Chapter 2

Is Extracellular Matrix a Castle Against to Invasion of Cancer Cells?

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Additional information is available at the end of the chapter

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Abstract

Metastasis is a complicated course that involves the spread of a neoplasm to distant parts of the body from its original site. A cancer cell must complete a series of steps before it becomes a clinically detectable lesion for successful colonization in the body. These are separation from the primary tumor, invasion and penetration of their basement membranes, entry into the blood vessels and survival within blood, and entry into lymphatics. A major challenge in extracellular matrix (ECM) biology is to understand the roles of the ECM and how disruption of ECM dynamics may contribute to cancer. A noteworthy area of forthcoming cancer research will be to determine whether abnormal ECM could be an effective cancer therapeutic target. We should understand how ECM composition and organization are normally maintained and how they may be deregulated in cancer. So the aims of this chapter were to focus on extracellular matrix. Invasion and metastatic skills, properties and functions of the ECM, abnormal ECM dynamics, tumor microenvironment and ECM, details of ECM invasion, role of ECM and ECM-associated proteins in metastasis, tumor dormant and metastatic process, essential component of the niches, role of the ECM in tumor angiogenesis and lymphangiogenesis are be briefly explained in this chapter.

Keywords: extracellular matrix, niche, tumor dormancy, metastasis, cancer

1. Introduction

Extracellular matrix (ECM) was synthesized and secreted by embryonic cells starting from the early stages of its development. Our knowledge on the composition, structure, and function of ECM increased significantly in recent years. The most prominent among these is that extracel-

lular microenvironment holds a critical importance in cellular growth, survival, differentiation, and morphogenesis [1].

The major role played by the local microenvironment or niches in the arrangement of cellular behavior is gradually accepted more and more in cancer biology [2–5]. The fact that extracellular matrix is a dynamic source in the progression of cancer became the center of attention for researchers [1, 5–7].

ECM affects negatively multiple proteases in remodeling, but it should be debated whether proteolysis constitutes a mandatory step in tissue invasion [8]. Many groups reported that the crossed structural barriers of cancer cells may be transferred to ECM only via the proteolytic pathway. Yet, others suggested that the neoplastic cells progressed toward the matrix by pushing or suppressing without proteases [9–12]. No matter what the route is, neoplastic cells invade the two major subtypes of ECM, namely basal membrane and interstitium [13–17].

2. Invasion and metastatic skills

The dissemination of tumors is a complex process occurring in a sequential series which can be named as a sequence of invasive-metastatic events (**Figure 1**). These phases are composed

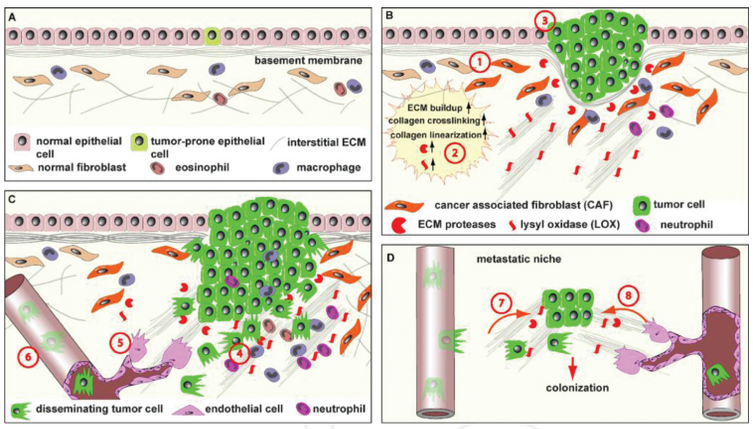

Figure 1. Abnormal ECM promotes cancer progression. (A) ECM remodeling is tightly controlled to ensure organ homeostasis and functions. Normal ECM dynamics are essential for maintaining tissue integrity and keep rare tumor-prone cells, together with resident fibroblasts, eosinophils, macrophages, and other stromal cells, in check by maintaining an overall healthy microenvironment. (B) With age or under pathological conditions, tissues can enter a series of tumorigenic events. One of the earlier events is the generation of activated fibroblasts or CAFs (stage 1), which contributes to abnormal ECM buildup and deregulated expression of ECM remodeling enzymes (stage 2). Abnormal ECM has profound impacts on surrounding cells, including epithelial, endothelial, and immune cells and other stromal cell types. Deregulated ECM promotes epithelial cellular transformation and hyperplasia (stage 3). (C) In late-stage tumors, immune cells are often recruited to the tumor site to promote cancer progression (stage 4). In addition, deregulated ECM affects various aspects of vascular biology and promotes tumor-associated angiogenesis (stage 5). Creation of a leaky tumor vasculature in turn facilitates tumor cell invasion and metastasis to distant sites (stage 6). (D) At distant sites, cancer cells leave the circulation and take hold of the local tissue. Together with local stromal cells, cancer cells express ECM remodeling enzymes and create a local metastatic niche. Abnormal niche ECM promotes extravasation, survival, and proliferation of cancer cells (stage 7). At later stages when cancer cells awake from dormancy, abnormal ECM turns on the angiogenic switch (stage 8), presumably using a mechanism similar to that used at the primary site (stage 5), and promotes the rapid growth of cancer cells and an expansion of micrometastasis to macrometastasis (see ref. [5]).

of local invasion, entry into blood and lymphatic vessels (intravasation), intravenous journey, exit from the veins (extravasation), development of micrometastases, and finally the growth of the micrometastases into macroscopic tumors [18, 19]. As it might be expected, any one of these phases may be interrupted by factors associated with the tumor or host. The series of metastatic events may also be divided into two phases, namely (1) ECM invasion and (2) intravenous dissemination of tumor cells and their homing in distant tissues/organs [20].

2.1. Characteristics, function, and invasion of ECM

As known, human tissues are composed of a series of compartments separated from each other by two types of ECMs, namely basal membranes and interstitial connective tissues. Although organized in different manners, each ECM type is composed of collagens, glycoproteins, and proteoglycans [21].

In addition to the ECM molecules, the general critical functions are important also for developmental events (**Figure 2**). Extracellular compartment comprises various ECM components

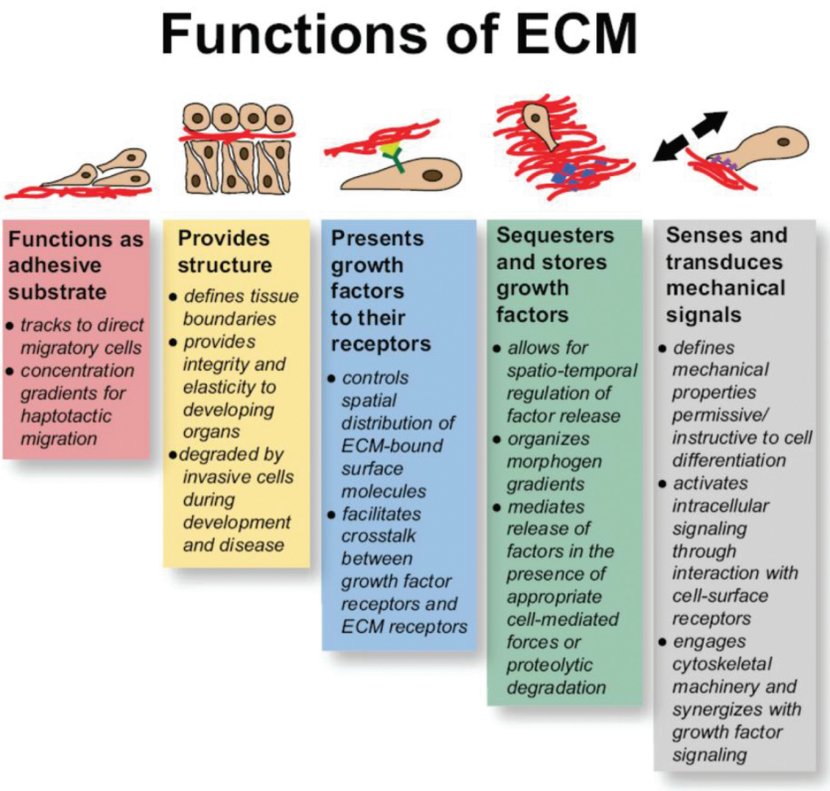

Figure 2. Summary of ECM functions in development. The ECM is multi-functional and can influence multiple biochemical and mechanical processes simultaneously. This figure illustrates different functional states of the ECM and their biological contexts. The five categories are not mutually exclusive. When interpreting ECM loss-of-function phenotypes, one should consider that multiple processes may be compromised thus specific roles of individual ECM components are difficult to glean. A couple of important properties of ECM are not illustrated in this cartoon. First, ECMs are highly dynamic and can be modified by the cells that come into contact with them creating a bi-directional mode of cell-matrix communication. Second, ECM-ECM interactions vary the chemical and mechanical composition of the extracellular microenvironment. In this review, we incorporate several examples of how the functions of ECM are utilized during embryonic development (see ref. [1]).

and this organization and composition modifies the development with the initiation of fertilization. The most prominent characteristic of cell-ECM interaction is that it is mutual. On one hand, cells are continuously formed, destroyed, or rearranged. ECM components modify one or multiple characteristics of ECM. On the other hand, as ECM arranges different cellular behaviors, this will impact adjacent cells as a result of any different cellular activity and modify its behaviors [22]. This feedback regulating mechanism between the cells and ECM enables rapid adaptation to the surrounding of cells and tissues [23].

The extracellular matrix (ECM) structure is dynamic and may be destroyed by the enzyme family known as the matrix metalloproteinases (MMPs). These enzymes are actually secreted by stromal cells or heparinase (this is an endoglycosidase enzyme which separates heparin sulfate chains expressed and secreted particularly by tumor cells). Thus, the microenvironment may contribute to tumor dormancy or metastatic growth with the impact of MMPs. The expression and secretion of MMPs by leukocytes and macrophages may lead to the release of angiostatic factors inhibiting angiogenesis and metastatic growth from ECM. These anti-angiogenesis factors comprise endostatin, restin, arrestin, three chains of collagen IV, and macrophage elastase [24]. Similarly, stromal MMPs may release cytokines and angiogenic factors affiliated with ECM such as fibroblast growth factor (FGF) and vascular endothelial growth factor (VEGF) and may initiate the angiogenic switch required for the transition from micrometastatic dormancy to metastatic growth. MMPs may also contribute to the formation of a suitable place for the transition from dormancy to metastatic growth. For instance, modifications in ECM components, such as the arrangement and production of type I collagen and fibronectin, were detected in gene expression signals associated with metastasis and poor prognosis in breast cancer [25]. Furthermore, the leukocyte secretion of MMP2 and MMP9 may activate latent transforming growth factor (TGF)-beta localized in ECM. The activation of TGF-beta may enhance the Type I collagen and lysyl oxidase expression synthesis and thus provide a suitable setting for metastatic growth [26]. On the other hand, the heparinase synthesis of tumor cells regulates the re-arrangement and destruction of ECM in association with angiogenic factors promoting angiogenesis and tumor cell migration [27]. In summary, the crosstalk between dormant tumor cells and ECM regulated by stromal and tumor cells may control the initiation or termination of the dormant status of the cell.

2.2. Dormancy of tumor cells and the metastatic process

Tumor dormancy may be defined as the long-term asymptomatic, non-detectable and latent state of disseminated tumor cells (DTCs). This period is a stage where the residual disease exists, but is not clinically visible. The cells in dormant state avail of the capacity to grow slowly, escape treatment and the immune system of the host and to renew themselves. Tumor dormancy may contribute to the progression and relapse of the tumor metastatically both in local and distant sites. Cancer cells go into a dormant stage at the beginning of the disease or following the first treatment and may remain dormant even for years or for decades after the first treatment. The mechanisms and the sleep markers regulating the transition between the dormancy and proliferation phases have not been fully designated [28]. A part of the latent period in all patients may take place as the slow accumulation of the genetic modifications

leading to immortality (TP53, RB1, P16 loss, and/or telomerase gain, etc.) and the transformations during and/or following carcinogenesis (Ras-activating mutations, ERBB2 amplification, BRAF-activating mutations, etc.). Breast and prostate tumors, melanoma, B-cell lymphoma, and leukemia are malignancies displaying dormant cancer cells [29, 30].

The metastatic growth of disseminated tumor cells (DTCs) from the primary tumor constitutes the main reason of cancer-associated deaths. DTCs should survive around the circulation when they mix with blood and avoid physical damage and immune system attacks. Thus, DTCs adapt themselves to the new microenvironment of the secondary site and the reprogramming periods to the micrometastasis or quiescent state begin according to the characteristics of the microenvironment [31].

Various metastasis suppressant genes responding to microenvironmental stress may regulate the dormancy. Metastasis suppressant genes have the capacity to encourage apoptosis or the dormancy of cells and prevent the development of metastasis. KISS-1 is a tumor suppressor gene contained inside kisspeptins, and it has been demonstrated that the cells expressing kisspeptins remain dormant in many organs. Kangai 1 (Kai1/CD82) is a cell surface transmembrane protein which joins the inhibition of invasion and cancer cell migration by forming complexes with integrins. Furthermore, Kai 1 reduces the formation of distant metastasis upon binding to duffy antigen-chemokine receptor on the surface of vascular endothelial cells [32]. It was demonstrated that in melanoma, colon, breast, and lung cancer models that the metastasis is suppressed via the Nm23–1H (NME1) protein [33, 34]. Mitogen-activated protein kinase 4 (MKK4) is a specific kinase which plays a role in dormancy in the micrometastatic stage. MKK7 and MKK6 are other kinases with less metastasis suppressor effects. BRMS1, SMAD7, SSeCKS, RhoGD12, and CTGF are metastasis suppressor genes which play a potential role in dormancy. In case of more activated P38 in the cell, tumor cells may be encouraged to enter dormancy [35].

2.3. ECM's invasion and the stages of invasion

It is necessary for a carcinoma first to pass through the basal membrane beneath and then through the interstitial tissue and consequently reach the circulation upon penetrating into the basal membrane in the veins. The referred cycle is repeated also when the tumor cells embolisms extravasate from a different site. Due to these reasons, a tumor cell may metastasize only when they pass through different and high number of basal membranes and at least two interstitial matrices [36, 37]. The ECM invasion is achieved in four steps.

The first step of the series of metastatic events is the relaxation of tumor cells. E-cadherins act as intercellular adhesives and their parts within the cytoplasm bind to β-catenin. Neighboring E-cadherin molecules hold together cells, and as also explained earlier, they may send antiproliferative signals over the β-catenin sequestration [38]. The E-cadherin function in almost all epithelial-derived cancers is lost due to the mutations achieved via the β-catenin gene activation of the E-cadherin genes or the inadequate expression of the SNAIL and TWIST transcription factors suppressing the E-cadherin expression [39, 40].

The second step in the invasion is composed of the local disintegration of the basal membrane and the interstitial connective tissue. The tumor cells themselves secrete proteolytic enzymes or stimulate stroma cells such as fibroblasts and inflammatory cells so that they secrete proteases. It was expressed indirectly that many different protease families, such as matrix metalloproteinases (MMPs), cathepsin D and urokinase plasminogen activators, play a role in the achievement of the invasive characteristic in the tumor cell. Matrix metalloproteinases regulate the tumor invasion not only by reshaping the insoluble components of the basal membrane and interstitial matrix but also by releasing the growth factors at ECM [41]. Actually, the cleavage collagens and proteoglycans also have effects which promote chemotactic, angiogenic, and growth. For instance, MMP-9 is a gelatinase which may release type IV collagen in the basal membrane of the epithelial and the veins; furthermore, it also stimulates the VEGF secretion of ECM from sequestered pools. Type IV collagenous activity, which is very rare in the benign tumors of the breast, large intestine, and stomach is at an abundant amount in the malignant tumors of the same organs. Meanwhile, indeed, an overexpression of metalloproteases and other proteases was reported for many tumors [42–44].

The third step of tumor invasion involves changes in the adhesion of tumor cells to ECM proteins. There are receptors in the normal epithelial cells, such as integrin, which belong to the basal membrane laminin polarized on the basal surfaces and to collagens, and these help the cell to maintain its undifferentiated status at rest. While the loss of adhesion initiates apoptosis in normal cells, tumor cells are resistant to the death of cells to take place via this path [36]. Furthermore, the matrix itself is changed in a manner so as to promote invasion and the occurrence of metastasis. For instance, the cleavage of basal membrane proteins (collagen IV and laminin) by MMP-2 or MMP-9 creates new sites to which the receptors in the tumor cells may bind and stimulate the migration [42, 44].

The final step of the tumor invasion is the locomotion of malignant cells. During the locomotion process, the tumor cells pass through fragmented basal membranes and proteolyzed matrix regions and translocate. The migration of cancerous cells is a multi-phased process, which impacts the cytoskeleton in the actin structure at the end and where many receptor families and the signalization protein family play a role (**Figure 3**). This last step appears to be a process which is promoted and directed by cytokines deriving from the tumor cell such as the autocrine motility factor. Furthermore, the cleavage products of the matrix proteins (such as collagen and laminin) and some growth factors (such as insulin-like growth factor I and II) have a chemotaxis effect on these cells.

Moreover, the stroma cells also produce paracrine effector factors such as HGF/SCF (hepatocyte growth factor/diffusion factor) which bind to the receptors on the tumor cells. HGF inhibition is as effective as standard chemotherapy in inhibiting local tumor growth [45]. The fact that the concentration of these factors is high in the peripheral region of glioblastoma multiform, which is a strong brain tumor with advanced invasion skills, supports the view that they play a role in motility [46].

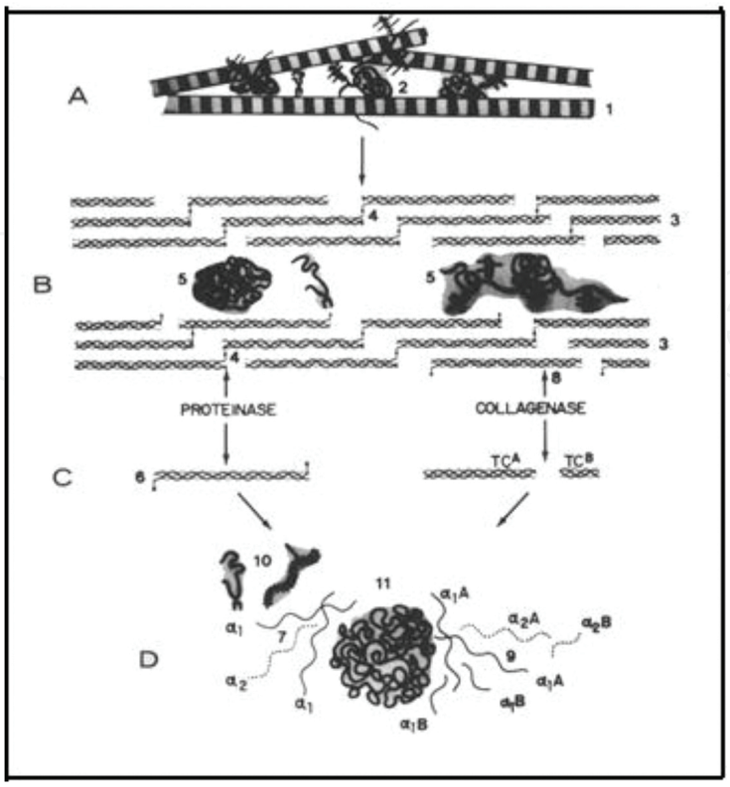

Figure 3. Schematic model of enzymatic disruption of extracellular matrix at the tumor invasion zone: (A) The tumor-surrounding extracellular matrix consists of a meshwork of collagen fibers (1) and interdispersed glycosaminoglycan-containing proteoglycans (2) that provide swelling pressure to maintain tissue volume; (B) collagen molecules (3) are aligned in staggered fashion overlapping by one quarter of their length to form a cross-striated collagen fiber (1). Covalent cross links (4) between neighboring collagen molecules are responsible for tensile strength and insolubility of the fiber meshwork. The interdispersed proteoglycans shows limited aggregation with hyaluronate (5). It is restricted from swelling by an intact collagen network; (C) collagen fibers are degraded by two enzymatic pathways: (a) proteinases (i.e., cathepsins, elastase, plasmin, thrombin) act as "cross-linkases" (4) to liberate collagen monomers from fibers (6). Collagen monomers then denature (7), solubilize, and become susceptible to many proteinases. (b) Vertebrate collagenases specifically cleave the collagen triple helix at the ¾-¼ point between the NH2- and COOH-termini (8). The resulting TCA and TCS fragments denature (9) and are further cleaved by neutral proteinases: (D) Collagen and concomitant proteoglycan degradation (10) transforms the matrix from an insoluble (solid) to a liquified (fluid) state. The remaining proteoglycans swell (11) due to breakdown of the restricting collagen network. These physical changes may allow locomotion and tumor cell penetration (see ref. [6]).

2.4. How does ECM deregulation signal cancer?

The structure of tumor-associated ECM is basically different than that of the normal tissue stroma. Relaxed, non-oriented fibrils and collagen I are significantly oriented with epithelia which are significantly linearized and attached in the breast tissue or are designed vertically to the tissue [41].

Abnormal ECM dynamics have been well documented in clinical studies as a sign of many diseases and cancer. For instance, excessive ECM production or decreased ECM destruction is evident in many organ fibroses [47]. The storage of various collagens containing collagen I, II, III, V, and IX increases during tumor formation [48]. These abnormal changes in the composition and rate of ECM may significantly modify the biochemical characteristics of ECM

and potentialize the oncogenic effects of various growth factor signal pathways [49]. Increased collagen deposition or ECM stiffness may support cell survival and proliferation alone or with the upregulation of the integrin signal [50, 51].

Increased collagen cross links and ECM stiffness stimulate ERK and PI3 kinase signal as a result of LOX overproduction and facilitate oncogenic transformation [47].

2.5. May ECM prevent cancer cell invasion?

Studies have demonstrated that ECM is essential in the protection and achievement of tissue polarity and structure. Abnormal ECM dynamics may cause basal membranes to compromise as a physical barrier and facilitate tissue invasion of cancer cells by supporting epithelial mesenchymal transition [52, 53].

The changes in ECM topography may facilitate the migration of cancer cells. Thickening and linearization are observed in collagen fibers in cancer cases, and these are mostly seen in tissue invasion and vascular tumor sites, which demonstrates that they may play an active role in this cancer cell invasion [41, 54].

2.6. Tumor microenvironment and ECM

Tumor microenvironment plays a critical role in the progression of cancer and is the main factor determining the growth and survival of DTCs in prioritized metastatic sites [43, 55]. It was recently revealed that the stroma cells surrounding tumor cells constitute a variable environment which promotes or prevents tumor formation of mutual signalizations between the tumor and stroma cells and not as a static barrier that prevents the motility of tumor cells [56]. The congenital and adaptive immunity cells as well as fibroblasts are among stroma cells which interact with tumors. It was revealed in various studies that tumor-accompanying cells contain ECM molecules, proteases, protease inhibitors, and genes encoding various growth factors in modified forms [57]. Dormant tumor cells are in close contact via the extracellular matrix via the integrin signalization pathway regulating tumor cell growth, migration, differentiation, and survival. Metastasis-associated urokinase receptor (uPAR) causes tumor growth via fibronectin receptor alfa5beta1-integrin activation and interaction. This complex enables the functioning of EFGR which promotes focal adhesion kinase (FAK) and adhesion to fibronectin and transfers mitogenic signals via Ras extracellular signal-regulated kinase (ERK), respectively. In an *in vitro* study, the downregulation of uPAR and the loss of function of integrin reduced the proliferative signals from a fibronectin-rich microenvironment which led to the transition from a tumorigenic status to a dormant status in human carcinoma cells. Furthermore, the blockade of uPAR, beta1-integrinler, FAK or EGFR alone or in combination results in *in vivo* tumor suppression which is demonstrated to be associated with the induction of tumor cell dormancy [58, 59].

In vivo ERK1/2 signalization revealed that dormancy derives from an almost complete full inhibition of the Raf-MEK-ERK pathway and triggers the stopping of cell cycle in the G0-G1 phase as in the dormant cells. It was demonstrated that the mitogen-activated protein kinase (MAPK) signalization cascade activated with (P38/c-Jun N-terminal kinase (JNK) has an

impact as a tumor suppressor via various tumor suppressor (TP53 and Rb-mediated) pathways and by the decrease of various oncogenic signals and become responsible for the stopping of the growth. The disruption of the UPAR complex activates the p38 MAPK signalization pathway. Proliferation in primary and secondary tumors requires a high ERK1/2/p38 MAPK, pathway activation—contrary to tumor dormancy. Thus, the molecular mechanisms of growth inhibition have become comprehensible during dormancy, which is observed both I the p38 MAPK pathway activation and ERK1/2 pathway inhibition [60].

Thus, tumor cells maintain their existence within a complex and constantly changing environment in which ECM, fibroblasts, and the immunity system cells communicate with one another. The cells which cooperate with the referred environment in order to fulfill their bad intentions and may adapt themselves to this environment can be the most successful tumor cells.

2.7. May fibroblasts play a role in tumor invasion?

When cells are transformed and the solid tumor mass formation is initiated, they lead to a modification in the phenotypes of the cells surrounding them. A transformation occurs in the extracellular matrix in addition to the modification in the cellular phenotype, and this tumor formation occurs simultaneously [57, 61]. It was recently discovered that increased matrix stiffness may also lead to the increase in the oncogenic YAP/TAZ complex increased in association with signal regulators comprising the Hippo signal pathway, enhanced cellular proliferation, decreased contact inhibition, increased cancer stem cell phenotype, and increased metastasis [62]. However, it was demonstrated in the recent publication by the authors that YAP/TAZ was not activate only at CAFs: Cancer associated fibroblasts, but that YAP/TAZ was necessary also for CAF development [63]. The authors demonstrated that CAF activation led to a matrix remodeling developing to increased stiffness with the myosin light-chain 2 (MYL9/MLC) expression which plays a vital role in the formation of ECM. Another point pinpointed by these authors was that the YAP/TAZ activation was not specific only to CAFs, but that it was also revealed in the normal tissue fibroblasts surrounding the cancerous tissue.

2.8. Role of ECM in vascular dissemination and homing of tumor cells

The growth of a tumor size requires an increase in the need for nutrients, oxygen, and waste exchange. Tumor vascularization constitutes the main path in metastases of cancer cells [36, 64].

When tumor cells reach the circulatory system, the host is likely to be destroyed by immune cells. Some tumor cells in the blood circulation aggregate and adhere on leukocytes in the circulation, particularly on thrombocytes, and cause embolism; thus, part of tumor cells in the circulation achieves a certain degree of protection against the antitumor effects of the effector cells of the host. However, the majority of tumor cells circulate alone in the circulation. During the extravasation of free tumor cells or the development of tumor embolism, the referred cells first adhere on the vascular endothelium and then enter the organ parenchyma upon passing through the basal membrane via mechanisms similar to those in the invasion process [64].

It is possible to estimate the site of extravasation of tumor cells and the distribution of the metastases in the organs by looking at the site of the primary tumor and the vascular or lymphatic drainage (**Figure 4**). Most tumors metastasize in the first organ they encounter in the capillary bed upon entering the circulation. However, natural drainage paths may not easily explain the distribution of metastases in many cases. Some tumors such as lung cancers frequently metastasize in the adrenals, while they almost never spread to the skeletal muscle [65]. This organ tropism may be associated with the following described mechanisms:

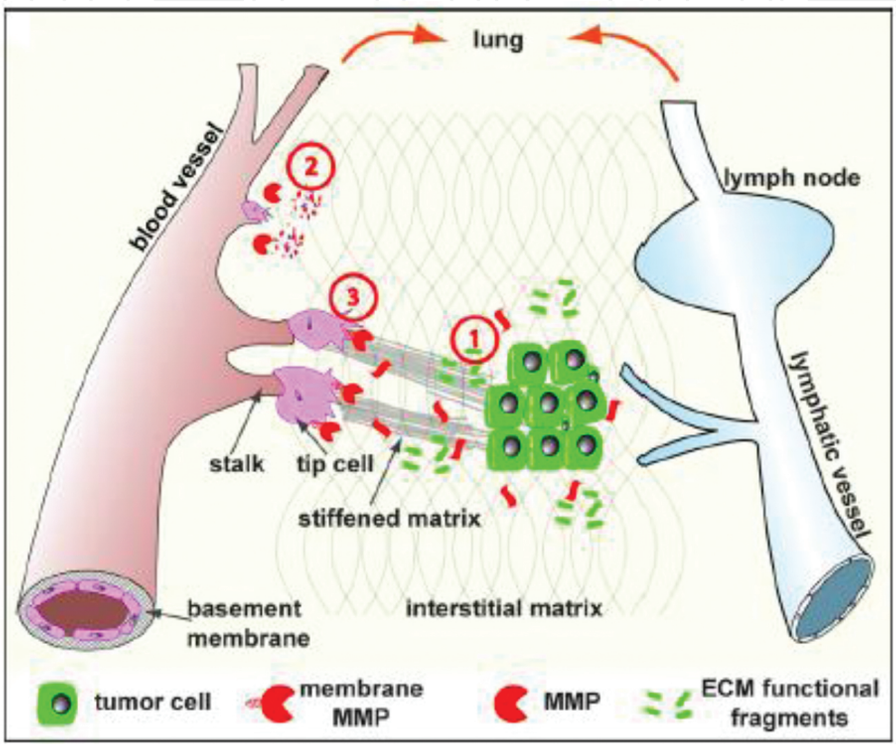

Figure 4. ECM role in tumor angiogenesis, lymphangiogenesis. Angiogenesis and lymphangiogenesis depend on the ECM. Tumor cells produce various components, including VEGF and angiogenic and antiangiogenic ECM fragments, to regulate blood vessel formation (stage 1). During branch initiation, endothelial cells secrete proteases to break down the basement membrane to grow out (stage 2). The outgrowth process of endothelial branching is propelled by at least two groups of cells: tip cells, which lead the migration toward the angiogenic chemoattractant source, and stalk cells, which depend on the ECM and its derivatives to survive and proliferate to provide building blocks for vessel formation (stage 3). Additionally, ECM components participate in cell migration and other aspects of tubulogenesis of blood vessels. Although details remain unclear, lymphangiogenesis depends on the ECM and, together with angiogenesis, provides routes for cancer cell metastasis and immune cell infiltration (see ref. [5]).

1. The expression of the ligands in the tumor cells and preferably of the adhesion molecules present in the endothelium of the target organs. 2. The expression of chemokines and their receptors. Chemokines contribute to the guided movements of leukocytes (chemotaxis), and cancer cells appear as cells which utilize similar tricks in order to settle in special tissues. Chemokine receptors named CXCR4 and CCR7 have a high expression in human breast cancer. The ligands of these receptors (CXCK12 and CCL21) are present in high amounts only in the organs where breast cancer cells have metastasized. Based on this observation, it was claimed that the blockage of chemokine receptors may limit metastases [44, 66].

When tumor cells reach their target, they may be colonized in that target. The factors regulating the referred colonization have not yet been fully understood. However, in order for tumor cells to proliferate after extravasation, they need a stroma that will accept them. In some cases, the target tissue may not carry a suitable environment identity for metastasis and is not the suitable soil, so to say, for the development of the tumor seeds. For instance, although the skeletal muscle is not rich in terms of vessels, it rarely becomes a stage for metastases [44].

Because, the biochemical characteristics of ECM, which play an important role in tubulogenesis during tumor angiogenesis [67, 68] in the vein lumen formation blood vessel lumen formation [69], are different in terms of displaying different branching patterns and various elasticities in these fields [70].

2.9. Details of ECM invasion

Willis et al. drew attention to the astuteness in the invasion of the devilish hidden cancer cells in the review they published [7]. Many groups reached the conclusion that cancer cells acquire an amoeboid phenotype characterized by insensitivity to proteinous inhibitors and surpass type I collagen barriers [12]. Now we know that a wide spectrum of types of cancer cells are definitely dependent on MT1-MMP when they are faced with cross-linked Type I collagen barriers [12, 71].

Still, when cancer cells encounter structural barriers, they hold the potential to adapt themselves to a protease-dependent position. Although there is limited information on the size of ECM pores, it is estimated via confocal reflection microscope that micropores range between of 40–10 μm^2 and macropores of 40–1000 μm^2 inside *in vivo* tissues [72–74]. These results increase the probability indicating that the collagen structure combined in an *in vitro* setting may not be repeated in a complex *in vivo* setting.

However, it should be noted that the defects in the migration of vascular smooth muscle cells, adipocytes differentiation, and stem cell origin displayed an *in vitro* setting duplication with the use of dense acid extracted type I collagen hydrogels in MT1-MMP-targeted mice [75–77]. Interestingly, the diameter of collagen fibers at *in vivo* neoplastic fields matched with the self-polymerized collagen hydrogels prepared in acid extractor type I collagen under standard conditions [78].

These results led to the thought that cancer cells may rapidly migrate to precleared tunnels via the proteolytic pathway through proteinaceous-independent processes similar to those in the *in vitro* setting [11, 79–81].

2.10. Role of ECM and ECM-associated proteins in metastasis

As cancer cells accumulate mutations or other molecular signals during the metastatic process, they are predisposed to become more easily malignant and lose contact with the surrounding cells and ECM in the primary tumor. These surrounding cells provide the opportunity for invasion. Thus, ECM and ECM-associated adhesion proteins play a critical role in the metastatic process [82]. Therefore, Zacharia et al. [83] published a review describing roles of the

new molecules named migfilin, mitogen-inducible gene-2 (Mig-2), and Ras suppressor-1 (RSU-1) in the cell-ECM adhesion fields. The authors reached the conclusion that cell-ECM adhesion proteins are predisposed to function such as adaptor proteins in the form of multiple protein-protein interaction in the cell-ECM adhesion fields.

Even though the different effects in various types of cancer cells were discussed, they added that cell adhesion, which is crucial in terms of cell metastasis in many cases, supported cell invasion and apoptosis.

2.11. Is ECM the main constituent of niches?

Despite the "skill" they display in moving away from the site in which they were first formed, tumor cells are rather ineffective in terms of forming colonies in distant organs. Millions of cells drop off even from small tumors every single day; even though macroscopic metastases have not developed, it is possible to identify these cells in the blood circulation and in small foci in the bone marrow. The dormant state of micrometastases, which is defined as the capacity to preserve their existence for a long period without any progression, was observed in breast and prostate cancer [29, 30, 44].

In the studies demonstrating that ECM undertook a dynamic niche role in the progression of cancer in recent years [5–7], investigators indicated that the microenvironment or niche played a major role in the development of cancer. Abnormal ECM directly promotes cellular transformation and metastasis and impacted the progression of cancer [84].

A successful metastasis does not require local niche supporting only cancer cell development in the primary focus, but also necessitates the survival, colonization of the cancer cells invading the metastatic niches and their achievement of macrometastasis [85–87].

The molecular mechanisms of colonization have just begun to be enlightened in mice models, but the view claiming that tumor cells impact normal stroma cells and secrete cytokines, growth factors and proteases, which transform the site of metastasis into an environment where cancer cells may live, appears to be suitable [88, 89].

3. Concluding remarks

As metastatic mechanisms are better understood at a molecular level, it will be significantly easier for physicians to use these mechanisms as a treatment goal [90]. The identification of tissue-specific signals involved in metastatic progression will open the way to new therapeutic strategies. For this purpose, the authors [91] reviewed recent progress in the field, with particular emphasis on the mechanisms of organ-specific dissemination and colonization of breast cancer (**Figure 5**). Despite what has been described so far, it may not be possible to estimate exactly which cancer type may metastasize. But a noteworthy area of forthcoming cancer research will be to determine whether abnormal ECM could be an effective cancer therapeutic target. So we should understand how ECM composition and organization are

normally maintained and how they may be deregulated in cancer. Then, we may protect the ECM as a castle against to invasion of cancer.

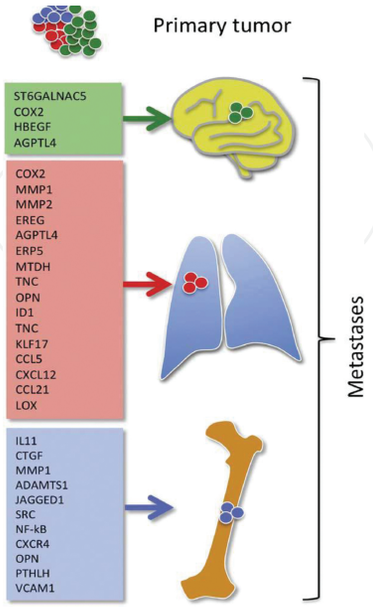

Figure 5. Gene mediating organ-specific breast cancer metastasis. Breast cancer genes promoting organ-specific metastasis to bone, lung, and brain have been identified. They include proinflammatory molecules and chemokines/receptors (e.g., COX-, CXCL12/CXCR4), matrix-degrading and modifying enzymes (e.g., MMP1/2, LOX), adhesion and extracellular matrix molecules (e.g., VCAM-1, TNC, OPN), transcription factors (e.g., ID1, KLF17), intracellular signaling proteins (e.g., SRC, NF-_B), and cell communication proteins (JAGGED1, CTGF). Some genes promote seeding (e.g., ST6GALNAC5, AGPTL4), whereas others promote colonization (e.g., OPN, CXCR4) (see ref. [91]).

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