CHAPTER ONE

Force balancing ACT-IN the tumor microenvironment: Cytoskeletal modifications in cancer and stromal cells to promote malignancy

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Abstract

The tumor microenvironment is a complex milieu that dictates the growth, invasion, and metastasis of cancer cells. Both cancer and stromal cells in the tumor tissue encounter and adapt to a variety of extracellular factors, and subsequently contribute and drive the progression of the disease to more advanced stages. As the disease progresses, a small population of cancer cells becomes more invasive through a complex process known as epithelial-mesenchymal transition, and nearby stromal cells assume a carcinoma associated fibroblast phenotype characterized by enhanced migration, cell contractility, and matrix secretion with the ability to reorganize extracellular matrices. As cells transition into more malignant phenotypes their biophysical properties, controlled by the organization of cytoskeletal proteins, are altered. Actin and its associated proteins are essential modulators and facilitators of these changes. As the cells respond to the cues in the microenvironment, actin driven mechanical forces inside and outside the cells also evolve. Recent advances in biophysical techniques have enabled us to probe these actin driven changes in cancer and stromal cells and demarcate their role in driving changes in the microenvironment. Understanding the underlying biophysical mechanisms that drive cancer progression could provide critical insight on novel therapeutic approaches in the fight against cancer.

1. Overview

The dynamic progression of the tumor microenvironment (TME) requires the participation of a wide variety of cell types, facilitating a complex network of chemical and physical crosstalk (Balkwill et al., 2012; Fukumura and Jain, 2007; Quail and Joyce, 2013; Stroka and Konstantopoulos, 2014; Whiteside, 2008). A multitude of cell types are recruited to the tumor under the influence of tumor secreted growth factors and chemokines; this includes immune cells, endothelial cells, mesenchymal stem cells (MSCs), and fibroblasts that play important roles in tumor growth by modulating the immune response, promoting angiogenesis, and forming the stroma. These cells crosstalk with cancer cells through direct cell contacts and paracrine signaling, in order to restructure the TME to one that is permissible for tumor growth and metastasis. Increased understanding of the molecules in the TME and their interactions with cancer cells may be critical in identifying novel targets for therapeutic intervention.

Along with selective influences within the TME, such as extracellular matrix (ECM) remodeling and abnormal vascularization, cancer cells within the tumor develop transcriptionally and phenotypically heterogeneous subclones with different levels of malignancy (Marusyk et al., 2012; Meacham and Morrison, 2013). Although recent technological advancements in deep-sequencing at the single-cell level have allowed researchers to obtain preliminary insights into cancer heterogeneity (Patel et al., 2014; Tirosh et al., 2016), more studies are needed to probe how this intratumor heterogeneity affects the development of chemoresistant subpopulations, cancer recurrence after therapy, and cancer metastasis (Meacham and Morrison, 2013; Sharma et al., 2010). Heterogeneity in cancer cell phenotypes is thought to rely on the inherent variation in the rate of stochastic mutations (Lawson et al., 2018). As genomic instability increases, the cell cycle becomes more abnormal and cancer cells with diverse malignant characteristics begin to form, including cells with high metastatic potential (Burrell et al., 2013; Cifone and Fidler, 1981; Joung et al., 2017). However, cancer therapy also contributes to intratumor heterogeneity. Single-cell sequencing analysis demonstrated that there were distinct subpopulations of cancer cells with different genomic and transcriptomic profiles in Paclitaxel treated cells/tumors compared to control (D'Alterio et al., 2020; Lee et al., 2014). TME itself could also confer selective pressures to cancer cells. For instance, stromal cell-secreted growth factors and cytokines have been found to profoundly influence phenotypic developments in cancer cells by promoting epithelial to mesenchymal transition (EMT) (Polyak and Weinberg, 2009). In conjunction with ECM remodeling (changes in matrix rigidity), environmental pressures could largely alter transcriptomic and proteomic properties, and subsequently, the phenotypic and biophysical properties of cancer cells (Emon et al., 2018; Hanahan et al., 2011; Lu et al., 2012; Spill et al., 2016). Lastly, histology studies have consistently reported spatial heterogeneity in the tumor architecture, which could have profound implications on cancer biophysics (Ramón y Cajal et al., 2020). This heterogeneity includes regional differences in the collagen architecture, level of vascularization, stromal cell incorporation, and cancer cell phenotype (Malandrino et al., 2018; Yamauchi et al., 2020). For instance, some regions of the tumor may have collagen-rich basement membrane; whereas, other regions have very little collagen (Case et al., 2017; Conklin et al., 2011). Also, some regions of the tumor are highly vascularized; whereas, other regions are hypoxic (Fukumura et al., 2010; Petrova et al., 2018). Cells near the collagen-rich basement membrane may be more migratory; whereas, cells at the tumor core may be under solid stress, which drives the collective migration of surrounding cells (Tse et al., 2012). Taken together, cancer is a highly heterogeneous disease that requires additional characterization, particularly through the lens of cancer biophysics, to better elucidate drivers of phenotypic heterogeneity in the tumor.

This review will highlight recent work from our lab and other labs demonstrating the importance of biophysical properties in identifying aggressive cell populations. Additionally, we will show how chemical and physical cues from the TME alter cell shape and cytoskeletal organization to dynamically affect cell function and cell-cell interactions. The complexity of the TME is a major barrier in understanding the molecular and mechanical interactions of cells in the tumor. Quantitative biophysical analysis allows us to probe the biomechanical properties of cells with an unprecedented level of detail to enhance our understanding of cancer.

2. Actin cytoskeleton

Cytoskeletal proteins mechanically support the cell structure and spatially organize the contents of the cell (Fletcher and Mullins, 2010). This group of filamentous proteins is categorized into three main families: actin microfilaments, microtubules, and intermediate filaments. While microtubules and intermediate filaments contribute significantly to the organizational integrity of cells, actin and its associated proteins enable cells to respond and adapt to dynamic changes in the microenvironment. The hierarchical structure of the actin network is controlled by small Rho GTPases, myosin motor proteins, and a large group of cytoplasmic mediators known as actin binding proteins (ABPs) (illustrated in Fig. 1) (Hall, 1998; Parsons et al., 2010; Winder and Ayscough, 2005). Dynamic changes in the organization of the cytoskeleton transform cell shape and generate mechanical forces required for numerous cellular processes, including adhesion, migration, division, molecular transport, and differentiation (DuFort et al., 2011; Eyckmans et al., 2011; Humphrey et al., 2014; Iskratsch et al., 2014). The cytoskeletal network responds dynamically to soluble or mechanical cues from the tumor ECM and is connected directly to canonical signal transduction pathways important in cancer (Chin et al., 2016; Huang and Ingber, 2005; Shieh, 2011; Stroka and Konstantopoulos, 2014). The family of RhoGTPases and ABPs have been strongly implicated in multiple stages of cancer progression to metastasis (Sahai and Marshall, 2002; Stevenson et al., 2012; SUN et al., 2015; Vega and Ridley, 2008). For example, Arp2/3—a protein facilitating actin branch formation, is overexpressed in malignant tumors, such as breast carcinomas (Molinie and Gautreau, 2018). Another actin binding protein Filamin that crosslinks actin bundles and provide mechanical strength has been detected in the blood from metastatic breast cancer patients (Yue et al., 2013).

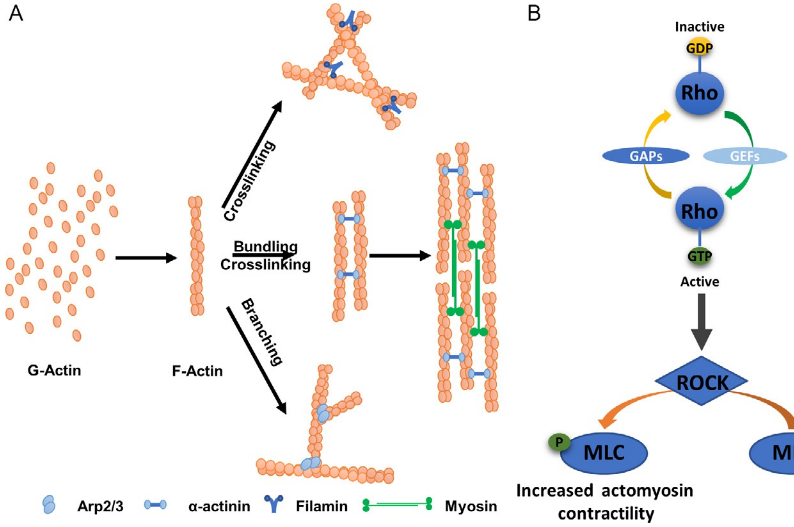


Fig. 1 (A) Globular actin (G-actin) subunits polymerize to form filamentous actin structure (F-Actin), which can be organized into multitude of networks to support location specific function in cells. Actin binding proteins (ABPs) facilitate the assembly of F-Actin into various forms including bundling, crosslinking, and branching examples shown here. Additionally, myosin motor proteins can bind between two adjacent bundles of crosslinked actin structures to generate contractile actin stress fibers. (B) The family of small Rho-GTPases such as RhoA is converted from a GDP-bound inactive form to GTP-bound active form by guanine nucleotide exchange factors (GEFs) and the reverse process of inactivation is mediated by GTPase-activating proteins (GAPs). Activation of Rho GTPases leads to activation of ROCK which can trigger multitude of downstream cytoskeletal reorganization processes including blocking myosin light chain (MLC) phosphatase activity and facilitate MLC phosphorylation leading to increased actomyosin contractility.

3. Measuring intracellular and extracellular forces

A growing body of evidence has emerged highlighting the importance of mechanical cues in both normal tissue development and cancer (DuFort et al., 2011; Kumar and Weaver, 2009). Despite highly divergent chemical signaling cascades, a highly conserved feature of mechanical signaling is that it requires transmission of force from the ECM to the internal cytoskeleton, which forms the structure of the cell. Forces from the external environment activate Rho/Rho associated protein kinases (ROCK) signaling pathways that regulate the actin cytoskeleton and cytoskeletal tension. Upregulation of ROCK, which increases actomyosin contractility, results in tissue stiffening and malignant transformation. Actin bundling (e.g., α-actinin, fascin) and crosslinking (e.g., filamin) proteins give rise to actin stress fibers that link the cytoskeleton to focal adhesions and actin networks that modulate intracellular stiffness (Hall, 1998; Winder and Ayscough, 2005). Since cytoskeletal alterations depend on the mechanical environments and vice versa, it is necessary to use biophysical tools to probe essential forces at both the intracellular and extracellular levels.

3.1 Intracellular particle tracking microrheology

Cytoskeletal actin forms a mesh-like structure in the cell cytoplasm that regulates the intracellular tension (Fletcher and Mullins, 2010; Hale et al., 2009). Parallel actin bundles provide tensile strength and strong contractile activity, whereas crosslinked bundles of actin filaments increase intracellular elasticity. Depending on the location in the cell, actin architecture can vary drastically and manifest heterogeneous local mechanical properties (Tseng et al., 2002). To measure cell mechanical properties, multiple techniques have been developed over the years, including, atomic force microscopy (AFM), magnetic bead twist, optical tweezers, micropipette aspiration, hydrodynamic stretching and particle-tracking microrheology (Kollmannsberger and Fabry, 2011; Moeendarbary and Harris, 2014). Intracellular particle tracking microrheology (IPTM) allows direct and rapid measurement of the local microrheological properties throughout the cell (Crocker and Hoffman, 2007; Dawson et al., 2014; Li et al., 2009; Wirtz, 2009). Briefly, fluorescent particles are ballistically injected into the cell and their thermal energy driven movements captured at a high magnification with a high-speed camera to obtain information about the local polymeric network. The 2D Brownian motion of these submicron probe particles is then used to calculate particle mean square displacements (MSDs). MSDs of particles moving in a viscous liquid vary linearly ($\text{slope} \approx 1$) with time scale. However, for viscoelastic fluids, the motion of the embedded particles becomes more restricted due to the presence of mesh-like structures. Because of the sub-diffusive restricted motion of particles, the time-dependent MSD curves flatten ($\text{slope} \ll 1$). In a viscous liquid, diffusivity due to thermal energy driven motion can be described using the Stokes-Einstein Eq. (1), where D is the diffusion coefficient, k_B is Boltzmann's constant, T is temperature, a is particle radius, and η is the fluid viscosity.

$$D = \frac{\langle \Delta r^2(\tau) \rangle}{4\tau} = \frac{k_B \mathrm{T}}{6\,\pi\,\mathrm{a}\,\eta} \tag{1}$$

To describe viscoelastic properties of complex fluids, Mason et al. derived complex shear modulus of the viscoelastic fluid using a modified Stokes-Einstein equation in the frequency domain (Eq. 2), where G^* is the frequency-dependent complex shear modulus and Γ is the gamma function. The in-phase component of the complex shear modulus (G^*) is known as the elastic modulus (G'), and out-of-phase component is known as the viscous modulus (G'') (Eq. 3).

$$G^*(\omega) = \frac{2\,\mathrm{k}_B\mathrm{T}}{3\,\pi\,\mathrm{a} < \Delta r^2(1/\omega) > \Gamma[1+\alpha(\omega)]} \tag{2}$$

$$G'(\omega) = \left|G^*(\omega)\right| \mathit{cos}\left(\frac{\pi\alpha(\omega)}{2}\right);\ G''(\omega) = \left|G^*(\omega)\right| \mathit{sin}\left(\frac{\pi\alpha(\omega)}{2}\right) \tag{3}$$

The IPTM approach is illustrated for analyzing MDA-MB-231 breast cancer cells in Fig. 2. At lower time scales, particle transport in the cytosol remains restricted and MSD varies almost independent of time scale ($\alpha \ll 1$); whereas at longer time scales, as the structures around the particles begin to relax, particles are able to move longer distances, and MSDs vary more linearly ($\alpha \approx 1$). For the more restricted transport regime of embedded particles, cells typically resemble a viscoelastic fluid with comparable magnitudes of both viscous and elastic moduli. At linear regime of particle motion, cells properties are very similar to a viscous liquid with a highly dominant viscous modulus. Individual location specific particle MSDs can be used to calculate local viscoelastic properties; whereas, all MSDs from a cell can be ensemble-averaged to evaluate overall viscoelastic behavior. Particle tracking microrheology (PTM) has been successfully adapted to characterize a great range of complex biological fluids, including mucus, reconstituted actin solutions, and the cell cytoplasm (Dawson et al., 2014; Mason et al., 1997; Wirtz, 2009).

Although IPTM is primarily conducted on 2-D cultures, the application of this method in 3D and in vivo has been investigated (Baker et al., 2010; Daniels et al., 2006; Panorchan et al., 2006; Zhou et al., 2008). IPTM does not require an external probe unlike other techniques like AFM, thus provides an advantage in tracking cell mechanics in 3D. Panorchan et al. embedded human umbilical vein endothelial cells ballistically injected with 100 nm fluorescent particles in 3D peptide hydrogels and monitored changes

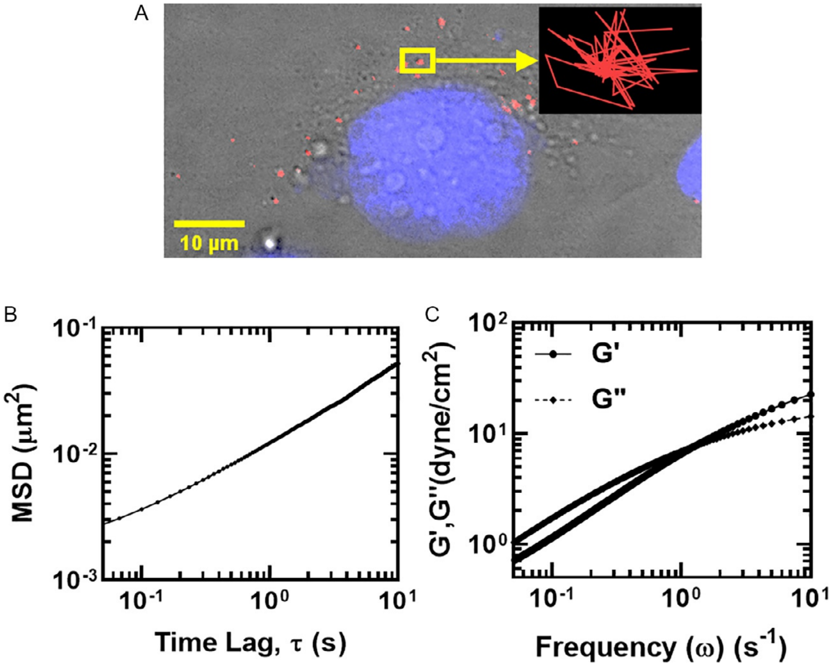


Fig. 2 Illustration of intracellular particle tracking microrheology (IPTM). (A) Fluorescent submicron (200 nm) probe particles were injected into MDA-MB-231 breast cancer cells using PDS-100 ballistic particle injection system. The representative trace of a single particle undergoing Brownian motion in the cell cytoplasm is shown in the inset. (B) The x-y displacements of particles are used to calculate ensemble average MSDs. (C) Frequency-dependent viscous (G′) and elastic (G″) moduli are then calculated from the MSDs as described by Mason et al. (1997). *Adapted from Dawson, M.R., Tseng, Y., Lee, J.S.H., McAndrews, K.M., 2014. Intracellular particle tracking microrheology, In: Handbook of Imaging in Biological Mechanics. CRC Press, pp. 381–388. https://doi.org/10.1201/b17566-40.*

in cell mechanics after stimulation with vascular endothelial growth factor (VEGF). Exposure to VEGF led to softening of the cytoplasm highlighted by significant reduction in the elastic modulus (Panorchan et al., 2006). Other studies in 3D have used microbeads and nanotubes or endogenous organelles to track changes in cell mechanics in more physiologically relevant microenvironment. Wu et al. recently demonstrated the combination of IPTM and intravital imaging to measure biophysical parameters of live cells in mice (Wu et al., 2020). In short, 200 nm fluorescent particles were ballistically injected into EGFP-labeled MDA-MB-231 breast cancer cells.

The cells were then implanted in mice using the dorsal skinfold chamber window and examined using intravital fluorescent microscopy. Similar to 2-D IPTM, the thermal motion of particles that were embedded in the GFP-labeled cells were captured and used to determine cell microrheology. Using this method, in vivo cell biophysical properties can be more accurately captured along with effects of the surrounding tissue microenvironment. However, there were several limitations to this method. First, a stringent correction was needed for animal movement, as the rhythmic breathing motion was several magnitudes higher than the tracked particle motion. Secondly, in vivo imaging requires increased working distances to see deep into the tissue, limiting spatial and temporal resolution, which are critical in IPTM. Similar resolution limits may apply when using IPTM to determine cell microrheology in 3-D gels.

3.2 Traction force microscopy

Physical interactions between cells and the surrounding ECM regulate the reciprocal forces via adhesion molecules linking the cell cytoskeleton to the ECM. The magnitude of traction forces generated at these adhesion sites, along with the strength of adhesions, are critical in regulating cell processes. Traction forces have been quantified in both 2D and 3D environments (Koch et al., 2012; Kraning-Rush et al., 2011; McGrail et al., 2015b; Munevar et al., 2001; Sabass et al., 2008). Studies on 2D elastic substrates formed from synthetic materials, including silicon, polyacrylamide, and polydimethylsiloxane allow for cell-generated force measurements on a wide range of stiffnesses. Traction force microscopy (TFM) has been combined with other techniques for simultaneous characterization of cell adhesion machinery using total internal reflection microscopy (TIRF) or intracellular rheology using IPTM (Gutierrez et al., 2011; McAndrews et al., 2014). To mimic more physiologically relevant microenvironments, TFM has been performed in 3D hydrogels and collagen matrices (Legant et al., 2010, 2013; Steinwachs et al., 2016). Due to non-linear elastic properties of collagen quantitative analysis of traction forces is limited/not possible, but particle displacements are still useful in understanding collagen matrix deformations. In elastic hydrogels, the algorithm to derive cell-generated forces is extremely complex, limiting the usefulness of this approach (Legant et al., 2013). A more detailed review on the current 2D and 3D TFM techniques and their limitations can be found here (Cho et al., 2016; Hur et al., 2020).

2D traction forces are characterized for cells cultured on polyacrylamide substrates with rigidities tuned to mimic specific biological tissues (Kim et al., 2009; Nerger et al., 2017; Plotnikov et al., 2014). Fluorescent nanoparticles embedded in the substrates are displaced under cell-exerted stress. When cells are removed, the particles revert to their unstressed locations. Thus, cell-induced displacements from stressed and unstressed particle images can be used in traction force calculations (illustrated in Fig. 3). In Boussinesq theory, the displacement field $(\vec{\mathrm{u}})$ of an elastic substrate is correlated to the traction field $(\vec{\mathrm{T}})$ (Eq. 4), where G is the Green's function (Munevar et al., 2001; Sabass et al., 2008).

$$\vec{u}\left(\vec{x}\right) = \mathbf{G}\left(\vec{x} - \vec{x}\,'\right) \otimes \vec{T}\left(\vec{x}\,'\right) \tag{4}$$

The estimation of the Green's function is critical for inverse calculation of the traction field (Eq. 5). The Green's function includes displacement vector $r = \vec{x} - \vec{x}'$ components (r_x, r_y), the Young's modulus E, and the Poisson ratio υ

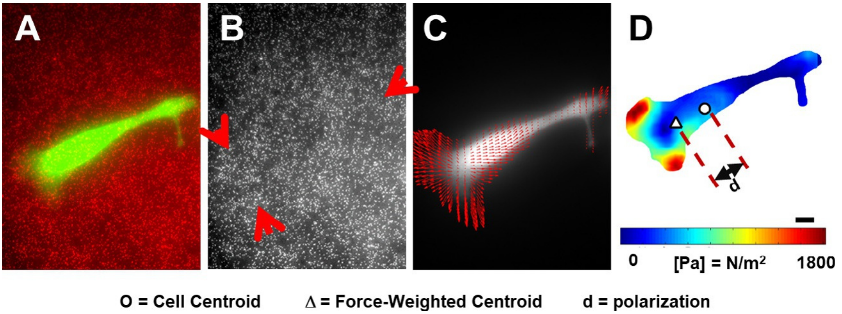


Fig. 3 Traction force microscopy (TFM). (A–B) Fluorescently labeled cells (SKOV-3 epithelial ovarian cancer cells shown in green) were cultured on collagen-coated polyacrylamide substrates embedded with fluorescent red particles (200 nm). Images of the embedded nanoparticles were taken before (A) and after (B) detaching the cells. Red arrows point to the zones with high displacements. (C–D) Previous images were used to calculate displacement vectors for each particle and followed by the estimation of traction force field and polarization. (D) Heatmap of traction forces is overlaid with symbols indicating the cell's center of mass (○) and force-weighted center of mass (Δ). *Adapted from Mcgrail, D.J., 2015. Mechanics & Malignancy: Physical Cues And Changes That Drive Tumor Progression.*

$$\mathbf{G}\left(\vec{\mathrm{r}}\right)=\frac{(1+\upsilon)}{\pi E r^3}\begin{bmatrix}(1-\upsilon)r^2+\upsilon r_x^2 & \upsilon r_x r_y\\ \upsilon r_x r_y & (1-\upsilon)r^2+\upsilon r_y^2\end{bmatrix}\tag{5}$$

Furthermore, polarization is calculated as the difference in un-weighted center of mass of the cell and the traction force-weighted center of mass of the cell (Eq. 6), where M_i^{unwt} is the un-weighted center of mass and M_i^{wt} is the weighted center of mass.

$$Polarization\ (d)=\sqrt{\left(M_x^{unwt}-M_x^{wt}\right)^2+\left(M_y^{unwt}-M_y^{wt}\right)^2}\tag{6}$$

The aforementioned techniques are two of the most commonly used methods to measure forces inside and outside of living cells. However, other techniques exist and could be useful in collecting similar measurements. Our chapter focuses on how our lab has combined the two methods we previously described with cell fate analysis to understand cell behavior in tumor and tissue microenvironments.

4. Utilizing force measurements to distinguish non-invasive and invasive cancer cells

The transformation of cancer cells to highly invasive phenotypes allows cells to distort their shape and generate forces to navigate through dense stroma. Epithelial cancer cells undergoing EMT lose some epithelial characteristics, including reduced expression of cadherins responsible for cell-cell junctions and increased expression of ECM binding integrins important in cell-ECM adhesion (Kalluri and Weinberg, 2009). Invasive and migratory properties acquired through EMT are prerequisites for metastasis; thus, it is imperative to identify or even predict which cancer cells undergo EMT. Using biophysical approaches to interrogate actin cytoskeletal modifications in cancer cells, we previously examined the phenotype of cancer cells undergoing EMT.

4.1 Genetically induced EMT makes cancer cells more deformable

The breast cancer cell line MCF7 was modified to constitutively express Snail, a zinc-finger transcription factor that triggers EMT by suppressing *E*-Cadherin expression. While cells transformed with an empty vector (MCF7-NEO)

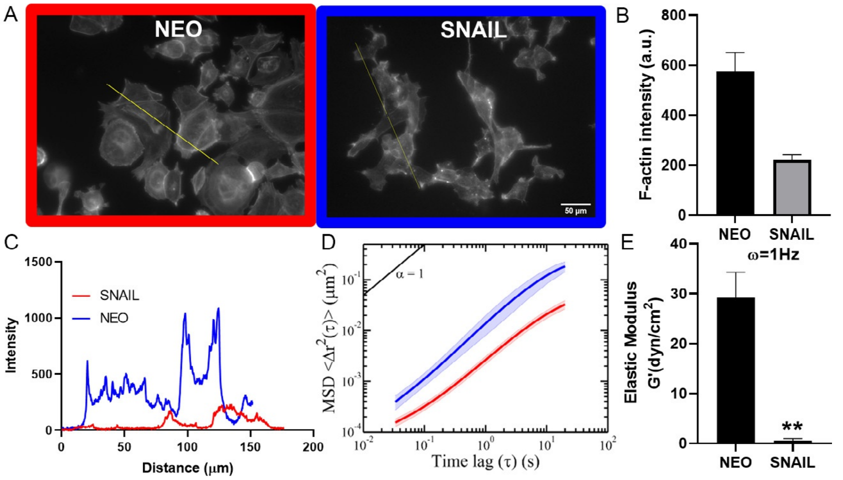


Fig. 4 SNAIL-induced EMT alters cytoskeletal and mechanical properties of MCF-7 cells. (A–C) Immunostaining for actin, showed change in cytoskeletal organization of MCF7-SNAIL cells compared control (Scale bar = 50 μm). Analysis of actin intensity revealed that overexpression of SNAIL reduced actin polymerization (B). Gray intensity distribution along the line (A) across the images were quantified in image J to look at differences in actin (C). (D–E) MSDs of 200 nm particles injected into the cytoplasm were increased for MCF7-SNAIL cells across all time lags suggesting relatively softer cytosol. Elastic modulus of MCF7-SNAIL cells was reduced significantly at ω = 1 Hz. To calculate statistical significance student's *t*-test was used and *P*-values of less than 0.05 was considered significant ($^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$) (McGrail et al., 2015b).

were more epithelial with mostly round morphology, cells transformed with Snail (MCF7-SNAIL) exhibited a more mesenchymal phenotype. MCF7-SNAIL cells displayed an elongated morphology, downregulation of *E*-Cadherin, and upregulation of N-Cadherin and Vimentin, characteristics of a mesenchymal phenotype (McGrail et al., 2015b). To understand the underlying changes in the cytoskeletal organization, we analyzed the actin structure using immunostaining (Fig. 4A). The integrated fluorescence intensity of actin in MCF7-SNAIL was 3-fold lower in comparison to MCF7-NEO cells (Fig. 4B). Analysis of actin distribution across individual MCF7-NEO cells showed high intensity in cortical regions indicating presence of polymerized actin stress fibers. In contrast, MCF7-SNAIL cells displayed significantly lower actin intensity suggesting dissolution of polymerized actin structure (Fig. 4C). Using IPTM, we confirmed that the intracellular mechanical properties were markedly altered. Embedded nanoparticles in MCF7-SNAIL cell cytosol displayed higher MSDs at all timescales and subsequently revealed a significant reduction in elastic modulus (Fig. 4D–E). Together, these results confirmed that MCF7-SNAIL cells possess a more deformable cytosol in comparison to MCF7-NEO. Results from our study corroborated with the results reported by Craene et al. in colon cancer cells (De Craene et al., 2005). Expression of Snail in these cells led to significant loss in cytoskeletal proteins, including ABPs. Additionally, highly invasive cancer cells from different tissues including breast and ovarian cancer have been shown to display less actin stress fibers compared to the normal cells (Alibert et al., 2017). However, other EMT studies with cancer and normal epithelial cells treated with transforming growth factor β (TGF-β)—a known inducer of EMT, have shown increased actin stress fiber formation (Haynes et al., 2011; Nalluri et al., 2015; Sousa-Squiavinato et al., 2019; Zhitnyak et al., 2020). This EMT response may vary in different cell types, perhaps due to intrinsic differences in the cells undergoing EMT or differences in the surrounding environment. Similarly, while most studies have reported that more invasive cancer cells are often softer than less invasive cells, a few studies have reported stiffening of invasive cancer cells (Alibert et al., 2017). Different probing techniques can contribute to the reported differences in cancer cell mechanics. Measurement with techniques that use external probe at local regions of the cell can be influenced by cortical actin structure, which is more polymerized in invasive cells that exhibit high traction forces. For example, studies using AFM can be measuring a specific region of the cell, not the intracellular mechanics (Alibert et al., 2017). Together these results highlight the need for a more comprehensive biophysical analysis of cancer cells undergoing malignant transformation.

In addition to the intracellular changes, MCF7-SNAIL also displayed significantly different traction force profile on a polyacrylamide substrate (Fig. 5A–B). As the cells assumed a more elongated phenotype, it generated higher traction forces localized at cell periphery. MCF7-SNAIL-generated peak traction forces were threefold higher than those exerted by NEO cells. Consequently, MCF7-SNAIL demonstrated significantly higher migratory behavior with more than 2-fold increase in cell velocity (Fig. 5C). Coefficient of variation, calculated by dividing the standard deviation with the mean, provides a measure of heterogeneity in the population and was significantly increased in SNAIL cells for both traction force and migration (Fig. 5D).

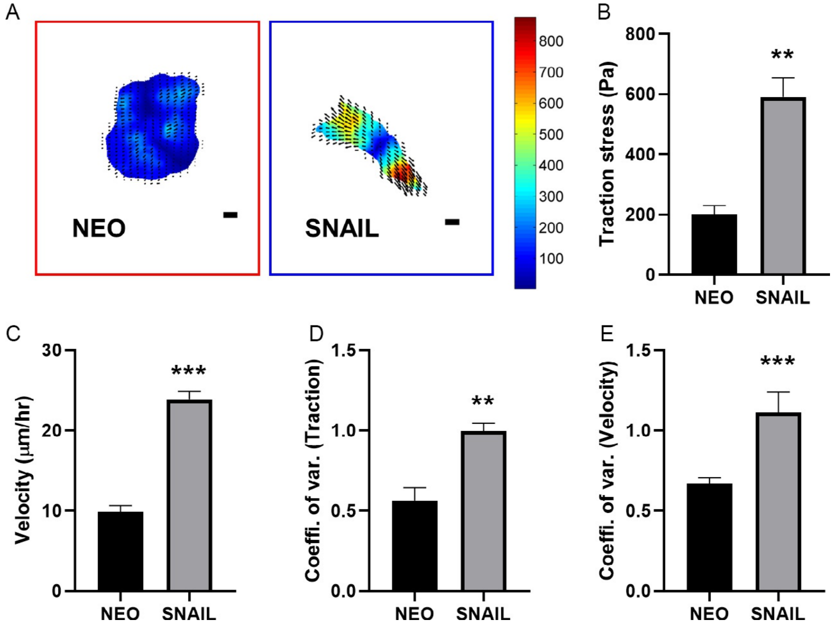


Fig. 5 (A) Traction heat maps of MCF7-NEO and MCF7-SNAIL cells are overlaid with matrix displacements with force range is specified in Pascals (Scale bars = 10 μm). (B) Peak traction stresses in SNAIL cells were significantly higher than NEO cells. (C) Mean velocity of MCF7-SNAIL cells was significantly increased. (D–E) Coefficient of variation calculated for both traction force (D) and cell velocity (E) was significantly higher in MCF7-SNAIL cells. To calculate statistical significance student's *t*-test was used and *P*-values of less than 0.05 was considered significant ($*P < 0.05$, $**P < 0.01$, $***P < 0.001$) (McGrail et al., 2015b).

4.2 Invasive cancer cells exert increased and polarized traction forces in a context dependent manner

In addition to the biochemical signals, the biomechanical properties of the ECM can also dictate the traction force profile of the cancer cells. Solid tumors are generally stiffer compared to their surrounding tissues (Chang et al., 2011; Egeblad et al., 2010; Youk et al., 2014). This increased rigidity has been shown to promote an invasive behavior in cancer cells from multiple tissues, including breast, liver, and prostate (Acerbi et al., 2015; Kostic et al., 2009; Leight et al., 2017; Pickup et al., 2014; Tilghman et al., 2010; Ulrich et al., 2009). Inversely, invasive tumor cells often exhibit some form of durotaxis or response to increased substrate rigidity (Acerbi et al., 2015; Lachowski et al., 2017; Samuel et al., 2011). This observation is certainly true in highly metastatic MDA-MB-231 breast cancer cells. We have shown that when MDA-MB-231 cells were cultured on hard polyacrylamide-collagen-coated substrates (~35 kPa), they exhibited characteristic of malignancy including, significantly increased proliferation rate and resistance to the chemotherapeutic, compared to those of soft (~3 kPa) substrates (Fig. 6A–B) (McGrail et al., 2015a). Other characteristic properties of invasiveness also followed the same trend. MDA-MB-231 cells showed a significantly higher migration velocity (Fig. 6C) and increased cell spreading when cultured on hard substrates relative to soft substrates. However, metastatic SKOV-3 ovarian cancer cells displayed an opposite mechanical response to substrate stiffness. When SKOV-3 cells were cultured on soft

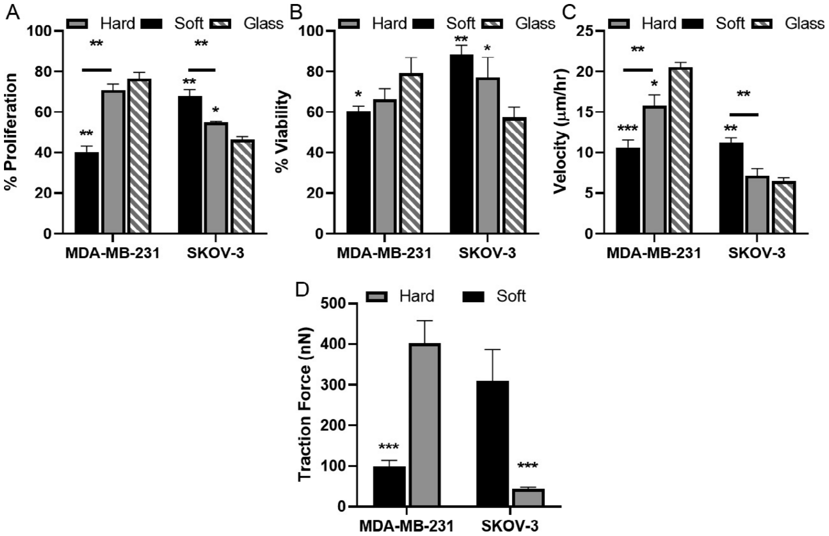


Fig. 6 Context dependent response of cancer cells. MDA-MB-231 and SKOV-3 cells were cultured on soft (~3 kPa) and hard (~35 kPa) collagen-coated polyacrylamide substrates or collagen-coated glass. (A) Percent proliferation was determined by BrdU incorporation. (B) Viability was determined by MTT assay after 2 μM (MDA-MB-231) or 0.1 μM (SKOV3) Doxorubicin—treatment. (C) The average cell velocity was determined by tracking cell nuclei at 5-min intervals over an 8-h period. (D) Average traction force was quantified from displacement of fluorescent nanoparticles embedded in substrates showed Increased traction forces were correlated with increased migration velocity, increased proliferation, and treatment resistance for SKOV-3 cells on soft substrates and MDA-MB-231 cells cultured on hard substrates or glass. To calculate statistical significance student's *t*-test was used and *P*-values of less than 0.05 was considered significant (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$) (McGrail et al., 2015a).

matrices, they exhibited greater proliferation rates, migration velocity, and cell spreading compared to those cultured on hard matrices (Fig. 6A–C). This discrepant behavior between MDA-MB-231 and SKOV-3 cells demonstrated that mechanical responses to substrate stiffness are cell specific, most likely due to differences in their physiological environment in the primary tumor or metastatic niche (Kostic et al., 2009; Kraning-Rush et al., 2012). Moreover, MDA-MB-231 and SKOV-3 cells became highly polarized and exerted greater traction force on hard and soft substrates, respectively (Fig. 6). The mechanism of this progression has been well-studied in breast cancer, where increased ECM stiffness leads to integrin activation followed by focal adhesion formation and increased actomyosin contractility (Levental et al., 2009). However, the increased forces exerted by SKOV-3 on soft matrices had not been characterized before our studies. Rigidity dependent behavior of cancer cells has been correlated to their ability to metastasize to specific locations in vivo (Kostic et al., 2009). Indeed, studies in murine models found that MDA-MB-231 cells yielded significantly more bone (stiff) metastases compared to lung (soft) metastases (Kang et al., 2003; Kostic et al., 2009). Though the idea of different cancer cell types invading sites with contrasting mechanical properties may seem counterintuitive, our studies demonstrated that cells adapted their response using Rho-ROCK mediated actomyosin contractility and intracellular cytoskeletal tension (McGrail et al., 2014, 2015a,b).

4.3 Rho-ROCK signaling regulates distinct mechanical response of differing cancer cell types

Mechanotransduction signaling pathways can create a mechanically-induced positive feedback loop, whereby increased ECM deposition and rigidity enhances malignant properties in cancer cells (Chin et al., 2016; Lu et al., 2012). For cancer cells to find balance between intracellular cytoskeletal tension and extracellular adhesion, optimal levels of Rho-ROCK pathway activation must be maintained (McGrail et al., 2015a). We demonstrated the salience of optimal Rho-ROCK signaling activation in MDA-MB-231 and SKOV-3 cells cultured on matrices of different rigidities by measuring traction forces before and after introducing Rho-ROCK pathway inhibitor (Y27632). Using TFM, we first found that both MDA-MB-231 and SKOV-3 cells exerted significantly larger (23-fold increase) cell-substrate traction forces when cultured on their respective preferential substrates, hard and soft (Fig. 7). In this paper preferred substrate referred to the

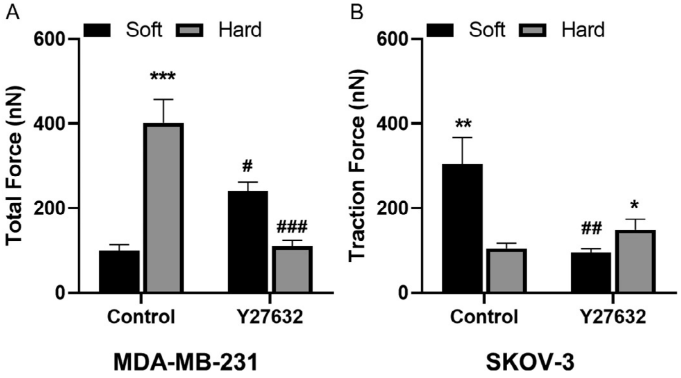


Fig. 7 Rho-ROCK pathway controls cancer cell response. (A–B) SKOV-3 and MDA-MB-231 cells cultured on soft (~3 kPa) and hard (~35 kPa) polyacrylamide substrates were treated with ROCK inhibitor (Y27632). Quantification of traction forces for MDA-MB-231 (A) and SKOV-3(B), respectively. To calculate statistical significance student's *t*-test was used and *P*-values of less than 0.05 was considered significant ($*P < 0.05$, $**P < 0.01$, $***P < 0.001$) (McGrail et al., 2015a).

substrate rigidity that elicited more malignant properties in mechanosensitive cancer cells. SKOV-3 ovarian cancer cells were more proliferative, migrated more rapidly, and exhibited reduced sensitivity to chemotherapeutic drugs on soft substrates, which were considered their preferred rigidity. Generally, increased Rho-ROCK signaling correlates with increased traction force, so we sought to further understand how this pathway was linked to contractility in the context of ECM rigidity. When the cells were treated with ROCK inhibitor Y27632 on their preferred substrates, we saw no increase in traction force for both MDA-MB-231 and SKOV-3 lines. Interestingly, when MDA-MB-231 tumor cells were cultured on their nonpreferred soft substrates, we observed a gain-of-function (slightly increased traction force), compared to those without Y27632 treatment. This phenomenon can be largely explained by the idea that cancer cells' inherent contractility needs to be matched with substrates of optimal rigidity to generate maximum traction forces — larger cell-intrinsic contractility matched with a stiffer ECM or lower cell-intrinsic contractility matched with a softer ECM results in optimal cell-ECM traction. Since our past studies have shown that MDA-MB-231 cells are inherently more contractile than SKOV-3 cells, a reduction in MDA-MB-231 cells' inherent contractility with Y27632 rescued its function on its nonpreferred, soft substrates. Therefore, it is evident that optimal Rho-ROCK signaling inherent in individual cancer cell types governs their actomyosin contractility, which in effect defines their predisposed matrix compliances. Taken together, these findings highlight the complexity of cancer progression, and drive the need to take a nuanced approach in examining the biophysical landscapes of different tumors.

4.4 Utilizing force profiles to characterize chemoresistant subpopulations

Often, a small subpopulation of cells can survive initial treatment, through efficient drug efflux or quiescence. Polyploidal giant cancer cells (PGCCs) are thought to be able to survive chemotherapy via quiescence (Zhang et al., 2014). Despite their apparent dormancy and morphological similarities, PGCCs are distinct from senescent cells, as they can give rise to daughter cells trough amitotic budding (Lv et al., 2014; White-Gilbertson et al., 2020). These morphologically enlarged and often multinucleated cells are often seen in tumors that have undergone treatment, or in late stage and aggressive disease (Fei et al., 2015; Lopez-Sánchez et al., 2014; Zhang et al., 2014). Furthermore, injection of PGCCs into mouse xenograft models have led to the growth of new tumors, highlighting the tumorigenic potential of this unique subpopulation (Niu et al., 2017). Previous studies conducted in our lab have shown that MDA-MB-231 PGCCs have increased migratory persistence and migrate more readily into the scratch wound (Xuan et al., 2018). In order to understand exactly how PGCCs maintain their enlarged morphology and sustain high migratory persistence despite their increased size, we performed single cell IPTM and TFM (Fig. 8). We found that PGCCs on average had increased cytoplasmic stiffness, and a stiffer but more deformable nuclei. This is evidenced in the MSD plots of particle motion embedded within the cytoplasm of the cell and heterochromatic foci within the nucleus, where PGCCs had a lower MSD, indicating higher levels of constraint. Furthermore, we examined the MSD traces of individual cells and noticed a higher spread in PGCC populations. This indicates that PGCCs are more heterogeneous than non-PGCCs; this increased heterogeneity has been observed in chemoresistant and highly invasive cancer cells. When we stained for and quantified the actin cytoskeleton of our PGCCs, we found that PGCCs expressed both thicker and longer actin stress bundles (Fig. 8A–B), which is associated with higher traction forces and increased migration. Indeed, when we performed TFM on our MDA-MB-231 cells, we found that PGCCs on average had over twice the exerted traction force compared to our non-PGCCs (Fig. 8C). In order to see if the unique organization of their actin structure was responsible for PGCCs cytoplasmic stiffness,

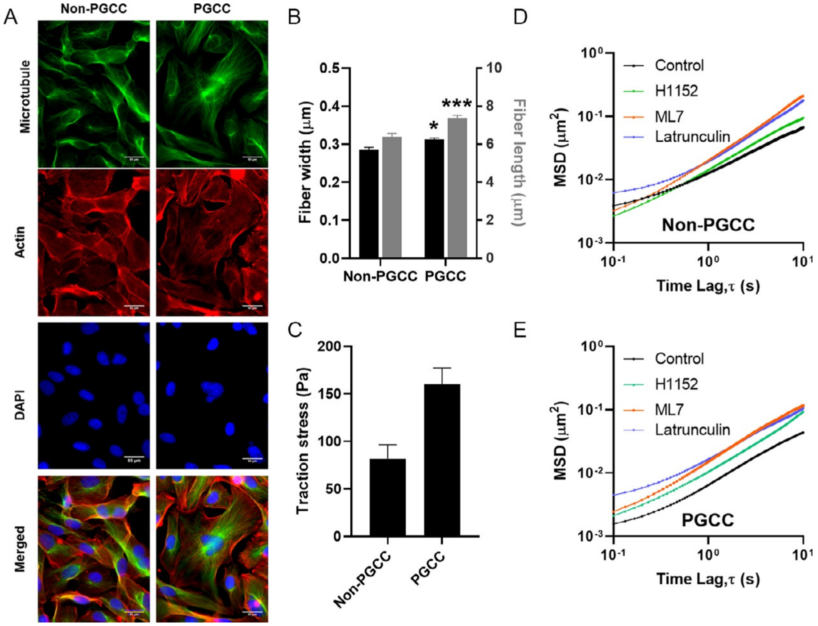


Fig. 8 (A) Fluorescent images of non-PGCCs and PGCCs stained for microtubule (green), actin (red), and DNA (blue) (Scale bar = 50 μm). (B) Quantification of average stress fiber width and length. (C) Mean traction forces exerted by non-PGCC and PGCC cancer cells on a 10 kPa stiffness polyacrylamide substrate. (D–E) Ensemble averaged MSDs of tracked particles of Non-PGCCs (D) and PGCCs (E) in control and inhibitor treated conditions. To calculate statistical significance student's *t*-test was used and *P*-values of less than 0.05 was considered significant (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$) (Xuan et al., 2018).

we inhibited parts of the RhoA/ROCK pathway, which is responsible for controlling actin cytoskeletal organization. Using inhibitors ML7 (MLCK inhibitor), H1152 (ROCK inhibitor), and latrunculin A (actin polymerization inhibitor), we observed consistent reductions in cytoplasmic stiffnesses in our polyploid cells (Fig. 8D–E). Taken together, these results demonstrate the biophysical characterization of a unique and highly chemoresistant subpopulation, which is more invasive as well as highly tumorigenic.

5. Utilizing force measurements to study tumor and stromal cell crosstalk

Interactions between cancer and stromal cells and the surrounding TME, with its diversity in cell types and matrix mechanics, play a critical role in directing cancer progression. Carcinoma associated fibroblasts (CAFs) are one major stromal contributor to TME malignancy (Karagiannis et al., 2012; Luo et al., 2015; Tao et al., 2017). They mediate hallmark cancer cell behaviors by secreting paracrine factors that alter tumor growth and cell survival, ECM proteins for matrix stiffening, and pro-inflammatory signals important in cancer progression. A CAF-like phenotype is characterized by changes in cytoskeletal architecture, motility, and adhesion, along with increased expression of α-smooth muscle actin (αSMA) and fibroblast activated protein (FAP) (Mishra et al., 2008). These myofibroblast-like cells arise from normal fibroblasts and MSCs that have been activated by tumor-secreted factors to form CAFs. MSCs that spontaneously home to tumors from the bone marrow may persist as stem cells in the tumor or differentiate into stromal cells (Bergfeld and DeClerck, 2010; Spaeth et al., 2009; Torsvik and Bjerkvig, 2013). Thus, MSCs serve as important tools in the study of the stroma-cancer crosstalk and are utilized extensively in our studies.

5.1 Cancer cell invasiveness determine direct intercellular interaction with stromal cells

Recruitment and engraftment of stromal cells, including fibroblasts and MSCs in TME are critical for cancer progression to malignancy and are associated with poor prognosis (Oudin and Weaver, 2016). As the disease become more invasive, the cell adhesion molecules on tumor cell surface are significantly altered and these altered interactions can subsequently modify both initial engraftment and long-term fate of stromal cells (Janiszewska et al., 2020). We elucidated the role of altered adhesion molecule repertoire on stromal cell engraftment with monolayers of cancer cells with varying levels of aggressiveness (McAndrews et al., 2015). Stromal cells were more adherent and spread more readily on more aggressive breast, ovarian, prostate, and taxol resistant cell lines. The aggressive cell lines expressed EMT associated cell-cell adhesion markers cadherin 2 (N-cadherin) and/or cadherin 11 (OB-cadherin) to a different degree. Both of these proteins and especially the OB-cadherin were also expressed by stromal cells. Subsequently blocking cadherin 11 on stromal cells reversed the enhanced adhesion to invasive cancer cells even with the cancer cells with low level of OB-cadherin expression. This suggests that OB-cadherin on stromal cells enabled them to bind to the cancer cells via homotypic (OB-cadherin) or heterotypic (N-cadherin) interaction and can be used as a therapeutic target to abrogate cancer cell-stromal cell interaction. Through the extensive bidirectional crosstalk between cancer cells and stromal cells, there is a feedback loop wherein stromal cell recruitment increases cancer cell invasion and malignancy, which in turn increases stromal cell recruitment.

When stromal cells are initially recruited to the TME, they undergo a multitude of changes due to the close interaction with tumor cells. These cells serve to prime the TME and create a supportive environment for cancer cells, which in turn enhances their invasive and metastatic potential. To understand how tumor cells can modulate MSC behavior in order to induce a CAF phenotype, we must first examine the multitude of factors that they are exposed to upon initial exposure to the TME. MSCs recruited to the tumor are exposed to a wide variety of soluble factors (SFs), including platelet derived growth factor (PDGF), TGF-β1, and the cocktail of pro-migratory molecules released by tumor cells (Fig. 9). To study the

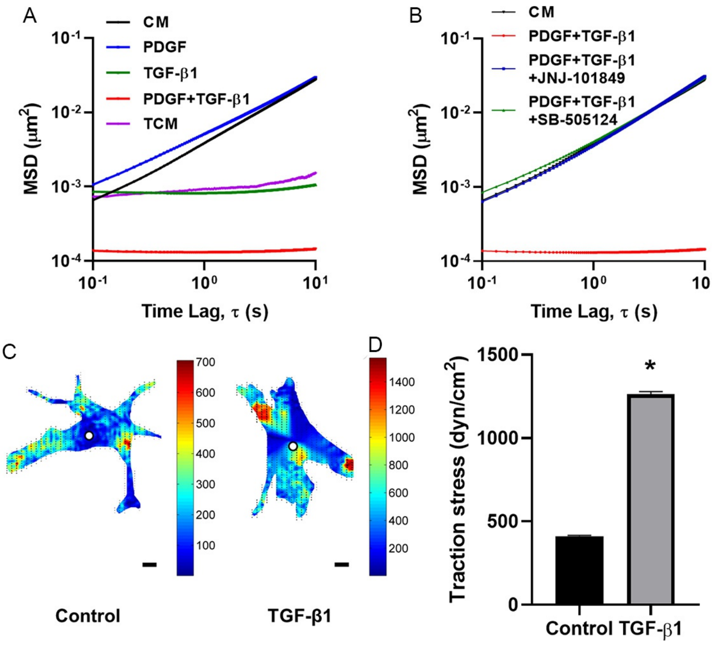


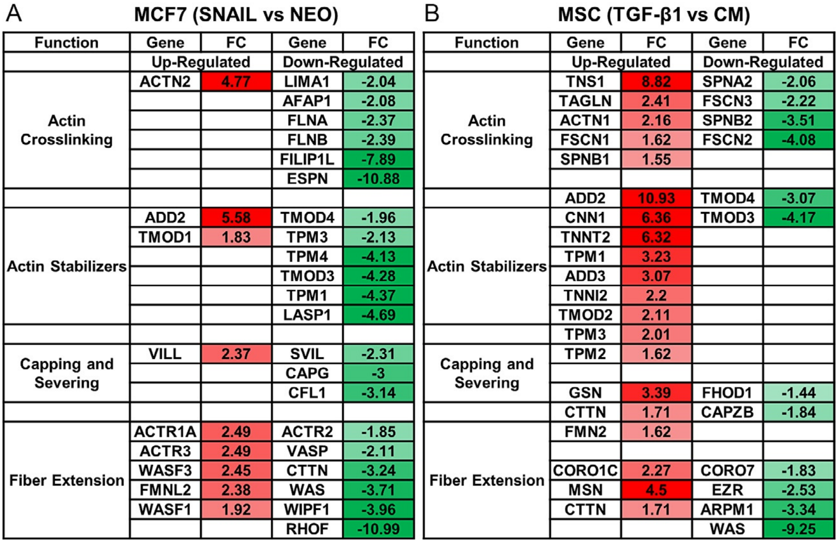
Fig. 9 Tumor-secreted soluble factors (SFs) Alter MSC Mechanics. (A) Time-dependent ensemble average MSDs for MSCs in control media (CM), tumor conditioned media (TCM), or media supplemented with PDGF and/or 5 ng/mL TGF-β1. MSCs stiffen in response to TCM, TGFB1, and the combination of PDGF and TGF-β1, but not PDGF. (B) SF treatment was then combined with small molecule inhibitors of PDGF (JNJ-10198409) and TGF-β1 (SB-505124). Inhibition of these signaling pathways reversed this stiffening response. (C) The mean traction stresses were determined for MSCs pre-treated with CM or CM supplemented with 5 ng/mL TGF-β1 (Scale bar = 10 μm). Treatment with TGF-β1 resulted in higher traction stresses. To calculate statistical significance student's *t*-test was used and *P*-values of less than 0.05 was considered significant (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$) (Dawson et al., 2014; Ghosh et al., 2014; McGrail et al., 2012).

biophysical changes that occur when MSCs are exposed to the tumor-secreted factors, we exposed MSCs to tumor conditioned media in order to simulate the tumor stromal cell paracrine signaling occurring in vivo. We combined biophysical characterization to elucidate the changes in actin structure and mechanical response of MSCs (Ghosh et al., 2014; McGrail et al., 2012).

5.2 MSCs and cancer cells undergo dramatic changes in cell mechanics in response to SF crosstalk

IPTM revealed that MSC treatment with soluble factors in tumor conditioned media (TCM) results in sudden cytoskeletal stiffening (characterized by a change in the slope of the MSD), which completely changed the intracellular mechanical phenotype of MSCs. TCM treatment resulted in increased expression of all Rho GTPases, with dramatic effects on the expression of Cdc42, indicating that this molecule was largely responsible for the altered mechanical response. Based on previous studies, TGF-β1 has been shown to be an important pleiotropic factor that contributes to cytoskeletal stiffening (Nalluri et al., 2015). In addition, it is also an important part of the paracrine signaling molecules within TCM. Indeed, when treating MSCs, TGF-β1 alone was sufficient to induce the biophysical changes observed with TCM. Furthermore, when treated in conjunction with PDGF, TGF-β1 can enhance cell stiffening in MSCs. Like TCM, TGF-β1 alone and in combination with PDGF profoundly increased condensed and elongated microtubules and actin filaments. Although PDGF alone did not result in any significant biophysical changes, the addition of PDGF to TGF-β1 amplified this cellular response, indicating possible interaction between these two signaling pathways. Subsequently, blocking PDGF signaling in TGF-β1 treated cells was enough to abrogate the stiffening, similar to the expected effect of TGF-B receptor inhibitor. This result demonstrates the integral role of PDGF signaling in regulating TGF-β1-mediated cell stiffening and further highlights the complexity of SF interactions in mediating cell mechanics responses. This suggested that TGF-β1 was working in conjunction with various other factors in order to induce the changes. TGF-β1 treated MSCs were also able to generate significantly larger traction force but were unable to polarize them.

Overall, as cancer cells become more aggressive, they become more deformable, while paracrine factors from these aggressive cancer cells make



A MCF7 (SNAIL vs NEO)

Function	Gene	FC	Gene	FC
	Up-Regulated		Down-Regulated	
Actin Crosslinking	ACTN2	4.77	LIMA1	-2.04
			AFAP1	-2.08
			FLNA	-2.37
			FLNB	-2.39
			FILIP1L	-7.89
			ESPN	-10.88
Actin Stabilizers	ADD2	5.58	TMOD4	-1.96
	TMOD1	1.83	TPM3	-2.13
			TPM4	-4.13
			TMOD3	-4.28
			TPM1	-4.37
			LASP1	-4.69
Capping and Severing	VILL	2.37	SVIL	-2.31
			CAPG	-3
			CFL1	-3.14
Fiber Extension	ACTR1A	2.49	ACTR2	-1.85
	ACTR3	2.49	VASP	-2.11
	WASF3	2.45	CTTN	-3.24
	FMNL2	2.38	WAS	-3.71
	WASF1	1.92	WIPF1	-3.96
			RHOF	-10.99

B MSC (TGF-β1 vs CM)

Function	Gene	FC	Gene	FC
	Up-Regulated		Down-Regulated	
Actin Crosslinking	TNS1	8.82	SPNA2	-2.06
	TAGLN	2.41	FSCN3	-2.22
	ACTN1	2.16	SPNB2	-3.51
	FSCN1	1.62	FSCN2	-4.08
	SPNB1	1.55		
	ADD2	10.93	TMOD4	-3.07
Actin Stabilizers	CNN1	6.36	TMOD3	-4.17
	TNNT2	6.32		
	TPM1	3.23		
	ADD3	3.07		
	TNNI2	2.2		
	TMOD2	2.11		
	TPM3	2.01		
Capping and Severing	TPM2	1.62		
	GSN	3.39	FHOD1	-1.44
	CTTN	1.71	CAPZB	-1.84
Fiber Extension	FMN2	1.62		
	CORO1C	2.27	CORO7	-1.83
	MSN	4.5	EZR	-2.53
	CTTN	1.71	ARPM1	-3.34
			WAS	-9.25

Fig. 10 Actin binding protein profile of genetically induced EMT in cancer cells and soluble factor (TGF-β1) treated MSCs (Ghosh et al., 2014; McGrail et al., 2015b).

MSCs less deformable. Whole genome microarrays used to probe for transcriptional differences in for genetically induced EMT in cancer cells and SF treated MSCs showed that the number of differentially expressed ABP genes were significantly altered (Fig. 10). For cancer cells undergoing EMT, actin cross-linking and stabilizing protein genes were down-regulated corroborating with our observation of depolymerized actin and softening of the cytoplasm. Conversely, SF treated MSCs that underwent cytosolic stiffening displayed significant upregulation in crosslinking and stabilizing proteins, along with downregulation of capping and severing proteins. This highlights the incredibly complex crosstalk that occurs within the TME, that collectively enhance the CAF phenotype and promote a microenvironment that favors cancer invasion and metastasis.

6. Conclusions

We have combined quantitative analysis of intracellular mechanics and surface traction forces with analysis of cell fate processes to study the malignant transformation of cancer cells and their interaction with stromal cells. Multivariable analysis is critical in determining the role of mechanical forces in cancer progression and in analyzing heterogeneity in cancer cell populations. This heterogeneity makes it difficult to target and kill all cancer cells. In 3D microenvironments that are non-uniform in structure, such as human tumors and 3D tissue culture models, the ability for invasive cancer cells to respond to gradients in soluble factor and matrix mechanics may further contribute to the heterogeneity in cancer cells. Metastasis is a highly selective process with less than 0.1% of tumor cells capable of forming metastatic tumors. Thus, it is critical to understand how heterogeneity in the primary tumor gives rise to metastatic disease.

7. Experimental challenges and future research efforts

Despite advances in the field of cancer biophysics, such as single-cell biophysical characterization in 3D models and patient samples, additional developments are needed to reproducibly characterize cancer cells in these more complex conditions. More importantly, novel studies and methodologies are needed to characterize and isolate malignant subpopulations of cancer cells. This will allow for a better understanding of cancer cell heterogeneity from biophysical measurements. Finally, developing high-throughput ways of quantifying cancer biophysical properties, while maintaining high spatial and temporal resolution, would increase the accuracy of these measurements.

References

Acerbi, I., Cassereau, L., Dean, I., Shi, Q., Au, A., Park, C., Chen, Y.Y., Liphardt, J., Hwang, E.S., Weaver, V.M., 2015. Human breast cancer invasion and aggression correlates with ECM stiffening and immune cell infiltration. Integr. Biol. (Camb) 7, 1120–1134. https://doi.org/10.1039/c5ib00040h.

Alibert, C., Goud, B., Manneville, J.B., 2017. Are cancer cells really softer than normal cells? Biol. Cell 109, 167–189. https://doi.org/10.1111/boc.201600078.

Baker, E.L., Lu, J., Yu, D., Bonnecaze, R.T., Zaman, M.H., 2010. Cancer cell stiffness: integrated roles of three-dimensional matrix stiffness and transforming potential. Biophys. J. 99, 2048–2057. https://doi.org/10.1016/j.bpj.2010.07.051.

Balkwill, F.R., Capasso, M., Hagemann, T., 2012. The tumor microenvironment at a glance. J. Cell Sci. 125, 5591–5596. https://doi.org/10.1242/jcs.116392.

Bergfeld, S.A., DeClerck, Y.A., 2010. Bone marrow-derived mesenchymal stem cells and the tumor microenvironment. Cancer Metastasis Rev. 29, 249–261. https://doi.org/10.1007/s10555-010-9222-7.

Burrell, R.A., McGranahan, N., Bartek, J., Swanton, C., 2013. The causes and consequences of genetic heterogeneity in cancer evolution. Nature 501, 338–345. https://doi.org/10.1038/nature12625.

Case, A., Brisson, B.K., Durham, A.C., Rosen, S., Monslow, J., Buza, E., Salah, P., Gillem, J., Ruthel, G., Veluvolu, S., Kristiansen, V., Puré, E., Brown, D.C., Sørenmo, K.U., Volk, S.W., 2017. Identification of prognostic collagen signatures and potential therapeutic stromal targets in canine mammary gland carcinoma. PLoS One 12, e0180448. https://doi.org/10.1371/journal.pone.0180448.

Chang, J.M., Moon, W.K., Cho, N., Yi, A., Koo, H.R., Han, W., Noh, D.Y., Moon, H.G., Kim, S.J., 2011. Clinical application of shear wave elastography (SWE) in the diagnosis of benign and malignant breast diseases. Breast Cancer Res. Treat. 129, 89–97. https://doi.org/10.1007/s10549-011-1627-7.

Chin, L., Xia, Y., Discher, D.E., Janmey, P.A., 2016. Mechanotransduction in cancer. Curr. Opin. Chem. Eng. 11, 77–84. https://doi.org/10.1016/j.coche.2016.01.011.

Cho, Y., Park, E.Y., Ko, E., Park, J.S., Shin, J.H., 2016. Recent advances in biological uses of traction force microscopy. Int. J. Precis. Eng. Manuf. 17, 1401–1412. https://doi.org/10.1007/s12541-016-0166-x.

Cifone, M.A., Fidler, I.J., 1981. Increasing metastatic potential is associated with increasing genetic instability of clones isolated from murine neoplasms. Proc. Natl. Acad. Sci. U. S. A. 78, 6949–6952. https://doi.org/10.1073/pnas.78.11.6949.

Conklin, M.W., Eickhoff, J.C., Riching, K.M., Pehlke, C.A., Eliceiri, K.W., Provenzano, P.P., Friedl, A., Keely, P.J., 2011. Aligned collagen is a prognostic signature for survival in human breast carcinoma. Am. J. Pathol. 178, 1221–1232. https://doi.org/10.1016/j.ajpath.2010.11.076.

Crocker, J.C., Hoffman, B.D., 2007. Multiple-particle tracking and two-point microrheology in cells. In: Wang, Y.L., Discher, D.E. (Eds.), Methods in Cell Biology. Academic Press, pp. 141–178, https://doi.org/10.1016/S0091-679X(07)83007-X.

D'Alterio, C., Scala, S., Sozzi, G., Roz, L., Bertolini, G., 2020. Paradoxical effects of chemotherapy on tumor relapse and metastasis promotion. Semin. Cancer Biol. 60, 351–361. https://doi.org/10.1016/j.semcancer.2019.08.019.

Daniels, B.R., Masi, B.C., Wirtz, D., 2006. Probing single-cell micromechanics in vivo: the microrheology of *C. elegans* developing embryos. Biophys. J. 90, 4712–4719. https://doi.org/10.1529/biophysj.105.080606.

Dawson, M.R., Tseng, Y., Lee, J.S.H., McAndrews, K.M., 2014. Intracellular particle tracking microrheology. In: Neu, C.P., Genin, G.M. (Eds.), Handbook of Imaging in Biological Mechanics. CRC Press, pp. 381–388, https://doi.org/10.1201/b17566-40.

De Craene, B., Gilbert, B., Stove, C., Bruyneel, E., Van Roy, F., Berx, G., 2005. The transcription factor snail induces tumor cell invasion through modulation of the epithelial cell differentiation program. Cancer Res. 65, 6237–6244. https://doi.org/10.1158/0008-5472.CAN-04-3545.

DuFort, C.C., Paszek, M.J., Weaver, V.M., 2011. Balancing forces: architectural control of mechanotransduction. Nat. Rev. Mol. Cell Biol. 12, 308–319. https://doi.org/10.1038/nrm3112.

Egeblad, M., Nakasone, E.S., Werb, Z., 2010. Tumors as organs: complex tissues that interface with the entire organism. Dev. Cell 18, 884–901. https://doi.org/10.1016/j.devcel.2010.05.012.

Emon, B., Bauer, J., Jain, Y., Jung, B., Saif, T., 2018. Biophysics of tumor microenvironment and cancer metastasis—a mini review. Comput. Struct. Biotechnol. J. 16, 279–287. https://doi.org/10.1016/j.csbj.2018.07.003.

Eyckmans, J., Boudou, T., Yu, X., Chen, C.S., 2011. A hitchhiker's guide to mechanobiology. Dev. Cell 21, 35–47. https://doi.org/10.1016/j.devcel.2011.06.015.

Fei, F., Zhang, D., Yang, Z., Wang, S., Wang, X., Wu, Z., Wu, Q., Zhang, S., 2015. The number of polyploid giant cancer cells and epithelial-mesenchymal transition-related proteins are associated with invasion and metastasis in human breast cancer. J. Exp. Clin. Cancer Res. 34, 1–13. https://doi.org/10.1186/s13046-015-0277-8.

Fletcher, D.A., Mullins, R.D., 2010. Cell mechanics and the cytoskeleton. Nature 463, 485–492. https://doi.org/10.1038/nature08908.

Fukumura, D., Jain, R.K., 2007. Tumor microenvironment abnormalities: causes, consequences, and strategies to normalize. J. Cell. Biochem. 101, 937–949. https://doi.org/10.1002/jcb.21187.

Fukumura, D., Duda, D.G., Munn, L.L., Jain, R.K., 2010. Tumor microvasculature and microenvironment: novel insights through intravital imaging in pre-clinical models. Microcirculation 17, 206–225. https://doi.org/10.1111/j.1549-8719.2010.00029.x.

Ghosh, D., Lili, L., McGrail, D.J., Matyunina, L.V., McDonald, J.F., Dawson, M.R., 2014. Integral role of platelet-derived growth factor in mediating transforming growth factor-β 1-dependent mesenchymal stem cell stiffening. Stem Cells Dev. 23, 245–261. https://doi.org/10.1089/scd.2013.0240.

Gutierrez, E., Tkachenko, E., Besser, A., Sundd, P., Ley, K., Danuser, G., Ginsberg, M.H., Groisman, A., 2011. High refractive index silicone gels for simultaneous total internal reflection fluorescence and traction force microscopy of adherent cells. PLoS One 6, e23807. https://doi.org/10.1371/journal.pone.0023807.

Hale, C.M., Sun, S.X., Wirtz, D., 2009. Resolving the role of actoymyosin contractility in cell microrheology. PLoS One 4, e7054. https://doi.org/10.1371/journal.pone.0007054.

Hall, A., 1998. Rho GTPases and the actin cytoskeleton. Science 279, 509–514. https://doi.org/10.1126/science.279.5350.509.

Hanahan, D., Weinberg, R.A., Pan, K.H., Shay, J.W., Cohen, S.N., Taylor, M.B., Clarke, N.W., Jayson, G.C., Eshleman, J.R., Nowak, M.A., et al., 2011. Hallmarks of cancer: the next generation. Cell 144, 646–674. https://doi.org/10.1016/j.cell.2011.02.013.

Haynes, J., Srivastava, J., Madson, N., Wittmann, T., Barber, D.L., 2011. Dynamic actin remodeling during epithelial-mesenchymal transition depends on increased moesin expression. Mol. Biol. Cell 22, 4750–4764. https://doi.org/10.1091/mbc.E11-02-0119.

Huang, S., Ingber, D.E., 2005. Cell tension, matrix mechanics, and cancer development. Cancer Cell 8, 175–176. https://doi.org/10.1016/j.ccr.2005.08.009.

Humphrey, J.D., Dufresne, E.R., Schwartz, M.A., 2014. Mechanotransduction and extracellular matrix homeostasis. Nat. Rev. Mol. Cell Biol. 15, 802–812. https://doi.org/10.1038/nrm3896.

Hur, S.S., Jeong, J.H., Ban, M.J., Park, J.H., Yoon, J.K., Hwang, Y., 2020. Traction force microscopy for understanding cellular mechanotransduction. BMB Rep. 53, 74–81. https://doi.org/10.5483/BMBRep.2020.53.2.308.

Iskratsch, T., Wolfenson, H., Sheetz, M.P., 2014. Appreciating force and shape-the rise of mechanotransduction in cell biology. Nat. Rev. Mol. Cell Biol. 15, 825–833. https://doi.org/10.1038/nrm3903.

Janiszewska, M., Primi, M.C., Izard, T., 2020. Cell adhesion in cancer: beyond the migration of single cells. J. Biol. Chem. 295, 2495–2505. https://doi.org/10.1074/jbc.REV119.007759.

Joung, J.G., Oh, B.Y., Hong, H.K., Al-Khalidi, H., Al-Alem, F., Lee, H.O., Bae, J.S., Kim, J., Cha, H.U., Alotaibi, M., Cho, Y.B., Hassanain, M., Park, W.Y., Lee, W.Y., 2017. Tumor heterogeneity predicts metastatic potential in colorectal cancer. Clin. Cancer Res. 23, 7209–7216. https://doi.org/10.1158/1078-0432.CCR-17-0306.

Kalluri, R., Weinberg, R.A., 2009. The basics of epithelial-mesenchymal transition. J. Clin. Invest. 119, 1420–1428. https://doi.org/10.1172/JCI39104.

Kang, Y., Siegel, P.M., Shu, W., Drobnjak, M., Kakonen, S.M., Cordón-Cardo, C., Guise, T.A., Massagué, J., 2003. A multigenic program mediating breast cancer metastasis to bone. Cancer Cell 3, 537–549. https://doi.org/10.1016/S1535-6108(03)00132-6.

Karagiannis, G.S., Poutahidis, T., Erdman, S.E., Kirsch, R., Riddell, R.H., Diamandis, E.P., 2012. Cancer-associated fibroblasts drive the progression of metastasis through both paracrine and mechanical pressure on cancer tissue. Mol. Cancer Res. 10, 1403–1418. https://doi.org/10.1158/1541-7786.MCR-12-0307.

Kim, D.-H., Wong, P.K., Park, J., Levchenko, A., Sun, Y., 2009. Microengineered platforms for cell mechanobiology. Annu. Rev. Biomed. Eng. 11, 203–233. https://doi.org/10.1146/annurev-bioeng-061008-124915.

Koch, T.M., Münster, S., Bonakdar, N., Butler, J.P., Fabry, B., 2012. 3D traction forces in cancer cell invasion. PLoS One 7, e33476. https://doi.org/10.1371/journal.pone.0033476.

Kollmannsberger, P., Fabry, B., 2011. Linear and nonlinear rheology of living cells. Annu. Rev. Mat. Res. 41, 75–97. https://doi.org/10.1146/annurev-matsci-062910-100351.

Kostic, A., Lynch, C.D., Sheetz, M.P., 2009. Differential matrix rigidity response in breast cancer cell lines correlates with the tissue tropism. PLoS One 4, e6361. https://doi.org/10.1371/journal.pone.0006361.

Kraning-Rush, C.M., Carey, S.P., Califano, J.P., Smith, B.N., Reinhart-King, C.A., 2011. The role of the cytoskeleton in cellular force generation in 2D and 3D environments. Phys. Biol. 8, 015009. https://doi.org/10.1088/1478-3975/8/1/015009.

Kraning-Rush, C.M., Califano, J.P., Reinhart-King, C.A., Thirumurthi, U., Dembo, M., 2012. Cellular traction stresses increase with increasing metastatic potential. PLoS One 7, e32572. https://doi.org/10.1371/journal.pone.0032572.

Kumar, S., Weaver, V.M., 2009. Mechanics, malignancy, and metastasis: the force journey of a tumor cell. Cancer Metastasis Rev. 28, 113–127. https://doi.org/10.1007/s10555-008-9173-4.

Lachowski, D., Cortes, E., Pink, D., Chronopoulos, A., Karim, S.A., Morton, J.P., Del Río Hernández, A.E., 2017. Substrate rigidity controls activation and durotaxis in pancreatic stellate cells. Sci. Rep. 7, 1–12. https://doi.org/10.1038/s41598-017-02689-x.

Lawson, D.A., Kessenbrock, K., Davis, R.T., Pervolarakis, N., Werb, Z., 2018. Tumour heterogeneity and metastasis at single-cell resolution. Nat. Cell Biol. 20, 1349–1360. https://doi.org/10.1038/s41556-018-0236-7.

Lee, M.C.W., Lopez-Diaz, F.J., Khan, S.Y., Tariq, M.A., Dayn, Y., Vaske, C.J., Radenbaugh, A.J., Kim, H.J., Emerson, B.M., Pourm, N., 2014. Single-cell analyses of transcriptional heterogeneity during drug tolerance transition in cancer cells by RNA sequencing. Proc. Natl. Acad. Sci. U. S. A. 111, E4726–E4735. https://doi.org/10.1073/pnas.1404656111.

Legant, W.R., Miller, J.S., Blakely, B.L., Cohen, D.M., Genin, G.M., Chen, C.S., 2010. Measurement of mechanical tractions exerted by cells in three-dimensional matrices. Nat. Methods 7, 969–971. https://doi.org/10.1038/nmeth.1531.

Legant, W.R., Choi, C.K., Miller, J.S., Shao, L., Gao, L., Betzig, E., Chen, C.S., 2013. Multidimensional traction force microscopy reveals out-of-plane rotational moments about focal adhesions. Proc. Natl. Acad. Sci. U. S. A. 110, 881–886. https://doi.org/10.1073/pnas.1207997110.

Leight, J.L., Drain, A.P., Weaver, V.M., 2017. Extracellular matrix remodeling and stiffening modulate tumor phenotype and treatment response. Annu. Rev. Cancer Biol. 1, 313–334. https://doi.org/10.1146/annurev-cancerbio-050216-034431.

Levental, K.R., Yu, H., Kass, L., Lakins, J.N., Egeblad, M., Erler, J.T., Fong, S.F.T.T., Csiszar, K., Giaccia, A., Weninger, W., Yamauchi, M., Gasser, D.L., Weaver, V.M., 2009. Matrix crosslinking forces tumor progression by enhancing integrin signaling. Cell 139, 891–906. https://doi.org/10.1016/j.cell.2009.10.027.

Li, Y., Schnekenburger, J., Duits, M.H.G., 2009. Intracellular particle tracking as a tool for tumor cell characterization. J. Biomed. Opt. 14, 064005. https://doi.org/10.1117/1.3257253.

Lopez-Sánchez, L.M., Jimenez, C., Valverde, A., Hernandez, V., Peñarando, J., Martinez, A., Lopez-Pedrera, C., Muñoz-Castañeda, J.R., De La Haba-Rodríguez, J.R., Aranda, E., Rodriguez-Ariza, A., 2014. CoCl2, a mimic of hypoxia, induces formation of polyploid giant cells with stem characteristics in colon cancer. PLoS One 9, e99143. https://doi.org/10.1371/journal.pone.0099143.

Lu, P., Weaver, V.M., Werb, Z., 2012. The extracellular matrix: a dynamic niche in cancer progression. J. Cell Biol. 196, 395–406. https://doi.org/10.1083/jcb.201102147.

Luo, H., Tu, G., Liu, Z., Liu, M., 2015. Cancer-associated fibroblasts: a multifaceted driver of breast cancer progression. Cancer Lett. 361, 155–163. https://doi.org/10.1016/j.canlet.2015.02.018.

Lv, H., Shi, Y., Zhang, L., Zhang, D., Liu, G., Yang, Z., Li, Y., Fei, F., Zhang, S., 2014. Polyploid giant cancer cells with budding and the expression of cyclin E, S-phase kinase-associated protein 2, stathmin associated with the grading and metastasis in serous ovarian tumor. BMC Cancer 14, 1–9. https://doi.org/10.1186/1471-2407-14-576.

Malandrino, A., Mak, M., Kamm, R.D., Moeendarbary, E., 2018. Complex mechanics of the heterogeneous extracellular matrix in cancer. Extreme Mech. Lett. 21, 25–34. https://doi.org/10.1016/j.eml.2018.02.003.

Marusyk, A., Almendro, V., Polyak, K., 2012. Intra-tumour heterogeneity: a looking glass for cancer? Nat. Rev. Cancer 12, 323–334. https://doi.org/10.1038/nrc3261.

Mason, T.G., Ganesan, K., Van Zanten, J.H., Wirtz, D., Kuo, S.C., 1997. Particle tracking microrheology of complex fluids. Phys. Rev. Lett. 79, 3282–3285. https://doi.org/10.1103/PhysRevLett.79.3282.

McAndrews, K.M., McGrail, D.J., Quach, N.D., Dawson, M.R., 2014. Spatially coordinated changes in intracellular rheology and extracellular force exertion during mesenchymal stem cell differentiation. Phys. Biol. 11, 056004. https://doi.org/10.1088/1478-3975/11/5/056004.

McAndrews, K.M., Yi, J., McGrail, D.J., Dawson, M.R., 2015. Enhanced adhesion of stromal cells to invasive cancer cells regulated by cadherin 11. ACS Chem. Biol. 10, 1932–1938. https://doi.org/10.1021/acschembio.5b00353.

McGrail, D.J., Ghosh, D., Quach, N.D., Dawson, M.R., 2012. Differential mechanical response of mesenchymal stem cells and fibroblasts to tumor-secreted soluble factors. PLoS One 7, e33248. https://doi.org/10.1371/journal.pone.0033248.

McGrail, D.J., Kieu, Q.M.N., Dawson, M.R., 2014. The malignancy of metastatic ovarian cancer cells is increased on soft matrices through a mechanosensitive Rho-ROCK pathway. J. Cell Sci. 127, 2621–2626. https://doi.org/10.1242/jcs.144378.

McGrail, D.J., Kieu, Q.M.N., Iandoli, J.A., Dawson, M.R., 2015a. Actomyosin tension as a determinant of metastatic cancer mechanical tropism. Phys. Biol. 12, 026001. https://doi.org/10.1088/1478-3975/12/2/026001.

McGrail, D.J., Mezencev, R., Kieu, Q.M.N., McDonald, J.F., Dawson, M.R., 2015b. SNAIL-induced epithelial-to-mesenchymal transition produces concerted biophysical changes from altered cytoskeletal gene expression. FASEB J. 29, 1280–1289. https://doi.org/10.1096/fj.14-257345.

Meacham, C.E., Morrison, S.J., 2013. Tumour heterogeneity and cancer cell plasticity. Nature 501, 328–337. https://doi.org/10.1038/nature12624.

Mishra, P.J., Mishra, P.J., Humeniuk, R., Medina, D.J., Alexe, G., Mesirov, J.P., Ganesan, S., Glod, J.W., Banerjee, D., 2008. Carcinoma-associated fibroblast-like differentiation of human mesenchymal stem cells. Cancer Res. 68, 4331–4339. https://doi.org/10.1158/0008-5472.CAN-08-0943.

Moeendarbary, E., Harris, A.R., 2014. Cell mechanics: principles, practices, and prospects. Wiley Interdiscip. Rev. Syst. Biol. Med. 6, 371–388. https://doi.org/10.1002/wsbm.1275.

Molinie, N., Gautreau, A., 2018. The Arp2/3 regulatory system and its deregulation in cancer. Physiol. Rev. 98, 215–238. https://doi.org/10.1152/physrev.00006.2017.

Munevar, S., Wang, Y., Dembo, M., 2001. Traction force microscopy of migrating normal and H-ras transformed 3T3 fibroblasts. Biophys. J. 80, 1744–1757.

Nalluri, S.M., O'Connor, J.W., Gomez, E.W., 2015. Cytoskeletal signaling in TGFβ-induced epithelial-mesenchymal transition. Cytoskeleton 72, 557–569. https://doi.org/10.1002/cm.21263.

Nerger, B.A., Siedlik, M.J., Nelson, C.M., 2017. Microfabricated tissues for investigating traction forces involved in cell migration and tissue morphogenesis. Cell. Mol. Life Sci. 74, 1819–1834. https://doi.org/10.1007/s00018-016-2439-z.

Niu, N., Mercado-Uribe, I., Liu, J., 2017. Dedifferentiation into blastomere-like cancer stem cells via formation of polyploid giant cancer cells. Oncogene 36, 4887–4900. https://doi.org/10.1038/onc.2017.72.

Oudin, M.J., Weaver, V.M., 2016. Physical and chemical gradients in the tumor microenvironment regulate tumor cell invasion, migration, and metastasis. Cold Spring Harb. Symp. Quant. Biol. 81, 189–205. https://doi.org/10.1101/sqb.2016.81.030817.

Panorchan, P., Lee, J.S.H., Kole, T.P., Tseng, Y., Wirtz, D., 2006. Microrheology and ROCK signaling of human endothelial cells embedded in a 3D matrix. Biophys. J. 91, 3499–3507. https://doi.org/10.1529/biophysj.106.084988.

Parsons, J.T., Horwitz, A.R., Schwartz, M.A., 2010. Cell adhesion: integrating cytoskeletal dynamics and cellular tension. Nat. Rev. Mol. Cell Biol. 11, 633–643. https://doi.org/10.1038/nrm2957.

Patel, A.P., Tirosh, I., Trombetta, J.J., Shalek, A.K., Gillespie, S.M., Wakimoto, H., Cahill, D.P., Nahed, B.V., Curry, W.T., Martuza, R.L., Louis, D.N., Rozenblatt-Rosen, O., Suvà, M.L., Regev, A., Bernstein, B.E., 2014. Single-cell RNA-seq highlights intratumoral heterogeneity in primary glioblastoma. Science 344, 1396–1401. https://doi.org/10.1126/science.1254257.

Petrova, V., Annicchiarico-Petruzzelli, M., Melino, G., Amelio, I., 2018. The hypoxic tumour microenvironment. Oncogene 7, 1–13. https://doi.org/10.1038/s41389-017-0011-9.

Pickup, M.W., Mouw, J.K., Weaver, V.M., 2014. The extracellular matrix modulates the hallmarks of cancer. EMBO Rep. 15, 1243–1253. https://doi.org/10.15252/embr.201439246.

Plotnikov, S.V., Sabass, B., Schwarz, U.S., Waterman, C.M., 2014. High-resolution traction force microscopy. In: Waters, J.C., Wittman, T. (Eds.), Methods in Cell Biology. Academic Press Inc, pp. 367–394, https://doi.org/10.1016/B978-0-12-420138-5.00020-3.

Polyak, K., Weinberg, R.A., 2009. Transitions between epithelial and mesenchymal states: acquisition of malignant and stem cell traits. Nat. Rev. Cancer 9, 265–273. https://doi.org/10.1038/nrc2620.

Quail, D.F., Joyce, J.A., 2013. Microenvironmental regulation of tumor progression and metastasis. Nat. Med. 19, 1423–1437. https://doi.org/10.1038/nm.3394.

Sabass, B., Gardel, M.L., Waterman, C.M., Schwarz, U.S., 2008. High resolution traction force microscopy based on experimental and computational advances. Biophys. J. 94, 207–220. https://doi.org/10.1529/biophysj.107.113670.

Sahai, E., Marshall, C.J., 2002. RHO–GTPases and cancer. Nat. Rev. Cancer 2, 133–142. https://doi.org/10.1038/nrc725.

Samuel, M.S., Lopez, J.I., McGhee, E.J., Croft, D.R., Strachan, D., Timpson, P., Munro, J., Schröder, E., Zhou, J., Brunton, V.G., Barker, N., Clevers, H., Sansom, O.J., Anderson, K.I., Weaver, V.M., Olson, M.F., 2011. Actomyosin-mediated cellular tension drives increased tissue stiffness and β-catenin activation to induce epidermal hyperplasia and tumor growth. Cancer Cell 19, 776–791. https://doi.org/10.1016/j.ccr.2011.05.008.

Sharma, S.V., Lee, D.Y., Li, B., Quinlan, M.P., Takahashi, F., Maheswaran, S., McDermott, U., Azizian, N., Zou, L., Fischbach, M.A., Wong, K.K., Brandstetter, K., Wittner, B., Ramaswamy, S., Classon, M., Settleman, J., 2010. A chromatin-mediated reversible drug-tolerant state in cancer cell subpopulations. Cell 141, 69–80. https://doi.org/10.1016/j.cell.2010.02.027.

Shieh, A.C., 2011. Biomechanical forces shape the tumor microenvironment. Ann. Biomed. Eng. 39, 1379–1389. https://doi.org/10.1007/s10439-011-0252-2.

Sousa-Squiavinato, A.C.M., Rocha, M.R., Barcellos-de-Souza, P., de Souza, W.F., Morgado-Diaz, J.A., 2019. Cofilin-1 signaling mediates epithelial-mesenchymal transition by promoting actin cytoskeleton reorganization and cell-cell adhesion regulation in colorectal cancer cells. Biochim. Biophys. Acta, Mol. Cell Res. 1866, 418–429. https://doi.org/10.1016/j.bbamcr.2018.10.003.

Spaeth, E.L., Dembinski, J.L., Sasser, A.K., Watson, K., Klopp, A., Hall, B., Andreeff, M., Marini, F., 2009. Mesenchymal stem cell transition to tumor-associated fibroblasts contributes to fibrovascular network expansion and tumor progression. PLoS One 4, e4992. https://doi.org/10.1371/journal.pone.0004992.

Spill, F., Reynolds, D.S., Kamm, R.D., Zaman, M.H., 2016. Impact of the physical microenvironment on tumor progression and metastasis. Curr. Opin. Biotechnol. 40, 41–48. https://doi.org/10.1016/j.copbio.2016.02.007.

Steinwachs, J., Metzner, C., Skodzek, K., Lang, N., Thievessen, I., Mark, C., Münster, S., Aifantis, K.E., Fabry, B., 2016. Three-dimensional force microscopy of cells in biopolymer networks. Nat. Methods 13, 171–176. https://doi.org/10.1038/nmeth.3685.

Stevenson, R.P., Veltman, D., Machesky, L.M., 2012. Actin-bundling proteins in cancer progression at a glance. J. Cell Sci. 125, 1073–1079. https://doi.org/10.1242/jcs.093799.

Stroka, K.M., Konstantopoulos, K., 2014. Physical biology in cancer. 4. Physical cues guide tumor cell adhesion and migration. Am. J. Physiol. Cell Physiol. 306, C98–C109. https://doi.org/10.1152/ajpcell.00289.2013.

Sun, B., Fang, Y., Li, Z., Chen, Z., Xiang, J., 2015. Role of cellular cytoskeleton in epithelial-mesenchymal transition process during cancer progression. Biomed. Rep. 3, 603–610. https://doi.org/10.3892/br.2015.494.

Tao, L., Huang, G., Song, H., Chen, Y., Chen, L., 2017. Cancer associated fibroblasts: an essential role in the tumor microenvironment. Oncol. Lett. 14, 2611–2620. https://doi.org/10.3892/ol.2017.6497.

Tilghman, R.W., Cowan, C.R., Mih, J.D., Koryakina, Y., Gioeli, D., Slack-Davis, J.K., Blackman, B.R., Tschumperlin, D.J., Parsons, J.T., 2010. Matrix rigidity regulates cancer cell growth and cellular phenotype. PLoS One 5, e12905. https://doi.org/10.1371/journal.pone.0012905.

Tirosh, I., Izar, B., Prakadan, S.M., Wadsworth, M.H., Treacy, D., Trombetta, J.J., Rotem, A., Rodman, C., Lian, C., Murphy, G., Fallahi-Sichani, M., Dutton-Regester, K., Lin, J.R., Cohen, O., Shah, P., Lu, D., Genshaft, A.S., Hughes, T.K., Ziegler, C.G.K., Kazer, S.W., Gaillard, A., Kolb, K.E., Villani, A.C., Johannessen, C.M., Andreev, A.Y., Van Allen, E.M., Bertagnolli, M., Sorger, P.K., Sullivan, R.J., Flaherty, K.T., Frederick, D.T., Jané-Valbuena, J., Yoon, C.H., Rozenblatt-Rosen, O., Shalek, A.K., Regev, A., Garraway, L.A., 2016. Dissecting the multicellular ecosystem of metastatic melanoma by single-cell RNA-seq. Science 352, 189–196 (80-.). https://doi.org/10.1126/science.aad0501.

Torsvik, A., Bjerkvig, R., 2013. Mesenchymal stem cell signaling in cancer progression. Cancer Treat. Rev. 39, 180–188. https://doi.org/10.1016/j.ctrv.2012.03.005.

Tse, J.M., Cheng, G., Tyrrell, J.A., Wilcox-Adelman, S.A., Boucher, Y., Jain, R.K., Munn, L.L., 2012. Mechanical compression drives cancer cells toward invasive phenotype. Proc. Natl. Acad. Sci. 109, 911–916. https://doi.org/10.1073/pnas.1118910109.

Tseng, Y., Kole, T.P., Wirtz, D., 2002. Micromechanical mapping of live cells by multiple-particle-tracking microrheology. Biophys. J. 83, 3162–3176. https://doi.org/10.1016/S0006-3495(02)75319-8.

Ulrich, T.A., De, E.M., Pardo, J., Kumar, S., 2009. The mechanical rigidity of the extracellular matrix regulates the structure, motility, and proliferation of glioma cells. Cancer Res. 69, 4167–4174. https://doi.org/10.1158/0008-5472.CAN-08-4859.

Vega, F.M., Ridley, A.J., 2008. Rho GTPases in cancer cell biology. FEBS Lett. 582, 2093–2101. https://doi.org/10.1016/j.febslet.2008.04.039.

White-Gilbertson, S., Lu, P., Jones, C.M., Chiodini, S., Hurley, D., Das, A., Delaney, J.R., Norris, J.S., Voelkel-Johnson, C., 2020. Tamoxifen is a candidate first-in-class inhibitor of acid ceramidase that reduces amitotic division in polyploid giant cancer cells—unrecognized players in tumorigenesis. Cancer Med. 9, 3142–3152. https://doi.org/10.1002/cam4.2960.

Whiteside, T.L., 2008. The tumor microenvironment and its role in promoting tumor growth. Oncogene 27, 5904–5912. Nature Publishing Group https://doi.org/10.1038/onc.2008.271.

Winder, S.J., Ayscough, K.R., 2005. Actin-binding proteins. J. Cell Sci. 118, 651–654. https://doi.org/10.1242/jcs.01670.

Wirtz, D., 2009. Particle-tracking microrheology of living cells: principles and applications. Annu. Rev. Biophys. 38, 301–326. https://doi.org/10.1146/annurev.biophys.050708.133724.

Wu, P.H., Gambhir, S.S., Hale, C.M., Chen, W.C., Wirtz, D., Smith, B.R., 2020. Particle tracking microrheology of cancer cells in living subjects. Mater. Today. https://doi.org/10.1016/j.mattod.2020.03.021.

Xuan, B., Ghosh, D., Cheney, E.M., Clifton, E.M., Dawson, M.R., 2018. Dysregulation in actin cytoskeletal organization drives increased stiffness and migratory persistence in polyploidal giant cancer cells. Sci. Rep. 8, 1–13. https://doi.org/10.1038/s41598-018-29817-5.

Ramón y Cajal, S., Sesé, M., Capdevila, C., Aasen, T., De Mattos-Arruda, L., Diaz-Cano, S.J., Hernández-Losa, J., Castellví, J., 2020. Clinical implications of intratumor heterogeneity: challenges and opportunities. J. Mol. Med. 98, 161–177. https://doi.org/10.1007/s00109-020-01874-2.

Yamauchi, M., Gibbons, D.L., Zong, C., Fradette, J.J., Bota-Rabassedas, N., Kurie, J.M., 2020. Fibroblast heterogeneity and its impact on extracellular matrix and immune landscape remodeling in cancer. Matrix Biol. 91–92, 8–18. https://doi.org/10.1016/j.matbio.2020.05.001.

Youk, J.H., Son, E.J., Gweon, H.M., Kim, H., Park, Y.J., Kim, J.A., 2014. Comparison of strain and shear wave elastography for the differentiation of benign from malignant breast lesions, combined with b-mode ultrasonography: qualitative and quantitative assessments. Ultrasound Med. Biol. 40, 2336–2344. https://doi.org/10.1016/j.ultrasmedbio.2014.05.020.

Yue, J., Huhn, S., Shen, Z., 2013. Complex roles of filamin-A mediated cytoskeleton network in cancer progression. Cell Biosci. 3, 7. https://doi.org/10.1186/2045-3701-3-7.

Zhang, S., Mercado-Uribe, I., Xing, Z., Sun, B., Kuang, J., Liu, J., 2014. Generation of cancer stem-like cells through the formation of polyploid giant cancer cells. Oncogene 33, 116–128. https://doi.org/10.1038/onc.2013.96.

Zhitnyak, I.Y., Rubtsova, S.N., Litovka, N.I., Gloushankova, N.A., 2020. Early events in actin cytoskeleton dynamics and E-cadherin-mediated cell-cell adhesion during epithelial-mesenchymal transition. Cell 9, 578. https://doi.org/10.3390/cells9030578.

Zhou, X., Rowe, R.G., Hiraoka, N., George, J.P., Wirtz, D., Mosher, D.F., Virtanen, I., Chernousov, M.A., Weiss, S.J., 2008. Fibronectin fibrillogenesis regulates three-dimensional neovessel formation. Genes Dev. 22, 1231–1243. https://doi.org/10.1101/gad.1643308.