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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. Mechanistically, 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 towards that exhibited by a stem-like cell.

Keywords

Mechanobiology; cancer stem cells; microenvironment; cancer aggression; cell fate

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

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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 include solid stress and compression forces resulting from the expanding tumor mass, matrix stiffening and desmoplasia, and an increase in interstitial fluid pressure that adversely impacts lymphatic drainage and blood vessel integrity (5,12–14). Coupled to these dynamically evolving tissue stresses, cancer cells and stromal cells tune their cell tension and remodel their physical microenvironments to realign their orientation to the newly interpreted tensional homeostasis (Fig. 1). Consequently, the evolving mechanical properties of cancerous tissues do not only reflect the genotype of the tumor, but also the physical phenotype of the cellular and non-cellular stroma. For instance, while K-Ras initiates squamous cell carcinoma, malignant transformation depends upon Ras/Rho Associated, Coiled-coil Containing Protein Kinase (ROCK)-induced keratinocyte contractility and matrix remodeling and stiffening (15). Likewise, pancreatic tumors with mutant Mothers Against Decapentaplegic Homolog 4 (SMAD4) express abundant cytokines that activate ROCK through G Protein Coupled Receptors (GPCRs) and the elevated ROCK activity induces matrix remodeling and stiffening that then promotes the assembly of focal adhesions and Signal Transducer and Activator of Transcription 3 (STAT3) signaling to drive tumor progression (16). These and other recent data argue that biomechanical forces may contribute to increased tumor aggression (17–21).

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 Twist Family BHLH Transcription Factor 1 (TWIST1) to mediate formation of the dorsal-ventral axis required for continued development. In human Embryonic Stem Cells (hESCs), alterations to the balance of tension and compression generated through cell-cell and cell-matrix adhesions contributes 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 Wingless-type MMTV Integration Site Family Member (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 (MSCs) can be directed toward different lineages based on the elasticity of their underlying matrix, as soft matrices promote adipogenic and neurogenic cell fates, while 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 modelled in three-dimensional (3D) patterned cultures of mammary epithelium, where traction force microscopy measurements conducted in 3D 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 Ras Homolog Family Member A (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 β 1-integrin from basal mammary epithelial cells in mice (32). Loss of β 1-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 lead 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 suspension, severely compromise bone matrix deposition, resulting in diminished bone mineral density and bone strength (39,40). The mechanical loads associated with exercise lead to an increase of proteoglycan content in articular cartilage, thereby maintaining joint mobility (41). Conversely, joint degeneration resulting from immobilization or strenuous exercise is accompanied by loss of proteoglycan expression (42). In particular, expression of the proteoglycan, Proteoglycan 4 (PRG4) or Lubricin, is induced by mechanical stress and may mark a cartilage progenitor population that undergoes expansion in response to mechanical loading (43,44).

Tissues have adapted robust mechanisms to respond to disrupted tensional homeostasis resulting from acute injury or pathological states. As previously alluded to, in such circumstances tissues must be able to tightly regulate pools of multipotent stem and progenitor cell populations, and mechanical forces are integral to the dynamic control of their self-renewal and differentiation required to restore tissue structure and function. For instance, the self-renewal of muscle stem cells is only efficiently maintained *in vitro* using substrates with a stiffness that resembles the *in vivo* elasticity of muscle, and only cells propagated in such a manner are capable of mediating muscle regeneration when implanted orthotopically into mice (45). In another example, mechanical load bearing strategies that simulate intrinsic mechanisms of bone tissue regeneration have been exploited to expedite stem cell-initiated bone healing (46,47).

Even among healthy, mechanically static adult tissues, such as the breast or the brain, tissue homeostasis is dynamic and requires the establishment of a tensional homeostasis specific to each tissue. Each cell within a tissue is constantly exposed to isometric forces due to active engagement with neighboring cells or the ECM and such forces exert control over cell behavior (5). For example, mammary epithelial cells form polarized acini with cleared lumens in compliant matrices, but form invasive mesenchymal-like structures when grown within a stiffer matrix (48). Indeed, it is increasingly evident 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 (49). 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 (50). The ECM may be composed of fibrillar collagens, proteoglycans, hyaluronic acid, laminins, fibronectin and other components whose content and arrangement is specific to each tissue (51). Through its structural nature and capacity for hydration, the ECM acts as a major determinant of tissue compressive resistance and viscoelasticity (52). Local adjustments to ECM quantity and composition, or ECM organization through crosslinking and fibril reorientation, can alter cell survival, growth and migration (51,52). 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 crosslinking enzymes, can lead to chronic conditions of fibrosis and inflammation with potential ramifications for the regulation of resident pools of stem and progenitor cells (51).

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 (53), Discoidin Domain Receptors (DDR) (54), growth factor receptors (55), and stretch activated ion channels (56,57). 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 folded to stretched conformational change when bound to ECM ligand (58). Force further modifies adhesions by enhancing the stretched unfolding of talin and vinculin to nucleate the recruitment of a suite of intracellular plaque proteins at the cytoplasmic tail of the β -integrins and foster the assembly of focal adhesions (59–61). Other focal adhesion associated proteins also exhibit force induced conformations, such as p130 Crk-associated Substrate (CAS), which is extended by mechanical stress to reveal a domain that can be phosphorylated by Src family kinases (62).

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 (48). 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 (63,64). 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 Extracellular Signal-Related Kinase (ERK) and other Mitogen Activated Protein Kinases (MAPKs) to promote the proliferation and survival of keratinocytes, lung and mammary epithelial cells in response to mechanical strain (48,65,66). ERK phosphorylation is also enhanced in endothelial cells in response to cyclical strain (67). 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 tenascin, as well as ECM modifying enzymes such as Matrix Metalloproteinases (MMPs) and Lysyl Oxidase (LOX) to remodel and stiffen the surrounding ECM (68). This mechanism of mechanoreciprocity 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 HOMESTASIS MAY PREDISPOSE TISSUES TO TRANSFORMATION

Perturbations to tensional homeostasis may facilitate the later development of tumorigenic lesions. Pathological 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 to malignancy (8,10,11). Likewise, chronic pancreatitis that is characterized by a striking fibrosis, as well as age-associated liver fibrosis, elevate 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 modelled experimentally. For example, the matrix deposited by adipose cells taken from obese mice induces mechanosignaling and Yes Associated Protein (YAP) / WW Domain Containing Transcription Regulator 1 (WWTR1; commonly referred to as TAZ) nuclear localization, and can enhance the tumorigenesis of pre-malignant human breast epithelial cells (69). Obese adipose tissue produces a stiffer matrix compared to 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 MECs cultured in glycosylated and stiffened collagen hydrogels (21). Positive mechanical feedback is not only stimulated 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 non-tumorous 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 (HIF)-1 α and stimulation of angiogenesis (13,73,74). Activity of the transcription factor HIF-1 α 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, while 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, picrosiruis red staining and polarized imaging, correlate 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 (PLODs) (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, Beta-aminopropionitrile (BAPN), 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 K-Ras/p53-induced model of murine pancreatic cancer (21,83). Inhibiting collagen cross-linking and reducing ECM stiffening also inhibited Polyoma Middle T (PyMT) induced mammary tumor metastasis (84). 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 hyaluronic acid (HA) functions as a scaffold for the aggregation of other ECM components including tenascin and versican (52). Glioblastoma multiforme (GBM) is an especially aggressive brain tumor that displays aberrant HA secretion and elevated expression of its receptor, CD44 (85). High CD44 levels are associated with aggressive tumor cell characteristics and its ligation to HA in GBM causes tissue stiffening, greater mechanosensing, and tumor cell invasion (85). Consistently, 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 HIF-1 α . HIF-1 α 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 TGF β activity (86), LOXL2 inhibitors to prevent collagen cross-linking (87,88), hedgehog inhibitors to reduce collagen deposition (89,90) and most recently vitamin D receptor manipulations to convert pancreatic stellate cells back into a quiescent state (91).

However, contrary to expectations derived from these anti-fibrotic treatments, studies using transgenic mouse models of pancreatic cancer revealed that genetic ablation of α -Smooth Muscle Actin (α -SMA) positive fibroblasts accelerated cancer progression (92). Similarly, genetic ablation of hedgehog signaling in the stromal compartment enhanced tumor growth by promoting tumor angiogenesis (93). At least part of the explanation for these findings resides in the heterogeneity of the stromal fibroblasts within a tumor (94). Thus, ablation of the Fibroblast Activation Protein (FAP) positive stromal fibroblasts repressed pancreatic tumor progression (95,96). In addition, we and others found that the nature of the stromal fibrotic response is profoundly influenced by the tumor genotype (16). Transgenic mouse studies revealed that pancreatic cancer lesions with abrogated epithelial Transforming Growth Factor β (TGF β) signaling were significantly more contractile with higher activation of STAT3-mediated cytokine secretion, which led to the induction of a unique periductal stromal fibrosis that was highly enriched in matricellular ECM proteins including TNC (16). Importantly, pancreatic cancer patients with mutated SMAD4 exhibited a strikingly similar, mechanically activated tumor phenotype (16).

Integrin mediated mechanotransduction and actomyosin contractility

Enhanced focal adhesion assembly and activation of FAK are particularly evident at tumor margins where the extracellular stroma is much stiffer (17,21,97). The importance of integrin-mediated focal adhesion assembly for tumor cell mechanotransduction has been illustrated through the use of a unique mutant of β 1-integrin that consists of a single amino acid substitution (V737N) in the transmembrane domain which enhances focal adhesion plaque formation possibly by potentiating talin recruitment (48). V737N expression enhances integrin mechanosignaling in cells even in the absence of a stiffened ECM (48). Targeted expression of this mutant in mouse pancreatic cells promoted pancreatic tumor cell tension and a fibrotic response with ECM stiffening, and the *in vivo* application of a FAK inhibitor revealed that FAK signaling was necessary for the accelerated tumor progression induced by elevated mechanosignaling (16). A separate study identified an important role for the focal adhesion component vinculin in mediating tumor cell invasion and metastasis (97). Vinculin behaves as a mechanical clutch and in breast cancer, ECM stiffness stabilizes vinculin at focal adhesions to alter membrane curvature and nucleate membrane phospholipids for the activation of Phosphatidylinositol-4,5-Bisphosphate 3-Kinase (PI3K)/Protein Kinase B (AKT) induced cell invasion (97). These data compliment and expand upon previous findings that mechanically induced integrin clustering leads to enhanced growth factor dependent ERK and PI3K/AKT activation (21,48,97). Ongoing biochemical inquiries into focal adhesion components coupled with mechanical force manipulations should yield improved understanding of their individual molecular functions that contribute to tumor cell mechanosignaling and aggressive behavior.

Cell generated forces also rely on actomyosin contractility, which requires RhoA GTPase and ROCK activity (48). Rho GTPases are regulated by numerous Guanine Nucleotide Exchange Factors (GEFs) and GTPase Activating Proteins (GAPs) and their dysregulation in cancer is well documented (98). Importantly, tumors that exhibit high rates of metastasis typically possess higher levels of Rho GTPases and other molecules important for

contractile force generation, and an increase in Rho/ROCK activity has been shown to stimulate tumor cell invasion and metastasis (99–101).

Mechanical control of gene expression

Mechanotransduction pathways can regulate the levels and activity of transcription factors to direct large-scale gene expression programs. For example, high ECM stiffness was shown to indirectly activate the nuclear translocation of TWIST1 in breast cancer cells by releasing it from a cytoplasmic binding partner (102). TWIST1 represses the transcription of E-cadherin to promote mesenchymal-like tumor cell invasion and metastasis, indicating an additional link between mechanosignaling and the breakdown of cell-cell adhesions. Interestingly, a subsequent study found that TWIST1 controls tumor cell maintenance and survival independently of its EMT function, raising doubts about the role of EMT in invasion and metastasis in this context (103). However, ECM stiffness was shown to stabilize the nuclear accumulation of the EMT transducer, Snail Family Transcriptional Repressor 1 (SNAIL1) in breast cancer associated fibroblasts through ROCK and ERK2 activation, and a fibrogenic response was dependent on this mechanotransduction pathway (104). 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 microRNAs to control gene expression and cell behavior. For example, stiff substrates were found to control cell contractility by downregulating the microRNA, miR-203, through a Roundabout Guidance Receptor 1 (ROBO1)/ Rac1 GTPase/FAK signaling axis (105). 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 (105).

While 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 (106). To date, YAP1 and TAZ transcriptional activity has been correlated with tumor aggressiveness in a number of different solid tumors (107). For example, microenvironmental stiffness induces YAP1/TAZ nuclear activity to confer resistance of HER2-positive breast cancer cells to the targeted kinase inhibitor lapatinib (108). These transcriptional 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 (107,109).

MECHANICAL FORCES AND TUMOR CELL FATE

Tumors exhibit extensive genetic and behavioral heterogeneity between patients, even among those originating at the same site. This intertumor heterogeneity, typically characterized by specific marker expression or gene expression 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 (23,110). In many cases, targeted treatment approaches are confounded by genetically and phenotypically distinct

subpopulations of cells within an individual tumor. Clonal evolution and Cancer Stem Cell (CSC) models have been proposed to account for this intratumor heterogeneity, with no reason to dismiss the idea that they simultaneously contribute to tumor progression (111). 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 (111). Evidence suggests that CSCs are resistant to conventional chemo- and radiotherapies and represent major contributors to disease relapse and metastasis (111,112). Further characterization of CSC gene expression and function has revealed that they possess properties similar to cells which have undergone an EMT (113). 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 resistance, it is plausible that corrupted tensional homeostasis somehow leads to an accumulation of aggressive mesenchymal or stem-like tumor cells. How might force favor these aggressive cell fates? Potentially, mechanical forces could induce the proliferative expansion of premalignant or transformed 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). While 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/HIF-1 α

Mechanically challenged tumor tissue is frequently accompanied by increased hypoxia. A build-up of solid stress, desmoplasia and compression in an expanding tumor may force vessel occlusion and hypoxia, resulting in decreased nutrient availability, impaired drug delivery, and resistance to treatment (13,72–74). The induction of hypoxia stabilizes HIF-1 α protein levels by protecting it from degradation to allow its nuclear translocation and transcriptional activity, and HIF-1 α upregulates several genes involved in promoting an EMT and stem-like characteristics in tumor cells (114,115). Interestingly, the forced depletion of pericytes in mouse models of breast cancer impaired vasculature function akin to changes induced by solid stress, thereby enhancing hypoxia and HIF-1 α activity to drive an EMT and tumor cell metastasis through the transcriptional upregulation of the c-MET receptor and TWIST1 (116). These data also suggest a possible feed forward mechanism where HIF-1 α -induced LOX expression could contribute to matrix stiffening and the further development of hypoxia.

In aggressive Glioblastomas (GBM), which are characterized by greater abundance of mesenchymal and stem-like tumor cells, elevated TNC expression contributes to matrix stiffening, presumably through HA crosslinking (78,110). In this context, hypoxia and matrix stiffness worked synergistically to activate HIF-1 α to induce TNC levels. Moreover, a mechanism of positive feedback was uncovered whereby ECM stiffness suppressed expression of the HIF-1 α and TNC targeting microRNA, miR-203 (78). Absence of this mechanism was implicit in the reduced ECM stiffness and better prognosis associated with GBMs characterized by Isocitrate Dehydrogenase 1 (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 glycoprotein that plays an important organizational and signaling role in stem cell niches and during cancer progression (117). Thus, future investigation should determine whether this HIF-1 α and TNC mediated mechanotransduction pathway underscores aggressiveness and stemness in the context of additional tumors.

Mechanical activation of TGF β signaling

As in the case for HIF-1 α , ECM stiffness can induce the action of TGF β to control a myriad of effects that promote tumor aggression. Broadly, TGF β can establish an immunosuppressive 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 (118). For instance, increased matrix rigidity switches the TGF β responsiveness of epithelial cells from apoptosis to an EMT, suggesting that force regulated TGF β signaling fosters mesenchymal behavior, migration and invasion in tumor cells (119). TGF β is initially maintained in an inactive state in complex with latent binding proteins that associate with the ECM (118), and the mechanical activity of cells, generated through matrix adhesion and cell contractility, can activate TGF β through its mechanical liberation from the matrix (120). In this way, mechanical stress also increases TGF β availability. Other reports have indicated that mechanical stress can activate the release of TGF β from different cell types (121,122). Together, these results suggest a feed forward mechanism, where mechanical stress may enhance both the availability and mesenchymal transition promoting activity of TGF β .

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 (123). Recent evidence suggests that ECM stiffness may directly stimulate β -catenin and MYC activity in breast cancer cells to modify the expression of microRNAs, 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 Phosphatase and Tensin Homolog (PTEN) and Homeobox A9 (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 crosslinking *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 (15,20). Once again, these data are indicative of a feed forward mechanism where actomyosin contractility induces WNT signaling to promote further ECM deposition and stiffening. Additionally, ROCK activation was found to augment the incidence, growth and progression of spontaneous carcinogen-induced papilloma (15). Given that WNT signaling promotes stem/progenitor activity in multiple tissues (123), these data suggest that a perturbed mechanical environment may act to expand stem/progenitor populations prior to overt malignancy, while its proper regulation may help to prevent oncogene induced transformation.

Mechanical regulation of Hippo signaling

The hippo pathway transcriptional coactivators YAP1 and TAZ play an integral part in controlling growth and organ size in development and their activity is regulated by mechanical stress and an active interplay with factors that mediate cell polarity (106,124). YAP1 and TAZ transcriptional activity has now been associated with aggressive metastatic tumors in a number of different cancers (107). In Pancreatic Ductal Adenocarcinoma (PDAC), for example, patients with shorter survival periods (less than 10 months) display greater periductal collagen accumulation, and elevated tumor cell expression of YAP1 and Sex Determining Region Y-Box 2 (SOX2), which are indicative of a more mesenchymal, stem-like tumor population (16). Mechanical disruption of cell polarity can also enhance YAP/TAZ transcriptional activity. Mechanical stress stimulates the mislocalization of SCRIB, a member of the basal polarity complex, to elicit nuclear translocation of TAZ and gene expression that drives the acquisition of stem-like properties in breast cancer cells (125,126). 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 Figure 4.

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 Nucleocytoskeleton to the Cytoskeleton (LINC)

complexes to the nuclear lamina (127,128). 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 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 (129). 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 (130–133). A recent study found that a bulky glycocalyx, 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 (133). These data suggest that mechanically induced changes to glycocalyx 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 have suggested (134,135). Certainly, more extensive characterization of the mechanical niches that control the functional behaviors of stem-like tumor cells, including their quiescence and self-renewal versus proliferation and differentiation, will be critical for developing strategies aimed at suppressing their accumulation and persistence in aggressive solid tumors.

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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.

TEXT BOX: FORCE DEFINITIONS

Force is a push or pull on an object that causes an object with mass to change its velocity. It has magnitude, direction and a temporal component, and is measured in force per unit area (N/m^2 , where N is Newtons and m is meters)

Mechanical stress can be of three types: tensile, compression and shear. It is a description of the internal resistance of a material to distortion by an external force.

Tensile and compression stresses occur when an object is subjected to linear forces normal to the cross-sectional area of the body. A tensile load will result in a decrease in cross-sectional area and an increase in body length. **Shear stress** occurs when two forces are acting tangentially across a section of the resisting object.

Tensile strain is the ratio of change in length to the original length of a material in the loading direction.

Elasticity is the ability of a deformed object to return to its original shape upon removal of a load. It is time-independent. The modulus of elasticity is the ratio of stress to strain and **Young's modulus** (E) can be used to report the elasticity of an object experiencing a tensile or compression load. **Shear modulus** (G) describes the elasticity of an object exposed to shear loading. E and G are related by the equation, $E = 2G(\mu)$ where μ is Poisson's ratio, a ratio of lateral to linear strain for an object with a linear load.

Stiffness (force per unit area) is a term related to the elasticity of a material. Measurements of stiffness can be derived from the slope of the load-displacement curve in the direction of a load. It is inversely related to compliance.

Viscoelasticity relates to most biological materials that possess both elastic and viscous properties when deformed. This property adds a time dependent component to strain.

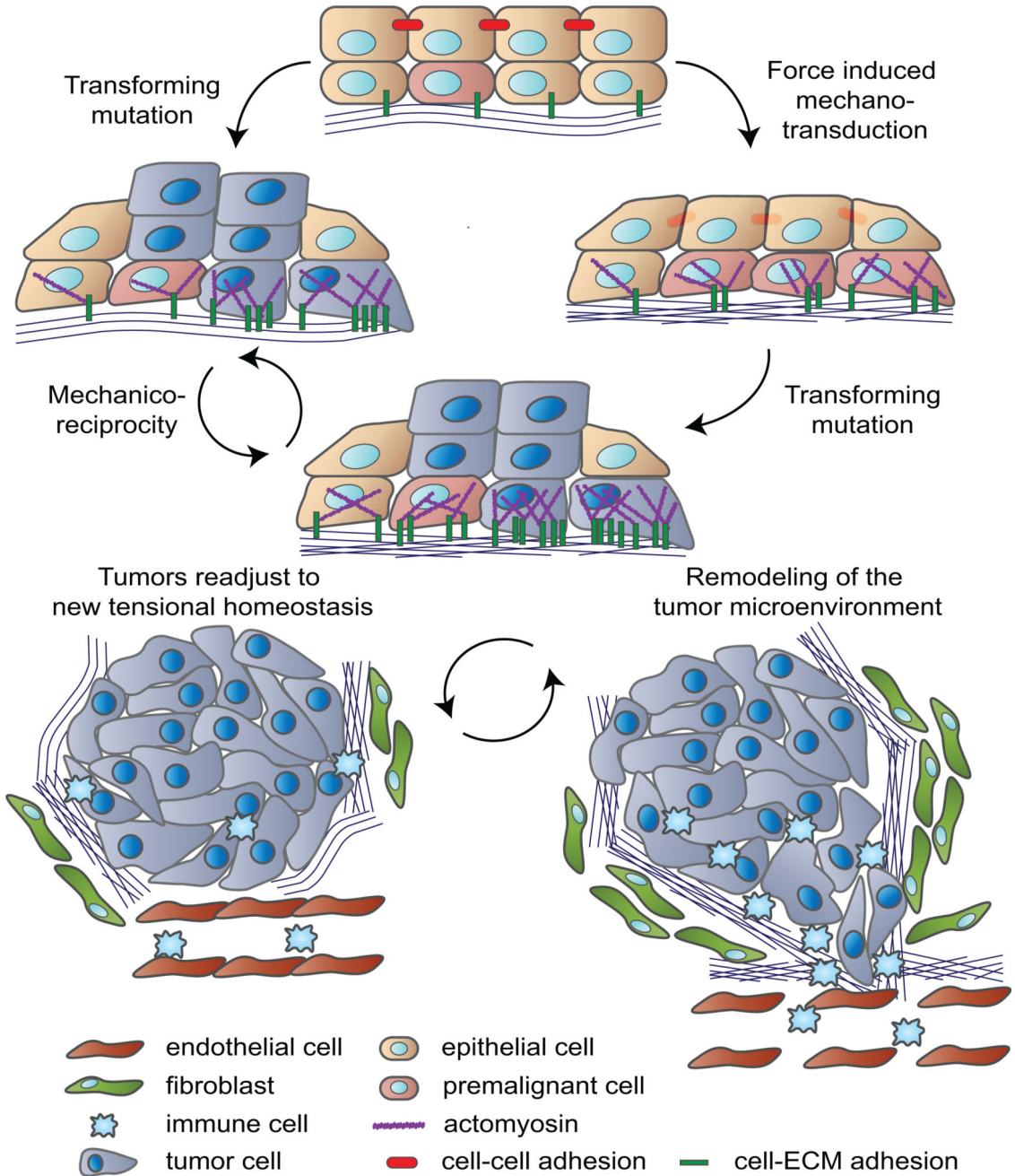


Figure 1. Corrupted tensional homeostasis accompanies tumor progression

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 mechanosignaling in the tumor cells and surrounding stromal cells. This vicious feedforward 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.

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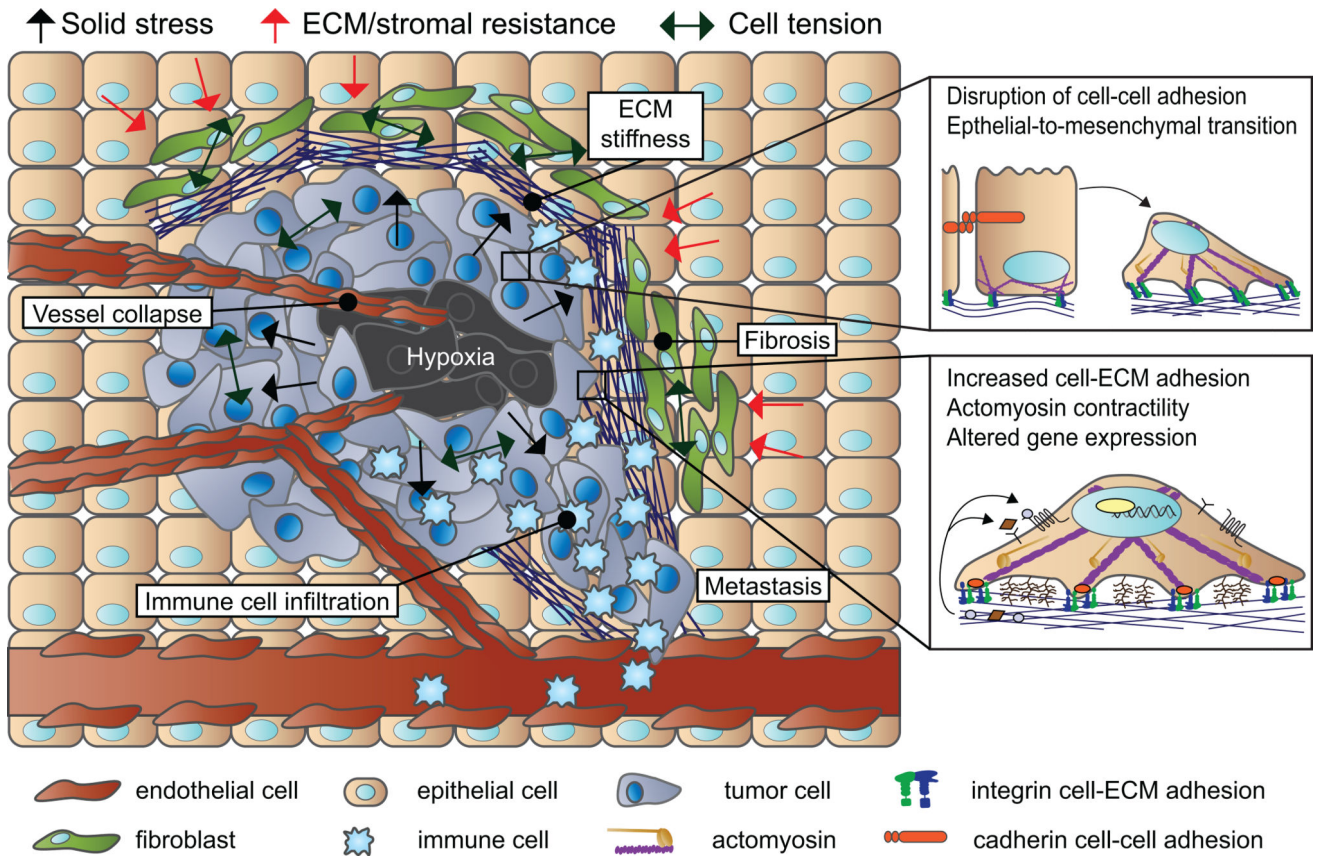


Figure 2. Mechanical forces can promote tumor aggression

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 extracellular matrix (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 which 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 Tenascin 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 crosslinking enzymes. A linearized and stiffened ECM provides tracks for immune infiltration and may facilitate tumor cell invasion and metastasis.

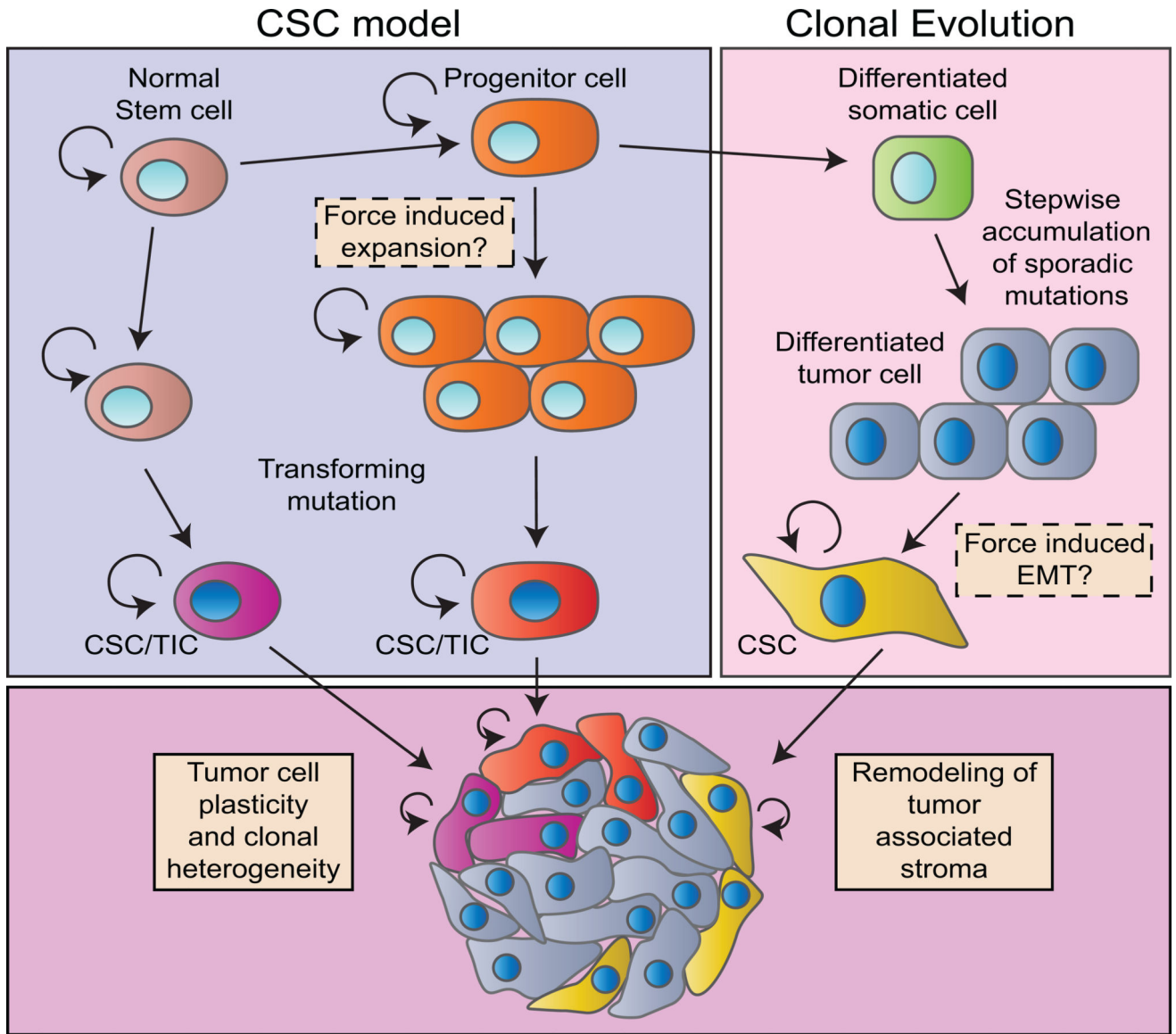


Figure 3. Biomechanical force may promote tumor progression by establishing an aggressive tumor cell hierarchy

In a hierarchical model of tumorigenesis, transformation may originate from among any of the different lineages that form a tissue including stem cells, progenitor cells, or their differentiated progeny. Normal stem and progenitor cells are intrinsically programmed for self-renewal and survival; therefore, their dysregulation could generate cancer stem cells (CSCs) or tumor initiating cells (TICs) with similar capacities for self-renewal and the propagation of differentiated tumor cells. Alternatively, CSCs may be derived from oncogenic events occurring in mature somatic cells that enable the acquisition of CSC properties. Alterations to biomechanical forces through a transformed physical and genetic landscape may contribute to CSC formation by favoring the proliferative expansion of a specific stem/progenitor population, or by inducing an epithelial-to-mesenchymal transition (EMT) and the dedifferentiation of more differentiated transformed cells. An expanded progenitor population represents an attractive long-lived target for the accumulation of

oncogenic mutation and tumor initiation. A stochastic model of tumor progression suggests the stepwise acquisition of sporadic mutations and clonal evolution through competitive selection. In all likelihood, tumors develop through mechanisms that include both hierarchical and stochastic models and force induced tissue remodeling and programming of tumor cells may play a significant role in regulating tumor heterogeneity and tumor cell plasticity.

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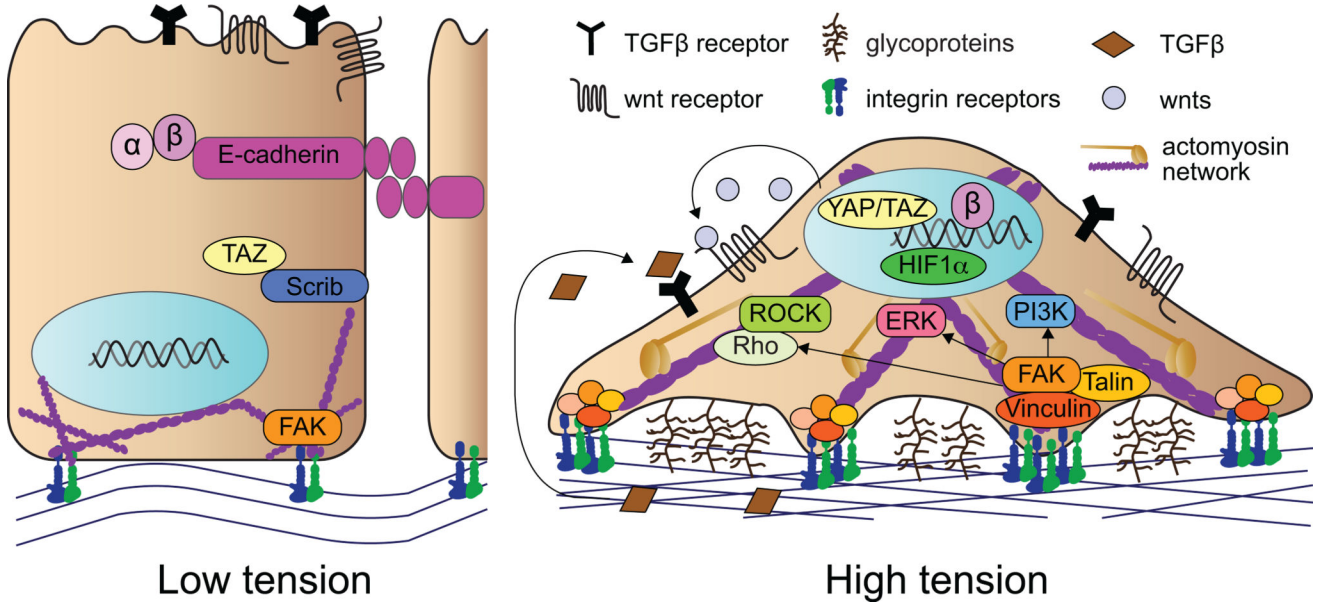


Figure 4. Mechanical control of tumor cell fate
 This illustration summarizes some of the mechanisms by which a stiff ECM or augmented cellular tension may alter tumor cell fate. A stiffened matrix strengthens cell-ECM interactions in tumor cells and prompts the disruption of E-cadherin mediated cell-cell 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 SCRIB 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-TGF β from the ECM, allowing it to potentially stimulate tumor cell Epithelial-to-mesenchymal transition (EMT), invasion and metastasis. High cell tension might also alter the activity of the transcription factor, HIF-1 α , in addition to YAP/TAZ and β -catenin, to promote gene expression patterns associated with an EMT and the acquisition of stem-like properties.