Chapter 7
Mechanotransduction, Metastasis and Genomic Instability

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Abstract Cells translate mechanical forces in the environment into biochemical signals in a process called mechanotransduction. In this way, mechanical forces direct cell behavior, including motility, proliferation, and differentiation, and become important in physiological processes such as development and wound healing. Abnormalities in mechanotransduction can lead to aberrant cell behavior and disease, including cancer. Changes in extracellular mechanical forces or defects in mechanosensors can result in misregulation of signaling pathways inside the cell, and ultimately lead to malignancy. Here, we discuss the ways in which physical attributes of the tumor microenvironment can promote metastasis and genomic instability, two hallmark features of cancer.

Keywords Mechanical stress · EMT · Stiffness

Abbreviations

2D	Two-dimensional
3D	Three-dimensional
αSMA	α-smooth muscle actin
bFGF	Basic fibroblast growth factor
ECM	Extracellular matrix
EGF	Epidermal growth factor
EMT	Epithelial-mesenchymal transition
ERK	Extracellular-signal-regulated kinase
FAK	Focal adhesion kinase
FGF	Fibroblast growth factor
GIN	Genomic instability

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IFP	Interstitial fluid pressure
ILK	Integrin-linked kinase
MET	Mechanoelectrical transduction
MLC	Myosin light chain
MMP	Matrix metalloproteinase
PDGF	Platelet-derived growth factor
PI3K	Phosphoinositide 3-kinase
PTEN	Phosphatase and tensin homolog
ROCK	Rho-associated kinase
ROS	Reactive oxygen species
RTK	Receptor tyrosine kinase
TAZ	transcriptional co-activator with PDZ-binding motif
TGF-β	Transforming growth factor β
VEGF	Vascular endothelial growth factor
YAP	Yes-associated protein

Introduction

Over a decade ago, Hanahan and Weinberg defined several features of cancer that they considered essential for the acquisition of a malignant phenotype, including replicative immortality, evasion of growth suppressors, evasion of apoptosis, stimulation of angiogenesis, stimulation of proliferation, and invasion and metastasis [1]. Since then, a flood of cancer research has led to modification and expansion of the proposed hallmarks; metastasis and genomic instability are two that persist [2]. Cancer is widely regarded as a disease of the cell, and cell behavior is directed by both biochemical and physical cues, which can work independently or synergistically [3]. Accordingly, the tumor microenvironment has been shown to affect tumor progression [4, 5]. This chapter focuses on the physical factors and mechanical forces that tumor cells encounter in the tumor microenvironment, which can in turn alter their behavior. Cells convert the physical signals they receive into biological responses via a process known as mechanotransduction [6].

Mechanotransduction involves both the external environment and internal signaling [7]. The transmission of external forces to intracellular signaling is centered on proteins that are activated by force, such as integrins [8, 9] and T-cell receptors [10]. Many cellular phenotypes, including morphology, motility, and proliferation, are governed by external mechanical forces [11–13]. Thus, mechanotransduction is central to a variety of physiologically normal processes, including embryonic development, differentiation, wound healing, and angiogenesis [14, 15]. Defects in mechanotransduction are known to be involved in several diseases, including cancer [16]. Understanding how defects in mechanotransduction affect tumor progression will add to our fundamental knowledge of cancer biology and may suggest new approaches for treatment.

How Mechanotransduction Regulates Normal Cell Behavior

Extracellular Factors Affecting Mechanotransduction in Normal Cells

Most cells are anchorage dependent: they need to adhere to a substratum to prevent apoptosis and promote cell cycle progression [17]. Thus, the mechanical microenvironment is important for cell survival. Cells sense their environment via conformational changes in mechanically responsive proteins, known as mechanosensors. Physical forces induce these conformational changes, which result in downstream signaling inside the cell [18, 14]. Forces can originate from a variety of features, including the rigidity of the extracellular matrix (ECM), static or dynamic fluid flow, and tissue growth [6]. These forces are further classified into specific types of loads that cells can detect. For example, forces incurred by blood flow include hydrodynamic pressure, shear stress, and cyclic strain, and all of these help regulate endothelial cell behaviors [19] such as cell reorientation [20].

Cells can also respond to mechanical loads by secreting biochemical factors, some of which result in subsequent ECM remodeling. Growth factors comprise one class of proteins that are important in this respect. Transforming growth factor β (TGF-β) is sequestered in the ECM, and is released when internal contractility of myofibroblasts is balanced externally by a stiff matrix, causing conformational changes in protein complexes embedded in the ECM. Free TGF-β starts a feed-forward loop, causing increased deposition of ECM proteins and additional (increased) expression of TGF-β [21]. Various other growth factors increase activity as a result of mechanical load, as evidenced by endothelial secretion of basic fibroblast growth factor (bFGF) in response to shear stress and hydrostatic pressure [22, 23]. Mechanical forces also regulate the expression of matrix remodeling proteins such matrix metalloproteinases (MMPs). This is seen in human monocytes/macrophages, which have been shown to increase expression of MMPs under cyclic strain, and thus contribute to ECM degradation [24].

Intracellular Factors Affecting Mechanotransduction in Normal Cells

There are several intracellular components involved in receiving mechanical signals and eliciting a response (Fig. 7.1). A feature that is particularly important to mechanical sensing is contractility; all cells have a network of cytoskeletal proteins (actin, microtubules, intermediate filaments) that aid in cell structure and mobility [17]. Cytoskeletal contractility creates a balance between intra- and extracellular forces acting on the cell, and thus is important for cells to be able to respond to forces in the surrounding microenvironment [25]. This balance exists so that

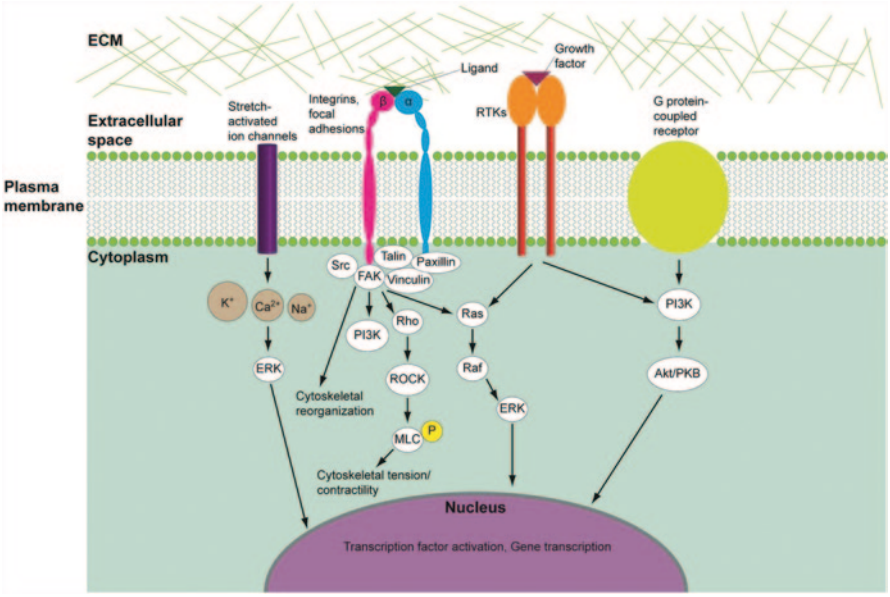

Fig. 7.1 Schematic of intracellular mechanotransduction pathways connecting the *ECM* to the *cytoplasm* and *nucleus.*

mechanical forces in the microenvironment and internal cellular tension can work together to regulate cell behavior, evident, for example, in changes in fibroblast proliferation when matrix stiffness and actomyosin contractility are decoupled [26]. Moreover, external mechanical stimuli help define the state of the cytoskeletal components through various pathways. For example, it has been shown that tensile forces regulate the expression of α-smooth muscle actin (αSMA), a gene important for cytoskeletal contractility, in osteoblasts [27], and that cytoskeletal tension in fibroblasts changes to match the stiffness of the substratum [28].

Communication between ECM and the cytoskeleton is mediated by mechanosensors, proteins or structures that can sense physical changes in the microenvironment and translate these into chemical signals inside the cell [15]. Mechanosensors are diverse and exist everywhere in the body, from ears to kidneys: mechanoelectrical transduction (MET) channels in cochlear hair cells respond to sound vibrations to induce the signaling necessary in auditory sensation [29], and primary cilia in renal epithelia respond to fluid flow to maintain homeostasis [30]. Yet the sensing mechanisms of many mechanosensors remain poorly understood.

The most well-studied mechanosensors are integrins, which contain extracellular, transmembrane, and cytoplasmic domains [31]. Integrins are composed of α- and β-subunits that form heterodimers [32]. Different types of integrins can bind to various ligands present in the ECM and induce signaling to regulate a variety of processes including attachment, migration, proliferation, and differentiation [33]. Through detection of external mechanical stresses, integrins promote changes in cytoskeletal structure and can activate signal transduction cascades [34–36]. Integrin

activity is also essential for the formation of focal adhesions, which act as centers of mechanotransduction [37]. Focal adhesions are protein complexes localized at the plasma membrane that link the ECM to the actin cytoskeleton. In addition to integrins, focal adhesions include hundreds of proteins, the most well-characterized of which are talin, paxillin, vinculin, focal adhesion kinase (FAK) and Src family kinases, which act as signaling molecules [38]. The formation of focal adhesions is regulated by both external forces and cytoskeletal contractility [39].

Other intracellular components involved in mechanotransduction include G proteins, receptor tyrosine kinases (RTKs), extracellular-signal-regulated kinases (ERKs), and stretch-activated ion channels [6].

G proteins are localized at focal adhesion sites and can undergo conformational changes induced by mechanical stress to promote cell growth. G proteins are activated in cardiac fibroblasts in response to stretch, as well as in endothelial cells and osteocytes in response to shear stress [40–42].

*RTK*s are transmembrane proteins that dimerize to become activated, and are involved in integrin-mediated mechanotransduction downstream of G proteins. Dimerization is triggered by binding of the receptor to extracellular ligands such as epidermal growth factor (EGF) and platelet-derived growth factor (PDGF), leading to further signaling [43]. RTKs can also activate ERKs, which are important for gene expression and protein synthesis [44].

*ERK*s are kinases that play an important role in intracellular signaling, such as the activation of cytoplasmic and nuclear regulatory proteins. These kinases can be activated in response to mechanical stimuli. Shear stress and stretch have been shown to activate ERKs in aortic endothelial cells and pulmonary epithelial cells, respectively [45, 46].

Stretch-activated ion channels allow ions such as $Ca^{2+}$to move in and out of cells, which regulates several cellular processes. Cell stretching has been shown to increase intracellular levels of Ca^{2+} in several cell types [47, 48]. Intracellular Ca^{2+} levels are also important for the activation of other proteins in the mechanotransduction signaling cascade, such as ERKs [49].

Mechanotransduction and Metastasis

The invasion of primary tumors into their surrounding tissue and subsequent metastatic spread to other organs are among the largest obstacles to cancer treatment, and metastasis is the main cause of cancer-related deaths [50]. Metastasis relies on the ability of tumor cells to migrate from the primary tumor and form new lesions at distant locations [51]. Invasion and metastasis require physical interactions between malignant cells and the microenvironment, a process that inherently involves mechanosensing and mechanotransduction [16]. Both extracellular factors in the physical tumor microenvironment and intracellular factors within cancer cells contribute to mechanotransduction during invasion and metastasis. Identifying how mechanotransduction becomes abnormally regulated in cancer cells is necessary to understand the mechanisms that underlie invasion and metastasis.

Extracellular Factors Affecting Mechanotransduction in Tumors

The physical microenvironment within a solid tumor differs from that of normal tissue in several ways (Fig. 7.2): uncontrolled proliferation results in increased mechanical compression in a spatially restricted environment [52]; there is an increase in the production of ECM components (of which collagen is the most prevalent structural protein), which exhibit increased alignment, crosslinking, bundling, and stiffening [53, 54]; poorly formed blood vessels and the absence of functional lymphatics lead to increased interstitial fluid pressure (IFP) [155]. These changes in the extracellular environment can alter the behavior of tumor cells via mechanotransduction pathways, which are important for both invasion and metastasis. For example, mechanical compression can promote invasion and metastasis [55]. Compression has been shown to enhance cell-substratum adhesion in two-dimensional (2D) cell culture compression assays [52]. Moreover, compression can facilitate invasion by increasing the release and activation of ECM-degrading MMPs [56]. Mechanical loading can also alter cell shape and motility through compression-dependent changes in cytoskeletal dynamics [57].

The ECM is the framework for intercellular crosstalk, adhesion, and migration [58]. Solid tumors exhibit increased ECM stiffness and crosslinking, and changes in the structural components and mechanical properties of the ECM can promote an invasive phenotype in cancer cells [7, 16, 59]. For example, the mode by which tumor cells migrate is strongly dependent on the physical properties of the ECM [60].

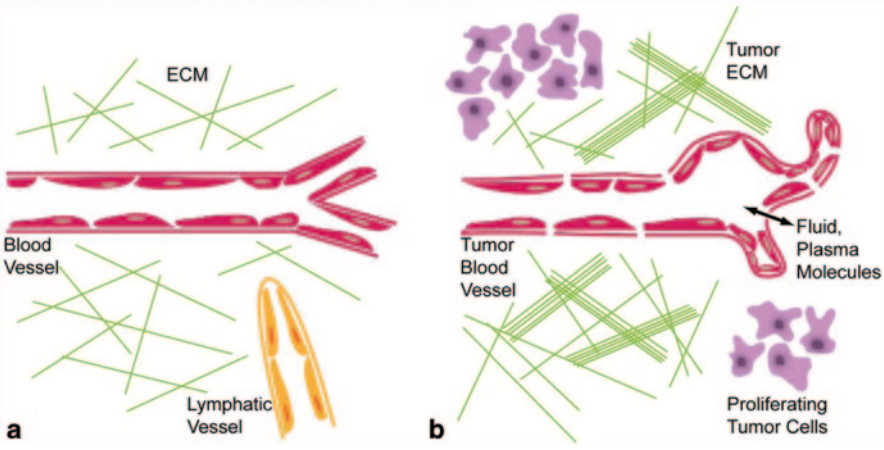

Fig. 7.2 Cartoon illustrating the physical changes in the tumor microenvironment compared to that of normal tissue. **a** Normal tissue microenvironment. The microenvironment in normal tissues contains linearized blood vessels that perfuse the tissue. *Lymphatic vessels* are present to drain excess fluids and maintain fluid homeostasis. *ECM* proteins make up the loose connective framework. **b** Tumor microenvironment. Poorly formed blood vessels *leak fluid* and *plasma macromolecules* into the interstitium. Many solid tumors lack a functioning lymphatic system. There are larger amounts of *ECM* proteins that are highly aligned, crosslinked, bundled, and stiffened. In addition, uncontrolled proliferation of cells in a confined space results in mechanical compression.

Changes in ECM composition and architecture also affect the distribution and activation of soluble factors (e.g., growth factors, cytokines, MMPs) that are themselves involved in cell behavioral changes and mechanotransduction [61]. ECM stiffness can promote the malignant behavior of tumor cells by increasing the expression and activity of adhesion receptors, thereby also activating mechanotransduction pathways [12]. For example, force has been shown to influence the development of focal adhesions since maturation of these complexes requires mechanical tension [62].

Increased ECM stiffness also directs cell behavior by increasing external resistance forces experienced by the cell [63]. Links to the ECM via integrins and focal adhesions can relay these stresses to the cytoskeleton, alter the balance of intracellular forces, and stimulate signal transduction cascades that influence cell behavior [7]. Moreover, increased ECM stiffness can disrupt epithelial polarity and induce migration and metastasis [64]. Cells have also been shown to migrate preferentially to regions of increased ECM stiffness via mechanotaxis/durotaxis [65, 66]. Finally, the crosslinking of ECM by lysyl oxidase, which can also stiffen the matrix and induce fibrosis, can promote tumorigenesis via enhanced integrin signaling [58].

ECM remodeling by tumor and stromal cells is important for both invasion and metastasis. For example, migrating tumor cells exhibit pericellular proteolytic degradation to make room for further migration [67]. Proteases such as MMPs are recruited to integrin assemblies and other adhesion receptors at the leading edge of a migrating cell to model and degrade the ECM [68]. Cancer cells have also been shown to realign their surrounding ECM perpendicular to the tumor boundary, altering its architecture for improved adhesion and migration, creating diverse routes for dissemination [69]. Migration is mediated by several types of proteolytic structures enriched with F-actin, β1-integrins, and MMPs, which are key players in mechanotransduction [70]. Single cell migration can also occur without proteolytic degradation under the mode of amoeboid migration [71]. The microscale architecture of the ECM, including the alignment of fibers and the location and size of pores, dictates the mechanisms of invasion and metastasis applied by cancer cells [72].

IFP and interstitial fluid flow have also been shown to affect the migratory and invasive behaviors of tumor cells [73, 156, 157]. In a three-dimensional (3D) culture model in which single tumor cells were suspended in ECM, fluid flow was shown to increase the percentage of migratory cells as well as their speed [73]. In a similar study, interstitial fluid flow was shown to result in the upstream migration of cancer cells as a result of asymmetry in matrix adhesion stresses needed to balance drag from fluid flow [74]. The stresses induced by flow created a gradient of integrin activation across the cells. Components of focal adhesions, including FAK, paxillin, and vinculin, localized at the upstream side of the migrating cells.

Intracellular Factors Affecting Mechanotransduction in Tumors

It is well known that changes in mechanotransduction promote invasion and metastasis [75]. The intracellular factors affecting mechanotransduction pathways in tumor cells may be altered in response to changes in the tumor microenvironment,

or to genetic mutations and changes in gene expression within the tumor cells. Intracellular mechanotransduction can, in turn, lead to changes in gene expression to promote invasion and metastasis.

Cytoskeletal reorganization is important for changes in cell shape and motility, and therefore migration and metastasis [16]. Cytoskeletal tension is primarily regulated by ERKs and the Rho family of small GTPases. One effector of Rho is Rho-associated kinase (ROCK), which regulates actin cytoskeletal contractility via myosin light chain (MLC) phosphorylation [64]. Rho activity has been shown to be elevated in some tumors, though decreases in its activity have also been reported [76, 77]. Cytoskeletal tension is also affected by the mechanical properties of the ECM, such as stiffness and crosslinking [7]. Increased matrix stiffness promotes the clustering of integrins and the formation of focal adhesions, in addition to increasing activation of FAK and ERK, and enhancing ROCK-mediated cytoskeletal contractility [64]. ROCK is also involved in the disruption of adherens junctions and moving the tail end of the cell behind the leading edge to assist in cell locomotion [78–80]. Moreover, cell migration involves the extension of membrane protrusions resulting from the cycling of actin polymerization and depolymerization, which are regulated by Rho GTPases via the cofilin pathway [81, 82].

ECM crosslinking has also been shown to result in the aggregation and clustering of integrins as well as enhanced signaling via phosphoinositide 3-kinase (PI3K) to induce invasion [58, 64]. Other components of focal adhesions have also been implicated in tumor progression, including Src, the activity of which has been shown to influence proliferation, invasion and metastasis [83, 84]. Src activation is required for ECM degradation during migration [85]. In 3D culture studies of breast tumor cells, Src activity increases the strength of cellular forces on the ECM as well as the duration and length of cell membrane protrusions [86].

Whereas some cells in the tumor become stiffer, metastatic cells are more deformable and exhibit reduced cytoskeletal stiffness [87]. Lower levels of integrin expression along with decreased adhesion to the ECM have been associated with oncogenic transformation [88, 60]. This increased deformability is correlated with enhanced metastatic potential. For example, enhanced deformability enables metastatic cells to move through tight spaces, such as between endothelial cells, during intravasation and extravasation [89].

In addition to regulating the cytoskeleton and associated proteins, mechanotransduction can lead to gene expression changes that promote invasion and metastasis. Cancer cells undergo a variety of genetic mutations and gene expression changes during tumor progression, which can affect their interactions with the microenvironment and subsequent mechanotransduction. Mechanotransduction itself is one source of changes in gene expression in cancer cells. A major way that mechanotransduction can affect gene expression is via the epithelial-mesenchymal transition (EMT). EMT, in which epithelial genes are downregulated and mesenchymal genes are upregulated, is thought to be an important mechanism in both invasion and metastasis [90, 91]. ECM stiffness has been shown to promote EMT, through which cancer cells acquire a migratory phenotype via a variety of pathways, some of which include key players in mechanotransduction, such as RTKs [92]. In one pathway,

EMT results from stiffness-mediated localization and signaling of Rac GTPases downstream of MMPs [93]. Mechanical stress and matrix rigidity can also induce EMT downstream of TGF-β [94, 95]. Furthermore, the activation of Rho GTPases is thought to contribute to EMT via the loss of adherens junctions between cells and the gain of mesenchymal characteristics [96].

Induction of EMT in tumor cells, which affects cytoskeletal organization and cell-cell and cell-matrix adhesions, can also alter how the cells sense exogenous forces, and therefore their responses to those forces [97, 98]. The downregulation of epithelial keratins results in reduced cytoskeletal stiffness and greater cell deformability, directly influencing the metastatic potential of tumor cells [99]. In addition to being more deformable than non-metastatic cells, metastatic cells also lose their anchorage dependence [100, 101]. Anoikis, or apoptosis induced by the loss of adhesion to the ECM, is suppressed in metastatic cells, allowing them to migrate and traverse through the bloodstream to distant organs [102, 103]. Anoikis is believed to be mediated by integrin signaling [104]. The activation of integrins and their associated proteins, including FAK and integrin-linked kinase (ILK), can suppress anoikis, indicating that mechanotransduction and apoptotic pathways are linked [105]. EMT can also suppress anoikis [106]. In particular, the downregulation of E-cadherin can protect cells against anoikis [107]. It is clear that several extracellular and intracellular components of mechanotransduction are altered in tumors, which promotes progression to invasive disease. Mechanotransduction, it seems, is another mechanism that can be hijacked to support malignant transformation.

Mechanotransduction and Genomic Instability

The term genomic instability (GIN) broadly describes the inability of a cell to pass on a copy of its DNA with fidelity. GIN can manifest itself in several ways, each the result of replicative stress caused by errors in DNA replication or the DNA damage response [108]. Microsatellite instability is the expansion or contraction of oligonucleotide repeats and results from mutations in mismatch repair genes [109, 110]; nucleotide excision-repair-related instability results from an impaired ability of the cell to remove and replace damaged nucleotides [111]; and chromosomal instability is a change in the structure or number of chromosomes, which typically occurs as a result of errors in DNA replication or mitosis [112, 113].

GIN is a defining feature of cancers, and is believed to be the driving force behind tumor progression. Various errors in DNA replication or repair processes lead to an abnormal genotype that continues to change with each generation of cells. As a result of GIN, tumors that originate from the same tissue and cell type can have wildly varying genetic profiles [114]. This intertumor heterogeneity, as well as subclonal heterogeneity within a single tumor, has been largely attributed to the Darwinian characteristics of cancer; that is, the evolution and adaptation of a cancer clone in response to external selective pressures [115]. Ultimately, this results in the acquisition of survival-enhancing features that allow a cancer to develop.

The local microenvironment is one source of pressure that results in GIN [116] and increased survival. Mouse embryonic stem cells exposed to radiation develop a high frequency of mutation *in vivo* but not in culture, suggesting that the microenvironment of the cells contributed to their development [117]. More specifically, both physical features of the tumor microenvironment as well as onslaughts by external agents have been shown to increase the frequency of mutation, thus increasing the chances that one of these mutations will affect maintenance of genomic integrity. Hypoxia is one hallmark characteristic of the tumor microenvironment known to play a role in promoting GIN. Hypoxia induces an elevated frequency of mutation in tumorigenic mammalian cell lines [118]. Similarly, exposure to heat and serum-starvation increases mutations in mouse mammary carcinoma cells [119]. Little is known about how GIN may arise from mechanical aspects of the microenvironment; the following describes a body of work that supports this idea.

Mechanical Forces Affect Mitosis and Cell Cycle Progression

One risk factor for the development of GIN is an increase in cellular proliferation, and hence the chance for DNA copy errors to arise. Recently, the mechanical properties of the microenvironment have been considered a major factor in its influence on cell behavior, specifically the regulation of cell cycle progression and mitosis and subsequent maintenance of the genome. Several studies have shown that modulating mechanical forces acting on cells can affect proliferation: mechanical stretch can reduce proliferation of podocytes [120], enhance differentiation and reduce proliferation of preadipocytes [121], and in endothelial cells, directed mechanical forces (specifically, shear and stretch) promote homeostasis but non-uniform forces can result in sustained pro-inflammatory and proliferative signaling [122]. These effects can be mediated by cell-cell contact, such as through VE-cadherin in endothelial cells [123].

The adhesion of a cell to its surroundings can alone induce changes in proliferation. Micropatterning techniques have been used to isolate the effects of cell spreading and cell-cell junctions from the effects of substratum adhesion on cell behavior. Such studies have revealed that E-cadherin is sufficient to induce epithelial cell proliferation via Rac1 signaling, and both proteins are required for cell-cell contact-dependent proliferation [124]. Similar findings hold for endothelial or smooth muscle cells via PI3K signaling [125]. Cytoskeletal structure and associated signaling have also proven to be important in cell-cell adhesion-mediated proliferation, based on studies regarding the role of VE-cadherin in vascular endothelial cells [126]. Additionally, simply varying the nature of the substratum also affects proliferative behavior. The basement membrane interacts differently with normal or cancerous epithelial cell lines, affecting growth and differentiation [127].

There is also evidence that mechanotransduction can influence various aspects of mitosis, and thus the segregation of the genome into daughter cells. Physical features of the microenvironment are one avenue of mechanical influence on

mitosis. For example, in HeLa cells (human cervical cancer cells), retraction fibers, which bind mitotic cells to the substratum, exert forces on the cell that dictate the orientation of the spindle during mitosis. This is mediated by regulation of the subcortical actin network [128]. Another study in HeLa cells similarly showed that the spatial distribution of ECM proteins helps determine the axis of division by regulating actin dynamics [129].

It would follow from these studies that mechanosensors and other intracellular mechanotransduction machinery are involved in the regulation of mitosis, and indeed this has been shown. Integrin-mediated adhesion is required for the cells to reorient the mitotic spindle parallel to the substratum [130]. Here again, cytoskeletal components are key communicators. G proteins and the motor protein dynein, both important in transmitting mechanical force, are also known to direct orientation of the spindle in development [131]. One can imagine that abnormal mechanical signaling, common to many diseases including cancer, could disrupt mitosis in a cell and thus generate genomically unstable progeny.

Mechanotransduction Regulates Biochemical Cues That Promote GIN

One way that mechanical stimuli ultimately promote changes in cell behavior is through intracellular signaling pathways that conclude with control of gene transcription. Genes regulated by mechanotransduction can affect a myriad of both normal and pathological processes in the body [14]. In the context of cancer, recent studies have suggested that important molecular targets of mechanotransduction include mitotic checkpoint genes and other cell-cycle regulators, which have long been associated with maintaining genomic stability [112, 132].

To discover mechanically-regulated genes associated with GIN, several studies have used polyacrylamide gels of varying stiffness to mimic the mechanical properties of the ECM, and thus determine the effects of substratum stiffness on cell behavior in culture [133]. Recent findings from these experiments show that the transcription factors YAP (Yes-associated protein) and TAZ (transcriptional coactivator with PDZ-binding motif), which have implications in growth, proliferation, and differentiation, become activated in response to cytoskeletal tension and cell spreading induced by a stiff substratum [134]. In human mammary epithelial cells, expression of tumor suppressor phosphatase and tensin homolog (PTEN) is reduced in the presence of microRNA miR-18a, which is modulated by ECM stiffness [135]. PTEN is antagonistic to PI3K, a protein involved in many pathways important for cell growth and survival that promotes cancer when misregulated [136]. Polyacrylamide gels were also used to show that matrix rigidity induces integrin clustering in mammary epithelial cells, which induces the formation of focal adhesions and generates cytoskeletal tension. This in turn activates ERK and enhances EGF-dependent pathways that activate ERK, which is known for its involvement in cell cycle regulation [137, 64].

Other cell cycle-regulators are activated by adhesion to or disruption of the substratum. The protein p38 is best known for its role as a tumor suppressor, but also regulates mitotic entry and the spindle assembly checkpoint [138], and negatively regulates cell proliferation through a reactive oxygen species (ROS)-mediated response to stress [139]. When mammary epithelial cells lose adhesion to the substratum, p38 is activated and can induce apoptosis [140]. NM23-H1 is another protein associated with growth arrest, and this function was shown to be correlated with basement membrane assembly in human breast cancer cells [141].

Aside from the effects of mechanically-regulated gene transcription, cytokines and other signaling factors that contribute to cancer progression are often triggered by mechanical forces, and can induce GIN. For example, lung cancer cells show an increased production of ROS in response to shear stress [142]. ROS are well known to promote genetic mutations and cancer progression [143]. Furthermore, a xenograft of human skin overexpressing bFGF (in a cocktail with stem cell factor and endothelin-3) causes replication stress [144], the major source of GIN [108]. As previously described, bFGF is regulated by shear stress and hydrostatic pressure [23, 22].

Restructuring of the Stroma Results in GIN

In addition to signaling mediated by mechanosensors, cells can communicate with the microenvironment through various soluble factors that serve to restructure the surrounding stroma. In cancer, misregulation of these proteins has been linked to GIN. MMPs make up one class of proteins that remodel the ECM. Overexpression of MMPs can induce cell cycle progression, activate genotoxic pathways, and inhibit cytokinesis [145]. Furthermore, cells overexpressing MMPs often exhibit patterns of genomic irregularities [146]. The stroma is also heavily remodeled during the formation of new vasculature. Both cyclic and constant static stretch of endothelial cells increase the expression of vascular endothelial growth factor (VEGF) receptor and promote VEGF-induced proliferation, vasculogenesis, and angiogenesis [147]. VEGF has been shown to regulate the axis of division in endothelial cells, potentiating GIN [148]. Thus, through restructuring of the stroma, in addition to control of the cell cycle and associated proteins and cytokines by external forces, GIN is mediated by mechanotransduction in cancer cells.

Synopsis and Outlook

Aberrant mechanotransduction is a major contributor to tumor progression, metastasis, and GIN. Both mechanosensing and subsequent intracellular signaling alter properties of the cell that can lead to malignant transformation in cancer. Mechanotransduction is therefore important to study in order to understand the progression of this disease. Developing improved 2D and 3D cell culture models to mimic

the tumor microenvironment will enable us to determine the effects of abnormal mechanotransduction in cancer progression. Beyond experimental models, computational models can characterize the effects of mechanical stretch on cell behavior [121]. Others begin to account for intratumor heterogeneity when predicting therapeutic response [149]. However, current computational models cannot cope with mutational frequency of cancer cells, and thus there is a disconnect between investigations of the causes and consequences of this feature.

Although many of the proteins involved in mechanotransduction are known (e.g. integrins, cytoskeleton, myosins, kinases), the precise mechanisms by which a cell perceives the mechanical information of its environment remain unclear [150]. In addition, mechanical forces in the microenvironment are known to affect the cell cycle, and abnormal expression of cell-cycle regulators can result in GIN [132]; however, a clear mechanotransduction pathway linking these two events has not been elucidated. Similarly, current knowledge on the mechanosensing capabilities of stem cells is limited; verifying which forces, molecular pathways, and mechanosensing proteins are most important in directing construction of the stem cell niche and stem cell differentiation could lead to clinical applications (for example, targeting cancer stem cells) [151, 152].

Components of mechanotransduction pathways are starting to be considered as potential therapeutic targets. For example, it has been shown that the disruption of Rho or ERK signaling results in a reduction of cytoskeletal tension that leads to a decrease in tumor cell proliferation and the repression of malignant progression [16, 64]. Targeting Src activity could reduce proliferation, invasion, and metastasis [153]. Restoring anoikis response might curb metastasis [154], and the inhibition of collagen crosslinking and integrin signaling might reduce invasion. In addition, the mechanical properties of isolated metastatic cancer cells could be diagnostic indicators for prognosis. As we broaden our current understanding of mechanotransduction as it relates to both normal cell functions and disease, we will be able to integrate this knowledge into a synergistic treatment strategy for cancer.

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