METASTASIS, THE SPREAD OF CANCER to distant organs, is responsible for 90% of cancer patient deaths. The genetic composition and behavior of metastatic cells are often very different from cells in the primary tumor, making them extremely difficult to target and treat. Therefore, much effort is going into understanding the metastatic process to develop new therapeutic possibilities for patients with metastatic cancer.

When cancer cells metastasize to distant organs, they are dependent on the successful interaction with the new microenvironment they enter. The structure and composition of microenvironments in the distant organs are different than in the organ that is the “home” of the primary tumor. The extracellular matrix (ECM) is a crucial component of that distant microenvironment, and increasing amounts of evidence suggest that the ECM, and modifications thereof, are key factors determining whether metastatic tumors form or not. Because of this, ECM components and their effects on metastatic cell behavior have been under intense investigation during the past 15 years.

Some ECM components can initiate cellular responses in several ways; some examples include: by binding cell surface receptors (e.g., integrins and heparan sulfate proteoglycans), by sequestering and presenting growth factors and chemokines, or by providing tensile strength cues (51, 73, 107). The specific ECM molecules that have been implicated in creating a favorable, as well as unfavorable, structural microenvironment for metastatic colonization are the focus of this review. Successful targeting of metastatic cell-ECM interactions is therefore an important avenue in the treatment of metastatic cancer. The importance of the ECM in primary tumor progression has been reviewed extensively elsewhere (36, 42, 73, 95), and it is now widely accepted that the ECM can affect many of the hallmarks of cancer.

Structurally, the ECM is divided into two main categories, the basement membrane and the interstitial matrix (24). The basement membrane is produced and organized by epithelial, endothelial, and stromal cells, and it has a distinctive composition consisting mainly of laminins, collagen IV, fibronectin (FN), and linker proteins connecting the structural proteins with various other components. The interstitial matrix is mainly produced by stromal cells and is highly charged and hydrated due to the major presence of fibrillar collagens, proteoglycans, and glycoproteins like hyaluronan. This charged environment creates favorable conditions to bind growth factors (29). The interstitial ECM thus plays an important role as a signal reservoir by limiting the diffusion range and accessibility of these factors. Remodeling of the ECM can therefore affect cellular responses by releasing or sequestering more or less soluble factors.

The specific protein components of the ECM have been termed the “matriosome” (81). The matriosome has further been subdivided into “core matriosome” and “matriosome-associated” proteins. The core matriosome consists of the more “traditional” structural ECM proteins, namely, proteoglycans, collagens, and glycoproteins. Matriosome-associated proteins are further subdivided into ECM-affiliated proteins (galectins, semaphorins, etc.), secreted proteins (growth factors, cytokines, etc.), and ECM regulators [matrix metalloproteinases (MMPs), cross-linking enzymes, etc.] (49, 81).

The ECM provides biophysical and biochemical cues regulating cellular proliferation, differentiation, migration, and invasion (51, 95). It creates a dynamic environment constantly influencing cellular responses. The ECM biochemical cues can be direct or indirect—indirect by sequestering, presenting, or releasing growth factors as described above, and direct by acting as precursors that can be cleaved into functional fragments by proteases and thereby initiate signaling events (51, 73). The biophysical properties of the ECM include its stiffness, porosity, solubility, and topography (73).

In many solid cancer types, the increased expression of matrisomal proteins has been associated with increased mortality in patients (28, 95). Furthermore, changes in matrix
stiffness and fibrosis, the excessive deposition of fibrous ECM components (mainly collagen), have been implicated in tumor progression (21). The process of converting physical signals into biological responses is known as mechanotransduction (115). By sensing and regulating the ECM, cells maintain the mechanical homeostasis of tissues to sustain proper structure and function (47). Changes in ECM structure and stiffness lead to altered cellular mechanotransduction, and the molecular pathways involved are increasingly being considered as therapeutic targets in cancer therapy (107). Increased deposition, cross-linking, and linearization of collagen lead to stiffer tissue, and this has been shown to be required for tumorigenesis and malignant transformation in models of mammary cancer (68, 70). In the primary tumor the combined effects of leaky blood and lymphatic vessels, proliferation of cancer cells, and elevated deposition and remodeling of ECM components hinder proper fluid drainage, raising the interstitial pressure leading to increased stiffness (47, 73, 87, 108, 117). A detailed discussion of changes in stiffness and in mechanotransduction upon matrix changes in tumorigenesis has been covered elsewhere (21, 28, 47, 54, 72, 107) and is beyond the scope of this review.

The stiffness of the premetastatic or the metastatic niche is, however, an understudied area. Any measurements of premetastatic niches will be difficult as any changes in structure are minute and because it will only become a metastatic niche upon the arrival and colonization of metastatic cells, which is challenging to monitor (21). The careful dissection of components and structural changes in premetastatic and metastatic niches will possibly be aided by the development of nanoparticles, such as polymer dots, coupled to sensors that are able to measure alterations in force and stiffness in the future.

Another aspect that is under investigation is whether the ECM plays a major part in the concept of organ-specific metastasis (45). Certain cancer types seem to have preferential sites to where they metastasize. For instance, if metastasis occurs in patients with primary breast tumors, metastases often form in lungs, liver, bone, and brain, whereas liver and lung are the most common sites of metastasis for patients with primary colorectal or pancreatic cancer (84, 112). Several reports have investigated the genetic signatures and the contribution of specific genes and the regulation of these in organ-specific metastasis in different cancer types (9, 22, 45, 63, 83). A number of these reports have highlighted the importance of matrisomal proteins in the establishment of premetastatic and metastatic niches of various cancer types, as detailed below. The therapeutic opportunities in this area are now under intense investigation. However, we are only beginning to understand how and which components of the matrisome are involved. As with most biological responses, the cellular response to changes in the matrisome is not one-sided. In addition to promoting metastatic niche formation and metastatic growth, matrisomal proteins have also been shown to actively suppress metastatic cell survival and growth (11, 40). Therefore, more research is needed to understand how to effectively target metastatic colonization and growth. Here, we review the ECM proteins implicated in metastasis with a particular focus on structural components that are a part of the ECM “core” and ECM “regulators,” as defined by the matrisome.

**Glossary**

- **BMP1** Bone morphogenic protein 1
- **C1QTNF5** C1q and tumor necrosis factor-related protein 5
- **COMP** Cartilage oligomeric matrix protein
- **CTGF** Connective tissue growth factor
- **ECM** Extracellular matrix
- **EGLN1** egll homolog 1
- **ER** Estrogen receptor
- **FasL** Fas ligand
- **FN** Fibronectin
- **FNDC1** FN type III domain containing 1
- **HIF** Hypoxia-inducible factor
- **HPX** Hemopexin
- **IGFBP4** Insulin-like growth factor binding protein 4
- **IGAFSLS** Insulin-like growth factor binding protein, acid labile subunit
- **IL-6** Interleukin 6
- **L1CAM** L1 cell adhesion molecule
- **LLC** Lewis lung carcinoma
- **LM2** MDA-MB-231-LM2
- **LOX** Lysyl oxidase
- **LOXL** LOX-like
- **MIF** Migration inhibitory factor
- **LTBP3** Latent transforming growth factor-β-binding protein 3
- **MMP** Matrix metalloproteinase
- **PDAC** Pancreatic ductal adenocarcinoma
- **PDX** Patient-derived xenograft
- **PIGF** Placental growth factor
- **PR** Progesterone receptor
- **SERPIN** Serine peptidase inhibitor, clade A (α-1 antitrypsin, antitrypsin) member 1
- **SNED1** Sushi nidogen and EGF-like domains 1
- **SPARC** Secreted protein acidic and rich in cysteine
- **SPP1** Secreted phosphoprotein 1
- **TGF-β** Transforming growth factor-β
- **TINAGL1** Tubulointerstitial nephritis antigen-like 1
- **TNC** Tenascin-C
- **TSP1** Thrombospondin 1
- **VCAM-1** Vascular cellular adhesion molecule 1
- **VEGFR1** Vascular endothelial growth factor receptor 1
- **VLA-4** Very-late antigen 4

**The Multistep Process of Metastasis**

The importance of preparing distant sites for metastatic colonization, before disseminated tumor cells actually arrive, has become apparent over the last few years. The primary tumor secretes factors and vesicles (below) that are able to travel and primes these “premetastatic” niches to become future homing sites for disseminated tumor cells (Fig. 1, A and B) (94). These secreted factors may directly alter the microenvironment or instruct local or recruited distant stromal cells to create a permissive niche for further metastatic progression. For instance, bone marrow-derived hematopoietic progenitor cells are attracted to premetastatic niches and, together with reprogrammed stromal cells and changes in ECM structure and composition, these distant sites will become hospitable environments awaiting the arrival of disseminated tumor cells (26, 33, 59, 76).
The chances that a cancer cell from the primary tumor locally invades, intravasates into either the blood or lymphatic vessels, survives during circulation, and successfully extravasates and colonizes a distant site are very small (Fig. 1, C–E) (12, 30, 75). From the very beginning of the establishment and expansion of a primary tumor, millions of cancer cells escape and enter the circulation, but most die (12, 64). Mounting evidence suggests that extracellular proteins in the distant microenvironment and their cognate receptors expressed on the disseminated tumor cell surface are crucial for deciding whether a cell is able to form a metastatic tumor or not (6, 45, 59).

A major group of cell-surface receptors important for cellular sensing and interaction with the environment is the integrin family, and ECM proteins are the main group of integrin ligands. Integrins are a family of 24 heterodimeric cell surface receptors that can regulate cellular processes such as cell adhesion, migration, survival, proliferation, and differentiation,
by bidirectional signaling between the cell and its extracellular milieu (46, 50). Expression of integrins are cell-type specific and expression levels are often deregulated in cancer and other diseases (103). To further complicate the matter, integrins often have overlapping ligand specificity and can in many cases compensate for the loss of a family member (48). ECM-integrin binding is instrumental for cell adhesion and migration during a normal immune response (52). A similar process is believed to mediate the arrest and intravasation of circulating tumor cells at distant sites (Fig. 1E). For instance, integrin α4-positive macrophages were shown to associate with VCAM-1-positive lung-tropic human breast cancer MDA-MB-231-LM2 (LM2) cells in the bloodstream, protecting the cancer cells from apoptosis (14). Treatment with an α4-blocking antibody reduced macrophase adhesion to LM2 cells, resulting in loss of protection from apoptosis (14). Furthermore, functional blocking antibodies against integrin subunits α2, α6, and β4 reduced extravasation and migration of a set of colorectal cancer cell lines in in vivo models (100). The idea to target and disrupt the adhesion and extravasation process between circulating tumor cells, immune cells, and endothelial cells could potentially prevent distant metastasis from occurring altogether. Although this idea is exciting, such therapies are hampered by the fact that many of the integrins and ECM proteins are normally present in various cell and tissue types, calling for careful development of highly specific inhibitors to avoid unwanted side effects.

Several scenarios can come into play after the successful arrival of disseminated tumor cells at a premetastatic niche. The disseminated cells may be cleared by immune cells or undergo apoptosis if the new microenvironment does not express the right proteins to support their growth (specific examples are given below). If the disseminated tumor cells survive colonization, the metastatic cells might remain in a dormant state for a period of time until the cells are reactivated and enter a growth phase. Clinically, patients who have had their primary tumor removed can years later present with relapse in distant organs, a phenomenon known as metastatic dormancy. Understanding the metastatic dormancy process and the reactivation of dormant cells could help to maintain patients in a controlled or disease-free state (39). Successful metastatic cells might also form micrometastases that stay as micrometastases by keeping an equilibrium between proliferating and apoptotic cells. Or, this equilibrium may be shifted in favor of a proliferative state after inducing an angiogenic switch, giving rise to overt macrometastases (Fig. 1F) (5, 97).

Preparing for Colonization: Conditioning of the Premetastatic Niche by the Primary Tumor

The primary tumor releases soluble factors that mobilize bone marrow-derived cells (myeloid cells in particular) into the blood and recruits them to premetastatic niches (11, 26, 59). There is a growing list of matrisome proteins implicated in the establishment of metastatic niches, including: FN, tenasin-C (TNC), periostin, osteopontin, versican, collagen IV, collagen VI, lysyl oxidase (LOX), MMPs, and galectins (Table 1).

It has been proposed that one of the initiating events in transforming a distant site into a premetastatic niche is the reprogramming of local fibroblast by tumor-derived factors to produce FN (59). Hematopoietic progenitors expressing vascular endothelial growth factor receptor 1 (VEGFR1) and integrin α4β1 (often referred to as very late antigen 4, VLA-4), a FN receptor, are recruited to the premetastatic niche (59). In line with the hypothesis that sites of metastasis are predetermined (84, 89, 114), it was shown that the upregulation of FN in organs varied depending on the treatment with tumorspecific-conditioned medium (59). Conditioned medium from Lewis lung carcinoma (LLC) cells contained high levels of VEGF and upregulated FN expression in the lungs alone, while conditioned medium from B16 melanoma cells containing high levels of both VEGF and placental growth factor (PIGF) upregulated the expression of FN in the kidney, spleen, intestine, and ovotestis (59).

Similarly, bone marrow-derived CD11b+Gr1+ myeloid progenitor cells were recruited to premetastatic lung niches in the PyMT spontaneous breast cancer model (33). In these niches, a specific subpopulation of myeloid cells, CD11b+ Ly6Chigh, produced the ECM proteoglycan versican that stimulated mesenchymal-to-epithelial transition of metastatic tumor cells, which induced proliferation and hence development of macrometastases (33). Both FN and versican were detected in patient samples (33, 59), suggesting that they potentially could be antimetastatic targets.

Preparing for Colonization: Exosomes From the Primary Tumor Educate Metastatic Niches

Cancer-derived exosomes have received much attention recently because of their ability to transport tumor-promoting factors and to engage with metastatic niches (19, 45, 77, 94). Exosomes are membrane vesicles of endocytic origin ranging from 40 to 120 nm in diameter (25). Exosomes can contain cell surface receptors, such as integrins, which bind to and modulate the ECM (45). Importantly, exosomes were recently shown to determine organotropism of metastasis based on the integrin profile (45). The exosomes secreted by the primary tumor fuse with resident cells at distant sites, initiating premetastatic niches.

Matrix remodeling components can also be transported in exosomes. For instance, enzymatically active MT1-MMP was found in exosomes from G361 human melanoma cells (41). That exosomes can contain enzymatically active MMPs suggests another mechanism whereby the primary tumor can remodel the niche directly upon arrival. The modulated ECM and the released factors can promote tumor cell adhesion, metastasis, and activation (exit from dormancy) (5, 28). Exosomes from pancreatic ductal adenocarcinoma (PDAC) were shown to initiate premetastatic niche formation in the liver (19). The exosomes contained macropathic migration inhibitory factor (MIF), which induced transforming growth factor-β (TGF-β) production by Kupffer cells, subsequently stimulating hepatic stellate cells to produce FN. As previously shown for breast cancer metastasis to the lung (59), bone marrow-derived cells bind to these FN-rich environments, creating a premetastatic niche (19). Interestingly, preconditioning of the liver by PDAC-derived exosomes leads to a decrease in vitronectin and TNC expression, while collagen I expression was unaltered (19). These results suggest that changes in the matrisome expression profile and corresponding metastatic colonization upon conditioning of the premetastatic niche depend both on tumor and secondary organ type. Whether exosomes can ini-
### Table 1. ECM proteins implicated in the premetastatic and metastatic niche

<table>
<thead>
<tr>
<th>ECM Protein</th>
<th>Matrisome Classification</th>
<th>Primary Tumor Type</th>
<th>Source of ECM Protein</th>
<th>Metastasis</th>
<th>Premetastatic Niche</th>
<th>Dormancy Switch</th>
<th>Patient Evidence</th>
<th>Ref</th>
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<tbody>
<tr>
<td>COMP</td>
<td>Core matrisome ECM glycoprotein</td>
<td>Colon cancer</td>
<td>Metastatic cancer cells</td>
<td>+</td>
<td>x</td>
<td>(83)</td>
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<td></td>
<td>(91)</td>
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<td>Core matrisome ECM glycoprotein</td>
<td>Lung cancer</td>
<td>Stromal fibroblasts</td>
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<td>x</td>
<td>(59)</td>
<td></td>
<td></td>
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<td>Fibronectin</td>
<td>Core matrisome ECM glycoprotein</td>
<td>Melanoma cells</td>
<td>Stromal fibroblasts</td>
<td>+</td>
<td>x</td>
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<td></td>
<td></td>
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<td>Fibrinogen</td>
<td>Core matrisome ECM glycoprotein</td>
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<td>Stromal S100A4+ fibroblasts</td>
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<td>(85)</td>
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<td></td>
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<td>FNDC1</td>
<td>Core matrisome ECM glycoprotein</td>
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<td>Metastatic cancer cells</td>
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<td>x</td>
<td>(82)</td>
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<td>IGFALS</td>
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<td>Cancer cells</td>
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<td>x</td>
<td>(82)</td>
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<td>SPARC</td>
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<td>Stromal S100A4+ fibroblasts</td>
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<td>(85)</td>
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<td>SPP1</td>
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<td>Tenascin-C</td>
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<td>Timp1</td>
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<td>CD11b+ Lys6Chigh cells</td>
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<td>Collagen IV</td>
<td>Core matrisome Collagen</td>
<td>Breast cancer</td>
<td>Resident collagen IV</td>
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<td>x</td>
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<td>Matrisome associated ECM regulator</td>
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<td>+</td>
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<td>(26)</td>
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Continued
Hypoxia can regulate the gene expression of collagen, but also ECM by increasing expression of MMPs in tumor cells (35). In addition, hypoxia has been shown to indirectly modulate the ECM in the metastatic niche [reviewed by Gilkes et al. (35)].

Directly influences the composition and organization of the metastasis (35, 104). Several recent papers show that hypoxia expressing genes necessary for neoangiogenesis, invasion, and reprogramming of cancer cells in the primary tumor towards many developmental processes that are reactivated or induced behavior and differentiation (105). Hypoxia is also a potent development, and hypoxia-induced proteins regulate cellular

Themes

C960 ECM COMPONENTS IN THE TUMOR NICHE

Table 1.—Continued

<table>
<thead>
<tr>
<th>Protein</th>
<th>Matrisome Classification</th>
<th>Primary Tumor Type</th>
<th>Source of ECM Protein</th>
<th>Metastasis Long</th>
<th>Liver</th>
<th>Bone</th>
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<td>SERPIN1</td>
<td>ECM regulator</td>
<td>Breast cancer</td>
<td>VEGFR1+ HPC</td>
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<td>Cancer cells</td>
<td>(59)</td>
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<td>S100A2</td>
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<td>VEGF</td>
<td>Secreted factor</td>
<td>Cancer cells</td>
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”+” indicates a positive effect on metastatic growth. “–” indicates a negative effect on metastatic growth. “x” indicates present or detected.

Time events that inhibit the formation of metastatic niches or create unfavorable niches is currently not known, and the roles of exosomes secreted by the primary tumor in educating distant sites are only beginning to become elucidated. In the future it will be of interest to investigate cancer-specific exosome contributions and, in particular, whether it will be possible to predict if and where metastasis will occur based on patient samples, as is indicated in a recent study where increased levels of integrin α5β5 were found on exosomes from a small group of patients with lung or liver metastasis respectively (45), indeed, suggesting that exosome integrin profile could be used to predict organ-specific metastasis in a clinical setting (45).

Preparing for Colonization: Hypoxic Conditioning of the Premetastatic Niche

Hypoxia (low oxygen) is a condition critical during normal development, and hypoxia-induced proteins regulate cellular behavior and differentiation (105). Hypoxia is also a potent regulator of ECM composition and remodeling (35). Like many developmental processes that are reactivated or induced during tumorigenesis, so is hypoxia signaling. Hypoxia leads to reprogramming of cancer cells in the primary tumor towards expressing genes necessary for neoangiogenesis, invasion, and metastasis (35, 104). Several recent papers show that hypoxia directly influences the composition and organization of the ECM in the metastatic niche [reviewed by Gilkes et al. (35)]. In addition, hypoxia has been shown to indirectly modulate the ECM by increasing expression of MMPs in tumor cells (35). Hypoxia can regulate the gene expression of collagen, but also of intra- and extracellular collagen-modifying enzymes which then can alter collagen architecture and organization by, for instance, cleaving or cross-linking the collagens (35). One prominent example is the ECM-modifying enzyme LOX (27). LOX is a secreted amine oxidase that catalyzes the cross-linking of collagens and elastin by oxidatively deaminating lysine or hydroxylysine residues (38).

Recently, a LOX/hypoxic signature was shown to be associated with metastasis in pancreatic ductal adenocarcinoma (PDAC) and estrogen receptor negative (ER–) breast cancer patients (22, 78). LOX was initially proposed as a tumor suppressor gene because of its effect on HRAS-mediated transformation (18, 60). The current evidence supports that potential tumor-suppressing effects of LOX are mediated by the 18-kDa propeptide that is cleaved from pro-LOX by bone morphogenic protein 1 (BMP1) and not by the mature LOX protein itself (60, 90). In contrast, LOX mRNA and protein expression levels are elevated in high grade tumors of various cancer types, including breast, colorectal, prostate, and pancreatic cancer, and head and neck squamous cell carcinoma (7). Secreted LOX has also been shown to create permissive metastatic niches in the lung and liver by recruiting bone marrow-derived inflammatory cells through its enzymatic activity (26). Secretion of LOX by MDA-MB-231 human mammary carcinoma cells and 4T1 mouse mammary cancer cells, present as orthotopic primary tumors in mice, is associated with accumulation of LOX at premetastatic sites, where it modifies basement membrane collagen IV and thereby recruits CD11b+ myeloid cells to the niche (26). Both LOX and CD11b+ cells were detected in metastatic patient samples (26),
and targeting LOX has been shown to suppress metastasis in colon cancer, PDAC, and breast cancer models in vivo (2, 22, 78). More recently, it was shown that LOX-mediated premetastatic focal osteolytic lesions generate niches within the bone microenvironment, supporting the colonization of disseminated tumor cells and the formation of overt metastasis (22). Here it was further shown that LOX directly modulates bone homeostasis by acting on osteoclasts and osteoblasts (22). Interestingly, FN was shown to interact with and increase the catalytic activity of LOX (32). Moreover, it has been shown that knockdown of LOX leads to an increase in FN expression by KPC cells derived from the PDAC mouse model Pdx1-Cre Kras^G12D/+ Trp53R172H/+ (78). The increase in FN could rescue the decrease in proliferation seen with LOX inhibition (78), suggesting that FN could be a compensatory mechanism in the KPC cells. In these studies it was shown that LOX inhibition could completely abrogate metastasis in the KPC model but did not impact primary tumor burden. However, a striking and significant increase in survival was observed when LOX inhibition was combined with standard chemotherapy (gemcitabine).

These studies highlight both the importance of hypoxia-regulated ECM proteins and of factors released by the primary tumor in creating the metastatic niche.

### Setting Up Camp: Structural Rearrangements in the Metastatic Niche

The focus on changes in ECM structure during tumorigenesis has primarily been on collagen remodeling in primary tumors, and several reports have investigated the collagen structure in this setting. The current evidence for structural changes in the ECM at the metastatic niche is still only in its infancy. Increased collagen deposition, desmoplasia, is a well-known and well-studied ECM event in the development of primary tumors (breast cancer in particular). In primary tumors the normally curly structure of collagen I becomes linearized and often perpendicular to the tumor boundary (68, 96). In fact, in breast cancer patients it was shown that increased linearized collagen at the tumor boundary is an independent predictor of poor survival (17). As described above, the secretion and deposition of several core ECM proteins in the premetastatic and metastatic niche are rather well documented. However, there is a paucity in data concerning the organization of ECM components in the premetastatic and metastatic niche. What is currently known is that LOX and MMPs exert proadhesive and proinvasive functions (below). In addition to cross-linking collagens, LOX also cross-links elastin, contributing to elastin stability (57). Increased cross-linking of collagens and elastin leads to a stiffer extracellular environment (28). Cross-linking by LOX enables conversion of curly collagen fiber to linearized collagen fibers that are stiffer than curly collagen (68). This occurs in part through the promotion of MMP-driven remodeling. Increased stiffening of the ECM, and thus mechanical force, by LOX has been shown to increase the infiltration of tumor-promoting immune cells and the colonization of cancer cells (26). Further, LOX activity can stimulate angiogenesis in colon cancer (1), and hence it is feasible that the modulation of LOX at the metastatic niche could trigger the angiogenic switch needed for the development of macrometastases from micrometastases. After injury-induced fibrosis in the lung, LOX plays an important role in establishing an advantageous premetastatic niche by increasing fibrillar collagen (20). In response to the preinduced fibrosis in the lungs and liver, the average size and number of lung and liver metastases, respectively, increased in mice bearing 4T1 tumors (20). Fibrosis did not alter primary tumor growth but specifically altered the metastatic niche to enhance outgrowth of metastases, as shown in tail vein colonization assays in mice with fibrotic lungs. This fibrosis-enhanced metastasis was dependent on LOX activity (20).

In the premetastatic niche when myeloid CD11b^+ cells adhere to LOX-modified collagen IV they produce MMP2, whose collagenase activity leads to enhanced invasion of disseminating tumor cells (26). It has also been shown that VEGF secreted by the primary tumor leads to the upregulation of MMP9 by binding the VEGFR1 receptor on lung endothelial cells and macrophages, preceding metastasis to the lung (44). Similarly, MMP9 was found to be expressed in premetastatic clusters, potentially as a result of integrin α4β1 signaling after binding to FN in VEGFR1^+ hematopoietic progenitor cells (59). The expression of MMP9 could possibly accelerate the establishment of metastases by aiding the extravasation of bone marrow-derived cells and circulating tumor cells by degrading the basement membrane (58). In MDA-MB-231 orthotopic tumors, knockdown of LOX and the LOX-like (LOXL) family member LOXL4 led to a reduction of cross-linked collagen and CD11b^+ cells in both premetastatic and metastatic lungs compared with control cells (116). The total metastatic burden was also less upon LOX and LOXL4 knockdown (116). In another human cancer cell line, MDA-MB-435, LOXL2 knockdown had the same effects on collagen cross-linking, CD11b^+ recruitment, and lung metastasis when injected into the mammary fat pad, as it was shown for LOX and LOXL4 in MDA-MB-231 premetastatic and metastatic lungs (116).

### To Grow or Not To Grow

Upon arrival at a distant site, most disseminated tumor cells do not colonize and form metastases. Rather, they die or enter a stage of dormancy. It has been speculated that the lack of engagement with the ECM is a leading cause of dormancy in metastatic cells (5). Therapy blocking cancer cell-ECM engagement has long been conceived as a favorable target in antitumor treatment. However, the dual function of ECM molecules and receptors being both supportive and inhibitory depending on the setting complicates the matter further. For instance, the knockdown of β1 integrin in E-cadherin triple-negative breast cancer orthotopic models reduced primary tumor growth, but it enhanced metastatic growth by switching on a TGF-β-miR200-Zeb signaling pathway (111). The contrasting response in primary tumor and metastatic growth upon disrupting certain cell-ECM engagements calls for caution when developing targeted therapy. Combined with the lack of specificity seen with the failure of first generation drugs targeting MMPs (109), the drug development of therapies against cell-ECM engagements have been impeded (42). Increased specificity, and a thorough understanding of when a molecule enhances or inhibits tumor progression, will be crucial for developing future therapies.

Solitary disseminated tumor cells may undergo apoptosis, enter autophagy or senescence, as long-term survival strate-
gies. Inflammation or continued primary tumor growth can eventually induce permissive changes in the ECM expression, composition, and structure surrounding the dormant cells, leading to activation of proliferative signals. In particular, FN has been implicated in the switch from dormant micrometastases to proliferative macrometastases in breast cancer (6). FN binding to integrin β1 induced phosphorylation of myosin light chain kinase, resulting in changes in the actin cytoskeleton that reactivated the proliferative growth of the cancer cells (6). The induction of fibrosis, associated with collagen I deposition, could also induce metastatic growth of dormant cancer cells by collagen I binding to and activating integrin β1 signaling pathways through Src and focal adhesion kinase (4). In a bone metastasis dormancy model, the aberrant upregulation of VCAM-1 could, by chemotaxis, interact with its receptor integrin α4β1 on osteoclasts, promoting the switch from indolent micrometastasis to macrometastasis (74). Treatment with blocking antibodies against either VCAM-1 or α4β1 inhibited bone metastasis progression in this model (74).

TNC is a large oligomeric ECM glycoprotein that exhibits unique time- and tissue-specific expression patterns during development and in the adult. De novo expression of TNC in the adult is a hallmark of injury, regeneration, and cancer (15). Both cancer and stromal cells can initiate TNC production, and this has been shown to be important for successful metastatic colonization. Micrometastatic growth was induced by TNC in an autocrine manner in lung metastasis from both orthotopic and tail-vein colonization models using the highly lung-tropic human metastatic breast cancer cell lines, LM2 and CN34-LM (86). The expression of TNC by the colonizing cancer cells supported the expression of stem cell markers in the metastatic niche. This is in accordance with data showing that TNC is detected in stem cell niches (23, 34) and could imply that TNC helps to maintain a metastatic cancer stem cell population in the metastatic niche. TNC knockdown also led to a decrease in bone metastasis, but not in brain metastasis (86), suggesting that not all metastases are dependent on TNC for successful colonization. In immunohistochemistry stainings of metastatic melanoma, TNC was found to localize to channel-like structures together with FN and procollagen I (56). It was speculated that these structures or “channels” help to disseminate tumor cells into the niche. This is similar to the idea that linearized collagen aids tumor cells to migrate away from the primary tumor and eventually intravasate into the blood or lymphatic vessels.

That stromal cells also contribute to metastatic progression by producing TNC was observed after genetic ablation was used in an effort to determine the effects of stromal S100A4+ fibroblasts on metastasis. S100A4 is a calcium-binding protein that has been shown to have high prognostic significance for metastasis and it is associated with poor prognosis in a variety of cancer patients (80). It was shown that the ablation of S100A4+ fibroblasts attenuated lung metastasis in the 4T1 orthotopic model of breast cancer. Reduced liver metastases were also seen after intrasplenic injections of CT26 mouse colon carcinoma cells following S100A4+ fibroblast removal. Mechanistically, the attenuated metastasis could be explained by TNC production by the S100A4+ fibroblasts, which was lost upon S100A4+ fibroblast ablation (85). In addition to TNC, the matrisomal proteins secreted protein acidic and rich in cysteine (SPARC), vitronectin, and EDA-containing FN were downregulated in metastatic lungs where S100A4+ fibroblasts were ablated compared with control metastatic lungs. The study also found ECM proteins not regulated by S100A4+ fibroblasts, hence regulated by other cell types, that were upregulated in metastatic lungs compared with normal lungs, specifically, collagen I, III, XVIII, and thrombospondin-1 (TSP1) (85).

In contrast, upon investigating why some cancers are metastatic-incompetent through comparing parental MDA-MB-231 and the human prostate cancer cell line PC3 with their more metastatic counterparts LM2 and PCRM-LN4, respectively, TSP1 expression was shown to be induced in bone marrow-derived CD11b+ Gr1+ myeloid cells and to create a metastasis-suppressive niche (11). Prosaposin, a precursor of sphingolipid activator proteins, secreted by MDA-MB-231 cells was suggested to be the stimulator of TSP1 expression in the CD11b+ Gr1+ myeloid cells (11). TSP1 was the first endogenously occurring antiangiogenic protein that was discovered (37), and it has since been shown to bind multiple receptors and therefore affect a range of cellular processes depending on cell and tissue context (79).

Another member of the core matrisome that has been implicated in creating a favorable metastatic niche is periostin. Cancer stem cells release TGF-β3, which stimulates the production of periostin by resident fibroblasts in the lungs of PyMT mice, and this was found to be permissive for metastatic colonization and growth (76). The periostin laid down by the fibroblast bound and presented Wnt, thereby creating a favorable environment for a particular subset of disseminated tumor cells expressing Thy-1/CD24+, a subpopulation with cancer stem cell properties (76). Interestingly, periostin can form matrix networks together with TNC (61). This combination might synergistically promote metastasis through Wnt signaling (15), as well as the maintenance of cancer or metastatic stem cell populations. Supporting the idea that induction of stem cell properties is supportive of successful metastatic growth, single-cell analysis of human metastatic breast cancer cells revealed that early-stage metastatic cells possessed a stem-like gene expression signature (67). Interestingly, a higher number of stem-like cells (coexpressing basal cell markers) was found in patient-derived xenograft (PDX) models with higher metastatic potential compared with PDX models with fewer stem-like basal cells and thus a corresponding reduced metastatic potential (67).

In a microarray platform with ECM proteins spotted in combinatorial patterns, murine metastatic lung adenocarcinoma cells were found to preferentially adhere to galectin-3 or galectin-8 only in combination with FN (99). Galectins, a family of β-galactoside-binding lectins, can act intracellularly but are also secreted into the circulation where they can be presented on cell surfaces or become incorporated into the ECM. Galectin-3 expression in cancer patient (breast, lung, gastrointestinal, ovarian, melanoma, and non-Hodgkin’s lymphoma) sera is higher than that of healthy controls (53). Additionally, mice bearing MDA-MB-435 xenograft tumors treated with a truncated version of galectin-3 acting as a competitive binding inhibitor displayed reduced metastasis (55). The expression of FN, galectin-3 and -8 was shown by immunohistochemistry to be upregulated in distant metastases in a murine genetic model of lung adenocarcinoma (KrasLSL-G12D+/−:p53lox/lox). Using the same genetic model, tumor-bearing mice were shown to exhibit elevated galectin-3 in the early metastatic niche (98). Tu-
mor-secreted IL-6 mobilized CD11b+ galectin-3+ leukocytes into the peripheral blood and then to the metastatic niches. As a result of altered glycosyltransferase activity the cancer cells exhibit elevated presentation of the galectin-3 ligand, Thomas-Friedenreich antigen, promoting adhesion in the early metastatic niche (98).

In addition to ECM proteins contributing to the metastatic niche, the ECM glycoprotein fibrinogen has been implicated in the successful survival of tumor emboli in the circulation and adhesion before extravasation into the distant organ (91, 92). Fibrinogen deficiency led to reduced spontaneous macroscopic metastases in the lungs and in regional lymph nodes in a LLC model (91). Fibrinogen can bind to multiple integrin and nonintegrin receptors, and it is speculated that fibrinogen promotes sustained adhesion and thereby survival of circulating and disseminated tumor cells, leading to metastasis.

**Metastasis-Specific ECM Signatures**

Several studies have recently taken advantage of quantitative mass spectrometry to assess differences between nonmetastatic and metastatic tumors and to assign metastatic-specific signatures to individual cancer types. An orthotopic xenograft model was employed to compare the difference in matrisomal protein signature between the parent MDA-MB-231 cell line and its lung-tropic counterpart, LM2. Forty-three matrisome proteins were found upregulated in LM2 tumors compared with MDA-MB-231 tumors (82). Based on these, ingenuity pathway analysis showed, in accordance with previous published data (35, 88), that the TGF-β and the hypoxia-inducible factor (HIF)/VEGF signaling pathways were upregulated in the highly metastatic LM2 cell line compared with MDA-MB-231. Knockdown of four of the candidate ECM proteins not previously investigated in metastasis in the LM2 cell line, LTBP3 (latent transforming growth factor-β-binding protein 3), SNED1 (sushi nidogen and EGF-like domains 1), EGLN1 (egl 9 homolog 1), and S100A2 (S100 calcium binding protein A2), showed reduced metastasis to the lung, liver, and spleen, but not reduced primary tumor growth (82). It will be interesting to see what differences exist when analyzing LM2 cells grown in 3D culture. Moreover, in vivo a cleaved COL6A3 domain, the C5 domain, was shown to enhance pulmonary metastasis in breast cancer by inducing TGF-β-dependent epithelial-mesenchymal transition (93), suggesting that both fibril-forming and microfibrillar collagens can affect metastasis.

**Metastasis to the Brain**

Metastasis to the brain has devastating consequences during cancer progression. However, experimental models show that it is difficult for disseminated tumor cells to reach the brain parenchyma by breaching the blood-brain-barrier and actually survive if they are successful. Recent genomic analysis shows that for clonally related cancer samples, a branched evolution is observed when comparing matched primary tumor and brain metastasis samples (10), meaning that if primary and metastatic sites have a common ancestor they continue to evolve independently of each other (10). The same study also showed that brain metastases were highly divergent from extracranial and lymph node metastases. Moreover, it has been shown that disseminated tumor cells that are able to colonize the brain do so by adhering to the capillary surfaces and coopting the vessels by growing as a sheath around them (43, 71). One other potential method of surviving in the foreign brain environment that recently was discovered, upon comparing the transcriptomic signatures of brain metastatic cell lines, is when cancer cells express antilaminin serpins, such as SERPIN1 [serine peptidase inhibitor, clade A (α-1 antiproteinase, antitrypsin) member 1], on the cell surface (113). Reactive brain stroma uses plasmin as a defense against metastatic cells either by inducing apoptosis by releasing Fas ligand (FasL) from astrocytes or by inactivating L1 cell adhesion molecule (L1CAM), an adhesion molecule expressed by metastatic cells to aid their adhesion to brain capillaries (113). SERPIN1 was one of two matrisomal genes found to be upregulated in the brain metastatic subpopulation of the human lung adenocarcinoma cell lines H2030 and PC9, and in the human mammary carcinoma cell lines MDA-MB-231 and CN34, but the only one to be associated with brain relapse in patients (113).
The second matrisomal gene found to be upregulated in brain metastatic cell lines in the same study was CTGF (connective tissue growth factor), a core matrisome glycoprotein (113). CTGF has previously been implicated as a possible therapeutic target in metastatic melanoma because it is induced by hypoxia and its expression correlates with melanoma patient tumor progression (31). High CTGF expression has also been shown to be important for pancreatic tumor growth (8) and to correlate with poorer survival in glioblastoma, esophageal adenocarcinoma, gastric cancer, and adult acute lymphoblastic leukemia (62, 69, 102, 118). In contrast, CTGF expression was attenuated with tumor progression patients with lung cancer and chondrosarcoma (13, 106). Altogether these studies highlight the importance of investigating the specificity of cancer and metastatic sites.

**Future Perspectives/Unanswered Questions**

We are only just in the beginning of unraveling the differences between the ECM components at the primary tumor site compared with the premetastatic and metastatic niches. The data so far for many of the ECM proteins suggest that the contribution of a specific protein to the metastatic niche is often cancer type specific. There are also a limited number of studies that have performed global analyses on patient samples. The presence and/or expression levels of many of the ECM proteins mentioned above have been investigated in cancer patient samples (Table 1). However, often the number of samples is limited and often restricted to primary tumor. To obtain data to test whether there is a consistent regulation of ECM proteins in the same cancer or multiple cancer types, a higher number of patient samples (particularly metastatic samples) are needed.

Moreover, many of the ECM proteins that are important for the establishment and development of the metastatic niche have similar functions in the progression of the primary tumor. In some cases, there has been conflicting evidence as to whether a protein promotes or inhibits metastatic growth. It will be important to investigate the similarities and differences in cancer- or organ-specific settings, but also to determine which stage of the metastatic process a protein actively contributes to metastatic progression, in order to develop new therapeutic possibilities.

As is seen during the restructuring of collagen during primary tumor progression, not only aberrant expression of ECM proteins but structural changes could affect the metastatic process. However, the ECM structure and remodeling in the metastatic microenvironment remain unknown. This information will be highly valuable, as it will give insight into how the various proteins interact, and which regions of the molecules should be targeted to disrupt cell-ECM interactions that are critical to permitting metastatic growth. Our view is that if we can identify the foundation on which metastatic tumors are critical to permitting metastatic growth, increase drug efficacy, and thereby increase patient survival.

**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the author(s).

**AUTHOR CONTRIBUTIONS**

A.M.H. prepared figures; A.M.H. drafted manuscript; A.M.H. and J.T.E. edited and revised manuscript; A.M.H. and J.T.E. approved final version of manuscript.

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