Review

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Extracellular matrix in cancer progression and therapy

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Abstract: The tumor ecosystem with heterogeneous cellular compositions and the tumor microenvironment has increasingly become the focus of cancer research in recent years. The extracellular matrix (ECM), the major component of the tumor microenvironment, and its interactions with the tumor cells and stromal cells have also enjoyed tremendously increased attention. Like the other components of the tumor microenvironment, the ECM in solid tumors differs significantly from that in normal organs and tissues. We review recent studies of the complex roles the tumor ECM plays in cancer progression, from tumor initiation, growth to angiogenesis and invasion. We highlight that the biomolecular, biophysical, and mechanochemical interactions between the ECM and cells not only regulate the steps of cancer progression, but also affect the efficacy of systemic cancer treatment. We further discuss the strategies to target and modify the tumor ECM to improve cancer therapy.

Keywords: cancer invasion; cancer metabolism; cancer progression; cancer therapy; cell-ECM interaction; extracellular matrix; metastasis.

Introduction

The study of solid tumors has traditionally centered on the individual tumor cells, the processes leading to their transformation or progression in their malignancy. In recent decades the tumor ecosystem with heterogeneous cellular compositions and the tumor microenvironment (TME) increasingly became the focus of cancer research. All components of the TME, including the tumor vasculature, infiltrating immune cells, tumor-associated fibroblasts and other cell types, and the extracellular matrix (ECM), have received increased attention. Many novel discoveries of the TME have led to new successful and potential cancer treatments.

The ECM provides critical signals to preserve tissue architecture, polarity, and homeostasis and to regulate cell growth and apoptosis. During cancer progression, alterations in tumor cell – ECM interactions drive malignant transformation, invasion, and metastasis, as well as treatment resistance [1–6]. The rapid expansion of tumor cells leads to the compression of adjacent tissue and the accumulation of stress within the tumor microenvironment [7]. This solid stress is compounded by dysregulated ECM deposition and remodeling. Degradation of the pericellular matrix and loss of basement membrane integrity due to both soluble matrix metalloproteinases (MMPs) [8] and membrane-localized MMPs [9] define a hallmark of invasive lesions.

In this review, we focus on the functional roles of the ECM in cancer initiation, growth, angiogenesis, invasion, and metastasis, as well as the ECM in the current cutting-edge tools to diagnose and treat invasive cancer. We will start by describing how tumor ECM differs from that in normal tissue.

The normal tissue ECM comprises a network of biochemically distinct components, including water, minerals, glycoproteins, proteoglycans, polysaccharides, and fibrous proteins such as collagen, elastin, and laminin, secreted by the resident cells. The ECM composition and structure are unique in every tissue to serve the tissue-specific functional needs. For example, the ECM in most glandular epithelial tissues, including breast, lung, and prostate, is composed of a relaxed meshwork of type I and II collagens, elastin, and fibronectin that allows for a mechanically compliant tensional homeostasis. By contrast, the ECM in ductal epithelial tissues is rich in proteoglycans, decorin, biglycan, and lumican associated with collagen to

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generate a structure that is essential for mechanical buffering and hydration [10, 11]. This unique composition arises through dynamic biophysical and biochemical feedback between cellular components and their evolving microenvironment [12]. The crosslinked fiber network of the ECM provides key tissue scaffolding structures so cells can adhere, grow, migrate within ECM. The cell-ECM cross-interaction is regulated in multiple scales, from genomic and epigenomic regulation to biochemical signaling from cell to ECM, and then biomechanical signaling from ECM to cell. The ECM is a highly dynamic structure, constantly undergoing a remodeling process where ECM components are deposited, degraded, or modified [13]. The ECM production by residence cells and degradation through metalloproteinases (MMPs) secreted by matrix modifying cells are balanced under normal conditions [14, 15], which is responsible for tensional homeostasis of the tissues.

In a normal wound healing process, ECM remodeling plays a critical role in not only providing structural integrity and scaffolding but also as storage and delivery of growth factors and cytokines that are important to tissue repair. E.g., degradation of ECM proteins by MMPs in response to wounds can locally release growth factors, including transforming growth factor β (TGF-β), vascular endothelial growth factor (VEGF), and epithelial growth factor (EGF), from their anchorage and modulate wound healing [16]. Normal skin wound healing ends with scars that have a denser and more rigid ECM, characterized by thicker aligned collagen fibers, excessive dermal fibrosis, lack of elastic fibers, and impaired tissue functions. Interestingly, early developing fetus can heal wounds by regenerating skin and restoring the normal ECM structure, strength, and function. The mechanisms of fetal scarless wound healing remains unknown, but are believed to involve the ECM [17]. Abnormal ECM reconstruction during wound healing results in the formation of pathological scars, including hypertrophic scars that are raised, painful, rigid, disfiguring, and functionally limiting form of dermal fibrosis, and keloid scars that are red, raised, fibrous, and can grow into large, tumorous (benign) neoplasms [18]. Similar to normal scars, both hypertrophic and keloid scars have collagen fibers parallel to the epithelial surface instead of a 3D network seen in normal skin tissue. There are structural and composition differences between normotrophic and abnormal scars and between hypertrophic and keloid scars. For example, in keloid scars, collagen forms disorganized, larger, thicker bundles than normal scars, which is absent in hypertrophic scars [19].

Tumors have been considered as wounds that do not heal [20, 21]. Under stress, tumors co-opted the wound healing response to induce the stroma they required for maintenance and growth, resulting in the formation of dense fibrous connective tissue, which is called scar tissue in wounds and desmoplasia in cancer. In essence, the tumor ECM is the worst form of abnormal scar, much denser and more rigid, and functionally less desirable than its counterpart in normal tissue. The tumor ECM differs from the normal tissue ECM not only in the amount of deposition but also in composition, organization, and post-translational modification. Many solid tumors express high levels of various ECM molecules like fibrillar collagens, fibronectin, elastin, and laminins [22, 23]. Pancreatic ductal adenocarcinomas are rich in hyaluronan [24]. These excess ECM molecules come from the tumor cells and cancer-associated fibroblasts (CAFs) [25, 26]. Increases in ECM deposition without the appropriately balanced degradation result in increased stromal density, including both fibrillar collagen [22, 27], which contributes to the overall increase in stiffness in mammary tumors [28], and fibronectin, which influences tumor growth, invasion, metastasis, and therapy resistance. Increased fibrillar collagen density and changes in architecture have been appreciated in various solid and malignant tumors [13, 22, 29–32]. For example, the increased mammographic density is associated with a 4- to 6-fold increased risk of breast cancer [33], and increased stromal collagen in mouse mammary tissue significantly increases tumor formation and results in a significantly more invasive phenotype with lung metastasis [22]. In addition to the changes in collagen density, changes in collagen compositions are indicative of cancer progression. Collagen production is shifted toward collagen type I (Col I) and type III (Col III) in invasive ductal carcinomas compared to benign mammary lesions [34]. Experiments using second harmonic generation microscopy show that increasing the Col V/Col I ratio reduces the length and organization of collagen fibers [35]. In melanoma, increased Col I expression is correlated with invasiveness, angiogenesis, and reduced survival [36]. Furthermore, the increased invasive phenotype of tumor cells that arose within collagen-dense mammary tissues remains after tumor explants are cultured within reconstituted 3D collagen gels [22]. These characteristic changes in collagen fiber organization have a demonstrated potential as an early diagnostic and prognostic marker in breast tumors [31].

The tumor ECM also is rich in matrix fragments resulting from the activation of proteases during cell migration and invasion. The matrix fragments act directly on cancer cells influencing their viability, apoptotic rate, and metastatic potential. These ECM fragments can often display opposite effects compared to the intact molecules of origin [37], making the study on the ECM microenvironment even more complex. Adding to the mix, tumor-associated hypoxia induces lysyl oxidase expression [38], along with other ECM

modifying enzymes, resulting in the fibrillar collagen crosslinking within the ECM and the synergy of biophysical and biochemical changes that allow cancer cells to more efficaciously invade and metastasize [1].

The dense and rigid tumor ECM not only serves as a diffusion barrier for drug molecules and therapeutic immune cells [39, 40], but also reduces the diffusion of nutrients and oxygen, resulting in a hypoxic and stressful TME such that tumor cells are less responsive to drugs [41]. The increased cell-ECM contact activates signaling pathways that increase epithelial-to-mesenchymal transition (EMT) for tumor cells, making cells more chemo-resistant [41]. These effects of tumor ECM on tumor progression and therapy (Figure 1 [42]) will be discussed in more detail below.

ECM in cancer progression

Tumorigenesis and growth

The ECM provides critical signals to maintain tissue architecture, polarity, and homeostasis, and regulate cell growth and apoptosis. In the omics era, matrisome is defined as the ensemble of 1,000+ genes encoding ECM and ECM-associated proteins [43]. Matrisome has two interactome layers: the core-matrisome, genes for the ECM building proteins including ECM glycoproteins, collagens, and proteoglycans, and the ECM-associated genes including ECM-affiliated proteins (e.g., mucins, glypicans), and ECM regulators, including ECM-crosslinking and modifying enzymes and their inhibitors (e.g., LOX, MMP, and tissue inhibitors of MMP or

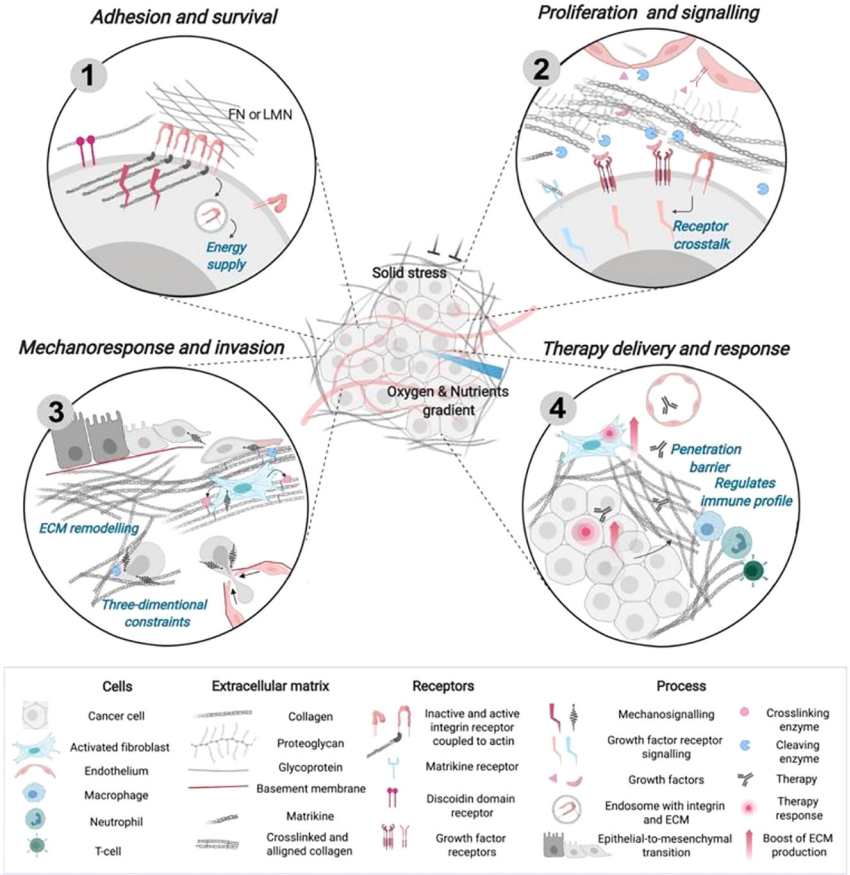

Figure 1: The ECM as a regulator of tumor progression and therapy.
(1) Adhesion to the ECM through the receptors (e.g., via integrin or discoidin domain receptor) promotes signaling that supports cancer cell survival. To supply metabolic pathways, cancer cells can use the ECM (laminin and fibronectin), internalized with an integrin. (2) Growth factors that are released upon cleavage by ECM proteases (mainly MMPs) can regulate cancer cells and other cells of the TME. Biologically active ECM fragments (peptides) released upon proteolysis can promote cell signaling in the TME and spread with the blood flow. (3) Recruitment of activated fibroblasts stimulates ECM production, alignment, and cross-linking (e.g., by LOX) of collagen fibrils resulting in fibrosis. Changes in mechanical properties can prompt cancer cells to adapt their signaling, gene expression, and cytoskeleton polarization, acquiring invasive phenotype (EMT). (4) Dense and rigid ECM creates a barrier to drug and T-cell penetration. Targeted cancer cells and fibroblasts can increase their resistance by promoting ECM production and deposition. Changes in the tumor ECM are associated with the formation of a tumor-permissive immune profile (Figure reproduced with permission [42]).

TIMPs), and other secreted factors as signaling proteins (e.g., Wnts, cytokines) [42, 44]. Mutations in ECM genes are causal of musculoskeletal, cardiovascular, renal, ocular, and skin diseases [45]. Conversely, excessive deposition or destruction of ECM is a hallmark of pathologies such as fibrosis, osteoarthritis, and cancer [11]. High ECM deposition and increased stiffness combined with integrin overexpression in various cancers trigger tumor promotion [46–48].

Dysregulation of the ECM genes has been found to relate to tumor initiation. ECM gene mutations and protein signatures were identified in many tumors, including breast, ovarian, colon cancer, and melanoma [49]. Recent studies also discovered more frequent copy number alteration and mutations in matrisome than that in the rest of the genome based on TCGA [50]. In addition, biologically active ECM fragments (peptides) released upon ECM proteolysis, termed matricryptins or matrikines, can promote cell signaling in the TME [37, 42], further promoting cancer progression.

During pre-tumorigenesis, diverse stromal cell types are activated [51]. Notably, the irreversible activation of the pro-carcinogenic fibroblasts (termed cancer-associated fibroblasts or CAFs) contributes to the ECM remodeling and the TME reprogramming [52–54]. Despite the origins, the CAFs are recognized as an essential driver of ECM alternation during tumor progression [55–59]. In pancreatic ductal adenocarcinoma (PDAC), CAFs near the tumor cells show active TGF-β signaling and deposit collagen. In contrast, CAFs at a greater distance are also activated by the tumor cells but are unresponsive to TGF-β, deposit hyaluronic acid, and establish a tumorigenic, pro-inflammatory environment through the expression of cytokines such as IL-6 or antigen presentation [60, 61]. This pro-inflammatory environment fosters the recruitment and activation of immune cells, contributing to the establishment of the cancer niche. Some CAFs additionally support tumor growth through mechanisms independent of ECM remodeling, such as promoting cancer stemness or preventing cancer cell recognition by T-cells [62, 63].

In addition to biochemically and biomechanically controlling a plethora of cellular functions, the ECM has also been shown to impinge on the cancer metabolism, as the cancer metabolism reprograms the ECM of the TME [64, 65]. The cell-ECM interactions via mechanotransduction pathways are found to regulate the metabolic interactions of cancer cells with the TME. In pancreatic tumors, the ATP/ADP and ATP/AMP ratios are modulated, leading to invasive migration, chemotaxis, and enhanced liver metastasis by tuning the creatine–phosphagen ATP-recycling system [66]. Furthermore, the cell-ECM adhesion transduces the neutral lipid synthesis. It enhances glycolysis while dysregulated glutamine metabolism in both cancer and stromal cells facilitates aspartate–glutamate exchange, supporting tumor growth and metastasis [66, 67]. In addition, increased ECM may promote fatty acid oxidation in ovarian cancer [68]. Reciprocally, cancer cells accumulate metabolic alterations that allow them to gain access to conventional nutrient sources as well as to unconventional nutrient sources, utilize these nutrients towards the creation of new biomass to sustain deregulated proliferation, and take advantage of the ability to select metabolites to affect the fate of cancer cells themselves as well as a variety of normal cell types within the TME [69].

Accompanying the secreted growth factors and alterations to the extracellular matrix and cell-cell interactions, proliferating cancer cells also alter the metabolic composition of the extracellular milieu. The high utilization of extracellular glucose and glutamine by cancer cells results in the accumulation of extracellular lactate, which was shown to affect a number of cell types within the TME. For example, the tumor-derived lactic acid promotes the emergence of an immune-permissive microenvironment by attenuating dendritic and T cell activation and monocyte migration [70–72]. Furthermore, increased lactate levels also stimulate hyaluronic acid production by fibroblasts, which may contribute to tumor invasiveness [73].

Tumorigenesis and tumor growth require stroma that the host provides. In this respect, tumors behave like parasites that prey on the host, leaving behind a messed-up microenvironment that is easier for tumor cells to survive and progress but stressful for host cells to survive.

ECM in tumor angiogenesis

Solid tumors need the vascularized stroma to survive, grow, and metastasize. A hallmark of cancer is angiogenesis, the formation of new blood capillaries from pre-existing vessels that enables oxygen and nutrients from the surrounding microenvironment to reach the tumor [74]. The list of ECM molecules that have been shown to influence angiogenesis has grown over the last two decades [75]. Several ECM components have been shown to play a critical role in cancer angiogenesis. A recent review presented a detailed list of these molecular players [75]. Here we highlight only a few major ones: fibronectin, collagen, and thrombospondins.

Collagen as a major component of the ECM is intimately involved in angiogenesis. Type I collagen interacts with α1β1, α2β1, αvβ3 and αvβ5 integrins and induces the activation of MAP kinase pathway supporting endothelial survival. The binding of type I collagen to α1β1 and α2β1

integrins suppresses cAMP-dependent protein kinase A, resulting in the actin cytoskeleton remodeling and cell shape changes [76]. Type I collagen is also important for lumen formation during angiogenesis [77]. Type IV collagen is one of the major components of the basement membrane [78]. Fragments of type IV collagen promote endothelial cell adhesion and migration in the early stages of tumor development [79] but inhibit angiogenesis later in tumor development. Type XV collagen and type XVIII collagen are also components of the vascular basement membrane important in stabilizing microvessels; their fragments have various anti-angiogenic effects [75].

Fibronectin binds to integrin receptors and, in particular, to integrin α5β1, which is markedly up-regulated during tumor-associated angiogenesis [80]. The deposition of fibronectin is necessary for matrix assembly and vascular morphogenesis [81]. Fibronectin promotes endothelial survival, whereas the blockage of its polymerization impairs endothelial cell proliferation and tube formation *in vitro* and *in vivo* [82, 83].

Thrombospondins (TSPs) are a family of large ECM glycoproteins, including five members (TSP-1 to TSP-5) [84]. TSP-1 and TSP-2 have been extensively studied for their anti-angiogenic properties. Accordingly, the over-expression of these two molecules by tumor cells is associated with impaired tumor growth in mice [85, 86].

The role of matrix metalloproteinases (MMPs) in angiogenesis is more complex than that in the remodeling of basement membranes by degrading ECM components. Specific MMPs have been shown to enhance angiogenesis by helping to detach pericytes from vessels undergoing angiogenesis, by releasing ECM-bound angiogenic growth factors, by exposing cryptic proangiogenic integrin binding sites in the ECM, by generating pro-migratory ECM component fragments, by activating other MMPs, and by cleaving endothelial cell-cell adhesions. MMPs can also contribute negatively to angiogenesis by generating endogenous angiogenesis inhibitors by proteolytic cleavage of certain collagen chains and plasminogen, modulating cell receptor signaling, and cleaving off their ligand-binding domains. A number of inhibitors of MMPs that show antiangiogenic activity are already in the early stages of clinical trial. A rather complete summary of these roles of MMPs in facilitating and inhibiting angiogenesis can be found in this classic review [87]. A more recent review on MMPs in cancer angiogenesis [88] emphasized the role of cytokines and growth factors inducing EMT in various types of cancer together with the role of MMPs.

In addition to matrix components and their fragments, matrix remodeling also leads to the release of growth factors and cytokines, of which the ECM serves as a reservoir [89]. Moreover, cell-matrix mechanics in the mechanical regulation of angiogenesis have been increasingly recognized. The motility, metabolism, proliferation, and differentiation of anchorage-dependent cells, including endothelial cells and pericytes, are highly regulated by mechanical stimuli received by cells via mechanotransduction through the ECM [90–93]. Angiogenic neovessels remodel the ECM through traction, proteolytic degradation, and cell-matrix adhesion, and the ECM properties influence vascular growth and alignment. The cell-ECM mechanical interaction was also found to regulate endothelial sprouting speed and proliferation, thereby regulate sprout stalk diameter [94].

Tumor angiogenesis has been a fertile ground for mathematical models since the early 1970s. Some of the first models used differential equations to represent a generic growth factor produced and released by a tumor, which as chemoattractant triggered endothelial cell growth and migration into the tumor [95–97]. More mathematical models have since included detailed vessel network formation [98], molecular-level interactions of VEGF and its receptors [99], rule-based cellular automaton models of vascular network growth [100], cell-based cellular Potts models of angiogenic sprouting [101, 102], a finite-element/level-set method for topological changes including tumor splitting [103], among many others [104, 105]. Together they give insight into the multiscale, multi-faceted conditions affecting tumor angiogenesis. The roles of ECM in tumor angiogenesis have also been modeled respectively and in combination: 1) ECM-bound VEGF and other factors are released when MMPs degrade the ECM [101, 106], 2) ECM-alignment and density guides capillary movement through the matrix and mediates vascular morphogenesis [104, 107, 108], and 3) ECM-mechanical feedback to endothelial cells explains the behavior of individual endothelial cells and the interactions of endothelial cell pairs in compliant matrices [109] and cell-ECM mechanics, including load initiation time, magnitude, and mode regulate microvascular growth, as well as upstream angiogenic and mechanotransduction signaling pathways [110].

Angiogenesis is another process that is shared between wound healing and tumor development. Microhemorrhages are common in both healing wounds and solid tumors, emanating from newly formed, fragile blood vessels. Abnormal intravascular hemostasis has a long historical association with cancer [111]. The biomechanical and biochemical roles the ECM plays in angiogenesis are similar in wound healing and in tumor development. The major differences are that the normal microenvironment allows the wound to heal, and the stressful tumor microenvironment renders tumors as wounds never heal.

ECM in cancer invasion and metastasis

Cancer metastasis is a multistep process that starts with invasion whereby cancer cells penetrate neighboring tissues and escape the primary compartment, intravasate and ride the bloodstream, extravasate to establish growth at distant sites [112]. The acquisition of invasive phenotype requires the abrogation of cell-cell contacts, remodeling of the ECM, and cell-ECM interactions. A recent review [113] summarized four distinguishing features of ECM as hallmarks of cancer for metastasis [114]: 1) motility and invasion; 2) the ability to modulate secondary sites and/or local microenvironments; 3) phenotypic plasticity; 4) the ability to colonize secondary tissues. Extensive research during the last decade has unraveled the significance of ECM remodeling at each stage of metastasis, from invasion and surviving in circulation to forming the pre-metastatic and metastatic niches.

The increased tissue stiffness in many carcinomas modifies the local forces experienced by the tumor cells through the ECM [115]. Tumor cells respond to this altered mechanical microenvironment by converting mechanical stimuli into downstream biochemical signaling changes in cytoskeletal dynamics, integrin signaling, and various mechano-sensitive signaling molecules such as the nuclear localization of b-catenin or Yes-associated protein 1 (Yap) [116]. In turn, cell-generated forces respond to this altered microenvironment by modifying the cellular response to growth factor cues, increasing cell growth and survival, destabilizing cell-cell adhesions, and compromising tissue integrity [117]. This destabilization of cell-cell adhesions and tissue integrity is necessary for cancer cells to break free from the confinements of the primary tumor [118–120]. Most carcinomas are epithelial in origin, and transition to an invasive phenotype requires drastic reprogramming on a genetic and physiological level, known as epithelial-to-mesenchymal transition (EMT). An epithelial cell in a normal situation is laterally linked to its akin neighbors via cell adhesion molecules (e.g., E-cadherin) and basally rooted in the ECM via integrins.

On the other hand, mesenchymal cells exhibit strong matrix interactions, the ability to migrate through ECM, and increased resistance to chemotherapeutics, facilitating cancer dissemination. Cancer cells or CAFs can also tunnel through the ECM and create micro-tracks for the follower cancer cells facilitating the migration through the ECM [121] before intravasation into the blood or lymphatic systems. Several lines of evidence revealed that CAFs produce a broad spectrum of MMPs [8] and plasminogen activators to degrade ECM directly [122], facilitating mesenchymal cell invasion. Other tumor cells going through EMT also have altered signaling pathways involving growth factors, cytokines, cell adhesion molecules, leading to upregulated motilities [123–127]. Moreover, MMPs can also trigger a cascade of molecular alterations that leads to EMT [125–127].

Traditionally, the mesenchymal cells are considered to migrate individually, appear as single cells in the circulation and directly initiate metastatic lesions by reverting to a proliferating epithelial phenotype [128]. Collective cancer invasion with leader-follower organization is increasingly recognized as a predominant mechanism in the metastatic cascade, e.g., in non-small-cell lung cancer (NSCLC) spheroids [129], head and neck squamous cell carcinomas (HNSCC) [130]. Leader cells support cancer invasion by creating invasion tracks, sensing environmental cues, and coordinating with follower cells biochemically and biomechanically (recently reviewed in Ref. [131]). This review proposed a set of definitions for leaders in collective cancer invasion and a framework to unify the cellular and molecular programs adopted by each leader cell subtype [131]. A recent study [132] on clinical invasive ductal carcinomas (IDC) and invasive lobular carcinoma (ILC) showed that the downregulation of E-cadherin and p120-catenin caused a transition from coordinated to uncoordinated collective movement along extracellular boundaries. In contrast, single-cell escape depended on locally free tissue space. These results provide a new understanding of 3D invasion plasticity on how cell-cell adhesion and cell confinement affect collective cell motion.

Next, tumor-induced angiogenesis and heightened MMP activity at the primary tumor site result in a leaky vasculature that facilitates invasive tumor cells to intravasate and extravasate. Circulating tumor cells (CTCs) in the blood upregulate the expression of common stroma-derived ECM proteins, such as collagens, TIMP-2, the proteoglycan decorin, the glycoprotein osteonectin, and fibronectin, as revealed by single-cell RNA-sequencing of pancreatic CTCs [133]. CTCs captured by neutrophil extracellular traps (NETs) have been shown to correlate with increased metastasis [134]. Potential sites of metastasis before the presence of the metastatic tumor cells, so-called pre-metastatic niches [135], involve ECM remodeling that is distinct from the primary tumor [136]. In contrast to increased collagen deposition in the primary TME, fibronectin is the leading player in the formation of the pre-metastatic niche along with glycoproteins and proteoglycans [136]. Primary tumor-derived exosomes activate stromal cells in the pre-metastatic site to secrete new ECM molecules or remodel and modify the ECM directly, creating a fibrotic and pro-inflammatory environment. Bone marrow-derived cells (BMDCs) are recruited to the pre-metastatic niche, attach to

the remodeled ECM via integrins, and contribute to further ECM remodeling in preparation for the arrival of disseminated tumor cells [137]. CTCs that extravasate through the disrupted vasculature into the distant tissue may be dormant. Proteases expressed in NETs, including neutrophil elastase and MMP-9 cleave laminin, generating a specific matrikine that can awaken dormant tumor cells [138]. Together, these ECM remodeling processes support the formation of metastasis [48].

Contrary to the common impression that 'malignent' cancer cells are formidable armies that invade and occupy new grounds with ease, cancer cells must adapt to a relatively inhospitable microenvironment, evade immune surveillance, and progress from the micro- to the macro-metastatic stage to generate a secondary tumor. Cancer invasion and metastasis involve cell migration and proliferation that are also central to wound healing. A recent review titled "Cancer metastasis as non-healing wound" [139] focused on cellular responses to inflammation in both wound healing and metastasis. We show here that the ECM as the major component of the microenvironment and ECM remodeling as a dynamic response to environmental stresses also support the parallel between metastasis and non-healing wound.

ECM as diagnostic markers

The expression profile of ECM-related genes is, in many cancers, a valuable prognostic factor. For example, besides indicators for immune suppression, high expression of collagens Col3a1, Col4a1, and Col5a2 is correlated with poor prognosis in glioblastoma [140]. According to their ECM expression profile, breast cancer was subdivided into four groups (ECM1–4) with significant correlations with prognosis [141]. Between tumor subtypes, ECM expression and deposition also differ significantly. Triple-negative breast cancer and, to a lesser extent, Her2 tumors show not only increased deposition of collagen but also enhanced invasion with CAFs [142, 143].

In addition, the stromal architectures were indicative of tumor progress status and prognosis. In breast cancer again, tumor-associated collagen signatures (TACS) were identified as TACS-1, -2, and -3. TACS-1 is the presence of locally dense collagen within the globally increased collagen concentration surrounding tumors. TACS-1 serves as a reliable hallmark for locating small tumor regions. TACS-2 is identified as straight (taut) collagen fibers stretched tangentially around the tumor, constraining the tumor volume, indicative of a growing tumor. TACS-3 is characterized by bundles of straightened and aligned collagen fibers that are oriented perpendicular to the tumor boundary and facilitate local invasion [29]. A rigorous examination of the relationship of TACS-3 to the long-term survival rate of human patients showed that the presence of TACS-3 was associated with poor disease-specific and disease-free survival [30].

Furthermore, TACS-3 was confirmed to be an independent prognostic indicator regardless of tumor grade and size, estrogen or progesterone receptor status, human epidermal growth factor receptor-2 status, node status, and tumor subtype [30]. Interestingly, TACS-3 was positively correlated to the expression of stromal syndecan-1, a receptor for several ECM proteins, including collagens [30]. Aligned ECM *in vivo* serves as natural trails on which cancer cells migrate [29, 144, 145]. The deposition of fibrillar collagen and collagen-induced release of pro-inflammatory COX-2 is sufficient to induce collective invasion in otherwise benign or less aggressive breast tumor lesions [146]. These studies strongly suggest that tumor ECM morphology is a biomarker for diagnosis and prognosis.

These ECM alterations can be visualized as tumor-associated collagen signatures that can be measured using second harmonic generation imaging [29, 31, 32, 147]. Several computational methods facilitate quantification of the fiber alignment based on the second harmonic generation images of collagen and have demonstrated clear discrimination of invasiveness in breast tumor and non-small cell lung cancer spheroids [32, 148, 149].

A major problem in effectively treating tumor metastasis is identifying tissues with micrometastases. Matrix proteins that participate in this process become activated and undergo conformational changes that expose biologically active cryptic-specific sites [150]. Monoclonal antibodies are recruited to activate vitronectin preferentially localized to malignant tumors [150]. Antibodies directed to the extra domain (ED-B) of fibronectin localized selectively to new blood vessel development sites and malignant tumors. At the same time, little has been associated with non-proliferative tissues [151]. Moreover, the cryptic epitope in laminin-1 recognized by the STQ-peptide was selectively exposed in malignant melanoma *in vivo* [152]. Taken together, these studies suggest that cryptic ECM elements may represent highly selective targets for the development of novel imaging reagents for the detection of early metastasis [153].

The cross-talk between cancer metabolic pathways and the tumor ECM offers another set of potential imaging targets for cancer. For example, the dynamic contrast enhancement (DCE) MRI has been applied widely in screening high-risk populations and diagnosis and therapy evaluation of breast cancer [154]. A recent low-dose multitasking DCE MRI imaging has enabled extravascular extracellular space (EES) volume fraction as a marker for fibrosis

in liver cancer [155]. Additionally, with the explosion of publications on deep learning applications for cancer diagnosis, prognosis, and treatment response [156–159], we expect to see real-world medical utility of these ECM markers in the near future.

ECM in cancer treatment

Drawing the parallel between tumor development and wound healing again, the tumor ECM is more abundant, denser, and stiffer than that in normal tissue, akin to but worse than pathological scars, which can negatively impact response to cancer therapy biophysically and biochemically. A recent review highlighted the current understanding of the physical, cellular, and molecular mechanisms by which the pathological tumor ECM affects the efficiency of radio-, chemo-, and immunotherapy [41] (Figure 2). The accumulation of dense and rigid ECM histologically often encapsulates tumors (e.g., the TACS in breast cancer [29]), acts as a barrier to reducing molecular diffusion, and shields the cells from chemotherapeutic agents. At the same time, reduced diffusion of oxygen, nutrients, and metabolites in and out of the tumor also increases hypoxia and metabolic stress, leading to activation of angiogenesis, antiapoptotic, and drug resistance pathways. Furthermore, via integrin-mediated FAK-signaling, increased tissue stiffness and different components of the ECM can contribute to chemoresistance by inhibiting cytotoxic stress response and triggering EMT of tumor cells. Adding to this complexity, ECM dynamics intersect other intracellular signaling networks through modulating activation thresholds, which have in turn become promising targets in increasing chemotherapy efficacy and improving patient outcome [160]. These mechanisms individually and in combination provide many potential targets for therapeutic tools.

The physical barrier for drug diffusion: Distribution of drugs in the tumor occurs mainly by diffusion. Therefore, an abundance and highly cross-linked ECM can significantly reduce drug transport, especially for large molecules. In addition, the ECM barrier can result in only a small volume of the surrounding tissue being supplied by the individual vessels. Solid tumors are already characterized by low microvessel density [161, 162]. The abundant and

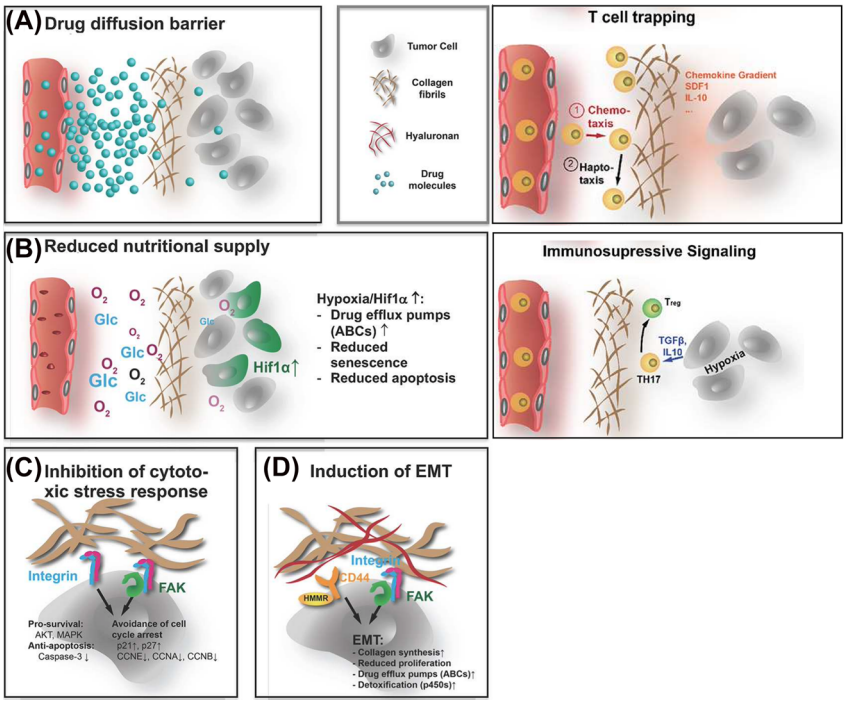

Figure 2: Four ways the ECM affects the efficacy of systemic treatment and immunotherapy.
(A) The abundant, rigid, and dense ECM is a diffusion barrier for drug molecules, thus shielding the tumor from the tumor therapy agents and T cells. (B) The ECM also reduces the diffusion of nutrients and oxygen, resulting in a hypoxic and stressful TME. Tumor cells increase the expression of drug efflux pumps, impair apoptosis and senescence, and activate and activate immunosuppressive signaling, rendering drugs that reach the cells less effective. (C) The cell-ECM contact mediated by integrin and FAK signaling inhibits the cytotoxic stress response that avoids cell cycle arrest. (D) Similarly, integrin, FAK, and hyaluronan-induced CD44/HMMR signals can lead to EMT. The mesenchymal state is less proliferative and more chemoresistant. The EMT further increases collagen production and crosslinking (Adapted from Henke et al. [41] with permission).

dense ECM further aggravates the supply situation, leading to hypoxia and metabolic stress, which are directly linked to resistance vs. various forms of cytotoxic therapy and radiotherapy [163–167].

Molecular mechanisms: The increased collagen production in the TME is strongly correlated with chemoresistance and reduced survival in triple-negative breast cancer patients [168]. The complex process of collagen synthesis and maturation offers many potential targets for treatment. Inhibition of LOX reduces tissue stiffness on overall collagen deposition, as the maturation process in the form of LOX-induced cross-links further stabilizes collagens and protects them from degradation. Treatment with 2-aminopropionitrile reduced collagen deposition and drug accumulation in murine allograft models [41]. Hyaluronic acid (HA) production is increased in many cancers [168]. Stromal fibroblasts are often identified as the primary source of hyaluronic acid in the tumor. Acting as a ligand for CD44 and playing a role in EMT, HA levels are correlated with poor prognosis [169, 170].

Cellular mechanisms: As carcinoma- or tumor-associated fibroblasts (TAFs) are the primary producer of the ECM in tumors, it is necessary to have a closer look at the particularities of these cells [171–173]. CAFs are found in all solid tumors [174, 175]. They differ substantially from the quiescent, metabolically inactive fibroblasts found in normal connective tissue, as they are migratory, growth and immune response promoting, and synthetically active [41]. TAFs further contribute to more malignant tumor phenotype by driving epithelial-to-mesenchymal transition (EMT), resulting in invasion and metastasis.

The increased understanding of these physical, molecular, and cellular mechanisms has led to increased interest in targeting the ECM for improved therapeutic efficacy. For example, the degradation of the ECM to remove the physical barrier has been shown to improve drug uptake and response in preclinical trials [176]. However, as the ECM in tumors is constantly remodeled, removing the existent ECM might not be necessary. The perpetual turnover is signified by the high-level synthesis of ECM macromolecules and degradation of the ECM by tumor-secreted hydrolytic enzymes like MMPs and cathepsins. Therefore, it is possible to shift the ECM remodeling toward degradation by blocking or reducing ECM synthesis. This can be accomplished by either reducing cues that lead to increased expression of ECM molecules, like TGFβ signaling or hypoxia-response pathways, or by inhibiting the various modifying enzymes necessary for proper production, secretion, and maturation of ECM molecules [41]. This approach can be further specified by targeting collagen synthesis and maturation and hyaluronan synthesis. For example, inhibition of NFκb reversed the typical CAF phenotype, strongly reduced collagen deposition in the tumor matrix, and improved response to cisplatin in ovarian cancer xenografts [177].

On the other hand, the ECM and ECM-like materials have great potential as cell and drug carriers. Modulated and engineered ECM is an effective approach to improve drug delivery and normalize the tumor ECM by reducing matrix stiffness or reversing the cell–ECM alignment. Modulating collagen alignment using anti-lysyl oxidase-like 2 (LOXL2) antibodies interfered with the adhesion and invasion properties of human breast cancer cells [178]. Although targeting specific stages of collagen assembly *in vivo* presents a great challenge, additional studies capitalizing on these ideas can potentially lead to a more comprehensive understanding of this promising type of approach [144]. ECM-like materials, e.g., thermally responsive elastin-like polypeptides (ELPs), were synthesized by recombinant DNA techniques and conjugated to doxorubicin to enable release of the drug in the acidic environment of lysosomes, which was delivered to solid tumors as a cancer therapy [179]. Electrospun scaffolds from synthetic polymers, natural proteins, or hybrid materials are often used to deliver drug [180].

While the research efforts targeting ECM to improve the outcome of cancer therapies are still mainly in the realms of primary and early translational research, a number of protodrugs are in various phases of clinical trial [41]. For example, LOXL2 antibody in combination with gemcitabine and FOLFIRI in PDAC and CRC patients, respectively, has completed phase II trials [181, 182]. Three major challenges in ECM-targeting therapies have been identified [41]. First, targeting ECM synthesis almost always affects other processes in the TME. Likewise, targeting other components of the TME, e.g., angiogenesis or immune cells, also affects the ECM. This mutual interdependence of various processes in the tumor often complicates the interpretation of experiments. Second, as the ECM is a complex mixture of macromolecules, the challenge is to select from this multitude of possible points of attack the right ones that promise the strongest effects and are least likely to cause problems with the functions of the ECM in physiological processes. Finally, the most likely application for ECM-targeted approaches is not a stand-alone therapy but as synergistic improvements in combination with other forms of treatment.

Summary

The concept of tumors as non-healing wounds helps to organize our understandings of the functional roles of ECM in cancer biology. It has long been recognized that tumors share properties with healing wounds. In the early 1970s,

Haddow proposed that tumor formation might represent an "overhealing" [183]. Surgeons have reported the tendency of tumors to recur in healing resection margins and that the wound healing environment provides an opportunistic matrix for tumor growth [184, 185]. We compare the ECM of normal tissue to that of normal and pathological scars and propose that tumor ECM is the worst form of abnormal tissue scar that promotes cancer progression.

A dynamic interlocking mesh of water, minerals, proteoglycans, and fibrous proteins such as collagen, elastin, and laminin, secreted by resident cells, the ECM is unique in every tissue to serve the tissue-specific functional needs [10]. In stressful microenvironments, tumor cells co-opt the wound healing response to induce survival and growth stroma. As a result, the tumor ECM resembles the worst form of abnormal scar tissue that differs drastically from normal organs in composition, architecture, and mechanical properties, due to disrupted balance between ECM synthesis and secretion and altered expression of matrix-remodeling enzymes. The tumor ECM provides physical, mechanical, biochemical, and cellular cues to promote tumor growth and malignancy, regulate the course of the disease, and influence the response toward therapy.

The more abundant, dense, and rigid ECM associated with the TME interacts with tumor cells and stromal cells, not only can induce tumorigenesis, regulate tumor metabolism, facilitates angiogenesis, induces EMT for invasion, forms pre-metastatic and metastatic niches to facilitate metastasis but can be a barrier for therapeutic agents. As our in this field grow, interfering with the synthesis and accumulation of ECM or related processes can potentially improve the outcome of concomitant therapeutic approaches, whether these are conventional cytotoxic treatments, radiotherapy, or targeted therapy, including immunotherapy. Furthermore, the ECM and ECM-like materials have great potential as cell and drug carriers, and modulating ECM and engineered ECM can improve drug delivery for enhanced therapeutic efficacy. Of course, the ECM not only affects the efforts to treat cancer but also is directly involved in tumor establishment and disease progression. Effects of ECM-targeted approach on the efficacy of antitumor therapy often cannot be distinguished from direct effects on tumor behavior or progression. Taken together, it is crucial that future studies further elucidate the role of ECM on all stages of cancer development to design therapies that most effectively treat or manage cancer.

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