Tissue Force Programs Cell Fate and Tumor Aggression

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ABSTRACT
Biomechanical and biochemical cues within a tissue collaborate across length scales to direct cell fate during development and are critical for the maintenance of tissue homeostasis. Loss of tensional homeostasis in a tissue not only accompanies malignancy but may also contribute to oncogenic transformation. High mechanical stress in solid tumors can impede drug delivery and may additionally drive tumor progression and promote metastasis. Mechanically, biomechanical forces can drive tumor aggression by inducing a mesenchymal-like switch in transformed cells so that they attain tumor-initiating or stem-like cell properties. Given that cancer stem cells have been linked to metastasis and treatment resistance, this raises the intriguing possibility that the elevated tissue mechanics in tumors could promote their aggression by programming their phenotype toward that exhibited by a stem-like cell.

Significance: Recent findings argue that mechanical stress and elevated mechanosignaling foster malignant transformation and metastasis. Prolonged corruption of tissue tension may drive tumor aggression by altering cell fate specification. Thus, strategies that could reduce tumor mechanics might comprise effective approaches to prevent the emergence of treatment-resilient metastatic cancers.

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INTRODUCTION
Biomechanical forces integrate with biochemical signals to control cell behavior and direct cell fate during embryogenesis and development. These forces exist at the tissue level and descend to the level of the cell and subcellular structures. For example, differential multicellular tension fields in colonies of cultured embryonic stem cells generated through the compressive and tensional forces mediated by cell-matrix and cell-cell adhesions can significantly modulate cell fate specification (1, 2). Mechanical forces are also implicated in regulating the branching morphogenesis that occurs during the development of mammary epithelium where branching points are characterized by extensive matrix remodeling and stretch-induced mechanical stress (3). At the cellular level, cells actively respond to externally applied forces through mechanically responsive sensors that then couple to intracellular biochemical signaling pathways and effectors. For instance, integrin-mediated adhesion of cells to a matrix stimulates the activity of RAS family GTP hydrolases (RHO GTPases) and actin remodeling to regulate cell contractility and modify cellular behaviors such as growth, survival, and migration (4). Mechanotransduction pathways converge at the level of gene expression to generate sustained responses to mechanical stress. In this manner, cells achieve a state of tensional homeostasis that depends upon a balanced response to force that is required to organize their fate and maintain their function and integrity within a heterogeneous tissue (5).

Mechanical corruption is a distinctive feature of malignant tissue (6), raising the intriguing possibility that chronic disruption of tensional homeostasis may act as a precursor to overt tumor development. Indeed, the inflammation and matrix stiffening associated with several pathologies, such as cystic fibrosis, chronic pancreatitis, and cirrhosis or fibrosis of the liver, are associated with increased risk to malignancy (7–11). Nevertheless, with a historical focus on the genetic and biochemical foundation of tumors, cancer research has often overlooked how chronic physical stress contributes to malignancy. The nature of the mechanical perturbations in a solid tumor includes solid stress and compression forces resulting from the expanding tumor mass, matrix stiffening and desmoplasia, and an increase in interstitial fluid pressure that adversely affects lymphatic drainage and blood vessel integrity (5, 12–14). Coupled to these dynamically evolving tissue stresses, cancer cells and stromal cells tune their cell...
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Figure 1. Corrupted tensional homeostasis accompanies tumor progression. The tensional balance required for the proper organization and function of adult tissues can be perturbed by oncogenic mutations that modify mechanosensitive signaling in cells (path going left). Alternatively, oncogenic mutations may be preceded by an increase in tissue mechanics that results from chronic fibrosis or injury (path going right). Cells are in a dynamic mechanoreciprocity with their environment such that newly transformed cells can remodel the extracellular matrix which will feed back and further stimulate mechanisms in the tumor cells and surrounding stromal cells. This vicious feed-forward mechanism feeds into and promotes tumor evolution until a new tensional homeostasis is established in the tumor. This process may favor the growth, survival, and expansion or transdifferentiation of stem-like tumor cells that are typically more aggressive given that they frequently display an enhanced survival phenotype and a predisposition to disseminate.

In solid tumors, aggressive subtypes typically exhibit elevated mesenchymal and stem-like cell properties, which have been associated with detrimental tumor characteristics such as treatment resistance, invasion, and metastasis (22–25). Given that mechanical forces direct cell fate in development and can promote tumor aggression, it is conceivable that the chronically augmented force landscape of a tumor might foster the expansion of stem-like tumor cells either by influencing the ability of premalignant stem/progenitor cells to self-renew and proliferate prior to overt transformation, or by reprogramming more differentiated tumor cells to confer them with mesenchymal and stem-like traits. In this review, we outline a role for force in regulating cell fate in development and tumorigenesis and discuss potential mechanisms whereby biomechanical forces could alter tumor cell fate to cultivate tumor aggression.

FORCE DIRECTS EMBRYOGENESIS AND TISSUE DEVELOPMENT

Force has a fundamental role in regulating the cell state transitions that drive embryogenesis (26). In one example, a micropipette was used to apply force directly to developing Drosophila melanogaster embryos in an attempt to mimic the forces present during normal embryogenesis (27). This manipulation was sufficient to induce nuclear translocation of the transcription factor Armadillo, which activates...
the expression of TWIST1 to mediate formation of the dorsal–ventral axis required for continued development. In human embryonic stem cells (hESC), alterations to the balance of tension and compression generated through cell–cell and cell–matrix adhesions contribute significantly to cell-fate changes. A recent study observed that hESCs cultured on more compliant substrates had strengthened cell–cell adherens junctions, which were critical for the maintenance of WNT levels to stabilize β-catenin for nuclear translocation and enhance the response of cells to morphogens that drive mesoderm differentiation (1). It remains uncertain how biomechanical forces integrate with temporally and spatially coordinated gradients of soluble morphogens for correct embryo patterning, yet these results clearly suggest a relationship between force and the priming of hESCs for subsequent fate transitions in the developing embryo.

Thus, stem cell shape and specification are directly linked to the mechanical properties of their immediate microenvironment. Indeed, mesenchymal stem cells (MSC) can be directed toward different lineages based on the elasticity of their underlying matrix, as soft matrices promote adipogenic and neurogenic cell fates, whereas stiffer matrices favor the formation of myogenic and osteogenic lineages (28). However, cells are not merely passive responders to applied force. Rather, they actively modulate their shape and behavior through molecular mechanisms that include RHO-dependent actomyosin contractility. For example, improper localization of the RHO GTPases, RHO and RAC, impairs blastula formation in Xenopus embryos (29, 30), and high versus low RHO activity is critical for the cell fate specification of human MSCs toward osteogenic and adipogenic lineages, respectively (31).

Force is similarly essential for the control of cellular behaviors, such as growth, survival, and migration, that manage the accurate development of adult tissues. Taking the mammary epithelium as an example, regulation of branch patterning and epithelial lineage specification during ductal elongation is highly dependent on extracellular matrix (ECM) remodeling and a corresponding induction of mechanical stress in adjacent epithelial cells. This process has been elegantly modeled in three-dimensional (3-D) patterned cultures of mammary epithelium, where traction force microscopy measurements conducted in 3-D were able to identify areas of high mechanical stress at points of sharp curvature that could be used to predict points of branch initiation (3). Branching at these sites was abrogated by inhibition of mechanotransduction through focal adhesion kinase (FAK), and the extent of mechanical stress and branching depended on matrix stiffness and RHOA-induced cell contractility. The importance of cell–matrix adhesion for maintenance of the correct distribution of mammary epithelial cell lineages during ductal outgrowth was demonstrated through the conditional deletion of β1-integrin from basal mammary epithelial cells in mice (32). Loss of β1-integrin resulted in irregular ductal morphogenesis characterized by aberrant cell divisions and depletion of the basal lineage in favor of luminal cell fate. Studies exploring the inhibition of RHO activity to impair cell contractility led to similar defects in branching morphogenesis, but with a slightly different presentation defined by a disconnected myoepithelial layer that permits hyper-branching and poorly developed ductal elongation (33, 34). Together, these data suggest that the pattern and magnitude of mechanical stress cooperate with biochemical signaling to determine overall branching morphology.

**TENSIONAL HOMEOSTASIS, ADULT STEM CELLS, AND THE ECM**

Adult tissue homeostasis requires a balance of forces to maintain and coordinate tissue function. For instance, vascular stability and maturation is highly dependent on the cyclic strain and fluid shear stress induced by blood flow (35). Interestingly, endothelial progenitor cells can be differentially directed toward endothelial lineages by shear stress and smooth muscle cell lineages by cyclic strain (36–38). Similarly, mechanical loading is critical for skeletal health, as extended periods under reduced mechanical loads, such as those experienced in microgravity or with unilateral lower limb
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Mechanotransduction refers to the process by which mechanistic forces, such as those generated by physical deformation or tension, influence cellular behavior. This phenomenon is crucial in understanding the role of extracellular matrix (ECM) in the regulation of cell fate and cancer aggression. The ECM, a complex network of proteins and carbohydrates, is dynamic and can change in response to mechanical forces. For example, the mechanical properties of the ECM, such as hydration and stiffness, can alter cell behavior. The ECM acts as a mechanical interface that facilitates tissue homeostasis and regulates cellular functions like proliferation, differentiation, and migration.

Through cross-linking and fibril reorientation, the ECM can alter cell to ECM quantity and composition, or ECM organization. The ECM is a major determinant of tissue compliance and rheology, which can adapt as necessary for a tissue to perform its functions effectively. The ECM is composed of fibrillar collagens, proteoglycans, and other ECM components whose content and arrangement are specific to each tissue. Each cell within a tissue is constantly exposed to mechanical forces due to active engagement with neighboring cells or the ECM, and such forces exert control over cell behavior. For example, mammary epithelial cells form polarized acini with cleared lumens in compliant matrices. Indeed, it is increasing evidence that each tissue possesses a characteristic stiffness and that each cell type within a tissue harbors a distinct rheology that can adapt as necessary for a tissue to perform its function, which may vary over the lifetime of an organism. The mammary gland illustrates such an adaptive function during lactation, when mammary epithelial progenitors must undergo extensive proliferation and differentiation to produce the contractile alveoli required for milk production. The stromal matrix is also significantly remodeled to facilitate this epithelial restructuring.

Therefore, the ECM is a major source of isometric forces that can profoundly alter the fate of cells to organize distinct cellular functions within a heterogeneous tissue. The ECM may be composed of fibrillar collagens, proteoglycans, hyaluronic acid (HA), laminins, fibronectin, and other components whose content and arrangement are specific to each tissue. Through its structural nature and capacity for hydration, the ECM acts as a major determinant of tissue compressive resistance and viscoelasticity. Local adjustments to ECM quantity and composition, or ECM organization through cross-linking and fibril reorientation, can alter cell survival, growth, and migration. These effects of the ECM on cell behavior may manifest gradually and chronically over time; consequently, an aberrant stiffening of tissue due to an overproduction of collagens and proteoglycans, or collagen cross-linking enzymes, can lead to chronic conditions of fibrosis and inflammation with potential ramifications for the regulation of resident pools of stem and progenitor cells.

MECHANOSENSING AND MECHANOTRANSDUCTION

To regulate cell fate and behavior during development and homeostasis, cells have evolved several specialized mechanisms designed to sense and respond to biomechanical forces from their surrounding environment. Examples of mechanosensing machinery include transmembrane proteins such as integrins, discoidin domain receptors, growth factor receptors, and stretch-activated ion channels. Many agents of mechanotransduction respond to mechanical strain by undergoing controlled conformational changes in molecular structure that promote protein-protein interactions. For instance, at the cell-ECM interface, mechanical forces are largely sensed and propagated intracellularly through integrin-ECM adhesion plaques. Integrin receptors themselves function as heterodimers of α and β subunits, and structural studies have revealed that their extracellular domain undergoes a domain that can be phosphorylated by SRC family kinases.

In order for the altered molecular state of the protein to effect a change in cell behavior, the mechanical cue must be amplified within the cell by altering the activity of enzymes and stimulating signaling mechanisms to adjust reciprocal intracellular tension. Force-induced integrin clustering initiates the recruitment of focal adhesion signaling molecules such as FAK, SRC, and paxillin, as well as the small GTPases RAC, RHO, and RAS, to trigger signaling cascades and cytoskeleton reorganization. Focal adhesion plaque proteins link integrins directly to actin filaments which interact with myosins to induce cell contractility. Such mechanisms permit cells to rapidly respond to dynamic forces and modify their shape and behavior accordingly. The GTPase RAS, in particular, leads to the activation of ERK and other MAPKs to promote the proliferation and survival of keratinocytes and lung and mammary epithelial cells in response to mechanical strain. ERK phosphorylation is also enhanced in endothelial cells in response to cyclic strain. Cells can generate sustained responses to mechanical stress by altering their gene expression. An upregulation of ECM-related proteins can create a positive feedback mechanism whereby cells responding to mechanical
force modify the composition, organization, and elasticity of their tissue microenvironment. For example, high mechanical tension can stimulate fibroblasts to become myofibroblasts that produce several ECM proteins, including collagens, fibronectin, and tenasin, as well as ECM-modifying enzymes such as matrix metalloproteinases and LOX to remodel and stiffen the surrounding ECM (68). This mechanism of mechanical anoreciprocity equips cells with the ability to fine-tune their behavior to correspond with the physical nature of the ECM and surrounding environment.

**DISRUPTION OF TENSIONAL HOMEOSTASIS MAY PREDISPOSE TISSUES TO TRANSFORMATION**

Perturbations to tensional homeostasis may facilitate the later development of tumorigenic lesions. Pathologic conditions of chronically elevated mechanical stress such as cystic fibrosis or cirrhosis of the liver, which often present with extensive inflammation and collagen accumulation, are associated with increased risk of malignancy (8, 10, 11). Likewise, chronic pancreatitis that is characterized by a striking fibrosis, as well as age-associated liver fibrosis, elevates an afflicted individual’s overall risk for subsequent tumor formation (7, 9).

The concept that the physical properties of the microenvironment could alter cell fate to initiate cell transformation has been modeled experimentally. For example, the matrix deposited by adipose cells taken from obese mice induces mechanosignaling and Yes-associated protein 1 (YAP1)/WW Domain Containing Transcription Regulator 1 (WWTR1; commonly referred to as TAZ) nuclear localization and can enhance the tumorigenesis of premalignant human breast epithelial cells (69). Obese adipose tissue produces a stiffer matrix compared with adipose taken from lean control animals. These data represent an intriguing area for further investigation given knowledge that obesity and diabetes are well-known risk factors for cancer (70). An earlier study also implicated matrix stiffness in promoting the loss of polarity and invasion of mammary epithelial cells cultured in gel-casted and stiffened collagen hydrogels (21). Positive mechanosignaling feedback is stimulated not only by cell matrix–mediated forces, but also through intercellular-generated tension. For instance, the disruption of cell-cell-mediated adhesions through overexpression of active NOTCH resulted in hyperproliferation of cells in the colon crypts of mice, which then increased mechanical stress and β-catenin nuclear accumulation in adjacent nontumorous epithelial cells to drive the formation of tumorous crypt foci (71). Moreover, the stimulation of cell-intrinsic force generation through ROCK-mediated actomyosin contractility in the epidermis caused an increase in the incidence, growth, and progression of spontaneous carcinogen-induced papilloma (15). These clinical and experimental data raise the intriguing possibility that enhanced screening of patients for disruptions to their tissue tensional homeostasis may aid in the identification of those patients at high risk for future cancer development. More provocatively, it suggests that strategies to prevent ECM stiffening or the hypercontractility of cells may prove effective as strategies for cancer prevention.

**FORCING TUMOR AGGRESSION**

The mechanical forces that develop coincident with oncogenic transformation and increase as a function of tumor progression create a microenvironment that can favor tumor cell growth, survival, migration, and invasion (6). These physical forces also modulate the phenotype and behavior of stromal cells and can even alter tumor cell responsiveness to treatment. Figure 2 depicts an overview of tissue-level forces, as well as their potential impact on individual tumor cells. As outlined in the figure and discussed throughout this review, these forces are both extrinsic and intrinsic and influence cellular behavior by altering signaling at the plasma membrane and gene transcription in the nucleus. Here, we describe mechanical forces that promote aggressive tumor characteristics, such as invasion, metastasis, and treatment resistance.

**Solid Stress, Interstitial Fluid Pressure, and Compression**

Tumors display greatly altered tensional homeostasis that develops in part through solid stress exerted by an expanding tumor mass (72). Solid stress–generated compression of tumor-associated vasculature, lymphatics, and interstitial space can lead to impaired lymphatic drainage and a leaky vasculature. These forces cause fluid to accumulate in the interstitial space, resulting in a gradual increase in interstitial pressure that can impede drug delivery to a tumor (14, 72). As the tumor force landscape becomes increasingly aberrant, compression forces can promote vasculature collapse, leading to regions of hypoxia, activation of hypoxia-inducible factor 1α (HIF1α), and stimulation of angiogenesis (13, 73, 74). Activity of the transcription factor HIF1α in colorectal cancer and hepatocellular carcinoma can promote an epithelial-to-mesenchymal transition (EMT), tumor cell invasion, and metastasis (75, 76).

**Matrix Stiffness and Desmoplasia**

Mapping the elastic modulus (stiffness) of tissue using atomic force microscopy (AFM) has revealed that the tissue of developing solid tumors and their local ECM is generally stiffer than that of their normal counterparts, albeit with notable underlying heterogeneity (16, 17, 77, 78). Taking the breast as an example, AFM indentation revealed that the stiffest regions of human and murine breast tumors were located at the invasive margins of the tumor. Moreover, although tumors are a compilation of stiff and compliant regions, overall, tumors harboring the stiffest regions were the most aggressive (17). In particular, breast tumors that contained the highest number of stiff regions within the stroma were those with a basal-like phenotype. Considering that these basal-like or triple-negative tumors also have the worst patient prognosis and that many of these tumors express mesenchymal markers, as well as a stem-like molecular signature that has been associated with treatment resistance, these findings imply that ECM stiffness may be linked to tumor aggression (24). Consistently, poor patient prognosis and a less differentiated mesenchymal phenotype were also correlated with increased periductal collagen deposition in patients with pancreatic cancer (16).
Tumor desmoplasia is characterized by the accumulation of several ECM proteins, including fibrillar collagens I, II, and III, fibronectin, tenascin C (TNC), and elastin (79, 80). Moreover, the extent of collagen abundance and its organization into thick linearized bundles, as revealed by second harmonic generation (SHG) imaging, picrosirius red staining, and polarized imaging, correlates with tumor aggression (16, 17). The presence of thick linearized collagen fibrils reflects an elevated activity of collagen cross-linking enzymes such as LOX, LOX-like enzymes (LOXL1/2), and procollagen lysyl hydroxylases (81, 82). The importance of collagen cross-linking and stiffening to malignant transformation was illustrated through in vivo studies which showed that premalignant HA-RAS transformed mammary epithelial cells transplanted into mouse mammary glands whose collagen cross-linking had been enhanced by prior seeding with fibroblasts ectopically expressing LOX, transformed into invasive, rapidly growing tumors (21). A direct link between collagen cross-linking and mammary tumor aggression was demonstrated by showing that the inhibition of LOX with a LOX-targeting antibody or the pharmacologic inhibitor β-aminopropionitrile was able to delay tumor progression, reduce tumor incidence, and decrease tumor grade in a murine transgenic model of NEU-induced mammary cancer and a KRAS/p53-induced model of murine pancreatic cancer (19, 21). Inhibiting collagen cross-linking and reducing ECM stiffening also inhibited polyoma middle T-induced mammary tumor metastasis (83). These findings not only illustrate the importance of ECM-mediated stiffening in malignant transformation but also implicate tumor mechanics in tumor aggression and metastasis.

The ECM of solid tumors can also be characterized by changes in the levels and composition of proteoglycans. Proteoglycans occupy interstitial space and become hydrated to generate a gel-like ECM, but they can also stiffen the ECM through the formation of hydrophilic associations (52). The brain ECM is a particularly proteoglycan-rich matrix in which HA functions as a scaffold for the aggregation of other ECM components, including collagen, fibronectin, and tenasin C. Fibroblasts can stimulate tumor cell growth through paracrine factors and, together with tumor cells, remodel the ECM through cell-generated tension and elevated production of ECM molecules and cross-linking enzymes. A linearized and stiffened ECM provides tracks for immune infiltration and may facilitate tumor cell invasion and metastasis.

Figure 2. Mechanical forces can promote tumor aggression. An expanding tumor mass results in increased solid stress. Solid stress refers to the force exerted by the solid structural components of a tissue experiencing growth. This stress, together with the mechanical resistance produced by the ECM and stromal cells, promotes an increase in interstitial pressure. Interstitial pressure relates to the interstitial fluid occupying the space between cells and containing water-soluble components of biological tissues. High hydrostatic pressure will force plasma to exit blood and lymphatic capillaries to enter the interstitial space. Conversely, when hydrostatic pressure in capillaries is decreased, interstitial fluid can enter these vessels. Thus, high solid stress and interstitial pressure can impair lymphatic drainage and drug delivery, and in severe cases can precipitate vessel compression and collapse. Insufficient blood supply generates regions of hypoxia within a tumor, a condition that can induce an epithelial-to-mesenchymal or stem-like transition and treatment-resistant qualities in tumor cells. To counter these mechanical stresses, tumors often develop a desmoplastic response characterized by the recruitment of fibroblasts and immune cells with increased deposition of ECM proteins including collagen, fibronectin, and tenasin C. Fibroblasts can stimulate tumor cell growth through paracrine factors and, together with tumor cells, remodel the ECM through cell-generated tension and elevated production of ECM molecules and cross-linking enzymes. A linearized and stiffened ECM provides tracks for immune infiltration and may facilitate tumor cell invasion and metastasis.
poor prognosis of patients with brain tumors is correlated with elevated tissue stiffness as determined through AFM measurements (78). But more intriguingly, GBMs are very hypoxic and consequently express elevated levels of HIF1α. HIF1α induces expression of TNC, which, when bound to HA, creates a stiffened, hydrated ECM that fosters GBM aggression and treatment resistance (78).

Not surprisingly, many approaches have been developed to ameliorate the desmoplastic response in an effort to reduce tumor aggression. This includes pirfenidone treatment, which reduces TGFβ activity (85), LOXL2 inhibitors to prevent collagen cross-linking (86, 87), hedgehog inhibitors to reduce collagen deposition (88, 89), and most recently vitamin D receptor manipulations to convert pancreatic stellate cells back into a quiescent state (90). However, contrary to expectations derived from these antifibrotic treatments, studies using transgenic mouse models of pancreatic cancer revealed that pancreatic cancer response is profoundly influenced by the tumor genotype (16). A separate study identified an important role for the tumor progression induced by elevated mechanosignaling revealing that FAK signaling was necessary for the accelerated stiffening, and the application of a FAK inhibitor in vivo of this mutant in mouse pancreatic cells promoted pancreatic cancer progression independently of its EMT function, raising doubts about the role of EMT in invasion and metastasis in this context (102). However, ECM stiffness was shown to stabilize the nuclear accumulation of the EMT transducer SNAIL1 in breast cancer–associated fibroblasts through ROCK and ERK2 activation, and a fibrogenic response was dependent on this mechanotransduction pathway (103). These data suggest that mechanical stresses can trigger the activity of EMT transcriptional regulators to support tumor fibrosis, tumor cell survival, and invasion.

In related studies, ECM stiffness was found to regulate the transcription of miRNAs to control gene expression and cell behavior. For example, stiff substrates were found to control cell contractility by downregulating the miRNA miR-203 through a ROBO1/RAC1 GTPase/FAK signaling axis (104). ROBO1 is involved in RHOA-mediated cell migration, and miR-203 targets ROBO1 transcripts for degradation. Thus, its downregulation represents a strategy for cells to maintain RHOA signaling, cell shape, and adhesion during periods of high mechanical pressure (104). Although the molecular mechanisms controlling their cytoplasmic retention and nuclear translocation continue to be elucidated, the Hippo pathway transcription factors YAP1 and TAZ have often been designated as bona fide mechanosensors (105). To date, YAP1 and TAZ transcriptional activity has been correlated with tumor aggressiveness in a
number of different solid tumors (106). For example, micro-
environmental stiffness induces YAP1/TAZ nuclear activity to
confer resistance of HER2-positive breast cancer cells to
the targeted kinase inhibitor lapatinib (107). These tran-
scriptional cofactors stimulate the gene expression of several
targets involved in proliferation and ECM production in both
tumor cells and their associated stroma, arguing that they
are force sensors with positive feedback to mechanical stress
(106, 108).

MECHANICAL FORCES AND
TUMOR CELL FATE

Tumors exhibit extensive genetic and behavioral het-
erogeneity among patients, even among those originating
at the same site. This intratumor heterogeneity, typically
characterized by specific marker expression or gene expres-
sion profiles, has led to the identification of molecular
subclasses of tumors that associate with different patient
outcomes and predict the success of different treatment
regimens (23, 109). In many cases, targeted treatment
approaches are confounded by genetically and phenotypi-
cally distinct subpopulations of cells within an individual
tumor. Clonal evolution and cancer stem cell (CSC) models
have been proposed to account for this intratumor het-
erogeneity, with no reason to dismiss the idea that they
simultaneously contribute to tumor progression (110). In
a clonal evolution model, subpopulations of cells emerge
from the sporadic step-wise acquisition of mutations. A
CSC model is based on a hierarchical organization of
tumor cells, where CSCs are stem-like in their capacity for
self-renewal and their ability to regenerate new tumors that
support the full heterogeneity of differentiated tumor cells
present within the parental tumor (110). Evidence suggests
that CSCs are resistant to conventional chemotherapies and
radiotherapies and represent major contributors to disease
relapse and metastasis (110, 111). Further characterization
of CSC gene expression and function has revealed that they
possess properties similar to cells that have undergone an
EMT (112). This mechanism of cell plasticity adds to the
current perplexity regarding the existence of CSCs and the
potential relationship between CSCs and cells responsible
for initiating a tumor (cell of origin).

Clearly, tumor genotype is a dominant factor driving tumor
evolution and heterogeneity. However, increasing evidence
points to a role for mechanical forces in modifying the tumor
phenotypes associated with different genetic aberrations,
suggesting that mechanical heterogeneity within a tumor
could collaborate with other hallmarks of cancer to influence
the intratumoral heterogeneity of tumor cells. Given evidence
demonstrating that elevated tissue forces promote aggressive
tumor characteristics such as invasion and treatment resist-
ance, it is plausible that corrupted tensional homeostasis
somehow leads to an accumulation of aggressive mesenchy-
mal or stem-like tumor cells. How might force favor these
aggressive cell fates? Potentially, mechanical forces could
induce the proliferative expansion of premalignant or trans-
formed normal stem/progenitor cells, or alternatively they
could drive the reprogramming of more differentiated tumor
cells to foster mesenchymal and stem-like behaviors (Fig. 3).

Although supporting evidence has yet to be fully developed,
in the following section, we will explore ways in which force-
regulated mechanisms might promote mesenchymal or stem-
like tumor cell fates.

Force-Induced Hypoxia/HIF1α

Mechanically challenged tumor tissue is frequently accom-
panied by increased hypoxia. A buildup of solid stress, des-
mosplasia and compression in an expanding tumor may force
vessel occlusion and hypoxia, resulting in decreased nutrient
availability, impaired drug delivery, and resistance to treat-
ment (13, 72–74). The induction of hypoxia stabilizes HIF1α
protein levels by protecting it from degradation to allow
its nuclear translocation and transcriptional activity, and
HIF1α upregulates several genes involved in promoting an
EMT and stem-like characteristics in tumor cells (113, 114).
Interestingly, the forced depletion of pericytes in mouse mod-
els of breast cancer impaired vasculature function akin to
changes induced by solid stress, thereby enhancing hypoxia
and HIF1α activity to drive an EMT and tumor cell metastasis
through the transcriptional upregulation of the c-MET recep-
tor and TWIST1 (115). These data also suggest a possible
feed-forward mechanism where HIF1α-induced LOX expres-
sion could contribute to matrix stiffening and the further
development of hypoxia.

In aggressive GBMs, which are characterized by greater
abundance of mesenchymal and stem-like tumor cells,
elevated TNC expression contributes to matrix stiffening,
presumably through HA cross-linking (78, 109). In this
context, hypoxia and matrix stiffness worked synergisti-
cally to activate HIF1α to induce TNC levels. Moreover, a
mechanism of positive feedback was uncovered, whereby
ECM stiffness suppressed expression of the HIF1α and
TNC-targeting miRNA miR-203 (78). The absence of this
mechanism was implicit in the reduced ECM stiffness and
better prognosis associated with GBMs characterized by
IDH1 mutation. Furthermore, experimental introduction
of mechanosignaling or high ECM stiffness in patients
resulted in restored IDH1-mutant GBM aggression and
clinical recurrence, respectively (78). TNC is an ECM glyco-
protein that plays an important organizational and signaling
role in stem-cell niches and during cancer progression
(116). Thus, future investigation should determine whether
this HIF1α and TNC-mediated mechanotransduction path-
way underscores aggressiveness and stemness in the context
of additional tumors.

Mechanical Activation of TGFβ Signaling

As in the case for HIF1α, ECM stiffness can induce the
action of TGFβ to control a myriad of effects that promote
tumor aggression. Broadly, TGFβ can establish an immuno-
suppressive and fibrotic milieu that would serve to aggravate
solid stress in the tumor, and it can directly induce the EMT
and invasion of tumor cells under different contexts (117).
For instance, increased matrix rigidity switches the TGFβ
responsiveness of epithelial cells from apoptosis to an EMT,
suggesting that force-regulated TGFβ signaling fosters mes-
enchymal behavior, migration, and invasion in tumor cells
(118). TGFβ is initially maintained in an inactive state in
complex with latent binding proteins that associate with
the ECM (117), and the mechanical activity of cells, generated through matrix adhesion and cell contractility, can activate TGFβ through its mechanical liberation from the matrix (119). In this way, mechanical stress also increases TGFβ availability. Other reports have indicated that mechanical stress can activate the release of TGFβ from different cell types (120, 121). Together, these results suggest a feed-forward mechanism, where mechanical stress may enhance both the availability and mesenchymal transition–promoting activity of TGFβ.

**Mechanical Activation of WNT Signaling**

The cellular production and release of WNTs is also stimulated by solid stress and compression in tumors (71). Canonical WNT signaling involves the release of the transcription factor β-catenin from a complex with adherens junction components to translocate to the nucleus and elicit gene expression changes (122). Recent evidence suggests that ECM stiffness may directly stimulate β-catenin and MYC activity in breast cancer cells to modify the expression of miRNAs, which fine-tune levels of gene transcripts in the cell (20). The β-catenin– and MYC-dependent induction of miR-18a targets the degradation of mRNAs encoding the tumor suppressors PTEN and HOXA9, and this mechanism was implicated in the formation of more highly aggressive metastatic mammary tumors (20). Importantly, these effects could be reversed through the inhibition of LOX-mediated collagen cross-linking in vivo.

A substantive connection between RHOA–ROCK-mediated cell contractility, WNT signaling, and stem-like tumor cells was also discovered through the expression of a conditionally activated form of ROCK in the skin of mice, which promoted actomyosin cytoskeleton contractility, collagen ECM thickening, and skin hyperplasia (15). ROCK-mediated cell contractility resulted in the nuclear accumulation of β-catenin in hyperplastic epidermis, potentially through the forced breakdown of cell–cell adhesions in a manner similar to the effect of elevated ECM stiffness on breast cancer cells.

![Figure 3. Biomechanical force may promote tumor progression by establishing an aggressive tumor cell hierarchy.](image.png)
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Mechanosensory cues from the extracellular matrix (ECM) control the fate of tumor cells through the mechanical regulation of Hippo signaling. This pathway is important in tissue homeostasis, organ size, and the control of growth and survival. It involves the transcriptional coactivators YAP1 and TAZ, which mediate cell polarity and play a critical role in controlling growth and survival. Under mechanical stress, YAP1/TAZ can be activated, leading to the stimulation of WNT signaling and the induction of an epithelial-to-mesenchymal transition (EMT) and the acquisition of stem-like properties.

**Mechanical Regulation of Hippo Signaling**

The Hippo pathway transcriptional coactivators YAP1 and TAZ are integral components of cell polarity complexes, which are involved in the regulation of cell fate. In pancreatic ductal adenocarcinoma, patients with shorter survival periods (less than 10 months) demonstrate greater periductal collagen accumulation and elevated tumor cell expression of YAP1 and SOX2, indicative of a more mesenchymal, stem-like tumor population. Mechanical stress can trigger the mislocalization of cell polarity proteins such as SCIB, leading to nuclear translocation of TAZ and the activation of YAP1/TAZ transcriptional activity. Mechanical disruption of cell polarity can also enhance tumor cell expression of YAP1 and SOX2, supporting the idea that YAP1/TAZ can act to expand stem/progenitor populations prior to overt malignancy.

**Mechanical Control of Tumor Cell Fate**

Mechanical stress regulates tumor cell behavior by altering the mechanical properties of the ECM and cell-generated tension. This results in changes to cell-ECM interactions, adhesion, and migration, which can drive stem-like phenotypes in tumor cells. For example, mechanical stress can activate the cellular production and secretion of WNTs, promoting stem-like phenotypes through stem cell activity. Moreover, the mechanical action of integrin adhesion and cell-generated tension releases latent TGFβ from the ECM, allowing it to potentially stimulate tumor cell EMT, invasion, and metastasis. High cell tension might also alter the activity of the transcription factor HIF1α, in addition to YAP1/TAZ and β-catenin, to promote gene expression patterns associated with an EMT and the acquisition of stem-like properties.

**Figure 4.** Mechanical control of tumor cell fate. This illustration summarizes some of the mechanisms by which a stiff ECM or augmented cellular tension may alter tumor cell fate. A stiffened matrix strengthens cell-ECM interactions in tumor cells and prompts the disruption of E-cadherin-mediated cell-cell junctions, thereby freeing β-catenin to relocate to the nucleus (β). Similarly, mechanical stress may cause the mislocalization of cell polarity proteins. Breast cancer cells cultured on a stiff matrix exhibit SCIB mislocalization, leading to nuclear translocation of the Hippo signaling pathway transcriptional coactivator TAZ, to induce stem-like programming of tumor cells. Integrin receptor clustering and adhesion plaque formation through the recruitment of vinculin, talin, and other focal adhesion components is another consequence of tumor cell interaction with a stiff matrix. Focal adhesion maturation may then stimulate RHO/ROCK-mediated actomyosin contractility and intracellular signaling through FAK, ERK, and PI3K to enhance cell growth and survival. Integrin clustering may be further modified by a bulky glycocalyx, which creates a membrane kinetic trap for integrin complex assembly. Mechanical stress on tumor cells also activates the cellular production and secretion of WNTs, which may drive stem-like phenotypes in tumor cells through β-catenin activity. Moreover, the mechanical action of integrin adhesion and cell-generated tension releases latent TGFβ from the ECM, allowing it to potentially stimulate tumor cell EMT, invasion, and metastasis. High cell tension might also alter the activity of the transcription factor HIF1α, in addition to YAP1/TAZ and β-catenin, to promote gene expression patterns associated with an EMT and the acquisition of stem-like properties.

**FUTURE PERSPECTIVES**

To better comprehend the mechanisms by which mechanical forces direct cell fate changes in tumor cells, it will be important to decipher the molecular pathways by which mechanical stress is propagated to the nucleus to program large-scale modifications to gene expression. Besides the considerable evidence that mechanically initiated cell signaling can feed into modulation of transcription factor activity, it is also appreciated that mechanical stress results in extensive reorganization of chromatin architecture. However, the molecular mediators controlling the remodeling and segregation of chromatin into silenced versus actively transcribed regions remain ill-defined. One possibility is that the cellular cytoskeleton transmits force directly to the nucleus through specific physical linkages such as those mediated by Linker of the Nucleoskeleton and Cytoskeleton complex to the nuclear lamina (126, 127). Alternatively, mechanical stress could modulate the activity of epigenetic regulating molecules such as histone-modifying enzymes. Both force and epigenetic modifications are critical for the regulation of gene expression that drives the acquisition of stem-like properties in breast cancer cells (124, 125). Collectively, these data provide compelling evidence that ECM stiffness regulates the transcriptional activity of YAP1 and TAZ to support the expansion of invasive stem-like tumor cells. A summary of the mechanically induced pathways described above that could potentially promote aggressive stem-like tumor cell fates is depicted in Fig. 4.
for the lineage specification that occurs during embryogenesis and development, suggesting a possible interaction between the two. Interestingly, RHO GTPase activity and actomyosin contractility have been implicated as major modifiers of chromatin histone acetylation (128). It is likely that precise regulation of chromatin remodeling enables cells to enact transient and reversible gene expression changes in response to mechanical stress, as well as long-term adaptations to a chronic elevation of biophysical forces.

ECM-cell interactions are also fine-tuned by mechanical regulation of membrane curvature and membrane topology (129–132). A recent study found that a bulky glycolaloyx, of which Mucin 1 (MUC1) is a prominent member, is able to form a kinetic trap in membrane topology to promote integrin clustering, focal adhesion–generated cell tension, cell survival, and numbers of circulating tumor cells in breast cancer (132). These data suggest that mechanically induced changes to glycolaloyx composition might prompt membrane redesigns that promote growth factor signaling and features of survival and dissemination that have been attributed to stem-like cancer cells.

Clearly, there is much to discover about the influence of biophysical forces on tumor cell fate, but as our understanding grows, so too does the potential for interventions that could normalize the tensional microenvironment and enforce a physical check on tumor progression. It is important to note that certain populations of CSCs may favor soft mechanical environments as opposed to the stiff environments that we make pancreatic cancer deadly: inhibition of LOX abrogates metastasis and enhances drug efficacy. EMBO Mol Med 2015;7:321–46.


REFERENCES


Mechanotransduction in Cell Fate and Cancer Aggression


