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Forcing Form and Function: Biomechanical Regulation of Tumor Evolution

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Abstract

Cancer cells exist in a constantly evolving tissue microenvironment of diverse cell types within a proteinaceous extracellular matrix. As tumors evolve, the physical forces within this complex microenvironment change, with pleiotropic effects on both cell- and tissue-level behaviors. Recent work suggests that these biomechanical factors direct tissue development and modulate tissue homeostasis, and, when altered, critically influence tumor evolution. In this review, we discuss the biomechanical regulation of cell and tissue homeostasis from the molecular, cellular and tissue levels, including how modifications of this physical dialogue could contribute to cancer etiology. Because of the broad impact of biomechanical factors on cell and tissue functions, an understanding of tumor evolution from the biomechanical perspective should improve risk assessment, clinical diagnosis and the efficacy of cancer treatment.

1. TUMOR EVOLUTION WITHIN A BIOMECHANICAL CONTEXT

Tumors are composed of a heterogeneous collection of cells surrounded by various soluble factors and an evolving extracellular matrix (ECM). In addition to the roles of genetic and biochemical events in tumor development, recent studies support the notion that biomechanical factors also critically direct tissue development, sculpt tissue organization and maintain tissue homeostasis [1]. Central to this assumption is the concept that every tissue component (e.g. cells, proteins) is a biomaterial possessing unique mechanical properties that respond specifically to various physical forces. Mechanical inputs, such as tumor expansion leading to tissue compression and increased interstitial pressure, can increase both cell and tissue tension within the confined stroma, leading to the release, concentration and activation of various growth factors, ultimately assisting in tumor progression [2,3]. Additionally, within the tumor, oncogene-mediated alterations in cellular actomyosin contractility and RhoGTPase activity can compromise cell-cell junction integrity to destroy tissue polarity and promote cell invasion while ECM remodeling and stiffening drive

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integrin clustering and actin remodeling to re-enforce focal adhesions. Taken together, these alterations enhance intracellular growth factor receptor signaling within the increased extracellular pool of activated growth factors, drive tumor cell growth and survival, and confer tumor drug resistance [4–6].

In this review, the effects of cell and tissue level forces on tissue behavior are discussed, with recent studies on the role of mechanics in tumor development and evolution highlighted. Because the composition and organization of tumors is continuously evolving, the influence of cell and tissue architecture on the material properties and physical behavior at the molecular, cellular and tissue levels are discussed. Finally, the clinical implications of current research on tumor mechanics, as well as future research directions, are discussed.

2. TISSUE MECHANICS AND MECHANOTRANSDUCTION

Cells and tissues experience various physical forces, which can be classified as externally applied or cell-generated. These physical forces can directly and indirectly affect many fundamental biological processes and, in turn, contribute to normal physiological and pathological phenomena. The direct impact of these forces on the cells and tissues subjected to these cues include displacement, deformation, and an alteration of tissue morphology and organization. For example, externally applied compression force can deform the ECM and decrease the interstitial space, which alters the transport and distribution of soluble factors within the ECM thereby modifying cell behavior [7]. Indirect effects of mechanical force on tissues include changes in levels and/or activity of various growth, differentiation and motility regulators, as well as ECM remodeling. The specificity of these force-induced effects can depend on the direction of the force (e.g. tension, compression and shear forces, see Glossary) as well as its magnitude and duration. For instance, transient tensile forces up-regulate TGF- 1 expression in smooth muscle cells whereas constant tensile forces up-regulate both TGF- 1 and collagen I expression [8]. Dynamic loading (Glossary) increases MMP-9 (Matrix metalloproteinase-9) in fibroblasts whereas static loading up-regulates MMP-2 [9]. Hydrostatic pressure decreases cell proliferation and increases hyaluronan production [10].

Importantly, cells can actively generate forces through multiple mechanisms including Rho-dependent actin-myosin contraction and actin assembly, and transmit these forces through cell-cell and cell-ECM interactions. These cell-generated forces contribute to the branching morphogenesis of both embryonic lungs and cultured epithelial cell cysts, facilitate blood vessel "sprouting" (angiogenesis), and influence convergent extension during embryonic development [11–13]. Accumulating data demonstrate that cell-generated forces play broad roles in regulating cell survival, growth, migration and differentiation, as well as cell-cell and cell-ECM communication and the spatial organization of cells and tissues [14]. Externally applied and cell-generated forces do not operate independently within normal tissues and instead are typically balanced through multiple mechanisms. This orchestrated behavior helps to maintain cell and tissue structure and homeostasis. To facilitate our understanding of how externally applied and cell-generated forces are transmitted in both normal physiological and pathological conditions, the basic concepts of the common mechanical properties of cells and tissues are reviewed below.

Mechanical properties of tissues, cells and ECM

Tissues are composed of multiple cell types, various ECM proteins and other constituents, each with unique mechanical characteristics such as elasticity, plasticity, viscosity, tensile strength and stiffness (Glossary). These physical properties collectively define the material properties of the tissue and dictate how the tissue responds to mechanical cues and how it will sense and transmit force. Specifically, the elasticity and stiffness of cells and tissues

have been implicated in cancer biology. Methods such as tissue level tensile, compression and shear testing, and cellular and subcellular level atomic force microscopy (AFM) have been employed to measure these properties. These techniques have yielded valuable information that demonstrates the unique mechanical properties of each cell type that are reflected in its function, cellular origin and microenvironment (Table 1). These parameters allow the monitoring of differentiation or activation status of cells, as well as a method of assessing the state of disease progression.

The viscoelasticity of a tissue is dictated by its ECM and individual cellular constituents. Differences in the elastic moduli of various cell types or cellular states are partly due to structural variations in the cytoskeletal elements including filamentous actin, intermediate filaments and microtubules, and their organization [15]. In response to biochemical and biomechanical cues from their local environment (e.g. ECM and adjacent interacting cells), cells can tune their elastic moduli by altering their transmembrane receptors, intracellular cytoskeletal organization, actomyosin contraction and cytoskeletal tension, and remodel the local microenvironment to achieve mechanical equilibrium (Figure 1). This active adaptation to the rigidity of the environment also contributes to the elasticity of a cell [16,17]. Cellular elasticity and cell-generated forces are therefore closely related; the intracellular and extracellular mechano-responsive elements are linked by these dynamic and reciprocal conversations (Figure 1). Together, the extracellular and intracellular forces, the mechano-responsive elements, their mechanical properties and the cross-talk with intracellular signaling pathways maintain mechanical equilibrium and regulate diverse cellular behaviors such as adhesion, spreading, receptor signaling, gene expression and extracellular microenvironment remodeling [7]. This mechanical regulatory paradigm is essential for normal tissue structure and function, as demonstrated by the fact that tissue-specific cells often prefer mechanical microenvironments that closely mimic those of their native tissues such as the improved growth of CNS neurites in neurite and glial cell co-cultures and the morphogenesis of normal mammary epithelial cells on soft matrices [18–20].

Mechano-sensing and transduction

To respond to a mechanical stimulus, the cell must possess elements capable of responding to the applied force and translating this mechanical information into a biochemical signal. These cellular mechano-signaling pathways often overlap, feed into, and are themselves regulated by biochemical cascades. Several mechano-sensors that respond to different magnitudes and types of force have been identified, including highly specialized structures such as the mechano-sensory apparatus of the auditory hair bundle and the primary cilia in tubular epithelial cells [21,22]. Biochemical signaling can be initiated by force-induced conformational changes and exposure of functional sites of signaling proteins (e.g. tension-induced conformation changes in fibronectin, intracellular talin and membrane ion channels) (Figure 2A) [23–25]. Alternatively, intracellular signaling cascades can be activated by force-mediated alterations in membrane curvature, tension, and the distribution of membrane signaling molecules as seen with the transactivation of Ephrin receptors [26] and with integrin clustering [27] (Figure 2B). Cells can also sense force via cell-cell junctions deformed by mechanical forces, leading to a global remodeling of their cytoskeletal filaments (Figure 2C) [28,29]. Many of these mechano-sensitive mechanisms operate concurrently, overlapping and interacting with simultaneously occurring biochemical pathways. When these mechano-sensing and transudation events at cellular and subcellular level are coordinated at the multicellular and organ levels, they contribute to tissue and organ functions, such as wound closure and muscle contraction (Figure 2, D and E).

3. DYNAMIC DIALOGUE BETWEEN BIOMECHANICAL AND BIOLOGICAL SIGNALING DURING TUMOR EVOLUTION

Normal tissue structure is disrupted during tumor initiation and progression. The microenvironment becomes both mechanically and biologically active, highlighted by continuous and progressive remodeling of the tumor mass and the stromal compartments. Within the tumor mass, the transformation of tumor cells is accompanied by increased cell division, reduced apoptosis, loss of tissue polarity, and alterations in the composition and organization of extracellular matrix components. Adjacent to the tumor mass, the tumor stroma assists tumor development via multiple stromal cells, ECM molecules, soluble factors and the circulatory systems (blood and lymphatic vessels). Tumor-associated stromal cells, including fibroblasts, myofibroblasts, endothelial cells, mesenchymal stem cells, inflammatory cells and immune cells are often recruited, locally differentiated or activated during different tumor development stages. These cells actively participate in ECM remodeling and tumor angiogenesis, providing growth factors and chemokines that promote tumor growth and metastasis [30,31]. The non-cellular components of the tumor stroma, such as collagens, fibronectin, tenascin and proteoglycans, can be abnormally expressed and remodeled, resulting in new biochemical and mechanical signals [32]. Soluble signals, produced by tumor or stromal cells, released from cleaved ECM molecules and delivered from the circulatory system, can re-distribute within this tumor stromal compartment and regulate cell functions and ECM composition during tumor progression [32].

Different from the well-maintained tissue homeostasis and mechanical equilibrium in normal physiological conditions, the loss of growth control and the disrupted tissue structure and organization during tumor evolution lead to unbalanced physical forces and altered material properties of each tumor component. As the behavior, structure and organization of tumors are continuously changing as the cancer evolves, the mechanical state in a tumor also evolves as the tumor develops (Figure 3). In breast cancer, the mechanical characteristics in a hyperplasia, carcinoma *in situ*, invasive lesion and metastasis lesion can be very different. A hyperplastic lesion typically involves the loss of normal cell polarization and organization, cell-cell contacts and cell-ECM interactions, which result in altered cellular tension and mechano-sensing and transduction [33,34]. Increased matrix deposition, cell proliferation and altered cell tension in hyperplastic lesions also result in the thickening and remodeling of the basement membrane (BM) architecture (Figure 3A) [35]. Carcinoma *in situ* is characterized by active cell growth within an intact BM and interstitial ECM. This uncontrolled cell growth confined by an intact BM leads to a restricted tumor volume expansion and corresponding reaction forces within the various BM and stromal components [36–38]. In turn, the resistance of the BM and ECM to the expanding tumor mass leads to compression of the tumor mass [39]. Simultaneously, ECM remodeling, as a consequence of tumor compression and stromal reaction, results in altered mechanical properties of the ECM that can further increase cell-generated forces and cell tension (Figure 3B) [40]. In advanced carcinoma *in situ* lesions, intra-tumor pressure can be further elevated due to hypoxia and necrosis. Tumor and stromal cells secrete soluble factors, facilitating matrix remodeling and angiogenesis [41]. Inefficient transport and dense ECM networks result in further increases in interstitial fluid pressure within the tumor [42]. Increased interstitial flow due to blood vessel and BM permeability in the tumor microenvironment can promote TGF-dependent myofibroblast differentiation [43]. In invasive tumors, cell-cell interactions further decrease and intracellular contractility increases, leading to the dissemination of tumor cells from the tumor mass and invasion through the BM and interstitial ECM. Invading cells are accompanied by non-transformed stromal cells (e.g. fibroblasts, macophages) and migrate through a progressively stiffened ECM and bio-chemical gradients towards the circulatory system [44–47]. Various mechanical forces, such as interstitial compression and shear,

interstitial fluidic pressure and ECM stiffness can critically influence the rate and direction of tumor cell migration (Figure 3C) [48]. During intravasation, transportation (in the bloodstream or lymphatic flow) and extravasation, tumor cells are exposed to shear forces from adjacent cells and hydrodynamic flow. These shear forces assist tumor cell transport and facilitate interactions with leukocytes and endothelial cells to permit extravasation (Figure 3D) [49]. Cancer cells display organ-specific metastasis which can depend on the intrinsic genetics of the tumor cells, the affinity of tissues to host a metastatic lesion and the pattern of circulation within the tissue [50–52]. Additionally, different organs exhibit very different mechanical properties (e.g. lung is soft whereas bone is very stiff). Since cells can selectively grow on and within specific substrates according to their mechanical properties, organ-specific mechanical properties may contribute to the preferential migration, attachment, survival and proliferation of cancer cells in specific organs (Figure 3E and 3F) (Table 1) [48,53–55].

Influence of the ECM and tumor cell biomechanics on tumor progression

ECM mechanics broadly impact cell transcription, cell cycle control, cell-cell interactions, cell differentiation and migration. The mechanical influences of the ECM in tumor progression directly depend on the ECM composition, structure and organization, as well as the mechanical dialogue with intracellular mechano-responsive elements and cell generated forces (Figure 1) [32,56,57]. Recent work demonstrates that breast tumor progression is often accompanied by increased deposition, cross-linking and de-regulated cleavage of type I collagen [58]. This collagen remodeling is largely due to the increased expression and activity of various enzymes (lysyl oxidase, transglutaminase and MMPs) in the active tumor stroma [40,59–62]. Increased collagen cross-linking and resulting tissue stiffening intensify the biomechanical feedback in breast tissue and promote breast tumor progression [40]. Disrupting this feedback by targeting the cross-linking of fibrillar collagen through inhibition of the enzyme lysyl oxidase could delay both malignant transformation and tumor progression (Figure 4) [40]. In addition, cell migration can be guided by the gradient of ECM stiffness (“durotaxis”) [63,64], indicating such a gradient may serve as an important cue leading the directional migration of cancer cells in the interstitial ECM toward the intravasation sites. Other ECM proteins, such as fibronectin, tenasin, decorin, fibromodulin, lumican, and osteopontin, are also involved in tumor development and modify the mechanical properties of the ECM; however, their roles in tumor mechanics and tumor development have yet to be clarified [32,65–67].

Growth factors bound by the ECM can be released and activated by mechanical perturbation. For example, mechanical stretch or contraction of the ECM can release ECM-bound TGF- into the extracellular space, increasing the availability of active TGF- [3,68]. TGF- plays a key role during tumor progression; it is a strong chemoattractant for both monocytes and macrophages, a stimulant for pro-angiogenic factors such as bFGF (basic Fibroblast Growth Factor), MCP-1 (Monocyte Chemotactic Protein-1), TNF- (Tumor necrosis factor) and IL-1 (Interleukin-1), an activator of ECM remodeling enzymes, a potent activating and differentiating signal for stromal fibroblasts, and a key regulator of the modes of cancer cell motility [69,70]. Fibroblasts differentiated into myofibroblasts are able to generate stronger adhesions and greater cellular contractility, which, in turn, increase ECM tension, remodeling, and deposition that potentiate the further release of TGF- from the ECM [68]. Indeed, TGF- signaling and myofibroblast differentiation associate with invasive human breast cancers, implying that the initiation of mechanical signaling by TGF- may be very important in tumor development. Similarly, the expression and release of other factors, such as PDGF (platelet-derived growth factor), VEGF (vascular endothelial growth factor) and bFGF, which are also involved in tumor cell growth, angiogenesis, cell invasion, and

matrix deposition, can also be regulated by mechanical loading and mechanical properties of substrates [71–74].

Tumor cells exhibit very different mechanical properties than their normal counterparts (Table 1). Studies with isolated cancer cells suggest that they become increasingly compliant as they transform, such that highly metastatic tumor cells are less stiff than normal cells [75,76]. However, this point is still under contention as all of these measurements were conducted in culture and the apparent viscoelasticity of living cells *in situ* and in isolation can be very different. Indeed, the viscoelasticity of living cells can be substantially modified by many factors present in the context of a three-dimensional tissue including heterotypic cell-cell interactions, localized effects of the vasculature and the ECM. In this respect, isolated cancer cells are hypersensitive to substrate stiffness [20,54,77] and exhibit elevated actomyosin-generated contractility when compared to matched normal cells [20]. The increased cell contractility exhibited by tumor cells is mediated by increased activation of MLCK (myosin light chain kinase) and acto-myosin contraction through elevated Rho GTPase activity and EGFR signaling (Figure 1). Pharmacologically or genetically inhibiting these pathways is sufficient to reduce cell tension and normalize tumor tissue phenotype [20]. These results suggest that the intrinsic adhesion and cytoskeletal behavior of cancer cells that participate in their tension behavior contribute to their tumor phenotype. This means that enhanced mechano-responsiveness coupled with increased stiffening of the tissue ECM could contribute to the progressive and incremental stiffening of tumor cells *in situ* (Lopez *et al*., unpublished). Conversely, inhibiting cell or ECM tension may inhibit tumor progression [40]. Notably, ECM stiffness varies quite dramatically within the same tumor and ECM organization is non-uniform, providing a provocative explanation for some of the variability noted in tumor cell behavior within a cancerous tissue *in vivo* (Table 1) [40] (Lopez *et al*., unpublished). The discrepancy between *in situ* analysis and those studies using isolated tumor cells underscores the influences of the tissue microenvironment on cellular mechanical properties and the intrinsic differences of the mechanical properties between transformed and normal cells.

Importantly, the stromal cells associated with tumors also exhibit changes in their viscoelastic properties as tumors progress. Activated, highly contractile myofibroblasts, which frequently appear quite early during tumor progression, are stiffer than their non-malignant counterparts [78]. Additionally, activated tumor-associated macrophages are more compliant than resting macrophages [79] and tumor-derived endothelial cells exhibit enhanced mechanosensing [80] (Table 1). These stromal cells, as well as infiltrating lymphocytes, monocytes and mesenchymal stem cells, frequently participate in the remodeling of interstitial collagen and produce a wide array of growth factors, cytokines and chemokines which help to establish the chemical and rigidity gradients for the growth, transformation and directional metastasis of tumor cells [46,63,81–84]. Indeed, intravital imaging has shown that cancer cells and leukocytes migrate rapidly in collagen-rich regions [47,85] and that paracrine signaling between cancer cells and leukocytes facilitate the directional migration of cancer cells [82]. Considering the many genetic and epigenetic modifications that occur in tumor-associated stromal cells [86] and the functional diversity of these cells, it is clear that it will be important to study the mechano-sensitivity of the vast array of tumor-related cells and to understand how ECM remodeling, mechanical regulation and stromal cell activities contribute to tumor progression.

Tissue mechanics and oncogenic transformation

Tumor cells encounter various ECM environments and physical forces during tumor initiation, progression, and metastasis (Figure 3). Concurrently, tumor cells undergo malignant transformation, adopting a series of genetic and epigenetic changes, including genetic mutations and expression changes of different ECM adhesion receptors, cell

adhesion receptors, growth factor receptors and intra-cellular signaling molecules. These changes modify the ability of tumor cells to sense and respond to external and internal forces, as well as to the mechanical properties of other cells and the ECM. One widely studied family of mechano-sensors is the cell surface integrin family [87]. Integrins can mediate the sensing of mechanical properties of the ECM by changing their avidity, conformation, clustering, and recruitment, and transducing these signals downstream to focal adhesion kinase (FAK), which then leads to the stabilization of focal adhesions and the activation of downstream intracellular signaling cascades. Stiff ECM substrates increase integrin clustering, and induce focal adhesion formation and FAK activation, which intensifies the oncogene ErbB2-mediated PI3K (Phosphoinositide 3-kinase) and ERK (extracellular signal-regulated kinase) signaling pathways and promotes tumor cell malignant transformation in both 3D culture and mouse models for breast cancer (Figure 4) [20,40]. Inhibiting Rho GTPase-induced contractility normalizes tumor cell behavior and the inhibition of ECM stiffening delays oncogene-induced tumor progression. Other studies have demonstrated that elevated Rho signaling via oncogene Ras-driven ERK activation or ErbB2-driven PI3K induce cytoskeletal contractility, cell growth, and destabilize tissue architecture [88]. These small GTPases and their effectors are key regulators of cytoskeleton dynamics, cell polarity and migration, and are often found over-expressed in different types of human tumors [89–93]. Thus, knocking out specific small GTPase effectors including Tiam (a Rac activation factor) and GEP100 (an Arf6 activation factor) in mice reduced tumor incidences and metastasis [94,95]. Taken together, these data suggest that the crosstalk between mechanical and oncogene signaling pathways is essential for oncogene-initiated tumor progression. Accordingly, targeting these abnormal mechanical stimuli and mechanotransduction pathways with pharmacological reagents could potentially be of benefit to clinical cancer therapies.

4. CONCLUSION AND FUTURE DIRECTIONS

Tumors are composed of heterogeneous tumor cell populations. This heterogeneity originates from genetic instability and/or the differentiation spectrum of cancer stem cells (CSCs), potentially leading to drug resistance in cancer therapies [96]. Therefore, an understanding of the properties and regulation of tumor heterogeneity might improve clinical cancer treatment. As discussed in this review, the tumor microenvironment is also heterogeneous and can induce a series of non-uniform biological and biomechanical modifications in tumor cells and the surrounding ECM. Particularly, the biomechanical changes in a tumor microenvironment caused by multiple variable factors including tumor growth and expansion, increased interstitial pressure, cell contraction and ECM deposition and remodeling can modify the biochemical and biomechanical properties of both the tumor stromal microenvironment and tumor cells. This biomechanically modified tumor microenvironment exerts a higher resistance to drug delivery and penetration [97], enhancing the survival of tumor cells due to mechano-chemical coupling in the three-dimensional context and rendering the cells further resistant to drug-induced cell death [98]. Drugs targeting certain oncogenes or signaling pathways may be less effective when these oncogenes and signaling pathways are connected to mechanical signaling at multiple levels (e.g. integrins, ERK, PI3K, Rho GTPase) (Figure 1, Figure 4).

Current work suggests CSCs contribute to drug resistance because of their special properties, including the ability to remain quiescent during tumor progression as well as their increased resistance to DNA damage and external environmental insults [99]. What remains unknown is whether the tumor microenvironment also regulates these special properties of CSCs. CSCs have similar properties as stem cells, such as self-renewal, lineage differentiation and residence within specific niches. Studies on embryonic and adult stem cells revealed that self-renewal and differentiation can be regulated by various mechanical

cues [100], suggesting that CSCs may also be similarly regulated by tissue mechanics. Indeed, the frequency of cancer stem cells is very sensitive to different microenvironments [101]. CSCs express abundant adhesion molecules including integrins and CD44 [102–104] and require specialized niches composed of soluble factors (e.g. Wnt, Notch, hedgehog, and TGF-) [105–107], stromal cells [108,109], and tension-regulated ECM proteins (e.g. tenascin, fibronectin and laminin) for their self-renewal and differentiation [110,111]. Many of these niche components can modulate and be modulated by cell- and tissue-level tension to regulate cell growth, survival and migration [112–114]. Therefore, the properties of CSCs may be directly and indirectly regulated by the tumor microenvironment. If so, the drug resistance of CSCs may be potentially addressed by targeting the mechanical links in the CSC niches (ECM, stromal cells, and soluble signals). Clearly, elucidating the composition and structure of tumor microenvironment and their local and global mechanical influence on tumor phenotype and pathogenesis will be important for the understanding of cancer biology and for the treatment of multiple cancer types.

Both biochemical and biomechanical factors contribute crucial information to tumor development and evolution. Integral to this dialogue is the complex interplay between soluble factors, cell-cell and cell-ECM interactions and the mechanical environment, which cooperatively drive tumor progression. Indeed, we and others have demonstrated that genetic and epigenetic changes in cells combine with alterations in matrix architecture and material properties, propelling tumor evolution. However, many questions linking biomechanics and tumor progression still require resolution. Traditional cell biology approaches may need supplementation with techniques from materials science, engineering and physics. It will be critical to clarify the molecular basis of mechanotransduction in the development and progression of tumors to identify novel anticancer therapeutic targets.

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GLOSSARY

Tension	a load that acts in the direction perpendicular to a surface and tends to pull an object apart
Compression	a load that acts in the direction perpendicular to a surface and tends to push an object
Tensile strength	the maximum amount of tensile stress that a material can be subjected to before failure. (Unit:force per unit area)
Stress	describes the internal resistance of a material to distortion by an external force (average force per unit area). There are three basic stresses:tensile, compression and shear stress. ***Tensile and compression stress*** are the stresses normal to the cross-sectional area of a body; ***Shear stress*** is the *stress* tangential to the cross-sectional area of a body. (Unit:forces per unit area)
Strain	the ratio of the change in length to the original length of a material in the loading direction (Dimensionless)
Stiffness	describes the elasticity of a material or the property of restoration to its original shape after deformation. The unit of stiffness is force per unit

	length, which can be determined by the slope of the load-displacement curve in the linear region of loading. (Unit:force per unit length). ***Compliance*** is inversely related to stiffness
Elasticity	describes the ability of the tissue to return to its original shape after a load is removed. Mathematically, elasticity is described by the ***Modulus of elasticity***, which is defined as the ratio of stress to strain. For example, ***Young's modulus (E)*** describes the elasticity of a material subjected to tensile or compression loading: ***Shear modulus (G)*** describes the shear elasticity of a material subjected to shear loading. E and G can be related by:E= 2G (1+) where is the Poisson's ratio (Ratio of lateral strain to axial strain in an axial-loaded material)
Viscoelasticity	is the property of materials that exhibit both elastic and viscous properties when undergoing deformation. Most biological materials are viscoelastic. The strain of ***viscous*** materials is time-dependent, whereas that of ***elastic*** materials is time-independent. A dynamic test of viscoelastic materials is often performed to determine the frequency-dependent ***complex shear modulus***, which contains the ***elastic storage modulus*** and the ***viscous loss modulus***
Dynamic tests	refers to material tests with periodic deformation or frequency-dependent loading, such as the shear rheometric test. ***Static tests*** refer to those tests with gradually increasing force at a slow speed, such as the classic tensile test

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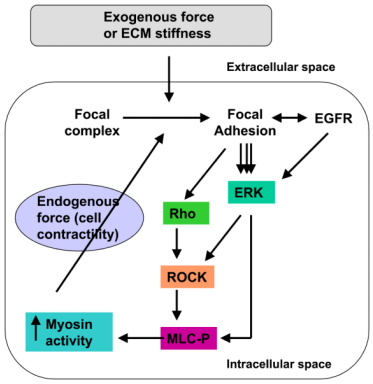

Figure 1. Dynamic and reciprocal conversation between matrix stiffness and cell tension

Non-malignant cells can respond to exogenous mechanical forces and matrix stiffness by enhancing focal complex maturation, resulting in Rho and ROCK activation, MLC phosphorylation and actomyosin contraction. Cell-generated forces from increased myosin contraction feed into focal adhesion maturation to adjust focal adhesion size and contract the ECM until the exogenous forces are balanced and the elastic modulus of cells are “tuned” to reflect the ECM stiffness. This mechano-signaling circuit is crucial in the dynamic and reciprocal conversation between exogenous and cell-generated mechanical factors. (ROCK, Rho-associated kinase; MLC, myosin light chain.) (Adapted with permission from [20].)

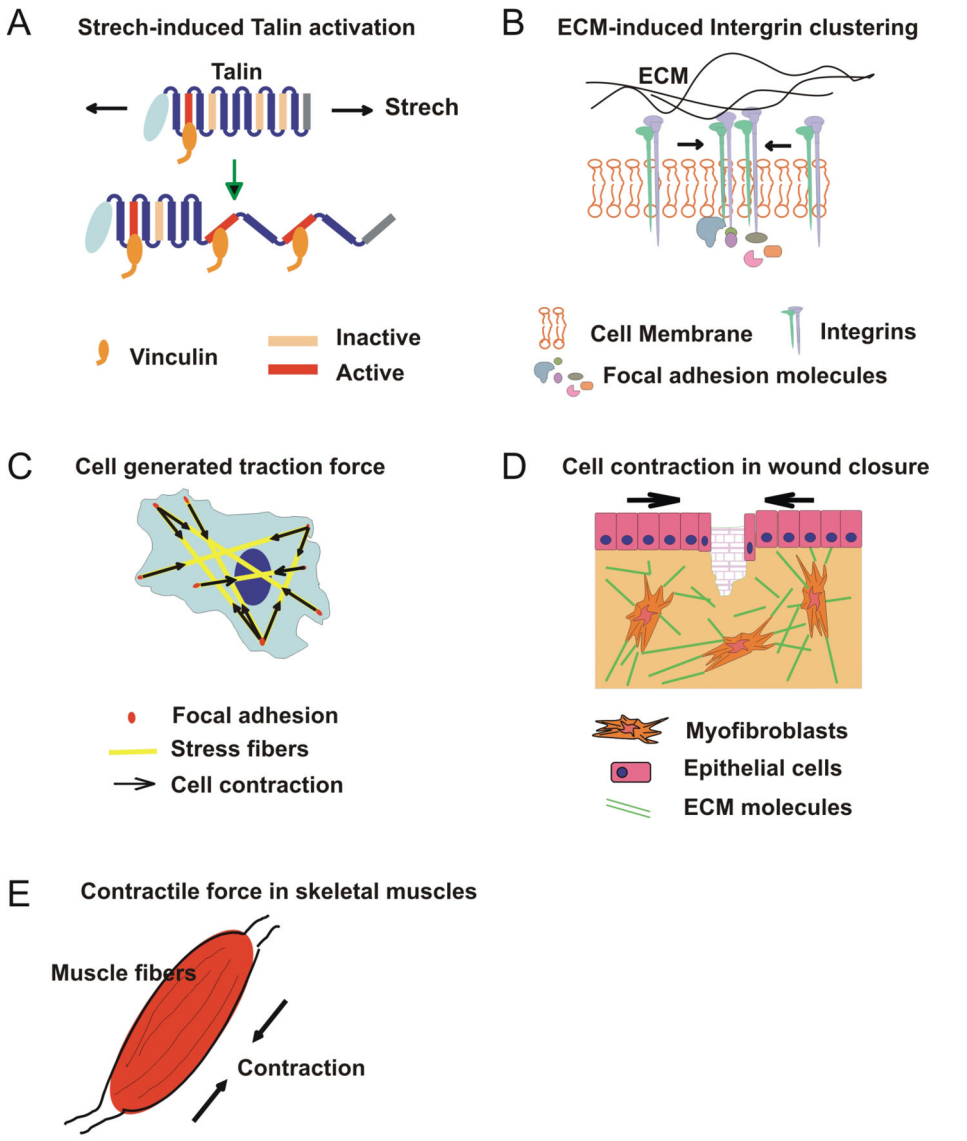

Figure 2. Examples of mechanical behaviors at different levels of biological systems

A) At the single-molecule level, mechanical stretching of talin rods exposes the cryptic binding sites for vinculin, which then activates downstream biochemical signaling pathways important in cell signaling, adhesion, and migration. **B)** At the multimolecule-level, increased matrix stiffness enhances integrin clustering, promotes large focal adhesions and activates downstream signaling cascades. **C)** At the single-cell level, cells can generate traction forces via actin polymerization (e.g. stress fibers) and actomyosin contraction between focal adhesions. **D)** At the tissue level, myofibroblasts differentiated from fibroblasts at wound sites exert contractile forces on the surrounding ECM and rearrange the ECM to close wounds. **E)** At the organ level, a muscle that contains multiple muscle fibers surrounded by connective tissues and sheaths can contract synchronously, generating tension and muscle motion upon the reception of signals from motor neurons.

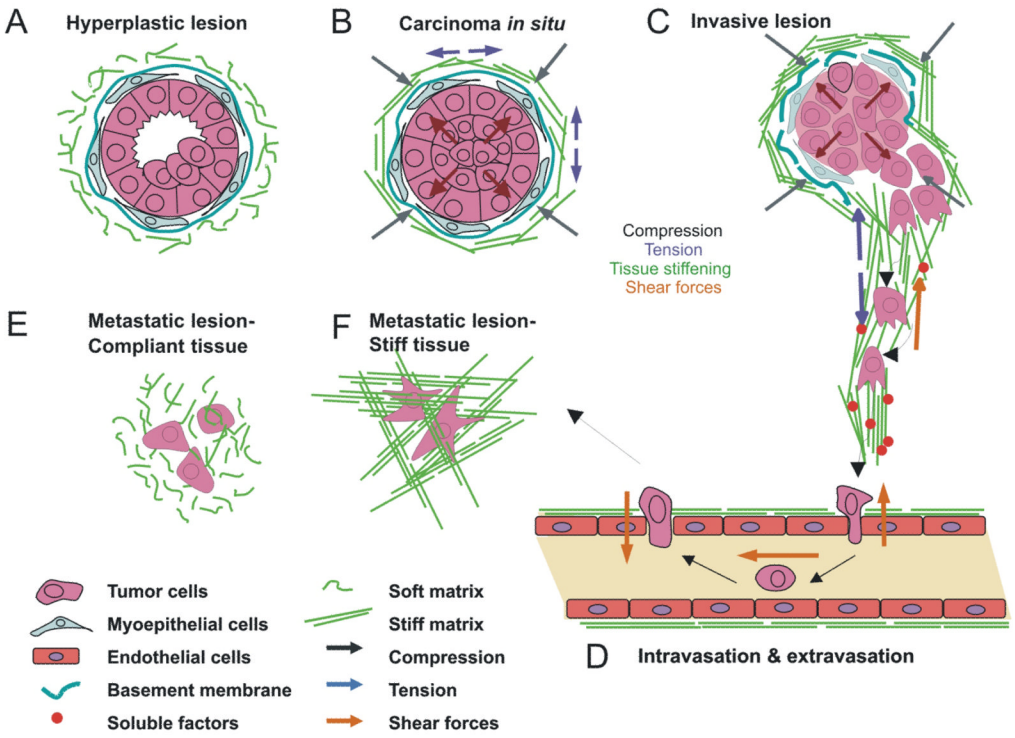

Figure 3. Tumor progression is associated with continuous alterations in tissue and cell mechanics

A) In hyperplastic lesions, cells gradually lose polarity and grow into the luminal space. The myoepithelial cell layer and the basement membrane (BM) remain intact and the surrounding ECM is compliant. **B)** In carcinoma *in situ* lesions, cell polarity is lost and the lumen is filled by proliferating cells. This volume expansion and resistance from the BM and interstitial ECM lead to increased forces between tumor cells and the stromal matrix. Simultaneously, ECM components are abnormally deposited and remodeled, which results in increased ECM and tissue stiffness, and in turn, cell-generated tension. **C)** In invasive lesions, tumor cells break down the BM and invade into the interstitial ECM. The reciprocal forces between tumor cells and the ECM continuously increase. Abnormal deposition and remodeling of ECM collagen further increase ECM and tissue stiffness. Tumor cells generate greater tension in response to this increased mechanical stimulation. As tumor cells invade through the BM and ECM, they experience a range of different forces from the dense ECM network. These external forces together with genetic and epigenetic events can change the contractility and viscoelasticity of tumor cells. **D)** During intravasation and extravasation, tumor cells experience various forces including shear forces exerted by the ECM, blood flow and neighboring cells, which facilitate their transport and attachment to the endothelium. **E)** and **F)** Cancer cells often metastasize to different organs, which can have very different microenvironmental and mechanical properties (e.g. E. soft tissue lung, F. stiff tissue bone). The mechanics of remote tissues and cancer cells may regulate cell dormancy, proliferation and differentiation in these organs.

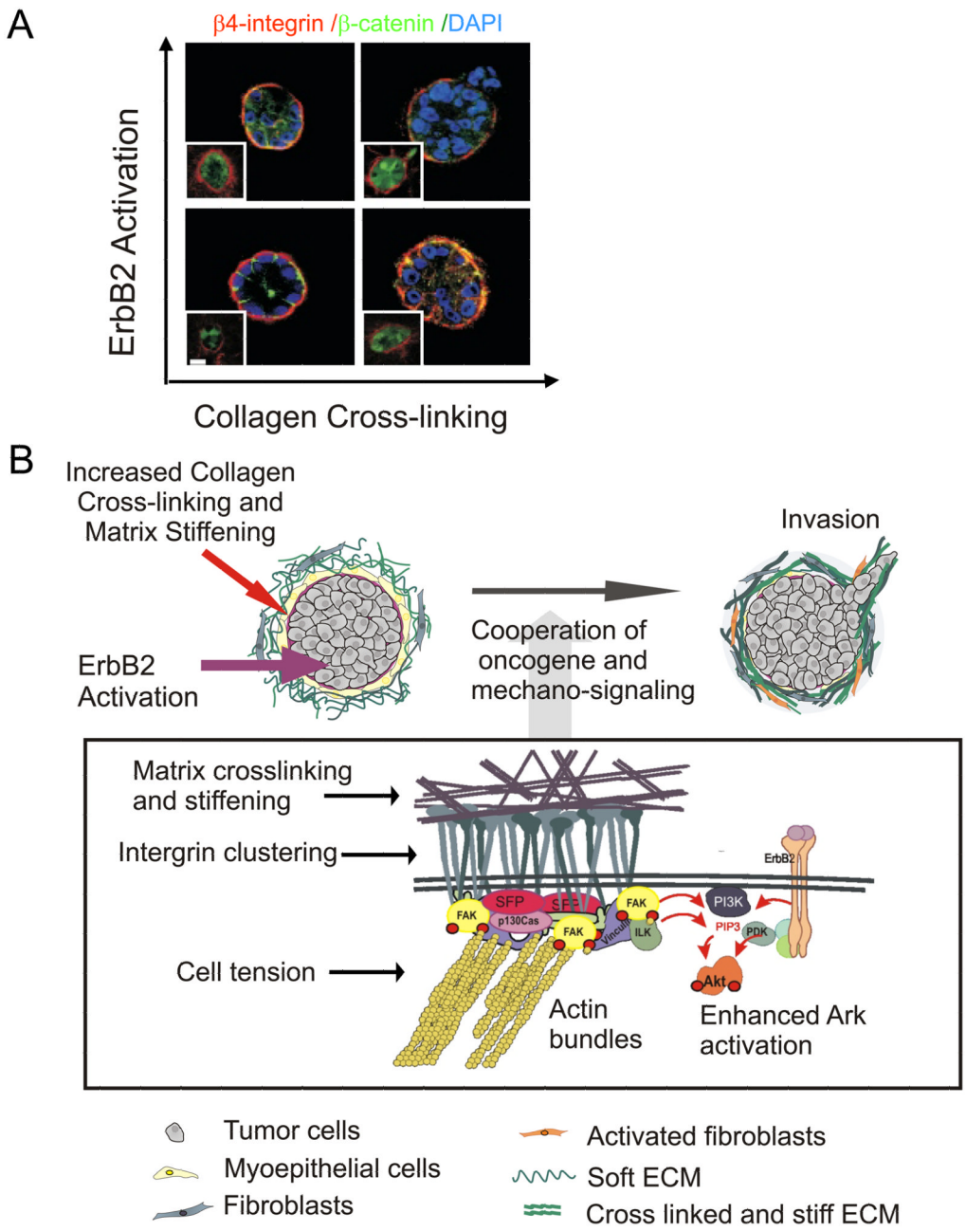

Figure 4. Increased collagen cross-linking and matrix stiffness modify the context of signaling and promote invasion of oncogene-transformed pre-malignant mammary cells

A) MCF10A cells (non-malignant mammary epithelial cells) expressing either a drug-activated ErbB2/NGFR (NGFR, neural growth factor receptor) chimera or a tetracycline-inducible ErbB2 construct form polarized, growth-arrested colonies in soft collagen/rBM gels (bottom left). Stiffening the collagen gel (by adding ribose to cross-link the collagen) or activating ErbB2 signaling increases cell proliferation but fails to drive cell invasion (bottom right and top left). MCF10A colonies start invading into collagen gels only when the collagen gels are stiffened and ErbB2 signaling is activated. (top right) (Bar 20 m;-catenin, green; 4 integrin, red; DAPI, blue.) Second harmonic generation images show the collagen alignment and bundling around the colonies (insert). **B)** Schematic presentation of the

cooperation between matrix stiffening and ErbB2 signaling in driving the invasive phenotype. Increased collagen cross-linking stiffens the ECM, which drives integrin clustering and promotes focal adhesion assembly, thereby activating PI3K and potentiating ErbB2/PI3K/Akt signaling. (Adapted with permission from [40].)

Table 1

Elastic Moduli of Tissues and Cells Involved in Cancer

		Normal or rest state	Pathological or activated state
Tissue	Breast [20,115,116]	0.4 ~ 2 kPa	4~12 kPa
	Lung [117]	10 kPa	25 ~ 35 kPa
	Brain [48]	0.26 ~ 0.49 kPa	7 kPa
	Bone [116,118,119]	2 ~ 14 G Pa	> 689 M Pa
	Liver [120,121]	0.3 ~ 0.6 kPa	1.6 ~20 kPa
Cells	Epithelial Cells [76,122]	~ 2 kPa	~ 0.4 kPa
	Fibroblasts [78]	~ 0.4 kPa	~1 kPa
	Mesenchymal stem cells [123]	0.25 ~ 0.9 kPa	N/A
	Macrophages [79]	1.5 kPa	0.5 kPa
	Myeloid [124]	N/A	0.17 ~ 1.5 kPa (HL60 cells)
	T lymphocyte [124]	0.013 ~ 0.083 kPa (Jurkat)	N/A
	Neutrophils [124]	0.07 ~ 0.24 kPa	N/A