Dependence of Tensional Homeostasis on Cell Type and on Cell–Cell Interactions

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(Received 6 February 2018; accepted 3 May 2018; published online 8 May 2018)

Associate Editor Yu-Li Wang oversaw the review of this article.

Abstract—Introduction—The ability to maintain a homeostatic level of cell tension is essential for many physiological processes. Our group has recently reported that multicellularity is required for tensional homeostasis in endothelial cells. However, other studies have shown that isolated fibroblasts also maintain constant tension over short time scales without the need of cell–cell contacts. Therefore, in this study, our aim was to determine how different cell types regulate tension as isolated cells or in small clustered groupings and to investigate the role of cell–cell adhesion molecules, such as E-cadherin, in this system.

Methods—Micropattern traction force microscopy was used to determine how bovine aortic endothelial cells, bovine vascular smooth muscle cells, mouse embryonic fibroblasts, and human gastric adenocarcinoma cells, with or without cell–cell interactions due to E-cadherin, maintain tensional homeostasis over time. Tension temporal fluctuations in single cells and cell clusters were evaluated.

Results—We found that only endothelial cells require clustering for tensional homeostasis. The same was not verified in fibroblasts or vascular smooth muscle cells. Of relevance, in adenocarcinoma cells, we verified that tensional homeostasis was dependent on the competence of the adhesion molecule E-cadherin at both the single cells and multicellular levels.

Conclusion—These findings indicate that cell–cell contacts may be critical for tensional homeostasis and, potentially, for barrier function of the endothelium. Furthermore, the cell–cell adhesion molecule E-cadherin is an important regulator of tensional homeostasis, even in the absence of cadherin engagement with neighboring cells, which demonstrates its relevance not only as a structural molecule but also as a signaling moiety.

Keywords—Traction force microscopy, E-cadherin, Tensional homeostasis.

INTRODUCTION

Cell contractility is essential to numerous cellular processes including migration, proliferation, and differentiation.^{8,14,34,47} The exact mechanism by which cells apply these forces has been extensively studied and much is known about the proteins and cytoskeletal elements involved, as well as the effect of outside stimuli on cellular traction forces.¹² Nevertheless, little is known about how cells reach and maintain a physiologically preferred level of cytoskeletal contractile prestress or tension. The ability to maintain this preferred tension has been shown to be essential for the preservation of healthy cells and tissues, and loss of the ability to maintain consistent tension may contribute to diseases such as atherosclerosis and cancer.^{7,17,38} We recently provided a first quantitative definition of tensional homeostasis as the ability of cells to maintain a consistent level of tension with low temporal fluctuations. Although this definition does not specify a threshold below which tensional homeostasis is achieved, it does permit quantitative comparison to determine how different factors such as multicellularity contribute to or disrupt tensional homeostasis.

Recent evidence suggests that tensional homeostasis may be cell type-dependent and that differences in the expression of cell–cell and/or cell–matrix adhesion proteins may be a key determinant in the maintenance of tensional homeostasis. Past studies have shown that fibroblasts exhibit tensional homeostasis at the multicellular level, as well as the single cell level, at least over

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short time scales of minutes.^{4,52} In contrast, we recently showed that endothelial cells are only capable of maintaining tensional homeostasis when organized in multicellular clusters and this ability increases as the number of cells in the cluster increases.⁵ This suggests that endothelial cells may require multicellular interactions to maintain a steady level of cytoskeletal tension and that the bonds formed between cells may play an important role in tensional homeostasis. Interestingly, tension fluctuations in monolayers of endothelial cells were recently shown to be predictive of gap formation and loss of barrier function.⁴⁸ In fact, in normal conditions, endothelial cells exist in monolayers, where cell–cell bonds create a multicellular structure that functions as a mechanical barrier and is fundamental for endothelial function.⁵⁴ Thus, there is a growing interest in measuring the dynamics of temporal and spatial variability of intracellular tension and also in how molecules pivotal for cell–cell adhesion may impact this process. These studies prompted the hypothesis that tensional homeostasis may be cell type-dependent and, furthermore, that phenotype-dependent differences in the expression of cell–cell and/or cell–matrix adhesion proteins may be a determining factor in whether single cells can maintain tensional homeostasis.

Strong evidence indicates that both cell–cell and cell–matrix adhesions may play a fundamental role in tensional homeostasis. Cadherins are important cell–cell adhesion molecules that exist in many forms across cell types. These proteins are mechanosensitive, as they have both the ability to sense and adapt to changes in environmental forces and to be used as a mechanical linkage to the cell cytoskeleton, transmitting actomyosin-generated stress to neighboring cells.^{9,22,31,49} In particular, it was demonstrated that stress transmitted across E-cadherin in epithelial clusters can fluctuate substantially during dynamic cell rearrangements, for example after cell division.³⁵ In support of this association between cell–cell adhesion and tensional homeostasis are some diseases, such as cancer, in which tension variability has been described.^{16,45,50}

Many epithelial cancers, such as metastatic breast and gastric cancers, are characterized by downregulation or loss of E-cadherin, the main cadherin molecule expressed in epithelial tissues.^{13,27} Interestingly, it was demonstrated that E-cadherin is under constitutive tension even in the single cell state.³ This finding suggests that E-cadherin does not need cell–cell binding to be mechanically coupled to actomyosin machinery, which could indicate that the presence or absence of E-cadherin may impact tensional homeostasis in epithelial cells, regardless of the presence of neighboring

cells. For this purpose, our study focuses on the effect that the presence of functional E-cadherin in epithelial cells has on tensional homeostasis.

Here, we sought to test the hypothesis that cell phenotype determines whether single cells are capable of attenuating temporal tensional fluctuations analogous to the fluctuation attenuation observed previously in endothelial cell clusters. We tested multiple cell types including bovine aortic endothelial cells, mouse embryonic fibroblasts, and bovine vascular smooth muscle cells. These cells express and depend upon a variety of cell–cell and cell–matrix adhesion molecules in addition to existing in different environments *in vivo* with varying levels of contact with other cells. Since endothelial cells,³⁰ fibroblasts,²⁶ smooth muscle cells, and epithelial cells^{2,29} all express different isoforms of cadherin, a direct comparison of cell–cell interactions of these cells is not possible. To unravel the relevance of cell–cell interactions, we have also evaluated tensional homeostasis in an isogenic model for the molecule. We used gastric adenocarcinoma cells stably expressing E-cadherin and cells without the molecule to model different levels of interactions between cells. Isogenic models of E-cadherin expression have been well characterized by our group.²⁸ Results of our studies demonstrated that the ability of isolated cells to maintain tensional homeostasis is heavily dependent upon cell phenotype. Moreover, the type of the adhesion molecules present in the cells impacts tensional homeostasis even at the single cell level, showing that signaling mediated by those proteins is key for this inside-out biophysical sensing.

MATERIALS AND METHODS

Cell Culture

All cells were maintained in a sterile incubator at 37 °C and 5% CO₂. Bovine aortic endothelial cells (BAECs) and bovine vascular smooth muscle cells (BVSMCs; Cell Applications) were cultured in DMEM with 1 g/L glucose (Corning) supplemented with 10% bovine calf serum (Sigma Aldrich) and 1% antibiotic–antimycotic solution (100×; Sigma Aldrich). BAECs were a generous gift from Dr. Matthew Nugent (University of Massachusetts Lowell). Cells were isolated as described elsewhere.¹⁵ Mouse embryonic fibroblasts (MEFs) were cultured in DMEM with 4.5 g/L glucose (Gibco) supplemented with 10% bovine calf serum and 1% antibiotic–antimycotic solution. The MEF cell line was a kind gift from Dr. Vesa Hytönen and has been previously described.⁵³ Gastric

adenocarcinoma (AGS) cells were stably transfected with a vector encoding the wild-type E-cadherin (E-cad) or with the corresponding empty vector (Mock) using Lipofectamine 2000 (Invitrogen), according to the manufacture procedure. Transfected cells were cultured in RPMI supplemented with 10% fetal bovine serum (Hyclone) and 1% penicillin–streptomycin (10,000 U/mL; Gibco) and maintained under antibiotic resistance to blasticidin (5 μ g/mL; Gibco, Invitrogen). For experiments, 30–40 $\times 10^3$ cells of each cell line were seeded onto a polyacrylamide (PAA) gel patterned with fluorescent fibronectin and allowed to adhere for 14–24 h, depending on cell type. Media was changed 1 h prior to imaging.

Micropatterning

An indirect patterning method was used to create PAA gels with a grid of covalently bound dots composed of extracellular matrix proteins, as previously described.^{40–42} Briefly, gels were patterned with 0.1 mg/mL of isolated fibronectin for experiments with BAECs, MEFs, and BVSMCs. AGS cells were plated onto a similar micropatterned PAA gel, however patterned dots were comprised of a protein mix of fibronectin and vitronectin (MTI GlobalStem), where each were present at 0.125 mg/mL. This combination was shown to be more suitable for adhesion of these cells (data not shown). Both protein concentrations used were well above previously described concentrations of ~ 0.05 mg/mL needed for saturating levels of protein.¹⁸ The pattern was made up of 2 μ m dots at 6 μ m center-to-center separation. The PAA gels had an elastic modulus of $E \approx 6.7$ kPa and a Poisson's ratio of $\nu = 0.445$, as we determined previously.^{40,42}

E-Cadherin Immunofluorescence

AGS cells were grown on glass coverslips for 72 h. Cells were washed in PBS, fixed in ice-cold methanol for 20 min and blocked with 3% BSA in PBS for 30 min. The staining was performed with the E-cadherin mouse monoclonal antibody (BD Biosciences), diluted at 1:300 in the blocking solution for 1 h. The secondary antibody Alexa Fluor 594 goat anti-mouse (1:250, Invitrogen) was applied for 1 h protected from light. Mounting was performed using Vectashield with DAPI (Vector Laboratories). Images were acquired with a Carl Zeiss Apotome Axiovert 200 M Fluorescence Microscope and an Axiocam HRm camera. Processing was carried out with the Zeiss Axion Vision 4.8 software.

Microscopy

Imaging was done using an Olympus IX881 microscope and a Hamamatsu Orca R2 camera controlled using Metamorph software. Cells were placed in an environmental microscope chamber that protect samples from outside light and maintains experimental conditions of 37°C and 5% CO₂. Fluorescent and DIC images were taken at 40 $\times$ magnification every 5 min, for 1 h. Cell number was then confirmed using a NucBlue (Life Technologies) live cell nuclear stain.

Image Processing

Time-lapse fluorescent images were analyzed using a MATLAB (Mathworks) custom script, as previously described.^{40,42} The program determines the displacement vector (**u**) of the geometrical center each patterned dot from its known traction-free position. Gel elastic properties (E and ν) and the micropatterned dot diameter are then used to calculate a corresponding traction force vector (**F**) as follows

$$\mathbf{F} = \frac{\pi E a \mathbf{u}}{2 + \nu - \nu^2} \quad (1)$$

where $a = 1$ μ m is the radius of the micropatterned dot.²⁵

Traction Field Metrics

The magnitude of the contractile moment (M) was applied as a scalar metric of the traction field. At a given time (t), it was calculated as follows⁵

$$M(t) = \sum_{i=1}^K [x_i(t)X_i(t) + y_i(t)Y_i(t)]. \quad (2)$$

Here x and y are the Cartesian components of the position vector of the center of the micropatterned dot, X and Y are the Cartesian components of the corresponding traction force **F** in the substrate plane, and K is the number of dots within a single cell or multicellular cluster. Traction measurements were carried out every 5 min over 1 h. For each image taken, traction forces were adjusted to satisfy mechanical equilibrium, as described previously.⁶ The significance of M is that, for a plane state of stress in the cell/cluster, it is equivalent to the mean normal stress within the cell/cluster times the cell/cluster volume. To the extent that, during the observed time, volumetric changes of cells may be regarded as negligible, M is indicative of the mean internal stress (tension) in the cluster. In order to eliminate cells that lost viability during the course of the 1 h experiment, data from cells presenting a 50% decrease in contractility (measured by

comparing the first time point to the last three) were excluded from analysis.

Quantification of Tensional Homeostasis

Tensional homeostasis was quantified as described by Canović *et al.*⁵ For comparison between time lapses of M and between different cells from distinct cell types, we normalized M with time-average value ($\langle M \rangle$) over the course of the 1-h experiment. As a quantitative measure of tensional homeostasis, we then computed the coefficient of variation (CV) of the normalized contractile moment [$M(t)/\langle M \rangle$] as follows

$$CV = \frac{1}{\langle M \rangle} \sqrt{\frac{1}{13} \sum_{i=1}^{13} [M(t_i) - \langle M \rangle]^2}, \quad (3)$$

where 13 indicates the number of 5-min time intervals within 60-min observation time. Cells were grouped into single cells or clusters containing 3 or more cells. As temporal fluctuations of the traction field decrease, CV approaches 0, which, according to our definition, would correspond to a stable, homeostatic state of cytoskeletal tension.

Statistical Analysis

Because samples did not exhibit a normal distribution, the experimental data was compared using Kolmogorov–Smirnov statistical tests. Significance was established at $p < 0.05$ or $p < 0.1$, as indicated. The relationship between $\langle M \rangle$ and CV for all sample types was studied with a Spearman's rank correlation test with a significance of $p < 0.05$.

RESULTS

To address whether multicellularity was necessary for maintaining tensional homeostasis in different cell types, we have studied the extent of temporal fluctuations of the traction field of BAECs, MEFs and BVSMCs. These measurements included both single cells ($n = 19$ BAECs, $n = 13$ MEFs, and $n = 12$ BVSMCs) and clusters of 3–17 cells ($n = 26$ BAECs, $n = 17$ MEFs, and $n = 8$ BVSMCs). We found that, over the 1 h observation time, $M(t)/\langle M \rangle$ in single BAECs exhibited substantially higher variability than single BVSMCs and MEFs (Figs. 1a, 1c, and 1e). Clusters did not show obvious qualitative differences between the cell types (Figs. 1b, 1d, and 1f).

We next quantified the time-average and the standard deviation values for M and CV for BAECs, MEFs, and BVSMCs, in order to determine whether multicellularity promoted tensional homeostasis

(Fig. 2). We found that $\langle M \rangle$ was higher in clusters than in single cells for all cell types tested (Fig. 2a). This was expected since M is an extensive quantity which depends on the cluster size (see Eq. 2). Thus, we calculated the contractile moment per cell by dividing M by the cluster size (i.e., the number of cells in the cluster). We found that the average contractile moment per cell was not significantly different from $\langle M \rangle$ in single cells of the same cell type (Fig. 2a). By comparing contractility of different cell types, we found that BVSMCs were significantly more contractile than other cell types for both single cells ($p = 0.001$, $p \ll 0.001$ for BAECs and MEFs respectively) and clusters, which is consistent with the literature.⁴⁴

We next compared CV values from single cells vs. multicellular clusters to determine the role of cell–cell interactions in tensional homeostasis (Fig. 2b). In the case of BAECs, a significant decrease in CV was noted when comparing clusters with single cells ($p = 0.0593$), which is consistent with our previous report.⁵ However, both MEFs and BVSMCs demonstrated no significant differences in CV values between single cells and multicellular clusters. Furthermore, both MEFs and BVSMCs had significantly lower values for CV at the single cell level when compared to BAECs ($p = 0.015$ and $p = 0.035$, respectively). Together, these results suggest that BAECs may need to cluster in order to maintain tensional homeostasis, whereas BVSMCs and MEFs may maintain tensional homeostasis regardless their contact with other cells.

E-cadherin immunofluorescence was performed on both AGS cell lines to confirm that AGS Mock cells had negligible levels of E-cadherin and AGS E-cad cells expressed the protein in a manner comparable with other epithelial cell types. Results showed that in transfected AGS cells, E-cadherin is expressed at the plasma membrane, which is consistent with its normal appearance and previous results (Fig. 3).³⁶

Measurements were next carried out on AGS cells in both single cells ($n = 10$ Mock and $n = 13$ E-cad) and clusters of 3–17 cells ($n = 10$ Mock and $n = 17$ E-cad) for both Mock and E-cad AGS cells. Time lapse measurements of $M(t)/\langle M \rangle$ of single Mock and E-cad cells qualitatively suggest that the traction field has higher temporal variability for AGS cells that lack E-cadherin (Fig. 4a, c). We found that single AGS E-cad cells were significantly more contractile than the AGS Mock single cells ($p = 0.042$; Fig. 5a). This finding was surprising, since single cells do not engage neighbors. The same tendency was observed for cell clusters. Interestingly, we did not find a significant decrease in CV values between single cells and cell clusters for either AGS cell line (Fig. 5b). Furthermore, AGS E-cad cells exhibited much less fluctuation, which suggests that E-cadherin may impact tensional homeostasis in

FIGURE 1. Time lapse of normalized contractile moment for endothelial cells, fibroblasts, and smooth muscle cells (a–f). Graphs show changes in normalized contractile moment for endothelial cells (BAECs) (a, b), fibroblast (MEFs) (c, d) and smooth muscle cells (BVSMCs) (e, f) over the course of a one-hour experiment. Both single cells and clusters are shown. For each graph, contractile moment (M) was normalized to its time-average value ($\langle M \rangle$). While the contractile moment of the BAECs varies widely over the course of the experiment, both MEFs and BVSMCs show a decrease in fluctuation. Each color represents a different cell. Representative images of a single cell and four-cell clusters for each type is also shown. Scale bars are 25 μm . All forces shown are in nN.

single cells, although they do not experience cell–cell contact.

Finally, we sought to determine whether fluctuations in M are related to the overall magnitude of M . Plots of CV vs. $\langle M \rangle$ for all cell types, including both single cells and cell clusters, demonstrated an inverse correlation (Spearman correlation, $\rho = -0.3705$, $p \ll 0.001$) in CV as $\langle M \rangle$ increased (Fig. 6). Interestingly, these data demonstrated a statistically ($p \ll 0.001$) significant fit to a power law according to $CV = 1.079\langle M \rangle^{-0.298}$ (Fig. 6). This power law represents an emergent phenomenon that exists globally across multiple cell phenotypes, but not for a single cell type, at least within the limited conditions of substrate ligand and PAA gel elastic properties used here.

DISCUSSION

Homeostasis of tissue tension is a fundamental requirement for normal physiological function, and loss of the ability to maintain steady tissue tension may contribute to numerous diseases.^{7,17,38} It has been speculated that tensional homeostasis is a length scale-

invariant phenomenon that may exist even at the subcellular length scale.^{7,17} In order to understand whether tensional homeostasis begins at the cellular, multicellular, or tissue level, we and others have measured temporal fluctuations of cell traction forces,^{5,52} and we found that endothelial cells were capable of maintaining stable traction only in multicellular clusters. To investigate if this phenomenon is unique to endothelial cells or it occurs across other cell types, we measured traction fluctuations in a variety of cell types in cohorts of single cells and cell clusters. We demonstrated herein the novel finding that the requirement of multicellularity for tensional homeostasis is cell type-dependent. Both smooth muscle cells and fibroblasts maintained a nearly steady level of tension at the single cell level over the 1 h observation time, whereas endothelial cells did not. Interestingly, the difference between the CV of single endothelial cells and clusters is not observed when comparing small clusters to large clusters. In fact, there is not a statistically significant difference between 3- and 4- cell clusters when compared to 8- and 10- cell clusters.

We next tested whether functional cell–cell adhesion molecules contribute to tensional homeostasis in gas-

FIGURE 2. Values of time-averaged contractile moment ($\langle M \rangle$) and coefficient of variation (CV) for endothelial, fibroblasts and smooth muscle cells. Single cells are shown in light grey, clusters in medium grey. Dark grey bars indicate the average contractile moment per cell in clusters. (a) In each cell type there is an increase in contractility of clusters. In addition, smooth muscle cells (BVSMCs) exhibit significantly higher levels of $\langle M \rangle$ than either endothelial cells (BAECs) or fibroblasts (MEFs). (b) BAECs show a significant decrease in CV when clusters of three or more cells are compared to single cells. This does not occur in MEFs or BVSMCs. Single BAECs have significantly higher values for CV than MEFs or BVSMCs. Samples were compared with a Kolmogorov-Smirnov test. * indicates a $p < 0.05$, † indicates $p < 0.1$.

AGS Mock

AGS E-cad

FIGURE 3. Immunofluorescent stains of nuclei (blue) and E-cad (red) in AGS Mock and AGS Ecad cells. The AGS Mock cells display negligible levels of cadherin while the AGS E-cad cells show high levels of E-cadherin localized to the cell membrane.

tric adenocarcinoma cells that either lack or express E-cadherin. Our results revealed that E-cadherin-expressing cells, even in isolation and without cell-cell contact, displayed more stable traction forces than E-

cadherin-negative cells. This indicates that besides a structural function, E-cadherin also has signaling properties namely through the cadherin-integrin pathway or the regulation of the actin cytoskeleton.

FIGURE 4. Time lapse of normalized contractile moment for gastric adenocarcinoma (AGS) cell lines (a–d). For each graph, contractile moment (M) was normalized to its time-average value ($\langle M \rangle$). The cells transfected with E-Cadherin (Ecad) show less fluctuation at both the single cell and multicellular levels (c, d) than AGS cells transfected with a mock vector (Mock) (a, b). Each color represents a different cell. Representative images of a single cell and four-cell clusters for each type is also shown. Scale bars are 20 μm . All forces shown are in nN.

FIGURE 5. Values of time average contractile moment ($\langle M \rangle$) and coefficient of variation (CV) for cadherin positive and negative adenocarcinoma cells. Single cells are shown in light grey, clusters in medium grey. Dark grey bars indicate the average contractile moment per cell in clusters. (a) In each cell type there is an increase in contractility of clusters. Single cells expressing E-cadherin (E-cad) are significantly more contractile than single cells expressing the mock vector (Mock). (b) Cells negative for E-cadherin show a significantly higher value of CV than those expressing E-cadherin. In both Mock and E-cad there is no significant difference between CV of single cells and cell clusters. Samples compared with a Kolmogorov-Smirnov test and $p < 0.05$ was required for significance.

The underlying mechanism resulting in temporal fluctuations of cell contractility in stable environments that lack external perturbations such as stretch or fluid flow remains unknown. We envision two possibilities that could account for traction fluctuations, and both mechanisms could explain cell phenotype-dependency for generation of stable traction. First, traction fluctuations could result from the biophysical process of cell–cell contact breakage, and subsequent remodeling of the actomyosin structure, which is required to

maintain applied tension to the extracellular matrix and neighboring cells. These cell–cell bonds occur periodically due to myosin cross bridge breakage from actin or due to reaching the end of the filament, which causes transient drops in tension until the filament forms new bonds with the cytoskeleton.^{19,23} This biophysical explanation of tension fluctuations could depend on cell phenotype due to different properties of actomyosin architecture such as distributions in the length of actin filaments, density and type of actin

FIGURE 6. Coefficient of variation vs. contractile moment relationship for all cell types and all cluster sizes. Coefficient of variation (CV) exhibits a statistically significant decreasing trend with increasing time-averaged contractile moment ($\langle M \rangle$) for all cell types and all cluster sizes (Spearman's rank correlation test, $\rho = -0.3705$, $p < 0.05$). This dependence follows a power law relationship (dashed line).

crosslinkers, as well as myosin filament size and density. Second, the variation in traction fluctuations from one cell type to another could be due to differences in the level and activity of regulators of cell contractility such as RHO kinase and myosin light chain kinase.^{24,39} Future studies that track the activity of these regulators of contractility, for example using FRET systems, with simultaneous measurement of cell traction would provide some insight into the coordination of myosin activity and traction fields.

The requirement of multicellularity for BAEC tensional homeostasis also suggests that intercellular adhesion molecules, such as cadherins, may play an important role. To explore this hypothesis, we have modulated the expression of E-cadherin in an epithelial system and studied tensional dynamics in the presence and in the absence of the molecule. We verified that the exogenous expression of E-cadherin significantly reduces fluctuations in traction field, which is in accordance with previous studies reporting that E-cadherin is under constitutive tension even in the absence of cell-cell interactions.³ A change in the magnitude of the traction forces was also verified: AGS E-cad cells present a significantly higher contractile moment than the AGS Mock cells, and it is possible that this change in contractile moment is also responsible for the change in tensional homeostasis. Past works have shown that there is a cross-talk between cadherin and integrins that can affect cell mechanical behaviors (e.g., motility), as both molecules are coupled to the actin cytoskeleton.^{32,43,51} According to these studies, this crosstalk also affects tension generation,^{32,33} which could serve as a possible explanation for the differences in contractile moment of cells that express E-Cadherin and those that do not.

Recently, evidence has emerged showing that the ability to maintain a stable level of tension increases as cells become more contractile.⁴⁸ To determine if this relationship scales across a broad range of contractile levels and cell phenotypes, we have addressed a possible relation between $\langle M \rangle$ and CV in single cells and cell clusters from the different cell lines included in this study (Fig. 6). Our data demonstrated that both variables were inversely associated and that this relationship was an emergent property of this cellular cohort that was not present if we looked only at each cell type individually. Although power-law dependences are relatively common in studies of cell and tissue mechanics,^{1,10,46} our finding was surprising due to the focus here on fluctuations in active contractility. Miller and colleagues demonstrated that tissue viscoelasticity arises due to the nature of molecular bonds³⁷ since bond lifetimes are distributed according to an inverse power law. Although speculative, it is possible that the power-law relationship found here could result from the formation and breakage of molecular bonds in the actomyosin backbone of cells, including bonds formed between myosin and actin, actin and focal adhesions, and crosslinkers of the actin cytoskeletal network. Remarkably, the data presented herein demonstrated that tensional fluctuations were not due entirely to differences in contractility. If that were the case, we would expect the lowest level of force fluctuation in the smooth muscle cells since they were the most contractile cells tested in this study. In fact, fibroblasts were the cells showing the lowest average CV , despite also having the lowest contractile moment than the endothelial cells, fibroblasts and smooth muscle cells. Furthermore, we did not see any decrease in fluctuation when fibroblasts and smooth muscle cell force was measured in clusters when compared to single cells. A broader range of investigation of a single cell type, for example by adding stimulators or inhibitors of cell contractility to increase the range of $\langle M \rangle$, would be of value to address whether individual cell types also possess a distinct power-law relationship between $\langle M \rangle$ and CV . Substrate stiffness may also be an important determinant of tensional homeostasis as it has been shown to impact both cell contractility and endothelial monolayer integrity,^{11,20,21} and thus future studies should determine if tensional homeostasis in endothelial and epithelial cells requires a sub-critical substrate stiffness even for large, multicellular clusters.

CONCLUSION

The evidence presented in this study shows that the ability of single cells to maintain tensional homeostasis is cell-type dependent. Our previous work have shown

that endothelial cells cannot maintain tensional homeostasis at the single cell level, and instead require multicellular clusters.⁵ The present work shows that both single fibroblasts and smooth muscle cells exhibit much lower tensional fluctuation at the single cell level, and no change is observed when cells are part of a cluster. Remarkably, we found that the presence of E-cadherin induces increased contractility and decreased traction field fluctuations. The impact of E-cadherin on tensional homeostasis is due to its role on cell architecture but also due to its relevance as a signaling-transduction molecule. In addition, we showed for the first time that the cell type is an important determinant in the ability of single cells to maintain tensional homeostasis and this could be explained, in part, by differences in the expression of cell-cell binding molecules.

ACKNOWLEDGMENTS

We thank Dr. Matthew Nugent for the generous gift of BAECs and Dr. Vesa Hytönen for the generous gift of wild type MEF cells. This study was supported by National Science Foundation Grants CEBET-115467 (M. L. Smith) and CMMI-1362922 (D. Stamenović) and by the Boston University Training Program in Quantitative Biology and Physiology. We also acknowledge FEDER funds through the Operational Programme for Competitiveness Factors (COMPETE) and National Funds through the Portuguese Foundation for Science and Technology (FCT), under the projects PTDC/BIM-ONC/0171/2012, PTDC/BIM-ONC/0281/2014 and Post-Doctoral grant SFRH/BPD/87705/2012-JF. We acknowledge the Programa IFCT (FCT Investigator) for funding J. Paredes' research.

CONFLICT OF INTEREST

A Zollinger, H. Xu, J. Figueiredo, J. Paredes, R. Seruca, D. Stamenović, M.L. Smith have no conflicts of interest to disclose.

ETHICAL APPROVAL

This article does not contain any studies involving humans or animals performed by any of the authors.

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