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# The extracellular matrix as hallmark of cancer and metastasis: From biomechanics to therapeutic targets

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**The extracellular matrix (ECM) is essential for cell support during homeostasis and plays a critical role in cancer. Although research often concentrates on the tumor's cellular aspect, attention is growing for the importance of the cancer-associated ECM. Biochemical and physical ECM signals affect tumor formation, invasion, metastasis, and therapy resistance. Examining the tumor microenvironment uncovers intricate ECM dysregulation and interactions with cancer and stromal cells. Anticancer therapies targeting ECM sensors and remodelers, including integrins and matrix metalloproteinases, and ECM-remodeling cells, have seen limited success. This review explores the ECM's role in cancer and discusses potential therapeutic strategies for cell-ECM interactions.**

## INTRODUCTION

Historically, cancer research has mostly focused on the genetic changes driving malignant transformation in cancer cells. Besides the development of successful therapeutic approaches for many types of cancer, this has also resulted in the identification of the main hallmarks of cancer. The recently updated hallmarks (*1*) do not include the deregulated extracellular matrix (ECM) surrounding the tumor, although the tumor's cellular compartment comprises a large part of the tumor microenvironment (TME) (Fig. 1). The important role of the ECM is generally underappreciated, although the impact on cancer progression, predominantly through biochemical and structural modulation, is profound (Fig. 2). Overall, the ECM can be classified into two main components besides the glycocalyx: the basement membrane (BM) and the stromal ECM. The BM primarily contains laminins and collagen IV, separating endothelial and epithelial cell layers from the fibrous stromal ECM. The stromal ECM comprises (glyco)proteins such as collagens, elastin, proteoglycans, hyaluronic acid (HA), laminins, periostin, and fibronectin, but this varies across tissues. Collagens, including types I, II, III, IV, and V, are the most abundant matrix proteins, providing tissue strength and limiting deformation. Elastin forms elastic fibers for shape recovery. Proteoglycans and HA bind to collagens, contributing to hydration and tissue compressive loads. Fibronectin and periostin connect cells to ECM components, facilitating ECM remodeling and mechanical signaling.

The ECM instructs cells directly by activation of receptors such as integrins, syndecans, and discoidin domain receptors (DDRs) or indirectly by modulation of receptor binding site availability and spacing (*2*). Integrin activity is regulated through inside-out and outside-in mechanisms. Inside-out activation involves cell-generated signals recruiting adaptor proteins to integrin cytosolic tails (*3*). Outside-in activation occurs when integrins bind to ECM ligands, leading to clustering and intracellular signaling (*4*). The focal adhesion (FA) complex connects the ECM to the intracellular actin cytoskeleton. The integrin-ligand interaction and the elicited intracellular signaling depend not only on the biochemical properties of the ligand (ligand type and concentration) but also on the forces that are transferred between the cytoskeleton and the ECM. The mechanical resistance of the ligand when traction forces are generated by actomyosin contractions dictates the integrin-ECM binding kinetics, integrin clustering, and subsequent intracellular signaling (*4*).

Syndecans, comprising four subtypes (syndecan-1 to syndecan-4), are another mechanosensitive transmembrane receptor with ecto-, transmembrane, and cytoplasmic domains. The ectodomain binds ECM molecules through heparan sulfate chains in all subtypes, with chondroitin sulfate binding exclusive to syndecan-1 and syndecan-3. The transmembrane domain drives syndecan homodimerization, crucial for downstream signal activation (*5*). Mechanotransduction and cascade signal activation occur through various mechanisms. Vinculin recruitment on syndecan-4 cytoplasmic domain triggers downstream protein kinase C-α (PKC-α), focal adhesion kinase (FAK), and extracellular signal–regulated kinase (ERK) activation, promoting FA formation and cell spreading (*6*). Another mechanism involves syndecan-4 integration with epidermal growth factor receptor (EGFR) and β1 integrin, inducing cell stiffening through the kindlin-integrin-RhoA (Ras homolog family member A) pathway (*7*). Syndecans can also mediate inside-out integrin activation by integrating with insulin-like growth factor-1 receptor and αvβ3 (and/or αvβ5) integrin (*8*).

In addition to integrins and syndecans, members of the DDR family (DDR1 and DDR2) are transmembrane receptors that may also transduce biophysical signals particularly from collagen-rich ECM (*9*). DDR1 is known to be mechanosensitive, by clustering after collagen binding, activation of its intracellular kinase domain, and interaction with myosin IIA filaments, linking it to the cytoskeleton in a force-dependent manner (*9*). Until now, DDR2 has not been shown to directly interact with myosin or cytoskeletal linkers, precluding direct mechanotransduction. Yet, DDR2 can modulate

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integrin-mediated mechanotransduction in breast cancer fibroblasts (*10*). DDR expression is associated with cancer progression (lung, breast, brain, gastric, and prostate) (*11*), making them an appealing anticancer research target.

Cells can also sense ECM biophysical changes through calcium-gated ion channels, Piezo, and transient receptor potential (TRP) channels. These channels respond to mechanical cues such as increased ECM stiffness and shear stress (table S1). The mechanism behind mechanical cue regulation of Piezo and TRP gating is not fully understood but may involve changes in phospholipid surface tension and intracellular regulatory proteins such as cytoskeletal or FA proteins (*12*). Channel opening brings in calcium ions, regulating cytoskeletal rearrangement, FA assembly, and gene expression (*13*). The expression of Piezo (including Piezo 1) and TRP (including TRPV3 and TRPM4) correlates with poor clinical outcomes in colon, head, and neck cancer and glioma (*14*). However, the precise functional role of these channels in tumor progression is yet to be elucidated.

The cell-ECM relationship is reciprocal (*15*). Cells remodel ECM by degradation, new ECM component deposition, posttranslational modifications such as hydroxylation, glycosylation, and cross-linking, and force-based changes (*16*). ECM-degrading proteins include the matrix metalloproteinase families (MMPs 1 to 23) and a disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS) (*17*). Degradation decreases ECM integrity, making space for cells or new matrix. During and after biosynthesis, ECM properties can be modified by posttranslational modifications, including the hydroxylation of collagen I, catalyzed by lysyl or prolyl hydroxylases, and glycosylation, catalyzed by different transferases. Additionally, cells can change mechanical and structural properties of the matrix by covalent cross-linking (*16*). Besides chemical remodeling, cells can modify the ECM by applying physical forces (pulling, pushing) onto the ECM, resulting in changes in ECM orientation, conformation, and access to binding or protease-cleavage sites (*16*). This reciprocal cell-ECM interaction maintains tissue homeostasis, modulating cellular states and ECM properties.

Most, if not all, of the hallmarks of cancer are controlled by the biochemical and physical ECM characteristics. Compositional ECM changes and increasing mechanical stiffness, which are present in most solid tumors, are known to induce cancer cell proliferation, resistance to cell death, and cell invasion. Through these changes, the ECM controls many facets of tumor progression, including tumor initiation, invasion into the surrounding tissue, secondary tumor formation at distant sites through metastatic colonization, and resistance to chemotherapeutic agents (*18*). Immune-targeting treatments are a great example of how increased knowledge of signaling in the TME has led to the development of successful clinical therapies (*19*). However, ECM-targeted therapies have so far seen limited success in clinical trials, despite promising results in preclinical studies (*20*). Thus, there is a need for a better understanding of the dynamic reciprocal nature of cancer cell–ECM and stromal cell–ECM interactions, including spatial and temporal profiles of the tumor ECM.

In this Review, we highlight the important role of the ECM in cancer, emphasizing the impact of both biochemical and physical ECM signals on tumor development, invasion, metastasis, and resistance to therapy. We also discuss the challenges and limited success of anticancer therapies targeting ECM-related factors and offer insights into potential therapeutic strategies focused on cell-ECM interactions.

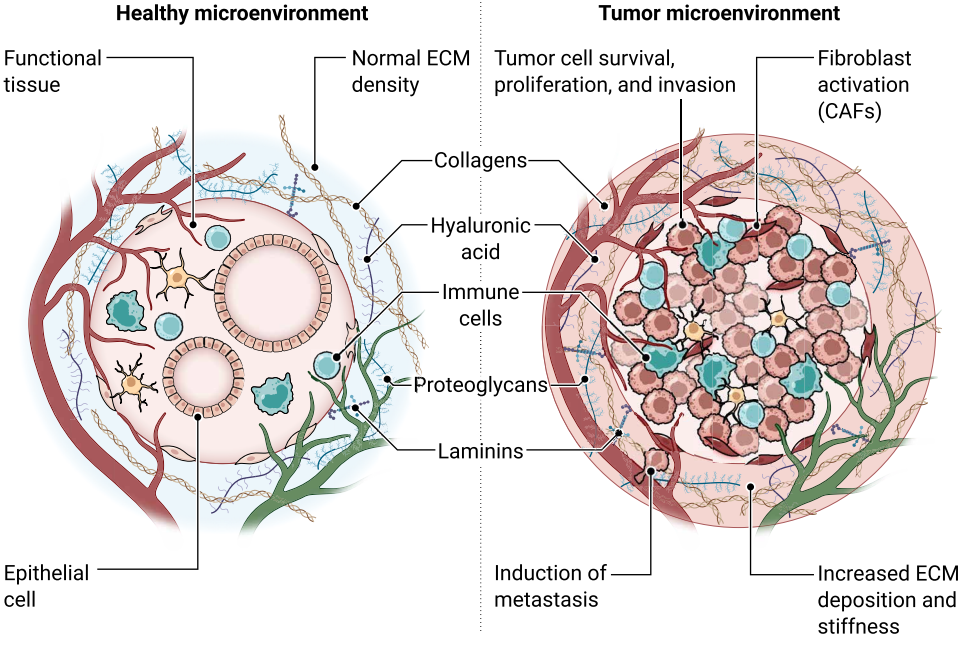


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**Fig. 1. The tumor microenvironment at a glance.** A healthy microenvironment provides a suitable niche for epithelial cell (yellow) function through interactions with neighboring cells, including immune cells, resident fibroblasts, and a homeostatic ECM composition. Oxygen delivery and nutrient transport is performed by the vascular (red) and lymphatic (green) systems (left panel). However, the healthy microenvironment can undergo drastic compositional changes to become a TME (right panel). These changes include, but are not limited to, transformation of fibroblasts into CAFs; induction of a high-stiffness fibrotic environment through ECM remodeling and increased deposition of collagen, laminin, HA, and proteoglycans; and initiation of an immunosuppressive environment. These ECM changes promote growth, survival, and invasion of tumor cells.

## LOSS OF MECHANICAL HOMEOSTASIS IN CANCER

Loss of biochemical and mechanical homeostasis is a hallmark of the tumor-associated ECM (Fig. 1). Overexpression of matrix components, ECM-degrading proteins, and cross-linkers and activation of mechanosensitive mechanisms lead to loss of ECM homeostasis favoring local and systemic cancer spread (*21*). ECM topology and biomechanics, such as stiffening through desmoplasia or fibrosis (Fig. 2), are changed in tumors (Table 1), promoting aggressive cell behavior (table S1). The difference in stiffness is highly pronounced in gliomas, which are around 10 times stiffer than nonmalignant gliosis, recurrent bladder tumors, which are four times stiffer than healthy bladder tissue, and breast tumors, which are around 20 times stiffer than healthy breast tissue (*22*, *23*).

There is growing evidence that mechanical signals from the stiff ECM are directly involved in tumor initiation. Malignant breast cancer cells revert to a

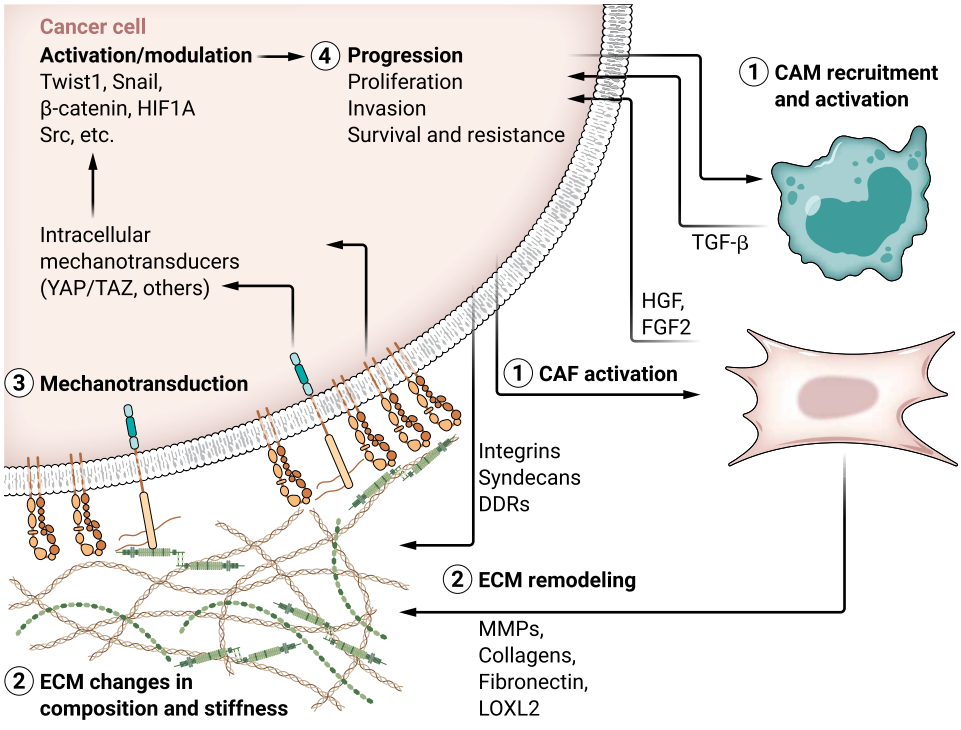


**Fig. 2. Reciprocal cell-ECM interactions in the TME.** Cancer cells recruit and activate CAFs, CAMs, and other stromal and immune cells (*1*). These cells stimulate cancer progression through paracrine factors such as fibroblast growth factor 2 (FGF2), hepatocyte growth factor (HGF), and transforming growth factor–β (TGF-β). In parallel, these cancer-associated cells induce an increase in ECM stiffness and changes in ECM composition by deposition of matrix components, including collagens and fibronectin, and secretion of cross-linkers such as lysyl oxidase homolog 2 (LOXL2) (*2*). The ECM compositional and mechanical changes lead to increased mechanical signaling (*3*), which can regulate downstream factors in the cancer cells including Twist-related protein 1 (Twist1), zinc finger protein SNAI1 (Snail), β-catenin, hypoxia-inducible factor 1α (HIF1α), and proto-oncogene tyrosine protein kinase Src (Src). This contributes to cancer progression through increased cell proliferation, invasion, survival, and therapeutic resistance (*4*). The growing and invading tumor leads to additional ECM remodeling and recruitment of CAFs, CAMs, and others, completing the signaling loop.

nonmalignant state in a three-dimensional (3D) hydrogel matrix when β1-integrin activity is inhibited. The involvement of the mechanosensitive receptor integrin indicates a role for mechanical matrix signaling in tumorigenesis (*24*). In the context of liver cancer, hepatocellular carcinoma (HCC) proliferation and activation of tumor-promoting hepatic stellate cells have been linked to increased ECM stiffness, implying that ECM mechanics is involved in liver tumorigenesis as well (*25*). More recently, ECM involvement in tumor progression was found in gastric cancer, where ECM stiffness was shown to reversibly regulate DNA methylation of the promotor region of Yes-associated protein (YAP), leading to stiffness-induced oncogenic activation (*26*).

In general, stiffening is associated with matrix cross-linking and compositional changes in the ECM by increased deposition of matrix proteins such as fibronectin and collagen I, as well as a decrease in proteoglycans such as decorin (*27*). Cross-linking enzymes [lysyl oxidase (LOX), lysyl hydroxylase (LOXL), and advanced glycation end product (AGE)], although providing potentially interesting targets, are not yet subjects of many (pre)clinical studies (Table 2). An in vitro model using a breast cancer cell line (MDA-MB-231) embedded in collagen I showed that the AGE-breaking drug (alagebrium chloride, ALT711) successfully disrupted the glucose-ECM cross-link and subsequently decreased ECM stiffness (*28*). In addition to cross-linking, the local ECM microstructure is affected in multiple ways, including by ECM pulling, increased fibrosis, and the formation of aligned microtracks along which tumor cells can efficiently migrate (*29*). These changes are induced by both the cancer cells and stromal cells such as cancer-associated fibroblasts (CAFs) and cancer-associated macrophages (CAMs) (Fig. 2) (*21*). CAFs are major producers of matricellular proteins and are thus heavily involved in the mechanical imbalance in solid tumors. Similar to cancer cells, CAFs are also reciprocally activated by tumor cells and ECM.

Increased TME stiffness activates many different transcription regulators through the mechanosensory receptors integrins, syndecans, and DDRs. This includes, but is not exclusive to, YAP and transcriptional coactivator with PDZ-binding motif (TAZ), catenin β-1 (β-catenin), Twist-related protein 1 (Twist1), zinc finger protein SNAI1 (Snail), and hypoxia-inducible factor 1α (HIF1α) (Fig. 2) (*21*, *30*). For most of these regulators, the underlying mechanisms for mechanosensitivity are not yet fully understood. HIF1α expression, for example, can be promoted by a stiffened matrix through

**Table 1. Stiffness in healthy and tumor tissue based on magnetic resonance elastography.**

| Tissue | Stiffness (kPa) | | References |
|---|---|---|---|
| | Healthy tissue | Tumor | |
| Breast | 1.5–7.5 | 15.5–33 | (*121*, *122*) |
| Liver | 2–5 | 6.5–10.5 | (*123*, *124*) |
| Pancreas | 2 | 6 | (*125*) |
| Prostate | 2–5.5 | 6.5–11 | (*126*, *127*) |

miR-203 repression in glioblastoma (*22*). In breast cancer, tamoxifen could reduce HIF1α expression by suppressing myosin-dependent contractility and matrix stiffness mechanosensing (*31*). The most studied mechanosensitive transcription coactivator YAP/TAZ is up-regulated in a large number of cancer types, including cervical, breast, pancreatic, colorectal, lung, liver, ovarian, and prostate cancer (*21*). Additionally, YAP signaling plays a key role in CAF differentiation and activation, triggered by increased matrix stiffness (*32*). For years, YAP activation was thought to be mainly regulated by large tumor suppressor proteins through the Hippo signaling pathway, which can suppress YAP translocation to the nucleus by YAP phosphorylation in the cytoplasm. However, a more direct and mechanosensitive activation mechanism has been proposed. In this mechanism, YAP translocation to the nucleus is directly driven by the actin cytoskeleton and mechanical modulation of nuclear pores, which seems to be a key regulatory mechanism in tumor signaling (*33*). Downstream effects of YAP/TAZ activation include increased proliferation, chemoresistance, and migration by triggering of epithelial to mesenchymal transition (EMT). Through EMT, cells of epithelial origin lose their apicobasal polarity and cell-cell adhesions and reorganize their cytoskeleton, eventually obtaining a mesenchymal phenotype enabling migration away from the tumor (*34*). As such, mechanically induced YAP/TAZ activation is highly relevant for cancer progression. Despite these findings, the role of ECM in tumor initiation is not yet fully understood.

## INVASION OF TUMOR CELLS INTO DEREGULATED ECM

Cancer cell invasion into the peritumor tissue is a basic step in the metastatic cascade. In many epithelial tumors, cell invasion starts after the breaching of the BM, which forms a barrier between the tumor and the adjacent stroma/parenchyma. After BM breaching, cancer cells can invade either as individual cells (single-cell invasion) or as multicellular groups (collective invasion). Collectively invading cells maintain cell-cell junctions, whereas individually invading cells fully detach from the rest of the tumor (*35*). Collective invasion is the typical mode of invasion for most epithelial tumors including breast, pancreatic, colorectal, and lung cancer (*36*). It may be initiated and guided by a subset of cells, originating either from the tumor or from the TME. These cells acquire protrusive/motile characteristic and function as leader cells (*37*).

In both single and collective invasion, the transition into an invasive cell is largely dependent on the extracellular signals within the TME. It is now well accepted that the ECM biomechanical properties have a large impact in determining invasive cellular characteristics (*38*). In patients with epithelial tumors, biochemical and physical changes in the ECM, such as increase in ECM density and mechanical stiffness, correlate with the invasive characteristics of the tumor and predict poor clinical outcome. On the basis of in vitro and in vivo studies, the changes in ECM biomechanics, such as an increase in collagen I contact or mechanical stiffness, are mechanotransduced by many epithelial cancer cells and drive key invasive properties, including polarized cytoskeletal organization and ECM remodeling (*39*).

Changes in ECM biomechanics can directly activate cytoskeletal reorganization and formation of actin-rich protrusions that are important for cell invasion. For instance, interactions with fibrillar collagen I and with stiff ECM induce the clustering of integrins and DDRs that increase FA assembly and signaling of small Ras homologous (Rho) guanosine triphosphatases (GTPases) (*9*). Active Rho-GTPases promote actin polymerization and polarized cytoskeletal protrusions (lamellipodia and filopodia). Similarly, fibronectin-rich ECM may induce protrusive activity such as through syndecan-mediated Rac 1 activation. In addition to lamellipodia, the small GTPases promote the formation of invadosomes, which are proteolytically active protrusions that focally degrade the ECM (*40*).

Rho GTPase activated by FAs also mediates actomyosin contraction in epithelial cells and CAFs (*41*). Actomyosin contraction generates pulling forces, resulting in a net translocation of cells toward the ECM. In collectively invading cells, the traction forces may be transmitted to the follower cells through cell-cell junction (*35*). The traction forces, in turn, result in the alignment of collagen I fibers to orientations that are parallel to the direction of cell invasion (*42*). The aligned fibers act as physical guidance cues that direct cytoskeletal protrusions and, as a consequence, enhance the persistence of cell invasion (*43*). The mechanisms that underlie collective invasion and leader cell behavior are more complex than described here and have been studied extensively (*37*). In the context of ECM-targeted treatments, it is most important to acknowledge that the mechanosensitive receptors (integrins, syndecans, DDRs) play a major role in orchestrating cancer cell invasion and are thus potential targets for inhibiting cancer invasion.

Besides posttranslational modifications, mechanotransduction may induce transcriptional changes such as the induction of the EMT and basal epithelial gene program, both of which have been implicated to be downstream of ECM biomechanics. The exact molecular mechanisms underlying the transcriptional regulation remain not fully understood but depend in many cases on the nuclear translocation of transcriptional regulators such as YAP/TAZ (*44*) and Twist (*45*). The regulation of gene expression by ECM mechanics may also involve epigenetic regulation and mechanical memory. This mechanism, in which cells maintain altered behavior induced by mechanical signals, even if the stimulus is removed, was first described in lung fibroblasts and mesenchymal stem cells (*46*). In these studies, expression of mechanosensitive genes was shown to be permanently affected by exposure to substrates of different stiffness. Both YAP/TAZ and Twist have been strongly implicated in driving EMT, which results in the reduction of cell-cell junctions and increase in cytoskeletal rearrangements, ECM interactions, and remodeling. Whereas EMT is a major driver of single-cell invasion, its role in collective invasion is not fully understood. Leader cells (cancer or stromal cells) maintain cell-cell junctions with the follower cells but may exhibit some EMT traits, such as the expression of vimentin, β1 integrin, and MMPs (*47*). MMPs mediate the proteolytic degradation of collagens and other ECM proteins and facilitate invasion not only into the peritumor stroma but also into the BM (*48*). In addition to ECM degradation, ECM cross-linking genes are up-regulated in response to deregulated ECM (*49*).

The biomechanical cues provided by the TME coexist with other paracrine factors that are present in or near the tumor. Exposure to transforming growth factor–β (TGF-β), for instance, increases MMP expression (particularly MT1-MMP or MMP-14, MMP-2, and MMP-9), which facilitates tumor cell invasion through proteolytic matrix degradation (*50*). Furthermore, in HCC, CAFs originating from hepatic stellate cells are activated by stiffened ECM and promote invasion by secreting fibroblast growth factor 2 (*51*). CAFs may also indirectly inhibit tumor growth, for example, by activation

**Table 2. Overview of the therapeutic targets of ECM for cancer treatment.** RR, response rate [less clearly defined for myelofibrosis than for solid tumors (ORR); typically, RR is based on a combination of bone marrow fibrosis score, hematological changes, and spleen size]; ORR, objective response rate, usually based on Response Evaluation Criteria in Solid Tumors (RECIST); DFS, disease-free survival.

| Target | Drug | Drug type/mode of action | Indications | Phase | Key patient outcomes | Ongoing/ failed | Reason of failure | NCT | Ref. |
|---|---|---|---|---|---|---|---|---|---|
| HA | PEGPH20 | PEGylated enzyme, directly degrading HA | PDAC HA-high | 2 | PFS, OS | Failed | No improved patient outcome/ suspended* | 03634332 | (*128*) |
| | | | | 3 | PFS, OS, ORR | Failed | No improved patient outcome/ suspended | 02715804 | (*129*) |
| | | | | 1/2 | PFS, OS | Failed | Increased toxicity | 01959139 | (*79*) |
| LOX | Copper depletion† | Reduction of LOX activity, which is copper dependent | Multiple solid tumors | – | | Ongoing | Often: adverse effects | 00176774 | (*130*) |
| | Simtuzumab | Blocking antibody against LOXL2 | PDAC | 2 | PFS, OS, ORR | Failed | No improved patient outcome | 01472198 | (*81*) |
| | | | Myelofibrosis | 2 | RR | Failed | No improved patient outcome | 01369498 | (*131*) |
| | | | Colorectal cancer | 2 | PFS, OS, ORR | Failed | No improved patient outcome | 01479465 | (*82*) |
| | PXS-5505 | Small-molecule pan-lysyl oxidase inhibitor | Myelofibrosis | 1/2 | RR | Ongoing | – | 04676529 | (*83*) |
| | | | HCC | 1/2 | PFS, OS | Ongoing | – | 05109052 | |
| MMPs | Prinomastat | Synthetic inhibitor of MMPs 2, 9, 13, and 14 | Prostate cancer | 3 | Not reported | Failed | | 00003343 | |
| | | | NSCLC | 3 | PFS, OS | Failed | No improved patient outcome | 00004199 | (*132*) |
| | | | Glioblastoma multiforme | 2 | Not reported | Failed | | 00004200 | |
| | | | esophageal adenocarcinoma | 2 | Not reported | Terminated | Unexpected thromboembolic events | unknown | (*98*) |
| | Andecaliximab | Monoclonal antibody inhibiting MMP9 | Gastric cancer | 2 | PFS, OS, ORR | Failed | No improved patient outcome | 02864381 | (*100*) |
| | | | Gastric or gastroesophageal junction adenocarcinoma | 3 | PFS, OS, ORR | Failed | No improved patient outcome | 02545504 | (*133*) |
| | BT1718 | Bicyclic peptide–toxin coupled drug, targeting MT1-MMP, releasing toxin DM1 | Multiple solid tumors | 1/2 | RECIST, estimated PFS and OS | Ongoing | – | 03486730 | (*134*) |

(Continued)

(Continued)

| Target | Drug | Drug type/mode of action | Indications | Phase | Key patient outcomes | Ongoing/ failed | Reason of failure | NCT | Ref. |
|---|---|---|---|---|---|---|---|---|---|
| ADAM | Aderbasib | Inhibitor of ADAM10 & ADAM17 by binding the active site of MMP domain | Breast cancer | 2 | Safety and tolerability, severity of adverse events | Terminated | Suspended by sponsor | 01254136 | |
| | | | Breast cancer | 1/2 | ORR | Terminated | Suspended by sponsor | 00864175 | |
| | | | High-grade gliomas in children | 1 | Estimated PFS & OS | Ongoing | | 04295759 | |
| Integrin blocking (type) | Abituzumab (av) | Monoclonal antibody against CD51 (integrin α-V), inhibiting its activity | Prostate cancer | 2 | Safety and tolerability, severity of adverse events | Failed | No improved patient outcome | 01360840 | (*103*) |
| | | | Colorectal cancer | 1/2 | PFS, OS | Failed | No improved patient outcome | 01008475 | (*135*) |
| | | | Colorectal cancer | 2 | PFS, OS, ORR | Terminated | Withdrawn for co-development decision | 03688230 | |
| | Volociximab (αvβ1) | Recombinant monoclonal antibody to αvβ1 integrin, inhibiting its activity | Pancreatic cancer | 2 | Confirmed tumor response, PFS, OS | Failed | No improved patient outcome | 00401570 | (*136*) |
| | | | Renal cell carcinoma | 2 | Confirmed tumor response | Terminated | Unknown | 00100685 | (*137*) |
| | | | Ovarian or peritoneal cancer | 2 | ORR | Terminated | Lack of efficacy | 00516841 | (*105*) |
| | | | Metastatic melanoma | 2 | PFS | Terminated | Insufficient clinical activity | 00369395 | (*138*) |
| | Etaracizumab (αvβ3) | Monoclonal antibody to αvβ3 integrin, inhibiting its activity | Metastatic melanoma | 2 | PFS | Failed | Insufficient clinical activity | 00066196 | (*106*) |
| Integrin targeting (type) | CEND-1 (αvβ3, αvβ5) | Enhances drug penetration by dual αv-integrin and neuropilin-1 targeting | PDAC | 1 | Safety, tolerability, preliminary PFS, OS, ORR | Ongoing | – | 05052567 | |
| | ProAgio (αvβ3) | Cytotoxin selectively binding to αvβ3 Integrin | Pancreatic cancer, solid tumors | 1 | Safety, tolerability, preliminary ORR | Ongoing | – | 05085548 | |
| | 7HP349 (α4β1, αLβ2) | Allosteric integrin activator, inducing immune response | Solid tumors | 1 | Safety and tolerability | Ongoing | – | 04508179 | |

(Continued)

| Target | Drug | Drug type/mode of action | Indications | Phase | Key patient outcomes | Ongoing/ failed | Reason of failure | NCT | Ref. |
|---|---|---|---|---|---|---|---|---|---|
| CAFs | Sibrotuzumab | Blocking antibody against FAP | Colorectal cancer | 2 | PFS | Failed | No improved patient outcome | 02198274 | (*114*) |
| | RO6874281 | Blocking antibody that blocks both FAP and IL-2 receptor variant | Multiple solid tumors | 2 | PFS | Ongoing | – | 02627274 | (*113*) |
| | | | Metastatic Melanoma | 1 | PFS, ORR | Ongoing | – | 03875079 | (*139*) |
| | All-trans retinoic acid (ATRA) | Vitamin A metabolite to restore retinoic acid storage in CAFs | PDAC | 2 | PFS, OS | Ongoing | – | 03307148 | (*120*) |
| | Paricalcitol | Synthetic form of vitamin D that binds to vitamin D receptor, which is up-regulated in CAFs | PDAC | 1/2 | PFS, OS, ORR | Ongoing | – | 04524702 | |
| Hedgehog pathway | IPI-926 | Antagonist of SMO receptor that inhibits Hedgehog pathway | PDAC | 2 | PFS, OS | Failed | Disease acceleration | 01130142 | |
| | Vismodegib | Antagonist of SMO receptor that inhibits Hedgehog pathway | Pancreatic cancer | 1/2 | PFS, OS, ORR | Failed | No improved patient outcome | 01064622 | (*140*) |
| | | | Pancreatic cancer | 2 | ORR, PFS | Failed | No improved patient outcome | 01195415 | (*141*) |
| | | | PDAC | 2 | PFS | Failed | No improved patient outcome | 01088815 | (*142*) |
| | | | Solid tumors, lymphomas, multiple myeloma | 2 | PFS, OS, ORR | Ongoing | – | 02465060 | |
| Fibrosis | Losartan | Antagonist of angiotensin II receptor inhibits collagen I production | PDAC | 2 | PFS, OS | Failed | No improved patient outcome | 03563248 | (*143*) |
| | | | Osteosarcoma | 1 | Safety and tolerability, preliminary PFS | Ongoing | – | 03900793 | |
| | Metformin | Activator of AMPK. Inhibits fibrosis through not fully understood mechanisms | Breast cancer | 3 | Local/regional invasion, PFS, OS | Failed | No improved patient outcome | 01101438 | (*94*) |
| | Pirfenidone | Small-molecule inhibitor of the production of growth factors and procollagens I and II | Non–small cell lung cancer | 1/2 | PFS, OS | Ongoing | – | 04467723 | |

(Continued)

(Continued)

| Target | Drug | Drug type/mode of action | Indications | Phase | Key patient outcomes | Ongoing/ failed | Reason of failure | NCT | Ref. |
|---|---|---|---|---|---|---|---|---|---|
| FAK | GSK2256098 | Highly selective ATP-competitive FAK inhibitor | Meningioma | 2 | PFS, OS | Ongoing | – | 02523014 | (*86*) |
| | | | PDAC | 2 | PFS, OS | Failed | No improved patient outcome | 02428270 | (*85*) |
| | CT-707 | Multikinase (FAK/ALK/ Pyk2) inhibitor | PDAC | 1/2 | PFS, OS, ORR | Ongoing | – | 05512208 | |
| | Defactinib | FAK inhibitor through phosphorylation at $Tyr^{397}$ | Malignant pleural meso-thelioma | 2 | PFS, OS, ORR | Failed | No improved patient outcome | 01870609 | (*89*) |
| | | | Cancers with NF2 genetic changes | 2 | PFS, OS, ORR | Modest effect | – | 04439331 | (*88*) |
| | | | NSCLC | 2 | ORR, PFS | Modest effect | – | 01778803 | (*87*) |
| | | | PDAC | 2 | PFS, OS, ORR | Ongoing | – | 04331041 | |
| | | | Ovarian cancer | 1/2 | PFS, OS, ORR | Ongoing | – | 03287271 | |
| | Defactinib + Avutometinib | Combination of FAK inhibitor and MAPK/ ERK pathway inhibitor | Metastatic uveal mela-noma | 2 | PFS, OS, ORR | Ongoing | – | 04720417 | |
| | | | Meso-nephric gynecologic cancer | 2 | ORR | Ongoing | – | 05787561 | |
| | | | Recurrent NSCLC | 2 | PFS, OS, ORR | Ongoing | – | 04620330 | |
| | | | Recurrent low-grade serous ovar-ian cancer | 2 | PFS, OS, ORR | Ongoing | – | 04625270 | |
| | | | Multiple solid tumors | 2 | PFS, OS, ORR | Ongoing | – | 05512208 | |
| | | | PDAC | 1/2 | PFS, OS | Ongoing | – | 05669482 | |
| | Defactinib + Pembrolizumab | Combination of FAK inhibitor and PD-1 blocking antibody | Resectable PDAC | 2 | OS, DFS | Ongoing | – | 03727880 | |
| | | | NSCLC, PDAC, meso-thelioma | 1/2 | PFS, ORR | Ongoing | – | 02758587 | |

*Drug development might be suspended for medical, financial, or other reasons, not always clearly stated for each trial.
†There is a wide array of drugs and trials focusing on copper depletion. As of yet, no clinical successes have been booked, mostly due to adverse effects because Cu is essential for physiological function (*130*).

of a tumor-suppressing immune response. This was demonstrated by depletion of smooth muscle actin ($SMA^+$) CAFs in a mouse model of pancreatic cancer, which then had detrimental effects on survival because of an immunosuppressive effect (*52*). From these and other studies, there is a knowledge gap about CAF subpopulations and their roles in disease progression and drug response.

Thus, the ECM plays a fundamental role in regulating cancer cells and surrounding stromal cells through posttranslational, transcriptional, genetic, and epigenetic mechanisms. These interactions lead to the induction of protrusive cancer cell phenotypes and ECM remodeling, creating a dynamic feedback loop that drives the penetration of cancer cells into the tissue and metastasis.

## VASCULARIZATION, INTRAVASATION, AND EXTRAVASATION

To enable dissemination to distant organs, tumor cells need to access the vasculature, survive in the circulation, and leave blood vessels at the distant site. Often, angiogenesis is induced to promote tumor growth. Driven by factors such as hypoxia-inducible factor (HIF) and increased ECM stiffness, new blood vessels can form through angiogenesis or vascular mimicry (VM).

Generally, a stiffer ECM and a hypoxic environment promote angiogenesis by inducing endothelial cell migration and increasing expression of vascular endothelial growth factor receptor 2 (VEGFR2) (*53*). YAP/TAZ activation leads to angiogenic sprouting and collective endothelial cell migration, resulting in a leaky tumor vasculature. In VM, hypoxia drives cancer cells to undergo the epithelial-endothelial transition by activating pathways that trigger expression of vimentin, fibronectin, vitronectin, and VE-cadherin, an endothelial cell-cell adhesion protein. Additionally, the hypoxic environment induces ECM remodeling through MMP-2, MMP-9, and MT1-MMP and deposition of matrix components as laminin 5 γ2 chain or chain fragments that facilitate VM (*54*).

During metastasis, cancer cells enter the circulation either through blood vessels or through the lymphatic system, a process called intravasation. During intravasation, cancer cells break through the BM and the endothelial or VM layer. This involves proteolytic degradation of BM by MMPs and opening of gaps between endothelial cells, where submicrometer-size junctions can block cells from passing (*50*). In an in vitro microfluidic model, cancer and cancer-associated cells were shown to modify these junctions to facilitate intravasation, although the exact mechanisms are still unknown (*55*). Intravasation has also been shown to depend on ECM stiffness because increased stiffness up-regulates CCN1 in endothelial cells, which activates β-catenin and subsequent N-cadherin expression, enhancing cancer cell adhesion to the epithelium (*56*). After reaching a secondary site, cancer cells may leave the circulation through a process called extravasation, which again involves crossing an endothelial barrier and BM. A microfluidic-based study demonstrated that cancer cells penetrate the endothelial barrier through small (~1 μm)–sized openings between endothelial cells, similar to intravasation (*57*). Another study showed that adhesion to vascular laminin through α3β1 and α6β1 integrins was necessary for endothelial transmigration, after which β1 integrin was essential for penetrating the BM (*58*). ECM mechanics and mechanosensitive receptors are essential in regulating vascularization, cancer cell intravasation, survival, and extravasation.

## METASTATIC COLONIZATION AND THE ROLE OF THE ECM

Once cancer cells have arrived in the distant organ, they experience a new microenvironment. ECM remodeling plays an important role in establishing the required environment for cancer cells. This process may already start before the cancer cells arrive, with the formation of a premetastatic niche (PMN). Priming of the metastatic niche occurs through complex interplays between tumor-derived (growth) factors, tumor-mobilized cell types, and the local stromal components present in the distant organ.

### Premetastatic niche

The mechanism of ECM remodeling in the PMN can be broadly classified into (i) production and deposition, (ii) cross-linking, (iii) degradation, and (iv) other modifications of ECM-related proteins. Bone marrow–derived cells (BMDCs), which are recruited to the PMN, are important players in ECM remodeling. BMDCs exert ECM remodeling through different mechanisms, often through TGF-β production. This causes accumulation of collagen I, for instance, in the lung, by releasing an inhibitor of metalloproteinase collagenolytic activity, tissue inhibitor of metalloproteinase–1 (TIMP-1) (*59*). In lung cancer, activation of stellate cells by BMDCs leads to increased ECM deposition in the liver, which enhances lung cancer cell attachment to the hepatic tissue (*60*). The process of metastasis requires an interplay between fibroblasts, immune cells, perivascular cells, and epithelial cells, which are all involved in homing of BMDCs, fibrosis formation, and development of the PMN (*61*). LOX plays a crucial role in ECM formation and degradation by mediating cross-linking of ECM components. Furthermore, LOX can induce extensive collagen cross-linking in the liver, establishing a fibrotic microenvironment that facilitates increased cell survival and proliferation (*62*). In bone metastasis of breast cancer, LOX leads to modulation of the bone microenvironment by creating osteolytic lesions, which subsequently provide a niche for colonizing circulating tumor cells (*63*). Unlike the molecular remodeling of the ECM occurring in PMN formation, less is known about the impact of altered ECM biomechanics. The BM rigidity seems crucial in metastasis, as demonstrated by increased stiffness in the alveolar lung BM promoting cancer cell invasion (*38*). It was shown that invasion was specifically related to this increased stiffness, rather than to pore size.

### Metastatic niche

After establishment, cancer cells may colonize the PMN and usually exhibit a state of dormancy. This includes down-regulation of MHC-1 expression, which allows cell survival through immune avoidance (*64*). Dormant cancer cells do not divide, making them resistant to chemotherapy. The metastatic niche is involved in the transition from dormancy to metastatic outgrowth, driven by integrin and DDR1 signaling. In turn, integrin signaling results in downstream activation of FAK/YAP-dependent pathways. This is vital for switching from dormant to proliferative states and subsequent secondary tumor formation (*65*, *66*). In addition to ECM remodeling, metastatic tumor growth is also influenced by collagen (I and III) hydroxylation. In the metastatic niche, hypoxia-induced HIF1α stabilization results in up-regulation of collagen prolyl-4-hydroxylase (P4HA). Combined with pyruvate uptake in cancer cells, P4HA induces addition of a hydroxyl group, thereby enhancing collagen stability and allowing twisting of the collagen helix, which results in outgrowth of metastatic breast cancer cells in the lung (*67*). The fibrotic microenvironment may also promote metastatic outgrowth. Clinically, the degree of liver fibrosis is a predictor for relapse-free survival in colorectal cancer, indicating that the fibrotic niche is important for metastasis (*68*). Furthermore, fibrosis-associated increased stiffness was shown to promote therapy resistance in colorectal cancer metastasis (*69*). It is clear that changing ECM properties are important in awakening dormant cells, yet the triggers for this remain unclear.

## TRANSLATIONAL STRATEGIES FOR ECM TARGETING

Biophysical alterations in their simplest form, such as palpable stiffer desmoplastic regions in the breast and prostate, are a hallmark of tumor ECM and are therefore a mainstay in tumor diagnosis. More local mechanical changes, however, are not yet widely used for

diagnostics, in part because of technical limitations. The current standard for quantifying local stiffness used is atomic force microscopy (AFM), which is limited to invasive ex vivo measurements and which requires a freshly isolated biopsy. Moreover, these measurements only provide information about the regions within the selected biopsy. Similar limitations are applicable to other probe-based techniques, including rheometry, magnetic tweezers, and optical tweezers.

An emerging technology to noninvasively quantify mechanics is magnetic resonance elastography (MRE), which combines magnetic resonance imaging (MRI) with a mechanical actuator to induce a shear wave in the tissue (*70*). This combination provides 3D spatial information about stiffness and viscoelastic properties, but standardization is still required before it can be used for diagnoses at a larger scale (Table 1). Clinical evaluation of ECM-related markers, such as periostin, fibronectin, and various integrin combinations, is used to classify solid tumors and association with poor prognosis (*71*, *72*). Furthermore, laminin γ2 chains can be detected in blood and are highly correlated with pancreatic ductal adenocarcinoma (PDAC) metastasis (*73*). Future research may allow for the development of more robust and specific tumor markers that are associated with the ECM, potentially improving patient stratification.

Although there is much knowledge concerning the ECM's involvement in cancer, this understanding has not yet translated into the development of highly successful therapeutic approaches. Although various strategies have been used, direct targeting through ECM depletion and/or degradation, as well as indirect targets more focused on the organization and cross-linking of ECM and associated signaling pathways (Fig. 3), has not yet been successful. Table 2 lists ECM-related treatment modalities used in clinical trials.

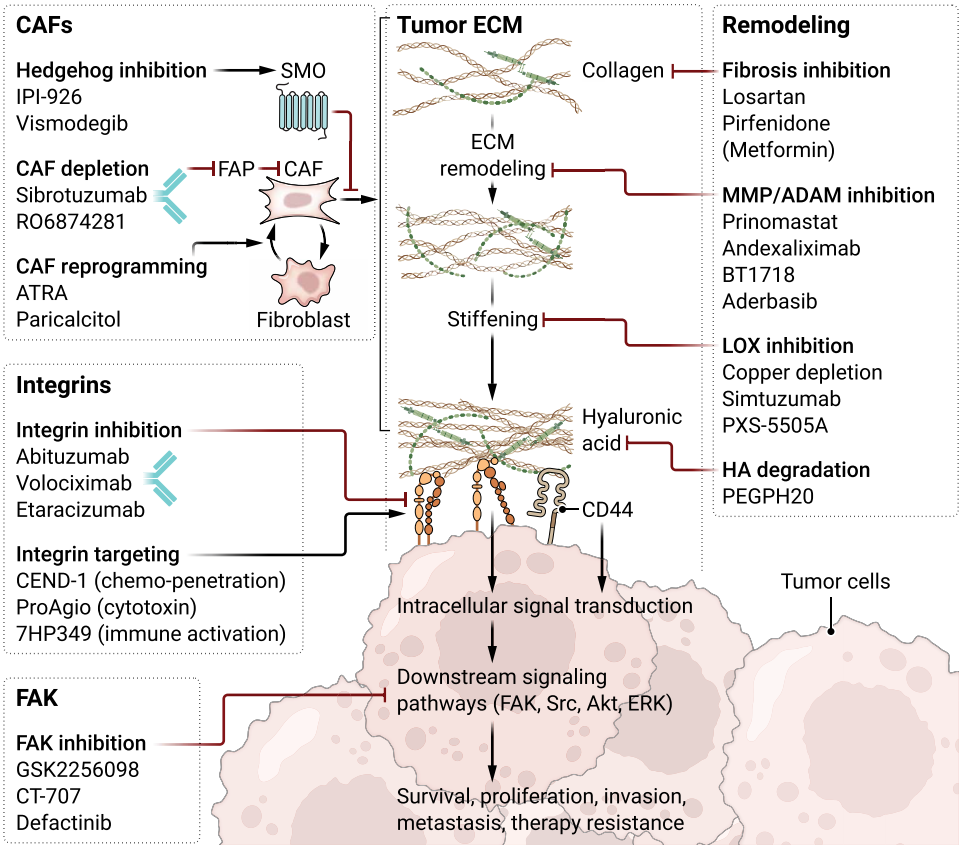


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**Fig. 3. Overview of targetable tumor characteristics regulated by ECM.** Various players in cancer cell–ECM mechanotransduction have been proposed as attractive therapeutic targets, including CAFs, integrins, focal adhesion kinase (FAK), and ECM remodelers. The common goal of these therapeutics is to reduce the downstream cellular signaling that results in tumor progression, down-regulating elements such as FAK, proto-oncogene tyrosine protein kinase Src, protein kinase B (Akt), and ERK. CAFs have been targeted through various compounds, such as by Hedgehog inhibition through the Smooth (SMO) receptor (IPI-926, Vismodegib), by antibody blocking of FAP (sibrotuzumab, RO6874281), and by CAF reprogramming (ATRA, paricalcitol). Integrins have been either inhibited by blocking antibodies (abituzumab, volociximab, etaracizumab) or used to target integrin-expressing cells for other purposes, including improved chemo-penetration (CEND-1), cytotoxin treatment (ProAgio), and immune activation (7HP349). FAK has only been targeted through inhibition (GSK2256098, CT-707, defactinib). ECM remodelers have been targeted by fibrosis inhibition, targeting collagen (losartan, pirfenidone) or through unknown mechanisms (metformin), by degradation inhibition through matrix metalloproteinases (MMPs; prinomastat, andecaliximab, BT1718) or a disintegrin and metalloproteinases (ADAMs; aderbasib), by ECM cross-linking inhibition targeting LOX (copper depletion, simtuzumab, PXS-5505A), or by HA (which signals to cells through the CD44 receptor) degradation (PEGPH20).

### Direct ECM component targeting

Because of their relatively high abundance and protumorigenic effects, fibrillar collagens and HA are attractive therapeutic targets. Besides directly affecting tumor cell behavior, they also have an indirect role in drug transport, partly by reducing drug delivery to the tumor as a result of increased ECM deposition (*74*). Collagenases and hyaluronidases showed to be potent enzymes that break down collagen I and HA in preclinical mouse models of breast cancer and prostate cancer (Table 2) (*75*, *76*). A phase 2 study in patients with pancreatic cancer combining hyaluronidase treatment (PEGPH20) with chemotherapy showed improvement in progression-free survival (PFS), particularly in a subset of patients whose tumors were classified as HA rich [NCT01839487, (*77*)]. However, a subsequent phase 3 trial, as well as a modified phase 2 trial, failed to show response in relevant clinical parameters and even showed increased toxicity (*78*, *79*). Besides targeting existing ECM components, using pathways involved in de novo synthesis of ECM molecules also has potential as a therapeutic target (Fig. 3). A potential strategy is to target the modifying enzymes necessary for the production, secretion, and maturation of different ECM components, including copper-dependent LOX enzymes (Table 2). A phase 2 study using copper depletion showed a decrease in LOX activity and suppression of metastasis in a preclinical mouse model but had no effect on overall survival (OS) in patients with breast cancer (*80*). Furthermore, direct inhibition with a LOXL2-binding antibody, simtuzumab, failed phase 2 trials in patients with colorectal or pancreatic

adenocarcinoma because of lack of efficacy (*81*, *82*). A different strategy using a small-molecule LOX inhibitor, PXS-5505A, in pancreatic cancer exhibited tolerability in a phase 1 trial [NCT04676529, (*83*)] and is currently being assessed in a phase 1/2 trial in combination with atezolizumab and bevacizumab in patients with HCC [NCT05109052, (*84*)].

In addition, FAK inhibition has led to limited success in cancer treatment. Highly selective adenosine triphosphate (ATP)–competitive FAK inhibitor GSK2256098, in combination with mitogen-activated protein kinase (MAPK) kinase (MEK) inhibitor trametinib, was tolerated but not active in patients with advanced PDAC (*85*). Ongoing studies in patients with progressive NF2-mutated meningiomas show an improved 6-month PFS rate (*86*). In this study, patients are treated with GSK2256098, Hedgehog pathway inhibitor vismodegib, Akt kinase inhibitor capivasertib, and cyclin-dependent kinase (CDK) inhibitor abemaciclib. An alternative is conteltinib (CT-707), a FAK inhibitor targeting multiple kinases (FAK/ALK/Pyk2), which is currently in phase 1b/2 trial in combination with PD-1 inhibitor toripalimab and chemotherapy gemcitabine. Currently, the most promising FAK inhibitor is defactinib (VS-6063). In early studies, treatment with defactinib in addition to chemotherapy or radiation therapy showed only limited clinical success in patients with non–small cell lung cancer (*87*) and cancers with NF2 genetic changes (*88*) or no efficacy at all in patients with malignant pleural mesothelioma (*89*). Ongoing clinical studies aim to improve the effectiveness of defactinib by combining it with other treatments. Other studies focus on combination therapy of defactinib with the MAPK/ERK pathway inhibitor avutometinib (NCT05512208, NCT04625270, NCT04620330, NCT05787561, NCT04720417, NCT05669482) or combination of defactinib with the PD-1 blocking antibody pembrolizumab (NCT02758587, NCT03727880). Additionally, there are multiple FAK inhibitors still under development, including advanced multitargeted types that coinhibit targets such as ALK, EGFR, and S6K1 (*90*).

An alternative approach that has also gained popularity in other therapeutic modalities is repurposing drugs with antifibrotic properties. The most potent options to target fibrosis are losartan, metformin, and pirfenidone (Fig. 3). Losartan, an antagonist of the angiotensin II receptor, inhibits collagen I production and was used in a phase 2 trial for patients with locally advanced pancreatic cancer. It showed considerable differences in the margin-negative resection rate, but to improve OS, combinational therapy is needed (*91*, *92*). Metformin, an activator of AMP-activated protein kinase (AMPK) normally used to treat type II diabetes, inhibits fibrosis through not fully understood mechanisms (*93*), whereas pirfenidone, which inhibits several growth factors and procollagens, has well-characterized antifibrotic properties. Metformin and pirfenidone remain to be validated in a cancer setting but have been successful in fibrosis-related diseases (*94*, *95*).

Additionally, ECM proteases are an attractive target for ECM degradation strategies, considering their widespread dysregulation in cancer cell invasion and metastasis. In preclinical models, these inhibitors have been shown to reduce tumor burden, inhibit angiogenesis, and prevent metastasis (*96*). Broad-spectrum MMP inhibitors are the most promising MMP-targeting therapeutics, but finding a balance between efficacy and toxicity is difficult (*97*). Development of broad-spectrum MMP inhibitors, such as BAY-12-9566 (Bayer Corporation), MMI-270 (Novartis), and SU5416 (SUGEN), has been halted because of disease acceleration or toxicities (*98*). After unsuccessful trials of broad-spectrum MMP inhibitors, focus has shifted toward the development of targeted inhibitors. Selective targeting approaches, mostly facilitated through monoclonal antibodies, can be especially beneficial for maintaining MMP activity required for normal physiological functions. Andecaliximab, an MMP-9–specific inhibiting antibody, showed clinical activity without toxicity in two phase 1 trials aimed at treating patients with advanced solid tumors but failed to provide efficacy in a phase 2 trial comparing andecaliximab and nivolumab (an immune checkpoint inhibitor) versus nivolumab monotherapy (*99*, *100*). Once thought of as promising, these MMP-targeting therapeutics have not yet been successfully translated to the clinic. The lack of success from early trials has created a negative bias toward MMP inhibition strategies, but a new generation of inhibitors has high potential for successful outcomes.

Regarding MMP therapeutic strategies, the start of treatment is highly dependent on the cancer stage. Application of marimastat combined with gemcitabine improved survival in patients with pancreatic cancer without metastases (*101*). Furthermore, presurgical treatment with the oral MMP inhibitor SD-7300 improved survival from 67 to 92% in a mouse breast cancer model and decreased the risk of recurrence (*102*). The "window of opportunity" for MMP inhibitor application seemingly is in premetastatic disease. Future MMP research should focus on temporal analysis of biological functioning in separate tumors to identify crucial targets and simultaneously develop high-specificity therapeutics for these targets. To this extent, microRNA-mediated MMP regulation might provide increasing specificity.

As a result of the highly complex nature of ECM, targeting therapies are not always successful and a high risk of off-target effects remains. This is reflected by the adverse events reported in various trials (*103*, *104*). Preclinical models that can encompass and recapitulate the highly complex nature of ECM are vital for future development. Regardless, reversing or inhibiting the dysregulation of the ECM, without affecting the delicate balance required for normal tissue homeostasis, is challenging.

### Integrin-targeting therapies

An alternative approach would be to target how cells sense changes in the pathological ECM. Integrin signaling is a logical first choice, because it is the primary conduit of reciprocal signaling between extracellular and intracellular inputs (Fig. 3). Over the years, antibodies, peptidic antagonists, and peptidomimetics have been used to target integrins in solid tumors; however, none has been successful yet (Table 2).

The $\alpha_V$ family has been a major focus for integrin-targeting therapies. Abituzumab, a pan-$\alpha_V$ inhibitor, has been used in prostate and colorectal cancer. Although metastatic bone lesions in patients with prostate cancer were reduced, it showed insufficient efficacy (*103*). Inhibitor strategies focusing on integrin combinations, including αvβ3 inhibitor etaracizumab and α5β1 inhibitor volociximab, showed no meaningful clinical improvement (*105*, *106*).

Although integrin-inhibiting therapies have been extensively studied, no breakthrough treatment has been developed. Alternative strategies might be to not inhibit, but activate, integrins on immune cells, for instance, with compound 7HP349, to increase the immune response (NCT04508179). Integrins can also be used to target cells that overexpress integrins and either kill them using a

linked cytotoxin (ProAgio, NCT05085548) or make them more susceptible to other tumor-targeting compounds (CEND-1, NCT05052567). Particularly, CEND-1, a bifunctional cyclic peptide containing integrin v and an RGD motif, already showed promise in a phase 1 trial (NCT03517176).

Integrin therapy failure is a nonexclusive multifactorial issue, already discussed 30 years ago. Many of the early integrin-focused therapies had less than ideal pharmacokinetics, meaning that the dosing regimens were not effective. This is directly related to the complex signaling pathways involved with integrins, particularly with their dual role in cell adhesion and inducing intracellular signaling pathways (*107*). Furthermore, integrin expression is heterogeneous, both within the tumor or between tumors (*108*). Integrin heterogeneity, combined with a lack of pharmacodynamic biomarkers to actually measure the efficacy, left many phase 3 trials to fail (*109*, *110*). Integrin therapies could therefore benefit from identification of subpopulations, for example, in patients with high expression of αvβ6. Therefore, interpretation of preclinical data seems difficult, because heterodimer-specific antibodies are required for accurate measurement of integrin expression. Like MMPs, integrins require precise timing protocols for optimal functioning. They can have different roles in different disease stages, as well as in primary and metastatic lesions. Pathophysiological and pharmacological insights have increased tremendously since the first integrin-targeted therapy, and future trials with solid biomarkers, subpopulation identification, correct timing strategies, and new drugs provide a hopeful future. Of note, other mechanosensitive receptors, such as syndecans and DDRs, have not yet clinically been targeted but might also provide potential therapeutic targets (*111*, *112*).

### CAF-targeted therapies

Targeting CAFs that produce the bulk of the dysregulated ECM components (Fig. 3) might be essential. Most CAF-targeting strategies focus on depletion by targeting classically associated markers, including fibroblast activation protein (FAP) (Table 2). Most classical CAF markers such as α-SMA, FAP, and vimentin seem to be not specific. Multiple FAP blocking antibodies (sibrotuzumab, RO6874281) have been tested in phase 1/2 trials, showing favorable toxicity profiles but no remission or stabilization of disease progression (*113*, *114*). However, RO6874281 is currently being assessed in combinational therapies in different solid tumor settings. Administration in combination with atezolizumab (an immune checkpoint inhibitor) in patients with cervical cancer (NCT02627274) seems very encouraging (*115*). Because CAF function is primarily driven by signaling pathways including TGF-β, Hedgehog, and nuclear factor κB (NFκB) inhibitors, these specific pathways are being pursued (*116*). The aberrant Hedgehog pathway activation, involved in embryonic development and tissue homeostasis, is an important driver of cancer progression (*117*). However, several phase 2 trials with Hedgehog inhibitors did not improve patient outcomes. One study, in which Hedgehog inhibitor IPI-926 was combined with chemotherapeutic gemcitabine, even showed a worse clinical outcome than that of gemcitabine alone [NCT01130142, (*118*)]. Alternative strategies to CAF depletion have been investigated, including reprogramming CAFs to a healthy phenotype. In a PDAC mouse model, calcipotriol treatment, a vitamin D analog, enhanced gemcitabine efficacy and increased OS (*119*). After a successful phase 1 trial in patients with PDAC, a phase 2 trial was initiated [NCT03307148, (*120*)]. Another vitamin D analog, paricalcitol, is now in phase 1 and 2 trials treating several solid tumors (NCT03520790 and NCT00637897). The reprogramming of CAFs, rather than depletion, seems to have the most potential for CAF targeting.

## CONCLUSION

Given the explicit role in tumor development, the deregulated ECM should be recognized as a hallmark of cancer. The ECM of solid tumors has been shown to be highly complex and undergoes dynamic temporal and spatial changes during disease progression. The dynamic aspect of the ECM complicates the development of matrix-targeted therapies, and the treatment success requires an intimate understanding of the reciprocal interaction between the transforming ECM, cancer cells, and cancer-associated stromal cells. Ideally, this understanding is obtained from preclinical studies that capture enough of the tumor's complexity to predict clinical outcome. Until now, ECM-targeting therapies have been limited in scope, with integrin and (pan-) MMP inhibition as focus points. However, new ways to promote or block specific integrins, target mechanosensitive receptors such as DDRs and syndecans, develop highly specific MMP inhibitors, and inhibit or modulate functionally distinct CAF subpopulations are approaches currently being pursued in the preclinical and clinical settings. Moreover, early studies have shown that interpatient variability in ECM composition and integrin expression can be determining factors in treatment effect. Therefore, these strategies should be combined with characterization of the patient-specific deregulated ECM. Only this allows for personalized medicine strategies and associated patient stratification. Accumulating clinical trial results have shown that monotherapy with an ECM target-specific agent is not sufficient. Therefore, dual- or multi-targeting therapy might be a more rational choice. It is key for ongoing and future clinical trials to report the exact reasons for failure. Potential factors contributing to the failure of targeting ECM molecular players include the complexity of the molecular interactions in the human body, challenges in dosage and delivery optimization, factors that may reduce the efficacy of the inhibitor, including immune responses, and patient heterogeneity or disease stage. Substantial preclinical work has been done to uncover targetable mechanisms of ECM remodeling in recent years and inevitably will boost development of interventions profitable to future patients.

## Supplementary Materials

**This PDF file includes:**

Table S1

References (*144*–*150*)

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