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Emerging roles of ECM remodeling processes in cancer

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Abstract: Extracellular matrix (ECM) plays a central and dynamic role in the creation of tumor microenvironment. Herein we discuss the emerging biophysical and biochemical aspects of ECM buildup and proteolysis in cancer niche formation. Dysregulated ECM remodeling by cancer cells facilitate irreversible proteolysis and crosslinking, which in turn influence cell signaling, micro environmental cues, angiogenesis and tissue biomechanics. Further, we introduce the emerging roles of cancer microbiome in aberrant tumor ECM remodeling and membrane bound nano-sized vesicles called exosomes in creation of distant pre-metastatic niches. A detailed molecular and biophysical understanding of the ECM morphologies and its components such as key enzymes, structural and signaling molecules is critical in successfully finding the next generation of therapeutic and diagnostic targets in cancer.

Key Words

Extracellular matrix; Tumor microenvironment; Cancer microbiome; Exosomes; Neoangiogenesis

Abbreviations

ECM, Extra cellular matrix; PDAC, Pancreatic ductal adenocarcinoma; MMP, Matrix Metalloproteinase; LOX, Lysyl oxidase; TIMP, Tissue inhibitor of metalloproteinase; CAF, Cancer associated fibroblast; TAM, Tumor associated macrophage; CSC, Cancer stem cell; VEGF, Vascular endothelial growth factor; TGF- β , Transforming growth factor beta

ECM homeostasis and molecular content

Extracellular matrix (ECM) is the non-cellular component of tissue, which is secreted by cells for its structural, and biochemical support. ECM plays a very crucial role in cell proliferation, differentiation and maintenance of tissue homeostasis [1]. In general, ECM is mainly composed of water, proteins, and polysaccharides. Each of these components assemble to form a unique niche, tailor-made for the parenchyma of that particular tissue type and helps it to survive, differentiate and carry out its characteristic functions. Among ECM proteins, collagen is the most abundant component of ECM, but its composition and structure vary across various tissue types [2]. For instance, the basement membrane (BM) mainly consists of collagen type IV, however, interstitial matrix/stroma is composed of collagen type I, II, and III [2, 3]. These various collagen types form polymeric assemblies and also contain non collagenous domains such as endostatin, a collagen type XVIII fragment known to control angiogenesis[4]. Thus, collagens invariably play a crucial role in a wide range of diseases and are prime targets in treating or managing them.

Another important protein component of the ECM is fibronectin, a glycoprotein that predominantly binds to transmembrane proteins and is important for fibro-proliferative condition in chronically injured or diseased tissues[5]. It has a unique arginine-glycine-aspartate (RGD) motif that helps in preferentially binding to $\alpha v \beta 1$ and $\alpha v \beta 3$ integrins [6], which regulates various aspects of focal adhesion and cell mechanical properties. Fibronectin exists in two different forms- soluble and fibrillary. The soluble form is also known as plasma fibronectin, and it is found circulating in blood and other tissue fluids of the body [7]. The fibrillar insoluble fraction of fibronectin sustains an intact fibronectin matrix within the ECM, which regulates cell-matrix adhesion and the mechanical properties of the ECM. It has been shown that fibronectin polymerization increases the retention capacity of various proteins like thrombospondin-1 and collagen I in the ECM [7, 8].

Along with these ECM proteins, there exists carbohydrate-rich ECM components, mainly including proteoglycans and their derivatives. They are classified into leucine-rich proteoglycans, modular proteoglycans, and cell-surface proteoglycans [9, 10]. Proteoglycans are mainly composed of glycosaminoglycans, which are polysaccharide chains with primary configurations consisting of an amino sugar or uronic acid [9, 11]. The known glycosaminoglycan chains are hyaluronan, chondroitin, dermatan, heparin and keratan [12, 13]. Except for hyaluronan, all other chains are sulfated at various positions. The negatively charged sulfate and uronic acid groups further aid in interaction with other ECM proteins and growth

factors [14]. Hyaluronan via its cell surface signaling molecule CD44 plays a significant role in cell proliferation, inflammation and activation of downstream signaling cascade [15, 16].

The various protein rich fibers and carbohydrates-rich polysaccharides of the ECM complement each other in the maintenance of tissue homeostasis and hydration. In normal conditions, a tightly regulated ECM provides architectural definition and anchoring points for mechanosensing and act as a signaling reservoir[17]. In dysregulated and diseased tissue environment, ECM forms a common thread driving aberrant signaling and resulting in a disorganized structure. It is thereby a treasure trove of clues to disease mechanisms, probable drug targets and diagnostic markers[18].

Dysregulated ECM remodeling generates molecular cues for cancer formation and progression

In cancer, the tight regulation of ECM is disturbed. Devoid of natural checks and balances, the malignant cells are free to manipulate the ECM, for an orchestrated roll out of their nefarious pursuits. This takeover of ECM, eventually leads to metastasis, which is often the primary reason behind cancer mortality[19]. In clinical samples of breast tumors, there is an elevated level of matrix remodeling enzymes such as matrix metalloproteinases (MMPs) which are directly related to poor prognosis and high risk of relapse [20, 21]. Apart from cancer cells, there are cancer-associated fibroblasts (CAFs), immune cells and other stromal cells, which play a decisive role in deregulation of ECM homeostasis [22, 23]. Tissue stiffening, collagen deposition and its crosslinking has been found in clinical samples of breast cancer carcinoma. It has also been observed that, in all of those patients, there is an upregulation of integrin signaling, which promotes downstream activation of signaling cascade associated with malignant phenotype, which includes, high Rho activity, loss of adherence junction, loss of tissue polarity and upregulation of EMT (epithelial–mesenchymal transition) markers [24, 25]. Along with cancer initiation and progression, tissue dynamics undergo indisputable change. An increase in tumor size is accompanied by proliferation and recruitment of cellular accomplices, such as CAFs, and stromal cells, which secrete various growth factors, like VEGF (vascular endothelial growth factor), and HIF (hypoxia-inducible factor). They promote angiogenesis and growth of blood vessels [26] and these out-branching neo angiogenic blood capillaries are a prerequisites for cancer growth, survival and eventually metastasis [27].

Recent reports have shown that proteoglycans and its derivatives mainly including, perlecan, syndecans, and glypicans perform multiple regulatory functions during the progression of malignancy [28]. Enhanced hydrophilic nature and capacity to modulate various receptor-signaling cascades make proteoglycans key regulators of tumor progression and

neovascularization. Another important polysaccharide, which plays a crucial role in the progression of cancer, is Hyaluronan (HA). It has been reported that in tumor microenvironment of breast, lung, and ovarian tissue, there is enhanced secretion of HA [29], leading to overexpression of signaling receptors mainly EGFR, ERBB2, TGF- β , and CD44 [30, 31]. Further, these stimulate downstream signaling pathways such as Akt-pathway and MAP kinase pathways that induce chemoresistance and cancer invasion [32, 33]. Overall, it is well understood that disorganization and deregulation of ECM occur during cancer progression and trigger various biochemical and biophysical cues, which promote cancer cell proliferation and invasion.

ECM crosslinking and stiffening gives rise to desmoplastic tumors

Solid tumors are often associated with excessive tissue fibrosis due to increased synthesis, crosslinking and deposition of fibrillar collagen, mainly collagen type I [34]. Fibrillar collagen accumulation in the stroma creates a very dense network of ECM fibrillar proteins, which gradually leads to tissue stiffening. Increase in matrix stiffness and alignment has been shown as a hallmark in many cancers, such as breast cancer, pancreatic cancer, and prostate cancer [35, 36]. Reports suggest that increase in tissue stiffness leads to upregulation of various outside to inside signaling cascades such as the Rho-ROCK-MLC pathway, which promotes overexpression of integrins, focal adhesion, cell contractility and EMT markers, and ultimately promotes the metastatic potential of cancer cells [37]. Collagen deposition, crosslinking and tissue stiffening have serious implication in maintenance of tissue homeostasis. It has been observed that dense accumulation of fibrillar collagen in the stroma leads to specific covalent intermolecular connections between collagen fibers, which results in masking of active sites for MMP activity, in turn leading to accumulation of MMP-resistant collagen fibers [38, 39]. This creates an imbalance between tissue degradation by proteases such as matrix metalloproteinases (MMPs) and their inhibitors – the tissue inhibitors of metalloproteinases (TIMPs).

During the early stage of cancer progression, there is the initiation of collagen type I crosslinking in the cancer microenvironment driven by the lysyl oxidase (LOXs) and LOX-like proteins (LOXLs) (Fig.1A) [40]. Proteomics analysis of clinical patient samples has revealed that these crosslinking enzymes were frequently overexpressed in various cancers including breast cancer, prostate cancer, and pancreatic cancer [41]. LOX-induced crosslinking results in alterations of ECM topology, directionality and its mechanical properties. Other reports suggest that in breast cancer and pancreatic cancer, these ECM crosslinking events

promote ECM stiffening, metastasis and infiltration of tumor-supporting immune cells [42, 43]. Clinical studies show that combinatorial effect of LOX-like proteins inhibitor along with cancer therapeutic drugs have shown better efficacy and reduction in tumor burden in pancreatic cancer mouse models [44], however, the overall survival rates of these mice have not improved significantly [45]. Presently, LOX inhibitors can only reduce the further crosslinking of collagen fibers but cannot restore the already cross-linked ECM, this is considered as one of the major hindrances in the successful implementation of these drugs in clinical trials. With the advancement in protein engineering skills and computation tools, there is a lot of scope for better application and design of inhibitors against LOX and its homologs [46]. These new inhibitors in combination with other anti-cancer drugs may lead to better efficacy in clinical trials.

Recent studies have revealed that modes of ECM crosslinking can also vary and it can affect the mechanical properties of tissue stroma (Fig. 1A). For example, during tumor progression, hyper hydroxylation of lysine residue in collagen telopeptide was shown to promote formation of hydroxylysylpyridinoline (HP) and lysylpyridinoline (LP), which gradually crosslinked with the ECM and increased its stiffness [47]. Reports from various groups have shown that during metastasis there is an overexpression of collagen stabilizing enzymes mainly consisting of lysyl hydroxylase-2, which promotes higher conversions of hydroxylysylpyridinoline and simultaneously stabilizes the crosslinked collagen network inside tumor stroma [48-50]. Lysyl hydroxylases and LOX-like enzymes have been shown to act in tandem inside tumor stroma. Under hypoxic condition, both these enzymes were found to be over-expressed and they play a critical role in hydroxylation of collagen telopeptide in response to HIFs [51, 52].

Apart from LOX-like enzymatic crosslinking of ECM, evidence exists for presence of non-enzymatic crosslinking mechanism of ECM, mainly via glycation and transglutamination [53]. In this process of ECM crosslinking and stiffening, an excessive deposition of proteoglycans in the stroma could be observed, which gradually leads to fibrosis [3, 54]. This process is also predictive of impending conditions. For instance, clinical studies have shown that patients with glycan-mediated crosslinking in their stroma have a higher chance of developing cancer in the later stages of their life [55]. Another method of crosslinking, which could be observed in tissue, is fibronectin-mediated collagen reorganization. *In-vivo* studies have shown that physical properties of fibronectin, mainly its density and size, dynamically regulate its interaction with collagen, which promotes tumorous microenvironment in the later stages of cancer [53, 56]. A highly conserved multifunctional divalent cation binding glycol-proteins known as secreted protein acidic and rich in cysteine (SPARC) or osteonectin/BM-40 has been

shown to promote cell migration and proliferation [57]. In cancerous conditions, these conserved non-structural secreted proteins take part in ECM reorganization and crosslinking via binding to collagen type I and collagen type IV. These events ultimately lead to creating of stiffer matrix and consequent promotion of metastatic environment[53]. As a matter of fact, matrix stiffening by various process initiated by cancer cells like those mentioned above, are fundamental for switch of resident fibroblasts and macrophages to CAFs and (Tumor associated macrophages) TAMs[58].

The lack of balance between ECM degradation by proteases and stiffening by crosslinkers contribute to cancer cell invasion[59]. Role of CAFs is central to both these processes. They produce TGF- β (transforming growth factor beta) for induction of stiffness and MMPs for ECM degradation [60, 61]. Along with CAFs, TAMs of M2 polarity also produce TGF- β and MMPs[62, 63]. Fibrotic ECM can prevent drug delivery and reduce drug efficacy[64]. It can also drive signaling processes that result in drug resistance[65]. In breast cancer, resistance against doxorubicin, 5-fluorouracil, paclitaxel as well as anti-estrogens and anti-Her2 antibodies is driven by cell-stroma interaction [66, 67]. Disorganized stroma in breast cancer biopsies is significantly associated with poor response to neoadjuvant therapy[68]. Normalizing fibrotic ECM may improve drug efficacy. Pirfenidone, a clinically approved anti-fibrotic drug was found to reduce both collagen and hyaluronan levels by disrupting TGF- β signaling and improved doxorubicin efficacy in orthotopic breast cancer model[69].

Keeping in mind the crucial role played by ECM stiffening and alignment, attempts are being made recently, to develop organotypic culture models that employ a variety of materials with distinctive properties to investigate how these microenvironmental features transform both tumor growth and effectiveness of therapeutic modalities[70]. Although prevailing evidence is sufficient to claim the irrefutable role of ECM stiffening as a tool for tumor progression, not much is known about various processes and molecular details of non-enzymatic crosslinking and its significance in ECM remodeling. In the pursuit for a holistic model for testing therapeutics, it is imperative that the dynamic and all-important ECM stiffness parameter is factored into the next generation of *ex-vivo* models.

Altering cell signaling and microenvironmental cues

Along with the aforementioned biophysical roles played by ECM in diseased tissue, it also plays an important biochemical role as a signaling mediator and reservoir. Once considered an inert scaffold bystander, ECM is now firmly placed as an active accomplice in conditions such as inflammation [71] and cancer[72]. ECM exerts both acute as well as chronic regulation of

cellular behavior, via activation or suppression of intracellular signaling processes. The route of downstream transduction of ECM cues are manifold. The most prominent route is direct transduction, which occurs via classical transmembrane gatekeeper proteins such as integrins. The dimerization of integrin subunits triggers phosphorylation of FAK/Src pathway that leads to increased migration and altered cell adhesion[73]. The substantial transformation of cell behaviors occurs due to activation of intermediary pathways, downstream of FAK/Src activation, such as the extracellular signal-regulated kinase 2 (ERK2)/mitogen-activated protein kinase (MAPK) cascade, small GTPases such as Rac and Rho[74] or β -catenin pathway (Fig.1B) [75]. Apart from integrin mediated transduction, the activation of phosphatidylinositol 3-kinase and Akt pathways can happen through cell surface glycoprotein receptor CD44[76]. Similar downstream transduction also occurs via discoidin domain receptor 2 (DDR2)[77] and elastin-binding protein receptor (EBPR)[72]. Upstream, CD44 is known to count hyaluronan, collagen, laminin, fibronectin, MMPs and osteopontin as ligands [78-81]. Likewise, integrins are known to bind fibronectin, vitronectin, collagen, Urokinase-type plasminogen activator receptor (uPAR) and laminin[73, 82].

In addition to above examples of convergence between ECM state and intracellular signaling, proteoglycans like decorin, biglycan, perlecan, syndecan and endostatin also transduce signals into cells using ligand receptors like EGFR, TLR2/TLR4, VEGFR2, integrin $\alpha_5\beta_1$ etc. This often affects tumor angiogenesis and endothelial cell autophagy[83]. Many of these pathways downstream activate many ECM remodeling enzymes creating an amplifying loop[84, 85].

In cancer, the extracellular matrix modulates the path towards achievement of cancer hallmarks by manipulating the existing mechanisms mentioned above. Besides, tumor cells and CAFs, in tandem, secrete various matrix remodeling enzymes such as MMPs, ADAMs, ADAMTs, uPAs, Cathepsins etc.[86]. These enzymes release active fragments from matrix components called matrikines[87]. MMP-1,-2,-8,-9,-12 regulate the matrikines Val-Gly-Val-Ala-Pro-Gly (elastin peptide) and proline-glycine-proline (PGP) and have implications in several cancers [88, 89]. MMP-9 along with MMP-8 contributes N-acetyl Pro-Gly-Pro (PGP) a bioactive collagen-1 fragment that functionally mimics chemoattractant CXCL8 on neutrophils [37].

MMPs could also act as ectodomain sheddases, often creating soluble receptors[90]. These soluble ligands or receptors include functional signal enhancers and nonfunctional decoys, which act to attenuate signal transduction. For example, cleavage of Fas Ligand (FASL) on effector cell surface by MMP-7 is known to protect tumor cells from chemotherapeutic drug cytotoxicity[91]. The E cadherin and integrin β -4[92] shedding by MMP-7 leads to increased cancer cell mobility. MMP-9 and MMP-2, both proteolytically cleave latent multifunctional

cytokine TGF- β , shedding light on the possible role of MMP-9 in tissue invasion and remodeling [93, 94]. Cancer signaling is also often found to converge with a metabolic shift, increasing tumor cells' capacity to maintain redox homeostasis and continue malignant advances[95].

The list of non-canonical sheddase targets of MMPs and other ECM proteases is growing by the day and umpteen number of feedback loops and decoy molecules are an indication of the complexity of the labyrinthine points of contact between ECM, its remodeling enzymes and ligands/receptors. Picking this web apart is sure to give rich dividends and can lead to improvement in efficacy of combination cancer therapy regimens. Evidence exists for pancreatic lesions having the ability to evolve through ECM stiffening in an oncogene independent manner to full blown pancreatic ductal adenocarcinoma (PDAC)[96, 97]. Just the recapitulation of rigid fibers in PDAC promotes elements of EMT[98]. But fibrosis in complex microenvironments, such as PDAC also plays a tumor antagonistic role[99]. Consequently, while matrix stiffening might be aiding tumors, it may also be a manifestation of body's defense mechanism to lock in and restrict the malignancy. The consensus seems to be that the targeting of ECM crosslinking, stiffening and remodeling as well as resultant aberrant cellular signaling, have to be context dependent.

ECM in Neo Angiogenesis

Angiogenesis is the process of sprouting of new blood vessels from pre-existing vasculature. Endothelial cell migration that precedes vascular tube formation is regulated by chemotactic, haptotactic and mechanotactic stimuli followed by ECM degradation[100]. All three stimuli have origin or close association with ECM or ECM regulating enzymes. The chemotactic stimuli is driven primarily by cytokines VEGF, bFGF, and angiopoietins [101-103]. These cytokines are often sequestered in the ECM and released by ECM remodeling enzymes such as MMPs[104]. Some evidence also points to the complex angiogenic and anti-angiogenic balance maintained by MMPs. MMP-7, for example, degrades human sVEGFR-1 (an endogenous VEGF receptor that traps VEGF), which increases VEGF bioavailability around the endothelial cells[105]. MMP-7 is also known to release VEGF stored by fibroblasts in its latent form in the ECM for urgent use[106]. Cathepsin B, a lysosomal cysteine protease, is known to promote and prevent angiogenesis. Its antiangiogenic effects is thought to be a result of its ability to cleave collagen XVIII to generate endostatin[107].

Haptotaxis on the other hand is more directly regulated by the ECM. It can be driven by integrins, ECM/cytokine scaffolds and even fibrillary collagen[100, 108]. Matrix stiffness with

concomitant rise in cytokine localization and MMP production, independent of matrix density is responsible for increased vascular growth[109]. Angiogenesis is heavily dependent on remodeling of sub endothelial ECM composed of collagen type IV and laminin[110]. As alluded to earlier, integrins help cells shake hand with the ECM. Integrin $\alpha v \beta 3$ on endothelial cells(EC) acts as adhesion receptor of vascular cells and bind to fibrinogen, fibronectin, vitronectin, and von Willebrand factor (Fig. 1C)[111, 112].

Neoangiogenesis in cancer is thought to be initially triggered by hypoxia. HIF-1 α induces both VEGF and glucose transporter-1 (GLUT-1) expression and initiates angiogenic processes[113]. The term angiogenic switch refers to the lack of balance between pro- and anti-angiogenic factors leading to capillary sprouting from previously quiescent vasculature[114]. In tumors that are beset with inflammatory responses to tumorigenesis, there is a perfect storm brewing. This inflammatory cyclone is critical for start of large-scale vascular sprouting. Tumor associated neutrophils and TAMs contribute matrix remodeling proteases and sustain chemokine signaling triggering neo angiogenesis and further recruitment of macrophages and neutrophils[115]. These chemokines originally secreted by tumor cells[116] are also contributed by TAMs including Interleukin 6(IL-6)[117], VEGF[118], Interleukin 8 (IL-8)[119] etc. and are also involved in paracrine signaling (Fig. 1C). The vasculature that results from this neo angiogenesis is far from normal. They lack contiguous basement membrane or an endothelial lining. They also have fewer than normal pericytes, smooth muscle cells and pharmacological receptors[120]. These physical peculiarities impart a primitive appearance to the tumor blood vessels. Apart from structure, they exhibit functional peculiarities as well. Tumor vasculature offer resistance to blood flow creating unstable speed and direction often resulting in vascular leakage. They also show increased RBC sledging and leucocyte adhesion to their walls[121]. All these vascular abnormalities coupled with high interstitial pressure in solid tumors render the microenvironment acidic and hypoxic[122]. Aberrant tumor vasculature also has far reaching implications on drug delivery. Even though leaky vasculature is supposed to result in enhanced permeability and retention in tumors, drug diffusion beyond small chemotherapeutics is considerably hindered by interstitial matrix surrounding the tumor vasculature. To improve drug delivery via circulation, corrective anti angiogenic therapy coupled with normalization of extravascular ECM is being considered[123]. Although it seems like common sense, this sort of intervention may lead to increased seeding of metastatic cells and open wider, deleterious implications and need to be studied in detail.

In addition to the tumor cells lining blood vessels that have noncontiguous endothelial lining, Periodic acid schiff positive (PAS) channels that mimic vascular tubes have been

observed[124]. These structures are lined up by ECM and tumor cells and provide alternate circulation. In essence aggressive cancer cells expressing VE-Cadherin (usually expressed in endothelium) mimic endothelial cells[125, 126]. Aggressive melanoma is known to express in excess fibrillin, collagen VI, collagen I and fibronectin. These highly invasive melanomas are known to reconstitute matrix rich vascular channels *in-vitro* while the non-invasive ones cannot reconstitute these channels [125, 126].

Essentially, ECM remodeling is imperative in acquiring blood supply through traditional or non-traditional channels, bringing in the raw materials for tumor progression and seeding tumor cells to distant body sites.

ECM remodeling in cancer niche formation in primary and distant sites

It is now a well-accepted paradigm that all cancer cells do not have equal potency to clonal expansion and repopulation. The subset of self-renewing cells called cancer stem cells (CSCs) are capable of constant clonal expansion and plasticity[127]. CAFs are known to enhance CSCs markers and results in therapeutic resistance via the action of proteases such as MMP-2,-3, and -9[128]. Beyond the primary site, cancer cells also prepare distant pre-metastatic niches in target organs. Hematopoietic progenitor cells, CAFs, TAMs are all known to assist in preparing the soil for incoming metastatic CSC seeds[129, 130]. Multiple stemness sustaining pathways such as WNT signaling, Hedgehog and NOTCH signaling are regulated via proteases like MMPs or via cell-cell contact[131, 132]. TGF- β , TNF- α (tumor necrosis factor alpha) and HIF-1 α are all known to be associated with the ECM in contexts explained earlier. All of these are known to also help sustain stemness in CSCs[133]. HIF inhibitors such as digoxin and acriflavine blocked breast cancer metastatic niche formation accompanied by reduction in expression of LOX and LOXL proteins, collagen crosslinking, CD11b⁺ bone marrow derived cell recruitment[134]. Chemokines like CXCL1, cell adhesion receptors for example ROBO1 and extracellular proteases such as MMP-1 expression in breast cancer was found to be associated with high tropism towards lung tissue for metastasis[135].

Apart from traditional cell-based effects on distant pre-metastatic sites, an exciting and emerging possibility of cancer cells directly communicating with target sites using membrane bound nano-sized vesicles called exosomes is being uncovered (Fig. 1D). Selective uptake of pancreatic ductal adenocarcinoma (PDAC) derived exosomes by Kupffer cells (KCs) in the liver was shown to cause activation of fibrotic pathways mediated by TGF- β [136]. Exosome uptake by cells in metastatic target organ trigger the formation of ECM components like fibronectin, as a first step of niche formation. Slowly but surely, a clearer picture regarding

organotropic metastasis is emerging and exosomes decorated with specific integrins and lipids might mediate the selective adhesion and fusion at the favored metastatic tissues sites [137]. The ECM is essentially a solid phase reservoir of ligands[138], a support scaffold and a boundary setter. To engineer cancer microenvironment along with its complex ECM *ex-vivo* is not an easy task, especially since all the complex cross talk between its components are not exhaustively described. Next generation of 3D bioprinting is attempting to recreate the cancer niches, factoring in, matrix-associated proteins, soluble factors, ECMs and already available nontoxic synthetic biomaterials[139]. It is now possible to fine tune the complex ECM biomechanical properties such as stiffness, viscoelasticity, architecture/fiber alignment, matrix pore size/geometry, topography curvature etc. to match primary cancer or metastatic tissue states[140]. A multiscale moving boundary model integrating microscale and macroscale adhesive dynamics have shown, how directed or undirected collagen fibers is of high importance when it comes to mesenchymal motion of tumor cells[141]. In the near future organotypic 3D models with integrated microfluidics[142] can be employed successfully for both drug testing and personalized medicine experiments.

Cancer microbiome and its possible role in ECM remodeling

Perhaps one of the most intriguing aspect of ECM remodeling in cancer is revealed by emerging reports about the critical role host-commensal bacteria play in regulating the tumor microenvironment. Specifically, the microbiome is now suspected to actively participate in creating niches and interact with multiple elements at the tumor microenvironment. With the advancement of genomics tools, there is a growing list of evidence, which suggests the presence of specific microbiome population in the tumor microenvironment. Recently, it has been shown that mycoplasma infected human dermal fibroblast can confer drug resistance in pancreatic ductal adenocarcinoma (PDAC). Further using genomics tools, it has been shown that the prevalence of microbiome population is found in around 75 % of human PDAC clinical samples [143]. Similarly, other groups have also reported the presence of various strains of tumor microbiome population in breast cancer samples. Their results suggest that bacterial strains vary with the different grades of the tumor [144, 145]. Presence of microbiome inside the tumor raises some very important fundamental questions like how are they surviving inside the tumor, how these foreign elements acclimatize with the host immune cells and how they modulate the tumor microenvironment to their benefit?

Recently, Miller and co-workers have shown that pancreatic tumor harbors certain species of bacteria which promotes cancerous growth by suppressing the host immune system. Their

results suggest that bacterial population in PDAC, modulates the host immune system via macrophage-mediated suppression of T-cell maturation [146]. Similarly, in breast cancer, colorectal cancer, and oral cancer there have been studies which reported the presence of certain species of bacteria which can promote tumor progression via suppressing activation of host immune cells [147, 148]. The exact molecular mechanism and underlying details is yet unclear. Clinical trials of chemotherapeutic drugs oxaliplatin and cyclophosphamide, have shown that reactive oxygen species created by resident microbiome is important for better efficacy of chemotherapeutics[149]. In immunocompetent mice, compared to germ-free mice, the efficacy of these drugs is very much reduced, along with significant reduction in immune cells activation and tumor clearance [150], thus indicating the critical role host-commensal bacteria play in the regulation of host immune system. Studies in primates have shown that presence of lactobacillus species in the breast stroma promotes anti-cancerous effects like reduction of oxidative stress and upregulation of bile salts and increased accumulation of bacteria modified compounds, which has been shown to act as an antagonist to the growth of cancer cells [151].

The presence of actively involved microbiome raise the question of what is the impact on ECM remodeling which may serve cancer niche formation, progression and drug resistance. For example, many bacterial enzymes are known to degrade host ECM and the repertoire includes collagenases, elastases, hyaluronidase etc. [152, 153]. Interestingly the activity of these enzymes is differently elicited at different growth conditions. For example, *Pseudomonas aruginosa*, secretes elastase under aerobic environs and in anaerobic conditions *in-vitro*, they predominantly produce alkaline proteases[154]. Bacterial proteases are also good imitators of host enzymes. They are known to shed various signaling moieties (Fig. 1D) like FASL, TNF, IL-6 and more[155, 156].

Bacterial collagenase activity and collagen degradation and its role in pathogenesis and virulence is well established[157], yet very little is known about any ECM remodeling activity mediated by the normal microbiome of human body. Studies have shown that in urothelial bladder cancer interaction between extracellular matrix components and host bacterial population regulates the microenviromental cues, which can determine the cancerous growth and disease progression [158]. There are studies where it has been shown that microbiome population can induce intestinal fibrosis by triggering the host immune cells [159]. All these results allude to the interaction of microbiota and ECM; however, the modes of these interactions are not clear. Recently, Blalock and co-workers have shown that pathogenic elements can trigger release of PMN (polymorphonuclear)-derived exosomes, which

downstream causes matrix degradation and promotes disease progression [160]. Another study showed that the release of outer membrane vesicles from *Group B Streptococcus* can cause ECM degradation in feto-maternal interface which leads to premature birth and fetal death in mice[161]. Taken together, all these reports suggest that microbiota can influence the host ECM and its homeostasis, but the ways of these interaction are yet to be elucidated. Considering recent reports, it appears release of exosomes or outer membranes vesicles might be one of the ways these interactions materialize.

Collectively, these results suggest critical elements of host microbiome and its diversity can play a significant role in the development of cancerous ECM, however, extensive further research is required to exactly elucidate the molecular and signaling cascades.

Conclusion

A plausible explanation for why we do not develop more cancer during our life time is associated with the fact that ECM in younger individuals is a stringent gatekeeper of normal tissue homeostasis. It is argued that as we age, oxidative damage leads to stromal activation via inflammatory signaling processes triggered by tissue damage or even tissue microbiome imbalance and results in progressive loss of control exercised by ECM homeostasis over dormant occult tumors.

Tumors are better understood if they are considered as organs with characteristic stromal cells, blood supply and ECM morphology[162]. But unlike normal organs, it is not restricted by laws governing healthy tissue. It is in a constant effort to override controls, originally set up to avoid rogue takeover of our body's cooperative balance. How these multiple gatekeepers fail to reign in an ever-expanding tumor is still not clear. What is clear is that if we want to target and wrest control of our system from this complex and maligned reflection of our normal self, a complete understanding of ECM and its dynamic remodeling that sustains and nourishes cancer niches should be factored in as a crucial component.

Through mechanisms described in this review, it is clear that extracellular matrix is the medium through which tumor cells acquire the essential hallmarks of cancer[163]. Remarkably, the ECM is rich in biomarkers for initial diagnosis and subsequent monitoring of treatment related prognosis. Soluble factors such as SPARC protein in plasma could be valuable to discriminate cancer patients from healthy but heavy smoker individuals[164]. Although screening procedures have improved in detection and reduced mortality in many cancer types, non-invasive procedures are still limited in accuracy, in predicting malignant disease of a tissue apart from benign or inflammatory conditions. MMP-2,-9,-7, TIMP-1, and TIMP-2 in renal

cell carcinoma, MMP-9, -2 and MMP degradation products of collagens -1,-3 and -4 in breast cancer, MMP-7 and -12 in pancreatic ductal adenocarcinoma, MMP-8 and TIMP-1 in colorectal cancer are all suspected to be candidate diagnostic or prognostic markers[165, 166]. As we look towards more noninvasive and powerful biomarker testing, circulating ECM molecules and derivatives are set to become more important in multi marker or fingerprint tests. The field is also exploring previously mentioned exosomes as good subpackets enriched in these tell-tale ECM markers in the plasma or serum[167]. Hence the matrisome[168] (an inventory of matrix constituents and associated proteins) could eventually become the Rosetta stone of tissue state and help us decipher the organ specific conditions that might develop during our life time including the most challenging of these maladies- cancer. The development of tumor ECM atlases may be proposed, similar to the cancer genome atlas, from all the tumor characterization studies and functional analyses so far. Such an endeavor will have the potential to impart great impetus to further mining the ECM as a reservoir of diagnostic, prognostic and therapeutic biomarkers and targets.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

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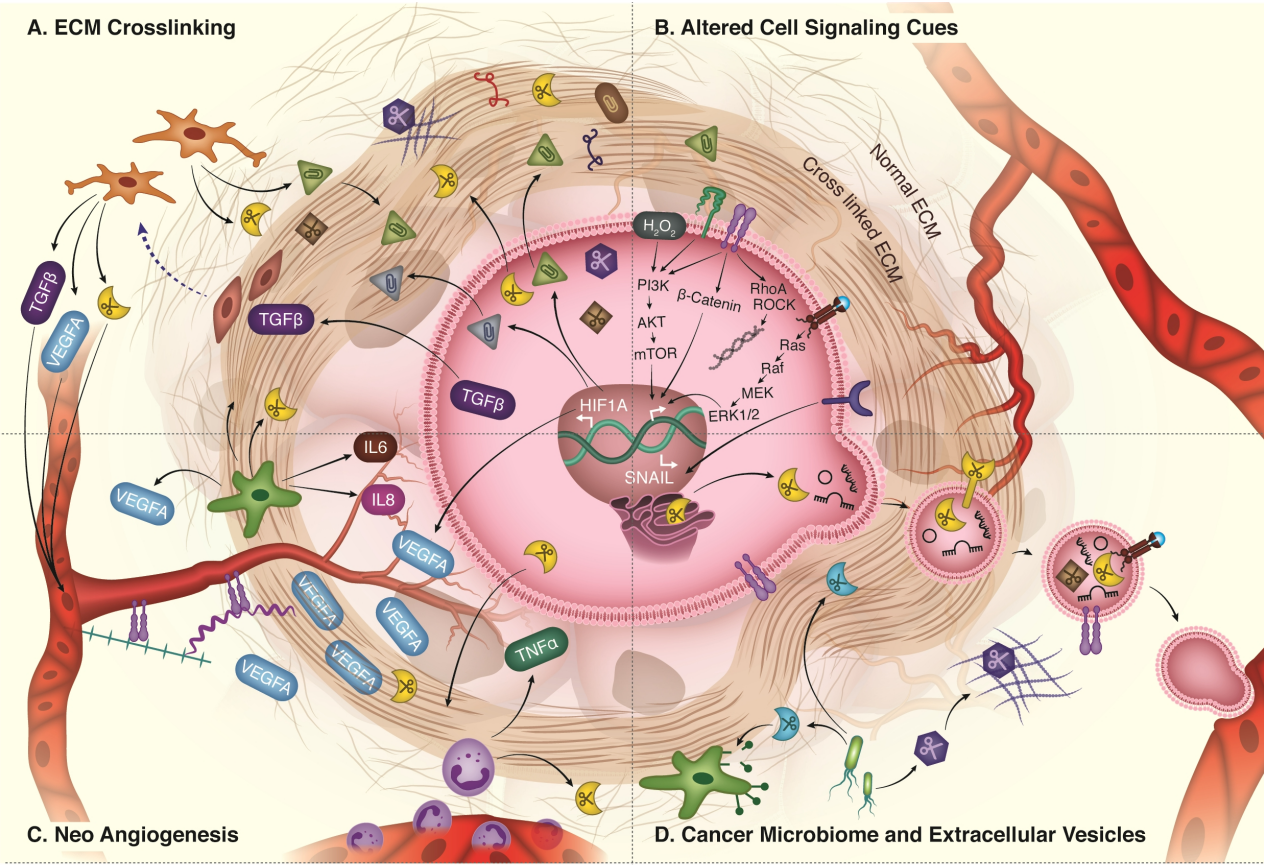
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Fig1. The dynamic and multifaceted interactions of the cancer cell and the ECM. **A**, Tumor cells induce a switch in the stromal cells towards cancer associated phenotype. Together they secrete enzymes such as proteases like MMPs, ADAMs and hyaluronidase, and crosslinkers like LOX, LOXLs and transglutaminase. **B**, Remodeled ECM results in altered cell signaling and environmental cues. Downstream transduction occurs via classical transmembrane proteins like integrins or via alternate pathways involving CD44 or DDR2. Substantial transformation of cell behaviors occurs due to activation of intermediary pathways such as ERK/MAPK cascade or RhoA/ROCK pathway. **C**, Endothelial cell migration in neo angiogenesis is regulated by chemotactic, haptotactic and mechanotactic stimuli from the ECM. The chemotactic stimuli are driven by cytokines such as VEGF, haptotactic stimuli by interactions of integrins with fibronectin, laminin etc. and mechanotactic stimuli by interaction of endothelial cytoskeleton via adhesion molecules to surrounding ECM. Macrophages recruited from circulation transform into TAMs and produce cytokines like IL6, IL8, VEGF-A for paracrine and autocrine signaling. Recruited neutrophils also secrete TNF- α and MMPs further supporting tumor progression and circulating cell recruitment and activation. **D**, Exosomes packaged with RNAs and matrix degrading enzymes are used to prepare distant metastatic niches. The cancer microbiome secretes collagenases which degrade structural molecules like collagen or signaling molecules like FASL.



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| | | | | | | | | | | |
| Neutrophil | Tumor Associated Macrophage (TAM) | Cancer Associated Fibroblast (CAF) | Fibroblast | Integrins | CD44 | DDR1/DDR2 | Receptor Tyrosine Kinase (RTK) | Growth Factor | Exosome | |
| | | | | | | | | | | |
| Microflora | Hydroxyllysylpyridinoline (HP) | Lysylpyridinoline (LP) | Blood vessel | Laminin | Fibronectin | Actin | Hyaluronic Acid (HA) | MT-MMPs | | |
| | | | | | | | | | | |
| MMPs | Bacterial Proteases | ADAMs | LOX | LOXs | Hyaluronidase | Transglutaminase | FASL | mRNA | circRNA | miRNA |