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The extracellular matrix modulates the hallmarks of cancer

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Abstract

The extracellular matrix regulates tissue development and homeostasis, and its dysregulation contributes to neoplastic progression. The extracellular matrix serves not only as the scaffold upon which tissues are organized but provides critical biochemical and biomechanical cues that direct cell growth, survival, migration and differentiation and modulate vascular development and immune function. Thus, while genetic modifications in tumor cells undoubtedly initiate and drive malignancy, cancer progresses within a dynamically evolving extracellular matrix that modulates virtually every behavioral facet of the tumor cells and cancer-associated stromal cells. Hanahan and Weinberg defined the hallmarks of cancer to encompass key biological capabilities that are acquired and essential for the development, growth and dissemination of all human cancers. These capabilities include sustained proliferation, evasion of growth suppression, death resistance, replicative immortality, induced angiogenesis, initiation of invasion, dysregulation of cellular energetics, avoidance of immune destruction and chronic inflammation. Here, we argue that biophysical and biochemical cues from the tumor-associated extracellular matrix influence each of these cancer hallmarks and are therefore critical for malignancy. We suggest that the success of cancer prevention and therapy programs requires an intimate understanding of the reciprocal feedback between the evolving extracellular matrix, the tumor cells and its cancer-associated cellular stroma.

Keywords ECM; hallmarks of cancer; mechanotransduction

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See the Glossary for abbreviations used in this article

Introduction

The extracellular matrix (ECM) regulates the development and maintains tissue homeostasis [1]. The ECM is composed of a complex network of macromolecules that assemble into three-dimensional

supramolecular structures with distinct biochemical and biomechanical properties that regulate cell growth, survival, motility and differentiation by ligating specific receptors such as integrins, syndecans and discoidin receptors [2,3]. The ECM also provides the structural foundation for tissue function and mechanical integrity, regulates the availability of growth factors and cytokines and maintains the hydration and pH of the local microenvironment. A critical aspect of the ECM is that it is dynamically remodeled and specifically tailored to the structure/function of each organ, and its composition, biomechanics and anisotropy are exquisitely tuned to reflect the physiological state of the tissue [4,5].

Tumors often display desmoplasia, and this fibrotic state is characterized by increased deposition, an altered organization and enhanced post-translational modifications of ECM proteins [6]. The cancer-associated ECM is not only an integral feature of a tumor but also actively contributes to its histopathology and behavior [7,8]. For instance, patients with pancreatic cancer show a marked stromal desmoplasia that often associates with tumor progression and poor disease outcome [9]. Similarly, expression of matrix remodeling genes such as MMPs and collagen cross-linkers is predictive of a poor prognosis for breast cancer patients [10,11]. Fibrosis can also predispose a tissue to malignancy; patients with cirrhosis of the liver or cystic fibrosis, conditions that are characterized by abnormal accumulation of collagen, have an increased risk of developing cancer [12,13]. Moreover, increased mammographic density, which associates with increased collagen deposition, correlates with an elevated risk of developing breast cancer [14]. Indeed, MMPs and high mechanical stress are predictive of tumor formation in breast cancer patients [15].

Originally described by Hanahan and Weinberg [16], the hallmarks of cancer encompass fundamental biological capabilities acquired during the development of human cancers including sustained proliferation, evasion of growth suppression, death resistance, replicative immortality, induced angiogenesis, and initiation of invasion and metastasis. In 2011, Hanahan and Weinberg [17] revisited the hallmarks, adding two emerging features: dysregulated cellular metabolism and the evasion of immune destruction. Importantly, the ECM regulates many of the same cellular responses that characterize the cancer hallmarks (Fig 1). This overlap suggests that the biochemical and biophysical properties of the ECM should be

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Glossary	
Anoikis	Form of programmed cell death, which is induced by anchorage-dependent cells detaching from the surrounding ECM
Akt	v-akt murine thymoma viral oncogene homolog
APC	Antigen-presenting cell
Bax	BCL2-associated X protein
Bcl2	B-cell CLL/lymphoma 2
Bim	BCL2-like 11
BRCA1	Breast cancer 1
CD3	Cluster of differentiation 3
CD28	Cluster of differentiation 28
CDK	Cyclin-dependent kinase
Chemoattractant	A cytokine that induces the movement of a cell toward a higher concentration of the chemical signal
DDR1	Discoidin domain receptor tyrosine kinase 1
Desmoplasia	The growth of fibrous or connective tissue
EGFR	Epidermal growth factor receptor
EMT	Epithelial to mesenchymal transition
ErbB2	v-erb-b2 avian erythroblastic leukemia viral oncogene homolog 2
Erk	Extracellular regulated MAP kinase
Fak	Focal adhesion kinase
FGF	Fibroblast growth factor
Extravasation	The movement of a cell out of the circulatory system
G1/S transition	A restriction point between the G1 phase and the S phase of the cell cycle, which must meet a specific set of requirements to be overcome. Progression through this point signifies a point of no return for cell cycle progression
GATA2	GATA binding protein 2
GLUT1	Glucose transporter 1
Hemidesmosome	A small bud-like structure attaching an epithelial cell to the basal lamina
HER2	Human epidermal growth factor receptor 2
IL-2	Interleukin 2
Intravasation	The invasion of cancer cells through the basement membrane and into the blood or lymphatic vessel
ITGB1	Integrin, beta 1
LAIR	Leukocyte-associated immunoglobulin-like receptor
LPS	Lipopolysaccharide
Mdm2	Transformed mouse 3T3 cell double minute 2, E3 ubiquitin ligase and proto-oncogene
Mechanotransduction	The means by which a cell converts mechanical stimulus from the ECM into downstream signaling changes
MMP	Matrix metalloproteinase
Myc	Myelocytomatosis oncogene
Neoadjuvant	The administration of a therapeutic agent before the standard treatment regimen, usually to enhance the efficacy of the conventional intervention
NF-κB	Nuclear factor kappa B
p130Cas	RAB3 GTPase-activating protein subunit 1
p21	Cyclin-dependent kinase inhibitor 1A
p27	Cyclin-dependent kinase inhibitor 1B
p53	Transformation-related protein 53
PI3K	Phosphatidylinositol 3-kinase
Posttranslational modifications	a step in protein biosynthesis in which the chemical or structural nature of the amino acids comprising a protein is altered after the translation of the protein
PTEN	Phosphatase and tensin homolog
Rac	Ras-related C3 botulinum toxin substrate 1
RAF	Raf-1 proto-oncogene
Ras	Rat sarcoma viral oncogene homolog
Rho	Rhodopsin
Shh	Sonic hedgehog
Smad	Mothers against DPP homologs
SPARC	Secreted protein acidic and rich in cysteine
Src	Rous sarcoma oncogene
TAZ	Transcriptional co-activator with PDZ binding motif
TCA cycle	Tricarboxylic acid cycle, also Krebs cycle
TFII-I	General transcription factor II-I
TGF-β	Transforming growth factor β
Th1 cell	Type 1 helper T cell
TNF-α	Tumor necrosis factor α
Tumor-associated ECM	Extracellular matrix that has been modified over the course of tumor progression to have altered composition, density and mechanical properties
VEGF	Vascular endothelial growth factor
VEGFR2	Vascular endothelial growth factor receptor 2
YAP	Yes-associated protein

considered when examining tumor behavior and therapeutic interventions [18]. In this review, we discuss how the composition and the mechanical properties of the ECM influence the acquisition and maintenance of each of the original and emerging cancer hallmarks.

Sustaining proliferative signaling

Cellular transformation and tumor progression require escape from proliferative suppression. Proliferation is initiated by the ligation of growth factor receptors whose activation promotes intracellular signaling that facilitates cell cycle progression. Cell cycle progression in turn is tightly controlled by the G1/S cell cycle checkpoint. G1/S cell cycle transition requires cellular adhesion to the ECM. Adhesion to the ECM permits growth factor-dependent activation of Ras, which through Erk signaling promotes G1/S transition [19–21].

Adhesion-dependent Fak phosphorylation stimulates Ras and PI3K signaling to activate Erk, promoting its nuclear translocation and cyclin D1 induction to sequester the growth suppressors CDK1 and CDK4 [22]. Although Erk mediates cell cycle progression in fibroblasts, recent work suggests that the Rac GTPases mediate adhesion-dependent cyclin D1 expression in epithelial cells. Interestingly, many malignantly transformed cells secrete their own ECM ligands and, in doing so, are able to escape proliferative suppression to grow and survive in hostile environments [23,24]. Tumor cells which acquire the ability to synthesize their own ECM proteins are shown to be highly metastatic [25]. Indeed, transformation by oncogenes such as Ras, which stimulates Erk signaling to promote anchorage independence for growth and survival, simultaneously induces expression of several ECM proteins [26]. In addition, a malignant tissue is typically stiffer than its normal counterpart, and this altered biomechanical property is largely mediated by a highly

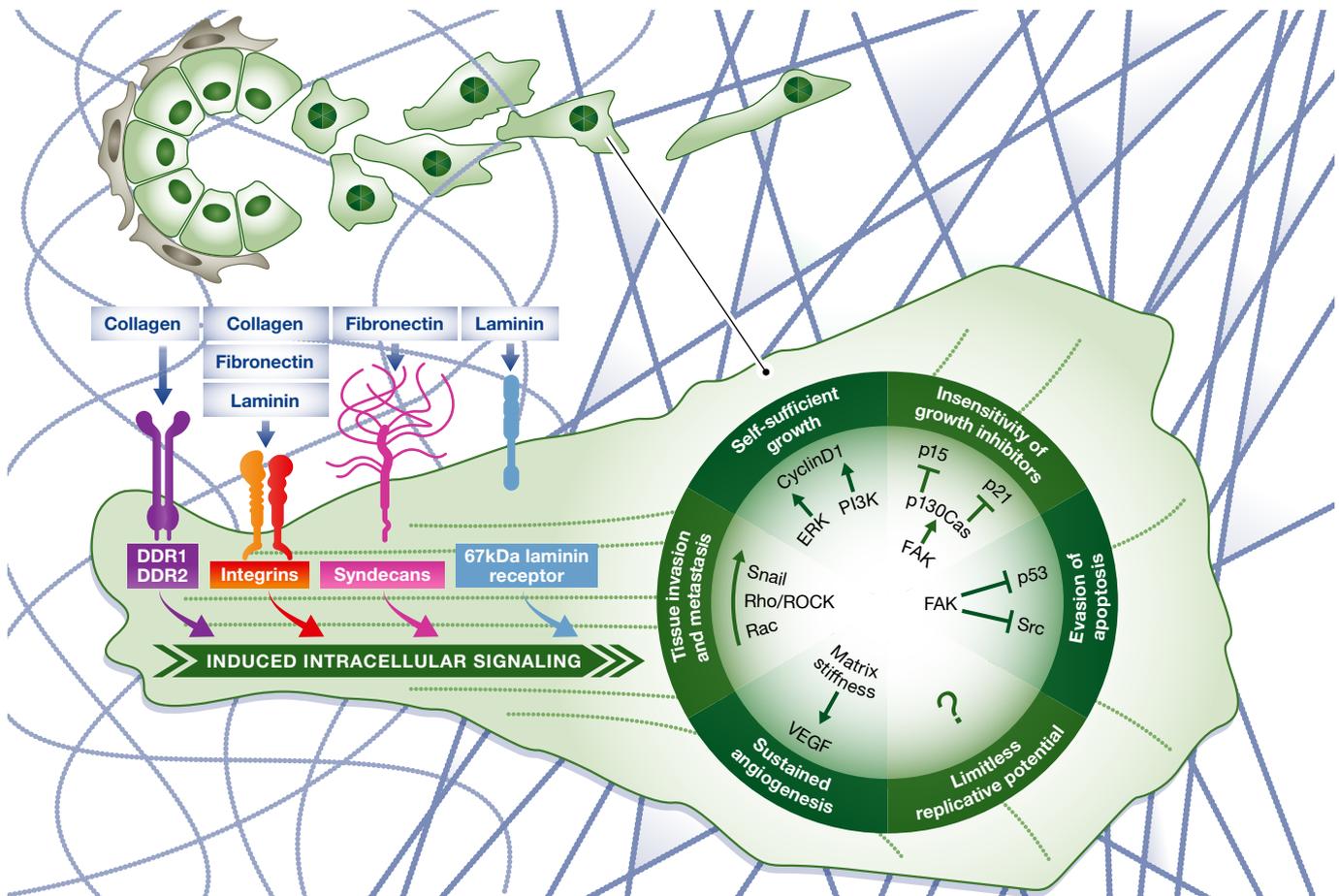


Figure 1. Influences of ECM on the hallmarks of cancer.

From tumor initiation to metastasis, the ECM influences each of the classically defined and emerging hallmarks of cancer as first described by Hanahan and Weinberg in 2000 and amended in 2011. ECM molecules bind to cell surface receptors, which activates intracellular signaling pathways. ECM adhesion-induced signals through ERK and PI3K promote self-sufficient growth [22]. FAK signaling inhibits growth suppressors p15 and p21 and limits the induction of apoptosis through p53 [42]. ECM components and biophysical properties promote EMT induction and enhance pro-migratory pathways, particularly TGF- β and Rho/Rac signaling [96]. ECM stiffness also enhances angiogenesis and increases VEGF signaling in endothelial cells [78]. At each phase of tumorigenesis, the ECM adapts to reinforce the progression of the disease through promotion of the hallmarks.

cross-linked and oriented collagenous ECM [6,8] (see [27–29] for review). This stiffened ECM associates with tumor aggression and correlates with an increased propensity toward metastasis and poorer patient outcome [6,30]. Consistently, cells interacting with a stiffer ECM proliferate more in response to growth factors and express genes that positively correlate with a proliferative signature [7,31]. Indeed, in response to a stiffened matrix, cells elevate Fak phosphorylation and stimulate Erk, PI3K and Rac, which accelerates cell cycle progression through increased expression of cyclin D1 [7,32–34]. Thus, the ECM and its receptors regulate cell proliferation, and corruption of these interactions modulates tumor progression.

Evading growth suppressors

Cellular quiescence must be overcome to establish a neoplastic lesion. Many tumor suppressors limit cell cycle transitions by blocking progression from G1 to S phase of the cell cycle [35,36].

Activated p53 and Smad phosphorylation by TGF- β induces proteins such as p21 and p27 that inhibit the activity of cyclin-dependent kinases that are critical for cell cycle progression, thus suppressing cell growth [37–40]. However, cell ligation to an ECM can temper the activity of many of these tumor suppressor pathways, thereby overriding this growth suppression mechanism [41]. For instance, cell–ECM interactions regulate TGF- β signaling by inducing p130Cas to prevent Smad3 phosphorylation and reduce p15 and p21 expressions to subvert cell cycle arrest [42]. ECM adhesion also directly and indirectly inhibits the function of tumor suppressors such as BRCA1, thereby compromising cell cycle checkpoint control [43]. In this manner, an increase in tumor cell adhesion to the ECM can circumvent many of the normal growth suppression pathways to foster malignant transformation. Indeed, the quiescence or dormant state observed in some extravasated metastatic cells may be due, at least in part, to their inability to actively engage the ECM and activate integrin and growth factor-dependent signaling at the secondary site [44,45]. Thus, in the absence of integrin-mediated adhesion to the ECM, Src and Erk signaling is not activated, and tumor

suppressor levels of cell cycle inhibitors such as p27 remain high, preventing cell proliferation [46,47]. Consistently, lung tumor metastasis is enhanced by ECM pre-conditioning, stiffening with lysyl oxidase and enhanced fibronectin deposition, which may foster tumor cell growth and survival by overriding the activity of these tumor suppressors. Indeed, a stiffened ECM reduces the expression of genes that typically inhibit cell cycle progression [27,48,49]. Matrix stiffness also induces the expression of microRNAs that lower expression of the tumor suppressor PTEN, thereby enhancing PI3K/Akt activity to promote cell growth and survival (Fig 2) [50]. In this respect, the HIPPO pathway components YAP and TAZ regulate cell proliferation and apoptosis to control organ development, and the activity of these transcription factors is exquisitely sensitive to mechanical cues from the ECM. Moreover, overexpression or mechanical activation of YAP permits tumor cells to overcome growth suppression by contact inhibition and to achieve uncontrolled proliferation [51,52]. These findings argue that tumor cells that are able to leverage interactions with the ECM should have a distinct growth advantage.

Resisting cell death

Malignant transformation is accompanied by enhanced cell survival. Cell death is mediated by the cleavage of cell death-associated caspases and the mitochondrial release of pro-apoptotic proteins such as cytochrome c, with tight regulation by a coterie of pro- and anti-apoptotic molecules including those from the Bcl2 family [53]. Oncogenic transformation is frequently accompanied by the acquisition of anchorage-independent survival (suppression of anoikis) as occurs when the receptor tyrosine kinase ErbB2 is overexpressed and Bim is inhibited through increased Erk activation [54]. Similarly, cell adhesion to an ECM inactivates pro-apoptotic

molecules such as Bax and induces expression of several anti-apoptotic genes including Bcl2 to promote cell survival [55–57]. Likewise, laminin ligation of $\alpha 6\beta 4$ integrin permits EGFR activation of Rac to promote anchorage-independent survival by stimulating NF- κ B [58].

Intriguingly, ECM ligation can also enhance a cell's ability to resist apoptosis induction. Indeed, breast tumor stiffness, a feature associated with elevated integrin signaling, associates positively with reduced chemotherapeutic responsiveness [59]. Although the molecular mechanisms underlying this phenotype remain poorly understood, prior studies suggest that activation of $\beta 1$ integrin and FAK via ECM ligation can suppress p53-induced apoptosis through Mdm2-mediated ubiquitination and p53 degradation in response to DNA damaging agents [60–62]. Moreover, tissue polarity, mediated through laminin ligation of $\alpha 6\beta 4$ integrin and hemidesmosome formation, permits NF- κ B activation and death resistance of mammary tumor cells in response to a plethora of chemotherapeutic and immune receptor death stimuli [63].

Enabling replicative immortality

The unrestrained growth observed in many tumors associates with replicative immortality. Normal cells demonstrate limited replicative ability due primarily to shortening of telomeres, which are the regions of noncoding nucleotide sequence at the end of each chromosome. Because conventional DNA polymerases are unable to replicate the entire DNA strand, the telomere at the end of each chromosome progressively shortens after each cell division. Cancer cells overcome this limitation by expressing the enzyme telomerase, which elongates the telomeres, and thereby, overcoming replicative senescence. Interestingly, patients with idiopathic pulmonary

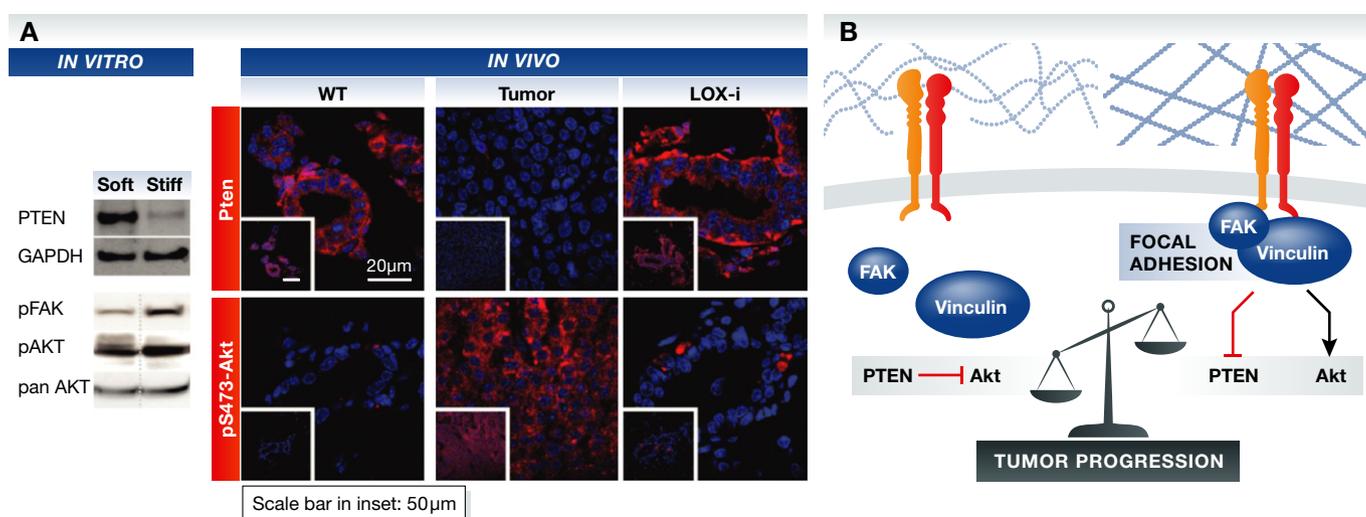


Figure 2. Effects of matrix rigidity on tumor progression.

Recent work from Mouw *et al* [50] shows how matrix rigidity impacts on tumor progression. (A) Culturing MCF10a cells on stiff polyacrylamide gels *in vitro* promotes FAK phosphorylation and suppresses the levels of the tumor suppressor PTEN. *In vivo* inhibition of collagen cross-linking (LOX-i) in the polyoma middle T (PyMT) mouse model of breast cancer results in the opposite phenotype, with PTEN levels being increased feeding into a suppression of Akt activity. (B) These data suggest that the stiffening of the ECM works through focal adhesions to inhibit tumor suppressors and promote tumor progression.

fibrosis, which is characterized by increased deposition of ECM proteins and tissue stiffening, exhibit elevated levels of telomerase, suggesting increased ECM adhesion may influence the replicative behavior of cells [64,65]. Indeed, epithelial cells expressing high levels of ITGB1 were enriched for telomerase activity [66].

Inducing angiogenesis

Tumors stimulate neo-vascularization to provide the oxygen and nutrients required for their growth and survival [67]. Angiogenesis is stimulated by growth factors such as VEGF and FGF and involves the proliferation and migration of endothelial cells into the nutrient-deprived tissue, specifically regions adjacent to the tumor followed by their assembly into patent blood vessels [68,69]. The ECM surrounding the tumor acts as a reservoir for pro- and anti-angiogenic factors, provides a conduit for the migration of endothelial cells, and fosters the growth and survival of newly recruited endothelial cells [70–75]. A stiffened tumor-associated ECM also favors angiogenesis by promoting endothelial cell migration and by inducing GATA2 and TFII-1 transcriptional programs to enhance expression of VEGFR2 receptors that support endothelial cell growth and survival [76–80]. Nevertheless, a highly rigid ECM can also compromise vascular integrity and activate MMPs that degrade the ECM by releasing anti-angiogenic factors. These data indicate that the ECM can both promote and inhibit angiogenesis [76,77,81].

Activating invasion and metastasis

Malignant transformation is defined as the invasion of transformed cells into the adjacent parenchyma and requires the acquisition of a motile, invasive phenotype [82]. Actin-rich

protrusions, termed invadopodia, which require integrin-mediated adhesion and focal adhesion formation, direct tumor cell invasion through localized MMP-mediated matrix degradation [83,84]. ECM stiffness promotes invadopodia formation and enhances tumor cell invasion by driving focal adhesion assembly [85]. Once the physical barriers surrounding a benign tumor are compromised, tumor cell migration is driven through elevated activity of Rho and Rac GTPases, which stimulate actin assembly and turnover and actomyosin-dependent cell tension [86]. Thereafter, the nature of the migratory phenotype is dictated by the activity of the dominant Rho-family GTPase; a mesenchymal migratory phenotype is largely dictated by the activity of Rac GTPases while elevated RhoA GTPase activity favors ameboid migration [87]. Consistently, the oncogene Ras stimulates Rho activity to promote an ameboid migratory phenotype, whereas the tumor suppressor p53 inhibits tumor cell migration by reducing RhoA activity [88,89]. Once within the parenchyma, tumor cell metastasis depends upon efficient navigation through the tissue to the vasculature, its successful intravasation into the circulatory system (blood or lymphatic vessels), where the tumor cell can disseminate throughout the body, eventual extravasation into a secondary tissue site and colonization (survival and growth) within the secondary site to form a viable tumor colony (Fig 3) [90]. In this regard, the metastatic potential of a transformed cell is favored by an epithelial to mesenchymal transition (EMT), which is fostered by exposure to TGF- β secreted by infiltrating immune cells or via localized degradation of the ECM [91–95]. A stiffened ECM promotes TGF- β -induced EMT and induces a basal-like tumor cell phenotype to stimulate cancer metastasis [96]; conversely, inhibiting collagen crosslinking and reducing matrix stiffening prevents tumor metastasis [10,95,97]. Thus, ECM stiffness promotes malignant transformation and metastasis by fostering integrin-dependent cell adhesion and migration and regulating tumor plasticity.

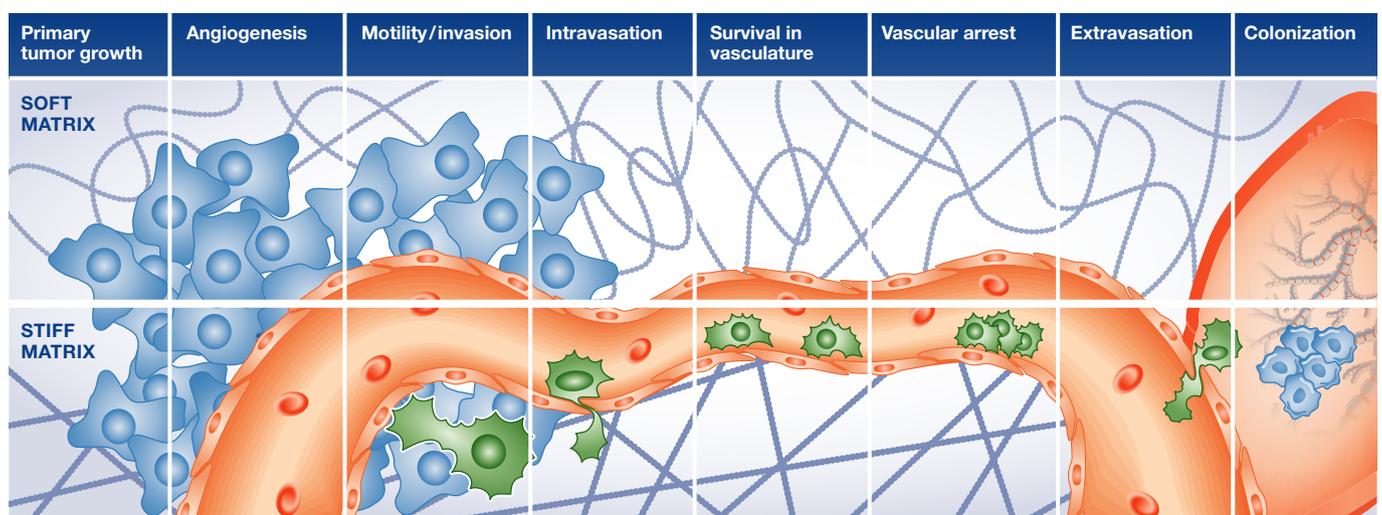


Figure 3. Influences of ECM on the metastatic cascade.

Tumor cell dissemination and establishment of metastatic lesions are controlled by several stringent processes that include induction of an invasive phenotype, migration through the tissue parenchyma, intravasation into the bloodstream, survival in the circulation, followed by extravasation and growth and survival at a secondary organ site. Adhesion to the ECM regulates each of these stages of tumor metastasis.

Emerging hallmark: avoiding immune destruction

Immune surveillance by the adaptive immune response is a key physiological mechanism that prevents tumor formation. Adaptive immunity relies on the ability of cytotoxic T cells to recognize foreign or mutated antigens displayed on transformed cells and to thereafter induce their demise through T-cell-mediated cell death. The ECM can both support and compromise the adaptive tumor immune response. The pro-immunogenic activity of the ECM is mediated in part through the provision of migratory 'highways' onto which T cells can invade into the tissue [98] in response to chemo-attractant ECM fragments released via MMP-mediated cleavage, as has been demonstrated for monocytes migrating into inflamed lung tissue following the release of digested elastin by MMP12 and elastase [99]. The ECM can also directly inhibit T-cell proliferation through type I collagen ligation of LAIR receptors, and T-cell activation from a naïve state [100]. However, T-cell activity can also be impeded by the ECM through the impairment of antigen presentation by APCs [101]. In this regard, a stiffened ECM can compromise T-cell activation by CD3 and CD28, possibly by impairing IL-2 production, which is necessary for T-cell proliferation and Th1-cell differentiation [102]. As such, a stiffened tumor-associated ECM could suppress anti-tumorigenic T-cell function.

Emerging hallmark: deregulating cellular energetics

The final emerging cancer hallmark is metabolic reprogramming of tumor cells. Termed the Warburg effect, tumor cells shift from predominantly aerobic glycolysis toward anaerobic glycolysis. While the overall yield of usable ATP from anaerobic glycolysis is less efficient, producing only 2 ATP compared to aerobic glycolysis which produces 36 ATP, this glycolytic switch permits the tumor cells to use glucose for other regulatory processes such as protein synthesis and cell division [103]. Effective partitioning of glucose consumption is essential for the rapid cell division required by a highly proliferative tumor cell, so that a shift toward anaerobic glycolysis provides the cells with a selective growth advantage compared with cells using aerobic glycolysis. Even in the presence of high oxygen tension, tumor cells exploit the anaerobic metabolic process to facilitate rapid cell division [104].

At its most basic level, the ECM is essential for the uptake of extracellular nutrients and production of functional ATP. Focal adhesion signaling mediates the transmission of ECM signals into the tumor cells which, in turn, promotes the activation of the PI3K pathway which increases glycolysis [105]. Facilitating the shift in metabolic processes, PI3K signaling increases the expression of GLUT1 and GLUT4 along with additional cell surface transport proteins to increase the cellular influx of glucose [106]. Additionally, FAK cooperation with oncogenic drivers such as Ras and Myc supports the conversion of glutamate to glutamine, which promotes cell survival through enhanced protein biosynthesis and feeds into the TCA cycle to maintain high tumor cell proliferation [107]. Intriguingly, tumor cells interacting with a stiffened ECM show a marked upregulation of growth factor-dependent PI3K/Akt signaling, which by virtue of its ability to increase aerobic glycolysis suggests that tissue tension may also directly regulate tumor cell metabolism [108].

Enabling characteristic: genomic mutation and instability

Cancer cells display genomic alterations due to mutations and chromosomal rearrangements as well as loss of tumor suppressors and compromised DNA repair mechanisms. These various acquired genetic alterations promote malignant transformation and facilitate tumor progression. Data suggest that an aberrant ECM may promote genetic instability and can even compromise DNA repair pathways necessary to prevent malignant transformation. For instance, inherited mutations in collagen components, such as the alpha 5 and 6 chains of collagen IV or collagen VII, increase the probability of developing smooth muscle tumors or skin cancer [109,110]. Consistently, ectopic expression of stromelysin or MMP3 in the mouse mammary gland induced mammary epithelial cell proliferation and precocious branching morphogenesis and promoted malignant transformation and genomic instability, even in the absence of oncogene expression [111,112].

Enabling characteristic: tumor-promoting inflammation

Tumors are characterized by tissue inflammation, and a chronically inflamed tissue has a heightened risk for malignant transformation. Intriguingly, a chronically inflamed tissue is frequently fibrotic and shows increased collagen and fibronectin deposition [113,114]. Furthermore, a fibrotic ECM and ECM receptor ligation can profoundly influence the recruitment of cellular components of the innate immune system. For instance, in the absence of expression of the laminin-binding receptor, $\alpha 6 \beta 1$ integrin, neutrophil recruitment into tissues is severely compromised [115]. Similarly, macrophages require expression of the collagen receptor DDR1 to infiltrate atherosclerotic plaques [116], whereas the ECM protein SPARC significantly inhibits macrophage infiltration [98]. The composition of the tissue ECM can also dramatically modify the activation state of the recruited innate immune cells. Thus, a collagen-rich ECM promotes macrophage proliferation and activation [117] and favors a pro-tumorigenic M2 polarization phenotype, whereas a fibronectin-rich ECM promotes the M1 or anti-tumorigenic potential of macrophages [118,119]. Indeed, the tumor-associated stiffened ECM is often enriched for type I collagen, and a rigid matrix promotes the M2 polarization of macrophages possibly by diminishing expression of the M1 macrophage regulator TNF- α in response to LPS [120].

Translation to the clinic

Conventional therapeutics typically target rapidly proliferating tumors and/or inhibit the activity of specific signaling pathways that drive malignancy, such as HER2, EGFR or RAF [121,122]. Such treatments often enjoy enormous initial success, yet are plagued by the emergence of resistant tumors. Cell adhesion to the ECM and the mechanical feature of the ECM can profoundly regulate many of the classic and emerging cancer hallmarks. As such, the ECM and its adhesion receptors constitute tractable therapeutic targets that might prove useful for preventing or treating cancer or at the very least might prove useful as a combinatorial treatment with classic chemotherapies or with targeted therapies. Indeed, the biochemical

and biophysical features of the ECM are not only essential for tumor progression, but can also regulate the efficacy of conventional therapies [123,124]. Thus, abrogation of ECM remodeling or deposition into the stroma of tumors in preclinical models of cancer progression has successfully retarded cancer progression [125–128]. The success of interventions targeting signaling molecules and remodeling enzymes, such as Shh, FAK and hyaluronidase inhibitors, has led to the development of clinical trials which utilize these interventions to neoadjuvantly enhance patient therapy (NCT01938443, NCT01130142, NCT01959139). Numerous FAK inhibitors have been developed and tested in phase I clinical trials [129]. Interestingly, preliminary studies in these patients show that these drugs show promise in slowing tumor growth as well as the metastatic nature of late stage cancers (clinicaltrials.gov, GSK2256098). Unfortunately, Shh inhibitors were ineffective in slowing the disease progression in pancreatic cancer patients [130]. This is surprising given the preclinical success of inhibition of the Shh pathway on pancreatic cancer progression, desmoplasia and mortality. Yet, such data do not negate therapeutically targeting pancreatic fibrosis as this discrepancy could be due to the systemic effects on various cell types within the tumor or genetic differences between preclinical models and patient disease. These results indicate that there is still significant work to be done to determine the genetic contexts and microenvironmental alterations, which would allow ECM targeting interventions to provide a therapeutic benefit to patients.

Conclusions and future perspectives

The hallmarks of cancer are driven by oncogenic mutations and influenced by biochemical and biomechanical properties of the extracellular matrix surrounding the developing tumor. During tumor progression, tumors develop from heterogeneous cell populations containing many different oncogenic mutations. These heterogeneous tumor populations are driven through very different oncogenic mutations and interact with the tumor microenvironment in different ways [131]. Heterogeneous development is also reflected in the tumor-associated ECM with a large degree of variability seen in ECM deposition and stiffening in a single tumor [132,133]. Heterogeneity within the ECM could explain why therapeutics targeting this feature of tumor development have not had significant success in clinical trials [130]. How ECM heterogeneity influences tumor development and therapeutic efficacy is one of the many unanswered questions yet to be addressed (see Sidebar A). A recent study abrogating stromal fibroblasts from pancreatic tumors suggests that the influences of this cell population on ECM composition and mechanical stiffening are inhibitory to tumor progression [134]. However, this is contrary to other studies, particularly in the breast, where increased deposition of ECM components results in enhanced tumor progression [7]. These data, which present opposing results for the role of the ECM in tumor progression, suggest that the influence of the ECM on the hallmarks of cancer cannot be broadly applied to all cancer types. These potentially conflicting results indicate that we must broaden our focus to encompass the various dynamic changes, outlined above, which occur to the biochemical and biophysical properties of the ECM during tumor progression. Given the essential need for matrix stiffness to drive

Sidebar A: In need of answers

- (i) What are the cellular sources inducing an altered ECM deposition and remodeling during cancer progression? And how do these various sources influence tumor progression?
In vitro and *in vivo* data show that the ECM can be deposited and altered by either epithelial or stromal cells in a tumor. However, the relative contribution of these actions to tumor progression is unknown. To answer these questions, novel transgenic models must be developed to specifically target either the expression of ECM proteins or remodeling capabilities from various cell populations in mouse models of tumor development. Additionally, modulating a tumor cells' response to an altered ECM at various time points in tumor progression would allow for the delineation of contribution of these ECM changes to the various steps in tumor cell progression to metastasis.
- (ii) Are the enhanced biomechanical properties of the ECM observed in tumors a causative or correlative factor to tumor development? Diseases associated with biochemical ECM changes are correlated with increased propensity for the development of cancers. The contribution of biomechanical processes to this development has yet to be addressed. Careful analysis of the mechanical properties of diseased tissue, like fatty liver or cystic fibrosis, would bring to answer this question.
- (iii) Is the influence of the ECM on tumor progression consistent through various cancer origins and subtypes?
Oncogenes have been shown to influence the intracellular signaling mechanisms, which control a cells' response to the ECM. As different tumors and subtypes within a given tumor display oncogenic drivers, the response to ECM cues is very different, thus dictating how intervening in this interaction could influence tumor progression. Determining what situations are applicable for what types of inhibitors through *in vitro* and *in vivo* analysis of oncogene-directed tumor–ECM interactions is critical to our understanding of when to target this aspect of the tumor microenvironment.
- (iv) At which point in a tumor cells' response to the altered ECM biochemical and biophysical properties it is most efficacious to therapeutically intervene?
Cellular adhesion to an ECM involves numerous extracellular and intracellular factors. Each of these factors represents an independent opportunity to intervene in the tumor cells' response to ECM changes. Determining whether intervention should be done through inhibiting collagen itself, the cell membrane mediators of tumor–ECM interactions or intracellular signaling in response to ECM adhesion is an essential question. *In vivo* examination of each of these components of a cells' response to the ECM through the use of transgenic knockouts as well as chemical inhibitors would provide invaluable information about efficacy of interventions during tumor progression.

many tumor-promoting effects of the ECM, it is essential to determine whether this ECM property is a correlative phenotype to tumor progression or a causative factor driving tumor initiation. While there are many unanswered questions with regard to how the ECM and its biophysical properties influence tumor progression, the potential for this component to be an efficacious target in treating cancer patients remains exceedingly high.

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Conflict of interest

The authors declare that they have no conflict of interest.

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