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CANCER

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

Jelle J. F. Sleeboom^{1,2†}, Gilles S. van Tienderen^{1†}, Katja Schenke-Layland^{3,4,5},
Luc J. W. van der Laan¹, Antoine A. Khalil^{6*}, Monique M. A. Versteeg^{1*}

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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 (DDR) 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). Mechano-transduction 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 β 1 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 α v β 3 (and/or α v β 5) 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

¹Department of Surgery, Erasmus MC Transplant Institute, University Medical Center Rotterdam, Postbox 2040, 3000CA Rotterdam, Netherlands. ²Department of Biomechanical Engineering, Delft University of Technology, Mekelweg 2, 2628CD Delft, Netherlands. ³Institute of Biomedical Engineering, Department for Medical Technologies and Regenerative Medicine, Eberhard Karls University Tübingen, 72076 Tübingen, Germany. ⁴NMI Natural and Medical Sciences Institute at the University Tübingen, 72770 Reutlingen, Germany. ⁵Cluster of Excellence iFIT (EXC 2180) "Image-Guided and Functionally Instructed Tumor Therapies," Eberhard Karls University Tübingen, 72076 Tübingen, Germany. ⁶Center for Molecular Medicine, University Medical Center Utrecht, Universiteitsweg 100, 3584 CG Utrecht, Netherlands.

*Corresponding author. Email: m.versteeg@erasmusmc.nl (M.M.A.V.); a.khalil@umcutrecht.nl (A.A.K.)

†These authors contributed equally to this work.

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.

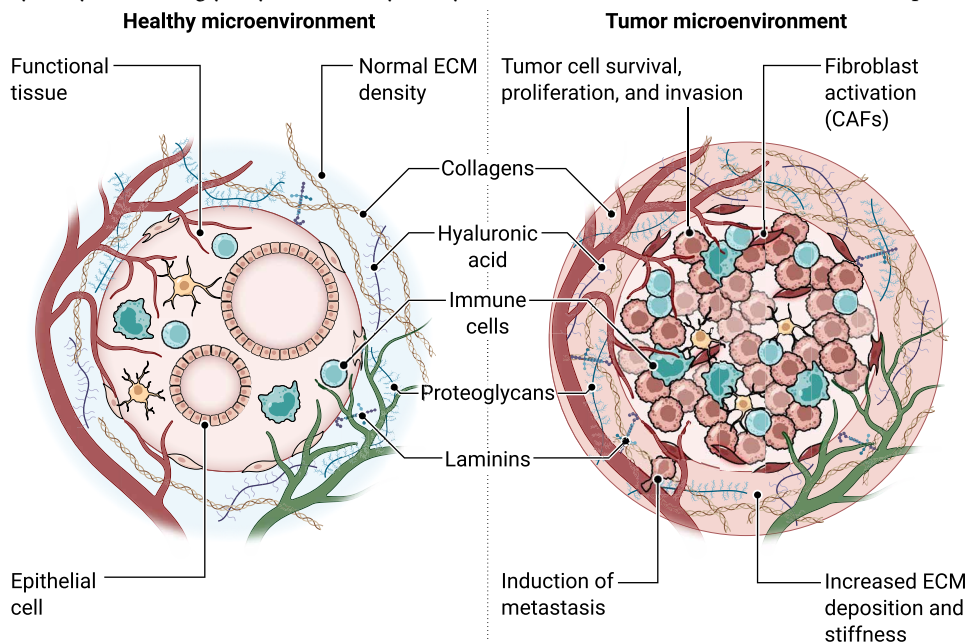


Fig. 1. The tumor microenvironment at a glance. A healthy microenvironment provides a suitable niche for epithelial cell (yellow) function through interactions with neighboring cells, including immune cells, resident fibroblasts, and a homeostatic ECM composition. Oxygen delivery and nutrient transport 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.

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 pre-clinical 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.

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

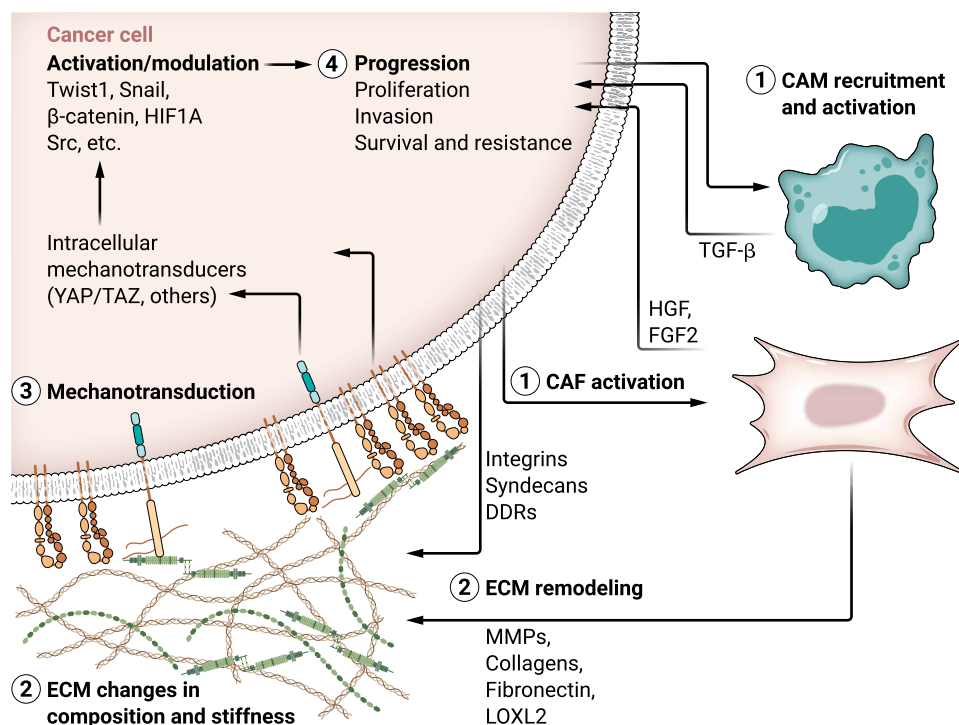


Fig. 2. Reciprocal cell-ECM interactions in the TME. Cancer cells recruit and activate CAFs, CAMs, and other stromal and immune cells (1). These cells stimulate cancer progression through paracrine factors such as fibroblast growth factor 2 (FGF2), hepatocyte growth factor (HGF), and transforming growth 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 1 α (HIF1 α), 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 β 1-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 promoter 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 matrix 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 1 α (HIF1 α) (Fig. 2) (21, 30). For most of these regulators, the underlying mechanisms for mechanosensitivity are not yet fully understood. HIF1 α 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 HIF1 α 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, β 1 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 panlysyl 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)
			Multiple solid tumors	1/2	RECIST, estimated PFS and OS	Ongoing	–	03486730	(134)

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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 (α v β 1)	Recombinant monoclonal antibody to α v β 1 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 (α v β 3)	Monoclonal antibody to α v β 3 integrin, inhibiting its activity	Metastatic melanoma	2	PFS	Failed	Insufficient clinical activity	00066196	(106)
	Integrin targeting (type)	CEND-1 (α v β 3, α v β 5)	Enhances drug penetration by dual α v-integrin and neuropilin-1 targeting	PDAC	1	Safety, tolerability, preliminary PFS, OS, ORR	Ongoing	–	05052567
ProAgio (α v β 3)		Cytotoxin selectively binding to α v β 3 Integrin	Pancreatic cancer, solid tumors	1	Safety, tolerability, preliminary ORR	Ongoing	–	05085548	
7HP349 (α 4 β 1, α L β 2)		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	

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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 mesothelioma	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		
Metastatic uveal melanoma			2	PFS, OS, ORR	Ongoing	–	04720417		
	Defactinib + Avutometinib	Combination of FAK inhibitor and MAPK/ERK pathway inhibitor	Meso-nephric gynecologic cancer	2	ORR	Ongoing	–	05787561	
Recurrent NSCLC			2	PFS, OS, ORR	Ongoing	–	04620330		
Recurrent low-grade serous ovarian cancer			2	PFS, OS, ORR	Ongoing	–	04625270		
Multiple solid tumors			2	PFS, OS, ORR	Ongoing	–	05512208		
PDAC			1/2	PFS, OS	Ongoing	–	05669482		
Resectable PDAC			2	OS, DFS	Ongoing	–	03727880		
	Defactinib + Pembrolizumab	Combination of FAK inhibitor and PD-1 blocking antibody	NSCLC, PDAC, mesothelioma	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 γ 2 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 μ m)-sized openings between endothelial cells, similar to intravasation (57). Another study showed that adhesion to vascular laminin through α 3 β 1 and α 6 β 1 integrins was necessary for endothelial transmigration, after which β 1 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 HIF1 α 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 $\gamma 2$ 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.

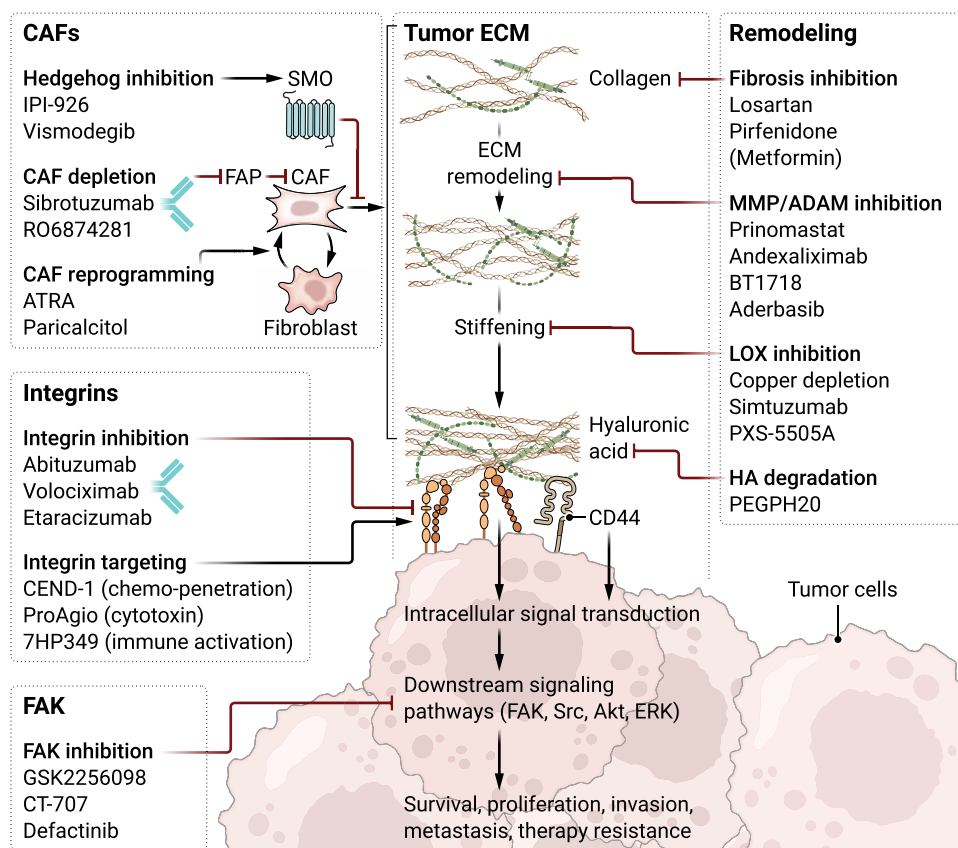


Fig. 3. Overview of targetable tumor characteristics regulated by ECM. Various players in cancer cell–ECM mechanotransduction have been proposed as attractive therapeutic targets, including CAFs, integrins, focal adhesion kinase (FAK), and ECM remodelers. The common goal of these therapeutics is to reduce 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 avotemetinib (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 multi-targeted 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 α_V family has been a major focus for integrin-targeting therapies. Abituzumab, a pan- α_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 $\alpha_V\beta_3$ inhibitor etaracizumab and $\alpha_5\beta_1$ 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 ν 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 $\alpha\beta6$. Therefore, interpretation of pre-clinical 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 κ B (NF κ B) 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 pre-clinical 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)

REFERENCES AND NOTES

1. D. Hanahan, Hallmarks of cancer: New dimensions. *Cancer Discov.* **12**, 31–46 (2022).
2. S. E. Amos, Y. S. Choi, The cancer microenvironment: Mechanical challenges of the metastatic cascade. *Front. Bioeng. Biotechnol.* **9**, 625859 (2021).
3. H. Hamidi, M. Pietilä, J. Ivaska, The complexity of integrins in cancer and new scopes for therapeutic targeting. *Br. J. Cancer* **115**, 1017–1023 (2016).
4. J. Z. Kechagia, J. Ivaska, P. Roca-Cusachs, Integrins as biomechanical sensors of the microenvironment. *Nat. Rev. Mol. Cell Biol.* **20**, 457–473 (2019).
5. N. Betriu, J. Bertran-Mas, A. Andreeva, C. E. Semino, Syndecans and pancreatic ductal adenocarcinoma. *Biomolecules* **11**, 349 (2021).
6. C.-P. Huang, C.-M. Cheng, H.-L. Su, Y.-W. Lin, Syndecan-4 promotes epithelial tumor cells spreading and regulates the turnover of PKC α activity under mechanical

- stimulation on the elastomeric substrates. *Cell. Physiol. Biochem.* **36**, 1291–1304 (2015).
7. A. Chronopoulos, S. D. Thorpe, E. Cortes, D. Lachowski, A. J. Rice, V. V. Mykuliak, T. Róg, D. A. Lee, V. P. Hytönen, A. E. del Río Hernández, Syndecan-4 tunes cell mechanics by activating the kindlin-integrin-RhoA pathway. *Nat. Mater.* **19**, 669–678 (2020).
 8. D. M. Beauvais, A. C. Rapraeger, Syndecan-1 couples the insulin-like growth factor-1 receptor to inside-out integrin activation. *J. Cell Sci.* **123**, 3796–3807 (2010).
 9. N. M. Coelho, C. A. McCulloch, Mechanical signaling through the discoidin domain receptor 1 plays a central role in tissue fibrosis. *Cell Adh. Migr.* **12**, 348–362 (2018).
 10. S. V. Bayer, W. R. Grither, A. Brenot, P. Y. Hwang, C. E. Barcus, M. Ernst, P. Pence, C. Walter, A. Pathak, G. D. Longmore, DDR2 controls breast tumor stiffness and metastasis by regulating integrin mediated mechanotransduction in CAFs. *eLife* **8**, e45508 (2019).
 11. V. Mehta, H. Chander, A. Munshi, Complex roles of discoidin domain receptor tyrosine kinases in cancer. *Clin Transl. Oncol.* **23**, 1497–1510 (2021).
 12. Y.-C. Chuang, C.-C. Chen, Force from filaments: The role of the cytoskeleton and extracellular matrix in the gating of mechanosensitive channels. *Front Cell Dev. Biol.* **10**, 886048 (2022).
 13. B. C. Coutino, R. Mayor, Mechanosensitive ion channels in cell migration. *Cells Dev.* **166**, 203683 (2021).
 14. J. Karska, S. Kowalski, J. Saczko, M. G. Moisescu, J. Kulbacka, Mechanosensitive ion channels and their role in cancer cells. *Membranes* **13**, 167 (2023).
 15. B. Martinez, Y. Yang, D. M. R. Harker, C. Farrar, H. Mukundan, P. Nath, D. Mascareñas, YAP/TAZ related biomechanical signal transduction and cancer metastasis. *Front Cell Dev. Biol.* **7**, 199 (2019).
 16. J. Winkler, A. Abisoye-Ogunniyan, K. J. Metcalf, Z. Werb, Concepts of extracellular matrix remodelling in tumour progression and metastasis. *Nat. Commun.* **11**, 1–19 (2020).
 17. Z. Piperigkou, K. Kyriakopoulou, C. Koutsakis, S. Mastrokolis, N. K. Karamanos, Key matrix remodeling enzymes: Functions and targeting in cancer. *Cancer* **13**, 1441 (2021).
 18. Y. Jiang, H. Zhang, J. Wang, Y. Liu, T. Luo, H. Hua, Targeting extracellular matrix stiffness and mechanotransducers to improve cancer therapy. *J. Hematol. Oncol.* **15**, 1–15 (2022).
 19. A. D. Waldman, J. M. Fritz, M. J. Lenardo, A guide to cancer immunotherapy: From T cell basic science to clinical practice. *Nat. Rev. Immunol.* **20**, 651–668 (2020).
 20. M. C. Lampi, C. A. Reinhart-King, Targeting extracellular matrix stiffness to attenuate disease: From molecular mechanisms to clinical trials. *Sci. Transl. Med.* **10**, eaa0475 (2018).
 21. S. Ishihara, H. Haga, Matrix stiffness contributes to cancer progression by regulating transcription factors. *Cancer* **14**, 1049 (2022).
 22. Y. A. Miroshnikova, J. K. Mouw, J. M. Barnes, M. W. Pickup, J. N. Lakins, Y. Kim, K. Lobo, A. I. Persson, G. F. Reis, T. R. McKnight, Tissue mechanics promote IDH1-dependent HIF1 α -tenascin C feedback to regulate glioblastoma aggression. *Nat. Cell Biol.* **18**, 1336–1345 (2016).
 23. H. Ghasemi, S. H. Mousavibahar, M. Hashemnia, J. Karimi, I. Khodadadi, F. Mirzaei, H. Tavilani, Tissue stiffness contributes to YAP activation in bladder cancer patients undergoing transurethral resection. *Ann. N. Y. Acad. Sci.* **1473**, 48–61 (2020).
 24. V. M. Weaver, O. W. Petersen, F. Wang, C. A. Larabell, P. Briand, C. Damsky, M. J. Bissell, Reversion of the malignant phenotype of human breast cells in three-dimensional culture and in vivo by integrin blocking antibodies. *J. Cell Biol.* **137**, 231–245 (1997).
 25. B. Yao, Y. Niu, Y. Li, T. Chen, X. Wei, Q. Liu, High-matrix-stiffness induces promotion of hepatocellular carcinoma proliferation and suppression of apoptosis via miR-3682-3p-PHLDA1-FAS pathway. *J. Cancer* **11**, 6188–6203 (2020).
 26. M. Jang, J. An, S. W. Oh, J. Y. Lim, J. Kim, J. K. Choi, J.-H. Cheong, P. Kim, Matrix stiffness epigenetically regulates the oncogenic activation of the Yes-associated protein in gastric cancer. *Nat. Biomed. Eng.* **5**, 114–123 (2021).
 27. E. Brauchle, J. Kasper, R. Daum, N. Schierbaum, C. Falch, A. Kirschniak, T. E. Schäffer, K. Schenke-Layland, Biomechanical and biomolecular characterization of extracellular matrix structures in human colon carcinomas. *Matrix Biol.* **68**, 180–193 (2018).
 28. M. M. Rowe, W. Wang, P. V. Taufalele, C. A. Reinhart-King, AGE-breaker ALT711 reverses glycation-mediated cancer cell migration. *Soft Matter* **18**, 8504–8513 (2022).
 29. P. Friedl, K. Wolf, Tube travel: The role of proteases in individual and collective cancer cell invasion. *Cancer Res.* **68**, 7247–7249 (2008).
 30. M. G. Ondeck, A. Kumar, J. K. Placone, C. M. Plunkett, B. F. Matte, K. C. Wong, L. Fattet, J. Yang, A. J. Engler, Dynamically stiffened matrix promotes malignant transformation of mammary epithelial cells via collective mechanical signaling. *Proc. Natl. Acad. Sci. U.S.A.* **116**, 3502–3507 (2019).
 31. E. Cortes, D. Lachowski, B. Robinson, M. Sarper, J. S. Teppo, S. D. Thorpe, T. J. Lieberthal, K. Iwamoto, D. A. Lee, M. Okada-Hatakeyama, Tamoxifen mechanically reprograms the tumor microenvironment via HIF-1A and reduces cancer cell survival. *EMBO Rep.* **20**, e46557 (2019).
 32. F. Feng, X. Feng, D. Zhang, Q. Li, L. Yao, Matrix stiffness induces pericyte-fibroblast transition through YAP activation. *Front. Pharmacol.* **12**, 698275 (2021).
 33. J. R. Davis, N. Tapon, Hippo signalling during development. *Development* **146**, dev167106 (2019).
 34. L. E. Scott, S. H. Weinberg, C. A. Lemmon, Mechanochemical signaling of the extracellular matrix in epithelial-mesenchymal transition. *Front Cell Dev. Biol.* **7**, 135 (2019).
 35. A. A. Khalil, J. de Rooij, Cadherin mechanotransduction in leader-follower cell specification during collective migration. *Exp. Cell Res.* **376**, 86–91 (2019).
 36. P. Friedl, J. Locker, E. Sahai, J. E. Segall, Classifying collective cancer cell invasion. *Nat. Cell Biol.* **14**, 777–783 (2012).
 37. A. A. Khalil, P. Friedl, Determinants of leader cells in collective cell migration. *Integr. Biol.* **2**, 568–574 (2010).
 38. R. Reuten, S. Zendeheroud, M. Nicolau, L. Fleischhauer, A. Laitala, S. Kiderlen, D. Nikodemus, L. Wullkopf, S. R. Nielsen, S. McNeilly, Basement membrane stiffness determines metastases formation. *Nat. Mater.* **20**, 892–903 (2021).
 39. G. M. Cramer, D. P. Jones, H. El-Hamidi, J. P. Celli, ECM composition and rheology regulate growth, motility, and response to photodynamic therapy in 3D models of pancreatic ductal adenocarcinoma. *Mol. Cancer Res.* **15**, 15–25 (2017).
 40. Y. Moshfegh, J. J. Bravo-Cordero, V. Miskolci, J. Condeelis, L. Hodgson, A Trio–Rac1–Pak1 signalling axis drives invadopodia disassembly. *Nat. Cell Biol.* **16**, 571–583 (2014).
 41. I. Belhabib, S. Zaghdoudi, C. Lac, C. Bousquet, C. Jean, Extracellular matrices and cancer-associated fibroblasts: Targets for cancer diagnosis and therapy? *Cancer* **13**, 3466 (2021).
 42. K. J. Cheung, E. Gabrielson, Z. Werb, A. J. Ewald, Collective invasion in breast cancer requires a conserved basal epithelial program. *Cell* **155**, 1639–1651 (2013).
 43. K. M. Ricking, B. L. Cox, M. R. Salick, C. Pehlke, A. S. Ricking, S. M. Ponik, B. R. Bass, W. C. Crone, Y. Jiang, A. M. Weaver, 3D collagen alignment limits protrusions to enhance breast cancer cell persistence. *Biophys. J.* **107**, 2546–2558 (2014).
 44. Z. Jiang, C. Zhou, L. Cheng, B. Yan, K. Chen, X. Chen, L. Zong, J. Lei, W. Duan, Q. Xu, Inhibiting YAP expression suppresses pancreatic cancer progression by disrupting tumor-stromal interactions. *J. Exp. Clin. Cancer Res.* **37**, 1–14 (2018).
 45. S. C. Wei, L. Fattet, J. H. Tsai, Y. Guo, V. H. Pai, H. E. Majeski, A. C. Chen, R. L. Sah, S. S. Taylor, A. J. Engler, Matrix stiffness drives epithelial-mesenchymal transition and tumour metastasis through a TWIST1–G3BP2 mechanotransduction pathway. *Nat. Cell Biol.* **17**, 678–688 (2015).
 46. C. Yang, M. W. Tibbitt, L. Basta, K. S. Anseth, Mechanical memory and dosing influence stem cell fate. *Nat. Mater.* **13**, 645–652 (2014).
 47. A. Pal, T. F. Barrett, R. Paolini, A. Parikh, S. V. Puram, Partial EMT in head and neck cancer biology: A spectrum instead of a switch. *Oncogene* **40**, 5049–5065 (2021).
 48. A. Castro-Castro, V. Marchesin, P. Monteiro, C. Lodillinsky, C. Rossé, P. Chavrier, Cellular and molecular mechanisms of MT1-MMP-dependent cancer cell invasion. *Annu. Rev. Cell Dev. Biol.* **32**, 555–576 (2016).
 49. T. Koorman, K. A. Jansen, A. Khalil, P. D. Haughton, D. Visser, M. A. K. Rätze, W. E. Haakma, G. Sakalaukaitė, P. J. van Diest, J. de Rooij, Spatial collagen stiffening promotes collective breast cancer cell invasion by reinforcing extracellular matrix alignment. *Oncogene* **41**, 2458–2469 (2022).
 50. H. Yamahana, M. Terashima, R. Takatsuka, C. Asada, T. Suzuki, Y. Uto, T. Takino, TGF- β 1 facilitates MT1-MMP-mediated proMMP-9 activation and invasion in oral squamous cell carcinoma cells. *Biochem. Biophys. Res. Commun.* **277**, 101072 (2021).
 51. Z. Liu, H. Mo, R. Liu, Y. Niu, T. Chen, Q. Xu, K. Tu, N. Yang, Matrix stiffness modulates hepatic stellate cell activation into tumor-promoting myofibroblasts via E2F3-dependent signaling and regulates malignant progression. *Cell Death Dis.* **12**, 1–9 (2021).
 52. B. C. Özdemir, T. Pentcheva-Hoang, J. L. Carstens, X. Zheng, C.-C. Wu, T. R. Simpson, H. Laklai, H. Sugimoto, C. Kahlert, S. V. Novitskiy, Depletion of carcinoma-associated fibroblasts and fibrosis induces immunosuppression and accelerates pancreas cancer with reduced survival. *Cancer Cell* **25**, 719–734 (2014).
 53. J. Liu, S. Agarwal, Mechanical signals activate vascular endothelial growth factor receptor-2 to upregulate endothelial cell proliferation during inflammation. *J. Immunol.* **185**, 1215–1221 (2010).
 54. B. Sun, D. Zhang, S. Zhang, W. Zhang, H. Guo, X. Zhao, Hypoxia influences vasculogenic mimicry channel formation and tumor invasion-related protein expression in melanoma. *Cancer Lett.* **249**, 188–197 (2007).
 55. I. K. Zervantonakis, S. K. Hughes-Alford, J. L. Charest, J. S. Condeelis, F. B. Gertler, R. D. Kamm, Three-dimensional microfluidic model for tumor cell intravasation and endothelial barrier function. *Proc. Natl. Acad. Sci. U.S.A.* **109**, 13515–13520 (2012).
 56. S. E. Reid, E. J. Kay, L. J. Neilson, A. T. Henze, J. Serneels, E. J. McGhee, S. Dhayade, C. Nixon, J. B. G. Mackey, A. Santi, Tumor matrix stiffness promotes metastatic cancer cell interaction with the endothelium. *EMBO J.* **36**, 2373–2389 (2017).
 57. M. B. Chen, J. A. Whisler, J. S. Jeon, R. D. Kamm, Mechanisms of tumor cell extravasation in an in vitro microvascular network platform. *Integr. Biol.* **5**, 1262–1271 (2013).
 58. M. B. Chen, J. M. Lamar, R. Li, R. O. Hynes, R. D. Kamm, Elucidation of the roles of tumor integrin β 1 in the extravasation stage of the metastasis cascade. *Cancer Res.* **76**, 2513–2524 (2016).
 59. A. Lebrun, S. Lo Re, M. Chantry, X. Izquierdo Carerra, F. Uwambayinema, D. Ricci, R. Devosse, S. Ibouaardaten, L. Brombin, M. Palmal-Pallag, CCR2+ monocytic myeloid-derived suppressor cells (M-MDSCs) inhibit collagen degradation and promote lung fibrosis by producing transforming growth factor- β 1. *J. Pathol.* **243**, 320–330 (2017).

60. Y.-L. Hsu, M.-S. Huang, J.-Y. Hung, W.-A. Chang, Y.-M. Tsai, Y.-C. Pan, Y.-S. Lin, H.-P. Tsai, P.-L. Kuo, Bone-marrow-derived cell-released extracellular vesicle miR-92a regulates hepatic pre-metastatic niche in lung cancer. *Oncogene* **39**, 739–753 (2020).
61. J. W. Lee, M. L. Stone, P. M. Porrett, S. K. Thomas, C. A. Komar, J. H. Li, D. Delman, K. Graham, W. L. Gladney, X. Hua, Hepatocytes direct the formation of a pro-metastatic niche in the liver. *Nature* **567**, 249–252 (2019).
62. T. R. Cox, D. Bird, A.-M. Baker, H. E. Barker, M. W. Y. Ho, G. Lang, J. T. Erler, LOX-mediated collagen crosslinking is responsible for fibrosis-enhanced metastasis. *Cancer Res.* **73**, 1721–1732 (2013).
63. T. R. Cox, R. M. H. Rumney, E. M. Schoof, L. Perryman, A. M. Høye, A. Agrawal, D. Bird, N. A. Latif, H. Forrest, H. R. Evans, The hypoxic cancer secretome induces pre-metastatic bone lesions through lysyl oxidase. *Nature* **522**, 106–110 (2015).
64. A. Pommier, N. Anaparthi, N. Memos, Z. L. Kelley, A. Gouronnet, R. Yan, C. Auffray, J. Albrengues, M. Egeblad, C. A. Iacobuzio-Donahue, Unresolved endoplasmic reticulum stress engenders immune-resistant, latent pancreatic cancer metastases. *Science* **360**, eaao4908 (2018).
65. E. E. Er, M. Valiente, K. Ganesh, Y. Zou, S. Agrawal, J. Hu, B. Griscom, M. Rosenblum, A. Boire, E. Brogi, Pericyte-like spreading by disseminated cancer cells activates YAP and MRTF for metastatic colonization. *Nat. Cell Biol.* **20**, 966–978 (2018).
66. T. Shibue, R. A. Weinberg, Integrin β 1-focal adhesion kinase signaling directs the proliferation of metastatic cancer cells disseminated in the lungs. *Proc. Natl. Acad. Sci. U.S.A.* **106**, 10290–10295 (2009).
67. I. Elia, M. Rossi, S. Stegen, D. Broekaert, G. Doglioni, M. van Gorsel, R. Boon, C. Escalona-Noguero, S. Torreken, C. Verfaillie, Breast cancer cells rely on environmental pyruvate to shape the metastatic niche. *Nature* **568**, 117–121 (2019).
68. X. Hu, A. Marietta, W.-X. Dai, Y.-Q. Li, X.-j. Ma, L. Zhang, S.-J. Cai, J.-J. Peng, Prediction of hepatic metastasis and relapse in colorectal cancers based on concordance analyses with liver fibrosis scores. *Clin. Transl. Med.* **9**, 1–15 (2020).
69. Y. Shen, X. Wang, J. Lu, M. Salfermoser, N. M. Wirsik, N. Schleussner, A. Imlé, A. F. Valls, P. Radhakrishnan, J. Liang, Reduction of liver metastasis stiffness improves response to bevacizumab in metastatic colorectal cancer. *Cancer Cell* **37**, 800–817.e807 (2020).
70. G. Pagé, P. Garteiser, B. E. Van Beers, Magnetic resonance elastography of malignant tumors. *Front. Phys.* **10**, 910036 (2022).
71. J. S. Desgrosellier, D. A. Cheresh, Integrins in cancer: Biological implications and therapeutic opportunities. *Nat. Rev. Cancer* **10**, 9–22 (2010).
72. K. Utispan, J. Sonongbua, P. Thuwajit, S. Chau-In, C. Pairijkul, S. Wongkham, C. Thuwajit, Periostin activates integrin α 5 β 1 through a PI3K/AKT-dependent pathway in invasion of cholangiocarcinoma. *Int. J. Oncol.* **41**, 1110–1118 (2012).
73. M. Katayama, A. Funakoshi, T. Sumii, N. Sanzen, K. Sekiguchi, Laminin γ 2-chain fragment circulating level increases in patients with metastatic pancreatic ductal cell adenocarcinomas. *Cancer Lett.* **225**, 167–176 (2005).
74. A. Hauge, E. K. Rofstad, Antifibrotic therapy to normalize the tumor microenvironment. *J. Transl. Med.* **18**, 1–11 (2020).
75. C. B. Thompson, H. M. Shepard, P. M. O'Connor, S. Kadhim, P. Jiang, R. J. Osgood, L. H. Bookbinder, X. Li, B. J. Sugarman, R. J. Connor, Enzymatic depletion of tumor hyaluronan induces antitumor responses in preclinical animal models. *Mol. Cancer Ther.* **9**, 3052–3064 (2010).
76. S. Shuster, G. I. Frost, A. B. Csoka, B. Formby, R. Stern, Hyaluronidase reduces human breast cancer xenografts in SCID mice. *Int. J. Cancer* **102**, 192–197 (2002).
77. S. R. Hingorani, A. J. Bullock, T. E. Seery, L. Zheng, D. Sigal, P. S. Ritch, F. S. Braithel, M. Zalupski, N. Bahary, W. P. Harris, J. Pu, C. Aldrich, S. Khelifa, X. W. Wu, J. Baranda, P. Jiang, A. E. Hendifar, Randomized phase II study of PEGPH20 plus nab-paclitaxel/gemcitabine (PAG) vs AG in patients (Pts) with untreated, metastatic pancreatic ductal adenocarcinoma (mPDA). *J. Clin. Oncol.* **35**, 4008 (2017).
78. G. J. Doherty, M. Tempero, P. G. Corrie, HALO-109–301: A phase III trial of PEGPH20 (with gemcitabine and nab-paclitaxel) in hyaluronic acid-high stage IV pancreatic cancer. *Future Oncol.* **14**, 13–22 (2018).
79. R. K. Ramanathan, S. L. McDonough, P. A. Philip, S. R. Hingorani, J. Lacy, J. S. Kortmanský, J. Thumar, E. G. Chiorean, A. F. Shields, D. Behl, Phase IB/II randomized study of FOLFIRINOX plus pegylated recombinant human hyaluronidase versus FOLFIRINOX alone in patients with metastatic pancreatic adenocarcinoma: SWOG S1313. *J. Clin. Oncol.* **37**, 1062–1069 (2019).
80. N. Chan, A. Willis, N. Kornhauser, M. M. Ward, S. B. Lee, E. Nackos, B. R. Seo, E. Chuang, T. Cigler, A. Moore, Influencing the tumor microenvironment: A phase II study of copper depletion using tetrathiomolybdate in patients with breast cancer at high risk for recurrence and in preclinical models of lung metastases. *Clin. Cancer Res.* **23**, 666–676 (2017).
81. A. B. Benson III, Z. A. Wainberg, J. R. Hecht, D. Vyushkov, H. Dong, J. Bendell, F. Kudrik, A phase II randomized, double-blind, placebo-controlled study of simtuzumab or placebo in combination with gemcitabine for the first-line treatment of pancreatic adenocarcinoma. *Oncologist* **22**, 241–e215 (2017).
82. J. R. Hecht, A. B. Benson III, D. Vyushkov, Y. Yang, J. Bendell, U. Verma, A phase II, randomized, double-blind, placebo-controlled study of simtuzumab in combination with FOLFIRI for the second-line treatment of metastatic KRAS mutant colorectal adenocarcinoma. *Oncologist* **22**, 243–e223 (2017).
83. R. Baker, J. Baskar, B. Charlton, G. S. Hobbs, W. Jarolimke, S.-E. Lee, L. Masarova, A.-M. Watson, Phase 1/2a study to evaluate safety, pharmacokinetic and pharmacodynamic dose escalation and expansion study of Pxs-5505 in patients with primary, post-polycythemia vera or post-essential thrombocythemia myelofibrosis. *Blood* **140**, 3947–3948 (2022).
84. J. How, Y. Liu, J. L. Story, D. S. Neuberger, K. Ravid, W. Jarolimke, B. Charlton, G. S. Hobbs, Evaluation of a pan-lysyl oxidase inhibitor, Pxs-5505, in myelofibrosis: A phase I, randomized, placebo controlled double blind study in healthy adults. *Blood* **136**, 16 (2020).
85. K. L. Aung, E. McWhirter, S. Welch, L. Wang, S. Lovell, L.-A. Stayner, S. Ali, A. Malpage, B. Makepeace, M. Ramchandran, A phase II trial of GSK2256098 and trametinib in patients with advanced pancreatic ductal adenocarcinoma. *J. Gastrointest. Oncol.* **13**, 3216–3226 (2022).
86. P. K. Brastianos, E. Twohy, E. R. Gerstner, T. J. Kaufmann, A. J. Iafrate, S. A. Jeyapalan, D. E. Piccioni, A. B. Lassman, C. E. Fadul, D. Schiff, Alliance A071401: Phase II trial of FAK inhibition in meningiomas with somatic NF2 mutations. *J. Clin. Oncol.* **38**, –2502 (2020).
87. D. E. Gerber, D. R. Camidge, D. Morgensztern, J. Cetnar, R. J. Kelly, S. S. Ramalingam, D. R. Spigel, W. Jeong, P. P. Scaglioni, S. Zhang, Phase 2 study of the focal adhesion kinase inhibitor defactinib (VS-6063) in previously treated advanced KRAS mutant non-small cell lung cancer. *Lung Cancer* **139**, 60–67 (2020).
88. D. M. Jackman, O. Jegede, M. G. Zauderer, E. P. Mitchell, J. Zwiebel, R. J. Gray, S. Li, L. McShane, L. Rubinstein, D. R. Patton, A phase 2 study of defactinib (VS-6063) in patients with NF2 altered tumors: Results from NCI-match (EAY131) subprotocol U. *J. Clin. Oncol.* **38**, –3087 (2021).
89. D. A. Fennell, P. Baas, P. Taylor, A. K. Nowak, D. Gilligan, T. Nakano, J. A. Pachter, D. T. Weaver, A. Scherpereel, N. Pavlakis, Maintenance defactinib versus placebo after first-line chemotherapy in patients with merlin-stratified pleural mesothelioma: COMMAND—A double-blind, randomized, phase II study. *J. Clin. Oncol.* **37**, 790–798 (2019).
90. X. Wu, J. Wang, Q. Liang, R. Tong, J. Huang, X. Yang, Y. Xu, W. Wang, M. Sun, J. Shi, Recent progress on FAK inhibitors with dual targeting capabilities for cancer treatment. *Biomed. Pharmacother.* **151**, 113116 (2022).
91. A. Kasi, J. Allen, K. Mehta, P. Dandawate, S. Saha, S. Bossmann, S. Anant, W. Sun, Association of losartan with outcomes in metastatic pancreatic cancer patients treated with chemotherapy. *J. Clin. Transl. Res.* **7**, 257 (2021).
92. J. E. Murphy, J. Y. Wo, D. P. Ryan, J. W. Clark, W. Jiang, B. Y. Yeap, L. C. Drapek, L. Ly, C. V. Baglini, L. S. Blaszkowsky, Total neoadjuvant therapy with FOLFIRINOX in combination with losartan followed by chemoradiotherapy for locally advanced pancreatic cancer: A phase 2 clinical trial. *JAMA Oncol.* **5**, 1020–1027 (2019).
93. M. Wu, H. Xu, J. Liu, X. Tan, S. Wan, M. Guo, Y. Long, Y. Xu, Metformin and fibrosis: A review of existing evidence and mechanisms. *J. Diabetes Res.* **2021**, 6673525 (2021).
94. P. J. Goodwin, B. E. Chen, K. A. Gelmon, T. J. Whelan, M. Ennis, J. Lemieux, J. A. Ligibel, D. L. Hershman, I. A. Mayer, T. J. Hobday, Effect of metformin vs placebo on invasive disease-free survival in patients with breast cancer: The MA. 32 randomized clinical trial. *JAMA* **327**, 1963–1973 (2022).
95. T. Komiya, J. Zhang, P. C. Neupane, K. Mehta, C. H. Huang, Combination of atezolizumab and pirfenidone in second-line and beyond NSCLC: A phase I/II study. *J. Clin. Oncol.* **39**, TP52678 (2021).
96. L. Devy, L. Huang, L. Naa, N. Yanamandra, H. Pieters, N. Frans, E. Chang, Q. Tao, M. Vanhove, A. Lejeune, Selective inhibition of matrix metalloproteinase-14 blocks tumor growth, invasion, and angiogenesis. *Cancer Res.* **69**, 1517–1526 (2009).
97. C. Lu, J. J. Lee, R. Komaki, R. S. Herbst, L. Feng, W. K. Evans, H. Choy, P. Desjardins, B. T. Esparaz, M. T., Chemoradiotherapy with or without AE-941 in stage III non-small cell lung cancer: A randomized phase III trial. *J. Natl. Cancer Inst.* **102**, 859–865 (2010).
98. E. I. Heath, B. A. Burtneis, L. Kleinberg, R. R. Salem, S. C. Yang, R. F. Heitmiller, M. I. Canto, J. P. Knisely, M. Topazian, E. Montgomery, Phase II, parallel-design study of preoperative combined modality therapy and the matrix metalloprotease (mmp) inhibitor prinomastat in patients with esophageal adenocarcinoma. *Invest. New Drugs* **24**, 135–140 (2006).
99. J. Bendell, S. Sharma, M. R. Patel, K. S. Windsor, Z. A. Wainberg, M. Gordon, J. Chaves, J. Berlin, C. B. Brachmann, M. Zavadovskaya, Safety and efficacy of andecaliximab (GS-5745) plus gemcitabine and nab-paclitaxel in patients with advanced pancreatic adenocarcinoma: Results from a phase I study. *Oncologist* **25**, 954–962 (2020).
100. M. A. Shah, D. Cunningham, J.-P. Metges, E. Van Cutsem, Z. A. Wainberg, E. Elboudwarej, K.-W. Lin, S. Turner, M. Zavadovskaya, D. Inzunza, Randomized, open-label, phase 2 study of andecaliximab plus nivolumab versus nivolumab alone in advanced gastric cancer identifies biomarkers associated with survival. *J. Immunother. Cancer* **9**, e003580 (2021).
101. S. R. Bramhall, J. Schulz, J. Nemanitis, P. D. Brown, M. Baillet, J. A. C. Buckels, A double-blind placebo-controlled, randomised study comparing gemcitabine and marimastat with gemcitabine and placebo as first line therapy in patients with advanced pancreatic cancer. *Br. J. Cancer* **87**, 161–167 (2002).

102. A. Winer, M. Janosky, B. Harrison, J. Zhong, D. Moussai, P. Siyah, N. Schatz-Siemers, J. Zeng, S. Adams, P. Mignatti, Inhibition of breast cancer metastasis by presurgical treatment with an oral matrix metalloproteinase inhibitor: A preclinical proof-of-principle study. *Mol. Cancer Ther.* **15**, 2370–2377 (2016).
103. M. Hussain, S. Le Moulec, C. Gimmi, R. Bruns, J. Straub, K. Miller, Differential effect on bone lesions of targeting integrins: Randomized phase II trial of abiraterone in patients with metastatic castration-resistant prostate cancer. *Clin. Cancer Res.* **22**, 3192–3200 (2016).
104. N. B. Leighl, L. Paz-Ares, J.-Y. Douillard, C. Peschel, A. Arnold, A. Depierre, A. Santoro, D. C. Betticher, U. Gatzemeier, J. Jassem, Randomized phase III study of matrix metalloproteinase inhibitor BMS-275291 in combination with paclitaxel and carboplatin in advanced non-small-cell lung cancer: National Cancer Institute of Canada–Clinical Trials Group Study Br. 18. *J. Clin. Oncol.* **23**, 2831–2839 (2005).
105. K. M. Bell-McGuinn, C. M. Matthews, S. N. Ho, M. Barve, L. Gilbert, R. T. Penson, E. Lengyel, R. Palaparthy, K. Gilder, A. Vassos, A phase II, single-arm study of the anti- $\alpha 5\beta 1$ integrin antibody volociximab as monotherapy in patients with platinum-resistant advanced epithelial ovarian or primary peritoneal cancer. *Gynecol. Oncol.* **121**, 273–279 (2011).
106. P. Hersey, J. Sosman, S. O'Day, J. Richards, A. Bedikian, R. Gonzalez, W. Sharfman, R. Weber, T. Logan, M. Buzoianu, A randomized phase 2 study of etaracizumab, a monoclonal antibody against integrin $\alpha 5\beta 3$, plus dacarbazine in patients with stage IV metastatic melanoma. *Cancer* **116**, 1526–1534 (2010).
107. M. Bharadwaj, N. Strohmeier, G. P. Colo, J. Helenius, N. Beerenwinkel, H. B. Schiller, R. Fässler, D. J. Müller, αV -class integrins exert dual roles on $\alpha 5\beta 1$ integrins to strengthen adhesion to fibronectin. *Nat. Commun.* **8**, 1–10 (2017).
108. A. Taherian, X. Li, Y. Liu, T. A. Haas, Differences in integrin expression and signaling within human breast cancer cells. *BMC Cancer* **11**, 1–15 (2011).
109. I. Eke, A. Y. Makinde, M. J. Aryankalayil, J. L. Reedy, D. E. Citrin, S. Chopra, M. M. Ahmed, C. N. Coleman, Long-term tumor adaptation after radiotherapy: Therapeutic implications for targeting integrins in prostate cancer. *Mol. Cancer Res.* **16**, 1855–1864 (2018).
110. T. Wang, G. Li, D. Wang, F. Li, D. Men, T. Hu, Y. Xi, X.-E. Zhang, Quantitative profiling of integrin $\alpha 5\beta 3$ on single cells with quantum dot labeling to reveal the phenotypic heterogeneity of glioblastoma. *Nanoscale* **11**, 18224–18231 (2019).
111. S. Guo, X. Wu, T. Lei, R. Zhong, Y. Wang, L. Zhang, Q. Zhao, Y. Huang, Y. Shi, L. Wu, The role and therapeutic value of syndecan-1 in cancer metastasis and drug resistance. *Front. Cell Dev. Biol.* **9**, 784983 (2022).
112. A. Elkmahawy, Q. Lu, H. Nada, J. Woo, G. Quan, K. Lee, The journey of DDR1 and DDR2 kinase inhibitors as rising Stars in the fight against cancer. *Int. J. Mol. Sci.* **22**, 6535 (2021).
113. V. Chung, A. Alistar, B. George, K. Kim, H. Kindler, D. Oh, S. Allen, H. Barak, B. Ci, J. Lau, SO-4 phase Ib/II, open-label, randomised evaluation of atezolizumab plus RO6874281 vs control in MORPHEUS—pancreatic ductal adenocarcinoma. *Ann. Oncol.* **31**, S218 (2020).
114. R. D. Hofheinz, S. E. Al-Batran, F. Hartmann, G. Hartung, D. Jäger, C. Renner, P. Tanswell, U. Kunz, A. Amelsberg, H. Kuthan, Stromal antigen targeting by a humanised monoclonal antibody: An early phase II trial of sibrutumab in patients with metastatic colorectal cancer. *Onkologie* **26**, 44–48 (2003).
115. A. Italiano, L. Verlingue, H. Prenen, E. M. Guerra, D. Tosi, R. Perets, I. Lugowska, V. Moiseenko, M. Gumus, C. Arslan, C. R. Lindsay, M. Richard, D. DeJardin, C. Boetsch, A. Kraxner, S. Evers, T. Vardar, N. Keshelava, V. Teichgräber, R. Dziadziszko, Clinical activity and safety of simlukafusp alfa, an engineered interleukin-2 variant targeted to fibroblast activation protein- α , combined with atezolizumab in patients with recurrent or metastatic cervical cancer. *J. Clin. Oncol.* **39**, –5510 (2021).
116. E. Sahai, I. Astsaturov, E. Cukierman, D. G. DeNardo, M. Egeblad, R. M. Evans, D. Fearon, F. R. Greten, S. R. Hingorani, T. Hunter, A framework for advancing our understanding of cancer-associated fibroblasts. *Nat. Rev. Cancer* **20**, 174–186 (2020).
117. Y. Kim, M.-o. Kim, J. S. Shin, S. H. Park, S. B. Kim, J. Kim, S. C. Park, C. J. Han, J. K. Ryu, Y. B. Yoon, Hedgehog signaling between cancer cells and hepatic stellate cells in promoting cholangiocarcinoma. *Ann. Surg. Oncol.* **21**, 2684–2698 (2014).
118. D. A. Richards, J. Stephenson, B. M. Wolpin, C. Becerra, J. T. Hamm, W. A. Messersmith, S. Devens, J. Cushing, T. Schmalbach, C. S. Fuchs, A phase Ib trial of IPI-926, a hedgehog pathway inhibitor, plus gemcitabine in patients with metastatic pancreatic cancer. *J. Clin. Oncol.* **30**, 213 (2012).
119. M. H. Sherman, T. Y. Ruth, D. D. Engle, N. Ding, A. R. Atkins, H. Tiriaci, E. A. Collisson, F. Connor, T. Van Dyke, S. Kozlov, Vitamin D receptor-mediated stromal reprogramming suppresses pancreatitis and enhances pancreatic cancer therapy. *Cell* **159**, 80–93 (2014).
120. H. M. Kocher, B. Basu, F. E. M. Froeling, D. Sarker, S. Slater, D. Carlin, N. M. deSouza, K. N. De Paepe, M. R. Goulart, C. Hughes, Phase I clinical trial repurposing all-trans retinoic acid as a stromal targeting agent for pancreatic cancer. *Nat. Commun.* **11**, 1–9 (2020).
121. A. L. McKnight, J. L. Kugel, P. J. Rossman, A. Manduca, L. C. Hartmann, R. L. Ehman, MR elastography of breast cancer: Preliminary results. *Am. J. Roentgenol.* **178**, 1411–1417 (2002).
122. J. Lorenzen, R. Sinkus, M. Lorenzen, M. Dargatz, C. Leussler, P. Röschmann, G. Adam, in *RöFo-Fortschritte auf dem Gebiet der Röntgenstrahlen und der bildgebenden Verfahren* (Georg Thieme Verlag, 2002), vol. 174, pp. 830–834.
123. P. Garteiser, S. Doblaz, J.-L. Daire, M. Wagner, H. Leitao, V. Vilgrain, R. Sinkus, B. E. Van Beers, MR elastography of liver tumours: Value of viscoelastic properties for tumour characterisation. *Eur. Radiol.* **22**, 2169–2177 (2012).
124. S. N. Araoz, MR elastography of liver tumors: Preliminary results. *Am. J. Roentgenol.* **190**, 1534–1540 (2008).
125. Y. Itoh, Y. Takehara, T. Kawase, K. Terashima, Y. Ohkawa, Y. Hirose, A. Koda, N. Hyodo, T. Ushio, Y. Hirai, Feasibility of magnetic resonance elastography for the pancreas at 3T. *J. Magn. Reson. Imaging* **43**, 384–390 (2016).
126. S. Li, M. Chen, W. Wang, W. Zhao, J. Wang, X. Zhao, C. Zhou, A feasibility study of MR elastography in the diagnosis of prostate cancer at 3.0T. *Acta Radiol.* **52**, 354–358 (2011).
127. R. Reiter, S. Majumdar, S. Kearney, A. Kajdacsy-Balla, V. Macias, S. Crivellaro, B. Caldwell, M. Abern, T. J. Royston, D. Klatt, Prostate cancer assessment using MR elastography of fresh prostatectomy specimens at 9.4 T. *Magn. Reson. Med.* **84**, 396–404 (2020).
128. D. B. Zhen, M. Whittle, P. S. Ritch, H. S. Hochster, A. L. Coveler, B. George, A. E. Hendifar, T. Dragovich, S. Green, B. Dion, A. C. Stoll-D'Astice, A. Lee, S. M. Thorsen, A. Rosenthal, S. R. Hingorani, E. G. Chiorean, Phase II study of PEGPH20 plus pembrolizumab for patients (pts) with hyaluronan (HA)-high refractory metastatic pancreatic adenocarcinoma (mPC): PCRT16-001. *J. Clin. Oncol.* **40**, 576 (2022).
129. M. A. Tempero, E. V. Cutsem, D. Sigal, D.-Y. Oh, N. Fazio, T. Macarulla, E. Hitre, P. Hammel, A. E. Hendifar, S. E. Bates, C.-P. Li, C. D. L. Fouchardiere, V. Heinemann, A. Maraveyas, N. Bahary, L. Layos, V. Sahai, L. Zheng, J. Lacy, A. J. Bullock, H.-, HALO 109-301: A randomized, double-blind, placebo-controlled, phase 3 study of pegvorhyaluronidase alfa (PEGPH20) + nab-paclitaxel/gemcitabine (AG) in patients (pts) with previously untreated hyaluronan (HA)-high metastatic pancreatic ductal adenocarcinoma (mPDA). *J. Clin. Oncol.* **38**, 638 (2020).
130. M. V. Babak, D. Ahn, Modulation of intracellular copper levels as the mechanism of action of anticancer copper complexes: Clinical relevance. *Biomedicine* **9**, 852 (2021).
131. S. Verstovsek, M. R. Savona, R. A. Mesa, H. Dong, J. D. Maltzman, S. Sharma, J. Silverman, S. T. Oh, J. Gotlib, A phase 2 study of simtuzumab in patients with primary, post-polycythaemia vera or post-essential thrombocythaemia myelofibrosis. *Br. J. Haematol.* **176**, 939–949 (2017).
132. D. Bissett, K. J. O'Byrne, J. Von Pawel, U. Gatzemeier, A. Price, M. Nicolson, R. Mercier, E. Mazabel, C. Penning, M. H. Zhang, Phase III study of matrix metalloproteinase inhibitor prinomastat in non-small-cell lung cancer. *J. Clin. Oncol.* **23**, 842–849 (2004).
133. M. A. Shah, G. Bodoky, A. Starodub, D. Cunningham, D. Yip, Z. A. Wainberg, J. Bendell, D. Thai, J. He, P. Bhargava, Phase III study to evaluate efficacy and safety of andecaliximab with mFOLFOX6 as first-line treatment in patients with advanced gastric or GEJ adenocarcinoma (GAMMA-1). *J. Clin. Oncol.* **39**, 990–1000 (2021).
134. N. Cook, U. Banerji, J. Evans, A. Biondo, T. Germetaki, M. Randhawa, L. Godfrey, S. Leslie, P. Jeffrey, M. Rigby, Pharmacokinetic (PK) assessment of BT1718: A phase I/II study of BT1718, a first in class bicycle toxin conjugate (BTC), in patients (pts) with advanced solid tumours. *Ann. Oncol.* **30**, v174 (2019).
135. E. Elez, I. Kocáková, T. Höhler, U. M. Martens, C. Bokemeyer, E. Van Cutsem, B. Melichar, M. Smakal, T. Csösz, E. Topuzov, Abiraterone combined with cetuximab plus irinotecan versus cetuximab plus irinotecan alone for patients with KRAS wild-type metastatic colorectal cancer: The randomised phase I/II POSEIDON trial. *Ann. Oncol.* **26**, 132–140 (2015).
136. T. Evans, R. Ramanathan, S. Yazji, R. Glynne-Jones, A. Anthonay, J. Berlin, J. Valle, Final results from cohort 1 of a phase II study of volociximab, an anti- $\alpha 5\beta 1$ integrin antibody, in combination with gemcitabine (GEM) in patients (pts) with metastatic pancreatic cancer (MPC). *J. Clin. Oncol.* **25**, –4549 (2007).
137. R. Figlin, G. Kondagunta, S. Yazji, R. Motzer, R. Bukowski, Phase II study of volociximab (M200), an $\alpha 5\beta 1$ anti-integrin antibody in refractory metastatic clear cell renal cell cancer (RCC). *J. Clin. Oncol.* **24**, –4535 (2006).
138. J. Barton, A multicenter phase II study of volociximab in patients with relapsed metastatic melanoma. *J. Clin. Oncol.* **26**, –9051 (2008).
139. I. Melero, E. C. Alvarez, M. Mau-Sorensen, U. Lassen, M. Lolkema, D. Robbrecht, C. Gomez-Roca, J. Martin-Liberal, J. Taberner, W. Ros, Clinical activity, safety, and PK/PD from a phase I study of RO6874281, a fibroblast activation protein (FAP) targeted interleukin-2 variant (IL-2v). *Ann. Oncol.* **29**, viii134–viii135 (2018).
140. D. V. Catenacci, M. R. Juntilla, T. Karrison, N. Bahary, M. N. Horiba, S. R. Nattam, R. Marsh, J. Wallace, M. Kozloff, L. Rajdev, Randomized phase Ib/II study of gemcitabine plus placebo or vismodegib, a hedgehog pathway inhibitor, in patients with metastatic pancreatic cancer. *J. Clin. Oncol.* **33**, 4284–4292 (2015).
141. E. J. Kim, V. Sahai, E. V. Abel, K. A. Griffith, J. K. Greenson, N. Takebe, G. N. Khan, J. L. Blau, R. Craig, U. G. Balis, Pilot clinical trial of hedgehog pathway inhibitor GDC-0449 (vismodegib) in combination with gemcitabine in patients with metastatic pancreatic adenocarcinoma. *Clin. Cancer Res.* **20**, 5937–5945 (2014).
142. A. De Jesus-Acosta, E. A. Sugar, P. J. O'Dwyer, R. K. Ramanathan, D. D. Von Hoff, Z. Rasheed, L. Zheng, A. Begum, R. Anders, A. Maitra, Phase 2 study of vismodegib, a hedgehog inhibitor, combined with gemcitabine and nab-paclitaxel in patients with untreated metastatic pancreatic adenocarcinoma. *Br. J. Cancer* **122**, 498–505 (2020).

143. T. S. Hong, B. Y. Yeap, N. K. Horick, J. Y.-L. Wo, C. D. Weekes, J. N. Allen, M. Qadan, P. E. Oberstein, R. K. Jain, L. S. Blaszkowsky, B. M. Wolpin, D. A. Laheru, W. A. Messersmith, L. Ly, L. C. Drapek, D. T. Ting, R. A. Burkhart, C. F.-d. Castillo, A. Kimmelman, D. P. Ryan, A multicenter, randomized phase II study of total neoadjuvant therapy (TNT) with FOLFIRINOX (FFX) and SBRT, with or without losartan (L) and nivolumab (N) in borderline resectable (BR) and locally advanced (LA) pancreatic ductal adenocarcinoma (PDAC). *J. Clin. Oncol.* **41**, 719 (2023).
144. M. J. Paszek, N. Zahir, K. R. Johnson, J. N. Lakin, G. I. Rozenberg, A. Gefen, C. A. Reinhart-King, S. S. Margulies, M. Dembo, D. Boettiger, Tensional homeostasis and the malignant phenotype. *Cancer Cell* **8**, 241–254 (2005).
145. R. W. Tilghman, C. R. Cowan, J. D. Mih, Y. Koryakina, D. Gioeli, J. K. Slack-Davis, B. R. Blackman, D. J. Tschumperlin, J. T. Parsons, Matrix rigidity regulates cancer cell growth and cellular phenotype. *PLOS ONE* **5**, e12905 (2010).
146. O. Chaudhuri, J. Cooper-White, P. A. Janmey, D. J. Mooney, V. B. Shenoy, Effects of extracellular matrix viscoelasticity on cellular behaviour. *Nature* **584**, 535–546 (2020).
147. E. Gachon, P. Mesquida, Stretching single collagen fibrils reveals nonlinear mechanical behavior. *Biophys. J.* **118**, 1401–1408 (2020).
148. M. S. Hall, F. Alisafaei, E. Ban, X. Feng, C.-Y. Hui, V. B. Shenoy, M. Wu, Fibrous nonlinear elasticity enables positive mechanical feedback between cells and ECMs. *Proc. Natl. Acad. Sci. U.S.A.* **113**, 14043–14048 (2016).
149. C. T. Mierke, Viscoelasticity acts as a marker for tumor extracellular matrix characteristics. *Front. Cell Dev. Biol.* **9**, 785138 (2021).
150. D. Huang, Y. Nakamura, A. Ogata, S. Kidoaki, Characterization of 3D matrix conditions for cancer cell migration with elasticity/porosity-independent tunable microfiber gels. *Polym. J.* **52**, 333–344 (2020).
151. M. W. Conklin, J. C. Eickhoff, K. M. Ricking, C. A. Pehlke, K. W. Eliceiri, P. P. Provenzano, A. Friedl, P. J. Keely, Aligned collagen is a prognostic signature for survival in human breast carcinoma. *Am. J. Pathol.* **178**, 1221–1232 (2011).

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