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Extracellular Matrix-Based Cancer Targeting

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Keywords

cancer; extracellular matrix; proteoglycans; hyaluronan; collagen; matrix remodeling enzymes

Abstract

Tumor extracellular matrix (ECM) operates in a coordinated mode with cancer and stroma cells to evoke the multistep process of metastatic potential. The remodeled tumor-associated matrix provides a point for direct or complementary therapeutic targeting. Here, we cover and critically address the importance of ECM networks and their macromolecules in cancer. We focus on the roles of key structural and functional ECM components, and their degradation enzymes and extracellular vesicles, aiming at improving our understanding of the mechanisms contributing to tumor initiation, growth, and dissemination, and discuss potential new approaches for ECM-based therapeutic targeting and diagnosis.

Extracellular matrix in cancer

Extracellular matrix (ECM) (see Glossary) has a pivotal role in patho(physio)logical processes. The ECM macromolecular networks, located extracellularly in all tissues, orchestrate numerous cellular processes and cancer propagation and metastasis. Altered expression of ECM macromolecules in the tumor microenvironment (TME) affects cancer cell growth and survival, adhesion, and migration. The creation of a provisional multitasking ECM that reinforces tumor cell capabilities emerges as a new hallmark in tumorigenesis [1,2]. The scope of this Review is to present the major ECM macromolecules in the context of their paramount importance in tumor initiation, propagation, and progression and to discuss recent advances and future perspectives of ECM-based cancer targeting. We first review the ECM networks based on the impact of **proteoglycans** (PGs)/**glycosaminoglycans** (GAGs), collagens, **laminins**, and their receptors in the TME. Second, we consider the roles of key partners in ECM remodeling, including **matrix metalloproteinases** (MMPs), cathepsins, heparanases and hyaluronidases, and their involvement in cancer progression. Third, we discuss the potential pharmacological targeting of ECM in cancer, with emphasis on **exosome** formation and epigenetics.

ECM Networks and Tumor Microenvironment

The integrity of the ECM networks is essential for normal tissue homeostasis. The networks, formed by interacting macromolecules and bioactive effectors, provide structural support to tissues and regulate, upon cell-matrix interactions, cell shape, viability and function. The ECM is the bioscaffold for tissues and organs. ECM supramolecular structures such as fibrils and sheet-like networks are composed of, among other constituents, collagens, PGs and GAGs, elastin and adhesive glycoproteins (laminin and fibronectin) [3]. These have an active role in several cell functions and properties such as cell morphology, survival, proliferation, morphogenesis, and differentiation [3-5]. The stability and functions of the ECM can be modified by enzymatic as well as non-enzymatic processes that alter its composition [5, 6].

The TME, apart from ECM, includes fibroblasts, immune cells and blood vessels (**Figure 1**). Cancer-associated fibroblasts (CAFs) and cancer cells synthesize and modify ECM composition and network organization [7, 8]. In this way, the nature of the TME is altered, and conversely, the TME can dictate the growth and spread of the tumor. The modification of TME composition and organization results in cancer cell drug resistance [8, 9]. ECM and TME serve also as a dynamic reservoir for numerous effectors such as growth factors,

cytokines, chemokines as well as various enzymes that bind to GAGs, primarily to **heparan sulfate** (HS) side chains of heparan sulfate proteoglycans (HSPGs) [10] ([Box 1](#)). Cleavage of HS by heparanase (HPSE) ultimately releases ECM-bound effectors. In this way, these effectors are converted to bioactive mediators which affect local signaling and tissue responses (**Figures 1 and 2**).

The formation of pre-metastatic niches (pMNs) in distant organs is induced by tumor cells. These pMNs are conducive to the proliferation and survival of cancer cells even before they arrive at these sites [11]. At the pMN the ECM is significantly altered in response to effectors released by the primary tumor, the activated stromal cells, and/or the recruited bone-marrow-derived dendritic cells. Such alterations involve deposition of new macromolecules as well as ECM fragments generated by enzymatic activity. Therefore, the physical properties of the pre-existing ECM at the pMN are remarkably modified (**Figure 1**) [11].

Proteoglycans and Glycosaminoglycans

PGs undergo post-translational modifications of their GAG chains to produce a live functional 'tree', with the bole being the protein core and the branches being the covalently attached GAG chains and oligosaccharides [12]. They perform their functional roles in the extracellular space, on the cell surface and even intracellularly [3]. Differential PG gene expression and GAG structural changes are tightly related to the availability of growth factors as well as cancer cell signaling. Research focused on the role of PGs/GAGs in cancer progression, highlighting their diagnostic value and therapeutic implications [12] ([Box 1](#)). The roles of secreted PGs and the intracellular serglycin in cancer propagation have been recently reviewed [13, 14]. Here we emphasize the role of hyaluronan and the cell membrane **syndecans**.

Hyaluronic acid (hyaluronan, HA) is present in almost all mammalian tissues, playing critical structural and biological functions ([Box 1](#)). To exert its roles, HA interacts with specific receptors, including lymphatic vessel endothelial HA receptor 1 (LYVE1), CD44, and receptor for HA-mediated motility (RHAMM) [12, 15]. The molecular size of HA is significant for its biological role, driving receptor clustering on the cell membrane that modulates cellular responses [3]. CD44 upregulation is a key element for cancer stem cells (CSC) triggering cell signal pathways [16], while HA-CD44 interactions mediate activation of leukocytes, stem cells and tumor cells [17]. In some cancer types, as head and neck tumors, the high expression of RHAMM has been associated with decreased cell survival and increased cancer cell proliferation [18].

HA has a critical role in solid tumor development [18], attributed to its ability to mediate cancer cell motility and spreading. It also acts as an immune shield against immune cell activity allowing the tumor to escape the control of the immune system. HA oligosaccharides promote angiogenesis which brings more nutrients to the tumor and facilitates systemic spread. Indeed, it has been demonstrated that the effects of HA in mammary cancer depend on its molecular size as well as the type of estrogen receptor (ER α / β) expressed [19-21]. Recent meta-analysis on several thousands of patients provides statistical evidence that the high level of HA can predict unfavorable breast cancer outcomes indicating HA as a new prognostic biomarker helpful for the selection of individual therapeutic strategies for breast cancer patients [22].

The syndecan family of transmembrane HSPGs serves as a prototype for PG dysregulation in cancer. Aberrant expression of its 4 family members contributes to tumor progression, prognosis and therapeutic resistance [12, 23]. Heterodimerization of individual syndecans and **shedding** expand the functional repertoire of syndecans [24, 25]. At the cell surface, the extracellular protein moiety can mediate cell- and integrin-binding, whereas the GAG chains of syndecans mediate a multitude of molecular interactions with ECM proteins (e.g., fibronectin and vitronectin), morphogens [e.g., bone morphogenetic proteins (BMPs), hedgehog (Hh)], mitogenic [e.g., fibroblast, hepatocyte and vascular endothelial growth factor (FGF, HGF and VEGF, respectively)] and inflammatory [e.g., interleukins IL-2, IL-8 and IL-10, tumor necrosis factor alpha (TNF)- α , monocyte chemoattractant protein-1 (MCP)-1] cytokines (**Figure 2**) [12, 26].

Syndecans modulate tumor progression by acting as co-receptors that facilitate signaling through transmembrane tyrosine kinase receptors including FGFR, insulin-like growth factor receptor (IGFR), and c-Met, heptahelical chemokine receptors, and stemness-associated pathways, such as Wnt and Notch [26, 27]. As co-receptors, syndecans are thought not to signal autonomously, but to rather substantially enhance signaling through a given receptor-ligand pair. As syndecans act as a binding interface for numerous pathways, they enhance and integrate these pathways in a given cell type, resulting in modulation of cell proliferation, invasion, angiogenesis, CSC phenotype, and cancer cell-immune cell communication [12, 27, 28]. Syndecans also act as regulators of exosome formation as well as integrin and receptor tyrosine kinase recycling (**Figure 2**) [29, 30, 31]. As syndecans are mechanistically involved in tumor progression, they have emerged as novel cancer drug targets in approaches using antibodies, inhibitory peptides, glycomimetics and chimeric antigen receptor T-cells (CAR-T-cells) [3].

Collagens, Laminins and their Cell Receptors

Fibrous ECM proteins are major components of the TME mostly including fibril-forming collagens I and III that play a major role in tumor tissue stiffness (Box 2). The evolution of the thick collagen network surrounding cancer cells increases the risk of developing metastases, making it a potential indicator of cancer stages [32-34]. Multiphoton excitation-second harmonic generation imaging of mouse mammary tumors revealed a characteristic alignment of collagen in close proximity to the tumor cells [32]. Tumor-associated collagen signatures (TACS) referring to the collagen alignment patterns were determined. TACS-1 seen in early tumorigenesis refers to an increased curly-type collagen deposition. TACS-2 describes a TME where collagen architecture becomes aligned tangentially to the tumor. TACS-3 seen in advanced stage tumors, describes radially aligned collagen fibers oriented perpendicular to the tumor. Imaging approaches combined with computational methodologies and statistical analysis are currently being developed to precisely characterize, quantify and possibly score collagen fibers alignment and orientation from histological sections of different types of human tumors [34] (see Clinician's Corner). Cancer cells, CAFs, and tumor-associated macrophages work in concert to modulate the TME and exhibit excessive deposition of collagen and collagen-transforming enzymes, particularly at the invasive front [35, 36]. Collagens found within the primary tumor ECM are mainly produced by stromal cells, forming a matrix that may develop into a fibrotic phenotype generally linked to metastasis and poor prognosis [37]. This complex ECM restricts the motility of immune cells, preventing their anti-tumor activity [38], and forming a physical barrier resistant to therapy. The ECM density is further intensified by hypoxia and inflammation that stimulate collagen and collagen-modifying enzyme expression, therefore increasing its stiffness. The high stiffness imposes new mechanical constraints to resident and tumor cells, forming a tumorigenic niche that leads to altered mechanotransduction pathways and supports tumor progression and invasion [39].

Collagen stimulates signaling pathways in cancer cells mainly through discoidin domain receptors (DDR), in conjunction with integrins, notch, and cadherins. In this way, collagen regulates processes as diverse as ECM remodeling and cancer cell survival, proliferation, and migration [40]. Collagen fibers may be subjected to proteolytic degradation by MMPs, releasing bioactive fragments and collagen-bound factors [41, 42]. Another key factor in collagen remodeling is the cross-linking enzyme lysyl oxidase (LOX), which is often overexpressed by CAFs. It enhances tensile strength and reinforces collagen alignment, shaping its orientation from randomly oriented fibers into a network of rails aligned

perpendicular to the tumor edge towards the blood vessels, facilitating the passage of tumor and immune cells [43-46].

Laminins are ECM glycoproteins found in all **basement membranes** (BMs) [47]. Via cell membrane receptors, including integrins and syndecans, laminins influence cell functions. These events are supported by their major cell-binding domains or triggered by cryptic interaction sites revealed by MMP-induced proteolytic cleavage (Box 2). Expression of various laminin subunits is often upregulated in tumor or stromal cells of malignant tissues. Among all isoforms, the isoform 332 ($\alpha3\beta3\gamma2$) has attracted a lot of attention by its altered expression pattern in several human carcinomas and its positioning as a potential prognostic factor [48]. Heterotrimeric laminin-332, or its monomeric $\gamma2$ subunit, is markedly up-regulated in budding cells located at the invasive edge of many tumors [48, 49]. Through MMP-dependent cleavage processes, proteolytic fragments of laminin-332 induce pro-tumorigenic signals [48, 50]. The high ability of budding cells to degrade and invade the TME could result from laminin-332-dependent regulation of membrane type 1 (MT1)-MMP activity through the recruitment of its molecular partner CD44 [51].

Integrin Functions and Targeting in Cancer

Integrins via their extracellular domains interact in a subtype-specific manner with ECM constituents including collagens, laminins and fibronectin (**Figure 2**). The outside-in activation of integrins is triggered by clustering of integrins via binding with multivalent ECM ligands which activate signal transduction through numerous pathways including Src and focal adhesion kinase (FAK) [52]. Frequently dysregulated in cancer, these processes regulate leukocyte recruitment, angiogenesis, tumor cell adhesion, migration and metastatic spread.

Within the TME, tumor cell-CAF-interactions via integrin $\alpha3$ and $\alpha6$ modulate interstitial pressure by regulating matrix surrounding architecture, whereas mechanical forces and reactive oxygen species further promote integrin activation and drive tumor progression [53, 54]. CAFs interact via $\alpha3\beta1$ and $\alpha11\beta1$ integrins with collagen and laminin-332 and thus support cancer cells [55]. Notably, integrin function is modulated by transmembrane proteins of the tetraspanin and syndecan families. This has been exemplified by insulin-like growth factor receptor (IGFR)-mediated inside-out activation of $\alpha\nu\beta3$ integrin upon association with syndecan-1 and by the antitumoral activity of synstatin, a syndecan-1 fragment that blocks integrin-dependent angiogenesis and tumor growth in mouse models [56]. IGF-IR inhibition attenuates the levels of integrins $\alpha\nu\beta3$, $\alpha\nu\beta5$, $\alpha\nu\beta6$, and $\alpha5\beta1$ resulting in reduced adhesion

of MCF-7 mammary cancer cells [57]. Furthermore, MMP-mediated biophysical changes in tissue rigidity within the TME have an impact on integrin function within the TME, demonstrating the complex interplay of ECM constituents during tumor progression [58].

Matrix Remodeling Enzymes

The enzymatic activity of matrix remodeling enzymes, which has a strong impact on cancer progression, has long been a source of interest in oncology research. Proteolytic (i.e. MMPs and cathepsins) (Box 2) and non-proteolytic enzymatic partners [i.e. heparanases and hyaluronidases (HYALs)] (Box 3) are involved in metastasis and constitute targets for pharmaceutical interventions [3, 59] (Box 3).

Matrix Metalloproteinases

Tumor progression requires profound remodeling of the ECM leading to the dissemination of cancer cells to distant tissues and contributing to angiogenesis [5, 10, 43]. ECM remodeling and degradation are carried out mainly by MMPs, which are either soluble/secreted in the TME or anchored to the cell membrane (MT-MMPs) and trigger important modifications in cellular and ECM interactions [60] (Box 2). Their dysregulated expression in tumor cells, CAFs and infiltrating immune cells, represents a hallmark of cancer and thus they have potential as diagnostic biomarkers individually or in combination [61]. Among the 24 MMPs known in humans, membrane-anchored MMPs are particularly important in collagenolysis and BM breaking in cancer (**Figure 3**). Specifically, MT1-MMP is linked to malignancy of multiple tumor types where it is found in specific protrusive adhesion structures with proteolytic functions named invadopodia [62, 63].

Adding to the complexity, through ECM proteolytic cleavages, MMPs contribute to the release and activation of matrix-bound cytokines and growth factors. They are also responsible for the delivery of bioactive ECM fragments potentially revealing cryptic binding sites. Besides, MMPs are also involved in epithelial-mesenchymal transition (EMT) and may generate genetic alterations [59-61]. Some MMPs may also contribute to tumor progression through the interaction of their non-catalytic domains with cell surface receptors [64].

Developing inhibitors of the transcription, activation, or activity of MMPs thus remains an attractive therapeutic option at the level of both the tumor and tumor-associated immune cells [65]. The clinical trials carried out with the first generation of MMP inhibitors have brought many disappointments. However, novel findings on MMP activity in the TME and a deeper understanding of their modulated expression in animal models, have opened promising perspectives for better targeted and more specific therapeutic approaches [66, 67].

Cathepsins

Cathepsin activity within the TME, particularly those belonging to the cysteine-protease family (namely cathepsins B, H, K, L and S) have potent roles in driving cancer formation and progression (Box 2). This is due to their capability to degrade both intracellular and extracellular matrix proteins, favoring angiogenesis and increasing cancer cell dissemination and invasiveness [68, 69]. The aspartate protease cathepsin D also plays a role in cancer development; its elevated expression is indicative of cancer stage and early recurrence in node-negative breast cancer [70].

Due to their role in promoting malignancy, cathepsins are considered potential therapeutic targets and prognostic indicators of disease progression [68-70]. Although no cathepsin inhibitors are clinically available yet, several drug candidates that selectively inhibit cathepsins are under development given their promising effects in the prevention of tumor-induced angiogenesis (Box 2). A particularly interesting therapeutic strategy is the targeting of cathepsins produced by tumor-associated immune cells, known to contribute to tumor progression [71, 72].

Heparanase-1 and Heparanase-2

Heparanase (**heparanase-1**, HPSE) is the sole HS-degrading endoglycosidase expressed by mammalian cells (Box 3). Degradation of HS by HPSE contributes to ECM disassembly and hence facilitates cancer metastasis and inflammation. In addition, it affects diverse patho(physio)logical processes ranging from gene transcription to signal transduction, autophagy and DNA damage [73, 74]. A key mechanism by which HPSE accomplishes its multiple effects on cells and tissues is by regulating the bioavailability of HS-bound growth factors, chemokines, and cytokines, priming the TME. In this way, HPSE mediates tumor-host crosstalk and promotes basic cellular processes (i.e., exosome formation, autophagy, immune responses) that together orchestrate tissue remodeling [75, 76].

HPSE acting on HS chains of HSPGs creates a permissive environment for cell proliferation, activation and differentiation. Furthermore, HPSE is a key player in abundant pathologies including inflammation, autoimmunity, tissue fibrosis, kidney dysfunction, diabetes, viral infection and cancer [74, 77-79]. The development of HPSE inhibitors as anti-inflammatory and anti-cancer drugs is, therefore, a promising area for matrix-based pharmacological targeting [80]. HPSE resides primarily within endocytic vesicles, colocalizing with lysosomal markers and suggesting a role in the normal physiology of this organelle (i.e., autophagy) [81].

Heparanase-2 (Hpa2) is a close homolog of HPSE (heparanase-1) that, unlike HPSE, does not undergo proteolytic processing and hence lacks intrinsic HS-degrading activity. Notably, Hpa2 retains the capacity to bind heparin/HS and, importantly, exhibits an even higher affinity towards HS than HPSE, thus competing for HS binding and inhibiting HPSE enzymatic activity [82]. Hpa2 binding to HS clusters syndecans-1 and -4. However, it remains on the cell surface and fails to be internalized [82]. Hpa2 exhibits anti-tumor, anti-angiogenic and anti-inflammatory effects mainly due to its regulatory effects on normal cell differentiation, apoptosis, endoplasmic reticulum stress and fibrosis [76, 77, 83]. Ongoing studies indicate that Hpa2 expressed by both the tumor cells and the host TME functions as a tumor suppressor [84, 85].

Epigenetics, Stemness and Exosomes in ECM-related Cancer Research

Epigenetic alterations are involved in cancer initiation and propagation as they modulate matrix remodeling, stemness and EMT. Moreover, tumor-derived exosomes have critical roles in different stages of cancer and its metastatic potential (**Figure 1**). Therefore, novel diagnostic and therapeutic ECM-based approaches include TME targeting through epigenetic pathways and cancer cell-derived exosomes.

In cancer, ECM constituents are not only subject to epigenetic regulation, but they also have a profound influence on epigenetic regulators themselves. For example, the HS sulfotransferase HS3ST2, HPSE and syndecan-2 are silenced via promoter methylation in several cancers, affecting cell proliferation, invasion, metastasis and the CSC phenotype [75, 86, 87]. In turn, nuclear translocation of shed syndecan-1 and its regulation by HPSE affect the activity of histone acetyltransferase thereby modifying histone acetylation [88, 89] (**Figure 2**).

MicroRNAs affect the expression of numerous ECM constituents in cancer, including collagens, laminins, fibronectin, tenascins, integrins, PGs, and MMPs [90]. Notably, ER-dependent regulation of microRNAs miR-145 and miR-200b in breast cancer cells affects tumor cell proliferation and metastatic growth by targeting syndecan-1, fibronectin and various MMPs [91, 92].

Dysregulation of ECM constituents leads to increased resistance of CSCs to chemically and radiation-induced damage of stem cells, rendering these cells particularly long-lived and therapy-resistant [93]. The pro-tumorigenic properties of CSCs are enhanced by interactions with ECM constituents in the TME. This is exemplified by the interaction of the CSC marker

CD44 with its natural ligand HA, or by the impact of syndecan-1, HPSE and HS sulfotransferases on stemness-associated signaling pathways such as notch-, Wnt- and hedgehog signaling [27, 87, 93-95]. As the presence of therapy-resistant CSCs in numerous tumor entities has been associated with relapse, targeting of ECM constituents (i.e., collagen synthesis and crosslinking), signaling (i.e., transforming growth factor- β , and FAK) and ECM-cell interactions (i.e., inhibition of $\alpha\beta3$ and $\alpha\beta5$ integrins) emerges as a promising approach in cancer therapy [93, 96]. Notably, selected PGs and GAGs emerge as therapeutic targets to induce differentiation of CSCs, and to guide therapeutics to the CSC population in order to prevent recurrence after conventional therapy [93].

Stemness and Hyaluronan in Extracellular Vesicle Trafficking

HA acts as a sheet that covers most cells and forms a pericellular coat mediating cell interaction with the surrounding microenvironment. The existence of large HA-dependent pericellular zones has been described around many cell types including cancer cells. HA is critical in the recently described system of cell interaction constituted by **extracellular vesicles** (EVs). The production of EVs is considered a novel extracellular messenger system. EVs carry different molecules including proteins, nucleic acids, and polysaccharides. EV size and molecular composition are strongly modified by malignant transformation and EMT occurring in the cells of origin. This emerges as an intriguing opportunity for novel therapeutic approaches and prognostic and diagnostic tools [97]. HA is associated with cancer stemness [98] and tissue repair and is hence considered a key molecule in regenerative medicine and tissue engineering [99-101].

Exosomes in ECM-based Targeting

Exosomes produced by tumors initiate the formation of the pMN [102]. The cross-talk between exosomes and CAFs and the cancer cells of the primary tumor influences cancer cell migration and metastatic potential [103]. HPSE enhances exosome biogenesis as demonstrated initially by transfecting human myeloma cells with the cDNA for HPSE [104]. Mechanistically, this enhancement is related to the shortening of syndecan HS chains which facilitates syndecan clustering, binding of syntenin to the syndecan cytoplasmic domain and endocytosis of this complex. Following proteolytic cleavage of the syndecan, syntenin interacts with ALIX, promoting its interaction with ESCRT-III and intraluminal budding of vesicles [28, 29] (Box 3). Apart from its contribution to exosome biogenesis, HPSE stimulates the targeting of specific cargo to exosomes. This likely accounts for the biological function of extracellular vesicles to sustain tumor growth, invasion capacity, and cancer cell dissemination.

The interactions between cancer cells and exosomes enhance chemoresistance and decrease the effectiveness of anti-cancer therapy. This was demonstrated in myeloma cells treated with anti-myeloma drugs, where the stimulated secretion of heparanase-loaded exosomes led to activation of ERK signaling, increased shedding syndecan-1, enhanced tumor survival and drug resistance [105]. These 'chemoexosomes' carry HPSE, proteases and other enzymes on their surface and hence can degrade the ECM and remodel the tumor microenvironment, contributing, among other effects, to cancer and host cell migration [106]. Collectively, HPSE facilitates exosome production, secretion and docking and thereby impacts cancer and host cell properties, enhancing cancer metastasis, stemness and chemoresistance [104]. It is worth noting that proteases and glycosidases residing on the surface of exosomes (Boxes 2 and 3) could degrade their natural substrates, having in this way significant implications for cancer progression [106].

Concluding remarks

New knowledge on the evolution and role of ECM networks and their macromolecular components, as well as the proteolytic and glycosidic enzymes in the TME, considerably advance our understanding of tumor, CAF and immune cell activity and cross-talk. This increased knowledge is highly informative for the development of novel diagnostic tools, innovative therapies and biomarkers with the goal of improving cancer treatment and care.

Better understanding of the mechanisms by which the ECM macromolecular networks within the TME function would greatly facilitate the design and development of novel therapeutic approaches that target the tumor niche (see Outstanding Questions). Advances on inspired *in vivo* ECM three-dimensional platforms with the combination of lab-on-chip technologies and microfluidic devices, and on the ECM-related epigenetics and exosomal cell-cell communication, provide a promising area to simulate early and advanced cancer stages and to bridge the translational gap between preclinical and clinical settings. Future research should help to identify targets specific to the tumorigenic ECM modifications and stiffness, allowing the development of relevant innovative therapies.

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Resources

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Legends to Figures

Figure 1. Targeting the pre-metastatic niche. Major components of provisional matrix reprogram primary tumor cells and evoke EMT that boosts an aggressive phenotype. Platelets accompany circulating cancer cells to extravasate. The latter are transferred through the bloodstream together with secreted factors, cancer cell-derived EVs and exosomes mediating intracellular communication. Pre-metastatic niche formation in secondary tissues and distant organs following the extravasation of metastatic cancer cells creates a favorable microenvironment for metastasizing cancer cells. Pre-metastatic niche establishment is characterized by the dynamic interplay among cancer and stroma cells through differential expression of matrix components, including growth factors and their receptors, glycosaminoglycans (i.e., HA), PGs (i.e., syndecans), proteolytic (i.e., MMPs, cathepsins, etc.) and non-proteolytic (i.e., HPSE) enzymes as well as cancer-derived EVs and exosomes. ECM remodeling increased inflammatory response, hypoxia and autophagy are the main characteristics of the pre-metastatic niche that enables cancer cell colonization, angiogenesis and metastasis.

CAFs, cancer-associated fibroblasts; ECM, extracellular matrix; EMT, epithelial-to-mesenchymal transition; EVs, extracellular vesicles; HA, hyaluronan; HPSE, heparanase-1; MMPs, matrix metalloproteinases; PGs, proteoglycans.

Figure 2. Roles of Syndecans in tumor progression. (a) Syndecans act as classical co-receptors facilitating receptor tyrosine kinase (RTK) signaling, by growth factors (GFs), regulating tumor cell proliferation, angiogenesis and cell motility. These processes are also regulated by the functional interactions of syndecans with integrins, which bind to the same ECM ligands and activate similar pathways such as focal adhesion kinase (FAK) (b). Syndecans also act as chemokine co-receptors modulating signal transduction via G-protein coupled receptors (GPCR). In this way, they regulate leukocyte recruitment, phenotypic switch and angiogenesis within the inflammatory tumor microenvironment. (c) Syndecans cooperate with the notch signaling pathway, modulating cancer stem cell properties. Interaction of notch with ligands like delta on neighboring cells triggers two consecutive proteolytic steps that lead to nuclear translocation of the notch intracellular domain, where it activates transcription factors. (d) Proteases such as membrane-type matrix metalloproteinases (MT-MMPs) can cleave syndecans, converting the intact ectodomains into paracrine effector molecules. Further proteolytic cleavage of the remaining transmembrane and cytoplasmic domains generates biologically active cytoplasmic fragments. (e) Syndecan ectodomains can regulate DNA topoisomerase and histone

acetyltransferase in the nucleus in heparanase (HPSE)-dependent manner. (f) microRNA and syndecans enter cells via exosomes, generated by a mechanism dependent on syndecan, heparanase and syntenin (not shown). During biogenesis of exosomes, the extracellular domain of syndecan is proteolytically processed, resulting in a truncated form without heparan sulfate (HS) chains. (g) Syndecan (Sdc) as an epigenetic regulator. Syndecan mRNA is post-transcriptionally regulated by microRNAs (miRNA).

Figure 3. Collagen and basement membrane remodeling in the tumor microenvironment of metastatic carcinoma. The basement membrane has been deformed and perforated by carcinoma cells and cancer-associated fibroblasts (CAFs). Collagen fibers, which are scattered at random in the healthy ECM, are drastically re-organized in the tumor cell microenvironment. Both cancer cells and CAFs produce matrix metalloproteinases (MMPs) and lysyl oxidase (LOX). Remodeling of ECM proteins is caused by MMPs, and cross-linking of collagen and ECM is triggered by LOX. MMPs degrade ECM allowing the carcinoma cells to migrate and invade the stiff and oriented ECM.

Boxes

Box 1. PGs/GAGs are Important Players in ECM Organization

Cell surface PGs are key players in cell-cell interaction and signal transduction regulating cell adhesion, migration, proliferation, differentiation and stemness. The major cell-bound HSPGs, transmembrane syndecans and GPI-anchored glypicans, often form a ternary complex with a ligand and its high-affinity receptor, necessary for efficient signaling. The cytoplasmic domains harbor phosphorylation sites and interact with PDZ-domain scaffolding proteins, signaling kinases and lipids, thereby fulfilling roles that are functionally related to integrins.

ECM provides a storage depot for bioactive molecules that bind primarily to HSPGs [107]. Cleavage of HSPGs releases these HS-bound proteins in a given location and time thereby regulating their bioavailability and bioactivity. By interacting with ECM and basement membrane constituents, HSPGs contribute to the matrix structural integrity, self-assembly, and insolubility, thus intimately modulating cell-matrix interactions.

HA is present in almost all mammalian tissues, albeit in varying amounts. The enhanced expression of **hyaluronan synthases** HAS2 and HAS3 and increased EV secretion in wounded or growth factor-treated mesothelial cells demonstrate the relationship between HA and EV production during EMT and tissue healing [108]. Notably, ovarian cancer cells may transfer CD44 to mesothelial cells via EVs coupled with increased HA levels. The interactions between HA and EV likely involve the participation of other ECM molecules. Hence, besides HA-CD44 interactions, various molecules are engaged, including integrins, tetraspanins, lectins, fibronectin and HSPGs [109].

Box 2. ECM is Altered in the Tumor Microenvironment

The TME has profound impacts on tumor growth and metastasis. Increased deposition of collagen and cross-linking of fibrillar collagens and elastin are common tumorigenic alterations of ECM homeostasis [37]. The increased production and density of ECM confers the TME with tumor-promoting and cell-spreading properties. Tumor and stromal cells sense the increased ECM stiffness through specific mechanoreceptors and become more aggressive [39]. This environment supports sustained proliferation, death resistance, induction of angiogenesis, protection against the immune system, as well as activation of invasion and metastasis [41]. The collagen fiber architecture thus influences cancer and stroma cell behavior.

Aberrant proteolytic cleavage of ECM proteins by MMPs and secreted cathepsins affect their interactions with cellular receptors thus promoting cell survival and proliferation [64,68]. Amplified degradation of collagen and laminin generates ECM fragments carrying biological activities and releases soluble factors, which modify tumor, stromal and immune cell behavior and trigger pro-tumorigenic signaling cascades [60].

Most MMPs orchestrate tissue remodeling through proteolytic cleavage and ECM rearrangement thereby modulating signaling pathways. Yet, some MMPs function solely through their hemopexin domain without involving any proteolytic activity. Their activity, very low under physiological conditions, explodes in situations of tissue remodeling such as tissue repair and various diseases. MMPs and their regulators have always been targets of interest in oncology. Despite the disappointing results of the first wave of clinical trials with MMP inhibitors, current strategies are based on developing more specific and spatio-temporal targeting second-generation inhibitors [66].

Cathepsins are proteases whose main activity is intracellular but they can also act extracellularly when secreted [69]. Different types of cathepsins exist, including serine cathepsins, aspartic cathepsins, and cysteine cathepsins which may cause damage to the ECM and modify cellular interactions thereby contributing to the development of the malignant process.

Box 3. ECM Glycosidic Remodeling Enzymes in Cancer Progression

HPSE is closely connected with tumor initiation, growth, metastatic potential and chemoresistance. These are mainly attributed to HPSE enzymatic- and non-enzymatic activities. HPSE is a regulatory molecule that impacts the aggressiveness of cancer cells. However, its heparanase-2 close homolog functions as a tumor suppressor and, unlike HPSE, does not undergo proteolytic processing [76, 82]. HPSE belongs to the wider class of enzymes known as 'retaining glycosidases', which catalyze hydrolytic cleavage of glycosidic bonds [110]. It uses a conserved 'double displacement mechanism', involving two key catalytic amino acid residues – a nucleophile (Glu343) and a general acid/base proton donor (Glu225) [110]. The heparin/HS binding domains (HBD1 and HBD2) are situated close to the active site micro-pocket fold.

Structurally, HPSE is composed of triosephosphate isomerase (TIM)-barrel fold that contains the active site and a flexible C-terminal domain that is required for secretion and signaling function of the protein [76, 111]. HPSE-mediated breakdown of HS is restricted to

a small subset of glucuronic acids, reflecting a requirement for specific N- and O-sulfation patterns on neighboring sugar units. *HPSE* encodes a 61.2 kDa latent pro-enzyme. Cathepsin L liberates a linker segment yielding two subunits (8 kDa and 50 kDa) that form the active HPSE heterodimer [75-77]. Intense research efforts are devoted to exploring the significance of HPSE in human diseases and to improve its targeting through a rational, structure-based design of heparin/HS mimetics, small molecules, and neutralizing monoclonal antibodies [75].

Hyaluronidases classified as endo-beta-N-acetylglucosaminidases degrade HA. Six different HYALs have been identified in mammalian tissues. HYAL-1 and HYAL-2 activities are synergic; HYAL-2 degrades HA to fragments of ca 50 disaccharide units and HYAL-1 degrades these fragments into smaller fragments of around two disaccharide units. These fragments have important biological functions, including nuclear factor- κ B signaling and biosynthesis of matrix metalloproteinases [3].

Clinician's Corner

ECM alterations provide specific molecular patterns by the release of cancer-specific ECM fragments that favor tumor cell survival and growth, EMT transition, cancer cell invasion and metastasis to distant sites. These fragments as well as cancer cell-derived EVs modify the pre-metastatic niche and have diagnostic significance [11, 60, 101, 102].

TACS 1-3 define the altered deposition and alignment of collagen fibres at progressive stages of tumorigenesis and thus are potentially prognostic indicators. TACS-3, for example, was identified in a cohort of human invasive breast cancer samples [33].

Collagen is a founding element modulating tumor stiffness and the correlation of its spatial organization with the tumor stage in human tumors requires additional work at both fundamental and translational levels.

Compounds and antibodies that target collagen synthesis and crosslinking (i.e., halofuginone, fresolimumab^{i,ii}, GS-6624, and losartanⁱⁱⁱ), CD44, HA, and CD44–HA interaction (i.e., 4-MU, RO5429083^{iv,v}, bivatuzumab, and verbascoside), integrins (i.e., vitaxin, volociximab^{vi}, and cilengitide^{vii}) and TGF- β signaling (i.e., Ki26894, SB-431542, and AVID200^{viii}) are in clinical trial for metastatic breast cancer, advanced non-small cell lung cancer, pancreatic cancer and glioblastoma multiforme [96].

Inhibition of MMPs by therapeutic monoclonal antibodies and their potential as activators of targeted prodrug therapies are promising anti-cancer strategies [66]. For example, a function blocking anti-MMP-7 monoclonal antibody inducing pancreatic cancer cell apoptosis and

cancer cell sensitivity to chemotherapeutics is a promising candidate for pancreatic ductal adenocarcinoma care [112].

Heparanase inhibitors showed encouraging results in mouse tumor models and clinical trials^{ix,x,xi,xii} of multiple myeloma, recurrent glioma, brain metastatic breast cancer, hepatocellular carcinoma, and prostate cancer, although none, as yet, has been approved for cancer treatment [75].

Hyaluronidase increases the efficacy of chemotherapeutics, possibly by reducing intratumoral pressure or by breaking down the ability of HA to function as shield, and has been found to be effective in multiple tumor types, including breast, brain, melanoma, and sarcoma [113,114].

Glossary

Basement membrane (BM): thin layer of ECM at the interface between an epithelial or endothelial layer and the connective tissue or surrounding nerves, adipocytes and muscle cells.

Extracellular matrix (ECM): 3D highly dynamic macromolecular network present in all metazoans except sponges and placozoa. ECM provides structural support for cells and tissues and plays key roles in functional cell properties.

Extracellular vesicles (EV): extensive population of lipid-layered vesicles originating from all cell types. They can be classified into exosomes, microvesicles and apoptotic bodies.

Exosomes: nanovesicles of endosomal origin secreted by cells. They contain various membrane and cytoplasmic components (i.e., DNA, proteins, enzymes, mRNA, miRNA, lipids and activated receptors). Exosomes are endocytosed by recipient cells and release their contents.

Glycosaminoglycans: heteropolysaccharides composed of repeating disaccharide building blocks. The GAG chains are linear and negatively charged due to esterified sulfate groups and uronic acids.

Heparan sulfate: a GAG with basic repeating units composed of uronic acids (D-glucuronic acid and/or L-iduronic acid) and D-glucosamine (N-acetyl or N-sulfamino).

Heparanase-1: an endo- β -glucuronidase that degrades the HS side chains of HSPGs releasing oligosaccharides that interact with proteins affecting their functions.

Heparanase-2: an HS-binding protein encoded in humans by the *HPSE2* gene mapped to chromosome 10. It inhibits heparanase enzymatic activity and acts as a tumor suppressor.

Hyaluronan synthases: located on the cell membrane and synthesize HA. They use UDP sugar precursors from the cell cytoplasm without using the Golgi apparatus. HA synthases (HASes 1-3) produce HA with specific size ranges and biological activity.

Integrins: type 1 transmembrane glycoproteins. They are composed of a pair of non-covalently associated alpha and beta subunits. The 24 different heterodimers are formed by a combination of 18 alpha with 8 beta subunits. They modulate cell-matrix adhesion and cell signaling through a bridge that they create with the cytoskeleton, connecting the extracellular space with the inside of the cell.

Laminins: cell-adhesive matrix glycoproteins composed of a heterotrimeric assembly of alpha, beta and gamma chains with molecular sizes 140-400 kDa. Laminins are key components of BMs.

Matrix metalloproteinases: zinc-dependent proteases that cleave ECM components and major cell regulators. Most MMPs are secreted as soluble enzymes, while six remain membrane-anchored.

Proteoglycans (PGs): ubiquitous macromolecules composed of a core protein with covalently attached GAG side chain(s) and oligosaccharides.

Shedding: generation of a bioactive effector by proteolytic cleavage of membrane protein ectodomains.

Syndecans: transmembrane PGs with three to five HS and chondroitin sulfate chains. Syndecans coordinate mechano-transduction to the intracellular environment to regulate cellular alignment. They interact with several ligands, thereby actively contributing to several signaling pathways.

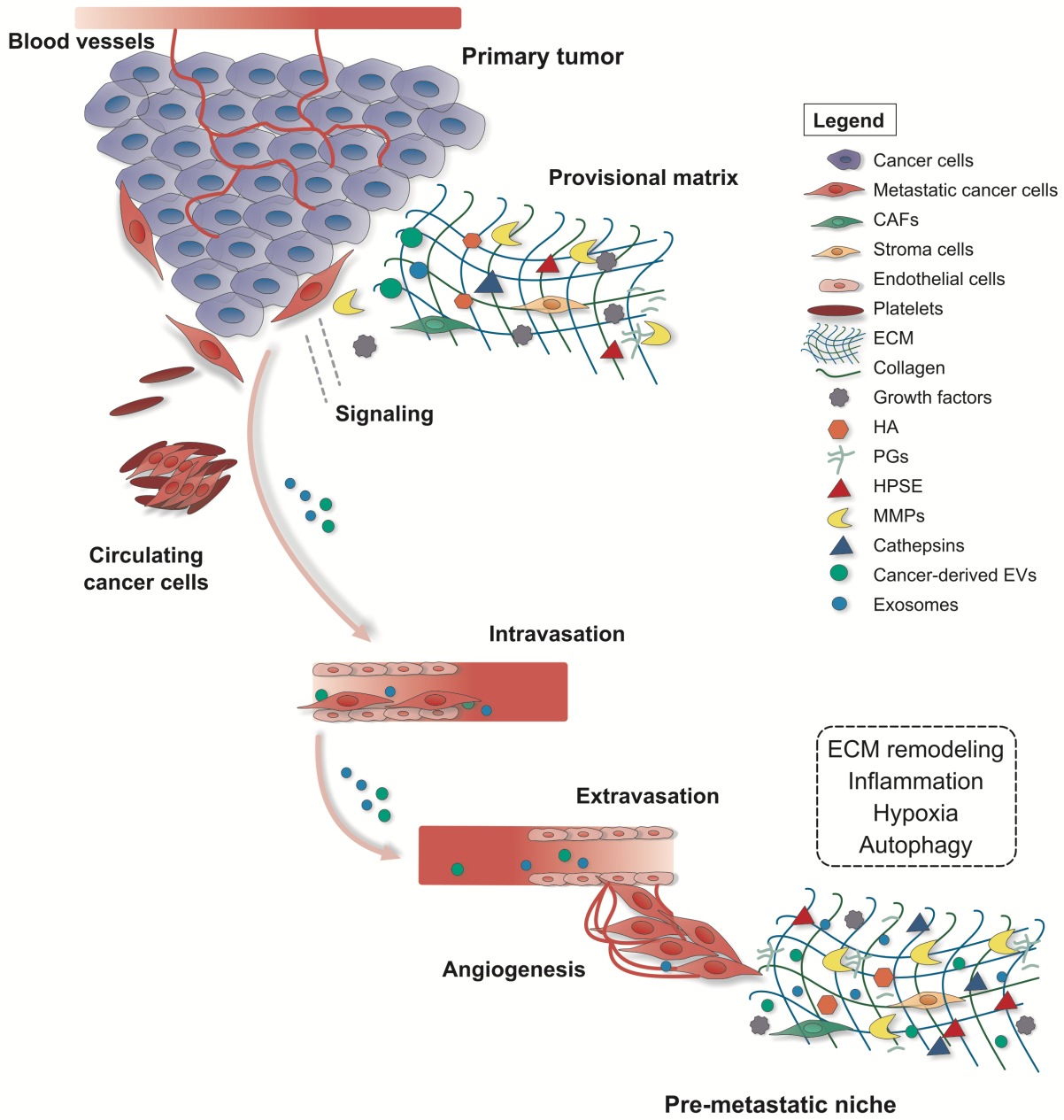


Figure 1

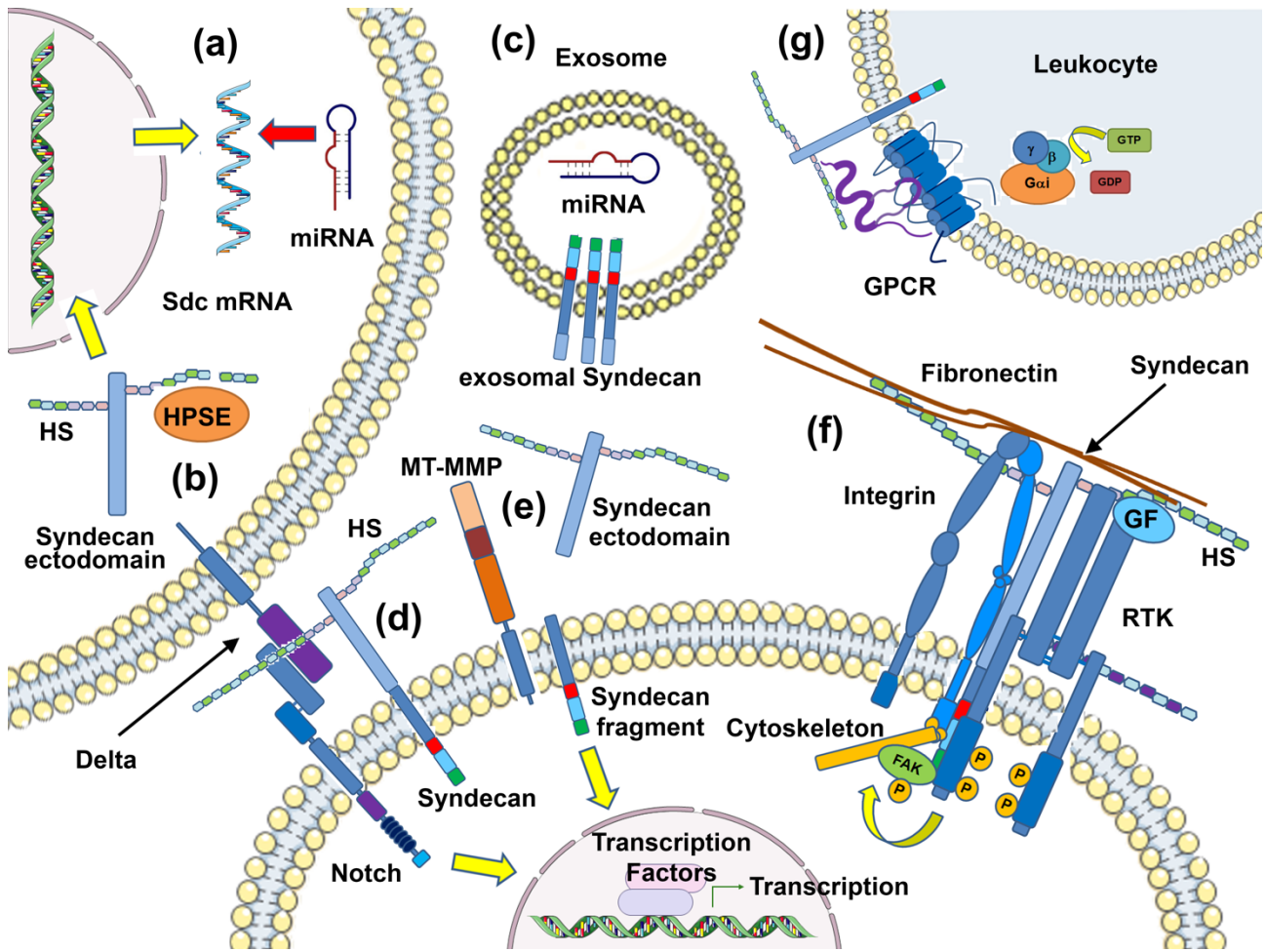


Figure 2

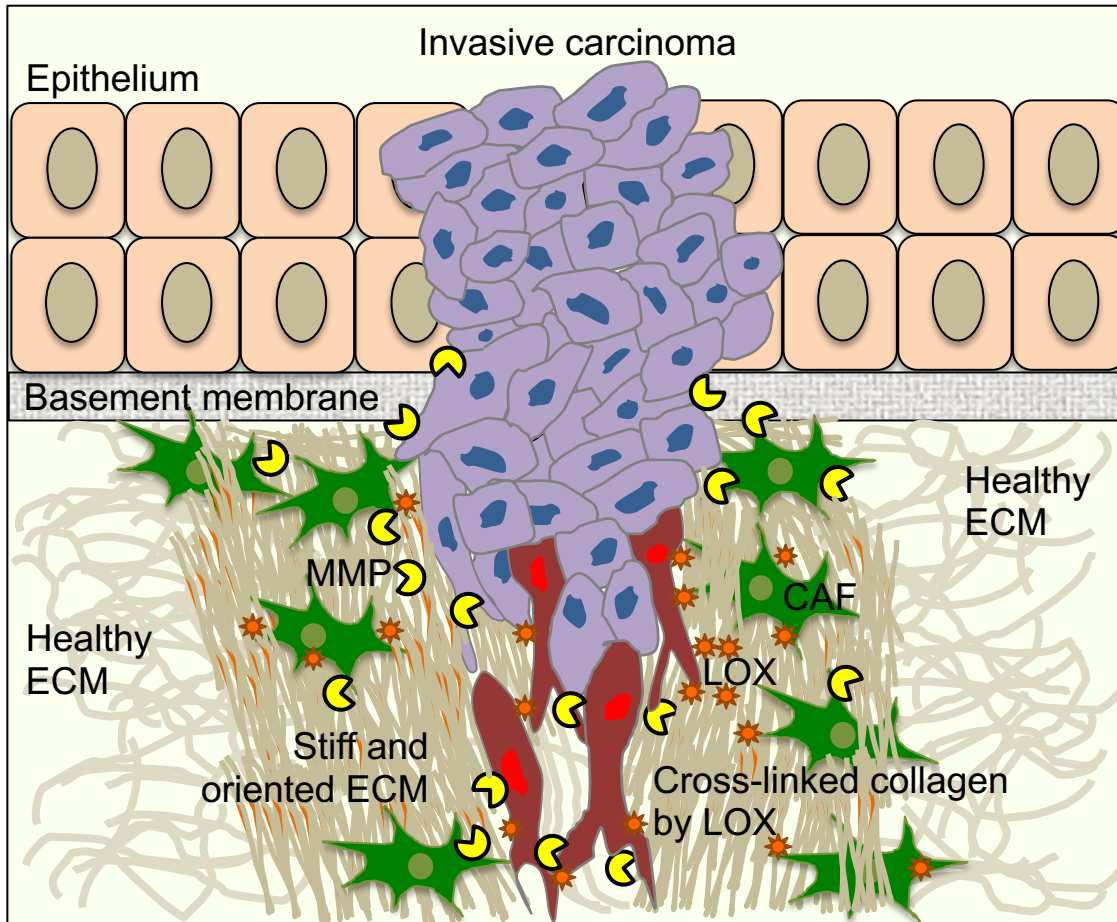


Figure 3