Morphodynamics facilitate cancer cells to navigate 3D extracellular matrix

Christopher Z. Eddy, Helena Raposo, Aayushi Manchanda, Ryan Wong, Fuxin Li & Bo Sun

Cell shape is linked to cell function. The significance of cell morphodynamics, namely the temporal fluctuation of cell shape, is much less understood. Here we study the morphodynamics of MDA-MB-231 cells in type I collagen extracellular matrix (ECM). We systematically vary ECM physical properties by tuning collagen concentrations, alignment, and gelation temperatures. We find that morphodynamics of 3D migrating cells are externally controlled by ECM mechanics and internally modulated by Rho/ROCK-signaling. We employ machine learning to classify cell shape into four different morphological phenotypes, each corresponding to a distinct migration mode. As a result, we map cell morphodynamics at mesoscale into the temporal evolution of morphological phenotypes. We characterize the mesoscale dynamics including occurrence probability, dwell time and transition matrix at varying ECM conditions, which demonstrate the complex phenotype landscape and optimal pathways for phenotype transitions. In light of the mesoscale dynamics, we show that 3D cancer cell motility is a hidden Markov process whereby the step size distributions of cell migration are coupled with simultaneous cell morphodynamics. Morphological phenotype transitions also facilitate cancer cells to navigate non-uniform ECM such as traversing the interface between matrices of two distinct microstructures. In conclusion, we demonstrate that 3D migrating cancer cells exhibit rich morphodynamics that is controlled by ECM mechanics, Rho/ROCK-signaling, and regulate cell motility. Our results pave the way to the functional understanding and mechanical programming of cell morphodynamics as a route to predict and control 3D cell motility.

Shape defines the cell. In the 1677 book Micrographia, Robert Hooke showed sections within a herbaceous plant under a microscope. The shape of those sections resembles cells in a monastery, so he named the structures cells. Many breakthroughs followed Hooke’s discovery, from the cell theory of Schwann and Schleiden, to the theory of tissue formation by Remak, Virchow and Kolliker, and the theory of cellularpathologie by Virchow, all of which are inspired by observations of cell shapes, or morphology in general.

In our modern view cell shape is determined by cell function. A nerve cell has long branched protrusions for communication with other neurons; while the cuboidal shape of epithelial cells allow them to tile the surface of organs. Loss of characteristic shape, on the other hand, is associated with functional abnormality. Thus morphological characterization has been an important tool for diagnosis such as in red blood cell disease, neurological disease, and cancer. More recently, cell shape analysis is boosted by techniques from computer vision. As a result, it becomes possible to obtain high content information of cellular states from morphological data alone.

While most research focuses on the static cell morphology, the dynamic fluctuation of cell shape is much less understood. However, shape fluctuation—namely morphodynamics, is of central importance for dynamic cellular functions. The abnormal diffusion of small protrusions—microvilli—on the surface of a T cell allows the T cell to efficiently scan antigen-presenting surfaces. For a migrating cancer cell, morphodynamics drives the motility of the cell in many ways similar to our body frame movements that enable swimming. In fact, just as there are different swimming styles, cancer cells have been observed to execute multiple programs—migration modes—during invasion in 3D tissue space. Each mode has distinct signatures of morphology and morphodynamics, and are usually classified based on cell morphology as filopodial, lamellipodial, lobopodial, blebbing, and actin-enriched leading edge. Cancer cell migration modes is controlled by intracellular signaling such as...

1Department of Physics, Oregon State University, Corvallis, OR 97331, USA. 2Department of Chemical, Biological, and Environmental Engineering, Oregon State University, Corvallis, OR 97331, USA. 3Molecular and Cellular Biology Program, Oregon State University, Corvallis, OR 97331, USA. 4School of Electrical Engineering and Computer Science, Oregon State University, Corvallis, OR 97331, USA. *email: sunb@onid.orst.edu
the Rho-ROCK-myosin pathways19,20, and extracellular factors such as the elasticity, and degradability of the extracellular matrix (ECM)18,21. The ability of a cancer cell to switch between migration modes is important for tumor prognosis. Many therapies, such as MMP inhibitors that target a particular mode of cell motility, fail to stop tumor metastasis largely because cells take other available migration programs22,23.

In this paper, we study the morphodynamics of MDA-MB-231 cells, a highly invasive human breast cancer cell line, in 3D collagen matrices. We devise machine learning techniques to classify cell shapes into morphological phenotypes that correspond to known migration modes. This approach provides a mesoscale mapping of cell morphodynamics into transitions among morphological phenotypes. We find individual cells are capable of rapidly sampling multiple morphological phenotypes, implying spontaneous migration mode transitions. We find ECM mechanics coupled with cell mechanosensing pathways regulate the stability and transition rates between morphological phenotypes. We also find that such transitions facilitate cancer cells to navigate ECM with inherent structural and mechanical heterogeneity. Our results reveal 3D cancer cell migration as a hidden Markov process and morphodynamics contribute to the changes of motility by ECM physical cues.

Results
We find 3D migrating cancer cells demonstrate rapid shape fluctuations (Fig. 1A, B). In order to quantify the cell morphodynamics, we take time-lapse fluorescent images of MDA-MB-231 cells migrating in collagen matrices. The GFP-labeled cells typically stay within the focal depth of the objective lens (20X, NA 0.7) for 10–20 h, while we obtain 2D cell images at a rate of 4 frames per hour (see SI Appendix section S1). After binarization and segmentation, we compute a total of twenty-one geometric measures which collectively quantify the shape of a cell (see SI Appendix section S1). These geometric measures characterize cell size (such as area and perimeter), deviation from circle (such as aspect ratio and form factor), surface topography (such as solidity), and backbone curvature (such as curl—the ratio between the major axis length and skeletonized contour length).

Figure 1. Three-dimensional migration of MDA-MB-231 cells show significant cell shape fluctuation. (A) A typical time lapse recording of 25 h is projected onto a single image with colors representing time. (B) The real space (x–y plane), and shape fluctuations of 3 cells shown in (A). (C) The mean square displacement (σ^2) of selected cell geometric measures. Dots: experimental measurements. Solid lines: linear fit. Dashed lines: 95% prediction interval. Here the form factor is defined as perimeter^2/area. Curl is defined as the ratio between the major axis length and skeletonized contour length. This figure is prepared with Matlab R2020a (www.mathworks.com) and ImageJ (https://imagej.net).
As shown in Fig. 2C, unseen data (15,000 data points) belonging to different morphological phenotypes form dimension of the (21-dimensional) geometric shape space to facilitate visualization of cellular morphodynamics. Following we mainly report the results from SVM algorithm. We also employ the t-SNE algorithm to reduce the overlapping. The SVM classifier particularly has a higher success rate of classifying unseen data (88%). In the other hand, demonstrate elevated actin polymerization that drive sharp protrusions. Neither AE nor BB cells show clear polarization of cytoskeleton and cytoskeleton-associated proteins. In contrast, FP and LA cells exhibit strong cell-ECM adhesions. The filopodial cells consist of distinguishable F-actin bundles extending across the polarized cell body, while the lamellipodial cells feature fan-shaped leading edges of migration. After quantitatively demonstrating the shape fluctuations of migrating cancer cells, we next investigate cell morphodynamics at a mesoscale that allows us to gain insights on cell migration modes. This is possible because different migration modes are associated with distinct characteristic cell morphologies (Fig. 2A). Using 3800 manually labeled single cell images, we have trained machine classifiers that classify cell morphology into four morphological phenotypes based on and named after their corresponding migration modes.

We consider four morphological phenotypes including two amoeboidal ones: actin-enriched leading edge (AE) and small blebbing (BB); as well as two mesenchymal ones: filopodial (FP) and lamellipodial (LA). These morphological phenotypes are associated with known migration modes exhibiting characteristic molecular fingerprints. For instance AE and BB cells do not rely on cell-ECM adhesions during migration. For BB cells, their cortical stress continuously drives the formation of rounded blebs at the cell membrane. Neither AE nor BB cells show clear polarization of cytoskeleton and cytoskeleton-associated proteins. In contrast, FP and LA cells exhibit strong cell-ECM adhesions. The filopodial cells consist of distinguishable F-actin bundles extending across the polarized cell body, while the lamellipodial cells feature fan-shaped leading edges of migration. Once the classifier is trained, morphological phenotypes are determined automatically from a cell image if a particular phenotype receives more than 60% probability score (Fig. 2B). For a small fraction of cells (~10%), none of the four phenotypes receive more than 60% probability score, we consider these cells to be in an intermediate state.

We have trained two classifiers (see SI Appendix S4). The first one is based on support vector machines (SVM) involving 21 geometric measures of binarized cell images. The second one is based on Random-Forest model using the same geometric properties. The two classifiers agree with each other well on test data sets (90% overlapping). The SVM classifier particularly has a higher success rate of classifying unseen data (88%). In the following we mainly report the results from SVM algorithm. We also employ the t-SNE algorithm to reduce the dimension of the (21-dimensional) geometric shape space to facilitate visualization of cellular morphodynamics. As shown in Fig. 2C, unseen data (15,000 data points) belonging to different morphological phenotypes form separable clusters in the embedding space.
By applying the SVM classifier to time lapse recordings of 3D migrating MDA-MB-231 cells we find cells spontaneously make transitions among different morphological phenotypes. Figure 3A shows snapshots of a typical cell. The cell switches directly from blebbing (B) mode to lamellipodial (L) mode via intermediate state (I). Therefore using machine learning technique we map cell morphodynamics into transitions between morphological phenotypes, or their associated migration modes.

In order to understand the mechanisms underlying cell morphological phenotype transitions, we examine the effects of manipulating Rho/ROCK-signaling, which is a master regulator that determines the mechanical state of a cell. Rho/ROCK-signaling controls key aspects of cell morphogenesis and migration, such as actomyosin contractility, actin polymerization, cell–cell and cell-ECM adhesion. By regulating the cell mechanotransduction pathways, Rho/ROCK-signaling has also been shown to control 3D cell migration phenotype plasticity.

We apply Y27632, a potent Rho kinase (ROCK) inhibitor, and CN03, a Rho-activator to MDA-MB-231 cells cultured in collagen ECM (see SI appendix S5). Y27632 reduces actomyosin contractility, promoting transitions from blebbing to mesenchymal phenotypes. On the other hand, CN03 elevates myosin II activity, leading to retraction of filopodia to rounded cell shapes (Fig. 3B). These results are consistent with previous reports on the molecular control of cell migration modes by Rho/ROCK-signaling.

While previous studies focus on the end points of manipulating Rho/ROCK-signaling, morphodynamic analysis offers insights to the transition paths between migration modes. In particular, we take advantage of a modified t-SNE algorithm, which projects a cell image in the embedding space defined by the training sets (Fig. 2C, SI appendix S4). This approach allows us to map the continuous shape change of a cell as a trajectory in the embedded shape space. Similar approaches have also been employed previously in studying complex body movements of other organisms such as fruit flies, where transition paths between different fly behaviors can be visualized.

Tracking the mesoscale morphodynamics of MDA-MB-231 cells under pharmacological perturbations, we find up and down regulation of Rho/ROCK-signaling do not lead to a reversal of morphodynamic trajectories. In particular, when treated with Y27632 blebbing cells turn to filopodial or lamellipodial via strongly converging trajectories most of which first visiting AE states (see also SI appendix S5). Y27632 reduces actomyosin contractility, promoting transitions from blebbing to mesenchymal phenotypes. On the other hand, CN03 elevates myosin II activity, leading to retraction of filopodia to rounded cell shapes (Fig. 3B). These results are consistent with previous reports on the molecular control of cell migration modes by Rho/ROCK-signaling.

Since Rho/ROCK-signaling is employed by cells to sense ECM physical properties, we next investigate the external control of mesoscale cell morphodynamics. In particular, we focus on the role of ECM physical properties in regulating cell morphological phenotype transitions. In order to control the microstructure of collagen...
Figure 4. Physical properties of collagen ECM regulate the morphological phenotype homeostasis of 3D migrating MDA-MB-231 cells. (A–D) Confocal reflection images and pseudo colored MDA-MB-231 cells for collagen matrices prepared at varying conditions. Scale bars: 20µm. A Collagen ECM prepared at room temperature (RT, or 25 °C) and collagen concentration of [col] = 1.5 mg/mL. B Collagen ECM prepared at 37 °C and [col] = 1.5 mg/mL. C Collagen ECM prepared at RT and [col] = 3.0 mg/mL. D collagen ECM prepared with flow-aligned collagen fibers. (E) Fraction of cells in each morphological phenotype. 8000 single cell images are analyzed under each ECM condition. (F) Dwell time of cells in each morphological phenotype. Errorbars in (E, F) represent 95% confidence intervals calculated from 1000 bootstrap iterations. (G–J) The transition matrix—morphological phenotype transition rates under varying ECM conditions. G Collagen ECM prepared at room temperature and [col] = 1.5 mg/mL. H Collagen ECM prepared at 37 °C and [col] = 1.5 mg/mL. I Collagen ECM prepared at RT and [col] = 3.0 mg/mL. J Collagen ECM prepared with flow-aligned collagen fibers. Under each ECM condition a total of more than 2000 h of single cell trajectories are analyzed. This figure is prepared with Matlab R2020a (www.mathworks.com) and ImageJ (https://imagej.net).
state than in AE state is consistent with both migratory measurements in vitro and metastasis measurements with the mechanical mechanism of blebbing formation. Blebs form when actomyosin contractility exceeds the AE to FP decreases by 52 percent, and rates from BB to AE decreases by 22 percent (Fig. 4G, H). As a result, we morphological phenotype (Fig. 4E). For instance, as gelation temperature increases from RT to 37 °C, the transition rates also offer insights to understand the ECM-dependence of the fraction of cells in each morphological phenotype (Fig. 4E). For instance, as gelation temperature increases from RT to 37 °C, rates from AE to FP decreases by 52 percent, and rates from BB to AE decreases by 22 percent (Fig. 4G, H). As a result, we observe more blebbing cells and less filopodial cells in collagen matrices prepared at 37 °C. This is consistent with the mechanical mechanism of blebbing formation. Blebs form when actomyosin contractility exceeds the binding between cortical actin and cell membrane. A blebbing cell turns to AE when actin polymerization causes sharp protrusion on the membrane. Our results suggest that homogeneous collagen ECM favors BB phenotype to AE, likely due to the reduced protrusive force associated with actin polymerization.

Conversely, as ECM becomes more anisotropic (Fig. 4G, J), the transition rate from LA to FP increases as much as 27 percent, while rate from AE to BB decrease by 44 percent. Together, these altered rates lead to a significant fraction of blebbing cells turning to filopodial as shown in Fig. 4E. Filopodial protrusions consist of elongated F-actin bundles supported by elevated actin polymerization and cross-linking by Ena/VASP proteins. Our results suggest that the mechanical barrier separating filopodia and blebbing protrusions is too high for actomyosin contractility to overcome directly. Instead, a blebbing cell turning into a filopodial one has to first transform into AE or LA states.

Because the morphological phenotype of a cell is linked to its 3D migration mode, we next investigate if the invasion potential of MDA-MB-231 cells depends on the mesoscale morphodynamics. Due to the short dwell times for each morphological phenotype, we only consider two coarse-grained classes of morphologies: mesenchymal (ME), which consists of FP and LA states; and amoeboidal (AM), which consists of AE and BB states. In particular, we measure for short time scales the step size distributions and for longer time scales the mean square displacement of the cells in randomly aligned collagen matrices gelled at room temperature (Fig. 5).

Interestingly we find the steps are better described by a log-normal, rather than Gaussian distribution. This means that an approximate detailed balance exists among morphological phenotypes. In comparison with other nonequilibrium stationary processes at mesoscale, we speculate morphological phenotype transitions are not gated by active processes such as ATP consumption.

The invasion potential of MDA-MB-231 cells depends on the mesoscale morphodynamics. Due to the short dwell times for each morphological phenotype, we only consider two coarse-grained classes of morphologies: mesenchymal (ME), which consists of FP and LA states; and amoeboidal (AM), which consists of AE and BB states. In particular, we measure for short time scales the step size distributions and for longer time scales the mean square displacement of the cells in randomly aligned collagen matrices gelled at room temperature (Fig. 5).

Interestingly we find the steps are better described by a log-normal, rather than Gaussian distribution due to frequent large steps. Figure 5A shows the mean and variance of the fitting parameters. (A) Figure 5A shows the mean and variance of the fitting parameters. It is clear that the steps in physical space are coupled with the corresponding mesoscale dynamics. For cells that dwell in the amoeboidal class, both mean and variance of the steps are the smallest. Correspondingly, the mean square displacement of amoeboid cells have a small slope, corresponding to an effective diffusivity of 6 μm²/h (for each spatial dimension, Fig. 5B). On the other hand, cells make larger steps when dwell in the mesenchymal class, and the effective diffusivity increases by three-fold to 19 μm²/h. The observed higher motility for cells in the ME state than in AE state is consistent with both migratory measurements in vitro and metastasis measurements in vivo.

For instance, using a mouse breast cancer model, it is shown that inhibition of mesenchymal phenotype by NEDD9-depletion significantly reduces the number of circulating tumor cells.

Our analysis shows that not only it is important to distinguish different morphological phenotypes in studying the motility of cancer cells, but also one may need to take into account of phenotype transitions. We find cell migration steps associated with different class-switching events have distinct statistical distributions (Fig. 5A, also very small (SI appendix S7). This means that an approximate detailed balance exists among morphological phenotypes. In comparison with other nonequilibrium stationary processes at mesoscale, we speculate morphological phenotype transitions are not gated by active processes such as ATP consumption.
Here the spatial frequency of transition (or dwell) events from state $i$ to state $j$ as $R(i \rightarrow j, x)$, which satisfies

$$P(i \rightarrow j, x) = R(i \rightarrow j, x)M(x)$$  \hspace{1cm} (1)$$

Here $P(i \rightarrow j, x)$ is the probability density of observing event $i \rightarrow j$ per unit time (1 hour), and $M(x)$ is the cell density (along x-axis). We use a 1-D Gaussian kernel to estimate $P(i \rightarrow j, x)$ and $M(x)$ (SI appendix S9). As a result, the spatial frequency $R(i \rightarrow j, x)$ represents the likelihood of a cell to undergo a specific type of transition over unit time (1 h) as a function of distance to the interface.

The spatial frequency of dwell events clearly show that while FP state is increasingly stable into the RT layer, LA and BB states are more stable in the 37 °C layer. AE state, on the other hand, is most stable at the interface.

To further quantify the effect of the interface in modulating cell morphodynamics, we calculate spatial frequencies of dwell events (Fig. 6E) and AE-originating transition events (Fig. 6F). We define a coordinate system where the y-axis is along the interface passing $x=0$ (Fig. 6A). This allows us to combine data from multiple repeating experiments where cell locations are seeded randomly. After aligning the coordinates, we define the spatial frequency of transition (or dwell) events from state $i$ to state $j$ as $R(i \rightarrow j, x, y)$, which satisfies

$$P(i \rightarrow j, x, y) = R(i \rightarrow j, x, y)M(x, y)$$  \hspace{1cm} (2)$$

Here $P(i \rightarrow j, x, y)$ is the probability density of observing event $i \rightarrow j$ per unit time (1 hour), and $M(x, y)$ is the cell density (along x-axis). We use a 2-D Gaussian kernel to estimate $P(i \rightarrow j, x, y)$ and $M(x, y)$ (SI appendix S9). As a result, the spatial frequency $R(i \rightarrow j, x, y)$ represents the likelihood of a cell to undergo a specific type of transition over unit time (1 h) as a function of distance to the interface.

The spatial frequency of dwell events clearly show that while FP state is increasingly stable into the RT layer, LA and BB states are more stable in the 37 °C layer. AE state, on the other hand, is most stable at the interface.
(Fig. 6E). Therefore AE state plays a special role in mediating the cell adaptation across distinct ECM layers. Indeed, we find a gradual shift of favorable AE-originating transitions as distance to the interface varies. The frequency of AE to LA events, the main amoeboidal to mesenchymal path, peaks in the RT layer. AE to BB events, which is mainly responsible of enriching blebbing cells, has peak frequency in the 37°C layer. Taken together, we find morphological phenotype transitions and the associated migration mode switching are integral parts of cancer cell invasion and adaptation to complex ECM.

Discussion
In this paper, we report the morphodynamics of MDA-MB-231 cells in type I collagen ECM as a model system of metastatic cancer cells migrating in 3D tissue. MDA-MB-231 cells rapidly change their geometry, exhibiting a subdiffusive random walk in the geometric shape space. This occurs simultaneously with their superdiffusive walks in the real space (Fig. 1).

The biological significance of the morphodynamics is further demonstrated by classifying cell shapes into morphological phenotypes corresponding to different migration programs (Fig. 2). This allows us to study cell morphodynamics at the mesoscale, in terms of morphological phenotype transitions. Utilizing machine learning and visualization techniques, we show that cell morphodynamics is regulated by Rho/ROCK-signaling (Fig. 3), which is a molecular control hub of cell mechanosensing and force generation. It has been shown previously that Rho/Rac signaling regulates the shift between mesenchymal and amoeboidal motility. Our analysis further reveals that instead of favoring a particular mode of motility, perturbations of Rho/ROCK-signaling alter the migration mode transition rates. In particular, down regulating Rho leads to overall amoeboidal-to-mesenchymal transition that routes through AE and LA states. Activation of Rho, on the other hand, leads to strongly fluctuating morphodynamics that enriches blebbing cells. The irreversibility of up and down regulating Rho/ROCK-signaling results suggest a complex phenotype landscape that controls 3D cancer cell motility.

We study morphological phenotype transitions in ECM of distinct physical properties and find ECM microstructure modulates the probabilities, dwell times, and transition rates of morphological phenotypes. Collagen matrices with homogeneous structure, as those prepared at higher temperature, enrich the population of blebbing cells. By comparing the transition matrices, we find the enrichment of blebbing cells is directly related with the reduced transition rate from BB to AE state, and also indirectly contributed by the mesenchymal-to-amoeboidal transition through LA and AE states. Similarly, collagen matrices with structural anisotropy enrich the population of filopodial cells. The enrichment is directly attributed to an increased LA to FP rate, and indirectly contributed by the amoeboidal-to-mesenchymal transition mediated by LA and AE states. These results show that it is possible to execute external control of cell morphodynamics (and the corresponding 3D migration modes) through ECM mechanics. Importantly, taking into account of the phenotype transitions allows us to better predict the outcome of manipulating cell migration mode through ECM physical properties.

In light of the rapid phenotype transitions exhibited by individual cells, 3D cancer cell motility may be considered as a hidden Markov process where each phenotype is associated with characteristic step size distributions (Fig. 5). Specifically, we find steps that occur simultaneously with a phenotype transition have distinct sizes compared with steps that occur while cells dwell in a particular morphological phenotype. This makes morphodynamics a crucial factor in determining the invasive potential of cancer cells. To our knowledge, this aspect has been so far largely overlooked in the literature.

In the lens of a hidden Markov process, morphodynamics may facilitate cancer invasion because phenotype transitions allow cancer cells to search for and commit to a more effective migration program. Using an ECM model consisting of two mechanically distinct layers, we show the cells gradually adjust their morphodynamics as they approach and cross the layer interface (Fig. 6). Therefore morphological phenotype transitions may be essential in cancer cell metastasis by enabling the cells to navigate non-uniform ECM.

The connection between morphological phenotypes and cell migration modes may be further strengthened by incorporating key molecular fingerprints. Using a small data set, we find the machine-classified morphological phenotypes have F-actin structures that are expected from the corresponding migration modes. The plasticity is controlled by the mode transition matrices, rather than a deterministic decision tree. In order to further exploit the information provided by the cell shape fluctuations, future research is needed to decode morphodynamics as a rich body language of cells, and to control morphodynamics as a route of mechanical programming of cell phenotype.

Materials and methods
GFP-labeled MDA-MB-231 cells are purchased from GenTarget Inc and are handled following the vendor’s recommendations. High concentration rat tail Type I collagen solutions are purchased from Corning Inc, and the collagen gels are prepared following standard protocol. Cells and collagen fibers are imaged using Leica SPE confocal microscope with fluorescent and reflection channels simultaneously. A streamline of utilizing ImageJ, Matlab, and Python scripts are developed to analyze raw images. See SI Appendix S1–S10 for details of 3D cell culture, microscopy, pharmacological treatments, and data analysis.
References

1. Hooke, R. Micrographia (The Royal Society, 1665).
2. Mazzarello, P. A unifying concept: The history of cell theory. Nat. Cell Biol. 25, E13–E15 (1999).
3. Maye, E. The Growth of the Biological Thought (Belknap, 1982).
4. Alberts, B. et al. Molecular Biology of the Cell (Garland Science, 2014).
5. Singhvi, R. et al. Engineering cell shape and function. Science 264, 696–698 (1994).
6. Drez-Silva, M., Dao, M., Han, J., Lim, C.-T. & Suresh, S. Shape and biomechanical characteristics of human red blood cells in health and disease. MRS Bull. Mater. Res. Soc. 35, 382–388 (2010).
7. Serrano-Pozo, A., Frosch, M. P., Masliah, E. & Hyman, B. T. Shape and biomechanical characteristics of human red blood cells in health and disease. Cold Spring Harbor Perspect. Med. 1, a006189 (2011).
8. Yin, Z. et al. A screen for morphological complexity identifies regulators of switch-like transitions between discrete cell shapes. Nat. Cell Biol. 15, 860–871 (2013).
9. Wu, P. H. et al. Evolution of cellular morpho-phenotypes in cancer metastasis. Sci. Rep. 5, 865 (2015).
10. Bakal, C., Aach, J., Church, G. & Perrimon, N. Quantitative morphological signatures define local signaling networks regulating cell morphology. Science 316, 1753 (2007).
11. Held, M. et al. Cell cognition: Time-resolved phenotype annotation in high-throughput live cell imaging. Nat. Methods 7, 747–754 (2010).
12. Lam, V. K., Nguyen, T. C., Chung, B. M., Nehmetallah, G. & Raub, C. B. Quantitative assessment of cancer cell morphology and motility using telecentric digital holographic microscopy and machine learning. Cytometry A 93, 334–345 (2017).
13. Caicedo, J. C. et al. Data-analysis strategies for image-based cell profiling. Nat. Methods 14, 849 (2017).
14. Pau, G. et al. Cell Cognition: time-resolved phenotype annotation in high-throughput live cell imaging. BMC Bioinform. 14, 1–10 (2013).
15. Gordonov, S. et al. Time series modeling of live-cell shape dynamics for image-based phenotypic profiling. Integr. Biol. 8, 73–90 (2016).
16. Cai, E. et al. Visualizing dynamic microvillar search and stabilization during ligand detection by t cells. Science 356, eaal31118 (2017).
17. Paul, C. D., Mistriotis, P. & Konstantopoulos, K. Cancer cell motility: Lessons from migration in confined spaces. Nat. Rev. Cancer 17, 131 (2017).
18. Petrie, R. J. & Yamada, K. M. At the leading edge of three-dimensional cell migration. J. Cell Sci. 125, 5917–5926 (2012).
19. Sanz-Moreno, V. et al. Rac activation and inactivation control plasticity of tumor cell movement. Cell 135, 510–523 (2008).
20. Wilkinson, S., Paterson, H. F. & Marshall, C. J. Cdc42-mrck and rho-rock signalling cooperate in myosin phosphorylation and cell invasion. Nat. Cell Biol. 7, 255–261 (2005).
21. Sanz-Moreno, V. & Marshall, C. J. The plasticity of cytoskeletal dynamics underlying neoplastic cell migration. Curr. Opin. Cell Biol. 22, 690–696 (2010).
22. Pavlaki, M. & Zucker, S. Matrix metalloproteinase inhibitors (MMPIS): the beginning of phase ii or the termination of phase iii clinical trials. Cancer Metastasis Rev. 22, 177–203 (2003).
23. Wolf, K. et al. Compensation mechanism in tumor cell migration. J. Cell Biol. 160, 267 (2003).
24. Liu, L. et al. Minimization of thermodynamic costs in cancer cell invasion. Proc. Natl. Acad. Sci. 110, 1686–1691 (2013).
25. Charras, G. & Palach, E. Blebs lead the way: how to migrate without lamellipodia. Nat. Rev. Mol. Cell Biol. 9, 730–736 (2008).
26. Lorentzen, A., Bamber, J., Sadok, A., Elson-Schwab, I. & Marshall, C. J. An ezrin-rich, rigid uropod-like structure directs movement of amoeboid bleeding cells. J. Cell Sci. 124, 1256–1267 (2011).
27. Yamazaki, D., Kurius, S. & Takenawa, T. Regulation of cancer cell motility through actin reorganization. Cancer Sci. 96, 379–386 (2005).
28. Petrie, R. J. & Yamada, K. M. At the leading edge of three-dimensional cell migration. J. Cell Sci. 125, 5917–5926 (2012).
29. Wycoff, J. B., Pinner, S. E., Gschmeissner, S., Condeelis, J. S. & Sahai, E. Rock- and myosin-dependent matrix deformation enables protease-independent tumor-cell invasion in vivo. Curr. Biol. 16, 1515–1523 (2006).
30. Nalbant, P., Hodgson, L., Kraynov, V., Toutchkine, A. & Hahn, K. M. Activation of endogenous cdc42 visualized in living cells. Science 305, 1615–1619 (2004).
31. Trinkaus, J. P. Surface activity and locomotion of fundulus deep cells during blastula and gastrula stages. Dev. Biol. 30, 68–103 (1973).
32. Abercrombie, M., Heaysman, J. E. & Pegrum, S. M. The locomotion of fibroblasts in culture I. movements of the leading edge. Exp Cell Res. 59, 393–398 (1970).
33. Petrie, R. J., Gavara, N., Chadwick, R. S. & Yamada, K. M. Polyolarnegative signaling reveals two distinct modes of 3D cell migration. J. Cell Biol. 197, 439–455 (2012).
34. Petrie, R. J., Harlin, H. M., Korsak, L. T. & Yamada, K. M. Activating the nuclear piston mechanism of 3D migration in tumor cells. J. Cell Biol. 216, 93–100 (2017).
35. Cortes, C. & Vapnik, V. N. Support-vector networks. Mach. Learn. 20, 1–10 (1995).
36. Ben-Hur, A., Horn, D., Siegelmann, H. & Vapnik, V. N. Support vector clustering. J. Mach. Learn. Res. 2, 125–137 (2001).
37. Breiman, L. Random forests. Mach. Learn. 45, 5–32 (2001).
38. Schwartz, M. Rho signalling at a glance. J. Cell Sci. 117, 5457–5458 (2004).
39. Matsubara, M. & Bissell, M. J. Inhibitors of Rho kinase (ROCK) signalling revert the malignant phenotype of breast cancer cells in 3D context. Oncotarget 7, 31602–31622 (2016).
40. Daoud, A., Gopal, U., Kaur, J. & Isaacs, J. S. Molecular and functional crosstalk between extracellular Hsp90 and ephrin A1 signaling. Oncotarget 8, 106807–106819 (2017).
41. Yeung, T. et al. Effects of substrate stiffness on cell morphology, cytoskeletal structure, and adhesion. Cell Motil. Cytoskeleton 60, 24–34 (2005).
42. van der Maaten, L. J. P. Learning a parametric embedding by preserving local structure. Proc. Twelfth Int. Conf. Artif. Intell. Stat. (JMLR W&CP 5, 384–391 (2009).
43. Berman, G. J., Choi, D. M., Bialik, W. & Shaevitz, J. W. Mapping the stereotyped behaviour of freely moving fruit flies. J. R. Soc. Interface 11, 20140672 (2014).
44. Yoshida, K. & Soldati, T. Dissection of amoeboid movement into two mechanically distinct modes. J. Cell Sci. 119, 3833–3844 (2006).
45. Wycoff, J. B., Pinner, S. E., Gschmeissner, S., Condeelis, J. S. & Sahai, E. Rock- and myosin-dependent matrix deformation enables protease-independent tumor-cell invasion in vivo. Curr. Biol. 16, 1515–1523 (2006).
46. Battle, C. et al. Broken detailed balance at mesoscopic scales in active biological systems. Science 352, 604–607 (2016).
47. Tinevez, J.-Y. et al. Role of cortical tension in bleb growth. Proc. Natl. Acad. Sci. 106, 18581–18586 (2009).
48. Petrie, R. J., Gavara, N., Chadwick, R. S. & Yamada, K. M. Nonpolarized signaling reveals two distinct modes of 3D cell migration. *J. Cell Biol.* **197**, 439–455 (2012).
49. Mejillano, M. R. *et al.* Lamellipodial versus filopodial mode of the actin nanomachinery: pivotal role of the filament barbed end. *Cell* **118**, 363–373 (2004).
50. Das, A., Monteiro, M., Barai, A., Kumar, S. & Sen, S. Mmp proteolytic activity regulates cancer invasiveness by modulating integrins. *Sci. Rep.* **7**, 14219 (2017).
51. Jones, R. C. *et al.* Dual targeting of mesenchymal and amoeboid motility hinders metastatic behavior. *Mol. Cancer Res.* **15**, 670–682 (2017).
52. Doyle, A. D. & Yamada, K. M. Mechanosensing via cell-matrix adhesions in 3d microenvironments. *Exp. Cell Res.* **343**, 60–66 (2016).
53. Gupta, P. R. *et al.* Stochastic state transitions give rise to phenotypic equilibrium in populations of cancer cells. *Cell* **146**, 633–644 (2011).

**Acknowledgements**

We thank Prof. Michelle Digman and Prof. Steve Pressé for helpful discussions. We also thank Prof. Willie Rochefort and Conor Harris for help with rheological measurements. The funding for this research results from a Scialog Program sponsored jointly by Research Corporation for Science Advancement and the Gordon and Betty Moore Foundation (award 6790.11). Part of this research was conducted at the Northwest Nanotechnology Infrastructure, a National Nanotechnology Coordinated Infrastructure site at Oregon State University which is supported in part by the National Science Foundation (grant NNCI-1542101) and Oregon State University. C. Eddy and B. Sun are supported by DOD award W81XWH-20-1-0444 (BC190068). B. Sun is also supported by the National Institute of General Medical Sciences award 1R35GM138179 and National Science Foundation award PHY-1844627.

**Author contributions**

B.S. perceived the research. C.E., H.R. and AM performed the experiments. C.E., H.R., R.W., F.L. and B.S. analyzed data. All authors wrote and reviewed the manuscript.

**Competing interests**

The authors declare no competing interests.

**Additional information**

**Supplementary Information** The online version contains supplementary material available at https://doi.org/10.1038/s41598-021-99902-9.

**Correspondence** and requests for materials should be addressed to B.S.

**Reprints and permissions information** is available at www.nature.com/reprints.

**Publisher’s note** Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

**Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article’s Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article’s Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by/4.0/.

© The Author(s) 2021, corrected publication 2021