

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Declaration of interests

The authors declare no competing interests.

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