AWARD NUMBER: W81XWH-20-1-0445

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

PRINCIPAL INVESTIGATOR: Dr. Wouter-Jan Rappel

CONTRACTING ORGANIZATION: The R egents of t he University of California, San Diego

REPORT DATE: SEPTEMBER 2021

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Development Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
SEPTEMBER 2021	Annual	8/15/2020-8/14/2021

4. TITLE AND SUBTITLE

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

5a. CONTRACT NUMBER
W81XWH-20-1-0445

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

Wouter-Jan Rappel

E-Mail:rappel@physics.ucsd.edu

5d. PROJECT NUMBER
0011487651

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

The Regents of the University of
California, San Diego
9500 Gilman Drive
La Jolla, CA 92093

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Development Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

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

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
Unclassified	Unclassified	Unclassified	Unclassified	10	19b. TELEPHONE NUMBER *(include area code)*

TABLE OF CONTENTS

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 60% 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 50% 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 60% 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 3 is yet to begin

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 yet to begin.

What was accomplished under these goals?

During the past reporting period, we made progress towards both specific aims. 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.

In a second study, Ghabache et al, **Coupling traction force patterns and actomyosin wave dynamics reveals mechanics of cell motion**, in second review at Molecular Systems Biology, We used traction force microscopy and fluorescent labeling of actin and myosin to quantify and correlate traction force

patterns and cytoskeletal distributions in eukaryotic cells that move and switch between keratocyte-like fan-shaped, oscillatory, and amoeboid modes. We found that the wave dynamics of the cytoskeletal components critically determine the traction force pattern, cell morphology, and migration mode. Furthermore, we developed a mathematical model that is able to recapitulate the traction force patterns and that uses the experimentally determined spatio-temporal distributions of actin and myosin forces and a viscous cytoskeletal network. Our results suggested that cell motion can be generated by friction between flow of this network and the substrate.

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

As part of this project, Mr. Christopher Eddy, a graduate student at Oregon State University, was trained in carrying out experiments and analyzing data. Furthermore, this project offered him training in writing scientific manuscripts. In addition, Dr. Ghabache and Dr. Karmakar, 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?

One study has been published in a prestigious, peer-reviewed journal (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) while another is currently under second review in Molecular Systems Biology (Ghabache et al, Coupling traction force patterns and actomyosin wave dynamics reveals mechanics of cell motion). Dr. Rappel described the results of these studies in two invited talks, one at the annual Biophysical Society meeting and one at the annual Society for Mathematical Biology conference. Obviously, both conferences were virtual. Dr. Sun described the results of these studies in two virtual invited seminars. One at Oregon Health and Science University, and another one at the Physical Science Oncology Center of University Pennsylvania.

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 recently submitted report (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.

What was the impact on other disciplines?

Our project is highly disciplinary and involves, aside from cancer biology, the field of cell biology and mathematical modeling. It will thus have an impact on these disciplines. For cell biology, for example, our experimental studies provide deeper insights how signaling and external environments can affect cell morphologies and cell migration. These insights should be applicable to other cell migration systems. Furthermore, our mathematical models developed as part of this project can be extended to address different 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, the lab shut down for 4 months, followed by a partial opening with only limited capacity (approximately 30%). This partial opening continues up to the present day. In addition, many research supporting functionality, such as shipping of reagents, staff hiring, facility access, and equipment maintenance were seriously impacted. As such, even with our best effort to address the challenges, we expect delays in the research output.
We are adherent to the local government policies in terms of resumption of research operation. If the pandemic continues to delay the full re-opening of facilities, we expect further and unavoidable delays. However, and hopefully with the pandemic in control, we will resume to normal research activities as soon as it is possible.

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.

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

Elisabeth Ghabache, Yuansheng Cao, Yuchuan Miao, Alex Groisman, Peter N. Devreotes & Wouter-Jan Rappel, Coupling traction force patterns and actomyosin wave dynamics reveals mechanics of cell motion, under review in Molecular Systems Biology, *acknowledgement of federal support: yes*

7. Participants & Other Collaborating Organizations

What individuals have worked on the project?

Participants at University of California San Diego

Name:	Elisabeth Ghabache
Project Role:	Postdoctoral Scholar
Researcher Identifier (e.g. ORCID ID):	0000-0001-9832-9354
Nearest person month worked:	2
Contribution to Project:	Dr. Ghabache is 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:	3
Contribution to Project:	Dr. Karmakar is 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

Name:	Wouter-Jan Rappel
Project Role:	Collaborating PI
Researcher Identifier (e.g. ORCID ID):	0000-0003-3833-7197
Nearest person month worked:	2
Contribution to Project:	Dr. Rappel is the collaborating PI on the project and responsible for the modeling efforts
Funding Support:	DOD, NSF, NIH

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:	1
Contribution to Project:	Oversee overall project progress, analyze data, write manuscript, coordinate with collaborating labs
Funding Support:	DOD, NSF, 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

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. Peter Devreotes from Johns Hopkins University

8. Special Reporting Requirements

9. Appendices