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TITLE: Decoding the Mechanoregulation of Breast Tumor Organoid Invasion, One Cell at a Time

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14. ABSTRACT

The vast majority of breast cancer deaths are related to metastasis, during which cell migrate and invade surrounding tissue. Attempts to design effective drug treatments for metastasis have largely failed. A major reason for this failure is the plasticity of migrating cancer cells: they are able to rapidly switch between different modes of migration when faced with different extracellular environment. As a consequence, drugs that target a single migration mode will not be effective in stopping metastasis. This plasticity is poorly understood but depends strongly on the mechanical properties of the extracellular matrix (rigidity, fiber alignment, pore size, etc.). In this project, we will carry out quantitative experiments which determine the modes of migration as a function of the extracellular matrix properties, quantify the transitions between migration modes, and determine how the remodeling of the extracellular matrix couples back to the migration mode and mode transitions.

15. SUBJECT TERMS
None listed.

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1. Introduction

We aim to address the overarching challenge to understand why some breast cancers become metastatic. Metastasis is enabled by cell migration during which cancer cells navigate through and negotiate space within the extracellular matrix (ECM). During metastasis, cancer cells can dynamically switch migration modes and these transitions between modes may significantly contribute to the invasive properties of tumors. To directly address the overarching challenge, we hypothesize that bidirectional and mechanical interactions in the cell-ECM system regulate the migration mode switching of breast cancer cells, which ultimately determines the metastatic potential of breast tumors. We will employ a combination of quantitative experiments, automated algorithmic data analysis, and computational modeling. Our project has two specific aims:

Aim 1: To quantify how breast cancer cell migration mode transitions are determined by extracellular matrix properties and mechanotransduction pathways.

Aim 2: To determine how the invasiveness and migration mode transitions of disseminating breast cancer cells depend on collective extracellular matrix remodeling and tumor geometry.

2. Keywords

Cancer, metastasis, migration, morphology, migration modes, modeling

3. Accomplishments

What were the major goals of the project?

The major goals of this project are to quantify how breast cancer cell migration mode transitions are determined by extracellular matrix properties and mechanotransduction pathways and to determine how the invasiveness and migration mode transitions of disseminating breast cancer cells depend on collective extracellular matrix remodeling and tumor geometry.

Aim 1: To quantify how breast cancer cell migration mode transitions are determined by extracellular matrix properties and mechano-transduction pathways

Major Task 1: To quantify the ECM micromechanical control of migrational mode transitions
Milestone of Major Task 1: establish how ECM micromechanical rigidity and anisotropy modulate the migration mode transition rates of breast cancer cells of different subtypes.
Major Task 1 is 100% accomplished.

Major Task 2 To identify main molecular pathways that regulate cell migrational mode transitions
Milestone of Major Task 2: establish how mechanosensing pathways modulate the migration mode transition rates of breast cancer cells. Examine the pathways with different subtypes of breast cancer cells
Major Task 2 is 90% accomplished

Major Task 3: Development of a comprehensive cell motility model
Milestone of Major Task 3: develop a validated cell motility model that can be validated using experimental data and that can generate experimentally testable predictions.
Major Task 3 is 100% accomplished

Aim 2: To determine how the invasiveness and migration mode transitions of disseminating breast cancer cells depend on collective extracellular matrix remodeling and tumor geometry

Major Task 4: To determine individual cell migrational mode transitions in disseminating tumor organoids
Milestone of Major Task 4: establish the spatial-temporal pattern of cancer cell migration mode transitions disseminating from tumor organoids. Test the effects of ECM micromechanics remodeling in modulating cell migration mode transitions in these dissemination processes.
Major Task 4 is 90% accomplished

Major Task 5: Development of a computational model for collective ECM remodeling and tumor organoid invasion

Milestone of Major Task 5: validate an efficient computational model for collective ECM remodeling and tumor organoid invasion

Major Task 5 is 100% accomplished.

What was accomplished under these goals?

Major Task 1: To quantify the ECM micromechanical control of migrational mode transitions

Milestone of Major Task 1: establish how ECM micromechanical rigidity and anisotropy modulate the migration mode transition rates of breast cancer cells of different subtypes.

In one published report, Kim et al, **The mechanics and dynamics of cancer cells sensing noisy 3D contact guidance**, Proceedings of the National Academy of Sciences, 118 (10), 2021, we examined how contact guidance, which is directly linked to the prognosis of cancer patients, modulates cancer cell morphology and motility, and is. Under physiological conditions, particularly in the three-dimensional (3D) extracellular matrix (ECM), the disordered assembly of fibers presents a complex directional bias to the cells. It is unclear how cancer cells respond to these noncoherent contact guidance cues. In this study, we combined quantitative experiments, theoretical analysis, and computational modeling to study the morphological and migrational responses of breast cancer cells to 3D collagen ECM with varying degrees of fiber alignment. We quantified the strength of contact guidance using directional coherence of ECM fibers, and found that stronger contact guidance causes cells to polarize more strongly along the principal direction of the fibers. Furthermore, we found that sensitivity to contact guidance was positively correlated with cell aspect ratio, with elongated cells responding more strongly to ECM alignment than rounded cells. Both experiments and simulations showed that cell–ECM adhesions and actomyosin contractility modulate cell responses to contact guidance by inducing a population shift between rounded and elongated cells. We also found that cells rapidly change their morphology when navigating the ECM, and that ECM fiber coherence modulates cell transition rates between different morphological phenotypes. Taken together, we found that subcellular processes that integrate conflicting mechanical cues determine cell morphology, which predicts the polarization and migration dynamics of cancer cells in 3D ECM.

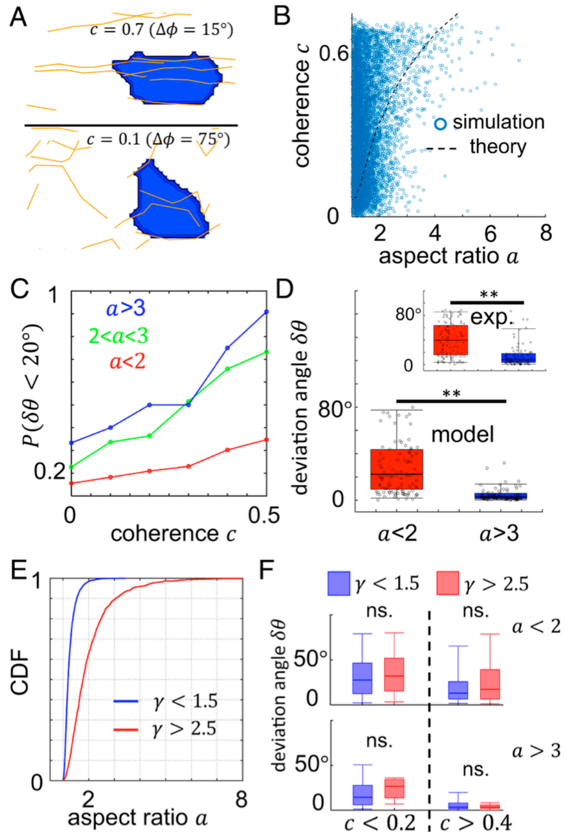

Fig. 1 Model of cell–fiber interactions. (*A*) Two examples of cell morphologies for fiber networks with high (c=0.7, $\Delta\varphi=15^0$) and low (c=0.1, $\Delta\varphi=75^0$) coherence. (*B*) Computed cell aspect ratio and fiber network coherence using 10,000 simulated networks. (*C*) Fraction of well-aligned cells for varying coherence values. Cells are divided into three subgroups based on their aspect ratios : red, 2; green, 2 3; and blue, 3. (*D*) Distribution of cell–fiber deviation angles for cells grouped using their aspect ratio. *Inset*: experimental result. Red, cells with aspect ratio less than two; blue, cells with aspect ratio greater than three. $^{**}P < 0.01$. (*E*) CDF of cell aspect ratio for two different values of the ratio of cell adhesion and cell contractility. (*F*) Distribution of the deviation angles for cells obtained in simulations with low (*Left*) and high (*Right*) coherence, and for cells with low (*Upper*) and high (*Lower*) values of the aspect ratio.

The modeling was carried using a cellular Potts model, shown in Fig. 1. Within this model, we incorporated cell-fiber interactions and varied the coherence of the fiber network. Panel A shows two examples of cell morphologies obtained using a cellular Potts model and fiber networks with high and low coherence. The highly coherent network exhibits fibers that are mostly aligned, while the network with low coherence has many fibers that cross at large angles. To determine how fiber architecture and model parameters affect cell morphology and alignment, we generated a large number of networks with values of that result in a computed coherence that is in the range of the experiments (c < 0.7). The results of simulations are shown in panel B of Fig. 1, where each dot represents the outcome of a single simulation. For fiber networks with low coherence, most cells are found to have aspect ratios close to one, corresponding to rounded shapes. Increasing the coherence, however, results in more and more cells with higher aspect ratio, corresponding to elongated cells. Panel C shows the distribution of cell–fiber deviation angles in the model, which was found to be qualitatively similar to experimental observations. When grouped as rounded and elongated shapes, cells in each group showed significantly different distributions of deviation angles (Panel D), which is consistent with experimental observations (Panel D, *Inset*). We also computed the CDF of the aspect ratio for two different values of the ratio of cell adhesion and cell contractility. We find that increasing this ratio, corresponding to a relative increase of the cell–fiber adhesion, leads to more elongated cells (Panel E). Importantly, as shown in Panel F, varying the ratio does not affect the sensitivity of cells to contact guidance. Thus, our model predicts that the relative strength of the cell–fiber adhesion and cell contractility only indirectly affects the alignment through its change of aspect ratio.

In another published report, Eddy et al, **Morphodynamics facilitate cancer cells to navigate 3D extracellular matrix**, Scientific Reports, 2021, 11:20434, we examined the migration phenotype of breast cancer cells in 3D extracellular matrix. We developed a computer algorithm that quantified the morphology of cells using a set of 18 values such as aspect ratio, solidity and circularity. Based on these shape measures, we trained a machine learning model to classify the cell into four established migration phenotypes based on cell morphology. We showed that breast cancer cells exhibit multiple mode of migration and the transition between these modes are highly dependent on the extracellular matrix (ECM) physical properties. In particular, we showed that collagen matrices with homogeneous structure enriched the population of blebbing cells (BB). The enrichment of blebbing cells was directly related with the reduced transition rate from BB to Actin Enriched Leading Edge (AE) state, and also indirectly contributed by the mesenchymal-to-amoeboidal transition through lamellipodial (LA) and AE states. Similarly, collagen matrices with structural anisotropy enriched the population of filopodial (FP) cells. The enrichment was directly attributed to an increased LA to FP rate, and indirectly contributed by the amoeboidal-to-mesenchymal transition mediated by LA and AE states.

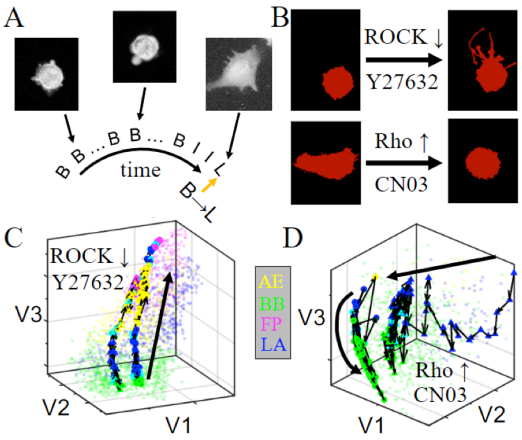

Fig. 2 Rho/ROCK-signaling internally controls mesoscale morphodynamics of 3D cultured breast cancer cells. (A) Sample time series of morphological phenotype. Insets: snapshots showing GFP-labeled cell morphology. Abbr.: B -- blebbing, I -- intermediate state, L -- lamellipodial. (B) Representative morphological changes under treatment of Y27632 and CN03, which downregulates and upregulates Rho/ROCK signaling respectively. (C-D) Morphodynamic trajectories of cells in the t-SNE embedded shape space. Trajectories start after introducing Y27632 or CN03, and ends after 12 hours of drug incubation. Forward time directions shown as thick curves. Two representative trajectories (circular/triangle symbols) per each treatment are shown as colored symbols connected by black lines (color represents the phenotype).

Major Task 2: To identify main molecular pathways that regulate cell migrational mode transitions

Milestone of Major Task 2: establish how mechanosensing pathways modulate the migration mode transition rates of breast cancer cells.

In one published report, Eddy et al, **Morphodynamics facilitate cancer cells to navigate 3D extracellular matrix**, Scientific Reports, 2021, 11:20434, we examined the migration phenotype of breast cancer cells in 3D extracellular matrix. We showed perturbations of

Rho/ROCK-signaling altered the migration mode transition rates (Fig. 2). In particular, down regulating Rho led to overall amoeboidal-to-mesenchymal transition that routed through AE and LA states. Activation of Rho, on the other hand, led to strongly fluctuating morphodynamics that enriched blebbing cells. Taking together, we showed that the migration of cancer cells in 3D ECM was a hidden Markov process, where morphodynamics facilitated cancer invasion because phenotype transitions allowed cancer cells to search for and commit to a more effective migration program in the presence of mechanical heterogeneity of the ECM.

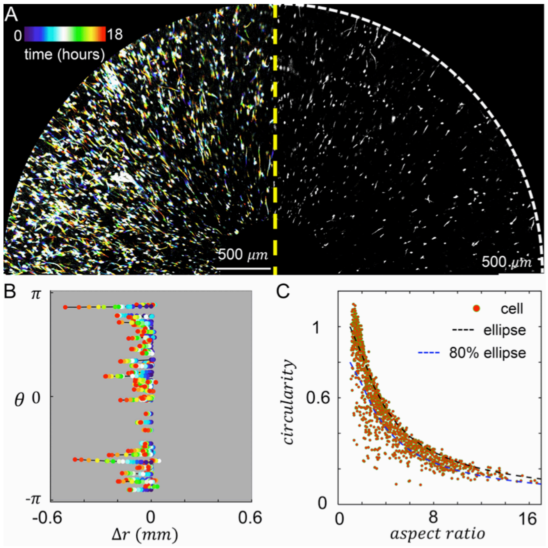

Fig. 3 Combined effects of chemotaxis and contact guidance on breast cancer cell morphology and motility. (A) Left: temporal projected time-lapse imaging of breast cancer cells showing the cell motility. Right: a snapshot at five hours showing the cell morphology. (B) Cell trajectories in the radial (Δr) and azimuthal (θ) coordinates. (C) A scattered plot showing cell aspect ratio and circularity. The black dashed line indicates the circularity of an ellipse at a given aspect ratio. The blue dashed line indicates 80% of circularity corresponding to an ellipse at a given aspect ratio. We empirically consider data points below the blue dashed line as strongly protrusive cells. In (B-C) 70 cells are tracked.

In another published report, Esfahani et al, **Patterning ECM microstructure to investigate 3D cellular dynamics under multiplexed mechanochemical guidance**, F1000Research 2022, 11:1071, we developed a technology to combine chemotaxis and contact guidance cues to study 3D breast cancer cell motility. The device allows generation of well-maintained chemotactic gradient in the radial direction, and collagen fiber alignment in the radial or circumferential direction. The dual guidance therefore will control breast cancer cell migration through cross-talking chemical sensing and mechanical sensing pathways. As shown in Fig. 3**,** when ECM fibers are aligned in parallel with the chemical gradient, cell migration is strongly biased to the radial direction, indicating strengthened chemical and mechanical guidance. Cell morphology, which is indicative of migration phenotype, shows a broad distribution with most cells following the ellipsoidal shape with varying aspect ratios. In particular, a cell with large aspect ratio and low circularity is likely in the lamellipodial (LA) state; a cell with large aspect ratio and large circularity cells is likely in the filopodial (FP) state; a cell with small aspect ratio is likely in the blebbing state (BB).

Major Task 3: Development of a comprehensive cell motility model

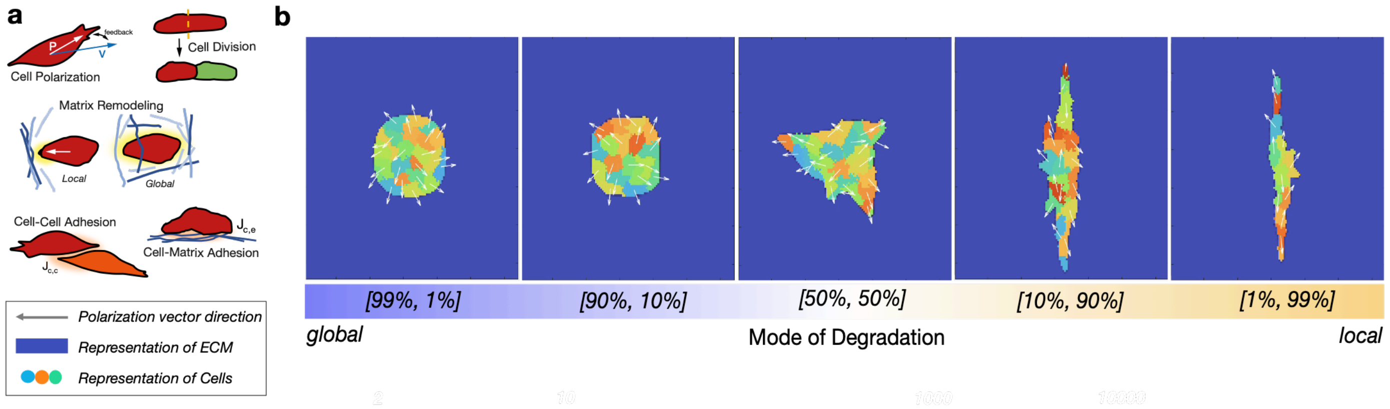

Fig. 4 (a) Schematic representation of the cellular Potts model, which includes cell-cell and cell-ECM interaction terms. (b) Simulated phenotypes of increasing global (left) or local (right) matrix remodeling. Local matrix remodeling results in invasive structures, while global remodeling leas to rotating acinar structures.

We developed a modeling framework, which is able to simulate how cell deformations, cell polarization, cell-cell adhesion, and matrix remodeling can contribute to collective migration in confining ECM conditions (Fig. 4). This framework is based on the cellular Potts Model that relies on minimization of the overall energy of the system using a Hamiltonian. In this Hamiltonian, we incorporated terms for cell motility, cell-ECM interactions, cell proliferation, and cell polarization. In addition, we defined a matrix-remodeling agent that is active either locally in the direction of the polarization vector or globally over the whole cell body. Our simulations showed that in the presence of strong local degradation, migration displays invasive behavior while in the presence of primarily global degradation, cells formed rotating acinar structures.

Major Task 4: To determine individual cell migrational mode transitions in disseminating tumor organoids

In a manuscript under review (Eddy et al, **Facilitating cell segmentation with the Projection-Enhancement Network**), we have developed a deep-learning based method to facilitate segmentation of individual cells at high cell density. The tool allows us to study migration phenotypes of breast cancer cells disseminating from tumor spheroids (Fig. 5). Applying the PEN algorithms, we have studied the motility and migration phenotypes of breast cancer cells disseminating from tumor spheroids. As shown in Fig. 6 we find that breast cancer cells

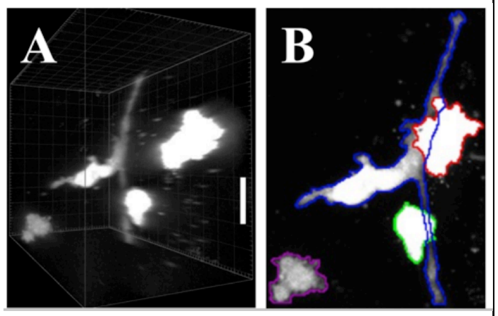

Fig. 5 Closely packed breast cancer cells in 3D ECM (A) can be segmented using our deep-learning based algorithm Projection Enhancement Network (PEN), as shown in (B).

rapidly leave the tumor spheroid and continuously invade in the 3D ECM. During the invasion process, cells make transitions between migration phenotypes. We have quantified the phenotype dynamics by computing the transition rates, which indicates typical transition happens once every two to four hours (Fig. 6C). We also find the breast cancer cell motility depends on the phenotype. In particular, the radial step sizes follow logistic distributions, with the filopodial phenotype being the most invasive (Fig. 6D&E). Furthermore, as time progresses, the number of FP cells found near the spheroid decreases dramatically and is almost zero after 24 hours. The spatial temporal map of phenotype probability of an invading tumor spheroid show that filopodial cells (FP) are enriched at the invasion front (Fig. 7). This implies that FP phenotype emerge as leader cells during the metastasis. Our results underscored the importance of fully characterizing the cancer cell migration phenotype in order to better understand invasion and metastasis.

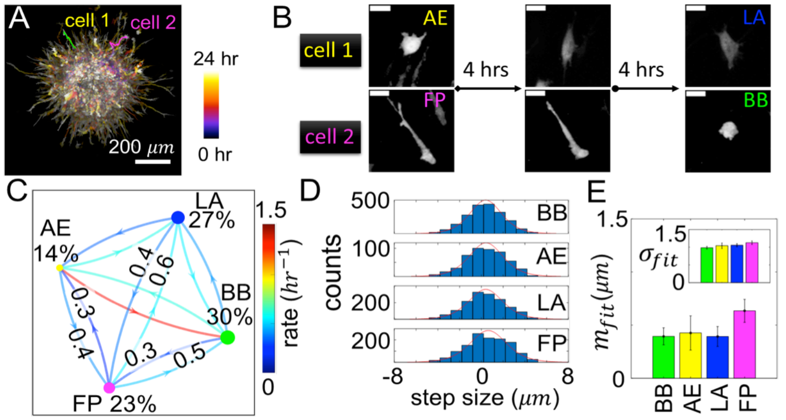

Fig. 6 Breast cancer cells disseminating from tumor spheroids exhibit transitions between different migration phenotypes and phenotype-dependent motility. (A) Temporal projection showing the trajectories of disseminating breast cancer cells. (B) Two representative cells showing phenotype transitions as they invade into the surrounding collagen-based ECM. (C) The transition rates of single breast cancer cells between different phenotypes. (D) Histogram showing the radial step size distribution for each migration phenotype. (E) The histograms can be fitted by Logistic models with means m and variances σ that parameterize the motility.

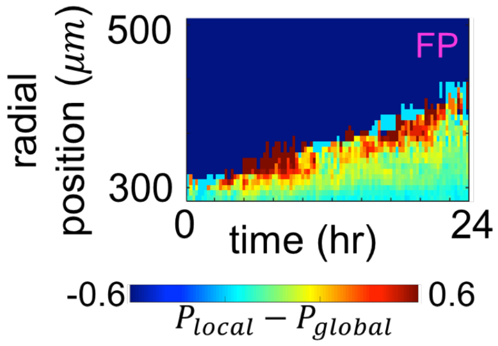

Fig. 7 Spatial-temporal evolution of breast cancer migration phenotype when disseminating from a tumor spheroid. The heatmap represents the elevated filopodial phenotype at the invasion front

Major Task 5: Development of a computational model for collective ECM remodeling and tumor organoid invasion

Motivated by our experimental results, we have developed a stochastic model in which discrete particles, corresponding to the four experimentally observed phenotypes, are introduced into the computational domain at fixed rates. Using experimentally observed transition rates and cell speeds, we computed the fraction of

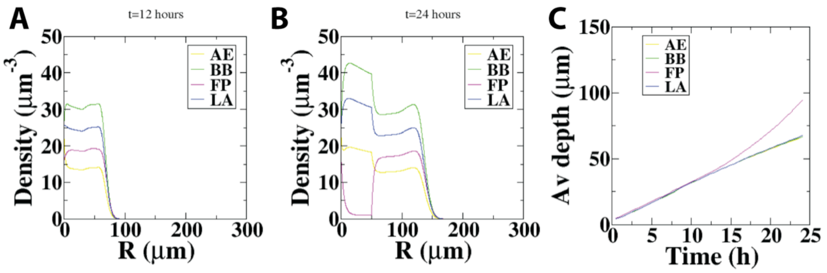

Fig. 8 Results of a model simulation. (A&B) Fraction of the 4 different phenotypes as a function of distance from the particle release point after 12 hours (A) and 24 hours (B). (B) Average invasion depth of the four phenotypes as a function of time.

different phenotypes as a function of the distance from the point of particle flux. We found that this fraction rapidly achieved equilibrium values, independent of the distance from this point. For this reason, we next focused on a deterministic model, in which the four phenotypes were considered to transition and move according to deterministic differential equations.

Using this model, we found that the fraction of FP cells is slightly larger at the leading edge than in the bulk, but at much smaller values than found in the experiments. This suggests that different transition or speed rules apply at this leading edge. We therefore implemented transition rates to the FP phenotype that are density dependent. For this, we assumed that as the density of migrating cells becomes larger, the transition rate to the FP phenotype decreases. The results of this model are shown in Fig. 8 and are consistent with the experiments. Specifically, the number of FP cells close to the organoid decreases dramatically after 12 hours. Furthermore, and as seen in the experiments, the invasion depth of the FP cells increases as time progresses.

What opportunities for training and professional development has the project provided?

As part of this project, Dr. Pedram Esfahani, a graduate student turned to postodoc at Oregon State University was involved in the project. He was trained in conducting live cell experiments, extracellular matrix engineering and data analysis. He was trained in writing and presenting his scientific communication skills that helped him to defend his PhD thesis. Mr. Austin Naylor, currently a graduate student, is also involved in the project. He was trained in mechanical characterization of soft materials, data processing, and scientific presentation.

In addition, Dr. Ghabache, Dr. Karmakar, and Dr. Elmi, post-doctoral researchers at UC San Diego, were supported by this grant and were able to further develop their modeling and analysis skills. Finally, all participants were given the opportunity to improve their presentation skills during our group meetings.

How were the results disseminated to communities of interest?

Aside from the publications listed in 5, Dr. Pedram Esfahani presented the research at invited seminars at the University of Chicago and Loyola University. Dr. Rappel presented results in two invited talks, one at the annual Biophysical Society meeting and one at the annual Society for Mathematical Biology conference. Dr. Sun presented results in two invited seminars, one at Oregon Health and Science University, and another one at the Physical Science Oncology Center of University Pennsylvania. Dr. Eddy and Mr. Esfahani both presented the research at the American Physical Society meeting in 2022. Dr. Eddy additionally presented the research at Allen Institute, and Oregon Health and Science University. Austin Naylor presented the research at Annual Meeting of American Physical Society in 2023. The title of his presentation was “Mechanosensing directs invasion and morphodynamics of spheroids”.

4. Impact

What was the impact on the development of the principal discipline(s) of the project?

Our results, reported in Kim et al, 2021, provide insights into cancer cell metastasis in realistic 3D extracellular matrices (ECMs). The spatial organization of ECM fibers biases the polarization and migration of cancer cells, a phenomenon known as contact guidance, which is directly linked to the clinical outcome of cancers. In physiological conditions, ECM fibers do not align perfectly in parallel. We identify the cell's aspect ratio as an integrated biomarker that determines its sensitivity to contact guidance cues. We also find that the level of ECM alignment modulates transitions between cells of differing morphology. Taken together, we show that cells integrate complex mechanical cues to determine their morphodynamics, thereby controlling polarization and migration in 3D ECM.

In our study (Ghabache et al, 2021), we develop a computational model that can address how motile cells can use and switch between different modes of migration. This model is critically compared to quantitative experimental data, including data from traction force microscopy and fluorescent labeling of actin and myosin.

We show that this model can reproduce the experimental data and can provide further insights into the correlation between signaling and force generation. Specifically, we show that cell motion is critically dependent on the flow of the cytosolic actomyosin network and that friction between this flow and the substrate can generate the required traction for movement.

Our results, reported in Esfahani et al, F1000Research 2022, provide insights into breast cancer cell metastasis in 3D extracellular matrices (ECMs) under physiological conditions where multiple external cues often exist simultaneously. Our results show a method to efficiently combine chemical gradient and ECM fiber alignment in 3D cultures. Our results demonstrate how cells integrate mechanical and chemical cues together to decide on the direction of motility. These results offer a generalizable experimental platform to understand and predict the metastasis of breast cancer cells in complex physiological environments.

Our result, reported in Naylor et al, Soft Matter 2023, provide insights into the ECM remodeling by metastatic breast tumors. Solid tumors, such as breast tumors, often significantly remodel the surrounding ECM. This is an important process that modulate the tumor growth, metastasis, and their interactions with immune cells. Our results show that the various biological functions of breast cancer cells, including active pulling, ECM degradation, and collective contraction each have distinct roles in remodeling the micromechanics of ECM. Breast cancer cells and are known to exhibit multiple mode of migration, which poses significantly challenges to the treatment of metastasis disease. To facilitate studying the processing during multicellular tumor spheroid invasion, we have developed deep-learning based algorithm to segment the cells from confocal imaging. The segmented cells can then be classified into their corresponding migration phenotypes. The work is published in Physical Biology.

In addition, we used a cellular Potts model to investigate the interactions necessary to produce emergent behaviors of individually seeded cancer cells. These cells are observed to form either cylindrical ductal tissues by invasive collective migration (ICM) or spherical acinar tissues by rotational collective migration (RCM). Our model simulations suggest that in 3D confinement, RCM and ICM emerge based on how cells localize their matrix remodeling activity, which can drive differences in cell polarization and protrusion dynamics. Quantitative microscopy experiments confirm that initial differences in cell protrusion length, lifetime, and rate are coupled to distinct matrix remodeling localization patterns that dictate cell shape, which propagates across cell division cycles and gives rise to RCM and ICM behavior. This study has been submitted to Nature Physics.

What was the impact on other disciplines?

Our project is highly muldisciplinary and involves, aside from cancer biology, the field of cell biology, computer vision and mathematical modeling. It will thus have an impact on these disciplines. For cell biology, for example, our experimental studies provide deeper insights how cell morphologies and cell migration are coupled to lead phenotype-dependent invasion. The insights can be applied to other motile cells such as fibroblasts and immune cells. For computer vision, our result provides a practical tool to detect individual cells from 3D image stacks with limited axial resolution. This tool can be applied to essentially any types of image stacks. In addition, our mathematical models are able to address morphological changes in migrating cells as well as the effect of matrix degradation factors. These models should be applicable to a wide variety of cell biology problems.

What was the impact on technology transfer?

Nothing to report

What was the impact on society beyond science and technology?

Nothing to report

5. Changes/Problems

Changes in approach and reasons for change

Nothing to report

Actual or anticipated problems or delays and actions or plans to resolve them

Due to the COVID-19 pandemic, lab operation encountered frequent disruptions while health issues of lab members, staff hiring, facility access, and equipment maintenance were occasionally impacted. This resulted in delays in the research output. However, these issues have been overcome and in year 3, the labs were operating close to normal.

Changes that had a significant impact on expenditures

Nothing to report

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Nothing to report

Significant changes in use or care of human subjects

Nothing to report

Significant changes in use or care of vertebrate animals.

Nothing to report

Significant changes in use of biohazards and/or select agents

Nothing to report

6. Products

Publications, conference papers, and presentations.

Journal publications.

Morphodynamics facilitate cancer cells to navigate 3D extracellular matrix, Christopher z Eddy, Helena Raposo, Aayushi Manchanda, Ryan Wong, Fuxin Li, Bo Sun, Scientific Reports, 2021, 11:20434 *acknowledgement of federal support: yes*

Three-dimensional Cancer Cell Migration Directed by Dual Mechanochemical Guidance, Pedram Esfahani, Herbert Levine*, Mrinmoy Mukherjee, Bo Sun*, Physics Review Research 2022, 4, L022007 *acknowledgement of federal support: yes*

The Mechanics of Fibrillar Collagen Extracellular Matrix, Bo Sun, Cell Reports Physical Science, 2021, 2, 100515 *acknowledgement of federal support: yes*

The mechanics and dynamics of cancer cells sensing noisy 3D contact guidance. Kim, J., Cao, Y., Eddy, C., Deng, Y., Levine, H., Rappel, W. J., & Sun, B. (2021). *Proceedings of the National Academy of Sciences*, *118*(10). *acknowledgement of federal support: yes*

Coupling traction force patterns and actomyosin wave dynamics reveals mechanics of cell motion, Elisabeth Ghabache, Yuansheng Cao, Yuchuan Miao, Alex Groisman, Peter N. Devreotes, and Wouter-Jan Rappel. Molecular systems biology, 17 2021, p.e10505. *acknowledgement of federal support: yes*

Micromechanical Remodeling of the Extracellular Matrix by Invading Tumors: Anisotropy and Heterogeneity, Austin Naylor, Yu Zheng, Yang Jiao* and Bo Sun*, Soft Matter, 2023, 19, 9 – 16 *acknowledgement of federal support: yes*

Patterning ECM microstructure to investigate 3D cellular dynamics under multiplexed mechanochemical guidance, Pedram Esfahani and Bo Sun, F1000Research 2022, 11:1071*acknowledgement of federal support: yes*

Distinct matrix remodeling programs drive divergent cell polarization and collective migration modes,

Sural Ranamukhaarachchim Alyssa Walker, Man-Ho Tang, Sophia Lam, Wouter-Jan Rappel, and Stephanie I. Fraley, Under review. *acknowledgement of federal support: yes*

Facilitating cell segmentation with the Projection-Enhancement Network, Christopher z Eddy, Austin Naylor, Christian Cummingham and Bo Sun, Physical Biology, *20*(6), p.066003. *acknowledgement of federal support: yes*

7. **Participants & Other Collaborating Organizations**

What individuals have worked on the project?

Participants at Oregon State University

Name:	Bo Sun
Project Role:	*PI*
Researcher Identifier (e.g. ORCID ID):	0000-0001-7001-8781
Nearest person month worked:	3
Contribution to Project:	Oversee overall project progress, analyze data, write manuscript, coordinate with collaborating labs
Funding Support:	DOD, NSF, NIH

Name:	Pedram Esfahani
Project Role:	Postdoc Scholar
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	6
Contribution to Project:	conduct experiment, analyze data, write manuscript
Funding Support:	DOD, NIH

Name:	Austin Naylor
Project Role:	Graduate student
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	9
Contribution to Project:	conduct experiment, analyze data, write manuscript
Funding Support:	DOD, NIH

Name:	Christopher Eddy
Project Role:	Graduate student
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	12
Contribution to Project:	conduct experiment, analyze data, write manuscript
Funding Support:	DOD W81XWH-20-1-0445

Name:	Christopher Eddy
Project Role:	Postdoc Scholar

Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	12
Contribution to Project:	conduct experiment, analyze data, write manuscript
Funding Support:	DOD W81XWH-20-1-0445

Rappel personnel

Name:	Wouter-Jan Rappel
Project Role:	Collaborating PI
Researcher Identifier (e.g. ORCID ID):	0000-0003-3833-7197
Nearest person month worked:	3
Contribution to Project:	Dr. Rappel is the collaborating PI on the project and responsible for the modeling efforts
Funding Support:	DOD, NSF, NIH

Name:	Dorsa Elmi
Project Role:	Postdoctoral Scholar
Researcher Identifier (e.g. ORCID ID):	0000-0002-9741-6816
Nearest person month worked:	16
Contribution to Project:	Dr. Elmi was responsible for model development for cells in ECMs with varying composition
Funding Support:	DOD, NSF, NIH

Name:	Elisabeth Ghabache
Project Role:	Postdoctoral Scholar
Researcher Identifier (e.g. ORCID ID):	0000-0001-9832-9354
Nearest person month worked:	3
Contribution to Project:	Dr. Ghabache was responsible for data analysis and model development and the first author of our submitted study
Funding Support:	DOD, NSF, Human Frontiers Program

Name:	Richa Karmakar
Project Role:	Postdoctoral Scholar
Researcher Identifier (e.g. ORCID ID):	0000-0002-9741-6816
Nearest person month worked:	8
Contribution to Project:	Dr. Karmakar was involved in the development of

	the model that can address how cells can use and switch between different migration modes
Funding Support:	DOD, NSF, NIH

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Nothing to report

What other organizations were involved as partners?

We collaborated the project with Dr. Herbert Levine from Northeastern University, Dr. Joe Gray from Oregon Health and Science University, and Dr. Stephanie Fraley from UC San Diego.

8. Special Reporting Requirements

9. Appendices