# Microenvironment Influences Cancer Cell Mechanics from Tumor Growth to Metastasis

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Deepraj Ghosh and Michelle R. Dawson

**Abstract**

The microenvironment in a solid tumor includes a multitude of cell types, matrix proteins, and growth factors that profoundly influence cancer cell mechanics by providing both physical and chemical stimulation. This tumor microenvironment, which is both dynamic and heterogeneous in nature, plays a critical role in cancer progression from the growth of the primary tumor to the development of metastatic and drug-resistant tumors. This chapter provides an overview of the biophysical tools used to study cancer cell mechanics and mechanical changes in the tumor microenvironment at different stages of cancer progression, including growth of the primary tumor, local invasion, and metastasis. Quantitative single cell biophysical analysis of intracellular mechanics, cell traction forces, and cell motility can easily be combined with analysis of critical cell fate processes, including adhesion, proliferation, and drug resistance, to determine how changes in mechanics contribute to cancer progression. This biophysical approach can be used to systematically investigate the parameters in the tumor that control cancer cell interactions with the stroma and to identify specific conditions that induce tumor-promoting behavior, along with strategies for inhibiting these conditions to treat cancer. Increased understanding of the underlying biophysical mechanisms that drive cancer progression may provide insight into novel therapeutic approaches in the fight against cancer.

**Keywords**

Cell mechanics · Deformation · Microrheology · Traction force · Epithelial to mesenchymal transition (EMT) · Motility · Adhesion · Metastasis

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## 5.1 Introduction

To improve cancer prevention and survival rates, the biology of cancer has been extensively an-



C. Dong et al. (eds.), *Biomechanics in Oncology*, Advances in Experimental Medicine and Biology 1092, https://doi.org/10.1007/978-3-319-95294-9_5

alyzed to find molecular targets at genetic and epigenetic levels. Yet, cancer remains a leading cause of death worldwide, with over 90% of cancer-related deaths due to metastasis [1]. Physical interactions of cells in the tumor microenvironment, along with the mechanical forces that modulate them, play a critical role in cancer metastasis [2–4]. The growth of metastatic tumors is also highly dependent upon the recruitment of host-derived stromal cells, such as fibroblasts, mesenchymal stem cells (MSCs), and immune cells, which secrete extracellular matrix proteins, soluble factors, and proteases critical for tissue remodeling and tumor microenvironment development [5]. Therapeutics targeting non-cancer cells in the tumor microenvironment have emerged as adjuvants to traditional chemotherapeutics [6, 7]. The complexity and heterogeneity of cancer are major challenges to the development of successful treatments. In this respect, our knowledge and understanding of the disease are incomplete, and new aspects are being researched more actively to influence the outcome of cancer.

Many of the hallmarks associated with cancer, including unlimited replicative potential, apoptotic evasion, and tissue invasion and metastasis, can be linked to abnormal cytoskeletal or matrix mechanics—important biophysical parameters [8–10]. A common feature of these biophysical interactions is the transmission of force from the extracellular matrix (ECM) to the internal cytoskeleton, which forms the structure of the cell. Groundbreaking work from the Weaver lab has demonstrated that mechanics play a critical role in cancer progression [3, 4, 11]. My lab also showed that increased traction forces (transmitted from the internal cytoskeleton to the external environment) correlate with increased cancer cell motility, proliferation, and chemoresistance; this was demonstrated in mechanosensitive breast and ovarian cancer cells that respond to changes in matrix stiffness [12, 13] and in a genetic model of induced epithelial to mesenchymal transition (EMT) [14]. We also showed that paracrine factors exchanged between cancer and stromal cells dramatically alter the mechanical properties of both cell types [15–19].

The field of physical oncology is aimed at exploring the role of mechanical forces in the tumor microenvironment during growth and metastasis [2–4, 20–22]. Mechanical forces in the primary tumor are caused by solid stress that results from the rapid proliferation of tumor cells and the recruitment of host-derived stromal cells. Matrix stiffening and high interstitial fluid pressure further contribute to this high-stress environment, which alters cells and the surrounding matrix to activate signaling pathways important in cancer [11, 23]. Mechanical forces are also critical in directing cancer metastasis. In fact, cancer cells undergo a cascade of biophysical changes throughout this process. First, cells undergo morphological elongation with reduced cell-cell adhesion during EMT. Next, cells go through multiple deformations as they cross the tumor stroma and surrounding basement membrane, then migrate through the bloodstream to the metastatic site, and finally invade the tissue to form metastases [2, 3, 21]. This chapter on cancer cell mechanics will explore changes in matrix mechanics, cytoskeletal and nuclear mechanics, cell traction forces, and motility at different stages of cancer progression, including growth of the primary tumor, local invasion, and metastasis (illustrated in Fig. 5.1).

## 5.2 Mechanical Forces in Cancer

Quantitative analysis of intracellular mechanics, surface traction, and matrix stiffness forces allow us to probe the biomechanical properties of the tumor with an unprecedented level of detail. These biophysical techniques can be used to systematically investigate the parameters in the tumor that control cancer cell interactions with the stroma and to identify specific conditions that induce tumor-promoting behavior, along with strategies for inhibiting these conditions to treat cancer. This section briefly outlines biophysical techniques and provides insight on how these techniques can be combined with cell fate analysis to study cancer.

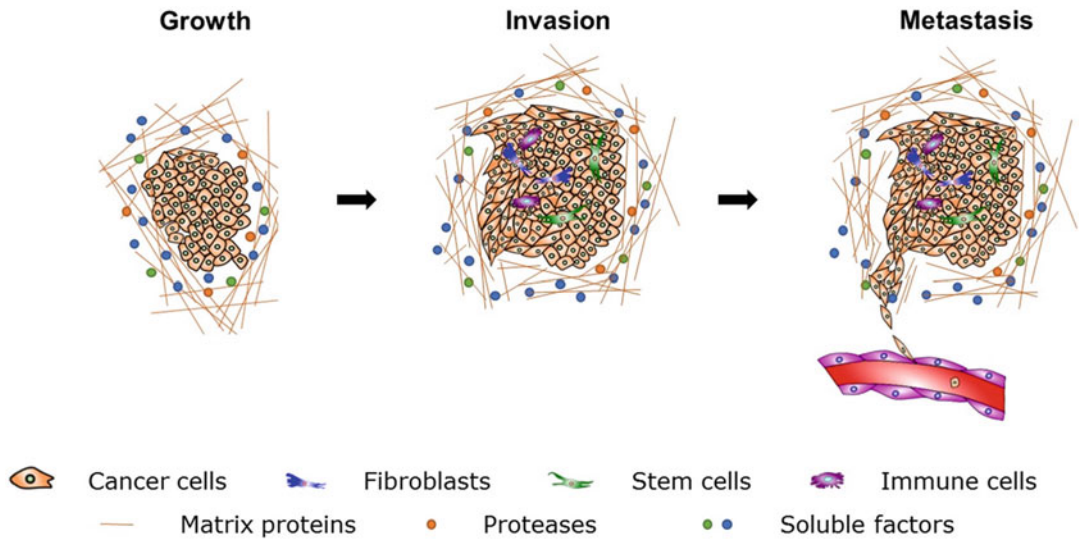


**Fig. 5.1** The progression of cancer from the development of the primary tumor, to the invasion of the surrounding tissue, and the formation of distal metastases are controlled by biophysical properties of the tumor microenvironment, including extracellular matrix mechanics, cell and nuclear mechanics, cell traction forces, and motility. This chapter will explore how these parameters are measured and used to increase our understanding of metastatic cancer

## 5.2.1 Intracellular Mechanics

**Actin and Rho GTPases**

Intracellular mechanical properties are largely determined by structure of filamentous actin. Actin filaments can organize in a myriad of hierarchical structures in a cell: parallel bundling of actin results in stress fiber formation to provide tensile strength and strong contractile activity, whereas cross-linking of actin filaments increase intracellular elasticity. Actin can interact with other structural complexes, like myosin motor proteins, to control actomyosin contractility which plays a key role in cell-generated forces [24]. The Rho family of GTPases and its downstream effectors play a pivotal role in regulating the structural dynamics of actin, and these proteins are overexpressed in tumors [25–29]. In particular, activation of small Rho GTPases such as RhoA with the help of guanidine exchange factors (GEFs) leads to the activation of Rho-associated kinases (ROCK) that block myosin light chain (MLC) phosphatase and activate myosin light chain kinase (MLCK) leading to MLC phosphorylation and actomyosin contractility. The actin cytoskeleton is also connected to the nucleus via LINC complexes that transmit mechanical signals to the nucleus to regulate transcription factors [30, 31].

(continued)

During progression of cancer, the dynamic microenvironment forces cancer cells to adapt and modify their mechanical properties in response to both chemical and mechanical stimulation. Characterization of cancer cell mechanics using deformability, defined as the resistance to deformation, at single-cell level has become increasingly important to design new diagnostic tools and treatment methods. Intracellular mechanical properties are regulated by the cytoskeleton, a complex network of filamentous actin, microtubules, and intermediate filaments extending

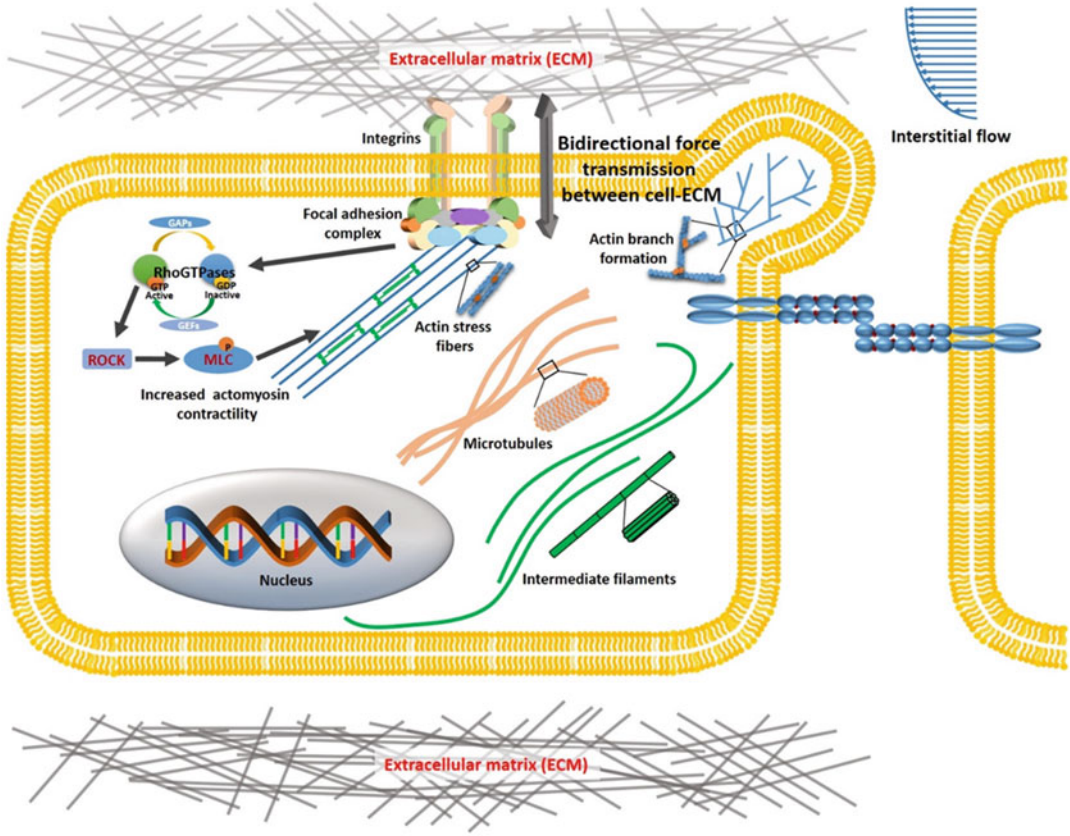


**Fig. 5.2** Schematic of cellular components contributing to cell mechanics and mechanotransduction. Cytoskeletal proteins actin, microtubule, and intermediate filaments act as load-bearing components of the cells. Cells can sense extracellular mechanical stimuli through transmembrane receptors (e.g., integrins) that activate downstream intracellular signaling pathways. Integrin dimerization results in recruitment of multiple structural proteins to the intracellular tail domain to form focal adhesion complex that activates pathways such as ERK and Rho-ROCK signaling. Furthermore, small Rho GTPases can activate myosin light chain phosphorylation and increase actomyosin assembly that generate and transmit contractile forces to the matrix

from the cell cortex to the nucleus (Fig. 5.2) [32–34]. Together these structures provide the principal resistance to an external deformation; and the role of each component in regulating mechanics is discussed briefly here. Contributions of actin filaments in regulating overall resistance to external deformation have been extensively investigated over many years [35]. F-actin, which undergoes rapid polymerization and depolymerization with the help of small Rho GTPases and actin-binding proteins, dynamically transforms cell shape and generates mechanical forces required for numerous cellular processes, including adhesion and migration [36–39]. The assembly and disassembly of microtubules are critical for processes such as cell division and molecular transport; however, the resistance of microtubules to external deformation is very low compared to the other cytoskeletal proteins [40]. Intermediate filaments can maintain cytoskeletal structure and resist deformation at a strain level where actin filaments break down [40]. Interestingly, vimentin, an intermediate filament protein, is upregulated during EMT, a critical step in the metastatic process where epithelial cancer cells transition to more invasive phenotype [41, 42]. The structure of cytoskeletal filaments can vary significantly between *2D* and *3D*. For example,

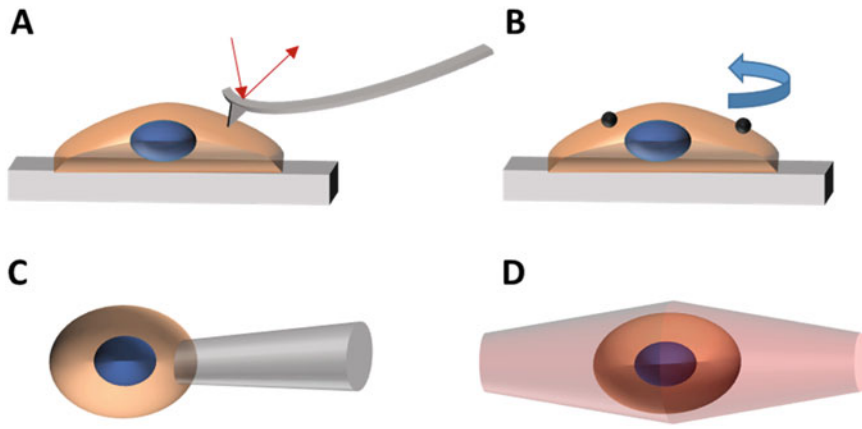


**Fig. 5.3** Biophysical tools to measure the mechanical properties of cells at different length scales. Tools that can probe at a local region of the cells include (**a**) atomic force microscopy and (**b**) magnetic bead twist rheometry, whereas techniques such as (**c**) micropipette aspiration and (**d**) optical trap measure the deformability of whole cell

actin forms large stress fibers, lamellipodia, and filopodial protrusions in 2D, whereas in 3D actin filaments primarily appear in cortical regions. Nonetheless, these cytoskeletal proteins play a significant role in regulating cell mechanics in both 2D and 3D.

Over the years different biophysical models have been proposed to characterize the mechanical properties of the cell including tensegrity, poroelasticity, glassy transition, and classic viscoelastic models [43, 44]. Cells are considered soft biomaterials that behave as complex viscoelastic fluids, and the study of microrheology is used to describe the viscoelastic properties of cells in terms of their response to applied external stress or strain [45, 46]. Recent advancements in microscopy have enabled the development of passive probing tools to analyze cell rheology. In this case, frequency-dependent viscoelastic properties are measured by monitoring internal energy-driven random motion of nanoparticles embedded inside the cell [47, 48]. However, the transport of embedded particles must be passive for these models to quantitatively assess rheology as active transport of ATP-dependent motor proteins provides inaccurate results [49].

The techniques that have been developed to probe the viscoelastic properties of individual cells can be grouped into two broad categories based on probing length scale varying between subcellular to whole-cell regions (Fig. 5.3). Techniques such as atomic force microscopy (AFM), intracellular particle tracking microrheology (IPTM), magnetic bead twist cytometry, and optical tweezers probe a local area on the membrane or cytosol to measure cell mechanics [50–53]. In case of AFM and magnetic bead twist cytometry, local stress is applied to membrane of adherent cells, and the measured parameters are dominated by the cortical and large actin structures and the rigidity of the plasma membrane. To capture the mechanics of an entire cell, mechanical stretch is applied to the cell in suspension using micropipette aspiration or optical stretching in suspension [54, 55]. Among the techniques, we have used IPTM to rapidly probe the heterogeneity as well as the short-term or transient mechanical response of the cell [47]. IPTM can use both injected inert nanoparticles or vesicles, and other granular materials inside the cells, to passively probe the microrheology. The basis of IPTM is described briefly below [56].

The displacements of fluorescent particles embedded in the filamentous network are captured using a fluorescence microscope with high magnification and a fast speed camera for good spatial and temporal resolution (Fig. 5.4). High resolution of particle displacements is obtained by tracking the intensity-weighted centroids of the

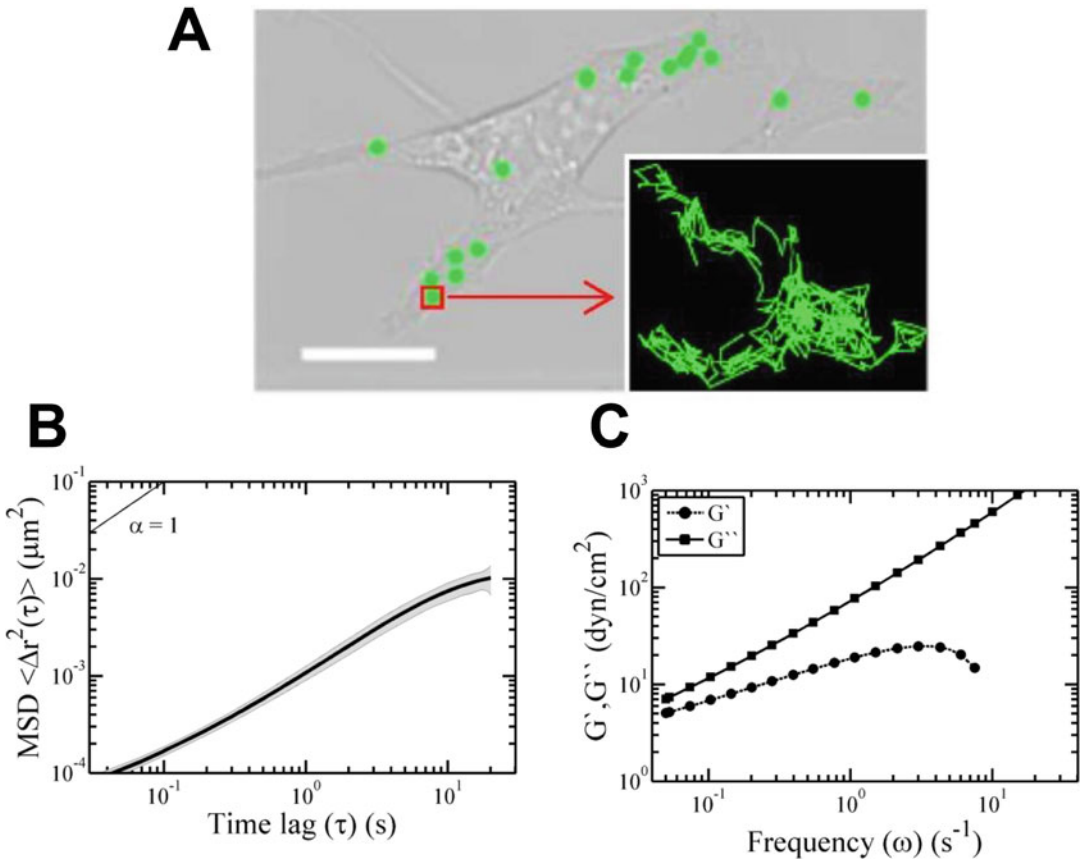


**Fig. 5.4** Intracellular particle tracking microrheology. (**a**) Phase-contrast image of SKOV3 cell injected with 100 nm green nanoparticles and Brownian motion of a single particle embedded in the cytoplasm (inset). (**b**) Time-dependent ensemble average MSDs of particles were used to calculate (**c**) frequency-dependent viscous (*G*') and elastic (*G*") moduli of SKOV3 cells. Adapted from Dawson et al. [56]

particles in the plane of focus of the objective. In 2D, the mean square displacement (MSD) of the particle is obtained by the following equation (Fig. 5.4b):

$$MSD =< r^2(\tau) > =< [x(t+\tau) - x(t)]^2 + [y(t+\tau) - y(t)]^2 > \quad (5.1)$$

The MSD of the particle is related to the local diffusivity of the network, which is determined:

$$D = \frac{< \Delta r^2(\boldsymbol{\tau}) >}{4\tau} \quad (5.2)$$

In a purely viscous fluid, such as water or glycerol, the thermal fluctuation-driven particle motion is only hindered by viscous drag with the Stokes-Einstein equation:

$$D = \frac{k_B T}{6\pi a \eta} \quad (5.3)$$

where $D$ is the diffusion coefficient, $k_B$ is Boltzmann's constant, $T$ is temperature, $a$ is particle radius, and $\eta$ is viscosity of the fluid. Therefore, the particle tracking data can be used to extract the viscosity of the fluid surrounding the particle.

However, in a complex filamentous network space when the particle size is greater than the pore size of the meshwork, which is the distance between filaments aligned in any direction, particle motion is not only affected by the viscosity of the fluid but also the elasticity of the filamentous network. The creep compliance ($J$), which measures the deformability of the meshwork, can be directly calculated from the particle MSD:

$$J(\boldsymbol{\tau}) = \left(\frac{3\pi a}{2k_B T}\right) MSD(\tau) \tag{5.4}$$

In order to facilitate analysis of additional viscoelastic properties of the cytoplasm, the MSD is transferred to the frequency-dependent Fourier domain. The frequency-dependent form of the Stokes-Einstein equation is used to determine the viscoelastic properties of the fluid:

$$G^*(\omega) = \frac{2k_B T}{3\pi a \Delta r^2 \left(\frac{1}{\omega}\right) \Gamma\left[(1+\alpha(\omega))\left(1+\beta(\omega)/2\right]\right.} \tag{5.5}$$

where $G^*$ is the frequency-dependent complex shear modulus, $\Gamma$ is the gamma function, $\alpha$ is the first derivative, and $\beta$ is the second derivative of the MSD curve. The complex shear modulus can be further divided into the in-phase component, or elastic (storage) modulus ($G'$), and the out-of-phase component, or viscous (loss) modulus ($G''$) (Fig. 5.4c):

$$G'(\omega) = \left|G^*(\omega)\right| cos\left(\frac{\pi\alpha(\omega)}{2}\right) \tag{5.6}$$

$$G'(\omega) = \left|G^*(\omega)\right| sin\left(\frac{\pi\alpha(\omega)}{2}\right) \tag{5.7}$$

These techniques have been utilized to measure the viscoelasticity of a variety of cancer cells e.g., breast, liver, prostate, kidney, glioma, ovary, and bladder [57]. It has been shown that malignant cancer cells are more deformable than their non-oncogenic counterpart [58]. For example, via optical stretching Guck et al. found that 12-O-tetradecanoylphorbol-13-acetate-treated MCF-7 cells, which display more tumorigenicity, are softer than the untreated MCF-7 cells, which were, in turn, more deformable than epithelial breast cells MCF-10A [55]. Furthermore, comparison between high-grade and low-grade breast, colon, and ovarian cancers and chondrosarcomas found softer cells in more metastatic lines. This drastic change in the viscoelastic property is often correlated with severe modification of cytoskeletal organization and intracellular tension of the cell.

### 5.2.2 Traction Forces

**Mechanotransduction and Cell Adhesion Molecules (CAMs)**

Mechanotransduction is defined as cells' ability to translate external mechanical stimulus into intracellular biochemical signaling. Adhesion molecules linking the cell cytoskeleton to the ECM play a critical role in reciprocating forces between cells and the surrounding environment. **Integrins** are heterodimeric transmembrane adhesion proteins that can act as a force sensor for cells. The extracellular domain of an integrin interacts with matrix proteins, including collagen and fibronectin, and the intracellular domain recruits focal adhesion proteins, including **talin, vinculin**, **paxillin**, **zyxin,** and **focal adhesion kinase (FAK)**, to form the **focal adhesion complex (FA)** which is directly connected to the actin cytoskeleton. Conformational changes in focal adhesions due to binding of the ECM lead to activation of various signaling pathways including Rho-ROCK and ERK. Cytoskeletal tension, which is highly dependent upon Rho GTPases, transmits signals through adhesion receptors that regulate adhesion, migration, and ECM remodeling [59–62].

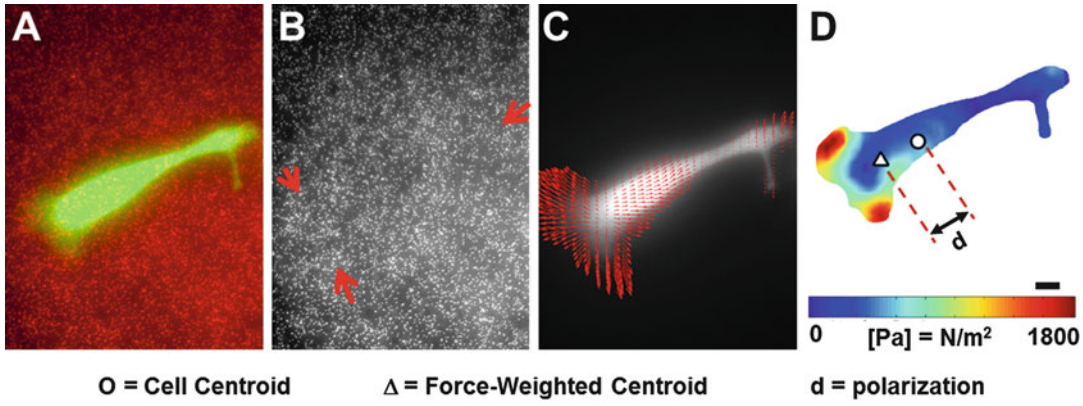


**Fig. 5.5** Traction force microscopy. (**a**) Calcein AM-labeled SKOV-3 cell (green) cultured on collagen-coated polyacrylamide substrate embedded with 200 nm fluorescent red particles was imaged. (**b**) Another image of the embedded nanoparticle was taken after detaching cells (red arrows point to the fields with high displacements). (**c**) Displacement vectors were calculated from previous images. (**d**) Heat map of the traction force field and polarization were estimated as previously described by Sabass et al. [64]. Adapted from McGrail et al. [65]

Physical interactions via adhesion between cancer cells and the surrounding matrix are necessary for vital cell processes, including cell deformation, migration, and mechanotransduction. The magnitude of traction forces generated at these adhesion sites, along with the strength of adhesions, are critical in regulating these processes [63]. Under normal static conditions, cells exert contractile forces on their ECM; however, the ECM is sufficiently rigid to resist elastic deformation. This balance between cellular forces and matrix rigidity is critical in maintaining homeostasis [2]. As normal breast epithelial cells transform into invasive cancer cells, they generate more force and secrete proteinases that breakdown ECM, which alters the force balance to favor invasion and metastasis [2].

To further understand the role of mechanotransduction in cancer cells, a number of methods have been used to study cell-generated traction forces in 2D and 3D. In this respect, hydrogels composed with synthetic polymeric materials that display linear elastic deformation have been used extensively. To determine cell-generated traction forces in 2D, cancer cells are often cultured on surfaces including hydrogels embedded with nanoparticles, micropatterned substrates, and micropost arrays. Analysis of cell traction forces on soft elastic substrates can be divided into two major steps: (1) determination of the substrate displacement field from image data and (2) calculation of cell traction stresses or forces (illustrated in Fig. 5.5). Traction forces are calculated using methods outlined by Sabass et al. [64]. In the Boussinesq solution, at any point $\vec{x}$, the displacement field $\vec{u}\left(\vec{x}\right)$ can be determined by the convolution of the Green function $G\left(\vec{x}-\vec{x}'\right)$ with the traction field $\vec{T}\left(\vec{x}'\right)$ as follows:

$$\vec{u}\left(\vec{x}\right) = \mathbf{G}\left(\vec{x}-\vec{x}'\right) \otimes \vec{T}\left(\vec{x}'\right) \tag{5.8}$$

where if one takes the two-element vector $\vec{r} = \left|\vec{x}-\vec{x}'\right|$ with components $(r_x, r_y)$ and magnitude $r$, $E$ as the Young's modulus, and $\upsilon$ as the Poisson ratio, the Green function is given by:

$$\mathbf{G}\left(\vec{r}\right) = \frac{(1+\upsilon)}{\pi E r^3} \begin{bmatrix} (1-\upsilon)\, r^2 + \upsilon r_x^2 & \upsilon r_x r_y \\ \upsilon r_x r_y & (1-\upsilon)\, r^2 + \upsilon r_y^2 \end{bmatrix} \tag{5.9}$$

Investigating cell-generated traction forces has provided new insights into cancer cell

invasion and metastasis. Kraning-Rush et al. reported that metastatic breast, prostate, and lung cancer cell lines generate higher traction forces compared to nonmetastatic cells [66]. Koch et al. examined traction force-induced strain distribution in 3D collagen gels with the same lung and breast cancer cell lines and determined that anisotropic and polarized distribution of traction force is integral for metastatic cancer cell invasion [67].Advent of new tools and defined in vitro models to study 3D traction forces has resulted in a better understanding of cancer cell functions that can be used either as a biophysical marker or manipulated to design new therapeutic targets.

### 5.2.3 Matrix Mechanics

Malignant breast, liver, and prostate tumors have been reported to be significantly stiffer than benign ones, and in most cases, increased tumor stiffness correlates positively with invasiveness (illustrated in Fig. 5.6) [70–73]. Additionally, other properties of ECM such as composition, pore size, and degree of cross-linking can play a critical role in regulating cell function in tissue microenvironments [10]. Chaudhuri et al. demonstrated that abnormal growth of the mammary epithelium was not only in part to increased tissue stiffness but reduced concentrations of laminin that normally interacts with α6β4 integrin; unbound integrin activates mechanosensitive Rac1 and PI3K contributing to malignancy [74]. Increased matrix stiffness combined with modified cell-ECM interaction can reinforce mechanotransduction in cancer and stromal cells and contribute to growth, survival, and invasion.

The progression of breast cancer marked by increased ECM stiffness in a tumor is due to excessive collagen secretion and lysyl oxidase(LOX)-dependent cross-linking of collagen [75]. This continual change in matrix properties profoundly alters the biophysical properties of the cancer cells. Paszek et al. reported that increased stiffness leads to enhanced clustering of integrins which upregulate cytoskeletal tension and focal adhesion assembly [11]. Increased actomyosin contractility and cytoskeletal tension help cells in the tumor ECM generate higher surface traction forces by promoting the maturation and turnover of focal adhesions [76, 77]. Disruption of actin or myosin in either 2D or 3D microenvironments disrupts cell-generated force and inhibits cell migration and invasion [78].

To study mechanotransduction and cell-generated forces, both synthetic and natural polymers have been used to create gels with controlled mechanical properties [79–81]. Among the synthetic 2D gels, polyacrylamide (PA)-based substrates have been utilized widely and can be used to produce a range of mechanical stiffness mimicking tissue rigidity by changing the ratio of monomer and cross-linker while maintaining ligand density [82]. PA substrates embedded with fluorescent tracer particles have been used to study cell-generated traction forces using traction force microscopy. Other synthetic polymers such as polydimethylsiloxane (PDMS), polyethylene glycol (PEG), poly (lactic-co-glycolic) acid (PLGA), and alginate have been engineered to synthesize materials with controlled physical, chemical, and biomolecular properties to recapitulate in vivo cell function. However, these synthetic matrices fabricated to model tumor microenvironment cannot be degraded and remodeled by the cells, thus limiting their role to study the dynamics between the cell and the ECM. Three-dimensional systems of natural biopolymers such as collagen I, reconstituted basement membrane, can overcome this limitation to capture the stiffness-dependent cell behavior in a more dynamic manner in vitro [83, 84]. Stiffness of these gels can be modified by altering gelation conditions with an increase in the total protein concentration or with the addition of cross-linkers; however, this change in stiffness is also intrinsically linked to pore size, ligand density, and fiber properties of the polymerized network. Despite their limitations, both 2D and 3D hydrogels have facilitated new biological insights into key features of tumor progression with greater physiological relevance.

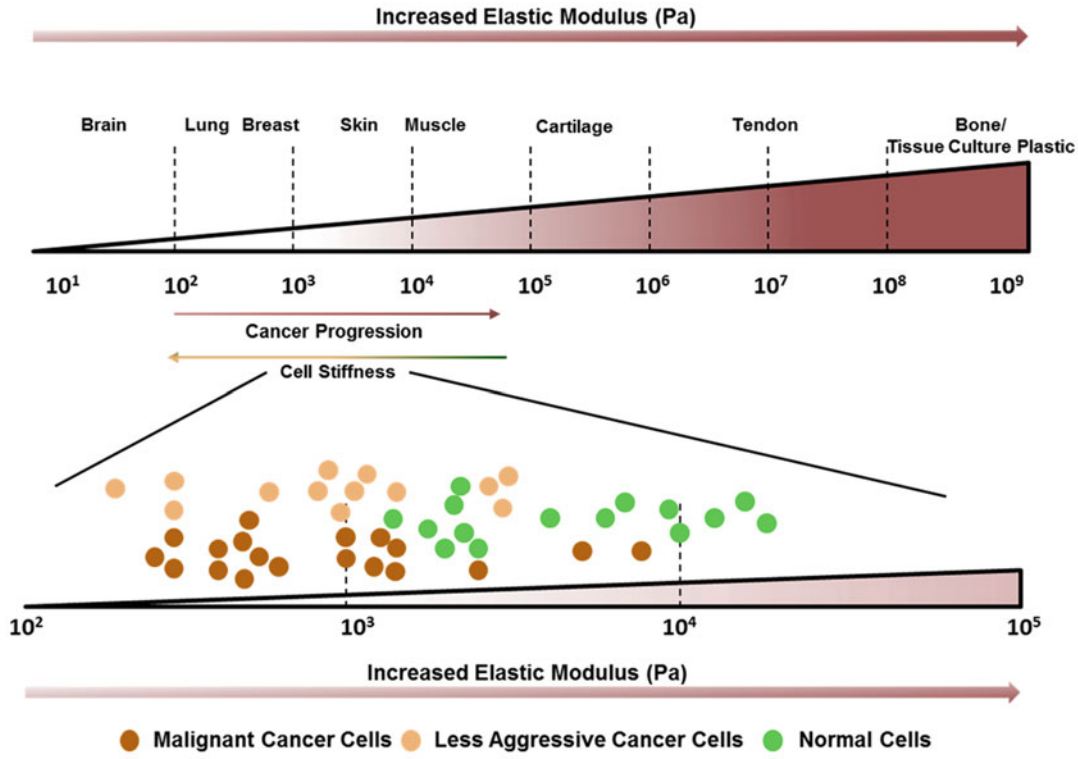


**Fig. 5.6** Matrix and cancer cell mechanics. Tissues stiffness, measured by the elastic modulus ($E$) in Pascals (Pa), varies significantly between soft tissues, such as brain (~100 Pa) to rigid tissues such as bone (>1 GPa) [68, 69]. Elastic or Young's modulus of cancer cells was measured by AFM and is reported to be lower compared to normal cells isolated from the breast, liver, ovarian, bladder, kidney [58]. Cancer cell stiffness decreases while tissue stiffness increases with cancer progression

### 5.2.4 Multivariable Analysis

Quantitative analysis of intracellular mechanics [14, 16] and surface traction forces [12, 13]has been combined with analysis of cell fate processes important in cancer; these cell fate processes include critical cancer hallmarks, such as adhesion, migration, proliferation, and chemoresistance. Multivariable analysis is critical in determining the role of mechanical forces in cancer progression and in analyzing heterogeneity in cancer cell populations. We previously used this approach to look at metastatic cancer cells originating from different locations which undergo inverse responses to altered matrix stiffness. Metastatic ovarian cancer cells (SKOV-3) prefer soft matrices, and metastatic breast cancer cells (MDA-MB-231) prefer hard matrices as characterized by tumor cell migration, proliferation, and chemoresistance (Fig. 5.7) [13]. SKOV-3 exerted higher traction forces on softer substrates, whereas MDA-MB-231 exerted higher traction forces on hard substrates (Fig. 5.7a, b). In both cases, these higher traction forces were correlated with an increased malignant phenotype, characterized by increased cell motility (Fig. 5.7c), proliferation (Fig. 5.7d), and resistance to doxorubicin (Fig. 5.7e). The observed correlation of increased traction forces with increased malignant phenotype agrees well with other studies across a variety of cell lines [66]. The mechanism of this progression has been well studied in breast cancer, where increased

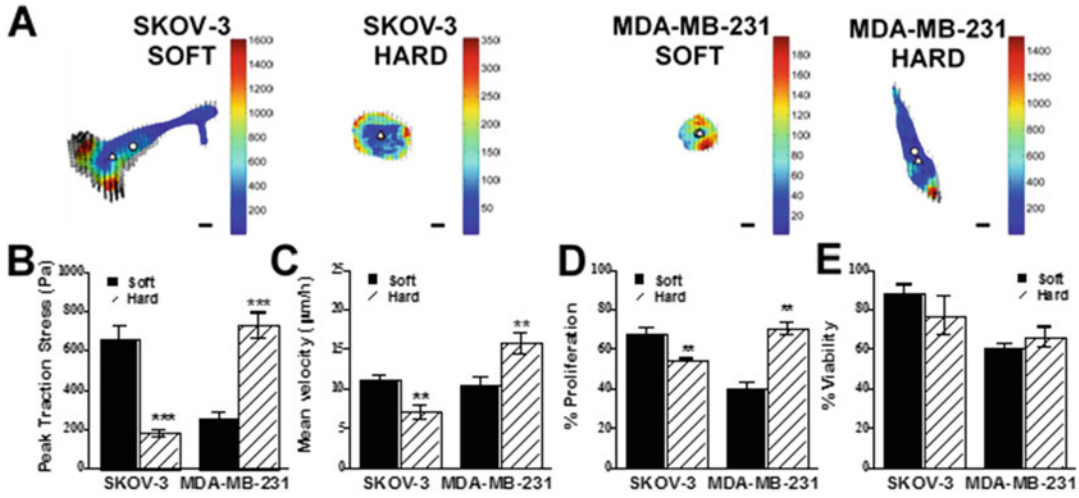


**Fig. 5.7** Cancer cell traction forces correlate with motility, proliferation, and chemotherapeutic resistance. SKOV-3 and MDA-MB-231 cells were cultured on soft (3 kPa) and hard (35 kPa) polyacrylamide substrate surface—modified with collagen or on collagen-coated glass. (**a**) Heat maps of traction stresses (in Pa) overlayed on cell-induced matrix displacements (black arrows) and (**b**) peak traction stresses (top 95th percentile of traction stresses). Heat maps also show force polarization, the distance between the cell center of mass (○) and force-weighted center of mass (Δ). (**c**) Mean cell velocity was determined by nuclear tracking over an 8-h period. (**d**) Cell proliferation was determined with BrdU. (**e**) The viability of cells treated for 24 h with 2 μM doxorubicin was determined by MTT. Increased traction stress was correlated with increased motility, proliferation, and chemoresistance for SKOV-3 ovarian cancer cells on soft and MDA-MB-231 breast cancer cells on hard substrates. Adapted from McGrail et al. [13]

matrix stiffness leads to integrin activation followed by focal adhesion formation and increased actomyosin contractility [3]. However, the increased forces exerted by SKOV-3 on soft matrices had not been previously characterized before our studies [12, 13]. Previous work showed that adipocytes in the omentum (primary metastatic site) act as a rich energy source and actively promote ovarian cancer cell homing via cytokines like interleukin-8 [85]. However, our findings show that metastatic ovarian cancer cells also display a mechanical tropism for softer environments. This tropism for matrices of specific mechanical rigidity was only observed in more invasive cancer cells; in fact, MDA-MB-231 and SKOV-3 showed larger functional differences across substrate rigidities than less invasive OVCAR-3, MCF-7, and MDA-MB-361 [13]. Multivariable analysis can also be used to study stromal cells in the tumor microenvironment [15, 16] or subpopulations of cancer cells [12, 86].

## 5.3 Mechanics of Primary Tumor Growth

This section outlines how cancer cell mechanics are affected by solid and fluid forces that accumulate in growing tumors. These physical forces intensify with tumor size and result in deformation of blood vessels and lymphatics critical in supplying fresh nutrients and draining depleted fluids. Although these physical forces play a critical role in regulating tumor growth, their effects on cancer cells are not completely understood. This section discusses the effects of solid and fluid stress on cancer cell behavior.

### 5.3.1 Solid Stress

Solid tumors grow under compressive stress from the local tissue, which corresponds with mechanical loads of 50–200 mm Hg [87, 88].

Compression accumulates from two sources, comprising (1) internal stress from rapidly dividing tumor cells and infiltrating stromal cells and (2) external stress from the surrounding matrix, which undergoes progressive stiffening [22]. In one of the earliest in vitro studies, Helmlinger et al. simulated the effects of compressive stress by growing tumor spheroids in varying concentrations of inert agarose gels and found that spheroids accumulate stress from 45 to 120 mm Hg, far greater than tumor microvascular pressure [87]. Stylianopoulos et al. recently developed a simple model to study tissue relaxation after removing external stress; this model was used to identify key contributors to growth-induced solid stress, e.g., cancer cells, stromal cells, collagen, and hyaluronan [88]. This study also showed that residual solid stress accumulated within the tumor from cell and matrix deformations and elastic strain is maintained even after excision of the tumor.

Despite the importance of solid stress in tumor growth, the role of the components of cancer cells that actively support this stress is not well defined. Although cytoskeletal proteins have been proposed to play an important role in cancer cell mechanics, we found that filamentous actin, microtubules, and intermediate filaments do not actively support compressive loads in breast, ovarian, and prostate cancer cell spheroids [89]. Instead, we found that the sodium channel NHE1 mediates ion efflux, to regulate intracellular tonicity and osmotic pressure, and rapidly alters spheroid size. Additionally, polymerized actin actively regulates sodium efflux and indirectly supports the compressive load. Thus, blocking sodium channel NHE1 or actin depolymerization led to compression-induced cell death [89]. The ability of cancer cells to use ion pumps to modulate their osmotic pressure is central to their survival under compressive solid stress.

Compression has been used to mimic solid stress in the tumor; compressing cells has been shown to alter cellular adhesion, migration, and matrix remodeling. Thamilselvan et al. reported that compressive load of 15 mm Hg for 30 min enhanced both cell-cell and cell-matrix adhesion of colon cancer cells on endothelial cells and collagen matrix, respectively [90]. Applied pressure was also shown to induce mechanotransduction, which increased colon cancer adhesion to matrix by activation of FAK and its binding affinity for β1 integrin [91]. Tse et al. also demonstrated that compressive stress mediates cytoskeletal reorganization and cell-matrix adhesion to promote coordinated migration of breast cancer cells [92]. Interestingly, blocking actomyosin contractility with Rho-kinase or myosin inhibitors did not affect leader cell formation prior to migration suggesting applied stress can actively sustain this transformation. Furthermore, the application of force to cells in vitro results in actin polymerization, cell stiffening, and increased matrix metalloproteinase (MMP) activity, important for ECM remodeling [53, 93].

### 5.3.2 Fluid Stress

Elevated interstitial fluid pressure (IFP) is a hallmark of solid tumors, which results from hyperpermeable or "leaky" blood vessels and nonfunctional lymphatics. Proliferating cancer cells compress blood and lymphatic vessels causing them to collapse; this reduces perfusion rates into the tumor, causing tumors to become hypoxic [22]. As fluid flux from leaky vessels increases, the fluid accumulates in the interstitial spaces due to the lack of draining lymphatics; this interstitial fluid leaking from the tumor forces drugs, growth factors, and cancer cells into the surrounding tissue, severely limiting drug delivery to the tumor and facilitating tumor metastasis [22]. Elevated IFP is linked to poor patient prognosis [94–96], and decreased IFP has been shown to reduce tumor cell proliferation [97, 98]. Although the cells are partially shielded by the matrix proteins against fluid-generated stress, the exposure can still lead to significant changes. Interstitial fluid velocity is very low compared to normal blood vessels, but the presence of small porous space in the stroma can create significant shear stress. Polachek et al. investigated the role of interstitial flow on the migration of breast cancer cells (MDA-MB-231) in a 3D collagen matrix and found that it can induce phosphorylation of FAK

and modulate tumor cell migration [99]. Additionally, the direction of interstitial flow from high-pressure tumor to low-pressure lymphatics or other vessels can create an autocrine chemotactic gradient and guide tumor cells to escape [100]. Elevated IFP and associated shear stress can profoundly modify the function of stromal cells including TGF-β1 signal upregulation, increased MMP-1 activity, and ECM remodeling and cell motility [101, 102]. Taken together, elevated IFP can lead to increased invasiveness of cancer cells [103–105]. In addition, these stresses can combine with biochemical factors to create an abnormal microenvironment that is conducive to malignant progression [102, 106].

## 5.4 Malignant Progression and Metastasis

Metastasis remains the main driver of cancer-related deaths. This complex cascade can be divided into two major steps: (1) physical translocation of a cancer cell from the primary tumor to the metastatic site and (2) colonization of the metastatic site [107]. Mechanical forces are critical in regulating the translocation step since cells must undergo multiple deformations and exert force as they migrate through the tumor stroma, blood vessel endothelium, vasculature system, and finally the tissue to enter the metastatic site. Previous studies have revealed that more invasive cancer cells are softer than less invasive cancer cells [14, 108] or nonmalignant epithelial cells [57] (Fig. 5.6). This change in cell stiffness may enable invasive cancer cells to contort their shape to navigate through dense tissue matrices throughout the metastatic process. Additionally, invasive cancer cells are more likely to respond to changes in matrix stiffness by exerting polarized traction forces [12, 13] (Fig. 5.7). This force response is important in directing cell migration through the tissues during metastasis [3]. This section discusses key issues that affect the mechanical properties of cancer cells throughout the metastatic process.

### 5.4.1 Epithelial-To-Mesenchymal Transition and Local Invasion

EMT is critical for cancer cell invasion and metastasis; this process whereby less motile epithelial cancer cells transition into a more motile elongated mesenchymal phenotype is largely attributed to the loss of E-cadherin that mediates cell-cell adhesions, along with an increase in matrix-binding integrins [107, 109–111]. Increased expression of basement membrane-degrading MMPs further contributes to the development of a more invasive mesenchymal phenotype [112, 113]. Thus, EMT enables cancer cells to escape from the primary tumor through loss of cell-cell adhesions and local invasion of the surrounding tissue, but it also induces a concerted series of biophysical changes to promote migration [14]. Additionally, cell-generated traction forces combined with secreted MMPs can remodel and realign the orientation of adjacent collagen fibers to facilitate the escape of cells from the primary tumor [114]. Inhibiting contractility and MMP activity has been shown abrogate cancer cell invasion [19, 115].

To understand cancer cell migration, motility studies have been performed in both 2D and 3D matrices; however, it is becoming increasingly clear that mechanisms of tumor cell migration through confined spaces in vivo can be better represented by 3D assays [59, 116–119]. Migration in 3D utilizes dynamic adhesion-dependent protrusions with localized actin to probe and invade the ECM, and 2D migration features such as the formation of focal adhesions, stress fibers, and protrusions at the leading edge are either absent or have limited function [120, 121]. In vivo, individual and collective migrations are both important for dissemination from the primary tumor [122–124]. Individual cancer cells can also switch between different modes of migration to invade the ECM [125, 126]. Cancer cells adopt an integrin-based mesenchymal mode of migration when matrix degradation is required to move the nucleus through smaller matrix pores, whereas

cells shift to a faster amoeboid mode of migration regulated by actomyosin contractility that necessitates rapid shape change when proteolysis is not required [127]. Furthermore, studies have reported cancer cells to undergo osmotic engine-driven migration that utilizes flow of water through the cell and pulsatile migration characterized by long periods of slow, random migration alternating with brief episodes of extremely fast, directed migration [128]. For collective cell migration, cancer cells display leader-follower migration dynamics at the edge of the tumor with leader cells exerting significantly higher force and aligning collagen fibers parallel or perpendicular to the tumor [129–131].

EMT-mediated detachment of cancer cells from the primary tumor and subsequent invasion of the basement membrane require changes in physical properties of the cell including cell shape, cytoskeletal organization, and cell-cell adhesion. We have previously reported how genetic induction of EMT in epithelial MCF-7 breast cancer cells via the constitutive activation of SNAIL directly affects cancer cell mechanics (Fig. 5.8). We have used IPTM to establish that genetically modified MCF-7 cells expressing SNAIL possess a softer cytosol (Fig. 5.8a, b) and nucleus (Fig. 5.8c, d) compared to the wild-type control [14]. Furthermore, this change in mechanical properties of the cell was coupled with structural changes including decreased polymerized actin, dramatic loss of cytokeratin, and increased expression of vimentin (Fig. 5.8e, f). In addition to these changes in intracellular compliance and structure, EMT also increased extracellular force exertion and contractile gene expression to further expedite migration (Fig. 5.8g, h) [14].

### 5.4.2 Intravasation, Extravasation, and Tropism to Distal Site

The passage of cancer cells into and from the vascular system across the endothelium barrier is known as intravasation and extravasation, respectively [132]. Cells must be highly deformable to migrate across tight junctions in the endothelium or for transmigration through endothelial cells. Nuclear deformation is critical and is found to be the rate-limiting step for this process; thus nuclear mechanics play a critical role in this step [133, 134].

In the circulatory system, cancer cells are exposed to a range of fluid shear stress that controls their mechanics and function [135]. To exit vasculature and extravasate into a secondary site, cancer cells must adhere to the vascular wall or get trapped in smaller vessels [136]. Invasive cancer cells can also metastasize by following other routes. For example, metastatic ovarian cancer cells disseminate through the intraperitoneal fluids before adhering to secondary site [137]. In both cases, adhesion remains key step in spreading of the disease. Adhesion molecules integrins and cadherins that control cell-ECM and cell-cell binding have been implicated in a wide range of cancers, causing increased tumor survival and metastasis [138–142]. For example, expression of α5β1 integrin regulates ovarian cancer invasion and metastasis, and cadherin 11 has been shown to promote prostate cancer metastasis [143, 144]. Adhesion molecules along with other factors help metastatic cancer cells to preferentially accumulate with higher probability for certain sites.

Massagué and colleagues compared breast cancer subclones that preferentially accumulate in one of the three main breast cancer metastatic sites, i.e., the brain, lung, and bone, to their parent lines to understand what gave these cells the ability to colonize specific sites [145–147]. These studies revealed location-specific adaptations like upregulation of key molecules for adhesion and extravasation into the secondary site as well as growth factor receptors that aid in homing and survival. Recent studies have highlighted that the response of cancer cells to matrix rigidity in vitro can correlate with tissue tropism in vivo: metastatic subclones of breast cancer cells that metastasized preferentially to lungs and bone in vivo displayed higher growth rate on soft and stiff substrates, respectively [148]. Furthermore, we found that after gaining the ability to invade the secondary site, adhere to the surrounding ECM, and respond to local

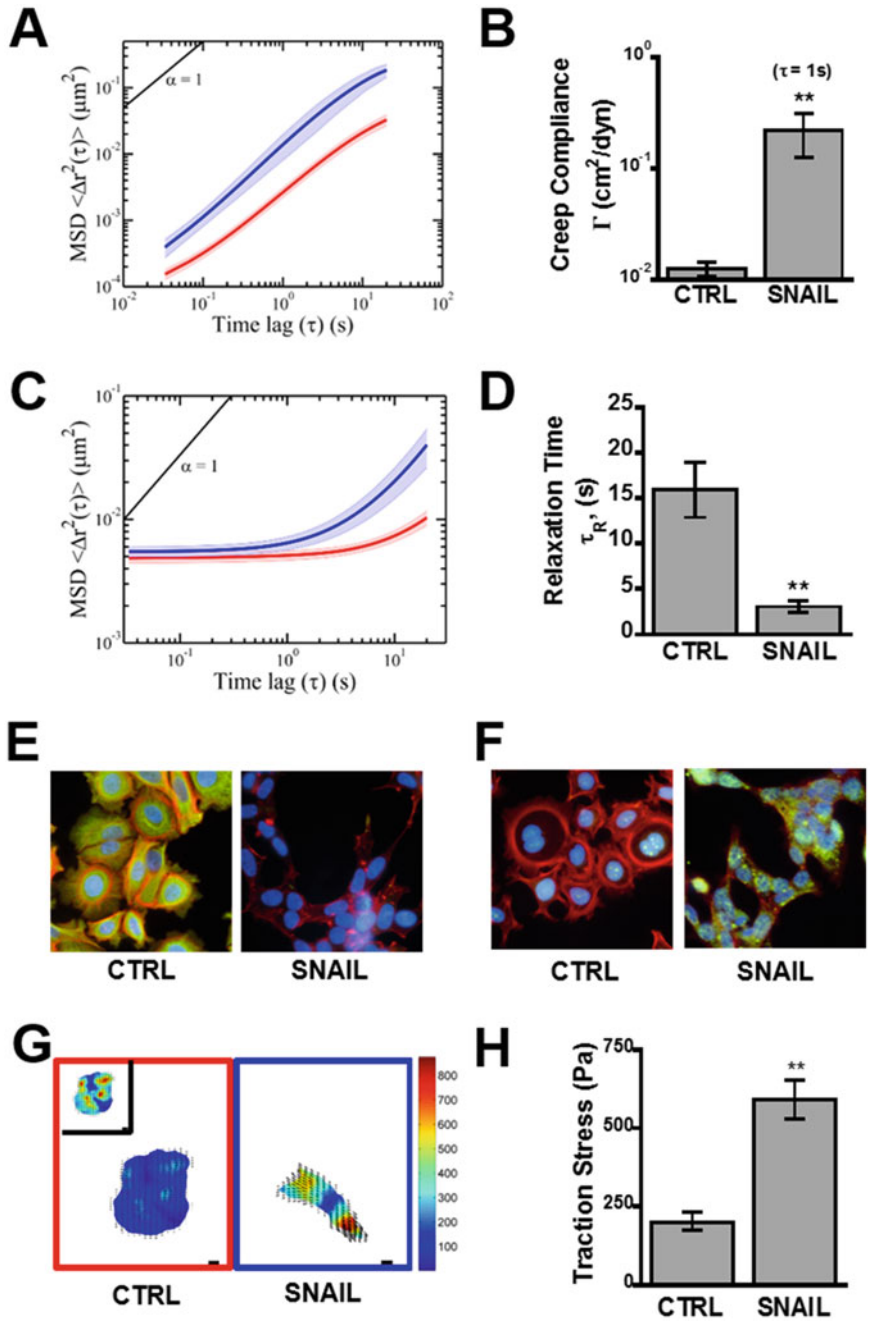


**Fig. 5.8** SNAIL-induced EMT drives malignant phenotype in MCF-7 cells. (**a**) MSDs of 200 nm particles injected into the cytoplasmic space are increased by nearly half a decade across all time lags. (**b**) Creep compliance of cytoplasm is also decreased by an order of magnitude at $t = 1$ s. (**c**) MSDs of Hoechst-labeled chromatin show that while at low scales, nuclei behave primarily as an elastic solid regardless of cell line; at later time MSDs begin to increase, indicative of more viscous diffusion. (**d**) Quantification of the time until this relaxation from elastic to viscous character shows that it happens over threefold faster in SNAIL cells. (**e**, **f**) To visualize intermediate filaments, cells were stained for cytokeratin (**e**) and vimentin (**f**), both shown in green, and then counterstained for F-actin (red) and nuclei (blue). (**g**) Traction heat maps in units of Pascals ranging from 0 (dark blue) to 850 (dark red) overlaid with matrix displacements (black arrows). (**h**) Peak traction stresses in SNAIL cells were threefold higher than those exerted by control cells

growth factors, MDA-MB-231 and SKOV-3 cells must also undergo adaptions to the rigidity of the secondary site, largely through regulation of cytoskeletal tension (Fig. 5.7) [13]. Rho-ROCK pathway activation was vital for both to assume contractile phenotype.

Endocrine factors secreted by cancer cells also modify the behavior of distant stromal cells to form a pre-metastatic niche [149–151]. Interestingly, LOX activity has been reported to be upregulated in the pre-metastatic niche [149]. More recently Hoshino and colleagues showed that tumor-secreted exosomes direct organ-specific colonization by fusing exosomal integrins with target cells in a tissue-specific fashion [152]. In a 3D co-culture model, we have reported that mesenchymal stem cells can increase the directional migration and force generation of metastatic breast cancer cells through TGF-β and downstream mechanosensitive pathways [19].

### 5.4.3 Chemoresistance

Chemotherapeutic drugs, e.g., cisplatin and taxol, are utilized to prevent and treat advanced-stage cancer. However, a subpopulation of tumor cells can acquire resistance to these drugs and become highly aggressive; thereafter, the disease progresses rapidly, causing mortality. Properties of the ECM such as composition and stiffness play a crucial role in modulating cancer cell response to chemotherapeutic drugs and developing chemoresistance [153]. For example, overexpression of multiple ECM proteins including collagen VI contributes to altered matrix properties to confer cisplatin resistance in ovarian cancer cells [154]. Additionally, mechanosensitive pathways regulated by integrins and Rho-ROCK signaling have been implicated in developing chemoresistance in multiple cancers [155–159]. To elucidate the correlation between adhesion and chemoresistance, we have analyzed taxol-resistant ovarian cancer cells that display enhanced microtubule dynamics, attachment rate, and β1-integrin expression compared to parent population [160]. Taxol-resistant cells also exerted her traction forces than parent ovarian cancer cell lines. We found a novel mechanism of FAK-driven control of microtubule dynamics that regulate ovarian cancer cell chemoresistance.

## 5.5 Conclusions and Future Directions

This chapter highlighted some of the biophysical tools used to study cancer cell mechanics, as well as the mechanical changes associated with cancer progression. These biophysical tools have increased our understanding of heterogeneous cancer cells; yet, a tremendous amount of work remains to be done. First, the wide-scale application of these biophysical tools in academic and clinical settings would require standardization of the techniques and simplification of the analysis. Next, high-throughput multivariable analysis would require a plate or chip-based system. Finally, combining single-cell biophysical analysis with single-cell RNA sequencing would allow us to develop drugs targeting the most invasive cancer cells based on changes in cell biophysical properties.

**Glossary**

**Stress ($\sigma$)** is defined as force ($F$) per unit area ($A$) of a surface. In the cases of normal and shear stresses, the force is acting on the perpendicular and tangential direction of the surface area, respectively.

$$\sigma = \frac{F}{A} \tag{5.10}$$

**Strain (ε)** is defined as deformation ($\Delta L$) compared to original length ($L$), and it is a dimensionless parameter.

$$\varepsilon = \frac{\Delta L}{L} \tag{5.11}$$

Modulus of a material is used to measured elasticity and it is defined as a ratio of

(continued)

stress to strain. In case of **Young's or elastic modulus (*E*)**, deformation is measured after applied tension or compression.

$$E = \frac{\sigma}{\varepsilon} = \frac{F \cdot L}{A \cdot \Delta L} \tag{5.12}$$

For fluids, rate of deformation ($\gamma$) is proportional to the applied stress, and **viscosity** is used to measure the resistance to applied force.

$$\mu = \frac{\sigma}{\gamma} \tag{5.13}$$

**Viscoelastic properties of cells** vary between Hookean solid that deforms linearly with applied stress and Newtonian fluid for which rate of deformation is proportional to the applied shear stress. Probing tools are used to apply either constant stress or strain to the cells, and resulting temporal changes in strain (creep) or stress (relaxation) are measured, respectively, to determine rheological properties.

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