The Interplay between Extracellular Matrix Remodeling and Cancer Therapeutics

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ABSTRACT

The extracellular matrix (ECM) is an abundant noncellular component of most solid tumors known to support tumor progression and metastasis. The interplay between the ECM and cancer therapeutics opens up new avenues in understanding cancer biology. While the ECM is known to protect the tumor from anticancer agents by serving as a biomechanical barrier, emerging studies show that various cancer therapies induce ECM remodeling, resulting in therapy resistance and tumor progression. This review discusses critical issues in this field including how the ECM influences treatment outcome, how cancer therapies affect ECM remodeling, and the challenges associated with targeting the ECM.

Significance: The intricate relationship between the extracellular matrix (ECM) and cancer therapeutics reveals novel insights into tumor biology and its effective treatment. While the ECM may protect tumors from anti-cancer agents, recent research highlights the paradoxical role of therapy-induced ECM remodeling in promoting treatment resistance and tumor progression. This review explores the key aspects of the interplay between ECM and cancer therapeutics.

INTRODUCTION

The extracellular matrix (ECM) is a complex and dynamic network of ~300 different molecules that provides structural support to cells and tissues within organs. It also regulates a variety of cellular processes including migration, proliferation, polarity differentiation, and apoptosis (1). Depending on function, composition, and location, the ECM mainly exists in two forms—interstitial matrix and basement membrane (2). While the interstitial matrix is composed of collagens, fibronectin, and elastin that interconnect cells to the stroma, the basement membrane is a sheet-like dense structure that lines endothelial and epithelial cells and segregates tissues. The basement membrane consists mainly of collagen IV and laminins, which are connected through bridging proteins such as nidogen, perlecan, and heparan sulfate proteoglycans (Fig. 1; ref. 1).

The ECM undergoes remodeling in a dynamic manner, in which different ECM components are produced, reassembled, and modified by ECM-associated enzymes (3). In cancer, in response to tumor cell growth and invasion, the host tissue exhibits a fibrotic reaction leading to high ECM deposition via a process known as desmoplasia (4). The deposition and remodeling of interstitial ECM are primarily driven by cancer-associated fibroblasts (CAF; ref. 5), whereas basement membrane ECM is generated by epithelial and endothelial cells (4). These latter cell types also produce the lysyl oxidase (LOX) enzymes which induce collagen crosslinking, thereby supporting ECM stiffness (4). Furthermore, immune cells are known to contribute to ECM remodeling, usually in collaboration with CAFs. Specifically, myeloid cells such as macrophages and neutrophils secrete metalloproteinase family enzymes and neutrophil elastase (NE), promoting degradation of both the interstitial ECM and the basement membrane (6).

The massive remodeling of interstitial ECM generally supports tumor progression, in part by inducing the biochemical and biophysical changes affecting cell signaling, cell migration, tumor progression, and ECM stiffness (2, 3). Alterations in ECM composition can enhance the availability of growth factors, cytokines, and other signaling molecules bound to the ECM, usually supporting tumor progression (2, 3). Modification of the mechanical properties of the ECM, such as elasticity, stiffness, and biophysical properties, can alter the characteristics of cancer cells. For example, high stiffness can

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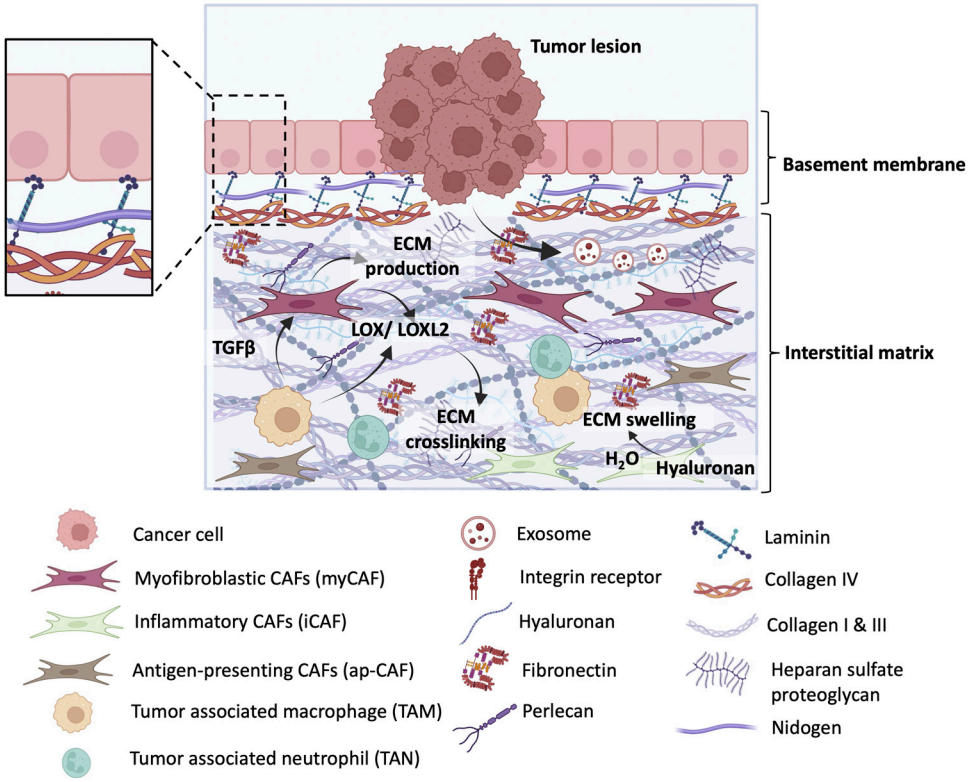

Figure 1. Tumor ECM remodeling. **A,** Tumor lesion showing basement membrane composed of laminin, collagen IV, and other components such as nidogen, while interstitial matrix contains collagen I/III and various proteoglycans. Infiltrating immune cells, such as TAMs and TANs, produce cytokines and growth factors that activate CAFs. In turn, CAFs produce the majority of the interstitial ECM. Collagens undergo crosslinking by LOX or LOXL2 enzymes, produced primarily by TAMs and CAFs, turning the ECM into a stiffened structure. ECM components, such as HA (hyaluronan), a polysaccharide that is produced by both tumor cells and stromal cells, absorb water causing the ECM to swell and enhance IFP. (Created with BioRender.com.)

facilitate the invasion and migration of cancer cells into the surrounding stromal tissue (2, 4). Conversely, ECM remodeling may restrict tumor cell dissemination both by creating physical barriers that prevent their escape as well as by disrupting signaling pathways that are crucial for tumor cell survival and growth (6).

In recent years, the interplay between ECM remodeling and cancer therapeutics has gained considerable attention. It is now evident that the ECM affects therapeutic outcomes of both chemotherapy and immunotherapy via different mechanisms such as hindering drug delivery to the tumor, restricting antitumor immune cell infiltration as well as conferring drug resistance to cancer cells. Furthermore, emerging studies have revealed that different cancer interventions including surgery, irradiation, and chemotherapy have an enormous impact on ECM remodeling. As anticancer therapeutics affect not only cancer cells but also the tumor stroma, including CAFs and immune cells (7), key questions arise as to how these therapies alter the ECM via their effect on stromal cells and how such changes affect tumor growth, relapse, and resistance.

In this review, we focus primarily on the reciprocal effects between the ECM and cancer therapy. To provide a comprehensive view, we discuss how cancer cells, CAFs, immune cells, and the reciprocities among these cells collectively regulate ECM remodeling. Furthermore, we shed light on how ECM composition affects response to cancer therapies, and conversely, how such therapies mechanistically remodel the ECM. Over the last years, major progress has been made with respect to therapeutic targeting of the ECM to improve efficacy of cancer treatments; unfortunately, such strategies are yet to be approved for clinical use. We herein discuss ECM-targeting strategies and highlight potential missing links limiting their progress to the clinic.

CELLULAR RECIPROCITY REGULATING ECM REMODELING

ECM remodeling results from the dynamic crosstalk among various cells within the tumor microenvironment (TME). Cancer cells recruit a large number of immune cells among other stromal cells to support their own survival, growth, and migration. Crosstalk between cancer cells, immune cells, and CAFs orchestrate the remodeling of the ECM during tumor development. The cell–cell reciprocity such as cancer cell–CAF, cancer cell–myeloid cell, CAF–immune cell reciprocities, and cell–ECM interactions create a feedforward loop to sustain the tumor ECM (Fig. 2).

Cancer-Associated Fibroblasts

Cancer cells secrete growth factors and cytokines that recruit and activate fibroblasts into CAFs. Recent studies have shown that CAFs can originate from resident cells or be recruited

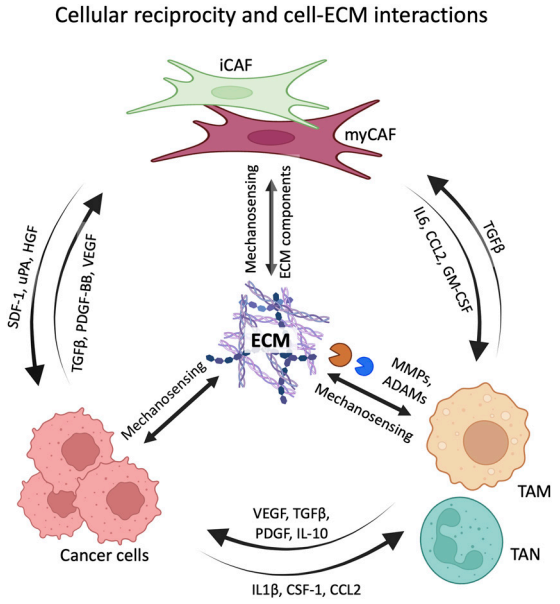

Figure 2. Cellular reciprocity and cell–ECM interactions. Cancer-associated myofibroblasts (mCAF), educated by cancer cells, produce abundant ECM components and stiffen it via their contractility. CAFs also secrete cytokines (IL6, IL8, GM-CSF) that activate TAMs and TANs into profibrotic/immunosuppressive M2 and N2 type, which in turn secrete TGFβ and activate and maintain myCAFs, creating a self-amplifying feedforward loop. ECM molecules undergo remodeling and degradation by enzymes such as MMPs, a disintegrin and metalloproteinases (ADAM), elastase, and cathepsins, largely secreted by TAMs and TANs. (Created with BioRender.com.)

from the bone marrow. CAFs appear in various forms, including inflammatory fibroblasts (iCAF), antigen-presenting fibroblasts, and myofibroblasts (myCAFs; refs. 5, 8). myCAFs are widely known to produce interstitial ECM (collagens, fibronectin, tenascin-C) in response to TGF-β and serve as a main source of ECM production (5). Cancer cells can also educate CAFs to secrete specific ECM components to support tumor invasion and migration. For example, a study has shown that pancreatic cancer cells with mutantp53 educate CAFs to secrete hyaluronic acid (HA) and perlecan, thereby supporting a prometastatic environment (9). In addition, CAFs remodel ECM and create tracks in the ECM executed via protease-induced ECM degradation or force-induced mechanisms via interaction with integrins. These effects support cancer cell invasion (10). The blockade of integrin α3, α5, or Rho-Rock function in CAFs reduces the formation of these tracks and hampers cancer cell invasion (10). Furthermore, different types of CAFs secrete matrix metalloproteinases (MMP) known to degrade ECM, thereby supporting metastasis. For example, CD26-positive iCAFs but not myCAFs exhibit induced expression of both MMP1 and MMP9 in triple-negative breast cancer, leading to cancer cell invasion (11). CAFs can also support cancer cells metabolically to stimulate their growth. For example, CAFs can metabolize collagen-rich ECM and secrete amino acids such as aspartate, which subsequently stimulates breast cancer cell proliferation (12). Furthermore, myCAFs have been shown to overexpress the enzyme PYCR1, which is responsible for the synthesis of proline, the main component of collagen. Accordingly, inhibition of PYCR1 reduces tumor growth and metastasis in various breast cancer mouse models (13). In addition, CAFs support cancer aggressiveness through ECM remodeling via different mechanisms. For example, they secrete lysyl hydroxydase 2, LOX, LOXL2, and LOXL4, which induce ECM crosslinking and stiffness, further supporting cancer metastasis (3). Overall, CAFs are a major producer of the ECM and remodel it, supporting tumor progression. Yet, there is a lack of studies on the role of different CAF subtypes in ECM remodeling that remains to be investigated.

Immune Cells

CAFs establish a reciprocal crosstalk with infiltrating immune cells such as tumor-associated macrophages (TAM) and tumor-associated neutrophils (TAN). Studies have shown that CAFs secrete cytokines such as SDF-1 to recruit monocytes into the TME as well as IL4, IL13, and CXCL14 which polarize TAMs and TANs into their profibrotic/immunosuppressive M2 and N2 phenotypes, respectively (14, 15). Likewise, TAMs and TANs can secrete profibrotic growth factors including TGF-β, which in turn stimulates CAFs to produce ECM components. Furthermore, TAMs can affect the organization and composition of the ECM by secreting proteases such as MMP2, MMP9, MMP11, and MMP14, which induce angiogenesis, cell migration, and cell invasion (16). In addition, TAMs instruct the deposition, crosslinking, and linearization of collagen fibers during tumor development, especially in regions of tumor invasiveness (16). M2-like TAMs have been shown to secrete LOX which further enhances stiffness. Neutrophils, similar to macrophages, also contribute to ECM remodeling by secreting neutrophil extracellular traps (NET), which contain proteases such as NE, MMP9, and cathepsin G, promoting tumorigenesis via ECM remodeling (17). Sustained inflammation can activate neutrophils to form NETs, which cleave laminin 111, further awaking dormant breast cancer cells by stimulating their proliferation via activation of α3β1 integrin signaling in preclinical models (18).

Emerging evidence shows that leukocytes may affect CAFs to support ECM remodeling. Specifically, it has been shown that activated $CD4^+$ T cells, but not $CD8^+$ T cells, can stimulate human lung fibroblast–mediated degradation of the ECM by MMP9, leading to pulmonary emphysema (19). Thus, adaptive immune cells may also have a direct role in the regulation of the ECM at metastatic sites. However, more evidence is needed to confirm the role of T lymphocytes in ECM remodeling.

Cancer Cells

Cancer cells play a crucial role in ECM remodeling (2). They recruit fibroblasts and immune cells into the tumor by secreting cytokines and chemokines including SDF-1 (2). Together with these cells, cancer cells produce ECM to support tumorigenesis. In metastatic melanoma, however, unlike in breast cancer (20), specific ECM molecules such as hyaluronan and proteoglycan protein link-1 were found to be deposited only by cancer cells (21). Likewise, studies showed that HA was produced by gastric and breast cancer cells and collagen-I was produced by non–small lung cancer cell and esophageal

squamous cancer cells (22–24). Moreover, cancer cells from pancreatic ductal adenocarcinoma (PDAC) were shown to produce different matrisomal proteins such as agrin, serine protease inhibitor B5, and Cystatin B, factors that have been associated with metastasis formation (25). Under hypoxic conditions, cancer cells produce LOX, which further supports leukocyte adhesion and invasion by crosslinking collagen IV (26). Moreover, breast cancer cells that have undergone epithelial-to-mesenchymal transition (EMT), attain a stromal cell phenotype and produce MMPs to degrade basement membrane facilitating the invasion of cancer cells (27). It is of interest, therefore, to further study the interactions between cancer cells and stromal cells within the TME, specifically in relation to ECM remodeling. For example, a recent study demonstrated that different mutations in tumor cells change the immune landscape (28), whether such changes affect the ECM remains to be investigated.

ECM REMODELING IN METASTASIS

A large body of evidence suggests that metastatic cancer cells maintain the ability to self-organize and recreate the histomorphology of the original tumor at the metastatic site (29). It has been proposed that this property, termed "tumor histostasis," is dependent on the concerted effects of 3D tissue architecture, cell–cell interactions, and cell–ECM interactions. Studies have shown that cancer cells at metastatic sites produce certain ECM components and that this process is further supported by stromal cells (2). The type of ECM produced by cancer cells and stromal cells likely depends on the metastatic status. For example, in poorly metastatic mammary tumors, cancer cells produce fibronectin and periostin (POSTN) whereas in highly metastatic tumors both cancer cells and stromal cells produce these ECM molecules (20). Similarly, POSTN is secreted by metastatic cancer cells in the lungs of breast cancer, while in primary breast cancer (and normal tissue) fibroblasts are the main source of POSTN (30). Furthermore, under hypoxic conditions, breast cancer cells secrete LOX. This supports the recruitment of $CD11b^+$ myeloid cells to the metastatic site, further facilitating invasion and migration of cancer cells (26).

Cancer cells create a premetastatic niche by secreting extracellular vesicles (EV) from the primary tumors, which can then induce ECM remodeling to facilitate metastatic growth. For example, EVs released by p53-mutant non–small cell lung cancer cells promote trafficking of α5β1 integrin in fibroblasts, influencing the organization and adhesive properties of ECM deposition, which in turn supports metastatic seeding of cancer cells in the lungs (31). Likewise, an increase in MMP activity (e.g., MMP2 and MMP9) in EVs secreted by prostate cancer cells under hypoxic conditions promotes ECM remodeling in pulmonary metastasis (32). Tumor-derived EVs may also contain MMP regulators such as CD147, a transmembrane protein that stimulates the expression of MMPs by CAFs (33). The role of EVs in ECM is an emerging avenue, especially with respect to metastasis. Further studies are required to better understand the composition and heterogeneity of tumor-derived EVs involved in ECM remodeling at the metastatic site (Fig. 3).

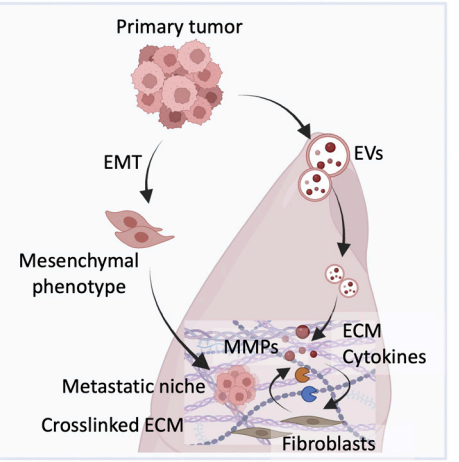

Figure 3. ECM remodeling in metastasis. Cancer cells release LOX that induces ECM crosslinking, while they can also produce ECM themselves. After undergoing EMT, cancer cells attain a mesenchymal phenotype capable of invading through the basement membrane by secreting MMPs and migrating to distant organs to form metastasis. Cancer cells at the primary tumor secrete exosomes, known as EVs, which are enriched with ECM molecules, MMPs, and cytokines responsible for ECM remodeling. EVs can travel to distant sites (lung, liver, bone), release their contents, and help create the premetastatic niche. (Created with BioRender.com.)

IMPACT OF ECM REMODELING ON CANCER PROGRESSION

ECM remodeling in tumor tissues can regulate cellular interactions by eliciting biochemical signals which can further support cancer progression, metastasis, and dormancy (34, 35). The physical modulation of the ECM results in enhanced tumor stiffness and intratumoral interstitial fluid pressure (IFP), which can limit tumor drug perfusion and intratumoral distribution, therefore affecting drug efficacy (Fig. 4; ref. 2).

Biochemical Signals from the ECM

The ECM elicits biochemical signals in three major ways: (i) direct signaling via intact binding to external receptors; (ii) signaling via ECM fragments; (iii) leveraging entrapped cytokines. Most ECM components primarily bind to integrin receptors and induce outside-in signaling, thus controlling cell adhesion, migration, and differentiation. Also, the ECM can induce intracellular signaling via other receptors such as collagen-binding discoidin domain receptors (DDR), laminin-binding dystroglycans, and HA-binding CD44 receptors. Notably, the signaling induced by these receptors may depend on the ECM type and architecture. For example, DDR1s can control head and neck squamous cancer cell dormancy at the metastatic niche by binding to "curly" collagen type III via the STAT1 pathway, while it awakens cancer cell proliferation by interacting with "straight" collagen type I via the STAT3 pathway (35).

Another way by which ECM elicits signals is via its bioactive fragments called matricryptins or matrikines (Fig. 4). These fragments are produced as a result of ECM proteolytic cleavage by proteases including MMPs, ADAMs, and cathepsins (36). Matricryptins and matrikines perform a vast range of functions such as tissue repair, angiogenesis, and inflammation,

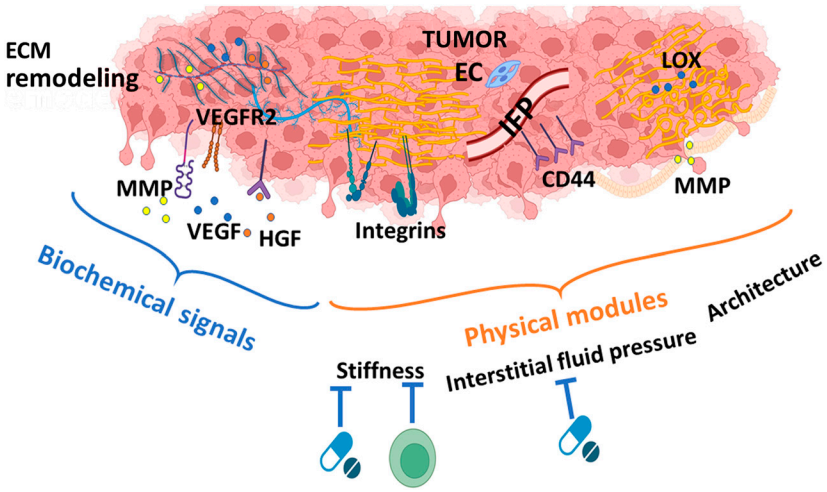

Figure 4. **ECM remodeling contributes to therapy resistance.** The illustration represents the mechanisms by which ECM remodeling affects therapy outcome. Biochemical signals are associated with stored factors within the ECM which can be released when the ECM undergoes remodeling. This includes ECM associated enzymes, e.g., MMPs, ADAMs and cathepsins which contribute to tumor invasion. In addition, growth factors such as VEGF, HGF, and matrikines contribute to cell signaling support tumor proliferation and growth. Physical modules also support resistance and aggressiveness through stiffness which supports cancer cell proliferation through the expression of integrins and focal adhesion molecules, as well as supporting cell invasion through mechanotranduction pathways. ECM stiffness leads to immunotherapy and chemotherapy resistance by restricting the perfusion of drugs and infiltration of immune cells to the tumor site; Interstitial fluid pressure (IFP) which compresses blood vessels and inhibits the ability of drugs to penetrate the tumor tissue. In addition, HA engages with CD44 to support cancer cell motility, invasion, and proliferation; and ECM architecture is altered via cross-linking enzymes, further supporting "wavy" collagen fibers. The wavy fibers support mechanical resistance contributing to cell-cell junction, tumor cell survival and growth. The less wavy collagen fibers contribute to cancer cell invasion, an effect associated with the secretion of ECM degrading enzymes. The figure was created with BioRender.com.

as summarized elsewhere (37). Matrikines act through various receptors such as integrins, growth factor receptors (e.g., VEGFR2, EGFR), a chemokine receptor (CXCR2), HSPG (glypican-1, -4, syndecans), and CD44 receptors. Furthermore, matrikines such as elastin-derived peptide (VGVAPG) enhance the activation of pro-MMP2 by occupying the elastin receptor, further promoting cancer cell invasion. Conversely, matrikines such as tetrastatin (derived from the NC1 domain of collagen IV) and endostatin (derived from collagen VXIII) have been shown to display antitumor and antiangiogenic functions (37).

The ECM also serves as a pool or sink for various growth factors and cytokines. In particular, proteoglycans have attached glycoaminoglycans (repeating polymers of disaccharides), such as heparan sulfates, that bind to various growth factors (e.g., FGFs, PDGF, HGF) due to their negatively charged carboxyl and sulfate groups (1). Enzymes that degrade glycoaminoglycans, such as heparanases and sulfatases, can modify the ECM proteoglycans, which have a major impact on the release of the attached growth factors, thus activating signaling pathways that support cancer cell proliferation, angiogenesis, and metastasis. Lastly, the ECM serves as a pool of numerous cytokines and growth factors. Unfolding of the ECM due to stretching or degradation can unleash entrapped soluble factors such as VEGF and latent TGF-β, which can facilitate angiogenesis and stimulate fibroblasts, respectively (38).

Biophysical Impact of the ECM

The ECM can attain different physical forms such as viscoelastic (a combination of fluid-like and solid-like) or stiffened forms. These physical forms affect cellular processes such as cancer cell proliferation and migration as well as serve as physical barriers for the infiltration of immune cells and drugs. Viscoelasticity is formed by abundant proteoglycans, enabling the ECM to respond to applied forces in a time-dependent manner and cause an impact on cell adhesion and migration (39). High ECM stiffness supports tumorigenesis, for example, by increasing the expression of oncogenes such as ZNF217 in breast cancer cells, and activating the AKT pathway inducing mammary cell proliferation (40). Likewise, stiffened ECM (12 kPa) compared to soft ECM (1 kPa) can induce hepatocellular carcinoma (HCC) proliferation via the PKB/AKT and STAT3 pathways (41). In pancreatic cancer, tissue transglutaminase induces collagen crosslinking and stiffness, which in turn conveys mechanical cues to cancer cells by activating the YAP/TAZ pathway, supporting their proliferation (42). Conversely, high matrix stiffness in the TME of breast carcinoma can also support cancer metastasis by inducing EMT and activating mechanotransduction pathways such as TWIST1-G3BP2 and EPHA2/LYN/TWIST1 (43, 44). Thus, ECM stiffness promotes a variety of tumor-supporting activities including cytoskeleton changes, increased metabolic pathways as well as cancer cell proliferation, invasiveness and aggressiveness (45).

The ECM acts as a physical barrier for the infiltration of $CD8^+$ T lymphocytes into the tumor by enforcing aligned collagen fiber formation via the interaction of extracellular domain of DDR1 receptor with collagen (46). Inhibition of this interaction with neutralizing antibodies has been shown to disrupt collagen fiber alignment and reverse immune exclusion, leading to reduced tumor growth in mammary mouse tumor models. Furthermore, activities of different immune cells within the

tumor microenvironment are affected by ECM stiffness. For example, antigen presentation as well as T-cell migration and infiltration to the tumor are reduced with stiff and remodeled ECM, while increased proliferation of T cells and antitumor immunity are associated with a less-stiff ECM (45).

Abnormal tumor growth causes hypoxia, angiogenesis, leaky blood vessels, and mechanical solid stress that collectively can cause blood vessel compression and impaired lymphatic drainage. This results in high IFPs, ranging from <1 kPa (7.5 mm Hg) in brain tumors to 5 kPa (37 mm Hg) in renal cell carcinomas (47). Deposition of ECM contributes to heightened intratumoral IFP. For example, HA may absorb and retain excess fluids and increase IFP (Fig. 4). Moreover, solid stress, due to ECM accumulation, can also lead to tumor vascular compression (48). Vascular compression reduces perfusion of drugs, affecting therapeutic efficacy. Degradation or reduction of ECM using various approaches reduces vascular compression and enhances tumor drug delivery (49). One may determine tumor perfusion using imaging techniques in patients in order to develop personalized approaches to improve therapeutic outcomes.

ECM REMODELING IN THERAPY RESISTANCE

ECM remodeling may promote resistance to different types of cancer therapeutics by various means. In general, the ECM serves as a physical barrier that inhibits the delivery of drugs to tumors. In addition, ECM interactions with different cell types within the TME may induce a range of mechanisms that ultimately contribute to therapy resistance. In this chapter, we discuss how ECM leads to resistance to specific types of cancer therapeutics.

Chemotherapy and Radiotherapy Resistance

The major mechanism of ECM-induced resistance is via the interaction with integrins overexpressed by cancer cells. ECM supports the survival and proliferation of cancer stem cells, a highly tumorigenic and resistant phenotype of cancer cells, by providing integrin-mediated autocrine and paracrine signaling. For example, stiffened ECM interacts with β1-integrin that activates the downstream ILK/PI3K/AKT pathway in cancer cells, thereby inducing stemness (50). A study demonstrated that upregulation of β1-integrin and its downstream FAK pathway support breast cancer cell proliferation and chemo- or radio-resistance (51). The inhibition of β1-integrin using a neutralizing antibody enhanced the efficacy of radiotherapy in mice bearing MCF-7 human breast cancer xenografts (51). Other integrins that support therapy resistance are αvβ3 and αvβ5, among others (50). The role of cell–ECM interaction via α2-integrin in radiotherapy resistance has been shown in glioblastoma. Interestingly, inhibition of α2-integrin reversed the resistance to both radiotherapy and chemotherapy (52). Unfortunately, to date, inhibitors of resistance-associated integrins have not yet been approved for clinical use, in part due to lack of clinical efficacy (53).

Immunotherapy Resistance

Recruitment of immune cells to tumors is a prerequisite for achieving therapeutic benefit from cancer immunotherapy. For example, the therapeutic activity of immune checkpoint inhibitors (ICI) depends heavily on the presence of T cells in the tumor core. In many fibrotic tumors such as pancreatic adenocarcinoma, dense ECM deposition and stiffness not only act as a barrier to T-cell infiltration but also support lower T-cell proliferation rate, downregulate cytotoxic factors, and upregulate regulatory T-cell markers (54). Likewise, metastatic breast cancers, that sometimes display highly desmoplastic morphology, are generally poorly perfused with restricted drug delivery. A study demonstrated that breast metastatic desmoplastic tumors are resistant to ICI therapy due to exclusion of $CD8^+$ T cells and immunosuppressive traits (55). Studies have suggested that these effects are mainly mediated by CXCL12/CXCR4-dependent immunosuppressive immune cells, suggesting that targeting CXCR4 along with ICI therapy may result in improved outcomes (56). In another preclinical study, researchers showed that the inhibition of ECM remodeling by targeting LOX reduced ECM content and led to enhanced migration of T cells into dense, solid epithelial tumor models including cholangiocarcinoma, breast carcinoma, and pancreatic adenocarcinoma. This approach enhanced efficacy of ICI therapy (54). Moreover, in mice with lung cancer, collagen was shown to promote resistance to ICIs, partly due to a decrease in total $CD8^+$ T cells and an increase in exhausted $CD8^+$ T-cell subpopulations (57). Specifically, the interaction of collagen with CD18 expressed by T cells resulted in overexpression of leukocyte-associated immunoglobulin-like receptor, which is known to suppress T-cell activity and induce T-cell exhaustion (58). These results suggest a link between ECM remodeling and tissue stiffness in response to immunotherapy. Collectively, such studies suggest that combining immunotherapy with agents that block ECM production represents a potential approach for improving immunotherapy outcomes.

ECM affects the phenotype of tumor myeloid cells in a manner that inhibits their antitumor immunity. For example, ECM affects TAM's function to promote both tumor growth and metastasis and suppress T-cell activity (59). High collagen density modulates macrophages to acquire an immunosuppressive phenotype in 3D cultures (60), suggesting a link between ECM density and poor prognosis. Specifically, a co-culture of T cells with macrophages in high-density collagen was less efficient in attracting cytotoxic T cells compared with cells co-cultured in low-density collagen (60). In addition, tenascin-C overexpressed in tumors supports immunosuppressive function by inhibiting the recruitment and/or activity of several immune cells including T lymphocytes, dendritic cells, and macrophages (61). Like immune cells, CAFs are also associated with impaired immunotherapy efficacy due to their interactions with immune cells and contribution to ECM stiffening (4, 62).

ECM-induced resistance is yet to be fully understood. There is a lack of systematic studies exploring interactions between different ECM components and their fragments with cancer cells and other cells in the TME in the context of resistance mechanisms. Furthermore, the effects of ECM-induced physical forces on therapy resistance remain unexplored. Understanding these aspects may lead to the development of better therapeutic options for cancer patients.

THERAPY-INDUCED ECM REMODELING

Therapy resistance is a consequence of intrinsic autonomous cancer cell mechanisms or extrinsic mechanisms mediated by the TME (63). Recent studies indicate that the host generates systemic protumorigenic effects in response to almost any type of anticancer therapy including chemotherapy, radiation, surgery, and targeted drugs, which in turn support tumor cell aggressiveness (7). These host protumorigenic and prometastatic effects counter the antitumor activity of the therapy and can therefore generate therapy resistance (7). As ECM contributes to resistance (outlined above), it is of interest to assess whether therapy influences the ECM, and whether such effects contribute to tumor progression and resistance. Here we cover some of the potential effects of therapy on tumoral or metastatic ECM specifically associated with cancer aggressiveness, as summarized in Fig. 5.

Surgery-Induced ECM Remodeling

Given that surgery induces inflammatory processes related to injury and wound healing, downstream effects on ECM remodeling are likely. For example, a side effect of laminectomy, i.e., the removal of small lamina bones to decrease spinal arthritis, is the development of epidural fibrosis. This effect is attributed to inflammatory process and activation of fibroblasts located near the surgical site (64). Indeed, use of an anti-inflammatory agent, resveratrol or quercetin, reduced fibrosis by inhibiting inflammatory processes and myofibroblast activity (64). It has been shown that surgery-induced ECM remodeling is associated with the activation of neutrophils that undergo NETosis, an inflammatory process associated with NETs. Some of the proteins associated with NETosis are proteolytic enzymes that alter the ECM, such as NE, MMP9, and cathepsin G (65).

The wound healing process that occurs after tumor resection involves the recruitment of immunosuppressive macrophages to the surgical site, which have been shown to activate endothelial cells, fibroblasts, and stem and progenitor cells, collectively contributing to tissue regeneration (66). These effects promote ECM restructuring via the induction of angiogenesis and inflammation that occur during the healing process (66). Furthermore, hypoxia induced at the surgical site due to vascular damage contributes to the upregulation of LOX, which in turn supports ECM remodeling at distant premetastatic sites e.g., the lungs (Fig. 5; refs. 26, 67). Specifically, overexpression of LOX at the surgical site in the mammary during breast carcinoma resection results in collagen crosslinking in the lungs, supporting a stiffer pulmonary tissue, which contributes to the seeding of circulating breast carcinoma cells to the lungs (67). Interestingly, LOX-induced changes in pulmonary ECM were not evident in other organs such as the spleen or liver, indicating a site-specific effect of LOX. It is plausible that the available oxygen in the organ may affect the activity of LOX. Nevertheless, this study showed that blocking LOX in mice subjected to surgical removal of breast carcinoma resulted in increased survival rates (67). The use of anti-inflammatory drugs and vasodilators pre-/post-surgery might be potential options to address key mechanisms i.e., inflammation and hypoxia, respectively, in order to overcome surgery-induced ECM remodeling in patients with cancer undergoing surgical resection.

Radiotherapy-Mediated ECM Remodeling

In response to radiotherapy, macrophages infiltrate into tumors and secrete TGFβ, which supports ECM remodeling and fibrosis leading to tumor growth (68). Along with macrophages, CAFs that support ECM remodeling overexpress focal adhesion contacts via integrins following exposure to radiation, increasing their activation and survival (69). These effects were also reported following radiation of brain tumors such as glioblastoma that secrete different ECM-associated factors including MMPs, heparanase, LOX, HA, as well as collagen I and fibronectin (69, 70). These molecules support changes in the ECM that further contribute to the invasiveness of cancer cells. Another study reported that upon radiotherapy of glioblastoma, the production of HA increased due to upregulation of the NFκB pathway with the mediation of IL1α. These effects resulted in glioblastoma cell aggressiveness, which manifested both by mechanically generating cell movement and through CD44 signaling, contributing to tumor relapse (71).

Radiation-induced tumor hypoxia can also support ECM remodeling by the production of reactive oxygen species (ROS) and the overexpression of HIF1α (26, 69, 70). The upregulation of HIF1α expression in activated CAFs supports the production of TGFβ, LOX, and EMT-associated proteins, all of which contribute to ECM remodeling and cancer aggressiveness (Fig. 5). Similarly, cancer cells produce proteolytic enzymes following exposure to radiation which support their aggressiveness and resistance to therapy (69). To address oxidative stress induced by radiotherapy, the use of antioxidants might be a potential counteractive way to reduce ECM remodeling.

Chemotherapy-Mediated ECM Remodeling

The damage caused by chemotherapy in tumor tissue induces a fibrotic response. Several reports have described how chemotherapy alters tumor ECM which was surprisingly found to support tumor growth and metastasis. For example, platinum chemotherapy has been shown to generate ECM modifications, fibrotic signaling, and immune cell activity (72). The activation of several kinds of immune cells by chemotherapy was shown to be mediated by immunogenic cell death, in which dying cancer cells release damage-associated molecular patterns as immunostimulating molecules. These effects, along with the induction of ROS and DNA damage induced by chemotherapy, contribute to ECM remodeling, in part by increasing the synthesis of ECM macromolecules by different cells (73). These effects suggest that the interplay between immune cells and cancer cells following exposure to chemotherapy leads to ECM restructuring and crosslinking (74). In addition, chemotherapy-induced ECM remodeling has been documented in other clinical indications such as pulmonary fibrosis. Specifically, lungs of mice exposed to bleomycin chemotherapy exhibited acute inflammation followed by ECM remodeling, leading to pulmonary fibrosis, further demonstrating that chemotherapy induces changes in the ECM that are mediated, in part, by immune cells (75). Another study exploring ECM remodeling at premetastatic sites demonstrated that following paclitaxel chemotherapy in mice bearing breast cancer, systemic $CD8^+$ T cells express

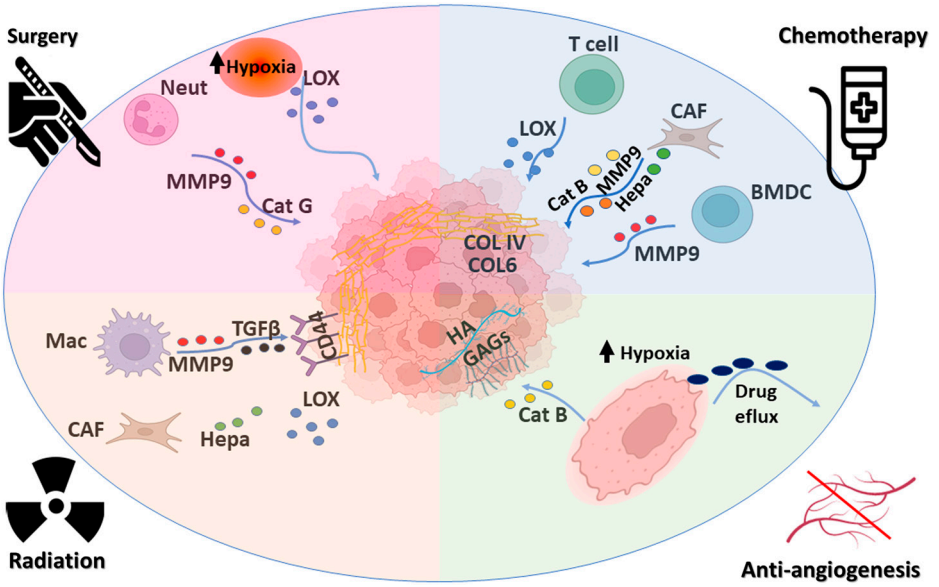

Figure 5. Mechanisms of therapy-induced ECM remodeling. The illustration represents the mechanisms by which therapy contributes directly to ECM remodeling. Surgery induces LOX expression at the surgical site, which contributes to ECM remodeling in the lungs. In addition, surgery activates neutrophils to secrete ECM-remodeling enzymes, e.g., MMP9. Radiation affects macrophages and CAFs, which infiltrate tumors and secrete ECM-remodeling enzymes including MMP9, heparanase, and cathepsins. In addition, it also contributes to the secretion of TGFβ by macrophages, which in turn affects fibrosis. Chemotherapy activates CAFs, which then support ECM remodeling. Furthermore, in response to chemotherapy, T cells secrete LOX, which then supports ECM remodeling at the premetastatic sites e.g., lungs. Chemotherapy can also increase ROS, which affects the activity of ECM-remodeling enzymes. Studies have demonstrated that bone marrow-derived cells infiltrate tumors in response to chemotherapy and secrete MMP9, which contributes to EMT and the degradation of the basement membrane, therefore supporting metastasis. Antiangiogenic therapy inhibits VEGF, which then contributes to changes in the expression of HA. These effects support ECM stiffness and contribute to metastatic cell seeding. In addition, it was demonstrated that hypoxia due to antiangiogenic therapy supports the secretion of cathepsins from the tumor tissue, leading to the activation of MMP9 and contributing to ECM degradation to support metastasis. (Created with BioRender.com.)

higher levels of LOX, which in turn reaches the lungs and contributes to ECM remodeling, therefore supporting cancer cell seeding at the metastatic sites (Fig. 5; ref. 76). Of note, similar ECM changes were not found in other organs, e.g., liver, for reasons that are not yet clear.

Chemotherapy also contributes to tumor ECM remodeling by directly altering CAF activity. For example, in a study of pancreatic cancer, areas in the TME enriched with spindle-shaped activated fibroblasts displayed immunosuppressive activity, leading to increased expression of ECM components, ECM signaling, and humoral immunity pathways. These unique areas, called the deserted subTME, increased in number following gemcitabine, nab-paclitaxel, and FOLFIRINOX chemotherapies (77). A preclinical study performed on triple negative breast cancer also found that ECM components were upregulated in response to paclitaxel and adriamycin chemotherapies (78). Specifically, collagen IV was highly enriched in treated tumors, resulting in increased tumor cell invasiveness through the Src and FAK signaling pathways. While the underlying mechanisms of the enrichment of collagen IV by these two chemotherapies remain unknown, researchers suggested that these chemotherapies stimulate the production of soluble signals that contribute to the communication between cancer and stromal cells, leading to increased production of ECM-associated enzymes that promote collagen IV trimerization (78). Similarly, in a study involving bulk RNA sequencing of high-grade serous ovarian carcinoma, platinum-based chemotherapy induced tumor stromal cells to upregulate COL6 and other matrisome-related genes, further promoting ECM remodeling especially at the metastatic sites (72). Contrary to the above studies, a single-cell atlas of colorectal cancer and its liver metastasis reveals that untreated tumors are rich in ECM-remodeling CAFs. Following chemotherapy, however, the number of these CAFs decreases while myofibroblasts and immunomodulatory CAFs increase (79). Thus, according to this study, the modulation of stromal cells and especially CAFs in tumors treated with chemotherapy can extend survival of cancer patients by inhibiting ECM-associated resistance.

Antiangiogenic Therapies and ECM Remodeling

There are some studies demonstrating a link between antiangiogenic therapy and ECM-related tumor progression. For example, patients with metastatic colorectal liver cancer who were treated with bevacizumab, an anti-VEGF neutralizing antibody, exhibited increased expression of HA and glycosaminoglycans at the metastatic sites, leading to increased tumor ECM stiffness (80). It was suggested that the induction of hypoxia due to antiangiogenic therapy contributed to these effects, thus supporting ECM remodeling. Indeed, targeting HA with hyaluronidase increased tumor perfusion and improved therapeutic efficacy due to reduced ECM stiffness (80). Another study found that long-term treatment of renal cell cancer with sunitinib, the antiangiogenic small-molecule receptor tyrosine kinase inhibitor, resulted in biosynthesis and release of lysosomes from the cancer cells (81). These

lysosomes contributed to the efflux of sunitinib from cancer cells, therefore increasing resistance to sunitinib. Furthermore, sunitinib-exposed cancer cells released cathepsin B, further activating MMP9 which degrades the matrix. While a direct link between sunitinib therapy and ECM-related resistance was not indicated, the release of cathepsin B and the degradation of the ECM supported cancer spread (Fig. 5; ref. 81).

Immune Checkpoint Inhibitors and ECM Remodeling

As both inflammation and CAFs have been shown to support ECM remodeling, a question that arises is, can targeted drugs that specifically alter the immune system contribute to ECM remodeling? Specifically, does ECM remodeling occur following ICI therapy, and if so, can it explain acquired resistance and short response duration reported in some ICI-treated patients? Indeed, a recent clinical study reported that among ~200 patients with non–small cell lung cancer treated with ICIs, 12% developed ICI-induced lung fibrosis, indicating that lung ECM undergoes massive remodeling (82). It would be of interest to further explore the effect of immunotherapy on the activity of immune cells in relation to ECM remodeling and cancer cell aggressiveness. While currently there is little direct evidence for this possibility, a chemotherapy study may provide some clues. Specifically, as discussed above, following chemotherapy, $CD8^+$ T cells express LOX which in turn supports pulmonary ECM remodeling (76). It would therefore be of interest to study whether LOX is upregulated in activated $CD8^+$ T cells in response to ICI-induced lung damage or other mechanisms, and if so, to explore its effect on ECM remodeling. Further studies in this direction will open a new area of research strengthening the link between cancer treatment and ECM remodeling.

ECM TARGETING APPROACHES AND MISSING LINKS

While several approaches targeting ECM components and remodeling have been proposed and validated using preclinical models, only a few have progressed to the clinical trial setting. In this section, we discuss approaches that have been tested in patients or validated in preclinical *in vivo* models. ECM-targeting strategies include enzymatic degradation of ECM components, inhibition of ECM production by CAFs, and reduction of ECM stiffness and density to improve therapeutic efficacy, as summarized in Table 1.

ECM Degradation using Enzymes

ECM-degrading enzymes such as MMPs and hyaluronidase (an HA-degrading enzyme) can remodel the ECM. HA plays a crucial role in enhancing solid stress, IFP, and tumor progressive mechanisms. In a human osteosarcoma xenograft mouse model, intratumoral injection of hyaluronidase reduced IFP by 50% and pretreatment with hyaluronidase enhanced tumor uptake of intravenously injected chemotherapy (Caelyx, liposomal doxorubicin), improving therapeutic outcome (83). A PEGylated version of this enzyme (PEGPH20) was developed to prolong circulation half-life, slow down elimination, and enhance tumor uptake. PEGPH20 was evaluated clinically in multiple clinical trials for safety and efficacy in combination with different chemotherapy regimens (84). Earlier trials showed a lack of therapeutic efficacy along with adverse events resulting in the termination of the trials. Later, patients with high HA and who were eligible for upfront thromboprophylaxis were selected for combination therapy. However, the trial still did not reach the clinical endpoint of overall survival or progression-free survival. Recently, PEGPH20 was tested in a clinical trial in combination with the ICI, pembrolizumab, the results of which reported improvement in overall survival but not progression-free survival (NCT02563548). These latter trial results strengthen the connection between ICIs and ECM remodeling.

Targeting CAFs to Inhibit ECM Remodeling

As CAFs are the main producers of the ECM, depletion of CAFs, inhibition of CAF activation signaling pathways, and reverting CAFs to normal fibroblasts serve as major ECM-modulating strategies. Genetic depletion of CAFs in a pancreatic cancer mouse model was shown to reduce collagen deposition and stiffness, while the depletion of CAFs augmented tumor growth and decreased mouse survival (85). These controversial results raised concerns about the role of CAFs. Recent studies have identified heterogeneity in CAFs, with different subtypes of CAFs that can act as tumor-promoting and tumor-restraining types (8). Thus, the modulation of CAFs, preferably of tumor-promoting subtypes, rather than depletion of all CAFs is a preferred strategy to selectively inhibit CAF-induced tumor progression. Over the years, studies have shown that the inhibition of signaling pathways, such as TGF-β, hedgehog pathway, PDGFβR, and CTGF/CCN2, inhibit CAF activation and ECM production (86). Antibody-mediated blocking of TGF-β, a key factor for myofibroblast differentiation, overcame the stroma barrier by reducing different ECM components. This enhanced the infiltration of $CD8^+$ T cells, thereby improving therapeutic efficacy of anti-PD-L1 ICI in an EMT6 mammary tumor model in mice (87). Furthermore, in colon and mammary carcinoma mouse models, blocking both TGF-β and PD-L1 using a bifunctional fusion protein called bintrafusp-alfa enhanced tumor infiltration and activation of $CD8^+$ T cells leading to longer survival rates and long-term protection (58). In locally advanced pancreatic cancer patients, anti-CTGF antibody (pamrevlumab) that targets CTGF, the matricellular signaling protein responsible for activation of fibroblasts and ECM production, also enhanced the surgical resection rate when administered in combination with neoadjuvant chemotherapy (88). Reversal of CAFs into normal fibroblasts is another strategy to reduce ECM production. Activation of vitamin D receptor by calcipotriol to reverse CAF activation and use of all-trans retinoic acid inhibited the activation of pancreatic stellate cells resulting in reduced ECM deposition, increased intratumoral gemcitabine levels and enhanced survival in pancreatic mouse tumor models (85). Similarly, all-trans retinoic acid in combination with gemcitabine enhanced tumor necrosis, increased vascularity, diminished hypoxia, and improved antitumor effects (89). These agents are currently being assessed in a phase II clinical trial (NCT03307148).

Table 1. Targeting ECM remodeling in clinical settings.

Mechanism of action	Target	Therapeutics	Combination standard of care therapy	Cancer type(s)	Phase	Status	Outcome	Trial no.
Prevent or inhibit ECM depositions	TGFβ	Galunisertib (LY2157299)	Capecitabine or Fluorouracil	Rectal cancer	II	Active	Improved the complete response rate to 32% and was well tolerated	NCT02688712
			Gemcitabine	Advanced PDAC	Ib/IIa	Completed	Improved overall 2-mo survival	NCT01373164
	Angiotensin II receptor	Losartan	FOLFIRINOX + chemoradiotherapy	Localized PDAC	II	Primary completed	High rate of R0 resection	NCT03563248
			FOLFIRINOX + 9-Ing-41 (glycogen synthase kinase-3β inhibitor)	Metastatic PDAC	II	Recruiting		NCT05077800
	Sonic Hedgehog	Vismodegib	Gemcitabine Hydrochloride	Advanced PDAC	II	Completed	Not superior to gemcitabine alone	NCT01195415
			No combination	Basal cell carcinoma	IV	Completed	Treatment preserved globe and visual function	NCT02436408
	CTGF/CCN2	Pamrevlumab (FG-3019)	Gemcitabine/nab-paclitaxel	Locally advanced unresectable PDAC	I/II	Completed	Enhanced resection rates	NCT02210559
	Vitamin D	All-trans retinoic acid	Nivolumab	Pancreatic cancer, advanced melanoma	I II	Not completed		NCT05482451 NCT02403778
Prevent ECM crosslinking	LOXL2	Simtuzumab (GS-6624)	Gemcitabine	Metastatic PDAC	II	Completed	No improvement	NCT01472198
	Pan-LOX	PXS-5505	Atezolizumab and Bevacizumab	Unresectable HCC	I/IIa	Recruiting		NCT05109052
Reduce ECM stiffness	Integrin α5β1	Volociximab	Gemcitabine	Metastatic PDAC	II	completed	No results posted	NCT00401570
	YAP/TAZ	IK-930	No combination	Advanced solid tumors	I	Recruiting		NCT05228015
	FAK	Defactinib (VS-6063)	Pembrolizumab + Neoadjuvant and Adjuvant chemotherapy	Resectable PDAC	II	Recruiting		NCT03727880
Degrade ECM	Hyaluronidase	PEGPH20	Gemcitabine	Stage IV previously untreated PDAC	1B/II	Completed	Multistage outcomes	NCT01453153
			Pembrolizumab	HA High Metastatic PDAC	II	Recruiting		NCT03634332

Inhibition of ECM Crosslinking

Numerous preclinical studies have demonstrated the therapeutic benefit of inhibiting LOX family proteins which represent the main collagen crosslinking enzymes. For example, inhibition of LOX has been shown to downregulate ITGA5/FN1 expression and inhibit FAK/Src signaling, thereby resensitizing chemotherapy in TNBC mouse models (90). Similarly, inhibition of LOXL2 has been shown to promote the efficacy of sorafenib and 5FU in chemoresistant liver cancer (91). Recently, inhibition of LOX enzymatic activity using beta-aminopropionitrile was shown to alter the mechanical properties of the ECM by reducing the tightly packed linearized collagen fibers responsible for stiffness. This treatment induced T-cell infiltration and improved response to anti-PD1 therapy in a mouse pancreatic KPC tumor model (54). Although preclinical therapeutic benefit of LOX inhibition has been widely demonstrated, the combination of simtuzumab (a LOXL2 inhibitor) and gemcitabine failed to improve clinical outcomes in patients with metastatic pancreatic adenocarcinoma (92). It is likely that the inhibition of LOXL2 was not sufficient to overcome the stroma barrier, probably due to other compensatory mechanisms.

One way of regulating ECM remodeling is to modulate the metabolic pathway for ECM synthesis. Indeed, using a small molecule glutamine analog (6-diazo-5-oxo-l-norleucine) to inhibit the hexosamine biosynthesis pathway, a shunt pathway of glycolysis responsible for promoting tumor cell survival and HA synthesis, resulted in decreased HA and collagen production and increased infiltration of $CD8^+$ T cells in an orthotopic pancreatic tumor mouse model (93). Reduced ECM content resulted in sensitization of tumors to anti-PD1 treatment and prolonged survival of mice (93).

Missing Links for ECM Modulating Therapeutics

Currently, there are no clinically approved ECM targeted therapies. However, the clinical studies so far have provided insights into the missing links that should be addressed when developing treatment approaches to target the ECM.

(i) Off-target effects on healthy ECM: Although tumor ECM is different from normal ECM, it is likely that ECM modulating therapies for cancer would also affect normal ECM in healthy organs. For example, the use of collagenase to degrade tumor ECM may cause collagen degradation elsewhere and potentially result in serious side effects. Therefore, this aspect should be carefully analyzed.

(ii) Compensatory mechanisms: Upregulation of compensatory pathways may explain the lack of efficacy of ECM-targeting drugs. Studies have shown that inhibition of FAK pathway resulted in resistance due to induction of STAT3 signaling (94). Proteomic and transcriptomic analyses may help identify compensatory mechanisms and therefore could be used to address this issue before progressing to clinical trials.

(iii) Tumor heterogeneity: Solid tumors exhibit high heterogeneity in ECM composition, density, and structure, both among different tumor types and also within the same tumor type. Patients with low expression levels of a target ECM component, receptor, or enzyme are likely to respond poorly to a specific targeted therapy, leading to treatment failure. Therefore, patient stratification approaches to confirm target abundance via biomarker testing (e.g., in plasma or biopsies) may help improve therapeutic outcome. Recent studies have revealed that proteomic and transcriptomic analyses of biopsies can indicate ECM changes in tumors (95). For example, PDAC patients with high HA in biopsies showed better outcomes with PEGPH20 treatment (96). An interesting approach to detect tumor ECM is to measure traces of ECM peptides and their biological pathways in peripheral blood; these measurements can help determine tumor fate. For example, plasma Pro-C3 has been recently shown to serve as a predictor of survival in patients with pancreatic cancer (97). Such biomarkers could be used to aid pre-treatment decisions as well as to monitor treatment effects on the ECM.

(iv) Optimization of treatment regimens: As ECM targeting approaches aim to enhance the therapeutic efficacy of chemotherapy or immunotherapy, it is crucial to optimize the sequence of drug administration. This could potentially lead to better therapeutic outcomes as well as limit the exposure to toxic chemotherapeutic agents. One limitation is that patients with aggressive tumors such as PDAC may risk tumor growth due to delayed treatment with chemotherapy.

(vi) Preclinical tumor models with human relevance: Most studies rely on mouse models such as syngeneic heterotypic models, genetically engineered spontaneous models, and human cell (patient-derived) xenografts. In these models, the ECM is derived from mice and does not necessarily represent human ECM composition and patient heterogeneity. Therefore, patient-relevant 3D *in vitro* models based on complex hetero-spheroids, cancer-on-chip models, 3D bioprinting techniques are required to recapitulate human tumor stroma interaction and human tumor ECM (98, 99). These advanced 3D models will enable a better evaluation of ECM modulating agents and increase the likelihood of translating the findings to clinical scenarios.

CONCLUSIONS AND PERSPECTIVES

While the ECM plays a crucial role in tissue homeostasis, in cancer, ECM remodeling affects tumor fate. Although most studies have focused on the protumorigenic role of the ECM and its contribution to metastasis, some studies have revealed that ECM remodeling can sometimes be antitumorigenic and can improve therapeutic outcome (54). In the context of immunotherapy, ECM remodeling and ECM stiffness support "cold" tumors and can, therefore, block the infiltration of antitumor immune cells into the TME (55). These effects may explain the *de novo* resistance of patients to immune checkpoint blockade. Using approaches that alter CAF or TAM activity, it is possible to change the architecture of the ECM and support the infiltration of T cells into tumors, thus enhancing cancer therapy (100). Furthermore, taking advantage of the tumor ECM, preclinical studies have demonstrated that conjugating immunotherapy drugs with agents that exhibit high-affinity binding to ECM components can reduce the cytotoxic activity of the drugs by enhancing their retention at the tumor site and reducing systemic adverse

events associated with the treatment. The conjugation of ICIs to the collagen-binding domain derived from the von Willebrand factor A3 domain represents one example of this approach (101).

The first ECM inhibitory drugs were clinically evaluated more than two decades ago and have been abandoned due to failure of advanced clinical studies. Specifically, the use of MMP inhibitors for the treatment of cancer was studied, but its evaluation in randomized clinical studies failed due in part to lack of efficacy (102). In these trials, MMP inhibitors were given in combination with standard of care, but at stages at which patients had already exhibited advanced metastatic disease. Based on this review, it might be useful to re-assess MMP inhibitors in combination with other treatment modalities such as immunotherapy, in which case the degradation of stiff ECM structures could potentially improve T-cell infiltration and overcome resistance, as outlined above.

Overall, this review summarizes some unique aspects of ECM remodeling with respect to tumor fate and treatment outcome. The growing number of studies exploring the ECM and its architecture in both normal and disease conditions reveals that this macroprotein architecture is critical in tissue growth and homeostasis. Studying the interplay between cancer therapy and the ECM can pave the way toward better treatments that take into consideration not only cancer cells and the tumor's cellular supporting stroma but also the critical role of the tumor scaffold.

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